

51° CONGRESSO NAZIONALE

DELLA SOCIETÀ ITALIANA DI MICROBIOLOGIA SIM

CAGLIARI | TEATRO DOGLIO - 24-27 SETTEMBRE 2023



ABSTRACT BOOK

Sommario

01 Approcci diagnostici	125
7 - The application of liquid colony in the sepsis diagnostic workflow: a preliminary overview on paediatric patients	125
Maddalena Calvo ⁽¹⁾ - Giuseppe Migliorisi ⁽¹⁾ - Guido Scalia ⁽¹⁾ - Stefania Stefani ⁽²⁾	125
Azienda Ospedaliera, Azienda Ospedaliera Universitaria Policlinico - San Marco, CATANIA, Italia ⁽¹⁾ - Università Di Catania, Dipartimento Di Scienze Biomediche E Biotecnologiche, CATANIA, Italia ⁽²⁾	125
9 - Extensive virologic characterization in an HIV-infected individual with apparent HIV remission for 2 years after allogeneic stem cell transplantation with CCR5 wild-type cells: a case study.	127
Illaria Vicenti ⁽¹⁾ - Simona Piemontese ⁽²⁾ - Lia Fiaschi ⁽¹⁾ - Camilla Biba ⁽¹⁾ - Adriana Cervo ⁽³⁾ - Ilenia Varasi ⁽¹⁾ - Francesco Saladini ⁽¹⁾ - Giovanna Leonardi ⁽⁴⁾ - Carlo Federico Perno ⁽⁵⁾ - Cristina Mussini ⁽⁶⁾ - Maurizio Zazzi ⁽¹⁾ - Vanni Borghi ⁽⁶⁾	127
University of Siena, Department of Medical Biotechnologies, Siena, Italia ⁽¹⁾ - Hematology and Bone Marrow Transplantation Unit, IRCCS San Raffaele Hospital, Milano, Italia ⁽²⁾ - Infectious Diseases Unit, Department of Health Promotion Sciences and Mother and Child Care "Giuseppe D'Alessandro" (PROMISE), University of Palermo, Palermo, Italia ⁽³⁾ - Hematology Unit and Chair, Department of Medical and Surgical Sciences, Azienda Ospedaliera Universitaria Di Modena, University of Modena and Reggio Emilia, Modena, Modena, Italia ⁽⁴⁾ - Multimodal Research Area, Unit of Microbiology and Diagnostics in Immunology, Bambino Gesù Children's Hospital, IRCCS, Rome, Rome, Italia ⁽⁵⁾ - UO of infectious Diseases, University of Modena and Reggio Emilia, Modena, Italia ⁽⁶⁾	127
16 - Characterization and classification of diagnostically unidentified mycobacteria by de novo sequencing	129
Giulia Gatti ⁽¹⁾ - Francesca Taddei ⁽²⁾ - Silvia Zannoli ⁽²⁾ - Giorgio Dirani ⁽²⁾ - Anna Marzucco ⁽²⁾ - Maria Sofia Montanari ⁽²⁾ - Irene Zaghi ⁽²⁾ - Martina Brandolini ⁽¹⁾ - Laura Grumiro ⁽²⁾ - Massimiliano Guerra ⁽²⁾ - Alessandra Mistral De Pascali ⁽¹⁾ - Monica Cricca ⁽¹⁾ - Vittorio Sambri ⁽¹⁾	129
Alma Mater Studiorum - University of Bologna, DIMEC - Department of Medical and Surgical Sciences, Bologna, Italia ⁽¹⁾ - The Great Romagna Hub Laboratory, O.U. Microbiology, Cesena, Italia ⁽²⁾	129
Characterization and classification of diagnostically unidentified mycobacteria by de novo sequencing	129
GIULIA GATTI, FRANCESCA TADDEI, SILVIA ZANNOLI, GIORGIO DIRANI, ANNA MARZUCCO, MARIA S. MONTANARI, IRENE ZAGHI, MARTINA BRANDOLINI, LAURA GRUMIRO, MASSIMILIANO GUERRA, ALESSANDRA M. DE PASCALI, MONICA CRICCA, VITTORIO SAMBRI.....	129
Department of Medical and Surgical Sciences (DIMEC), O.U. Microbiology, University of Bologna, Cesena, Italy; Local Health Authority, O.U. Microbiology, the Greater Romagna Area Hub Laboratory, Cesena, Italy; Local Health Authority, O.U. Microbiology, the Greater Romagna Area Hub Laboratory, Cesena, Italy; Local Health Authority, O.U. Microbiology, the Greater Romagna Area Hub Laboratory, Cesena, Italy; Local Health Authority, O.U. Microbiology, the Greater Romagna Area Hub Laboratory, Cesena, Italy; Local Health Authority, O.U.	
Microbiology, the Greater Romagna Area Hub Laboratory, Cesena, Italy; Local Health Authority,	

O.U. Microbiology, the Greater Romagna Area Hub Laboratory, Cesena, Italy; Department of Medical and Surgical Sciences (DIMEC), O.U. Microbiology, University of Bologna, Cesena, Italy; ; Local Health Authority, O.U. Microbiology, the Greater Romagna Area Hub Laboratory, Cesena, Italy; ; Local Health Authority, O.U. Microbiology, the Greater Romagna Area Hub Laboratory, Cesena, Italy; Department of Medical and Surgical Sciences (DIMEC), O.U. Microbiology, University of Bologna, Cesena, Italy; Department of Medical and Surgical Sciences (DIMEC), O.U. Microbiology, University of Bologna, Cesena, Italy; Department of Medical and Surgical Sciences (DIMEC), O.U. Microbiology, University of Bologna, Cesena, Italy.....	129
34 - Nocardia otitidiscavarium infection in an immunocompromised host: Importance of proper microbiological testing	131
SONIA DONEDDU ⁽¹⁾ - MARIA FRANCESCA SECCHI ⁽²⁾ - MARCO FOIS ⁽³⁾ - MAURA FIAMMA ⁽⁴⁾ - MARIA TERESA MURGIONI ⁽¹⁾ - CARLA BOI ⁽¹⁾ - FRANCO CARTA ⁽⁴⁾ - MARIA CRISTINA GARAU ⁽⁴⁾ - ANTONELLO PADDEU ⁽⁵⁾ - SILVIA PILLONI ⁽⁵⁾ - GIACOMO OLLA ⁽⁵⁾ - ALEXANDRA USAI ⁽⁵⁾ - MARINA CUBEDDU ⁽⁴⁾ - FRANCESCO MARCO LODDO ⁽⁵⁾	131
Sc Laboratorio Analisi, Nostra Signora Della Mercedes Asl Ogliastro, Lanusei, Italia ⁽¹⁾ - Sds Microbiologia Clinica Cagliari, Università Di Cagliari, Nuoro, Italia ⁽²⁾ - Sds Malattie Infettive E Tropicali S.C. Malattie Infettive, Università Degli Studi Di Sassari Dipartimento Di Medicina, Chirurgia E Farmacia, Sassari, Italia ⁽³⁾ - U.C. Laboratorio Analisi/Microbiologia, Ospedale San Francesco, Nuoro, Italia ⁽⁴⁾ - Sc Anestesia E Rianimazione, Nostra Signora Della Mercedes Asl Ogliastro, Lanusei, Italia ⁽⁵⁾	131
Nocardia otitidiscavarium infection in an immunocompromised host: Importance of proper microbiological testing.....	131
63 - Molecular surveillance of Candida auris colonization: our experience.	133
ALESSANDRO BONDI ⁽¹⁾ - ALESSIO LEONE ⁽²⁾ - FEDERICA ZULLO ⁽²⁾ - ANTONIO CURTONI ⁽¹⁾ - TERESA ZACCARIA ⁽²⁾ - TERESA ZACCARIA ⁽²⁾ - ROSSANA CAVALLO ⁽¹⁾ - CRISTINA COSTA ⁽¹⁾	133
Dipartimento di Scienze della Sanità Pubblica e Pediatriche, Università di Torino, Torino, Italia ⁽¹⁾ - SC Microbiologia e Virologia U, AOU Città della Salute e della Scienza, Torino, Italia ⁽²⁾	133
Molecular surveillance of Candida auris colonization: our experience.	133
74 - Evaluation of STANDARD M10 MDR-TB assay for rapid diagnosis of tuberculosis	134
LAURA RINDI ⁽¹⁾ - VINCENZO PUGLISI ⁽²⁾ - IACOPO FRANCONI ⁽¹⁾ - ANTONELLA LUPETTI ⁽¹⁾	134
Università di Pisa, Dipartimento di Ricerca Traslationale e Nuove Tecnologie in Medicina e Chirurgia, Pisa, Italia ⁽¹⁾ - Azienda Ospedaliero-Universitaria Pisana, S.D. Microbiologia Micologica, Pisa, Italia ⁽²⁾	134
Evaluation of STANDARD M10 MDR-TB assay for rapid diagnosis of tuberculosis.....	134
Laura Rindi, Vincenzo Puglisi, Iacopo Franconi, Antonella Lupetti	134
Dipartimento di Ricerca Traslationale e delle Nuove Tecnologie in Medicina e Chirurgia, S.D. Microbiologia Micologica, Azienda Ospedaliero-Universitaria Pisana, Pisa, Italy.....	134
89 - "Fast" viral respiratory diagnosis in Pediatric Patient	136
VITTORIO PANETTA ⁽¹⁾ - MARIA TERESA DELLA ROCCA ⁽¹⁾ - ADRIANA DURANTE ⁽¹⁾ - GIUSEPPINA TUCCI ⁽¹⁾ - STEFANO LABELLA ⁽¹⁾ - RITA GRECO ⁽¹⁾	136
UOSD Microbiology, AORN Sant'Anna and San Sebastiano, caserta, Italia ⁽¹⁾	136

"Fast" viral respiratory diagnosis in pediatric patients	136
VITTORIO PANETTA ¹ , MARIA T. DELLA ROCCA ¹ , ADRIANA DURANTE ¹ , GIUSEPPINA TUCCI ¹ , STEFANO LABELLA ¹ , RITA GRECO ¹	136
¹ UOSD MICROBIOLOGY – AORN SANT 'ANNA AND SAN SEBASTIANO, CASERTA, ITALY.....	136
100 - Preliminary results of valacyclovir efficacy in HCMV positive pregnant women.....	138
ENRICA MADDALENA RANIERI ⁽¹⁾ - PASQUALE D'AMBROSIO ⁽¹⁾ - RAFFAELE DEL PRETE ⁽²⁾ - MARIALUISA SCARASCIULLI ⁽²⁾ - ANNAMARIA COLACICCO ⁽²⁾ - ANGELA TRENTADUE ⁽¹⁾ - LUIGI SANTACROCE ⁽²⁾ - MARICA COLELLA ⁽¹⁾	138
Dipartimento Interdisciplinare di Medicina (DIM), Sezione. Microbiologia e Virologia, Università "Aldo Moro", Bari, Italia ⁽¹⁾ - Dipartimento Interdisciplinare di Medicina (DIM), Sezione. Microbiologia e Virologia, Università "Aldo Moro", Bari, Italia., UOC Microbiologia e Virologia, Azienda Ospedaliero-Universitaria Policlinico di Bari, Bari, Italia ⁽²⁾	138
Preliminary results of valacyclovir efficacy in HCMV positive pregnant women	138
ENRICA M. RANIERI ¹ , PASQUALE D'AMBROSIO ¹ , RAFFAELE DEL PRETE ^{1,2} , MARIALUISA SCARASCIULLI ^{1,2} , ANNAMARIA COLACICCO ^{1,2} , ANGELA TRENTADUE ¹ , LUIGI SANTACROCE ^{1,2} , MARICA COLELLA ¹	138
¹ Interdisciplinary Department of Medicine (DIM), Sect. Microbiology and Virology, University "Aldo Moro", Bari, Italy;	138
² UOC Microbiology and Virology, Azienda Ospedaliero-Universitaria Policlinico of Bari, Bari, Italy.	138
102 - Analytical evaluation of a novel Point Of Care (POCT) Loop Mediated Isothermal Amplification (LAMP) test for the detection of Sexually Transmitted Infections	140
MARTINA BRANDOLINI ⁽¹⁾ - LAURA GRUMIRO ⁽¹⁾ - PATRIZIA FARABEGOLI ⁽¹⁾ - GIORGIO DIRANI ⁽¹⁾ - IRENE ZAGHI ⁽¹⁾ - FRANCESCA TADDEI ⁽¹⁾ - GIULIA GATTI ⁽¹⁾ - ALESSANDRA MISTRAL DE PASCALI ⁽¹⁾ - ANNA MARZUCCO ⁽¹⁾ - MARIA SOFIA MONTANARI ⁽¹⁾ - SIMONA SEMPRINI ⁽¹⁾ - MONICA CRICCA ⁽¹⁾ - VITTORIO SAMBRI ⁽¹⁾	140
Unit of Microbiology, AUSL Romagna, Department of Laboratory and Transfusion Medicine, Cesena, Italia ⁽¹⁾	140
Analytical evaluation of a novel Point Of Care (POCT) Loop Mediated Isothermal Amplification (LAMP) test for the detection of Sexually Transmitted Infections	140
MARTINA BRANDOLINI ^{1,2} , LAURA GRUMIRO ^{1,2} , PATRIZIA FARABEGOLI ¹ , GIORGIO DIRANI ¹ , IRENE ZAGHI ^{1,3} , FRANCESCA TADDEI ^{1,2} , GIULIA GATTI ¹ , ALESSANDRA MISTRAL DE PASCALI ^{1,2} , ANNA MARZUCCO ¹ , MARIA SOFIA MONTANARI ¹ , SIMONA SEMPRINI ¹ , MONICA CRICCA ^{1,2} , VITTORIO SAMBRI ^{1,2}	140
Department of Laboratory and Transfusion Medicine (DiMeLabeT), Unit of Microbiology, The Greater Romagna Area Hub Laboratory, 47522 Cesena, Italy;	140
Department of Medical and Surgical Sciences (DIMEC), Unit of Microbiology, Alma Mater Studiorum University of Bologna, 47522 Cesena, Italy;	140
Department of Specialty Medicine, Unit of Infectious Diseases, Santa Maria delle Croci Hospital, 48100 Ravenna, Italy	140
123 - Dynamic expression of CD169 in blood cells and circulating microvesicles as a potential diagnostic marker of immune response in COVID-19 and PASC individuals.....	142

MARIALAURA FANELLI ⁽¹⁾ - VITA PETRONE ⁽¹⁾ - ROSSELLA CHIRICO ⁽¹⁾ - CHRISTIAN MARACCHIONI ⁽¹⁾ - MARTINA GIUDICE ⁽¹⁾ - ELISABETTA TETI ⁽²⁾ - LUIGI COPPOLA ⁽²⁾ - CHIARA SORACE ⁽³⁾ - MARCO IANNETTA ⁽²⁾ - MARTA ZORDAN ⁽²⁾ - PIETRO VITALE ⁽³⁾ - ALEXANDRE LUCAS ⁽⁴⁾ - CLAUDIA MARIA RADU ⁽⁵⁾ - LOREDANA SARMAI ⁽²⁾ - EMANUELA BALESTRIERI ⁽¹⁾ - SANDRO GRELLI ⁽⁶⁾ - CLAUDIA MATTEUCCI ⁽¹⁾ - ANTONELLA MINUTOLO ⁽¹⁾	142
University of Rome Tor Vergata, Department of Experimental Medicine, Roma, Italia ⁽¹⁾ - Tor Vergata Hospital, Infectious Diseases Clinic, Roma, Italia ⁽²⁾ - University of Rome Tor Vergata, Department of Systems Medicine, Roma, Italia ⁽³⁾ - Inserm UMR1297 and Université Paul Sabatier, We-Met platform, Institut des Maladies Métaboliques et Cardiovasculaires (I2MC), Toulouse, Italia ⁽⁴⁾ - University of Padua, Thrombotic and Hemorrhagic Diseases Unit Department of Medicine, Padova, Italia ⁽⁵⁾ - Policlinic of Tor Vergata, Virology Unit, Roma, Italia ⁽⁶⁾	142
Dynamic expression of CD169 in blood cells and circulating microvesicles as a potential diagnostic marker of immune response in COVID-19 and PASC individuals.	142
MARIALAURA FANELLI ¹ , VITA PETRONE ¹ , ROSSELLA CHIRICO ¹ , CHRISTIAN MARACCHIONI ¹ , MARTINA GIUDICE ¹ , ELISABETTA TETI ² , LUIGI COPPOLA ² , CHIARA SORACE ³ , MARCO IANNETTA ^{2,3} , MARTA ZORDAN ^{2,3} , PIETRO VITALE ³ , ALEXANDRE LUCAS ⁴ , CLAUDIA MARIA RADU ⁵ , LOREDANA SARMAI ^{2,3} , EMANUELA BALESTRIERI ¹ , SANDRO GRELLI ^{1,6} , CLAUDIA MATTEUCCI ¹ , ANTONELLA MINUTOLO ¹	142
¹ Department of Experimental Medicine, University of Rome Tor Vergata, Rome, 00133, Italy; ² Infectious Diseases Clinic, Tor Vergata Hospital, Rome 00133, Italy; ³ Department of Systems Medicine, University of Rome Tor Vergata, Rome 00133, Italy; ⁴ We-Met platform, Institut des Maladies Métaboliques et Cardiovasculaires (I2MC), plateau We-Met, Inserm UMR1297 and Université Paul Sabatier, Toulouse, France; ⁵ Thrombotic and Hemorrhagic Diseases Unit Department of Medicine – DIMED University of Padua, Italy; ⁶ Virology Unit, Policlinic of Tor Vergata, Rome, 00133, Italy;	142
133 - Fecal calprotectin values in pediatric patients with bacterial and viral diarrhoea: An observational study.....	144
BRUNELLA BRESCIA ⁽¹⁾ - CLAUDIA CICINO ⁽¹⁾ - AIDA GIANCOTTI ⁽¹⁾ - GIORGIO SETTIMO BARRECA ⁽¹⁾ - FRANCESCA TRIMBOLI ⁽¹⁾ - MARTA PANTANELLA ⁽¹⁾ - SIMONA GIGLIOTTI ⁽¹⁾ - EMANUELA LARATTA ⁽¹⁾ - CONCETTA ZANGARI ⁽¹⁾ - VALENTINA TANCRÈ ⁽¹⁾ - GRAZIA PAVIA ⁽¹⁾ - NADIA MARASCIO ⁽¹⁾ - ANGELA QUIRINO ⁽¹⁾ - GIOVANNI MATERA ⁽¹⁾	144
Università degli studi "Magna Graecia" di Catanzaro, Azienda Ospedaliero-Universitario "R. Dulbecco" presidio "Mater Domini", Dipartimento Scienze della Salute, Catanzaro, Italia ⁽¹⁾ ..	144
151 - Differential diagnosis for fever and rash as a key strategy to achieve the goal of eliminating Measles and Rubella.....	146
CLARA FAPPANI ⁽¹⁾ - MARIA GORI ⁽¹⁾ - SILVIA BIANCHI ⁽¹⁾ - MARA TERRANEO ⁽¹⁾ - ERICA BILARDI ⁽¹⁾ - DANIELA COLZANI ⁽¹⁾ - ELISABETTA TANZI ⁽¹⁾ - MARTA CANUTI ⁽²⁾ - ANTONELLA AMENDOLA ⁽¹⁾	146
Università degli Studi di Milano, Dipartimento di Scienze della Salute, Milano, Italia ⁽¹⁾ - Università degli Studi di Milano, Department of Pathophysiology and Transplantation, Milano, Italia ⁽²⁾	146
Differential diagnosis for fever and rash as a key strategy to achieve the goal of eliminating Measles and Rubella.....	146

CLARA FAPPANI ^{1,2,3} , MARIA GORI ^{1,3} , SILVIA BIANCHI ^{1,3} , MARA TERRANEO ¹ , ERICA BILARDI ¹ , DANIELA COLZANI ¹ , ELISABETTA TANZI ^{1,3,4} , MARTA CANUTI ^{3,4,5} , ANTONELLA AMENDOLA ^{1,3,4}	146
¹ Department of Health Sciences, Università degli Studi di Milano, Milan, Italy; ² Department of Clinical Sciences and Community Health, Università degli Studi di Milano, Milan, Italy; ³ Coordinate Research Centre EpiSoMI (Epidemiology and Molecular Surveillance of Infections), Università degli Studi di Milano, Milan, Italy; ⁴ Centre for Multidisciplinary Research in Health Science (MACH), Università degli Studi di Milano, Milan, Italy; ⁵ Department of Pathophysiology and Transplantation, Università degli Studi di Milano, Milan, Italy.	
174 - Fusarium Solani fungemia in pediatric patient with acute B-lymphoblastic leukemia	148
GIORGIA LA COSTA ⁽¹⁾ - VITTORIANA DE LAURENTIIS ⁽²⁾ - ENRICA M. RANIERI ⁽¹⁾ - MICHELE MASTRIA ⁽¹⁾ - MARA LORUSSO ⁽¹⁾ - STEFANIA GARZONE ⁽¹⁾ - FRANCESCO DE LEONARDIS ⁽³⁾ - VITTORIO GRECO MIANI ⁽³⁾ - RAFFAELE DEL PRETE ⁽¹⁾ - CARMELA DE CARLO ⁽²⁾ - LUIGI RONGA ⁽²⁾ - STEFANIA STOLFA ⁽¹⁾ - FEDERICA ROMANELLI ⁽²⁾ - ELEONORA SPARAPANO ⁽²⁾ - LUCIA NISI ⁽²⁾ - MARIA TERESA MONTAGNA ⁽¹⁾ - ADRIANA MOSCA ⁽¹⁾	148
Università degli studi di Bari Aldo Moro, Dipartimento interdisciplinare di Medicina sezione Microbiologia e Virologia, Università Aldo Moro, Bari, Italia ⁽¹⁾ - UOC Microbiology and Virology, University Aldo Moro, Dipartimento interdisciplinare di Medicina sezione Microbiologia e Virologia, Università Aldo Moro, Bari, Italia ⁽²⁾ - Pediatric Hematology and Oncology, University Hospital of Policlinico, Bari, Italia ⁽³⁾	
Fusarium solani fungemia in pediatric patient with acute B-lymphoblastic leukemia.....	148
Giorgia La Costa ³ , Vittoriana De Laurentiis ¹ , Enrica M. Ranieri ³ , Michele Mastria ³ , Mara Lorusso ³ , Stefania Garzone ³ , Francesco De Leonardis ² , Vittorio Greco Miani ² , Raffaele Del Prete ³ , Carmela De Carlo ¹ , Luigi Ronga ¹ , Stefania Stolfi ³ , Federica Romanelli ¹ , Eleonora Sparapano ¹ , Lucia Nisi ¹ , Maria Teresa Montagna ³ , Adriana Mosca ³	148
1 UOC Microbiology and Virology, University "Aldo Moro", Bari, Italy	148
2 Pediatric Hematology and Oncology - University Hospital of Policlinico - Bari Piazza G. Cesare,11, Bari, Italy	148
3 Section of Microbiology, Interdisciplinary Department of Medicine (DIM), School of Medicine, University of Bari " Aldo Moro", Bari, Italy	148
177 - SARS-CoV-2 infection in healthy school-age children.....	150
MATTEO DOMENICO MARSIGLIA ⁽¹⁾ - SILVIA ANCONA ⁽²⁾ - EMERENZIANA OTTAVIANO ⁽²⁾ - FRANCESCA TRIVA ⁽²⁾ - PRISCILLA PASUTTO ⁽²⁾ - MASSIMILIANO SERGIO FRANCESCO CAMPAGNOLI ⁽²⁾ - SILVIA BIANCHI ⁽²⁾ - ELISA BORGHI ⁽²⁾	150
Università degli Studi di Milano, Dipartimento di Scienze Biomediche e Cliniche, Milano, Italia ⁽¹⁾ - Università degli Studi di Milano, Dipartimento di Scienze della Salute, Milano, Italia ⁽²⁾	
Abstract.....	150
Title: SARS-CoV-2 infection in healthy school-age children	150
Authors:	150
MATTEO D. MARSIGLIA ^{1,2} , SILVIA ANCONA ¹ , EMERENZIANA OTTAVIANO ¹ , FRANCESCA TRIVA ¹ , PRISCILLA PASUTTO ¹ , MASSIMILIANO S. F. CAMPAGNOLI ¹ , SILVIA BIANCHI ¹ , ELISA BORGHI ¹	150
1. Department of Health Sciences, Università degli Studi di Milano, Milan (Italy);	150

2. Department of Biomedical and Clinical Sciences, Università degli Studi di Milano, Milan (Italy).....	150
CHIARA GIUBBI⁽¹⁾ - MARIANNA MARTINELLI⁽¹⁾ - MARIA LETIZIA DI MEO⁽²⁾ - RUTH CHINYERE NJOKU⁽³⁾ - FEDERICA PERDONI⁽¹⁾ - ERIKA BRUNO⁽¹⁾ - ROSARIO MUSUMECI⁽¹⁾ - FABIO LANDONI⁽²⁾ - ROBERT FRUSCIO⁽²⁾ - CLEMENTINA ELVEZIA COCUZZA⁽¹⁾.....	152
University of Milano-Bicocca, Department of Medicine and Surgery, Monza, Italia⁽¹⁾ - ASST Monza, Fondazione IRCCS San Gerardo dei Tintori, Monza, Italia⁽²⁾ - University of Sassari, Department of Biomedical Science, Sassari, Italia⁽³⁾.....	152
Self-sampling as alternative strategy to detect Human Papillomavirus (HPV) and other sexually transmitted pathogens in women referred to colposcopy	152
CHIARA GIUBBI¹, MARIANNA MARTINELLI¹, MARIA L. DI MEO², RUTH C. NJOKU³, FEDERICA PERDONI¹, ERIKA BRUNO¹, ROSARIO MUSUMECI¹, FABIO LANDONI^{1,2}, ROBERT FRUSCIO^{1,2}, CLEMENTINA E. COCUZZA¹	152
¹Department of Medicine and Surgery, University of Milano-Bicocca, 20900, Monza, Italy;	152
²Fondazione IRCCS San Gerardo dei Tintori, ASST Monza, 20900, Monza, Italy;	152
³Department of Biomedical Science, University of Sassari, 07100, Sassari, Italy.	152
200 - Comparison of two detection methods for the quantitative detection of circulating Human Papillomavirus (HPV) from plasma and saliva of patients with head and neck cancer.....	154
RUTH CHINYERE NJOKU⁽¹⁾ - MARIANNA MARTINELLI⁽²⁾ - CHIARA GIUBBI⁽²⁾ - FEDERICA PERDONI⁽²⁾ - CLEMENTINA ELVEZIA COCUZZA⁽²⁾ - CONSIGLIA PADUANO⁽³⁾ - DAVIDE SMUSSI⁽³⁾ - CRISTINA GURIZZAN⁽³⁾ - CLAUDIA ASSONI⁽³⁾ - LUIGI LORINI⁽³⁾ - PAOLO BOSSI⁽³⁾.....	154
University of Sassari, Department of Biomedical Science, Sassari, Italia⁽¹⁾ - University of Milano-bicocca, Department of Medicine and Surgery, Monza, Italia⁽²⁾ - University of Brescia, ASST Spedali Civili, Division of Medical Oncology, Department of Medical and Surgical Specialties, Radiological Sciences and Public Health, Brescia, Italia⁽³⁾	154
Topic: Approcci diagnostici.....	154
Comparison of two detection methods for the quantitative detection of circulating Human Papillomavirus (HPV) from plasma and saliva of patients with head and neck cancer.	154
RUTH C. NJOKU^{1,2}, MARIANNA MARTINELLI¹, CHIARA GIUBBI¹, FEDERICA PERDONI¹, CRISTINA GURIZZAN³, CONSIGLIA PADUANO³, LUIGI LORINI³, DAVIDE SMUSSI³, CLAUDIA ASSONI³, PAOLO BOSSI³, CLEMENTINA E. COCUZZA¹.....	154
¹ Department of Medicine and Surgery, University of Milano-Bicocca, Monza, Italy.....	154
² Department of Biomedical Science, University of Sassari, Sassari, Italy.	154
³ Division of Medical Oncology, Department of Medical and Surgical Specialties, Radiological Sciences and Public Health, University of Brescia, ASST Spedali Civili, Brescia, Italy	154
205 - Analysis of antibody titre decay in the mother-infant dyad exposed to congenital Toxoplasma gondii infection.....	156
SIMONA TRIMARCO⁽¹⁾ - ANGELA SPAGNUOLO⁽¹⁾ - FRANCESCO FRALLICCIARDI⁽¹⁾ - ADRIANO ROMANO⁽¹⁾ - ELENA SCAGLIONE⁽²⁾ - FRANCESCA CARRATURO⁽³⁾ - ILARIA CARUCCIO⁽¹⁾ - ASSUNTA RUSSO⁽¹⁾ - MARIA STABILE⁽¹⁾ - ROSA D'AURIA⁽¹⁾ - MARIA CONCETTA CUOMO⁽¹⁾ - CHIARA PAGLIUCA⁽⁴⁾ - ROBERTA COLICCHIO⁽²⁾ -	

MARIATERESA VITIELLO ⁽²⁾ - SERENA SALOMÈ ⁽³⁾ - FRANCESCO RAIMONDI ⁽³⁾ - PAOLA SALVATORE ⁽⁵⁾	156
Department of Integrated Activity of Laboratory Medicine and Transfusion, Complex Operative Unit of Clinical Microbiology, University Hospital Federico II, Naples, Italia ⁽¹⁾ - Dep. of Int. Act. of Lab. Med. and Tran. Complex Operative Unit of Clinic. Microb.; Department of Molecular Medicine and Medical Biotechnology, University of Naples Federico II, Naples, Italia ⁽²⁾ - Department of Translational Medical Sciences, Division of Neonatology, University Hospital Federico II, Naples, Italia ⁽³⁾ - Department of Molecular Medicine and Medical Biotechnology, University of Naples Federico II, Naples, Italia ⁽⁴⁾ - Dep. of Int. Act. of Lab. Med. and Tran. UOC Clinic. Microb.; Dep. of Mol. Med. and Med. Biotec.; CEINGE Franco Salvatore; Task Force on Microb. Stud., University of Naples Federico II, Naples, Italia ⁽⁵⁾	156
Analysis of antibody titre decay in the mother-infant dyad exposed to congenital <i>Toxoplasma</i> <i>gondii</i> infection.....	156
SIMONA TRIMARCO ¹ , ANGELA SPAGNUOLO ¹ , FRANCESCO FRALLICCIARDI ¹ , ADRIANO ROMANO ¹ , ELENA SCAGLIONE ^{1,2} , FRANCESCA CARRATURO ³ , ILARIA CARUCCIO ¹ , ASSUNTA RUSSO ¹ , MARIA STABILE ¹ , ROSA D'AURIA ¹ , MARIA C. CUOMO ¹ , CHIARA PAGLIUCA ² , ROBERTA COLICCHIO ^{1,2} , MARIATERESA VITIELLO ^{1,2} , SERENA SALOME ³ , FRANCESCO RAIMONDI ³ , PAOLA SALVATORE ^{1,2,4,5}	156
¹ Department of Integrated Activity of Laboratory Medicine and Transfusion, Complex Operative Unit of Clinical Microbiology, University Hospital Federico II, Naples, Italy; ² Department of Molecular Medicine and Medical Biotechnology, University of Naples Federico II, Naples, Italy; ³ Department of Translational Medical Sciences, Division of Neonatology, University of Naples Federico II, Naples, Italy; ⁴ CEINGE, Advanced Biotechnologies Franco Salvatore s.c.ar.l., Naples, Italy; ⁵ Task Force on Microbiome Studies, University of Naples Federico II, Naples, Italy.	156
209 - Comparative Analysis of miRNA Expression Profiles in HPV-Negative and HPV-Positive Cancer Cell Lines: Implications for Tumorigenesis	158
MARINA POTESÀ ⁽¹⁾ - VALENTINA ROGLIA ⁽¹⁾ - FEDERICO IACOVELLI ⁽¹⁾ - MARCO CIOTTI ⁽²⁾ - ANNA CRISCUOLO ⁽³⁾ - ANTONELLA MINUTOLO ⁽⁴⁾ - CARLA MONTESANO ⁽¹⁾	158
Università di Roma Tor Vergata, Dipartimento di Biologia, Roma, Italia ⁽¹⁾ - Policlinico Tor Vergata, Unità di Virologia, Roma, Italia ⁽²⁾ - Università di Roma Tor Vergata, Dipartimento di Ginecologia, Roma, Italia ⁽³⁾ - Università di Roma Tor Vergata, Dipartimento di Medicina Sperimentale, Roma, Italia ⁽⁴⁾	158
Comparative Analysis of miRNA Expression Profiles in HPV-Negative and HPV-Positive Cancer Cell Lines: Implications for Tumorigenesis.....	158
MARINA POTESÀ ^{1,2*} , VALENTINA ROGLIA ^{1*} , FEDERICO IACOVELLI ¹ , MARCO CIOTTI ³ , ANNA CRISCUOLO ⁴ , ANTONELLA MINUTOLO ⁵ AND CARLA MONTESANO ^{1,2}	158
1. Department of Biology University of Rome Tor Vergata, Rome, Italy.	158
2. UNESCO Interdisciplinary Chair in Biotechnology and Bioethics.....	158
3. Virology Unit, Polyclinic Tor Vergata, Rome, Italy.....	158
4. Department of Gynecology, University of Rome Tor Vergata, Rome, Italy.	158
5. Department of Experimental Medicine, University of Rome Tor Vergata, Rome, Italy.....	158
*These authors contributed equally to this work	158

216 - Contribution of T2 Magnetic Resonance (T2MR ®) technology for the early detection of ESKAPE pathogens in ICU patients: a two years experience in Palermo, Italy	160
DOMENICO GRACEFFA ⁽¹⁾ - SALVATORE ANTONINO DISTEFANO ⁽¹⁾ - GIOVANNI GIAMMANCO ⁽²⁾ - ANDREA CORTEGIANI ⁽³⁾ - SALVATORE CAMPANELLA ⁽⁴⁾ - ANGELICA ANCONA ⁽⁴⁾ - MARTA CAPUTO ⁽⁴⁾ - PIETRO MIRASOLA ⁽⁴⁾ - CELESTINO BONURA ⁽²⁾	
160	
Università degli Studi di Palermo, Azienda Ospedaliera Universitaria Policlinico “Paolo Giaccone” di Palermo - U.O.C Microbiologia e Virologia, Palermo, Italia ⁽¹⁾ - Università degli Studi di Palermo, Dipartimento di Promozione della Salute, Materno Infantile, Medicina Interna e Specialistica di eccellenza (PROMISE) “G. D’Alessandro”, Palermo, Italia ⁽²⁾ - Università degli Studi di Palermo, Azienda Ospedaliera Universitaria Policlinico “Paolo Giaccone” di Palermo - U.O.C Anestesia Rianimazione e Terapia Intensiva, Palermo, Italia ⁽³⁾ - Università degli Studi di Palermo, Dipartimento di Discipline Chirurgiche Oncologiche e Stomatologiche (Di.Chir.On.S.), Palermo, Italia ⁽⁴⁾	
160	
Contribution of T2 Magnetic Resonance (T2MR ®) technology for the early detection of ESKAPE pathogens in ICU patients: a two years experience in Palermo, Italy.....	160
Domenico Graceffa^{1,3}, Salvatore A. Distefano³, Giovanni Giammanco^{1,3}, Andrea Cortegiani^{2,4}, Salvatore Campanella⁴, Angelica Ancona⁴, Marta Caputo⁴, Pietro Mirasola⁴ and Celestino Bonura^{1,3}.....	
160	
¹Dipartimento di Promozione della Salute, Materno Infantile, Medicina Interna e Specialistica di eccellenza (PROMISE) “G. D’Alessandro” – Università degli Studi di Palermo.....	
160	
²Azienda Ospedaliera Universitaria Policlinico “Paolo Giaccone” di Palermo - U.O.C Anestesia Rianimazione e Terapia Intensiva	
160	
³Azienda Ospedaliera Universitaria Policlinico “Paolo Giaccone” di Palermo - U.O.C Microbiologia e Virologia.....	
160	
⁴Dipartimento di Discipline Chirurgiche Oncologiche e Stomatologiche (Di.Chir.On.S.) – Università degli Studi di Palermo	
160	
217 - Innovative phage-based sensor for the rapid detection of staphylococcus aureus	162
SARA BOLOGNINI ⁽¹⁾ - ZAHRAALSADAT YOUSEFNIAJEJAHROMI ⁽²⁾ - CLAUDIA CAMPOBASSO ⁽¹⁾ - NOEMI POMA ⁽¹⁾ - CATERINA FERRETTI ⁽¹⁾ - DARIA BOTTAI ⁽¹⁾ - ANDREA BONINI ⁽²⁾ - LAURA RINDI ⁽³⁾ - FABIO DI FRANCESCO ⁽²⁾ - ARIANNA TAVANTI ⁽¹⁾ - MARIAGRAZIA DI LUCA ⁽¹⁾	
162	
Università di Pisa, Dipartimento di Biologia, Pisa, Italia ⁽¹⁾ - Università di Pisa, Dipartimento di Chimica Industriale, Pisa, Italia ⁽²⁾ - Università di Pisa, Dipartimento di Ricerca Traslationale e delle nuove tecnologie in medicina e chirurgia, Pisa, Italia ⁽³⁾	
162	
Innovative phage-based sensor for the rapid detection of Staphylococcus aureus	162
SARA BOLOGNINI¹, ZAHRAALSADAT YOUSEFNIAJEJAHROMI², CLAUDIA CAMPOBASSO^{1,3}, NOEMI POMA¹, CATERINA FERRETTI¹, DARIA BOTTAI¹, ANDREA BONINI², LAURA RINDI⁴, FABIO DI FRANCESCO², ARIANNA TAVANTI¹, MARIAGRAZIA DI LUCA¹	
162	
¹Department of Biology, University of Pisa, Pisa, Italy; ²Department of Chemistry and Industrial Chemistry, University of Pisa, Pisa, Italy; ³Department of Biosystems, Laboratory of Gene Technology, KU Leuven, Leuven, Belgium; ⁴Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa, Pisa, Italy	
162	
225 - Evaluation of syndromic SeeGene Allplex GI panels for detection of pathogens associated with acute gastroenteritis.	164

ARIANNA SUCATO ⁽¹⁾ - FLORIANA BONURA ⁽¹⁾ - CHIARA FILIZZOLO ⁽¹⁾ - MARIANGELA PIZZO ⁽¹⁾ - FRANCESCA DI BERNARDO ⁽²⁾ - ANTONINA COLLURA ⁽²⁾ - DONATELLA FERRARO ⁽¹⁾ - GIOVANNI M. GIAMMANCO ⁽¹⁾ - SIMONA DE GRAZIA ⁽¹⁾	164
Università degli studi di Palermo, Dipartimento di Promozione della Salute, Materno-Infantile, di Medicina Interna e Specialistica di Eccellenza “G. D’Alessandro”, Palermo, Italia ⁽¹⁾ - ARNAS, Ospedale Civico e Di Cristina, Unità Operativa di Microbiologia e Virologia, Ospedale Civico e Di Cristina, ARNAS, Palermo, Italia ⁽²⁾	164
Evaluation of syndromic SeeGene Allplex GI panels for detection of pathogens associated with acute gastroenteritis.....	164
ARIANNA SUCATO¹, FLORIANA BONURA¹, CHIARA FILIZZOLO¹, MARIANGELA PIZZO¹, FRANCESCA DI BERNARDO², ANTONINA COLLURA², DONATELLA FERRARO¹, GIOVANNI M. GIAMMANCO¹, SIMONA DE GRAZIA¹	164
¹Department of Health Promotion Sciences, Maternal and Infant Care, Internal Medicine and Medical Specialties “G. D’Alessandro”, University of Palermo, Palermo, Italy;	164
²Unit of Microbiology and Virology, National Relevance and High Specialization Hospital Trust ARNAS Civico, Di Cristina, Benfratelli, Palermo, Italy.	164
246 - Multidrug-resistant bacteria surveillance in intensive care unit: the importance of a molecular approach.....	166
LIVIA TARTAGLIONE ⁽¹⁾ - ANDREA BUSNI ⁽¹⁾ - ELISA MATARAZZO ⁽¹⁾ - ELENA NICOLINI ⁽¹⁾ - ALESSANDRA ARMILLIS ⁽¹⁾ - ELENA PASTORI ⁽¹⁾ - DIANA FANTI ⁽¹⁾ - ALESSANDRA BIELLI ⁽¹⁾ - CHIARA S. VISMARA ⁽¹⁾.....	166
ASST Grande Ospedale Metropolitano Niguarda, ASST Grande Ospedale Metropolitano Niguarda, Milano, Italia ⁽¹⁾.....	166
Multidrug-resistant bacteria surveillance in intensive care unit: the importance of a molecular approach.....	166
LIVIA TARTAGLIONE, ANDREA BUSNI, ELISA MATARAZZO, ELENA NICOLINI, ALESSANDRA ARMILLIS, ELENA PASTORI, DIANA FANTI, ALESSANDRA BIELLI, CHIARA S. VISMARA	166
Clinical Microbiology, ASST GOM Niguarda, Milan, Italy.....	166
251 - Advanced Bacterial Detection in a Simulated Bloodstream Infections through Digital PCR: A Proof of Concept for Enhanced Diagnostic Accuracy	168
ALESSIA MIRABILE ⁽¹⁾ - CARMELO BONOMO ⁽¹⁾ - EMANUELE NICITRA ⁽¹⁾ - STEFANIA STEFANI ⁽¹⁾ - DAFNE SAMANTHA IRENE BONGIORNO ⁽¹⁾ - NICOLÒ MUSSO ⁽¹⁾	168
Università degli studi di Catania, Dipartimento di Scienze Biomediche e Biotechnologiche (BIOMETEC), Catania, Italia ⁽¹⁾.....	168
Advanced Bacterial Detection in a simulated Bloodstream Infections through Digital PCR: A Proof of Concept for Enhanced Diagnostic Accuracy.....	168
ALESSIA MIRABILE¹, CARMELO BONOMO¹, EMANUELE NICITRA¹, STEFANIA STEFANI¹, DAFNE S.I. BONGIORNO¹, NICOLO’ MUSSO².....	168
¹Department of Biomedical and Biotechnological Science (BIOMETC), Section of Microbiology, University of Catania, Via Santa Sofia 97, Catania, Italy ²Department of Biomedical and Biotechnological Science (BIOMETC), Medical Biochemistry, University of Catania, Via Santa Sofia 97, Catania, Italy	168

266 - Preventing early-onset group B streptococcus (GBS-EOD) invasive infection in the new-born: a national survey, Italy, 15 December 2022 to 12 February 2023.....	171
MICHELA SABBATUCCI ⁽¹⁾ - PIERANGELO CLERICI ⁽²⁾ - CRETI ROBERTA ⁽¹⁾.....	171
ISS, Department Infectious Diseases, Rome, Italia ⁽¹⁾ - Italian Clinical Microbiologists Association, AMCLI, ITALY, Italia ⁽²⁾	171
Preventing early-onset group B streptococcus (GBS-EOD) invasive infection in the new-born: a national survey, Italy, 15 December 2022 to 12 February 2023	171
MICHELA SABBATUCCI ^{1,3}, PIERANGELO CLERICI ², ROBERTA CRETI ³	171
Unit 5, Directorate General Health Prevention Communicable Diseases and International Prophylaxis, Ministry of Health, Rome, Italy.....	171
President of the Italian Clinical Microbiologists Association (AMCLI).....	171
Department of Infectious Diseases, Istituto Superiore di Sanità, Roma, Italy	171
269 - Laboratory strategy for the rapid diagnosis of leishmaniasis: experience in Palermo University hospital.	173
MARIANGELA PIZZO ⁽¹⁾ - TERESA MARIA ASSUNTA FASCIANA ⁽¹⁾ - ROBERTA VIRRUSO ⁽²⁾ - GIUSEPPE MINÌ ⁽²⁾ - GAETANO D'ALESSANDRO ⁽²⁾ - CHIARA MASSARO ⁽¹⁾ - LUCA PIPITÒ ⁽¹⁾ - ANTONIO CASCIO ⁽¹⁾ - GIOVANNI M. GIAMMANCO ⁽¹⁾ - CINZIA CALÀ ⁽¹⁾	173
Università degli studi di Palermo, Promozione della Salute, Materno-Infantile, di Medicina Interna e Specialistica di Eccellenza “G. D’Alessandro” (PROMISE), Palermo, Italia ⁽¹⁾ - A.O.U.P. “Paolo Giaccone”, Palermo, U.O.C di Microbiologia, Virologia e Parassitologia, Palermo, Italia ⁽²⁾	173
Laboratory strategy for the rapid diagnosis of leishmaniasis: experience in Palermo University hospital.....	173
MARIANGELA PIZZO*, TERESA M. A. FASCIANA*, ROBERTA VIRRUSO**, GIUSEPPE MINÌ**, GAETANO D’ALESSANDRO**, CHIARA MASSARO*, LUCA PIPITÒ*, ANTONIO CASCIO*, GIOVANNI M. GIAMMANCO*, CINZIA CALÀ*	173
*Department of Health Promotion, Mother and Child Care, Internal Medicine and Medical Specialties (PROMISE), University of Palermo, Palermo, Italy	173
** U.O.C. of Microbiology, Virology and Parassitology, A.O.U.P. “Paolo Giaccone”, Palermo, Italy	173
294 - Screening of carriage of Carbapenem-resistant Enterobacterales (CRE), Acinetobacter baumannii (CRAB), and Pseudomonas aeruginosa (CRPsA) in a hospital setting by culture and molecular tests.....	175
CALOGERO RASPANTI ⁽¹⁾ - WALTER RAZETE ⁽²⁾ - NOELE SPURIA ⁽²⁾ - CINZIA CALÀ ⁽¹⁾ - NICOLETTA DE LUCA ⁽²⁾ - RITA IMMORDINO ⁽²⁾ - ROBERTA VIRRUSO ⁽²⁾ - GIOVANNI M. GIAMMANCO ⁽¹⁾ - TERESA M.A. FASCIANA ⁽¹⁾	175
Department of Health Promotion, Mother and Child Care, Internal Medicine and Medical Specialties., University of Palermo, Palermo, Italia ⁽¹⁾ - Microbiology and Virology Unit., AOU Policlinico “P. Giaccone”, Palermo, Italia ⁽²⁾	175
300 - Extraction-free RT-qPCR for the detection of SARS-CoV-2: modification of a commercial assay protocol reduces turnaround time.	177

EDOARDO MANDALÀ⁽¹⁾ - CATERINA AMATO⁽²⁾ - WALTER RAZETE⁽¹⁾ - GAETANO D'ALESSANDRO⁽¹⁾ - NOELE SPURIA⁽¹⁾ - SHARON RUSSO⁽¹⁾ - SIMONA DE GRAZIA⁽²⁾ - GIOVANNI MARIA GIAMMANCO⁽²⁾.....	177
UOC Microbiologia e Virologia, Azienda Ospedaliera Universitaria Policlinico (AOUP) "P.Giaccone", Palermo, Italia⁽¹⁾ - Università degli studi di Palermo, Promozione della Salute, Materno-Infantile, Medicina Interna e Specialistica d'Eccellenza "G. D'Alessandro", Palermo, Italia⁽²⁾	177
Extraction-free RT-qPCR for the detection of SARS-CoV-2: modification of a commercial assay protocol reduces turnaround time.....	177
EDOARDO MANDALÀ¹, CATERINA AMATO², WALTER RAZETE¹, GAETANO D'ALESSANDRO¹, NOELE SPURIA¹, SHARON RUSSO¹, SIMONA DE GRAZIA², GIOVANNI M. GIAMMANCO²	177
¹U.O.C. of Microbiology and Virology, A.O.U.P. "Paolo Giaccone", Palermo, Italy;.....	177
²Department of Health Promotion Sciences, Maternal and Infant Care, Internal Medicine and Medical Specialties "G. D'Alessandro", University of Palermo, Palermo, Italy	177
307 - Assessment of increased blaKPC gene dosage by digital PCR for the rapid prediction of meropenem/vaborbactam resistance in Klebsiella pneumoniae clinical strains	179
NOEMI AIEZZA⁽¹⁾ - ALBERTO ANTONELLI⁽¹⁾ - MARCO COPPI⁽¹⁾ - VINCENZO DI PILATO⁽²⁾ - TOMMASO GIANI⁽¹⁾ - GIAN MARIA ROSSOLINI⁽¹⁾.....	179
University of Florence, Department of Experimental and Clinical Medicine, University of Florence; Microbiology and Virology Unit, Florence Careggi University Hospital, Florence, Italia⁽¹⁾ - University of Genoa, Department of Surgical Sciences and Integrated Diagnostics, University of Genoa; Microbiology and Virology Unit, Florence Careggi University Hospital, Florence, Italia⁽²⁾	179
Abstract.....	179
Assessment of increased blaKPC gene dosage by digital PCR for the rapid prediction of meropenem/vaborbactam resistance in Klebsiella pneumoniae clinical strains.....	179
NOEMI AIEZZA^{1,3}, ALBERTO ANTONELLI^{1,3}, MARCO COPPI^{1,3}, VINCENZO DI PILATO^{2,3}, TOMMASO GIANI^{1,3}, GIAN MARIA ROSSOLINI^{1,3}	179
¹Department of Experimental and Clinical Medicine, University of Florence, Florence, Italy;	
²Department of Surgical Sciences and Integrated Diagnostics, University of Genoa, Genoa, Italy;	
³Microbiology and Virology Unit, Florence Careggi University Hospital, Florence, Italy.....	179
314 - Concomitant syndromic diagnosis of monkeypox and other vesicular viruses in patients with skin and genital lesions.....	181
MARIA BEATRICE VALLI⁽¹⁾ - MARTINA RUECA⁽¹⁾ - GIULIA MATUSALI⁽¹⁾ - ENRICO GIRARDI⁽²⁾ - EMANUELE NICASTRI⁽³⁾ - ANDREA ANTINORI⁽⁴⁾ - FABRIZIO MAGGI⁽¹⁾ 181	
Istituto Nazionale Malattie Infettive "L.Spallanzani" I.R.C.C.S., Laboratorio di Virologia e laboratori di Biosicurezza, Roma, Italia⁽¹⁾ - Istituto Nazionale Malattie Infettive "L.Spallanzani" I.R.C.C.S., Direzione Scientifica, Roma, Italia⁽²⁾ - Istituto Nazionale Malattie Infettive "L.Spallanzani" I.R.C.C.S., UOC MI Alta Intensità e Cura, Roma, Italia⁽³⁾ - Istituto Nazionale Malattie Infettive "L.Spallanzani" I.R.C.C.S., UOC IMMUNODEFICIENZE VIRALI, Roma, Italia⁽⁴⁾	181
Concomitant syndromic diagnosis of monkeypox and other vesicular viruses in patients with skin and genital lesions.....	181

Valli Maria Beatrice, Martina Rueca, Giulia Matusali, Enrico Girardi, Emanuele Nicastrì, Andrea Antinori and Fabrizio Maggi.....	181
324 - Relationship between Cycle threshold value and N antigen amount in SARS-CoV-2 variants	
.....	183
MARIA ANTONELLA ZINGAROPOLI ⁽¹⁾ - ROBERTA CAMPAGNA ⁽²⁾ - BENEDETTA GENNENZI ⁽³⁾ - ELISABETTA LAZZARI ⁽⁴⁾ - CHIARA NONNE ⁽²⁾ - DANIELE EMANUELE COMPAGNINO ⁽²⁾ - MARIA GEMMA LEONE ⁽²⁾ - GERMANA SFARA ⁽²⁾ - SARA FARAONE ⁽³⁾ - GUIDO ANTONELLI ⁽²⁾ - OMBRETTA TURRIZIANI ⁽²⁾	183
Università La Sapienza, Dipartimento di Sanità Pubblica e Malattie Infettive, Roma, Italia ⁽¹⁾ - Università La Sapienza, Dipartimento di Medicina Molecolare, Roma, Italia ⁽²⁾ - Policlinico Umberto I, Unità di Microbiologia e Virologia, Roma, Italia ⁽³⁾ - INMI Lazzaro Spallanzani, Laboratorio di Virologia, Roma, Italia ⁽⁴⁾	183
Relationship between Cycle threshold value and N antigen amount in SARS-CoV-2 variants ...	183
MARIA A. ZINGAROPOLI 1, ROBERTA CAMPAGNA 2, BENEDETTA GENNENZI 3, ELISABETTA LAZZARI 4 CHIARA NONNE 2, DANIELE E. COMPAGNINO 2, MARIA G. LEONE 2, GERMANA SFARA 2, SARA FARAONE 3, GUIDO ANTONELLI 2, OMBRETTA TURRIZIANI 2	183
Affiliation	183
1 Department of Public Health and Infectious diseases, Sapienza University of Rome, Italy; 2 Department of Molecular Medicine, Sapienza University of Rome, Italy; 3 Microbiology and Virology Unit, Sapienza University Hospital Policlinico Umberto I, Rome, Italy; Virology Unit, INMI, Lazzaro Spallanzani, Rome, Italy	183
326 - RT-QuIC detection prion seeding activity in spiked human tears.....	185
SARAH VASCELLARI ⁽¹⁾ - CHRISTINA D. ORRÙ ⁽²⁾ - GIANLUIGI ZANUSSO ⁽³⁾ - ANNA POLEGGI ⁽⁴⁾ - FRANCO CARDONE ⁽⁴⁾ - GIOVANNI COSSU ⁽⁵⁾ - ANTONELLA MANDAS ⁽⁶⁾ - FABRIZIO ANGIUS ⁽¹⁾ - ALDO MANZIN ⁽¹⁾ - BYRON CAUGHEY ⁽²⁾	185
University of Cagliari, Department of Biomedical Sciences, Cagliari, Italia ⁽¹⁾ - National Institute of Allergy and Infectious Diseases (NIAID), National Institute of Health (NIH), Laboratory of Neurological Infections and Immunity, Rocky Mountain Laboratories, Hamilton, Montana, Stati Uniti D'america ⁽²⁾ - University of Verona, Department of Neuroscience, Biomedicine and Movement, Verona, Italia ⁽³⁾ - Istituto Superiore di Sanità, Department of Neuroscience, Roma, Italia ⁽⁴⁾ - A.O. Brotzu,, S. C. Neurology and Stroke Unit, Cagliari, Italia ⁽⁵⁾ - University of Cagliari, Department of Medical Sciences and Public Health, Cagliari, Italia ⁽⁶⁾	185
RT-QuIC detection prion seeding activity in spiked human tears	185
¹ SARAH VASCELLARI, ² CHRISTINA D. ORRÙ, ³ GIANLUIGI ZANUSSO, ⁴ ANNA POLEGGI, ⁴ FRANCO CARDONE, ⁵ GIANNI COSSU, ⁶ ANTONELLA MANDAS, ¹ FABRIZIO ANGIUS, ¹ ALDO MANZIN, ² BYRON CAUGHEY	185
¹ Department of Biomedical Sciences, University of Cagliari, Cagliari, Italy; ² Laboratory of Neurological Infections and Immunity (LNII), Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases (NIAID), National Institute of Health (NIH), Hamilton, MT, United States; ³ Department of Neuroscience, Biomedicine and Movement, University of Verona, Verona, Italy; ⁴ Department of Neuroscience, Istituto Superiore di Sanità, Rome, Italy; ⁵ S. C. Neurology and Stroke Unit, A.O. Brotzu, Cagliari, Italy; ⁶ Department of Medical Sciences and Public Health, University of Cagliari, Cagliari, Italy.	185
02 Controllo microbiologico dell'ambiente	187

26 - Antimicrobial activities of functionalized enamelled-coated surfaces	187
HAMZA UR REHMAN ⁽¹⁾ - FRANCESCA RUSSO ⁽²⁾ - MASSIMO CALOVI ⁽²⁾ - BERENICE FURLAN ⁽¹⁾ - STEFANO ROSSI ⁽²⁾ - ORIETTA MASSIDDA ⁽¹⁾	
Department of Cellular, Computational, and Integrative Biology, University of Trento, Trento, Italia ⁽¹⁾ - Department of Industrial Engineering, University of Trento, Trento, Italia ⁽²⁾	187
66 - Shaping the subway microbiome by a probiotic-based sanitation during the COVID-19 emergency.....	188
MARIA D'ACCOLTI ⁽¹⁾ - IRENE SOFFRITTI ⁽¹⁾ - FRANCESCA BINI ⁽¹⁾ - ELEONORA MAZZIGA ⁽¹⁾ - ANTONELLA VOLTA ⁽²⁾ - MATTEO BISI ⁽²⁾ - SANTE MAZZACANE ⁽²⁾ - ELISABETTA CASELLI ⁽¹⁾	
University of Ferrara, 1Department of Chemical, Pharmaceutical and Agricultural Sciences, and LTTA; 2 CIAS Research Center, University of Ferrara, Ferrara, Italia ⁽¹⁾ - University of Ferrara, CIAS Research Center, University of Ferrara, Ferrara, Italia ⁽²⁾	188
79 - Characterization of the pathogenic potential of the beach sand microbiome and assessment of quicklime as a remediation tool.....	189
FRANCESCA BINI ⁽¹⁾ - IRENE SOFFRITTI ⁽¹⁾ - MARIA D'ACCOLTI ⁽¹⁾ - ELEONORA MAZZIGA ⁽¹⁾ - ANTONELLA VOLTA ⁽²⁾ - MATTEO BISI ⁽²⁾ - SILVIA ROSSI ⁽³⁾ - FRANCESCO VIROLI ⁽⁴⁾ - MARCELLO BALZANI ⁽⁴⁾ - MARCO PETITTA ⁽⁵⁾ - SANTE MAZZACANE ⁽²⁾ - ELISABETTA CASELLI ⁽¹⁾	
University of Ferrara, Section of Microbiology, Department of Chemical, Pharmaceutical and Agricultural Sciences and LTTA; CIAS Research Center, Ferrara, Italia ⁽¹⁾ - University of Ferrara, CIAS Research Center, Ferrara, Italia ⁽²⁾ - Building and Construction Cluster of the Emilia Romagna Region, Building and Construction of the Emilia Romagna Region, Bologna, Italia ⁽³⁾ - University of Ferrara, TekneHub department of Architecture, Ferrara, Italia ⁽⁴⁾ - University 'La Sapienza', Department of Earth Sciences, Roma, Italia ⁽⁵⁾	189
Topic: Controllo microbiologico dell'ambiente	189
Characterization of the pathogenic potential of the beach sand microbiome and assessment of quicklime as a remediation tool	189
FRANCESCA BINI ^{1,2}, IRENE SOFFRITTI ^{1,2}, MARIA D'ACCOLTI ^{1,2}, ELEONORA MAZZIGA ^{1,2}, ANTONELLA VOLTA ², MATTEO BISI ², SILVIA ROSSI ³, FRANCESCO VIROLI ⁴, MARCELLO BALZANI ⁴, MARCO PETITTA ⁵, SANTE MAZZACANE ² AND ELISABETTA CASELLI ^{1,2}	
¹Section of Microbiology, Department of Chemical, Pharmaceutical and Agricultural Sciences, and LTTA, University of Ferrara, Ferrara, Italy; ²Cias Research Center, University of Ferrara, Ferrara, Italy; ³Building and Construction Cluster of the Emilia Romagna Region, Bologna, Italy; ⁴TekneHub, Department of Architecture, University of Ferrara, Ferrara, Italy; ⁵Department of Earth Sciences, University "La Sapienza", Rome, Italy	189
82 - Molecular surveillance of the pediatric hospital microbiome: strategies for infection prevention and control	191
CAROLINA CASON ⁽¹⁾ - GIUSEPPINA CAMPISCIANO ⁽¹⁾ - NUNZIA ZANOTTA ⁽¹⁾ - LUANA ALDEGHERI ⁽¹⁾ - SABRINA SCOLZ ⁽²⁾ - ELISABETTA CASELLI ⁽³⁾ - MANOLA COMAR ⁽¹⁾.....	
Institute for Maternal and Child Health – IRCCS "Burlo Garofolo", Department of Advanced Translational Microbiology, Trieste, Italia ⁽¹⁾ - Institute for Maternal and Child Health – IRCCS "Burlo Garofolo", Sanitary Direction, Trieste, Italia ⁽²⁾ - University of Ferrara, Section of	

Microbiology, Department of Chemical, Pharmaceutical and Agricultural Sciences, and LTTA, Ferrara, Italia ⁽³⁾	191
90 - Potential Use of a Combined Bacteriophage-Probiotic Sanitation System to Control Microbial Contamination and AMR in Healthcare Settings: A Pre-Post Intervention Study	193
ELEONORA MAZZIGA ⁽¹⁾ - MARIA D'ACCOLTI ⁽¹⁾ - IRENE SOFFRITTI ⁽¹⁾ - FRANCESCA BINI ⁽¹⁾ - LUCA ARNOLDO ⁽²⁾ - ANTONELLA VOLTA ⁽³⁾ - MATTEO BISI ⁽³⁾ - PAOLA ANTONIOLI ⁽⁴⁾ - PATRIZIA LAURENTI ⁽⁵⁾ - WALTER RICCIARDI ⁽⁵⁾ - SARA VINCENTI ⁽⁶⁾ - SANTE MAZZACANE ⁽³⁾ - ELISABETTA CASELLI ⁽¹⁾	193
University of Ferrara, Department of Chemical, Pharmaceutical and Agricultural Sciences and LTTA; CIAS Research Center, Ferrara, Italia ⁽¹⁾ - University of Udine, Department of Medicine, Udine, Italia ⁽²⁾ - University of Ferrara, CIAS Research Center, Ferrara, Italia ⁽³⁾ - S. Anna University Hospital, Department of Infection Prevention Control and Risk Management, Ferrara, Italia ⁽⁴⁾ - Department of Health Sciences and Public Health, Section of Hygiene, Catholic University of the Sacred Heart, Department of Woman and Child Health and Public Health, Fondazione Policlinico Universitario A. Gemelli IRCCS, Roma, Italia ⁽⁵⁾ - Fondazione Policlinico Universitario A. Gemelli IRCCS, Department of Woman and Child Health and Public Health, Roma, Italia ⁽⁶⁾	193
Topic: Controllo microbiologico dell'ambiente	193
Potential Use of a Combined Bacteriophage-Probiotic Sanitation System to Control Microbial Contamination and AMR in Healthcare Settings: A Pre-Post Intervention Study.....	193
ELEONORA MAZZIGA ^{1,2} , MARIA D'ACCOLTI ^{1,2} , IRENE SOFFRITTI ^{1,2} , FRANCESCA BINI ^{1,2} , LUCA ARNOLDO ³ , ANTONELLA VOLTA ² , MATTEO BISI ² , PAOLA ANTONIOLI ⁴ , PATRIZIA LAURENTI ^{5,6} , WALTER RICCIARDI ^{5,6} , SARA VINCENTI ⁶ , SANTE MAZZACANE ² , ELISABETTA CASELLI ^{1,2}	193
¹ Department of Chemical, Pharmaceutical and Agricultural Sciences, and LTTA, University of Ferrara, Ferrara, Italy; ² CIAS Research Center, University of Ferrara, Ferrara, Italy; ³ Department of Medicine, University of Udine, Udine, Italy; ⁴ Department of Infection Prevention Control and Risk Management, S. Anna University Hospital, Ferrara, Italy; ⁵ Department of Health Sciences and Public Health, Section of Hygiene, Catholic University of the Sacred Heart, Rome, Italy; ⁶ Department of Woman and Child Health and Public Health, Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy.....	193
131 - EVALUATION OF LEGIONELLA PNEUMOPHILA IN WATER SAMPLES AND COMPARISON WITH LEGIOLERT/REAL-TIME PCR	195
IGNAZIO ARRIGO ⁽¹⁾ - TERESA FASCIANA ⁽²⁾ - ELENA GALIA ⁽¹⁾ - MARIA RITA TRICOLI ⁽³⁾ - MARIO PALERMO ⁽⁴⁾ - LUCIA CANNOVA ⁽²⁾ - GIUSEPPE GALLINA ⁽⁵⁾ - ANNA GIAMMANCO ⁽³⁾	195
LABORATORIO DI RIFERIMENTO REGIONALE PER LA LEGIONELLOSI, A.O.U.P "P.GIACCONE", PALERMO, Italia ⁽¹⁾ - DIPARTIMENTO PROMISE, A.O.U.P "P.GIACCONE" /UNIVERSITA' DI PALERMO, PALERMO, Italia ⁽²⁾ - DIPARTIMENTO PROMISE, A.O.U.P "P.GIACCONE", PALERMO, Italia ⁽³⁾ - DASOE ASSESSORATO REGIONALE SALUTE, UNIVERSITA' DI PALERMO, PALERMO, Italia ⁽⁴⁾ - DISCIPLINE CHIRURGICHE, ONCOLOGICHE E STOMATOLOGICHE, A.O.U.P "P.GIACCONE" /UNIVERSITA' DI PALERMO, PALERMO, Italia ⁽⁵⁾	195
Evaluation of Legionella pneumophila contamination in water samples and comparison with Legiolert/real-time PCR	195

**IGNAZIO ARRIGO¹, TERESA FASCIANA², ELENA GALIA¹, MARIA RITA TRICOLI²,
MARIO PALERMO³, LUCIA CANNOVA², GIUSEPPE GALLINA⁴, ANNA GIAMMANCO². 195**

¹Regional Reference Laboratory for Clinical Environmental Surveillance and Control of Legionellosis, Palermo, Italy;..... 195

²Department of Health Promotion, Mother and Child Care, Internal Medicine and Medical Specialties, University of Palermo, Italy; 195

³Sicilian Health Department, Public Health and Environmental Risks Service, University of Palermo, Italy;..... 195

⁴Department of Surgical Oncological and Stomatological Disciplines, University of Palermo, Italy. 195

230 - Tracking SARS-CoV-2 RNA in urban wastewater through diverse biomarkers 197

ERIKA BRUNO ⁽¹⁾ - MARIANNA MARTINELLI ⁽¹⁾ - CHIARA GIUBBI ⁽¹⁾ - FEDERICA PERDONI ⁽¹⁾ - ARIANNA AZZELLINO ⁽²⁾ - ANDREA TUROLLA ⁽²⁾ - DANILO CEREDA ⁽³⁾ - EMANUELA AMMONI ⁽³⁾ - ELISABETTA SUFFREDINI ⁽⁴⁾ - GIUSEPPINA LA ROSA ⁽⁵⁾ - ANDREA FRANZETTI ⁽⁶⁾ - ROSARIO MUSUMECI ⁽¹⁾ - CLEMENTINA ELVEZIA COCUZZA ⁽¹⁾ - FRANCESCA MALPEI..... 197

⁽²⁾ 197

University of Milano - Bicocca, Department of Medicine and Surgery, Monza, Italia ⁽¹⁾ - Politecnico di Milano, Department of Civil and Environmental Engineering, Milano, Italia ⁽²⁾ - Regione Lombardia, Sicurezza alimentare, nutrizione e qualità delle acque destinate al consumo umano, Milano, Italia ⁽³⁾ - Istituto Superiore di Sanità, Department of Food Safety, Nutrition and Veterinary Public Health, Roma, Italia ⁽⁴⁾ - Istituto Superiore di Sanità, Department of Environment and Health, Roma, Italia ⁽⁵⁾ - University of Milano - Bicocca, Department of Earth and Environmental Sciences, Milano, Italia ⁽⁶⁾ 197

Title: Tracking SARS-CoV-2 RNA in urban wastewater through diverse biomarkers 197

ERIKA BRUNO^{1,2}, MARIANNA MARTINELLI¹, CHIARA GIUBBI¹, FEDERICA PERDONI¹, ARIANNA AZZELLINO³, ANDREA TUROLLA³, DANILO CEREDA⁴, EMANUELA AMMONI⁴, ELISABETTA SUFFREDINI⁵, GIUSEPPINA LA ROSA⁶, ANDREA FRANZETTI², ROSARIO MUSUMECI¹, CLEMENTINA E. COCUZZA¹, FRANCESCA MALPEI³..... 197

¹Department of Medicine and Surgery, University of Milano-Bicocca, Monza, Italy; ²Department of Earth and Environmental Sciences, University of Milano-Bicocca, Milano, Italy; ³Department of Civil and Environmental Engineering, Politecnico di Milano, Milano, Italy; ⁴Sicurezza alimentare, nutrizione e qualità delle acque destinate al consumo umano, UO Prevenzione, Regione Lombardia, Piazza Città di Lombardia, Milano; ⁵Department of Food Safety, Nutrition and Veterinary Public Health, Istituto Superiore di Sanità, Rome, Italy; ⁶Department of Environment and Health, Istituto Superiore di Sanità, Rome, Italy..... 197

318 - Investigation of SARS-CoV-2 RNA in wastewater and bivalve mollusks from the Campania Region 199

ANNALISA LOMBARDI ⁽¹⁾ - ANTONIA VOLI ⁽²⁾ - ANDREA MANCUSI ⁽³⁾ - SANTA GIRARDI ⁽³⁾ - YOLANDE THÉRÈSE ROSE PROROGA ⁽³⁾ - BIANCAMARIA PIERRI ⁽⁴⁾ - RENATO OLIVARES ⁽⁵⁾ - LUIGI COSSENTINO ⁽⁵⁾ - ELISABETTA SUFFREDINI ⁽⁶⁾ - GIUSEPPINA LA ROSA ⁽⁷⁾ - GIOVANNA FUSCO ⁽⁸⁾ - ANTONIO PIZZOLANTE ⁽⁴⁾ - AMALIA PORTA ⁽²⁾ - MARIA TRIASSI ⁽¹⁾ - FRANCESCA PENNINO ⁽¹⁾ - ALESSANDRA TOSCO 199

⁽²⁾ 199

Università degli Studi di Napoli "Federico II", Dipartimento di Sanità Pubblica, Napoli, Italia ⁽¹⁾ - Università di Salerno, Dipartimento di Farmacia, Fisciano, Italia ⁽²⁾ - Istituto Zooprofilattico Sperimentale del Mezzogiorno, Dipartimento Coordinamento di Sicurezza Alimentare, Portici, Italia ⁽³⁾ - Istituto Zooprofilattico del Mezzogiorno, Centro di Referenza Nazionale per l'Analisi e Studio di Correlazione tra Ambiente, Animale e Uomo, Portici, Italia ⁽⁴⁾ - Agenzia Regionale Protezione Ambientale Campania, ARPAC, Napoli, Italia ⁽⁵⁾ - Istituto Superiore di Sanità, Dipartimento di Sicurezza Alimentare, Nutrizione e Sanità Pubblica Veterinaria, Roma, Italia ⁽⁶⁾ - Istituto Superiore di Sanità, Dipartimento Ambiente e Salute, Roma, Italia ⁽⁷⁾ - Istituto Zooprofilattico Sperimentale del Mezzogiorno, Dipartimento di Sanità Animale, Portici, Italia ⁽⁸⁾	199
Investigation of SARS-CoV-2 RNA in wastewater and bivalve mollusks from the Campania Region	199
ANNALISA LOMBARDI ^{1,†} , ANTONIA VOLI ^{2,†} , ANDREA MANCUSI ³ , SANTA GIRARDI ³ , YOLANDE T. R. PROROGA ³ , BIANCAMARIA PIERRI ⁴ , RENATO OLIVARES ⁵ , LUIGI COSSENTINO ⁵ , ELISABETTA SUFFREDINI ⁶ , GIUSEPPINA LA ROSA ⁷ , GIOVANNA FUSCO ⁸ , ANTONIO PIZZOLANTE ⁴ , AMALIA PORTA ² , MARIA TRIASSI ¹ , FRANCESCA PENNINO ^{1,#} , ALESSANDRA TOSCO ^{2,#}	199
† These authors contributed equally to this work.	199
# These authors contributed equally to this work.	199
¹ Department of Public Health, University "Federico II", Via Sergio Pansini 5, 80131 Naples, Italy; ² Department of Pharmacy, University of Salerno, Via Giovanni Paolo II 132, 84084 Fisciano, Italy; ³ Department of Food Security Coordination, Istituto Zooprofilattico Sperimentale del Mezzogiorno, Via Salute 2, 80055 Portici, Italy; ⁴ Centro di Referenza Nazionale per l'Analisi e Studio di Correlazione tra Ambiente, Animale e Uomo, Istituto Zooprofilattico Sperimentale del Mezzogiorno, Via Salute 2, 80055 Portici, Italy; ⁵ Campania Regional Environmental Protection Agency (ARPAC), Via Vicinale Santa Maria del Pianto, 80143 Naples, Italy; ⁶ Department of Food Safety, Nutrition and Veterinary Public Health, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome, Italy; ⁷ Department of Environment and Health, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome, Italy; ⁸ Department of Animal Health, Istituto Zooprofilattico Sperimentale del Mezzogiorno, Via Salute 2, 80055 Portici, Italy	199
321 - Detection and characterization of carbapenemase producing Enterobacterales from wastewater samples	201
FABIO MORECCHIATO ⁽¹⁾ - LORENZO PACI ⁽¹⁾ - CLAUDIA NICCOLAI ⁽²⁾ - MARCO COPPI ⁽²⁾ - ALBERTO ANTONELLI ⁽²⁾ - SIMONA POLLINI ⁽²⁾ - MATTEO RAMAZZOTTI ⁽³⁾ - TOMMASO LOTTI ⁽⁴⁾ - CLAUDIO LUBELLO ⁽⁴⁾ - GIAN MARIA ROSSOLINI ⁽²⁾	201
Department of Experimental and Clinical Medicine, University of Florence, Firenze, Italia ⁽¹⁾ - Department of Experimental and Clinical Medicine; Microbiology and Virology Unit, University of Florence; Careggi University Hospital, Florence, Italia ⁽²⁾ - Department of Biochemical Sciences, University of Florence, Florence, Italia ⁽³⁾ - Department of Civil and Environmental Engineering - (DICEA), University of Florence, Florence, Italia ⁽⁴⁾	201
Detection and characterization of carbapenemase producing Enterobacterales from wastewater samples	201
Fabio Morecchiato ¹ , Lorenzo Paci ¹ , Claudia Niccolai ^{1,2} , Marco Coppi ^{1,2} , Alberto Antonelli ^{1,2} , Simona Pollini ^{1,2} , Matteo Ramazzotti ³ , Tommaso Lotti ⁴ , Claudio Lubello ⁴ , Gian Maria Rossolini ^{1,2}	201
¹ Department of Experimental and Clinical Medicine, University of Florence, Florence, Italy...	201

² Microbiology and Virology Unit, Careggi University Hospital, Florence, Italy	201
³ Department of Biochemical Sciences, University of Florence, Florence, Italy	201
⁴ Department of Civil and Environmental Engineering - (DICEA), University of Florence, Florence, Italy.....	201
03 Fisiologia e metabolismo microbico	203
25 - Characterization of Mutants Involved in the Regulation of Cell Wall Synthesis and Remodeling in <i>Acinetobacter baumannii</i>	203
BERENICE FURLAN ⁽¹⁾ - ALESSANDRA MARTORANA ⁽²⁾ - ALESSANDRA POLISSI ⁽²⁾ - MICHAEL WHALEN ⁽³⁾ - ORIETTA MASSIDDA ⁽¹⁾	203
Department of Cellular, Computational and Integrative Biology, Università di Trento, Trento, Italia ⁽¹⁾ - Department of Pharmacological and Biomolecular Sciences, Università di Milano, Milano, Italia ⁽²⁾ - Institute of Biophysics, National Research Council CNR, Trento, Italia ⁽³⁾	203
Characterization of Mutants Involved in the Regulation of Cell Wall Synthesis and Remodeling in <i>Acinetobacter baumannii</i>	203
Berenice Furlan ¹ , Alessandra Martorana ² , Alessandra Polissi ² , Michael B. Whalen ³ , Orietta Massidda ¹	203
¹ Department of Cellular, Computational and Integrative Biology (CIBIO), University of Trento, ...	203
Trento, Italy; ² Department of Pharmacological and Biomolecular Sciences, University of Milano, Milano, Italy; ³ Institute of Biophysics (IBF), National Research Council (CNR), Trento, Italy ..	203
195 - Culture-independent methods contributed to elucidate mechanisms for O ₂ tolerance in the bacterial genus <i>Sulfurimonas</i>	205
MASSIMILIANO MOLARI ⁽¹⁾ - GUNTER WEGENER ⁽¹⁾ - ANTJE BOETIUS ⁽¹⁾	205
HGF-MPG Joint Research Group for Deep-Sea Ecology and Technology,, Max Planck Institute for Marine Microbiology, Bremen, Germania ⁽¹⁾	205
Topic: Physiology and microbial metabolism	205
Culture-independent methods contributed to elucidate mechanisms for O ₂ tolerance in the bacterial genus <i>Sulfurimonas</i>	205
Massimiliano Molari ^{1,2} , Gunter Wegener ^{1,3} , and Antje Boetius ^{1,2,3}	205
¹ HGF-MPG Joint Research Group for Deep-Sea Ecology and Technology, Max Planck Institute for Marine Microbiology, Bremen, Germany.....	205
² HGF-MPG Joint Research Group for Deep-Sea Ecology and Technology, Alfred Wegener Institute for Polar and Marine Research, Bremerhaven, Germany.....	205
³ MARUM Center for Marine Environmental Sciences, University of Bremen, Bremen, Germany	205
329 - <i>Pseudomonas aeruginosa</i> exploits zinc-binding metallophores to cope with host nutritional immunity strategies.	207
EMMA MICHETTI ⁽¹⁾ - FRANCESCA PACELLO ⁽¹⁾ - VALERIO SECLI ⁽²⁾ - ANDREA BATTISTONI ⁽¹⁾ - SERENA AMMENDOLA ⁽¹⁾	207
Università di Roma Tor Vergata, Dipartimento di Biologia, Roma, Italia ⁽¹⁾ - Ospedale Bambino Gesù, Dipartimento di Oncoematologia, Terapia Genica e Cellulare, Roma, Italia ⁽²⁾	207

Pseudomonas aeruginosa exploits zinc-binding metallophores to cope with host nutritional immunity strategies.	207
Emma Michetti (1), Francesca Pacello (1), Valerio Secli (2), Andrea Battistoni (1), Serena Ammendola (1).....	207
(1) Department of Biology, Tor Vergata University of Rome, Rome, Italy; (2) Department of Onco-Hematology, Gene and Cell Therapy, Bambino Gesù Children's Hospital-IRCCS, Rome, Italy	207
04 Microbiota	209
20 - Repression of a potent genotoxin via amino acid supplementation	209
School of Infection and Immunity, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, Regno Unito ⁽¹⁾	209
Repression of a potent bacterial genotoxin via amino acid supplementation.....	209
SOFIA SANDALLI¹, JENNIFER C. HALLAM¹, GILLIAN R. DOUCE¹, ANDREW J. ROE¹..	209
¹School of Infection and Immunity, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, United Kingdom	209
35 - Characterization of the fecal microbiome in pediatric patients affected with chronic recurrent multifocal osteomyelitis: a pilot study	210
GIUSEPPINA CAMPISCIANO ⁽¹⁾ - SARA DELLA PAOLERA ⁽²⁾ - NUNZIA ZANOTTA ⁽¹⁾ - CAROLINA CASON ⁽¹⁾ - ANDREA TADDIO ⁽²⁾ - MANOLA COMAR ⁽¹⁾.....	210
Department of Advanced Translational Microbiology, Institute for Maternal and Child Health - IRCCS Burlo Garofolo, Trieste, Italia ⁽¹⁾ - Department of Pediatrics, Institute for Maternal and Child Health - IRCCS Burlo Garofolo, Trieste, Italia ⁽²⁾	210
Topic: Microbiota	210
Characterization of the fecal microbiome in pediatric patients affected with chronic recurrent multifocal osteomyelitis: a pilot study	210
GIUSEPPINA CAMPISCIANO^{1,3}, SARA DELLA PAOLERA², NUNZIA ZANOTTA¹, CAROLINA CASON^{1,3}, ANDREA TADDIO^{2,3}, MANOLA COMAR^{1,3}	210
¹Department of Advanced Translational Microbiology, Institute for Maternal and Child Health - IRCCS Burlo Garofolo, Trieste, Italy; ²Department of Pediatrics, Institute for Maternal and Child Health - IRCCS Burlo Garofolo, Trieste, Italy; ³Department of Medical Surgical and Health Sciences, University of Trieste, Trieste, Italy.	210
36 - Exploring the interplay between oral microbiome, inflammatory factors and glycemic control in Type 1 Diabetes Mellitus.	212
LUANA ALDEGHERI ⁽¹⁾ - EULALIA CATAMO ⁽²⁾ - NUNZIA ZANOTTA ⁽¹⁾ - GIUSEPPINA CAMPISCIANO ⁽¹⁾ - CAROLINA CASON ⁽¹⁾ - ANDREA CONTI ⁽²⁾ - GIANLUCA TORNESE ⁽³⁾ - ANTONIETTA ROBINO ⁽²⁾ - MANOLA COMAR ⁽¹⁾	212
SSD R Diagnostica Avanzata Microbiologica Traslazionale, Istituto materno infantile IRCCS Burlo Garofolo, Trieste, Italia ⁽¹⁾ - SC Genetica Medica, Istituto materno infantile IRCCS Burlo Garofolo, Trieste, Italia ⁽²⁾ - SS Endocrinologia, auxologia e diabetologia, Istituto materno infantile IRCCS Burlo Garofolo, Trieste, Italia ⁽³⁾.....	212
Title: Exploring the interplay between oral microbiome, inflammatory factors and glycemic control in Type 1 Diabetes Mellitus.....	212

LUANA ALDEGHERI ¹⁻² , EULALIA CATAMO ³ , NUNZIA ZANOTTA ¹ , GIUSEPPINA CAMPISCIANO ¹⁻² , CAROLINA CASON ¹⁻² , ANDREA CONTI ³ , GIANLUCA TORNESE ⁴ , ANTONIETTA ROBINO ³ , MANOLA COMAR ¹⁻²	212
¹ SSD R Diagnostica Avanzata Microbiologica Traslazionale, Istituto materno infantile IRCCS Burlo Garofolo, Trieste, Italia; ² Dipartimento di Scienze Mediche e Chirurgiche e della Salute, Università degli Studi di Trieste, Trieste, Italia; ³ SC Genetica Medica, Istituto materno infantile IRCCS Burlo Garofolo, Trieste, Italia; ⁴ SS Endocrinologia, auxologia e diabetologia, Istituto materno infantile IRCCS Burlo Garofolo, Trieste, Italia.	212
44 - Fecal sample collection and room-temperature storage using swabs with preservation medium enables deep characterization of the human gut metaproteome	214
ALESSANDRO TANCA ⁽¹⁾ - MARCELLO ABBONDIO ⁽¹⁾ - LAURA DE DIEGO ⁽¹⁾ - MARIA ANTONIETTA DELEDDA ⁽¹⁾ - SERGIO UZZAU ⁽¹⁾	214
Dipartimento di Scienze Biomediche, Università di Sassari, Sassari, Italia ⁽¹⁾	214
Fecal sample collection and room-temperature storage using swabs with preservation medium enables deep characterization of the human gut metaproteome.....	214
ALESSANDRO TANCA, MARCELLO ABBONDIO, LAURA DE DIEGO, MARIA ANTONIETTA DELEDDA, SERGIO UZZAU.....	214
Dipartimento di Scienze Biomediche, Università di Sassari, Sassari, Italy	214
54 - Effects of Clostridioides difficile infection on the structure and functions of the human gut microbiota	215
LAURA DE DIEGO ⁽¹⁾ - ALESSANDRO TANCA ⁽¹⁾ - MARCELLO ABBONDIO ⁽¹⁾ - SERGIO UZZAU ⁽¹⁾	215
Università degli Studi di Sassari, Dipartimento di Scienze Biomediche, Sassari, Italia ⁽¹⁾	215
Effects of Clostridioides difficile infection on the structure and functions of the human gut microbiota	215
LAURA DE DIEGO ¹ , ALESSANDRO TANCA ¹ , MARCELLO ABBONDIO ¹ , SERGIO UZZAU ¹	215
¹ Department of Biomedical Sciences, University of Sassari, Sassari, Italy	215
Preferred presentation method: Poster	215
Topic: Metaproteomics in Health and Disease	215
56 - Preliminary evaluation of fecal microbiota transplantation by oral capsules in patients with liver cirrhosis and hepatic encephalopathy refractory to conventional treatment.....	217
GIANLUCA QUARANTA ⁽¹⁾ - FRANCESCA ROMANA PONZIANI ⁽²⁾ - ALESSANDRA GUARNACCIA ⁽¹⁾ - CHIARA AGRILLO ⁽¹⁾ - FEDERICA IANNARELLI ⁽¹⁾ - CARLO AIROLA ⁽²⁾ - FRANCESCO SANTOPAULO ⁽²⁾ - GIOVANNI FANCELLO ⁽¹⁾ - FLAVIO DE MAIO ⁽¹⁾ - MAURIZIO SANGUINETTI ⁽¹⁾ - LUCA MASUCCI ⁽¹⁾	217
FONDAZIONE POLICLINICO A. GEMELLI, DIPARTIMENTO DI MICROBIOLOGIA E VIROLOGIA, ROMA, Italia ⁽¹⁾ - FONDAZIONE POLICLINICO A. GEMELLI, Digestive Disease Center-CEMAD, ROMA, Italia ⁽²⁾	217
Preliminary evaluation of fecal microbiota transplantation by oral capsules in patients with liver cirrhosis and hepatic encephalopathy refractory to conventional treatment.....	217
GIANLUCA QUARANTA ¹ , FRANCESCA R. PONZIANI ^{3,4} , ALESSANDRA GUARNACCIA ¹ , CHIARA AGRILLO ¹ , FEDERICA IANNARELLI ¹ , CARLO AIROLA ³ , FRANCESCO	

SANTOPAULO ³ , GIOVANNI FANCELLO ¹ , FLAVIO DE MAIO ¹ , MAURIZIO SANGUINETTI ^{1,2} , LUCA MASUCCI ^{1,2}	217
1 Department of Laboratory and Infectious Sciences, A. Gemelli University Hospital IRCCS, 00168 Rome, Italy.	217
2 Department of Basic Biotechnological Sciences, Intensivological and Perioperative Clinics, Catholic University of Sacred Heart, 00168 Rome, Italy.	217
3 Digestive Disease Center-CEMAD, Fondazione Policlinico Universitario Agostino Gemelli IRCCS, 00168 Rome, Italy.	217
4 Translational Medicine and Surgery Department, Università Cattolica del Sacro Cuore, 00168 Rome, Italy.	217
57 - Dynamics of Gut Microbiota and Clinical Variables after Medium (3 months) and Long-Term (6-12 months) Ketogenic and Mediterranean Diets in Drug-Naïve Patients with Type 2 Diabetes Mellitus and Obesity.....	219
VANESSA PALMAS ⁽¹⁾ - ANDREA DELEDDA ⁽²⁾ - VITOR HEIDRICH ⁽³⁾ - GIUSEPPINA SANNA ⁽¹⁾ - LORENZO PUGLIA ⁽²⁾ - MICHELE FOSCI ⁽²⁾ - GIULIA CAMBARAU ⁽²⁾ - ALESSIO LAI ⁽⁴⁾ - MARIETTA MELIS ⁽²⁾ - ELISABETTA LOI ⁽²⁾ - ANDREA LOVISELLI ⁽²⁾ - FERNANDA VELLUZZI ⁽²⁾ - ALDO MANZIN ⁽¹⁾	219
Università degli Studi di Cagliari, Dipartimento di Scienze Biomediche, MONSERRATO (CA), Italia ⁽¹⁾ - Università degli Studi di Cagliari, Department of Medical Sciences and Public Health, MONSERRATO (CA), Italia ⁽²⁾ - Instituto de Química, Universidade de São Paulo, Departamento de Bioquímica, São Paulo, Brasile ⁽³⁾ - P.O. Binaghi, DIABETOLOGIA, CAGLIARI, Italia ⁽⁴⁾ ..	219
TOPIC: MICROBIOTA	219
51th National Congress of the Italian Society of Microbiology (SIM), 24-27 September 2023, Cagliari	219
Dynamics of Gut Microbiota and Clinical Variables after Medium (3 months) and Long-Term (6-12 months) Ketogenic and Mediterranean Diets in Drug-Naïve Patients with Type 2 Diabetes Mellitus and Obesity.....	219
VANESSA PALMAS ¹ , ANDREA DELEDDA ² , VITOR HEIDRICH ³ , GIUSEPPINA SANNA ¹ , LORENZO PUGLIA ² , MICHELE FOSCI ² , GIULIA CAMBARAU ² , ALESSIO LAI ⁴ , MARIETTA MELIS ² , ELISABETTA LOI ² , ANDREA LOVISELLI ² , FERNANDA VELLUZZI ² , ALDO MANZIN ¹	219
¹ Department of Biomedical Sciences, Microbiology and Virology Unit, University of Cagliari, I-09042, Monserrato (CA), Italy;.....	219
² Department of Medical Sciences and Public Health, Obesity and Endocrinology Units, University of Cagliari, 09124 Cagliari, Italy;	219
³ Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo, São Paulo, Brazil;.....	219
⁴ P.O. Binaghi, Diabetologia, ASSL Cagliari, Italy.....	219
67 - Effect on sub-gingival microbiome of diverse subgingival air-polishing in the treatment of advanced periodontitis: a randomized controlled clinical trial.....	221
IRENE SOFFRITTI ⁽¹⁾ - MARIA D'ACCOLTI ⁽¹⁾ - MAGDA MENSI ⁽²⁾ - ROBERTO FARINA ⁽³⁾ - MARIA ELENA GUARNELLI ⁽³⁾ - CHIARA FABBRI ⁽³⁾ - FRANCESCA BINI ⁽¹⁾ - ELEONORA MAZZIGA ⁽¹⁾ - LEONARDO TROMBELLI ⁽³⁾ - ELISABETTA CASELLI ⁽¹⁾	221

University of Ferrara, Department of Chemical, Pharmaceutical and Agricultural Sciences, LTTA and CIAS Research Center, Ferrara, Italia ⁽¹⁾ - University of Brescia & ASST Degli Spedali Civili Di Brescia, Section of Periodontics, School of Dentistry, Department of Surgical Specialties, Radiological Science and Public Health & U.O.C. Odontostomatologia, Brescia, Italia ⁽²⁾ - University of Ferrara & Azienda Unità Sanitaria Locale (AUSL) di Ferrara, Research Centre for the Study of Periodontal and Peri-Implant Diseases & Operative Unit of Dentistry, Ferrara, Italia ⁽³⁾	221
Topic: Microbiota	221
Effect on sub-gingival microbiome of diverse subgingival air-polishing in the treatment of advanced periodontitis: a randomized controlled clinical trial.....	221
IRENE SOFFRITTI ¹, MARIA D'ACCOLTI ¹, MAGDA MENSI ^{2,3}, ROBERTO FARINA ^{4,5}, MARIA ELENA GUARNELLI ^{4,5}, CHIARA FABBRI ^{4,5}, FRANCESCA BINI ¹, ELEONORA MAZZIGA ¹, LEONARDO TROMBELLI ^{4,5}, ELISABETTA CASELLI ¹	221
¹ Department of Chemical, Pharmaceutical and Agricultural Sciences, LTTA and CIAS Research Center, University of Ferrara, Ferrara, Italy; ² Section of Periodontics, School of Dentistry, Department of Surgical Specialties, Radiological Science and Public Health, University of Brescia, Brescia, Italy; ³ U.O.C. Odontostomatologia - ASST Degli Spedali Civili Di Brescia, Brescia, Italy; ⁴ Research Centre for the Study of Periodontal and Peri-Implant Diseases, University of Ferrara, Ferrara, Italy; ⁵ Operative Unit of Dentistry, Azienda Unità Sanitaria Locale (AUSL), Ferrara, Italy	221
69 - Urinary microbiota and type II diabetes: a randomized clinical trial.....	222
MARCO CALVIGIONI ⁽¹⁾ - EDOARDO BIANCALANA ⁽²⁾ - DILETTA MAZZANTINI ⁽¹⁾ - FRANCESCO CELANDRONI ⁽¹⁾ - ANNA SOLINI ⁽²⁾ - EMILIA GHELARDI ⁽¹⁾	222
Universita' di Pisa, Dipartimento di Ricerca Traslationale e Nuove Tecnologie in Medicina e Chirurgia, Pisa, Italia ⁽¹⁾ - Universita' di Pisa, Dipartimento di Patologia Chirurgica, Medica, Molecolare e dell'Area Critica, Pisa, Italia ⁽²⁾	222
Urinary microbiota and type II diabetes: a randomized clinical trial	222
MARCO CALVIGIONI¹, EDOARDO BIANCALANA², DILETTA MAZZANTINI¹, FRANCESCO CELANDRONI¹, ANNA SOLINI², EMILIA GHELARDI¹	222
¹ Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa, Pisa, Italy; ² Department of Surgical, Medical, and Molecular Pathology and Critical Care Medicine, University of Pisa, Pisa, Italy.....	222
70 - Metaproteomic changes in the human gut microbiota following an antibiotic treatment for the eradication of Helicobacter pylori.....	223
MARCELLO ABBONDIO ⁽¹⁾ - ALESSANDRO TANCA ⁽¹⁾ - LAURA DE DIEGO ⁽¹⁾ - ROSANGELA SAU ⁽¹⁾ - GIOVANNI MARIO PES ⁽²⁾ - MARIA PINA DORE ⁽²⁾ - SERGIO UZZAU ⁽¹⁾	223
Università di Sassari, Dipartimento di Scienze Biomediche, Sassari, Italia ⁽¹⁾ - Università di Sassari, Dipartimento di Medicina, Chirurgia e Farmacia, Sassari, Italia ⁽²⁾	223
Metaproteomic changes in the human gut microbiota following an antibiotic treatment for the eradication of Helicobacter pylori.....	223
MARCELLO ABBONDIO¹, ALESSANDRO TANCA¹, LAURA DE DIEGO¹, ROSANGELA SAU¹, GIOVANNI M. PES², MARIA PINA DORE², SERGIO UZZAU¹	223
¹ Dipartimento di Scienze Biomediche, Università di Sassari, Sassari, Italy	223

² Dipartimento di Medicina, Chirurgia e Farmacia, Università di Sassari, Sassari, Italy	223
73 - Profiling gut microbiota in patients undergoing laparoscopic R-en-Y vs One Anastomosis Gastric Bypass	225
FLAVIO DE MAIO ⁽¹⁾ - CRISTIAN E. BORU ⁽²⁾ - GIULIA SANTARELLI ⁽³⁾ - DANILA CAPOCCIA ⁽²⁾ - NUNZIO VELOTTI ⁽⁴⁾ - ORNELLA VERRASTRO ⁽⁵⁾ - BRUNELLA CAPALDO ⁽⁶⁾ - MARIO MUSELLA ⁽⁴⁾ - MAURIZIO SANGUINETTI ⁽¹⁾ - MARCO RAFFAELLI ⁽⁵⁾ - FRIDA LEONETTI ⁽²⁾ - GIANFRANCO SILECCHIA ⁽²⁾ - GIOVANNI DELOGU ⁽⁷⁾	225
Fondazione Policlinico Universitario Agostino Gemelli, Department of Laboratory and Infective Sciences, Faculty of Medicine, Roma, Italia ⁽¹⁾ - Università di Napoli "Federico II", Department of Medico-Surgical Sciences and Biotechnologies, UOC Diabetologia Universitaria, SM Goretti Hospital, Latina, University "La Sapienza" of, Napoli, Italia ⁽²⁾ - Università Cattolica del Sacro Cuore, Department of Sciences of Basic Biotechnologies, Intensive and Perioperative Clinics, Section of Microbiology, Roma, Italia ⁽³⁾ - Università di Napoli "Federico II", Department of Advanced Biomedical Sciences, Napoli, Italia ⁽⁴⁾ - Fondazione Policlinico Universitario Agostino Gemelli, Division of Endocrine and Metabolic Surgery, Roma, Italia ⁽⁵⁾ - Università di Napoli "Federico II", Department of Clinical Medicine and Surgery, Napoli, Italia ⁽⁶⁾ - Mater Olbia Hospital, Mater Olbia Hospital, Olbia, Italia ⁽⁷⁾	225
Profiling gut microbiota in patients undergoing laparoscopic R-en-Y vs One Anastomosis Gastric Bypass	225
Authors	225
Flavio De Maio ¹ , Cristian E. Boru ³ , Giulia Santarelli ² , Danila Capoccia ³ , Nunzio Velotti ⁴ , Ornella Verrastro ⁵ , Brunella Capaldo ⁶ , Mario Musella ⁴ , Maurizio Sanguinetti ^{1,2} , Marco Raffaelli ⁵ , Frida Leonetti ³ , Gianfranco Silecchia ³ , Giovanni Delogu ^{2,7}	225
Affiliations	225
¹ Department of Laboratory and Infective Sciences, Faculty of Medicine, Fondazione Policlinico A. Gemelli IRCCS, Università Cattolica del Sacro Cuore, Rome, Italy; ² Department of Sciences of Basic Biotechnologies, Intensive and Perioperative Clinics, Section of Microbiology, Università Cattolica del Sacro Cuore, Rome, Italy; ³ Department of Medico-Surgical Sciences and Biotechnologies, UOC Diabetologia Universitaria, SM Goretti Hospital, Latina, University "La Sapienza" of Rome, Italy; ⁴ Department of Advanced Biomedical Sciences, University of Naples "Federico II", Naples, Italy; ⁵ Division of Endocrine and Metabolic Surgery, Fondazione Policlinico A. Gemelli IRCCS, Università Cattolica del Sacro Cuore, Rome, Italy; ⁶ Department of Clinical Medicine and Surgery, University of Naples "Federico II", Naples, Italy; ⁷ Mater Olbia Hospital, Olbia, Italy	225
75 - Comparison of methods for processing fecal protein extracts on a multiwell plate for metaproteomic analysis	227
MARIA ANTONIETTA DELEDDA ⁽¹⁾ - MARCELLO ABBONDIO ⁽¹⁾ - LAURA DE DIEGO ⁽¹⁾ - ALESSANDRO TANCA ⁽¹⁾ - SERGIO UZZAU ⁽¹⁾	227
Università degli studi di Sassari, Dipartimento di Scienze Biomediche, Sassari, Italia ⁽¹⁾	227
Comparison of methods for processing fecal protein extracts on a multiwell plate for metaproteomic analysis	227
MARIA ANTONIETTA DELEDDA, MARCELLO ABBONDIO, LAURA DE DIEGO, ALESSANDRO TANCA, SERGIO UZZAU	227
Dipartimento di Scienze di Scienze Biomediche, Università di Sassari, Sassari, Italy	227
147 - Altered oropharyngeal microbiota in common variable immunodeficiency (CVID) patients	228

MARIA GIUFRE' ⁽¹⁾ - FEDERICA PULVIRENTI ⁽²⁾ - TANCREDI M. PENTIMALLI ⁽³⁾ - ROMINA CAMILLI ⁽¹⁾ - CINZIA MILITO ⁽⁴⁾ - MARINA CERQUETTI ⁽¹⁾ - ANNALISA PANTOSTI ⁽¹⁾ - ISABELLA QUINTI ⁽⁴⁾	228
Istituto Superiore di Sanita', Malattie Infettive, Roma, Italia ⁽¹⁾ - Reference Centre for Primary Immune Deficiencies, Azienda Ospedaliera Universitaria Policlinico Umberto I, Roma, Italia ⁽²⁾ - Berlin Institute for Medical Systems Biology (BIMSB), Max Delbrück Center for Molecular Medicine (MDC), Berlin, Germania ⁽³⁾ - "Sapienza" University of Rome, Italy, Department of Molecular Medicine, Roma, Italia ⁽⁴⁾	228
Altered oropharyngeal microbiota in common variable immunodeficiency (CVID) patients	228
MARIA GIUFRE' ¹ , FEDERICA PULVIRENTI ² , TANCREDI M. PENTIMALLI ³ , ROMINA CAMILLI ¹ , CINZIA MILITO ⁴ , MARINA CERQUETTI ¹ , ANNALISA PANTOSTI ¹ , ISABELLA QUINTI ^{2, 4}	228
1Department of Infectious diseases, Istituto Superiore di Sanità, Rome, Italy; 2Reference Centre for Primary Immune Deficiencies, Azienda Ospedaliera Universitaria Policlinico Umberto I, Rome, Italy; 3Berlin Institute for Medical Systems Biology (BIMSB), Max Delbrück Center for Molecular Medicine (MDC), Berlin, Germany; 4Department of Molecular Medicine, "Sapienza" University of Rome, Italy	228
159 - Preliminary insights in the gut microbiome profile of immigrant patients from African countries	230
MARIANNA MARANGI ⁽¹⁾ - GIORGIA PALLADINO ⁽²⁾ - ROSELLA DE NITTIS ⁽³⁾ - SILVIA TURRONI ⁽²⁾ - SIMONE RAMPELLI ⁽²⁾ - MAURIZIO MARGAGLIONE ⁽¹⁾ - MARCO CANDELA ⁽²⁾	230
Università di Foggia, Dipartimento di Medicina Clinica e Sperimentale, Foggia, Italia ⁽¹⁾ - Università di Bologna, Dipartimento di Farmacia e Biotecnologie, Bologna, Italia ⁽²⁾ - Unità di Microbiologia e Virologia, Ospedale Riuniti, Foggia, Italia ⁽³⁾	230
Preliminary insights in the gut microbiome profile of immigrant patients from African countries	230
MARIANNA MARANGI ¹ , GIORGIA PALLADINO ² , ROSELLA DE NITTIS ³ , SILVIA TURRONI ² , SIMONE RAMPELLI ² , MAURIZIO MARGAGLIONE ¹ , MARCO CANDELA ²	230
¹ Department of Clinical and Experimental Medicine, University of Foggia, Viale Luigi Pinto, 71122, Foggia, Italy; ² Department of Pharmacy and Biotechnology, University of Bologna, Italy; ³ Microbiology and Virology Unit, Ospedali Riuniti, Viale Luigi Pinto, 71122, Foggia, Italy.	230
179 - Oral and blood microbiota relate to interferon and endothelial genes expression during COVID-19	232
LETIZIA SANTINELLI ⁽¹⁾ - GIULIA RADOCCHIA ⁽¹⁾ - MATTEO FRACELLA ⁽²⁾ - LEONARDO SORRENTINO ⁽²⁾ - MANOLA COMAR ⁽³⁾ - FEDERICA FRASCA ⁽²⁾ - LUCA MADDALONI ⁽¹⁾ - GINEVRA BUGANI ⁽¹⁾ - ALESSANDRA PIERANGELI ⁽²⁾ - ANNA TERESA PALAMARA ⁽⁴⁾ - GUIDO ANTONELLI ⁽²⁾ - CLAUDIO MARIA MASTROIANNI ⁽¹⁾ - GABRIELLA D'ETTORRE ⁽¹⁾ - CAROLINA SCAGNOLARI ⁽²⁾ - VALERIO IEBBA ⁽³⁾ - SERENA SCHIPPA ⁽¹⁾	232
Sapienza University of Rome, Department of Public Health and Infectious Diseases, Rome, Italia ⁽¹⁾ - Sapienza University of Rome, Department of Molecular Medicine, Laboratory of Virology, Rome, Italia ⁽²⁾ - University of Trieste, Department of Medical Sciences, Trieste, Italia ⁽³⁾ - National Institute of Health, Department of Infectious Diseases, Rome, Italia ⁽⁴⁾	232

Oral and blood microbiota relate to interferon and endothelial genes expression during COVID-19.....	232
LETIZIA SANTINELLI ¹ , GIULIA RADOCCHIA ¹ , MATTEO FRACELLA ² , LEONARDO SORRENTINO ² , MANOLA COMAR ³ , FEDERICA FRASCA ² , LUCA MADDALONI ¹ , GINEVRA BUGANI ¹ , ALESSANDRA PIERANGELI ² , ANNA T. PALAMARA ⁴ , GUIDO ANTONELLI ² , CLAUDIO M. MASTROIANNI ¹ , GABRIELLA d'ETTORRE ² , CAROLINA SCAGNOLARI ² , VALERIO IEBBA ³ AND SERENA SCHIPPA ¹	232
¹ Department of Public Health and Infectious Diseases, Sapienza University of Rome, Rome, Italy;	232
² Department of Molecular Medicine, Laboratory of Virology, Istituto Pasteur Italia, Sapienza University of Rome, Rome, Italy;.....	232
³ Department of Medical Sciences, University of Trieste, Trieste, Italy;	232
⁴ Department of Infectious Diseases, National Institute of Health, Rome, Italy.	232
186 - Phenotypic characterization and whole genome analysis of Staphylococcus aureus isolates colonizing the skin microbiota of patients with severe atopic dermatitis.	234
FRANCESCA SIVORI ⁽¹⁾ - ILARIA CAVALLO ⁽¹⁾ - MAURO TRUGLIO ⁽¹⁾ - GIORGIA FABRIZIO ⁽¹⁾ - FLAVIO DE MAIO ⁽²⁾ - MAURIZIO SANGUINETTI ⁽²⁾ - ALESSIA CAVALLO ⁽³⁾ - EMANUELA CAMERA ⁽³⁾ - ANTONIO CRISTAUDO ⁽⁴⁾ - FULVIA PIMPINELLI ⁽¹⁾ - ENEA GINO DI DOMENICO ⁽⁵⁾	234
Microbiology and Virology, San Gallicano Dermatological Institute, IRCCS, ROMA, Italia ⁽¹⁾ - Dipartimento di Scienze di Laboratorio e Infettivologiche, Fondazione Policlinico Universitario "A. Gemelli", IRCCS, ROMA, Italia ⁽²⁾ - Laboratory of Cutaneous Physiopathology, San Gallicano Dermatological Institute, IRCCS, ROMA, Italia ⁽³⁾ - Clinical Dermatology, San Gallicano Dermatological Institute, IRCCS, ROMA, Italia ⁽⁴⁾ - Department of Biology and Biotechnology "C. Darwin", Sapienza University of Rome, ROMA, Italia ⁽⁵⁾	234
Phenotypic characterization and whole genome analysis of Staphylococcus aureus isolates colonizing the skin microbiota of patients with severe atopic dermatitis.	234
Francesca SIVORI ¹ , Iliaria CAVALLO ¹ , Mauro TRUGLIO ¹ , Giorgia FABRIZIO ¹ , Flavio DE MAIO ² , Maurizio SANGUINETTI ² , Alessia CAVALLO ³ , Emanuela CAMERA ³ , Antonio CRISTAUDO ⁴ , Fulvia PIMPINELLI ¹ , Enea G. DI DOMENICO ⁵	234
¹ Microbiology and Virology, San Gallicano Dermatological Institute, IRCCS, Rome, Italy; ² Dipartimento di Scienze di Laboratorio e Infettivologiche, Fondazione Policlinico Universitario "A. Gemelli" IRCCS, Rome, Italy; ³ Laboratory of Cutaneous Physiopathology, San Gallicano Dermatological Institute, IRCCS, Rome, Italy; ⁴ Clinical Dermatology, San Gallicano Dermatological Institute, IRCCS, Rome, Italy; ⁵ Department of Biology and Biotechnology "C. Darwin" Sapienza University of Rome, 00185 Rome, Italy.	234
196 - Effect of food supplements on gut microbiota in obese patients: preliminary results of zimba trial.....	236
ALICE CARAMASCHI ⁽¹⁾ - SIMONETTA BELLONE ⁽²⁾ - VALENTINA ANTONIOTTI ⁽³⁾ - GIORGIA NOVELLO ⁽⁴⁾ - ROBERTA RICOTTI ⁽²⁾ - VALENTINA MANCIOPPI ⁽²⁾ - FLAVIA PRODAM ⁽³⁾ - ELISA BONA ⁽¹⁾	236
Università del Piemonte Orientale, Dipartimento per lo Sviluppo Sostenibile e la Transizione Ecologica, Vercelli, Italia ⁽¹⁾ - Università del Piemonte Orientale, Dipartimento di Scienze della Salute, SCDU Pediatrica, Novara, Italia ⁽²⁾ - Università del Piemonte Orientale, Dipartimento di	

Scienze della Salute, Novara, Italia ⁽³⁾ - Università del Piemonte Orientale, Dipartimento di Scienze e Innovazione tecnologica, Alessandria, Italia ⁽⁴⁾	236
Effect of food supplements on gut microbiota in obese patients: preliminary results of zimba trial	236
ALICE CARAMASCHI ¹ , SIMONETTA BELLONE ⁴ , VALENTINA ANTONIOTTI ³ , GIORGIA NOVELLO ² , ROBERTA RICOTTI ⁴ , VALENTINA MANCIOPPI ⁴ , FLAVIA PRODAM ³ , ELISA BONA ^{1,5}	236
¹ Dipartimento per lo Sviluppo Sostenibile e la Transizione Ecologica, Università del Piemonte Orientale, Vercelli, Italy	236
² Dipartimento di Scienze e Innovazione Tecnologica, Università del Piemonte Orientale, Vercelli, Italy	236
³ Dipartimento di Scienze della Salute, Università del Piemonte Orientale, Novara, Italy	236
⁴ Dipartimento di Scienze della Salute, SCDU Pediatrica, Novara, Italy	236
⁵ Centro Universitario per la Ricerca Traslazionale sulle Malattie Autoimmuni ed Allergiche, UPO-CAAD, Novara, Italy	236
207 - Mycobacterium avium subspecies paratuberculosis (MAP) infection, and its impact on gut microbiome of individuals with Multiple Sclerosis.....	237
HAJRA ASHRAF ⁽¹⁾ - UMER ZEESHAN IJAZ ⁽²⁾ - LEONARDO ANTONIO SECHI ⁽¹⁾	237
University of Sassari, Department of Biomedicine, Sassari, Italy ⁽¹⁾ - University of Glasgow, University of Glasgow, Glasgow, United Kingdom ⁽²⁾	237
Mycobacterium avium subspecies paratuberculosis (MAP) infection, and its impact on gut microbiome of individuals with Multiple Sclerosis.....	237
Hajra Ashraf ¹ , Umer Z. Ijaz ^{2,3,4} , Leonardo A. Sechi ^{1,5}	237
^{1*} Department of Biomedical Sciences; University of Sassari, Sassari, Italy	237
² Water & Environment Research Group, University of Glasgow, Mazumdar-Shaw Advanced Research Centre, Glasgow, United Kingdom	237
³ National University of Ireland, Galway, University Road, Galway, Ireland	237
⁴ Department of Molecular and Clinical Cancer Medicine, University of Liverpool, Liverpool, United Kingdom	237
⁵ Complex Structure of Microbiology and Virology; AOU Sassari, Sassari, Italy	237
243 - Gut microbiota profile in CDKL5 deficiency disorder patients.....	239
EMERENZIANA OTTAVIANO ⁽¹⁾ - ORNELLA XYNOMILAKIS ⁽²⁾ - CAMILLA CECCARANI ⁽³⁾ - SILVIA ANCONA ⁽¹⁾ - PAOLA TOGNINI ⁽⁴⁾ - AGLAIA VIGNOLI ⁽⁵⁾ - ELISA BORGHI ⁽¹⁾	239
Universita' Degli Studi Di Milano, Dip. Scienze Della Salute, Milano, Italia ⁽¹⁾ - Universita' Degli Studi Di Milano, Dip. Scienze Biochimiche E Cliniche, Milano, Italia ⁽²⁾ - Consiglio Nazionale Delle Ricerche (Cnr), Istituto Tecnologie Biomediche, Milano, Italia ⁽³⁾ - Università Di Pisa, Department Of Translational Research And New Technologies In Medicine And Surgery, Pisa, Italia ⁽⁴⁾ - Asst Gom Niguarda, Ospedale Niguarda Ca' Granda, Milano, Italia ⁽⁵⁾	239
Gut Microbiota Profile In Cdkl5 Deficiency Disorder Patients	239
Emerenziana Ottaviano ¹ , Ornella Xynomilakis ² , Camilla Ceccarani ⁵ , Silvia Ancona ¹ , Paola Tognini ³ , Aglaia Vignoli ⁴ , Elisa Borghi ¹	239
1- Department Of Health Sciences, Università Degli Studi Di Milano, Milan, Italy;	239

2- Department Of Biochemical And Clinical Sciences, Università Degli Studi Di Milano, Milan, Italy;.....	239
3- Department Of Translational Research And New Technologies In Medicine And Surgery, University Of Pisa, Pisa, Italy.	239
4- Neuropsychiatry Of Childhood And Adolescence, Asst Gom Niguarda, Ospedale Niguarda Ca' Granda, Milan, Italy;.....	239
5-Institute Of Biomedical Technologies, National Research Council, (Cnr), Segrate, Milan, Italy.	239
303 - Dynamics of nasopharyngeal tract mycobiota in COVID-19 patients	241
VERONICA FOLLIERO ⁽¹⁾ - CARLO FERRAVANTE ⁽¹⁾ - FEDERICA DELL'ANNUNZIATA ⁽¹⁾ - ROSARIO BRANCACCIO ⁽¹⁾ - ELENA ALEXANDROVA ⁽¹⁾ - DOMENICO DI ROSA ⁽¹⁾ - JESSICA LAMBERTI ⁽¹⁾ - DOMENICO PALUMBO ⁽¹⁾ - VALERIA CRUDELE ⁽²⁾ - ROBERTA MANENTE ⁽¹⁾ - RITA GRECO ⁽³⁾ - GIOVANNI BOCCIA ⁽¹⁾ - PASQUALE PAGLIANO ⁽¹⁾ - ALESSANDRO WEISZ ⁽¹⁾ - FRANCESCA RIZZO ⁽¹⁾ - GIANLUIGI FRANCI ⁽¹⁾.....	241
University of Salerno, Department of Medicine, Surgery and Dentistry, Scuola Medica Salernitana, Salerno, Italia ⁽¹⁾ - University Hospital of Campania "Luigi Vanvitelli, UOC of Virology and Microbiology, Napoli, Italia ⁽²⁾ - AORN Sant 'Anna and San Sebastiano, UOSD Microbiology, Caserta, Italia ⁽³⁾	241
Dynamics of nasopharyngeal tract mycobiota in COVID-19 patients	241
Veronica Folliero ¹ , Carlo Ferravante ¹ , Federica Dell'Annunziata ¹ , Rosario Brancaccio ¹ , Elena Alexandrova ¹ , Domenico Di Rosa ¹ , Jessica Lamberti ¹ , Domenico Palumbo ¹ , Valeria Crudele ² , Roberta Manente ¹ , Rita Greco ³ , Giovanni Boccia ¹ , Pasquale Pagliano ¹ , Alessandro Weisz ¹ , Francesca Rizzo ¹ , Gianluigi Franci ¹	241
1. Department of Medicine, Surgery and Dentistry "Scuola Medica Salernitana", University of Salerno, Salerno, Italy;	241
2. UOC of Virology and Microbiology, University Hospital of Campania "Luigi Vanvitelli", Naples, Italy.....	241
3. UOSD Microbiology - AORN Sant 'Anna and San Sebastiano, Caserta, Italy.	241
305 - Dynamics of nasopharyngeal tract mycobiota in COVID-19 patients	243
VERONICA FOLLIERO ⁽¹⁾ - CARLO FERRAVANTE ⁽¹⁾ - FEDERICA DELL'ANNUNZIATA ⁽¹⁾ - ROSARIO BRANCACCIO ⁽¹⁾ - ELENA ALEXANDROVA ⁽¹⁾ - DOMENICO DI ROSA ⁽¹⁾ - JESSICA LAMBERTI ⁽¹⁾ - DOMENICO PALUMBO ⁽¹⁾ - VALERIA CRUDELE ⁽²⁾ - ROBERTA MANENTE ⁽¹⁾ - RITA GRECO ⁽³⁾ - GIOVANNI BOCCIA ⁽¹⁾ - PASQUALE PAGLIANO ⁽¹⁾ - ALESSANDRO WEISZ ⁽¹⁾ - FRANCESCA RIZZO ⁽¹⁾ - GIANLUIGI FRANCI ⁽¹⁾.....	243
University of Salerno, Department of Medicine, Surgery and Dentistry, Scuola Medica Salernitana, Salerno, Italia ⁽¹⁾ - University Hospital of Campania "Luigi Vanvitelli, UOC of Virology and Microbiology, Napoli, Italia ⁽²⁾ - AORN Sant 'Anna and San Sebastiano, UOSD Microbiology, Caserta, Italia ⁽³⁾	243
Dynamics of nasopharyngeal tract mycobiota in COVID-19 patients	243
Veronica Folliero ¹ , Carlo Ferravante ¹ , Federica Dell'Annunziata ¹ , Rosario Brancaccio ¹ , Elena Alexandrova ¹ , Domenico Di Rosa ¹ , Jessica Lamberti ¹ , Domenico Palumbo ¹ , Valeria Crudele ² , Roberta Manente ¹ , Rita Greco ³ , Giovanni Boccia ¹ , Pasquale Pagliano ¹ , Alessandro Weisz ¹ , Francesca Rizzo ¹ , Gianluigi Franci ¹	243

1. Department of Medicine, Surgery and Dentistry "Scuola Medica Salernitana", University of Salerno, Salerno, Italy;	243
2. UOC of Virology and Microbiology, University Hospital of Campania "Luigi Vanvitelli", Naples, Italy.....	243
3. UOSD Microbiology - AORN Sant 'Anna and San Sebastiano, Caserta, Italy.	243
316 - In vitro study of various bacterial strains from human microbiota cultured in different types of advanced 3D substrates.....	245
EMANUELA PELUSO ⁽¹⁾ - DANIELA PACHECO ⁽²⁾ - SEBASTIAO VAN UDEN ⁽²⁾ - SONJA VISENTIN ⁽³⁾ - PAOLA PETRINI ⁽⁴⁾ - LIVIA VISAI ⁽⁵⁾	245
Università degli studi di Pavia, Department of molecular medicine, Pavia, Italia ⁽¹⁾ - Bac3Gel Lda, TagusPark—Edificio Inovacao II, Porto Salvo, Portogallo ⁽²⁾ - Università degli studi di Torino, Department of Molecular Biotechnology and Health Science, Torino, Italia ⁽³⁾ - Politecnico di Milano, Department of Chemistry, Materials and Chemical Engineering, Milano, Italia ⁽⁴⁾ - Università degli studi di Pavia, Department of Molecular Medicine, Pavia, Italia ⁽⁵⁾	245
In vitro study of various bacterial strains from human microbiota cultured in different types of advanced 3D substrates. EMANUELA PELUSO ¹ , DANIELA PACHECO ² , SEBASTIÃO VAN UDEN ² , SONJA VISENTIN ³ , PAOLA PETRINI ⁴ , LIVIA VISAI ^{1,5} ¹ Department of Molecular Medicine, Center for Health Technologies, Udr INSTM, University of Pavia, Pavia, Italy; ² Bac ³ Gel Lda, TagusPark—Edificio Inovacao II, Porto Salvo, Portugal;	245
³ Department of Molecular Biotechnology and Health Science, University of Torino, Torino, Italy; ⁴ Department of Chemistry, Materials and Chemical Engineering "Giulio Natta", Politecnico di Milano, Milano, Italy; ⁵ Medicina Clinica-Specialistica, UOR5 Laboratorio di Nanotecnologie, ICS Maugeri, IRCCS, Pavia, Italy.	245
323 - Bacterial, fungal and viral gut microbiota signatures predict immunotherapy response in cutaneous and mucosal melanoma patients	247
VALERIO IEBBA ⁽¹⁾ - SATORU YONEKURA ⁽²⁾	247
Università di Trieste, Dipartimento Universitario Clinico di Scienze Mediche Chirurgiche e della Salute, Trieste, Italia ⁽¹⁾ - Kyoto University Graduate School of Medicine, Kyoto, Japan., Department of Dermatology, Kyoto, Giappone ⁽²⁾	247
Bacterial, fungal and viral gut microbiota signatures predict immunotherapy response in cutaneous and mucosal melanoma patients	247
Valerio Iebba ¹ , Satoru Yonekura ²	247
AFFILIATIONS	247
1 Department of Medical, Surgical and Health Sciences, University of Trieste, Trieste, Italy.....	247
2 Department of Dermatology, Kyoto University Graduate School of Medicine, Kyoto, Japan..	247
325 - A metagenomic cross-cohort analysis of bacterial virulence factors in colorectal cancer	248
VALERIO IEBBA ⁽¹⁾ - SATORU YONEKURA ⁽²⁾	248
Università di Trieste, Dipartimento Universitario Clinico di Scienze Mediche Chirurgiche e della Salute, Trieste, Italia ⁽¹⁾ - Kyoto University Graduate School of Medicine, Kyoto, Japan., Department of Dermatology, Kyoto, Giappone ⁽²⁾	248
A metagenomic cross-cohort analysis of bacterial virulence factors in colorectal cancer	248
Valerio Iebba ¹ , Satoru Yonekura ²	248

AFFILIATIONS	248
1 Department of Medical, Surgical and Health Sciences, University of Trieste, Trieste, Italy.	248
2 Department of Dermatology, Kyoto University Graduate School of Medicine, Kyoto, Japan..	248
05 Modelli matematici ed epidemiologia/Epidemiologia molecolare	249
15 - Genomic epidemiology of K. pneumoniae carbapenemase-producing Klebsiella pneumoniae (KPC-Kp) strains from three Northern-Italy hospitals and investigation of intra- and inter-hospitals genetic diver	249
VALERIA FOX ⁽¹⁾ - DAVIDE MANGIONI ⁽²⁾ - AGNESE COMELLI ⁽³⁾ - SILVIA RENICA ⁽¹⁾ - MARIANNA ROSSI ⁽⁴⁾ - MADDALENA CASANA ⁽⁵⁾ - LUDOVICA MELA ⁽³⁾ - ALESSANDRA BIELLI ⁽⁶⁾ - CHIARA S. VISMARA ⁽⁶⁾ - PAOLO BONFANTI ⁽⁴⁾ - BARBARA BOTTAZZI ⁽⁷⁾ - BARBARA CASSANI ⁽⁷⁾ - PAOLA MORELLI ⁽⁵⁾ - CARLO F. PERNO ⁽⁸⁾ - ANDREA GORI ⁽⁹⁾ - ANTONIO MUSCATELLO ⁽³⁾ - ALESSANDRA BANDERA ⁽²⁾ - CLAUDIA ALTERI ⁽¹⁾	249
Department of Oncology and Hemato-Oncology, University of Milan, Milan, Italia ⁽¹⁾ - Infectious Diseases Unit, Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, &Department of Pathophysiology and Transplantation, University of Milan, Milan, Italia ⁽²⁾ - Infectious Diseases Unit, Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italia ⁽³⁾ - Infectious Diseases Unit, San Gerardo Hospital, Monza, Italia ⁽⁴⁾ - Infectious Diseases Unit, Humanitas Clinical and Research Center IRCCS, Rozzano, Italia ⁽⁵⁾ - Complex Unit of Clinical Microbiology, Niguarda Hospital, Milan, Italia ⁽⁶⁾ - Humanitas Clinical and Research Center, IRCCS, Rozzano, Italia ⁽⁷⁾ - Multimodal Research Area, Bambino Gesù Children Hospital IRCCS, Rome, Italia ⁽⁸⁾ - Department of Pathophysiology and Transplantation, University of Milan, &Division of Infectious Diseases, L. Sacco University Hospital, Milan, Italia ⁽⁹⁾	249
Category: Modelli matematici ed epidemiologia/Epidemiologia molecolare	249
Title: Genomic epidemiology of K. pneumoniae carbapenemase-producing Klebsiella pneumoniae (KPC-Kp) strains from three Northern-Italy hospitals and investigation of intra- and inter- hospitals genetic diversity	249
Authors: Valeria Fox¹, Davide Mangioni^{2,3}, Agnese Comelli², Silvia Renica¹, Marianna Rossi⁴, Maddalena Casana⁵, Ludovica Mela², Alessandra Bielli⁶, Chiara S. Vismara⁶, Paolo Bonfanti⁴, Barbara Bottazzi⁷, Barbara Cassani⁷, Paola Morelli⁵, Carlo F. Perno⁸, Andrea Gori^{3,9}, Antonio Muscatello², Alessandra Bandera^{2,3}, Claudia Alteri¹	249
¹Department of Oncology and Hemato-Oncology, University of Milan, Milan, Italy.....	249
²Infectious Diseases Unit, Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy	249
³Department of Pathophysiology and Transplantation, University of Milan, Milan, Italy	249
⁴Infectious Diseases Unit, San Gerardo Hospital – Monza, Italy	249
⁵Infectious Diseases Unit, Humanitas Clinical and Research Center IRCCS, Rozzano - Milan, Italy	249
⁶Complex Unit of Clinical Microbiology, Niguarda Hospital, Milan, Italy	249
⁷Humanitas Clinical and Research Center IRCCS, Rozzano – Milan, Italy	249
⁸Multimodal Research Area, Bambino Gesù Children Hospital IRCCS, Rome, Italy	249
⁹Division of Infectious Diseases, L. Sacco University Hospital, Milan, Italy	249
93 - Microorganisms from human clinical samples as a tool for technological innovation: creation of a shared platform of the UNITO collections.	251

LORENZA CAVALLO ⁽¹⁾ - JANIRA ROANA ⁽¹⁾ - ANTONIO CURTONI ⁽¹⁾ - ALESSANDRO BONDI ⁽¹⁾ - NARCISA MANDRAS ⁽¹⁾ - MATTIA GENCO ⁽¹⁾ - IOLANDA PERUGINI ⁽²⁾ - DANIELA SCALAS ⁽³⁾ - LUCA COCOLIN ⁽⁴⁾ - FRANCESCA MENOTTI ⁽¹⁾ - VALERIA PRIGIONE ⁽²⁾ - VIVIAN TULLIO ⁽¹⁾ - CRISTINA VARESE ⁽²⁾ - CRISTINA COSTA ⁽¹⁾	251
UNIVERSITA' DI TORINO, DIP SCIENZE DELLA SANITA' PUBBLICA E PEDITRICHE, TORINO, Italia ⁽¹⁾ - UNIVERSITA' DI TORINO, SCIENZE DELLA VITA E DEI SISTEMI, TORINO, Italia ⁽²⁾ - UNIVERSITA' DI TORINO, Dipartimento di Scienze Veterinarie, TORINO, Italia ⁽³⁾ - UNIVERSITA' DI TORINO, Dipartimento di Scienze Agrarie, Forestali e Alimentari, TORINO, Italia ⁽⁴⁾	251
Microorganisms from human clinical samples as a tool for technological innovation: creation of a shared platform of the UNITO collections.	251
LORENZA CAVALLO ¹ , JANIRA ROANA ¹ , ANTONIO CURTONI ^{1,2} , ALESSANDRO BONDI ^{1,2} , NARCISA MANDRAS ¹ , MATTIA GENCO ¹ , IOLANDA PERUGINI ³ , DANIELA SCALAS ⁴ , LUCA COCOLIN ⁵ , FRANCESCA MENOTTI ¹ , VALERIA PRIGIONE ³ , VIVIAN TULLIO ¹ , CRISTINA VARESE ³ , CRISTINA COSTA ^{1,2}	251
¹ Department of Public Health and Pediatrics, University of Turin, Italy; ² Microbiology and Virology Unit, University Hospital Città della Salute e della Scienza di Torino, Italy; ³ Department of Life Sciences and Systems Biology, University of Turin, Italy; ⁴ Microbiology and Infectious Diseases Unit, Department of Veterinary Sciences, University of Turin, Italy; ⁵ Department of Agricultural, Forest and Food Sciences, University of Turin, Italy.	251
106 - Human enterovirus surveillance in the Marche Region (Italy).....	253
KATIA MARINELLI ⁽¹⁾ - STEFANO MENZO ⁽²⁾ - SARA CAUCCI ⁽²⁾	253
SOSD VIROLOGIA, Azienda Ospedaliera Universitaria delle Marche, ANCONA, Italia ⁽¹⁾ - dipartimento di scienze biomediche e salute pubblica, università politecnica delle Marche, ANCONA, Italia ⁽²⁾	253
Human enterovirus surveillance in the Marche Region (Italy).....	253
KATIA MARINELLI ² , SARA CAUCCI ¹ AND STEFANO MENZO ^{1,2}	253
¹ Department of Biomedical Sciences and Public Health, Università Politecnica delle Marche, Ancona, Italy;	253
² Virology Laboratory, Azienda Ospedaliera Universitaria delle Marche, Ancona, Italy;	253
Abstract	253
114 - Frequency and Variation of Viral Respiratory Infections in Pre- and During -COVID-19 Pandemic: A 7-year experience in a teaching hospital of Southern Italy	255
GRAZIA PAVIA ⁽¹⁾ - GIUSEPPE GUIDO MARIA SCARLATA ⁽¹⁾ - CLAUDIA CICINO ⁽¹⁾ - MICHELE MANNO ⁽¹⁾ - MARIA GIUSEPPINA STILLITANO ⁽¹⁾ - GIORGIO SETTIMO BARRECA ⁽¹⁾ - ANGELO GIUSEPPE LAMBERTI ⁽¹⁾ - AIDA GIANCOTTI ⁽¹⁾ - LUIGIA GALLO ⁽¹⁾ - RAFFAELLA SINOPOLI ⁽¹⁾ - ELENA COLOSIMO ⁽¹⁾ - NADIA MARASCIO ⁽¹⁾ - ANGELA QUIRINO ⁽¹⁾ - GIOVANNI MATERA ⁽¹⁾	255
Università degli Studi "Magna Graecia" di Catanzaro, Azienda Ospedaliero-Universitaria "R. Dulbecco" Presidio "Mater Domini", Catanzaro, Dipartimento Scienze della Salute, Catanzaro, Italia ⁽¹⁾	255
Frequency and Variation of Viral Respiratory Infections in Pre- and During -COVID-19 Pandemic: A 7-year experience in a teaching hospital of Southern Italy	255

GRAZIA PAVIA ¹ , GIUSEPPE G.M. SCARLATA ¹ , CLAUDIA CICINO ¹ , MICHELE MANNO ¹ , MARIA G. STILLITANO ¹ , GIORGIO S. BARRECA ¹ , ANGELO G. LAMBERTI ¹ , AIDA GIANCOTTI ¹ , LUIGIA GALLO ¹ , RAFFAELLA SINOPOLI ¹ , ELENA COLOSIMO ¹ , NADIA MARASCIO ¹ , ANGELA QUIRINO ¹ AND GIOVANNI MATERA ¹	255
¹ Department of Health Sciences, Unit of Clinical Microbiology, “Magna Græcia” University Hospital of Catanzaro; Catanzaro, Italy	255
137 - A novel epidemiological tool: Fourier Transform infrared spectroscopy (FT-IR) for real time tracing of <i>Klebsiella pneumoniae</i> producing NDM-5 and NDM-1/OXA-48-like enzymes	257
ELENA ADDIS ⁽¹⁾ - MATTIA SCARAZZAI ⁽¹⁾ - ANNA BERTONCELLI ⁽¹⁾ - DAVIDE GIBELLINI ⁽¹⁾ - ANNARITA MAZZARIOL ⁽¹⁾	257
Università di Verona, Dipartimento di Diagnostica e Sanità Pubblica, Verona, Italia ⁽¹⁾	257
A novel epidemiological tool: Fourier Transform infrared spectroscopy (FT-IR) for real time tracing of <i>Klebsiella pneumoniae</i> producing NDM-5 and NDM-1/OXA-48-like enzymes	257
ELENA ADDIS. ¹ , MATTIA SCARAZZAI ¹ , ANNA BERTONCELLI ¹ , DAVIDE GIBELLINI ¹ , ANNARITA MAZZARIOL ¹	257
¹ Department of Diagnostics and Public Health, University of Verona, Verona, Italy	257
150 - The mosaic of Adenovirus variants circulating in 2022-2023 in hospitalized patients.....	258
FEDERICA ANNA MARIA GIARDINA ⁽¹⁾ - GUGLIELMO FERRARI ⁽²⁾ - ANTONINO MARIA GUGLIELMO PITROLO ⁽²⁾ - ANTONIO PIRALLA ⁽²⁾ - FAUSTO BALDANTI ⁽¹⁾	258
Department of Clinical, Surgical, Diagnostic and Pediatric Sciences, University of Pavia, Pavia, Italia ⁽¹⁾ - Microbiology and Virology Department, Fondazione IRCCS Policlinico San Matteo, Pavia, Italia ⁽²⁾	258
The mosaic of Adenovirus variants circulating in 2022-2023 in hospitalized patients.	258
Federica A.M. Giardina ¹ , Guglielmo Ferrari ² , Antonino M.G. Pitrolo ² , Antonio Piralla ² , Fausto Baldanti ^{1,2}	258
¹ Department of Clinical, Surgical, Diagnostic and Pediatric Sciences, University of Pavia, Pavia, Italy.	258
² Microbiology and Virology Department, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy.	258
172 - Genomic analysis of methicillin-resistant <i>Staphylococcus aureus</i> (MRSA) isolates from bloodstream infections in Italy: a pilot study	259
MICHELA PAGNOTTA ⁽¹⁾ - GIULIA ERRICO ⁽¹⁾ - CHRISTINA MERAKOU ⁽¹⁾ - FABIO D'AMBROSIO ⁽¹⁾ - SARA GIANCRISTOFARO ⁽¹⁾ - ROMINA CAMILLI ⁽¹⁾ - MARIA DEL GROSSO ⁽¹⁾ - MONICA MONACO ⁽¹⁾	259
Istituto Superiore di Sanità, Department of Infectious Diseases, Rome, Italia ⁽¹⁾	259
Genomic analysis of methicillin-resistant <i>Staphylococcus aureus</i> (MRSA) isolates from bloodstream infections in Italy: a pilot study	259
MICHELA PAGNOTTA ¹ , GIULIA ERRICO ¹ , CHRISTINA MERAKOU ^{1,2} , FABIO D'AMBROSIO ¹ , SARA GIANCRISTOFARO ¹ , ROMINA CAMILLI ¹ , MARIA DEL GROSSO ¹ , MONICA MONACO ¹ , AND THE MRSA GENOMIC SURVEILLANCE PILOT STUDY GROUP	259
¹ Department of Infectious Diseases, Antibiotic-Resistance and Special Pathogens Unit, Istituto Superiore di Sanità, Rome, Italy; ² ECDC fellowship Programme, Public Health Microbiology	

path (EUPHEM), European Centre for Disease Prevention and Control (ECDC), Stockholm, Sweden.	259
190 - Machine learning model based on MALDI-TOF mass spectra for prediction of ceftazidime resistance in Escherichia coli strains isolated from bloodstream infections.....	260
SIMONE AMBRETTI ⁽¹⁾ - DONATELLA LOMBARDI ⁽¹⁾ - ALESSIA BERTOLDI ⁽¹⁾ - CRISTINA MARIA CROVARA PESCE ⁽¹⁾ - ETTORE ROCCHI ⁽²⁾ - MAURO FACCIN ⁽²⁾ - CLAUDIA SALA ⁽²⁾ - GASTONE CASTELLANI ⁽²⁾	260
U.O. Microbiologia, IRCCS AOU di Bologna Policlinico S.Orsola, Bologna, Italia ⁽¹⁾ - Department of Medical and Surgical Sciences, Università di Bologna, Bologna, Italy, Bologna, Italia ⁽²⁾	260
Machine learning model based on MALDI-TOF mass spectra for prediction of ceftazidime resistance in Escherichia coli strains isolated from bloodstream infections.....	260
SIMONE AMBRETTI ^{1,2} , DONATELLA LOMBARDO ¹ , ALESSIA BERTOLDI ¹ , CRISTINA MARIA CROVARA PESCE ¹ , ETTORE ROCCHI ² , MAURO FACCIN ² , CLAUDIA SALA ² , GASTONE CASTELLANI ²	260
¹ Microbiology Unit, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy	260
² Department of Medical and Surgical Sciences, Università di Bologna, Bologna, Italy	260
214 - Emergence of high-level gentamicin resistance in Streptococcus agalactiae hypervirulent serotype IV strains.....	262
ROBERTA CRETI ⁽¹⁾ - MONICA IMPERI ⁽¹⁾ - SIMONA RECCHIA ⁽¹⁾ - GIOVANNA ALFARONE ⁽¹⁾ - GIOVANNI GHERARDI ⁽²⁾	262
Istituto Superiore di Sanità, Dipartimento di Malattie infettive, roma, Italia ⁽¹⁾ - Università Campus Bio-Medico, Facoltà Dipartimentale di Medicina e Chirurgia, roma, Italia ⁽²⁾	262
Emergence of high-level gentamicin resistance in Streptococcus agalactiae hypervirulent serotype IV strains.....	262
ROBERTA CRETI ¹ , MONICA IMPERI ¹ , SIMONA RECCHIA ¹ , GIOVANNA ALFARONE ¹ , GIOVANNI GHERARDI ²	262
¹ Dipartimento di Malattie Infettive, Reparto di Antibiotico-Resistenza e Patogeni Speciali, Istituto Superiore di Sanità, Roma, Italy; ² Unità di Scienze Batteriologiche Applicate, Facoltà Dipartimentale di Medicina e Chirurgia, Università Campus Bio-Medico, Roma, Italy.....	262
248 - Retrospective epidemiological study of candidemia at San Martino Hospital (HSM), Genoa, Italy, 2019-2022	264
ELISA COSTA ⁽¹⁾ - GIULIA CODDA ⁽¹⁾ - EDWARD WILLISON ⁽¹⁾ - LUCA CALABRESE ⁽¹⁾ - VINCENZO DI PILATO ⁽¹⁾ - ANNA MARCHESE ⁽¹⁾	264
DISC, Università di Gneova, Genova, Italia ⁽¹⁾	264
Retrospective epidemiological study of candidemia at San Martino Hospital (HSM), Genoa, Italy, 2019-2022	264
Elisa Costa ^a , Giulia Codda ^a , Edward Willison ^a , Luca Calabrese ^a , Vincenzo Di Pilato ^a , Anna Marchese ^a	264
^a DISC, Microbiology Unit, University of Genoa, Italy	264
287 - IMPACT OF THE SARS-COV-2 PANDEMIC ON THE CIRCULATION OF ENTERIC VIRUSES IN CHILDREN IN PALERMO	266
CHIARA FILIZZOLO ⁽¹⁾ - MARIANGELA PIZZO ⁽¹⁾ - GIUSEPPA LUISA SANFILIPPO ⁽¹⁾ - FLORIANA BONURA ⁽¹⁾ - CHIARA MASCARELLA ⁽¹⁾ - ARIANNA SUCATO ⁽¹⁾ - FRANCESCA	

DI BERNARDO ⁽²⁾ - ANTONINA COLLURA ⁽²⁾ - GIOVANNI MAURIZIO GIAMMANCO ⁽¹⁾ - SIMONA DE GRAZIA ⁽¹⁾	266
Dipartimento di Promozione della Salute, Materno-Infantile, di Medicina Interna e Specialistica di Eccellenza “G. D’Alessandro” (PROSAMI), Università degli Studi di Palermo, Palermo, Italia ⁽¹⁾ - Unità Operativa di Microbiologia e Virologia, Ospedale Civico e Di Cristina, ARNAS, Palermo, Italy, Palermo, Italia ⁽²⁾	266
IMPACT OF THE SARS-COV-2 PANDEMIC ON THE CIRCULATION OF ENTERIC VIRUSES IN CHILDREN IN PALERMO	266
CHIARA FILIZZOLO^a, MARIANGELA PIZZO^a, GIUSEPPA LUISA SANFILIPPO^a, FLORIANA BONURA^a, CHIARA MASCARELLA^a, ARIANNA SUCATO^a, FRANCESCA DI BERNARDO^b, ANTONINA COLLURA^b, GIOVANNI M. GIAMMANCO^a, SIMONA DE GRAZIA^a	266
^aDipartimento di Promozione della Salute, Materno-Infantile, di Medicina Interna e Specialistica di Eccellenza “G. D’Alessandro” (PROSAMI), Università di Palermo, Palermo, Italy	266
^bUnità Operativa di Microbiologia e Virologia, Ospedale Civico e Di Cristina, ARNAS, Palermo, Italy	266
301 - Epidemiological surveillance of SARS-CoV-2 variants using NGS during pandemic waves in Verona	268
RICCARDO CECCHETTO ⁽¹⁾ - EMIL TONON ⁽¹⁾ - ERICA DIANI ⁽¹⁾ - ANNA LAGNI ⁽¹⁾ - VIRGINIA LOTTI ⁽¹⁾ - NICOLETTA MEDAINA ⁽²⁾ - GIONA TURRI ⁽²⁾ - MOSÈ FAVARATO ⁽³⁾ - DAVIDE GIBELLINI ⁽¹⁾	268
Università Di Verona, Dip. Diagnostica E Sanità Pubblica, Verona, Italia ⁽¹⁾ - Ospedale G.B. Rossi, Uoc Microbiologia, Verona, Italia ⁽²⁾ - Aulss 3, Uosd Diagnostica Molecolare E Genetica, Venezia, Italia ⁽³⁾	268
Epidemiological surveillance of SARS-CoV-2 variants using NGS during pandemic waves in Verona	268
RICCARDO CECCHETTO^{1,2}, EMIL TONON^{1,2}, ERICA DIANI¹, ANNA LAGNI¹, VIRGINIA LOTTI¹, NICOLETTA MEDAINA², GIONA TURRI², MOSE’ FAVARATO³, DAVIDE GIBELLINI^{1,2}	268
¹ Department of Diagnostic and Public Health, Verona University, 37134 Verona, Italy; ² UOC Microbiology, AOUI Verona, 37134 Verona, Italy; UOSD of Molecular Diagnostics and Genetics, AULSS 3 Serenissima, Venice, Italy.	268
322 - SARS-CoV-2 variants in Marche region: evolution, mutations, and variability.	270
CARLA ACCIARRI ⁽¹⁾ - ROBERTA LONGO ⁽¹⁾ - BENEDETTA CORVARO ⁽¹⁾ - SARA CAUCCI ⁽¹⁾ - STEFANO MENZO ⁽¹⁾	270
Università Politecnica delle Marche, Dipartimento di Scienze Biomediche e Sanità Pubblica, Ancona, Italia ⁽¹⁾	270
SARS-CoV-2 variants in Marche region: evolution, mutations, and variability.	270
CARLA ACCIARRI¹, ROBERTA LONGO¹, SARA CAUCCI¹, BENEDETTA CORVARO¹, STEFANO MENZO^{1,2} 1. Dipartimento di Scienze Biomediche e Sanità Pubblica Università, Politecnica delle Marche, Ancona, Italia 2. SOSD Virologia, Azienda Ospedaliero Universitaria delle Marche, Ancona; Italia	270
330 - Characterization of a genome database of 837 isolates assigned to 72 distinct species in the Acinetobacter genus using the Pasteur Multi-locus sequencing type (MLST) scheme.	271

ANTONELLA MIGLIACCIO ⁽¹⁾ - JAMES BRAY ⁽²⁾ - MICHELE INTOCCIA ⁽³⁾ - MARIA STABILE ⁽¹⁾ - GIOVANNI SCALA ⁽³⁾ - KEITH A. JOLLEY ⁽²⁾ - SYLVAIN BRISSE ⁽⁴⁾ - RAFFAELE ZARRILLI ⁽¹⁾ 271

University of Naples “Federico II”, Department of Public Health,, Naples, Italia ⁽¹⁾ - University of Oxford, United Kingdom, Department of Biology,, Oxford, Stati Uniti D'america ⁽²⁾ - University of Naples Federico II, Italy, Department of Biology,, Naples, Italia ⁽³⁾ - Biodiversity and Epidemiology of Bacterial Pathogens,France, Institut Pasteur, Paris, Francia ⁽⁴⁾..... 271

Characterization of a genome database of 837 isolates assigned to 72 distinct species in the Acinetobacter genus using the Pasteur Multi-locus sequencing type (MLST) scheme..... 271

Antonella Migliaccio¹, James Bray², Michele Intoccia³, Maria Stabile^{4,5}, Giovanni Scala³, Keith A. Jolley², Sylvain Brisse⁶ and Raffaele Zarrilli¹ 271

¹ Department of Public Health, University of Naples “Federico II”, Naples, Italy, ² Department of Biology, University of Oxford, Oxford, United Kingdom, ³Department of Biology, University of Naples Federico II, Naples, Italy, ⁴ Department of Chemical Sciences, University of Naples Federico II, Naples, Italy, ⁵ Complex Operational Unit of Clinical Microbiology, University Hospital “Federico II”, Naples, Italy, ⁶Biodiversity and Epidemiology of Bacterial Pathogens, Institut Pasteur, Paris, France 271

06 Molecole naturali ad azione antimicrobica/Nuovi approcci antimicrobici..... 273

1 - Importin alpha/beta-dependent nuclear transport of human parvovirus B19 nonstructural protein 1 is essential for viral replication 273

GUALTIERO ALVISI ⁽¹⁾ - ELISABETTA MANARESI ⁽²⁾ - EMILY, M. CROSS ⁽³⁾ - MIKAYLA HOAD ⁽³⁾ - NASIM AKBARI ⁽¹⁾ - SILVIA PAVAN ⁽¹⁾ - DARYL ARIAWAN ⁽⁴⁾ - GLORIA BUA ⁽²⁾ - GAYLE, F. PETERSEN ⁽³⁾ - JADE FORWOOD ⁽³⁾ - GIORGIO GALLINELLA ⁽²⁾ 273

Università di Padova, Dipartimento di Medicina Molecolare, Padova, Italia ⁽¹⁾ - Università degli Studi di Bologna, Dipartimento di Farmacia e Biotecnologie, Bologna, Italia ⁽²⁾ - Charles Sturt University, School of Dentistry and Medical Sciences, Wagga Wagga, Australia ⁽³⁾ - Macquarie University, Dementia Research Centre, Sydney, Australia ⁽⁴⁾ 273

Importin alpha/beta-dependent nuclear transport of human parvovirus B19 nonstructural protein 1 is essential for viral replication 273

GUALTIERO ALVISI^{1*}, ELISABETTA MANARESI², EMILY M. CROSS³, MIKAYLA HOAD³, NASIM AKBARI¹, SILVIA PAVAN¹, DARYL ARIAWAN⁴, GLORIA BUA², GAYLE F. PETERSEN³, JADE FORWOOD³, GIORGIO GALLINELLA² 273

¹Department of Molecular Medicine, University of Padova, 35121 Padova, Italy; ² Department of Pharmacy and Biotechnology, University of Bologna, 40138 Bologna, Italy; ³ School of Dentistry and Medical Sciences, Charles Sturt University, Wagga Wagga, NSW, 2650, Australia; ⁴Dementia Research Centre, Macquarie University, Sydney, NSW, 2109, Australia 273

*** Correspondence to: gualtiero.alvisi@unipd.it 273**

Introduction: Human parvovirus B19 (B19V) is a major human pathogen causing a variety of diseases, characterized by a selective tropism to human progenitor cells in bone marrow. In similar fashion to all Parvoviridae members, the B19V ssDNA genome is replicated within the nucleus of infected cells through a process which involves both cellular and viral proteins. Among the latter, a crucial role is played by non-structural protein (NS)1, a multifunctional protein involved in genome replication and transcription, as well as modulation of host gene expression and function. Despite the localization of NS1 within the host cell nucleus during infection, little is known regarding the mechanism of its nuclear transport pathway. Methods: 273

In this study we undertake structural, biophysical, and cellular approaches to characterize this process. Results: Quantitative confocal laser scanning microscopy (CLSM), gel mobility shift, fluorescence polarization and crystallographic analysis identified a short sequence of amino acids (GACHAKKPRIT-182) as the classical nuclear localization signal (cNLS) responsible for nuclear import, mediated in an energy and importin (IMP) alpha/beta-dependent fashion. Structure-guided mutagenesis of key residue K177 strongly impaired IMP alpha binding, nuclear import, and viral gene expression in a minigenome system. Further, treatment with ivermectin, an antiparasitic drug interfering with the IMP alpha/beta dependent nuclear import pathway, inhibited NS1 nuclear accumulation and viral replication in infected UT7/Epo-S1 cells. Discussion: Thus, NS1 nuclear transport is a potential target of therapeutic intervention against B19V induced disease.

..... 273

11 - A heparan sulfate proteoglycan binding tetrapeptide successfully inhibits SARS-CoV-2 Omicron replication 274

CAMILLA BIBA ⁽¹⁾ - LORENZO DEPAU ⁽¹⁾ - LIA FIASCHI ⁽¹⁾ - JLENIA BRUNETTI ⁽¹⁾ - ILENIA VARASI ⁽¹⁾ - CHIARA FALCIANI ⁽¹⁾ - MAURIZIO ZAZZI ⁽¹⁾ - ILARIA VICENTI ⁽¹⁾ - LUISA BRACCI ⁽¹⁾ 274

Univesity of Siena, Department of Medical Biotechnologies, Siena, Italia ⁽¹⁾ 274

A heparan sulfate proteoglycan binding tetrapeptide successfully inhibits SARS-CoV-2 Omicron replication 274

CAMILLA BIBA, LORENZO DEPAU, LIA FIASCHI, JLENIA BRUNETTI, ILENIA VARASI, CHIARA FALCIANI, MAURIZIO ZAZZI, ILARIA VICENTI AND LUISA BRACCI..... 274

Department of Medical Biotechnologies, University of Siena, Siena, Italy 274

27 - All-Trans Retinoic Acid Effect on Candida albicans growth and biofilm formation 275

TERENZIO COSIO (1) - ENRICO SALVATORE PISTOIA (1) - GAETANA COSTANZA (1) - ELENA CAMPIONE (2) - ROBERTA GAZIANO (1) 275

Department of Experimental Medicine, Tor Vergata University of Rome, 00133 Rome, Italy;; Department of Experimental Medicine, Tor Vergata University of Rome, 00133 Rome, Italy;; Roma, Italia (1) - Department of Systems Medicine, Dermatology Unit, Tor Vergata University Hospital, 00133 Rome, Italy;; Department of Systems Medicine, Dermatology Unit, Tor Vergata University Hospital, 00133 Rome, Italy;; Roma, Italia (2)..... 275

All-Trans Retinoic Acid Effect on Candida albicans growth and biofilm formation..... 275

Terenzio COSIO1,2, Enrico S. PISTOIA3, Gaetana COSTANZA3, Elena CAMPIONE2, Roberta GAZIANO3 275

1Department of Experimental Medicine, PhD Course in Microbiology, Immunology, Infectious Diseases, and Transplants (MIMIT), University of Rome Tor Vergata, 00133 Rome, Italy;; 2Department of Systems Medicine, Dermatology Unit, Tor Vergata University Hospital, 00133 Rome, Italy;;..... 275

3Department of Experimental Medicine, University of Rome Tor Vergata, 00133 Rome, Italy;. 275

28 - Isolation and characterization of novel mycobacteriophages and evaluation of their lytic activity against Mycobacterium abscessus..... 276

MARIAGRAZIA DI LUCA ⁽¹⁾ - ENRICO CASU ⁽²⁾ - CLAUDIA CAMPOBASSO ⁽¹⁾ - CATERINA FERRETTI ⁽¹⁾ - SARA BOLOGNINI ⁽¹⁾ - FABIO FILIPPINI ⁽²⁾ - GIULIA FREER ⁽²⁾ - ARIANNA TAVANTI ⁽¹⁾ - LAURA RINDI ⁽²⁾..... 276

Università di Pisa, Dipartimento di Biologia, Pisa, Italia ⁽¹⁾ - Università di Pisa, Dipartimento di Ricerca Traslazionale e delle Nuove Tecnologie in Medicina e Chirurgia, Pisa, Italia ⁽²⁾..... 276

Isolation and characterization of novel mycobacteriophages and evaluation of their lytic activity against <i>Mycobacterium abscessus</i>	276
MARIAGRAZIA DI LUCA ¹ , ENRICO CASU ² , CLAUDIA CAMPOBASSO ^{1,3} , CATERINA FERRETTI ¹ , SARA BOLOGNINI ¹ , FABIO FILIPPINI ² , GIULIA FREER ² , ARIANNA TAVANTI ¹ , LAURA RINDI ²	276
¹ Department of Biology, University of Pisa, Pisa, Italy; ² Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa, Pisa, Italy; ³ Department of Biosystems, KU Leuven, Leuven, Belgium.....	276
31 - A promising antimicrobial peptide against some virulence features of <i>Acinetobacter baumannii</i> clinical strains	278
ROSANNA PAPA (1) - IRENE PARIS (1) - MARIKA TRECCA (1) - ESTHER IMPERLINI (2) - FRANCESCO BUONOCORE (2) - MARCO ARTINI (1) - LAURA SELAN (1).....	278
Sapienza Università, Dipartimento di Sanità Pubblica e Malattie Infettive, Roma, Italia (1) - University of Tuscia, Department for Innovation in Biological, Agro-Food and Forest Systems, Viterbo, Italia (2)	278
32 - Escape pathogens: looking for alternative strategies from an exotic source	279
ROSANNA PAPA (1) - MARIKA TRECCA (1) - IRENE PARIS (1) - CATERINA D'ANGELO (2) - ERMENEGILDA PARRILLI (2) - MARCO ARTINI (1) - LAURA SELAN (1).....	279
41 - Virucide activity of nanocomposites based on resin included clays for sanization of highly touched surfaces.....	280
FRANCESCO RICCHI (1) - FRANCESCO LIPANI (1) - ISABELLA MARCHESI (1) - ANNALISA BARGELLINI (1) - FABIO TATEO (2) - ANTONELLA MANSI (3) - CLAUDIO CERPELLI (1).....	280
Università degli Studi di Modena e Reggio Emilia, Facoltà di Medicina e Chirurgia, Modena, Italia (1) - Consiglio Nazionale delle Ricerche, Istituto di Geoscienze e Georisorse, Padova, Italia (2) - INAIL, Dipartimento di Medicina, Epidemiologia, Igiene del Lavoro e Ambientale, Roma, Italia (3).....	280
Virucide activity of nanocomposites based on resin included clays for sanization of highly touched surfaces	280
Francesco Ricchi ¹ , Francesco Lipani ¹ , Isabella Marchesi ² , Annalisa Bargellini ² , Fabio Tateo ³ , Antonella Mansi ⁴ , Roberta Bertani ⁵ , Claudio Cermelli ¹	280
43 - Isolation of Human Monoclonal Antibodies Against Pan-Drug Resistant <i>Klebsiella pneumoniae</i>	282
SORAYA SOLEDAD BOSCH (1) - VITTORIA ZUCCONI GALLI FONSECA (1) - EMANUELE ROSCIOLI (1) - GIULIA CARDINALI (1) - GIUSEPPE MACCARI (2) - GIAMPIERO BATANI (1) - IDA PACIELLO (1) - CLAUDIA SALA (1) - ANNA KABANOVA (1) - GIUSY TISEO (3) - FRANCESCO MENICHETTI (3) - MARCO FALCONE (3) - RINO RAPPUOLI (4)	282
Fondazione Toscana Life Sciences, Monoclonal Antibody Discovery Lab, Siena, Italia (1) - Fondazione Toscana Life Sciences, Data Science for Health (DASCH) Lab, Siena, Italia (2) - Department of Clinical and Experimental Medicine, Infectious Diseases Unit, University of Pisa, Pisa, Italia (3) - Fondazione Biotechnopolo di Siena, Monoclonal Antibody Discovery Lab, Siena, Italia (4)	282
Isolation of Human Monoclonal Antibodies Against Pan-Drug Resistant <i>Klebsiella pneumoniae</i>	282

SORAYA S. BOSCH ¹ , VITTORIA ZUCCONI GALLI FONSECA ^{1,2} , EMANUELE ROSCIOLI ¹ , GIULIA CARDINALI ^{1,2} , GIUSEPPE MACCARI ³ , GIAMPIERO BATANI ¹ , IDA PACIELLO ¹ , CLAUDIA SALA ¹ , ANNA KABANOVA ¹ , GIUSY TISEO ⁴ , FRANCESCO MENICHETTI ⁴ , MARCO FALCONE ⁴ , RINO RAPPUOLI ^{1,5}	282
¹ Monoclonal Antibody Discovery Lab, Fondazione Toscana Life Sciences, Siena, Italy; ² University of Siena, Siena, Italy; ³ Data Science for Health (DASCH) Lab, Fondazione Toscana Life Sciences, Siena, Italy; ⁴ Department of Clinical and Experimental Medicine, Infectious Diseases Unit, University of Pisa, Pisa, Italy; ⁵ Fondazione Biotechnopolo di Siena, Siena, Italy	282
49 - Azole-loaded mucosomes as multi drug delivery platform to counteract infections caused by Candida albicans.	284
NARCISA MANDRAS (1) - LORENZA CAVALLO (1) - OLGA VALENTINA GARBERO (2) - FABRIZIO CAREDDU (2) - FRANCESCA MENOTTI (1) - FABIO LONGO (1) - ELEONORA MANISCALCO (1) - VALERIA ALLIZOND (1) - CLAUDIA PAGANO (1) - JANIRA ROANA (1) - ANNA MARIA CUFFINI (1) - SONJA VISENTIN (2) - GIULIANA BANCHE (1)	284
Università di Torino, Dipartimento di Scienze della Sanità Pubblica e Pediatriche, Torino, Italia ⁽¹⁾ - Università di Torino, Dipartimento di Biotecnologie Molecolari e Scienze della Salute, Torino, Italia ⁽²⁾	284
Azole-loaded mucosomes as multi drug delivery platform to counteract infections caused by Candida albicans.	284
NARCISA MANDRAS ¹ , LORENZA CAVALLO ¹ , OLGA V. GARBERO ² , FABRIZIO CAREDDU ² , FRANCESCA MENOTTI ¹ , FABIO LONGO ¹ , ELEONORA MANISCALCO ¹ , VALERIA ALLIZOND ¹ , JANIRA ROANA ¹ , CLAUDIA PAGANO ¹ , ANNA M. CUFFINI ¹ , SONJA VISENTIN ² , GIULIANA BANCHE ¹	284
¹ Department of Public Health and Pediatrics, University of Torino, Turin, Italy; ² Department of Molecular Biotechnology and Health Science, University of Torino, Turin, Italy	284
50 - New inhibitors of Carbonic Anhydrases from Pseudomonas aeruginosa: antimicrobial and antibiofilm activities with respect to ciprofloxacin	286
BEATRICE MARINACCI ⁽¹⁾ - ANDREA ANGELI ⁽²⁾ - SIMONE CARRADORI ⁽¹⁾ - NOEMI MENCARELLI ⁽¹⁾ - VALENTINA PUCA ⁽¹⁾ - BENEDETTA PELLEGRINI ⁽¹⁾ - SANTOLO FRANCATI ⁽³⁾ - CLAUDIU T. SUPURAN ⁽²⁾ - ROSSELLA GRANDE ⁽¹⁾	286
Università degli Studi "G. d'Annunzio" Chieti-Pescara, Dipartimento di Farmacia, Chieti, Italia ⁽¹⁾ - Università degli Studi di Firenze, Dipartimento NEUROFARBA, Firenze, Italia ⁽²⁾ - Alma Mater Studiorum Università di Bologna, Dipartimento di Scienze e Tecnologie Agro-Alimentari, Bologna, Italia ⁽³⁾	286
New inhibitors of Carbonic Anhydrases from Pseudomonas aeruginosa: antimicrobial and antibiofilm activities with respect to ciprofloxacin	286
BEATRICE MARINACCI ^{1,2} , ANDREA ANGELI ³ , SIMONE CARRADORI ¹ , NOEMI MENCARELLI ¹ , VALENTINA PUCA ¹ , BENEDETTA PELLEGRINI ¹ , SANTOLO FRANCATI ⁴ , CLAUDIU T. SUPURAN ³ , ROSSELLA GRANDE ¹	286
¹ Department of Pharmacy, "G. d'Annunzio" University of Chieti-Pescara, Chieti, Italy;	286
² Department of Innovative Technologies in Medicine & Dentistry, "G. d'Annunzio" University of Chieti-Pescara, Chieti, Italy;	286
³ Neurofarba Department, University of Florence, Sesto Fiorentino (FI), Italy;	286
⁴ Department of Agricultural and Food Sciences, University of Bologna, Viale Fanin, 42, Bologna, 40127, Italy	286

52 - Silver-doped composite modified scaffolds to impart both anti-microbial features and enhanced biological properties in bone regenerative medicine. 288

FRANCESCA MENOTTI⁽¹⁾ - FABIO LONGO⁽¹⁾ - SARA COMINI⁽¹⁾ - ROSARIA SPARTI⁽¹⁾ - SARA SCUTERA⁽¹⁾ - LORENZA CAVALLO⁽¹⁾ - BARTOLOMEO COPPOLA⁽²⁾ - ELISA FIUME⁽²⁾ - ELEONORA MANISCALCO⁽¹⁾ - CLAUDIA PAGANO⁽¹⁾ - ANNA MARIA CUFFINI⁽¹⁾ - NARCISA MANDRAS⁽¹⁾ - PAOLA PALMERO⁽²⁾ - GIULIANA BANCHE⁽¹⁾ - VALERIA ALLIZOND⁽¹⁾ 288

University of Torino, Department of Public Health and Pediatrics, Torino, Italia⁽¹⁾ - Politecnico of Torino, Department of Applied Science and Technology, Torino, Italia⁽²⁾ 288

Silver-doped composite modified scaffolds to impart both anti-microbial features and enhanced biological properties in bone regenerative medicine..... 288

FRANCESCA MENOTTI¹, FABIO LONGO¹, SARA COMINI¹, ROSARIA SPARTI¹, SARA SCUTERA¹, LORENZA CAVALLO¹, BARTOLOMEO COPPOLA², ELISA FIUME², ELEONORA MANISCALCO¹, CLAUDIA PAGANO¹, ANNA MARIA CUFFINI¹, PAOLA PALMERO², GIULIANA BANCHE¹, VALERIA ALLIZOND¹ 288

¹Department of Public Health and Pediatrics, University of Torino, Turin, Italy; ²Department of Applied Science and Technology, Politecnico of Torino, Turin, Italy 288

55 - Small molecules and PROTACs to inhibit and degrade HPV oncoproteins 290

CHIARA BERTAGNIN⁽¹⁾ - LORENZO MESSA⁽¹⁾ - MATTEO PAVAN⁽²⁾ - JENNY DESANTIS⁽³⁾ - BEATRICE MERCORELLI⁽¹⁾ - MATTIA STURLESE⁽²⁾ - MARTA CELEGATO⁽¹⁾ - TOMMASO D'ANGELI⁽³⁾ - GABRIELE CRUCIANI⁽³⁾ - LAWRENCE BANKS⁽⁴⁾ - LAURA GORACCI⁽³⁾ - STEFANO MORO⁽²⁾ - ARIANNA LOREGIAN⁽¹⁾ 290

Department Of Molecular Medicine, University Of Padua, Padua, Italia⁽¹⁾ - Department Of Pharmaceutical And Pharmacological Sciences, University Of Padua, Padua, Italia⁽²⁾ - Department Of Chemistry, Biology And Biotechnology, University Of Perugia, Perugia, Italia⁽³⁾ - International Centre For Genetic Engineering And Biotechnology, Area Science Park, Trieste, Italia⁽⁴⁾ 290

Small molecules and PROTACs to inhibit and degrade HPV oncoproteins..... 290

CHIARA BERTAGNIN¹, LORENZO MESSA¹, MATTEO PAVAN², JENNY DESANTIS³, BEATRICE MERCORELLI¹, MATTIA STURLESE², MARTA CELEGATO¹, TOMMASO D'ANGELI³, GABRIELE CRUCIANI³, LAWRENCE BANKS⁴, LAURA GORACCI³, STEFANO MORO², ARIANNA LOREGIAN¹..... 290

¹ Department of Molecular Medicine, University of Padua, Padua, Italy; ² Department of Pharmaceutical and Pharmacological Sciences, University of Padua, Padua, Italy; ³ Department of Chemistry, Biology, and Biotechnology, University of Perugia, Perugia, Italy; ⁴ International Centre for Genetic Engineering and Biotechnology (ICGEB), Trieste, Italy..... 290

60 - A small-molecule inhibitor of flaviviral NS3-NS5 interaction with broad-spectrum activity and efficacy in vivo..... 292

BEATRICE MERCORELLI⁽¹⁾ - MARTA CELEGATO⁽¹⁾ - MATTIA STURLESE⁽²⁾ - VIVIAN VASCONCELOS COSTA⁽³⁾ - MARTA TREVISAN⁽¹⁾ - ANGELICA SAMER LALLO DIAS⁽⁴⁾ - INGREDY BEATRIZ SOUZA PASSOS⁽³⁾ - CELSO MARTINS QUEIROZ-JUNIOR⁽³⁾ - LORENZO MESSA⁽¹⁾ - ANNAGIULIA FAVARO⁽²⁾ - CHIARA BERTAGNIN⁽¹⁾ - STEFANO MORO⁽²⁾ - MAURO MARTINS TEIXEIRA⁽⁴⁾ - ARIANNA LOREGIAN⁽¹⁾ 292

Università di Padova, Dipartimento di Medicina Molecolare, Padova, Italia⁽¹⁾ - Università di Padova, Dipartimento di Scienze del farmaco, Padova, Italia⁽²⁾ - Universidade Federal de Minas Gerais, Belo Horizonte, Brazil, Departamento de Morfologia, ICB, Belo Horizonte, Brasile⁽³⁾ -

Universidade Federal de Minas Gerais, Belo Horizonte, Brazil, Departamento de Bioquímica e Imunologia, ICB, Belo Horizonte, Brasile ⁽⁴⁾	292
Categoria: Molecole naturali ad azione antimicrobica/Nuovi approcci antimicrobici	292
A small-molecule inhibitor of flaviviral NS3-NS5 interaction with broad-spectrum activity and efficacy in vivo	292
Beatrice Mercorelli ^a , Marta Celegato, ^a Mattia Sturlese, ^b Vivian Vasconcelos Costa, ^c Marta Trevisan, ^a Angélica Samer Lallo Dias, ^d Ingredy Beatriz Souza Passos, ^c Celso Martins Queiroz-Junior, ^c Lorenzo Messa, ^a Annagiulia Favaro, ^b chiara bertagnin, ^a Stefano Moro, ^b Mauro Martins Teixeira, ^d Arianna Loregian, ^a	292
^A Department of Molecular Medicine, University of Padua, Padua, Italy;	292
^B Molecular Modeling Section (MMS), Department of Pharmaceutical and Pharmacological Sciences, University of Padua, Padua, Italy;	292
^C Departamento de Morfologia, ICB, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil; ^D Departamento de Bioquímica e Imunologia, ICB, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil.	292
71 - Graphene Quantum Dots as promising nanomaterials against Mycobacterium tuberculosis infection	294
GIULIA SANTARELLI ⁽¹⁾ - FLAVIO DE MAIO ⁽²⁾ - GIORDANO PERINI ⁽³⁾ - VALENTINA PALMIERI ⁽³⁾ - ALESSANDRO SALUSTRI ⁽¹⁾ - ROBERTO ROSATO ⁽¹⁾ - IVANA PALUCCI ⁽²⁾ - MICHELA SALI ⁽²⁾ - MASSIMILIANO PAPI ⁽³⁾ - GIOVANNI DELOGU ⁽⁴⁾	294
Università Cattolica del Sacro Cuore, Department of Sciences of Basic Biotechnologies, Intensive and Perioperative Clinics, Section of Microbiology, Roma, Italia ⁽¹⁾ - Fondazione Policlinico Universitario Agostino Gemelli, Department of Laboratory and Infective Sciences, Faculty of Medicine, Roma, Italia ⁽²⁾ - Università Cattolica del Sacro Cuore, Dipartimento di Neuroscienze, Roma, Italia ⁽³⁾ - Mater Olbia Hospital, Mater Olbia Hospital, Olbia, Italia ⁽⁴⁾	294
Graphene Quantum Dots as promising nanomaterials against Mycobacterium tuberculosis infection.....	294
Authors	294
Giulia Santarelli ¹ , Flavio De Maio ² , Giordano Perini ^{3,4} , Valentina Palmieri ^{4,5} , Alessandro Salustri ¹ , Roberto Rosato ¹ , Ivana Palucci ^{1,2} , Michela Sali ^{1,2} , Massimiliano Papi ^{3,4} , Giovanni Delogu ^{2,6}	294
Affiliations	294
¹ Department of Sciences of Basic Biotechnologies, Intensive and Perioperative Clinics, Section of Microbiology, Università Cattolica del Sacro Cuore, Rome, Italy; ² Department of Laboratory and Infective Sciences, Faculty of Medicine, Fondazione Policlinico A. Gemelli IRCCS, Università Cattolica del Sacro Cuore, Rome, Italy; ³ Fondazione Policlinico Universitario “A. Gemelli”, IRCCS, Rome, Italy; ⁴ Dipartimento di Neuroscienze, Università Cattolica del Sacro Cuore, 00168 Rome, Italy; ⁵ Istituto dei Sistemi Complessi, CNR, Rome, Italy; ⁶ Mater Olbia Hospital, Olbia, Italy	294
78 - Citrus fruit polyphenols for fighting antibiotic resistance.....	296
DILETTA MAZZANTINI ⁽¹⁾ - MARIACRISTINA MASSIMINO ⁽¹⁾ - MARCO CALVIGIONI ⁽¹⁾ - FRANCESCO CELANDRONI ⁽¹⁾ - EMILIA GHELARDI ⁽¹⁾	296
Università di Pisa, Dipartimento di Ricerca Traslationale e delle Nuove Tecnologie in Medicina e Chirurgia, Pisa, Italia ⁽¹⁾	296
Citrus fruit polyphenols for fighting antibiotic resistance.....	296

DILETTA MAZZANTINI¹, MARIACRISTINA MASSIMINO¹, MARCO CALVIGIONI¹, FRANCESCO CELANDRONI¹, EMILIA GHELARDI¹	296
¹Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa, Pisa, Italy.	296
94 - Improving Gene-Editing delivery to fight viral infections	297
ALESSANDRO DE CARLI⁽¹⁾ - DOMENICO FAVARO⁽²⁾ - PIOTR BARSKI⁽³⁾ - VITTORIA RAFFA⁽⁴⁾ - MAURO PISTELLO⁽⁵⁾ - MICHELE LAI⁽²⁾.....	297
Università di Siena, Dipartimento di Biotecnologie Mediche, Siena, Italia⁽¹⁾ - Università di Pisa, Dipartimento di Medicina Traslazionale, Pisa, Italia⁽²⁾ - ProChimia Surfaces Sp. z o.o., Research and Development ProChimia Surfaces, Gdynia, Polonia⁽³⁾ - Università di Pisa, Dipartimento di Biologia, Pisa, Italia⁽⁴⁾ - U.O. Virologia Universitaria, Azienda Universitaria Ospedaliera Pisana, Pisa, Italia⁽⁵⁾	297
Improving Gene-Editing delivery to fight viral infections.....	297
ALESSANDRO DE CARLI^{1,5}, DOMENICO FAVARO¹, PIOTR BARSKI⁴, VITTORIA RAFFA³, MAURO PISTELLO^{1,2}, MICHELE LAI¹.....	297
¹Department of Translational Research and New Technologies in Medicine and Surgery, Retrovirus Center and Virology Section, University of Pisa, Pisa, Italy;	297
²Laboratory of Virology Unit, Pisa University Hospital, Pisa, Italy;	297
³ Department of Biology, Nanomedicine and Molecular Biology Section, University of Pisa, Pisa, Italy;.....	297
⁴ProChimia Surfaces, Gdynia, Poland;	297
97 - Effect of the Human Lactoferricin-Derived Peptide hLF 1-11 on Candida albicans and Staphylococcus spp. mixed biofilms.....	299
IACOPO FRANCONI⁽¹⁾ - COSMERI RIZZATO⁽¹⁾ - ARIANNA TAVANTI⁽²⁾ - ANTONELLA LUPETTI⁽¹⁾	299
Università di Pisa, Dipartimento di Ricerca Traslazionale e delle Nuove Tecnologie in Medicina e Chirurgia, Pisa, Italia⁽¹⁾ - Università di Pisa, Dipartimento di Biologia, Pisa, Italia⁽²⁾	299
Effect of the Human Lactoferricin-Derived Peptide hLF 1-11 on Candida albicans and Staphylococcus spp. mixed biofilms.	299
Iacopo Franconi¹, Cosmeri Rizzato², Arianna Tavanti², Antonella Lupetti¹	299
¹Dipartimento di Ricerca Traslazionale e delle Nuove Tecnologie in Medicina e Chirurgia, Università di Pisa, Pisa, Italy;	299
²Dipartimento di Biologia, Università di Pisa, Pisa, Italy.	299
99 - Humanized monoclonal antibody targeting a pre-fusion domain of the viral fusion protein stabilizes the extended state of the measles viral fusion process	300
ROBERTA DELLA MARCA⁽¹⁾ - OLIVIA HARDER⁽²⁾ - MASSIMILIANO GALDIERO⁽¹⁾ - STEFAN NIEWIESK⁽²⁾ - MATTEO POROTTO⁽¹⁾	300
Università degli studi della Campania Luigi Vanvitelli, Dipartimento di Medicina Sperimentale, Napoli, Italia⁽¹⁾ - Department of Veterinary Biosciences, College of Veterinary Medicine, The Ohio State University, Columbus, Ohio, Stati Uniti D'america⁽²⁾	300
Humanized monoclonal antibody targeting a pre-fusion domain of the viral fusion protein stabilizes the extended state of the measles viral fusion process.	300

Roberta Della Marca^{1,2,3}, Olivia Harder⁴, Massimiliano Galdiero³, Stefan Niewiesk⁴, Matteo Porotto^{1,2,3} *	300
¹Center for Host-Pathogen Interaction, Columbia University Vagelos College of Physicians and Surgeons, New York, New York, USA	300
²Department of Pediatrics, Columbia University Vagelos College of Physicians and Surgeons, New York, New York, USA	300
³Department of Experimental Medicine, University of Campania ‘Luigi Vanvitelli’, 81100 Caserta, Italy	300
⁴Department of Veterinary Biosciences, College of Veterinary Medicine, The Ohio State University, Columbus, Ohio, USA	300
110 - Pseudomonas spp.: a new ally to fight drug resistance?	302
MARCELLO MURGIA ⁽¹⁾ - LUISA MARRAS ⁽²⁾ - VALENTINA CORONEO ⁽¹⁾	302
Dipartimento di Scienze Mediche e Sanità Pubblica, Università di Cagliari, Cagliari, Italia ⁽¹⁾ - Servizio laboratorio analisi chimico cliniche-microbiologiche, ASL Cagliari, Cagliari, Italia ⁽²⁾	302
Pseudomonas spp.: a new ally to fight drug resistance?	302
MARCELLO MURGIA¹, LUISA MARRAS², VALENTINA CORONEO¹	302
¹Dipartimento di Scienze Mediche e Sanità Pubblica, Università di Cagliari, Italia;.....	302
²Servizio laboratorio analisi chimico cliniche-microbiologiche ASL Cagliari, Cagliari, Italia	302
111 - Antimicrobial activity of novel arginine- and proline-rich peptides obtained through a bioinformatic approach.....	303
TECLA CIOCIOLA ⁽¹⁾ - LAURA GIOVATI ⁽¹⁾ - LORENZA ARTESANI ⁽¹⁾ - STELLA GARCIA COLOMBAROLLI ⁽²⁾ - GIADA BIANCHETTI ⁽³⁾ - GIUSEPPE MAULUCCI ⁽³⁾ - STEFANIA CONTI ⁽¹⁾ - ALBERTO VITALI ⁽²⁾	303
University of Parma, Department of Medicine and Surgery, Parma, Italia ⁽¹⁾ - National Research Council of Italy, Istituto di Scienze e Tecnologie Chimiche (SCITEC-CNR), Rome, Italia ⁽²⁾ - Fondazione Policlinico Universitario A. Gemelli IRCCS; Università Cattolica del Sacro Cuore, Department of Neuroscience, Rome, Italia ⁽³⁾	303
Antimicrobial activity of novel arginine- and proline-rich peptides obtained through a bioinformatic approach	303
TECLA CIOCIOLA¹, LAURA GIOVATI¹, LORENZA ARTESANI¹, STELLA GARCIA COLOMBAROLLI², GIADA BIANCHETTI^{3,4}, GIUSEPPE MAULUCCI^{3,4}, STEFANIA CONTI¹, ALBERTO VITALI²	303
¹Department of Medicine and Surgery, Unit of Biomedical, Biotechnological and Translational Sciences, University of Parma, Parma, Italy; ²National Research Council of Italy, Istituto di Scienze e Tecnologie Chimiche (SCITEC-CNR), Rome, Italy; ³Department of Neuroscience, Section of Biophysics, Università Cattolica del Sacro Cuore, Rome, Italy; ⁴Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy.	303
112 - Characterization of four novel bacteriophages targeting multi-drug resistant Klebsiella pneumoniae strains of sequence type 147 and 307	305
GRETA PONSECCHI ⁽¹⁾ - LAURA SCARFONE ⁽¹⁾ - NOEMI POERIO ⁽¹⁾ - TOMMASO OLIMPIERI ⁽¹⁾ - ALBERTO ANTONELLI ⁽²⁾ - MARCO COPPI ⁽²⁾ - EUGENIO PACCAGNINI ⁽³⁾ - PIETRO LUPETTI ⁽³⁾ - GIAN MARIA ROSSOLINI ⁽²⁾ - MAURIZIO FRAZIANO ⁽¹⁾ - MARCO MARIA D'ANDREA ⁽¹⁾	305

University of Rome "Tor Vergata", Department of Biology, Rome, Italia ⁽¹⁾ - University of Florence, Department of Experimental and Clinical Medicine, Florence, Italia ⁽²⁾ - University of Sienna, Department of Life Sciences, Sienna, Italia ⁽³⁾	305
Characterization of four novel bacteriophages targeting multi-drug resistant <i>Klebsiella pneumoniae</i> strains of sequence type 147 and 307	305
GRETA PONSECCHI ^{1,2} , LAURA SCARFONE ¹ , NOEMI POERIO ¹ , TOMMASO OLIMPIERI ¹ , ALBERTO ANTONELLI ^{3,4} , MARCO COPPI ^{3,4} , EUGENIO PACCAGNINI ⁵ , PIETRO LUPETTI ⁵ , GIAN MARIA ROSSOLINI ^{3,4} , MAURIZIO FRAZIANO ¹ , MARCO M. D'ANDREA ¹	305
¹ Dept. of Biology, University of Rome "Tor Vergata", Rome, Italy; ² PhD Program in Evolutionary Biology and Ecology, Dept. of Biology, University of Rome "Tor Vergata", Rome, Italy; ³ Dept. of Experimental and Clinical Medicine, University of Florence, Florence, Italy; ⁴ Microbiology and Virology Unit, Florence Careggi University Hospital, Florence, Italy; ⁵ Dept. of Life Sciences, University of Sienna, Sienna, Italy.	305
113 - Evaluation of the antibiotic activity of grapefruit seed extract in edible mushrooms contaminations.....	307
MARCELLO MURGIA ⁽¹⁾ - ALESSANDRO BANCHIERO ⁽¹⁾ - LUISA MARRAS ⁽²⁾ - VALENTINA CORONEO ⁽¹⁾	307
Dipartimento di Scienze Mediche e Sanità Pubblica, Università di Cagliari, Cagliari, Italia ⁽¹⁾ - Servizio laboratorio analisi chimico cliniche-microbiologiche, ASL Cagliari, Cagliari, Italia ⁽²⁾	307
Evaluation of the antibiotic activity of grapefruit seed extract in edible mushrooms contaminations	307
MARCELLO MURGIA ¹ , ALESSANDRO BANCHIERO ¹ , LUISA MARRAS ² , VALENTINA CORONEO ¹	307
¹ Dipartimento di Scienze Mediche e Sanità Pubblica, Università di Cagliari, Italia;.....	307
² Servizio laboratorio analisi chimico cliniche-microbiologiche ASL Cagliari, Cagliari, Italia	307
121 - Effect of fish skin mucous derived from bathypelagic fish <i>Centrolophus niger</i> (Gmelin, 1789) on <i>Escherichia coli</i> strains	308
VIRGINIA FUOCHI ⁽¹⁾ - SALVATORE FURNARI ⁽¹⁾ - LUCIA LONGHITANO ⁽¹⁾ - FRANCESCO TIRALONGO ⁽²⁾ - DANIELE TIBULLO ⁽¹⁾ - PIO MARIA FURNERI ⁽¹⁾	308
Università di Catania, BIOMETEC, Catania, Italia ⁽¹⁾ - Università di Catania, Dip. di Scienze Biologiche, Geologiche e Ambientali, Catania, Italia ⁽²⁾	308
Effect of fish skin mucous derived from bathypelagic fish <i>Centrolophus niger</i> (Gmelin, 1789) on <i>Escherichia coli</i>	308
VIRGINIA FUOCHI ¹ , SALVATORE FURNARI ¹ , LUCIA LONGHITANO ² , FRANCESCO TIRALONGO ³ , DANIELE TIBULLO ² E PIO MARIA FURNERI ¹	308
1 Dipartimento di Scienze Biomediche e Biotecnologiche, Sez. di Microbiologia, Università di Catania, Catania, Italia; 2 Dipartimento di Scienze Biomediche e Biotecnologiche, Sez. di Biochimica medica, Università di Catania, Catania, Italia; 3 Dipartimento di Scienze Biologiche, Geologiche e Ambientali, Sez. Biologia Animale, Università di Catania, Catania, Italia.....	308
124 - Antimicrobial activity of in silico designed synthetic peptides against biofilms of antibiotic-resistant <i>Escherichia coli</i> isolates	309
LORENZA ARTESANI ⁽¹⁾ - TECLA CIOCIOLA ⁽¹⁾ - ALICE VISMARRA ⁽²⁾ - CRISTINA BACCI ⁽²⁾ - STEFANIA CONTI ⁽¹⁾ - LAURA GIOVATI ⁽¹⁾	309

University of Parma, Department of Medicine and Surgery, Parma, Italia ⁽¹⁾ - University of Parma, Department of Veterinary Science, Parma, Italia ⁽²⁾	309
Antimicrobial activity of in silico designed synthetic peptides against biofilms of antibiotic-resistant <i>Escherichia coli</i> isolates	309
LORENZA ARTESANI ¹ , TECLA CIOCIOLA ¹ , ALICE VISMARRA ² , CRISTINA BACCI ² , STEFANIA CONTI ¹ , LAURA GIOVATI ¹	309
¹ Department of Medicine and Surgery, Biomedical, Biotechnological and Translational Sciences Unit, University of Parma, Parma, Italy; ² Department of Veterinary Science, Parasitology Unit, University of Parma, Parma, Italy	309
128 - Novel fungicidal thiazole derivatives inhibit the growth of <i>Candida albicans</i> by ergosterol depletion.	311
GIANLUIGI CABIDDU ⁽¹⁾ - RITA MELEDDU ⁽²⁾ - LAURA DEMURU ⁽²⁾ - ELIAS MACCIONI ⁽²⁾ - ALESSANDRO DE LOGU ⁽¹⁾	311
Università degli Studi di Cagliari, Dipartimento di Scienze della Vita e dell'Ambiente - Sezione di Scienze Farmaceutiche, Farmacologiche e Nutraceutiche, Cagliari, Italia ⁽¹⁾ - Università degli Studi di Cagliari, Dipartimento di Scienze della Vita e dell'Ambiente - Sezione di Scienze del Farmaco, Cagliari, Italia ⁽²⁾	311
Novel fungicidal thiazole derivatives inhibit the growth of <i>Candida albicans</i> by ergosterol depletion.	311
GIANLUIGI CABIDDU ^a , RITA MELEDDU ^b , LAURA DEMURU ^b , ELIAS MACCIONI ^b , ALESSANDRO DE LOGU ^a	311
^a Department of Environmental and Life Sciences, Section of Pharmaceutical, Pharmacological and Nutraceutical Sciences, University of Cagliari, Cagliari, Italy; ^b Department of Environmental and Life Sciences, Section of Drug Sciences, University of Cagliari, Cagliari, Italy.....	311
138 - The inhibitory effects of <i>Lactobacillus gasseri</i> 1A-TV cell-free supernatant against ESBL and carbapenemase producing <i>Klebsiella pneumoniae</i>	313
GAIA VERTILLO ALUISIO ⁽¹⁾ - MARIA LINA MEZZATESTA ⁽¹⁾ - STEFANIA STEFANI ⁽¹⁾ - MARIA SANTAGATI ⁽¹⁾	313
Università di Catania, Dipartimento di Scienze Biomediche e Biotechnologiche, Catania, Italia ⁽¹⁾	313
The inhibitory effects of <i>Lactobacillus gasseri</i> 1A-TV cell-free supernatant against ESBL and carbapenemase producing <i>Klebsiella pneumoniae</i>	313
GAIA VERTILLO ALUISIO ¹ , MARIA LINA MEZZATESTA ¹ , STEFANIA STEFANI, MARIA SANTAGATI ¹	313
¹ Department of Biomedical and Biotechnological Sciences (BIOMETEC), Section of Microbiology, University of Catania, Catania, Italy	313
141 - Activity of a newly designed gallium-siderophore complex (GaS1) against lung isolates of <i>A. fumigatus</i> in an in vitro air-liquid interface lung infection model	314
SEMIH ESIN ⁽¹⁾ - BRUNELLA GRASSIRI ⁽²⁾ - MAGDALENA PIATEK ⁽³⁾ - LEWIS M. O'FERRALL ⁽⁴⁾ - JOHANNES A. SAKE ⁽⁵⁾ - CARSTEN EHRHARDT ⁽⁵⁾ - DARREN M. GRIFFITH ⁽⁴⁾ - KEVIN KAVANAGH ⁽³⁾ - ANNE MARIE HEALY ⁽⁵⁾ - ANNA M. PIRAS ⁽²⁾ - GIOVANNA BATONI ⁽¹⁾	314
Università di Pisa, Dipartimento di Ricerca Traslazionale e delle nuove tecnologie in Medicina e Chirurgia, Pisa, Italia ⁽¹⁾ - Università di Pisa, Dipartimento di Farmacia, Pisa, Italia ⁽²⁾ - Maynooth	

University, Department of Biology, Maynooth, Irlanda ⁽³⁾ - University of Medicine and Health Sciences, Department of Chemistry, RCSI, Dublin, Irlanda ⁽⁴⁾ - Trinity Collage Dublin, School of Pharmacy and Pharmaceutical Sciences, Dublin, Irlanda ⁽⁵⁾	314
Activity of a newly designed gallium-siderophore complex (GaS1) against lung isolates of <i>A. fumigatus</i> in an in vitro air-liquid interface lung infection model	314
SEMIH ESIN ¹ , BRUNELLA GRASSIRI ² , MAGDALENA PIATEK ^{3,6} , LEWIS M. O'FERRALL ^{4,6} , JOHANNES A. SAKE ⁵ , CARSTEN EHRHARDT ⁵ , DARREN M. GRIFFITH ^{4,6} , KEVIN KAVANAGH ^{3,6} , ANNE MARIE HEALY ^{5,6} , ANNA M. PIRAS ² AND GIOVANNA BATONI ¹	314
¹ Department of Translational Research and New Technologies in Medicine and Surgery and	
² Department of Pharmacy, University of Pisa, Pisa, Italy.....	314
³ Department of Biology, Maynooth University, Maynooth, Co Kildare, Ireland.	314
⁴ Department of Chemistry, RCSI, University of Medicine and Health Sciences, Dublin 2, Ireland.	314
⁵ School of Pharmacy and Pharmaceutical Sciences, Trinity College Dublin, Dublin, Ireland.....	314
⁶ SSPC The Science Foundation Ireland Research Centre for Pharmaceuticals, Ireland.	314
158 - Synergistic antibacterial effect of colistin in combination with human albumin nanoparticles on multidrug resistant <i>Acinetobacter baumannii</i> isolates.....	316
SARA SCUTERA ⁽¹⁾ - FRANCESCA MENOTTI ⁽¹⁾ - ELEONORA MANISCALCO ⁽¹⁾ - MONICA ARGENZIANO ⁽²⁾ - ILARIO FERROCINO ⁽³⁾ - GABRIELE BIANCO ⁽⁴⁾ - ROBERTA CAVALLI ⁽²⁾ - VIVIANA CAFISO ⁽⁵⁾ - TIZIANA MUSSO ⁽¹⁾	316
University of Torino, Department of Public Health and Pediatric Sciences, TORINO, Italia ⁽¹⁾ - University of Torino, Department of Drug Science and Technology, TORINO, Italia ⁽²⁾ - University of Torino, Department of Agricultural, Forest and Food Sciences, TORINO, Italia ⁽³⁾ - University Hospital Città della Salute e della Scienza di Torino, Microbiology and Virology Unit, TORINO, Italia ⁽⁴⁾ - University of Catania, Department of Biomedical and Biotechnological Sciences, CATANIA, Italia ⁽⁵⁾	316
Synergistic antibacterial effect of colistin in combination with human albumin nanoparticles on multidrug resistant <i>Acinetobacter baumannii</i> isolates	316
SARA SCUTERA ¹ , FRANCESCA MENOTTI ¹ , ELEONORA MANISCALCO ¹ , MONICA ARGENZIANO ² , ILARIO FERROCINO ³ , GABRIELE BIANCO ⁴ , ROBERTA CAVALLI ² , VIVIANA CAFISO ⁵ AND TIZIANA MUSSO ¹	316
¹ Department of Public Health and Pediatric Sciences, University of Torino, Torino, Italy; ² Department of Drug Science and Technology, University of Torino, Torino, Italy; ³ Department of Agricultural, Forest and Food Sciences, University of Torino, Torino, Italy; ⁴ Microbiology and Virology Unit, University Hospital Città della Salute e della Scienza di Torino, Torino, Italy; ⁵ Department of Biomedical and Biotechnological Sciences, University of Catania, Catania, Italy	316
160 - Bithiazole compounds in the treatment of virus/bacteria co-infections	318
GIULIA RADOCCIA (1) - MARTA DE ANGELIS (1) - SOFIA VENDITTI (1) - REBECCA DE GREGORIO (1) - FRANCESCA BARBIERI (2) - MARIA GRAZIA MARTINA (2) - MARCO RADI (2) - SERENA SCHIPPA (1) - LUCIA NENCIONI (1).....	318
La Sapienza University of Rome, Department of Public Health and Infectious Diseases, Roma, Italia (1) - University of Parma, Department of Food and Drug Sciences, Parma, Italia (2).....	318

Bithiazole compounds in the treatment of virus/bacteria co-infections.	318
GIULIA RADOCCIA ^{1,§} , MARTA DE ANGELIS ^{1,2,§} , SOFIA VENDITTI ¹ , REBECCA DE GREGORIO ¹ , FRANCESCA BARBIERI ³ , MARIA GRAZIA MARTINA ³ MARCO RAD ³ , SERENA SCHIPPA ^{1,*} , LUCIA NENCIONI ^{1,*}	318
1 Department of Public Health and Infectious Diseases, Sapienza University of Rome, Rome, Italy;.....	318
2 Department of Molecular Medicine, Laboratory of Virology, Sapienza University of Rome, Rome, Italy;.....	318
3 Department of Food and Drug Sciences, University of Parma, Viale delle Scienze, 27/A, 43124, Parma, Italy.....	318
165 - Antiviral activity of the non-steroidal anti-inflammatory drug indomethacin against Respiratory Syncytial Virus.....	319
CATERINA TRAMONTOZZI ⁽¹⁾ - SIMONE LA FRAZIA ⁽¹⁾ - ANNA RICCIO ⁽¹⁾ - SILVIA PAUCIULLO ⁽¹⁾ - MARIA GABRIELLA SANTORO ⁽¹⁾	319
Università di Roma "Tor Vergata", Dipartimento di biologia, Roma, Italia ⁽¹⁾	319
Antiviral activity of the non-steroidal anti-inflammatory drug indomethacin against	319
Respiratory Syncytial Virus.....	319
CATERINA TRAMONTOZZI ¹ , SIMONE LA FRAZIA ¹ , ANNA RICCIO ¹ , SILVIA PAUCIULLO ¹ and M. GABRIELLA SANTORO ¹	319
¹ Department of Biology, University of Rome Tor Vergata, Rome, Italy.	319
167 - Screening of a panel of compounds as anti-Sars-CoV-2 antiviral drugs	320
SILVIA NOTTOLI ⁽¹⁾ - CARLO DI MARZO ⁽²⁾ - PAOLA QUARANTA ⁽²⁾ - MICHELE LAI ⁽²⁾ - ROBERTA IBBA ⁽³⁾ - SIMONA SESTITO ⁽⁴⁾ - SANDRA PIRAS ⁽³⁾ - ANTONIO CARTA ⁽³⁾ - GIULIA FREER ⁽²⁾	320
unità operativa di virologia, Azienda Ospedaliera Universitaria Pisana, Pisa, Italia ⁽¹⁾ - Centro Retrovirus, Dipartimento di ricerca traslazionale e nuove tecnologie in medicina e chirurgia, Università di Pisa, Pisa, Italia ⁽²⁾ - Dipartimento di medicina, chirurgia e farmacia, Università di Sassari, Sassari, Italia ⁽³⁾ - Dipartimento di chimica, fisica, matematica e scienze naturali, università di sassari, Sassari, Italia ⁽⁴⁾	320
Screening of a panel of compounds as anti-Sars-CoV-2 antiviral drugs	320
Topic: Nuovi approcci antimicrobici.....	320
SILVIA NOTTOLI ¹⁻² , CARLO DI MARZO ² , PAOLA QUARANTA ² , MICHELE LAI ² , ROBERTA IBBA ³ , SIMONA SESTITO ⁴ , SANDRA PIRAS ³ , ANTONIO CARTA ³ , GIULIA FREER ²	320
¹ Virology Operative Unit, Pisa University Hospital, Pisa, Italy; ² Retrovirus Center, Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa, Pisa, Italy; ³ Department of Medicine, Surgery and Pharmacy, University of Sassari, Sassari, Italy; ⁴ Department of Chemical, Physical, Mathematical and Natural Sciences, University of Sassari, Sassari, Italy.	320
189 - Activity evaluation of phage SAU1593 with levofloxacin ot erythromycin against Staphylococcus aureus strains isolated from diabetic foot infections	322
ALESSIO CORRAINI ⁽¹⁾ - ROSA AURICCHIO ⁽¹⁾ - ANNA BERTONCELLI ⁽¹⁾ - ANNARITA MAZZARIOL ⁽¹⁾	322

Università di Verona, Dipartimento di Diagnostica e Sanità Pubblica, Verona, Italia ⁽¹⁾	322
Activity evaluation of phage SAU1593 with levofloxacin ot erythromycin against Staphylococcus aureus strains isolated from diabetic foot infections	322
ALESSIO CORRAINI, ROSA AURICCHIO ANNA BERTONCELLI, ANNARITA MAZZARIOL	322
Department of Diagnostics and, Public Health, University of Verona Italy	322
192 - Antibiotic resistance, a global health problem: search for alternative antimicrobial molecules and Superbug Awareness For Education (SAFE) activities.....	323
EMANUELA ROSCETTO ⁽¹⁾ - UMBERTO GALDIERO ⁽¹⁾ - MARTINA AVERSA ⁽¹⁾ - MARIATERESA MANZONI ⁽¹⁾ - ANNA FIORETTI ⁽¹⁾ - CIRO RAUCH ⁽¹⁾ - MARIA ROSARIA CATANIA ⁽¹⁾	323
Università Federico II di Napoli, Dipartimento di Medicina Molecolare e Biotecnologie Mediche, Napoli, Italia ⁽¹⁾	323
Antibiotic resistance, a global health problem: search for alternative antimicrobial molecules and Superbug Awareness For Education (SAFE) activities.....	323
EMANUELA ROSCETTO, UMBERTO GALDIERO, MARTINA AVERSA, MARIATERESA MANZONI, ANNA FIORETTI, CIRO RAUCH, MARIA R. CATANIA.	323
Department of Molecular Medicine and Medical Biotechnology, University Federico II of Naples, via Pansini 5, 80131 Napoli, Italy	323
203 - Zeno_omega: a novel therapeutic phage-based formulation evolved for the treatment of multi-drug resistant Staphylococcus aureus.....	325
CLAUDIA CAMPOBASSO ⁽¹⁾ - SARA BOLOGNINI ⁽¹⁾ - JEROEN WAGEMANS ⁽²⁾ - LAURA RINDI ⁽³⁾ - ARIANNA TAVANTI ⁽¹⁾ - ROB LAVIGNE ⁽²⁾ - MARIAGRAZIA DI LUCA ⁽¹⁾	325
Università di Pisa, Dipartimento di Biologia, Pisa, Italia ⁽¹⁾ - KU Leuven, Deapartment od Biosystems, Leuven, Belgio ⁽²⁾ - Università di Pisa, Dipartimento di Ricerca Traslazionale e delle Nuove Tecnologie in Medicina e Chirurgia, Pisa, Italia ⁽³⁾	325
Zeno_omega: a novel therapeutic phage-based formulation evolved for the treatment of multi-drug resistant Staphylococcus aureus	325
CLAUDIA CAMPOBASSO ^{1,2} , SARA BOLOGNINI ¹ , JEROEN WAGEMANS ² , LAURA RINDI ³ , ARIANNA TAVANTI ¹ , ROB LAVIGNE ² , MARIAGRAZIA DI LUCA ¹	325
¹ Department of Biology, University of Pisa, Pisa, Italy; ² Department of Biosystems, Laboratory of Gene Technology, KU Leuven, Leuven, Belgium; ³ Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa, Pisa, Italy.....	325
204 - In vitro antibacterial effects of Thyme essential oil against Salmonella enterica group B	327
MARTINA DI ROSARIO ⁽¹⁾ - GIUSEPPE MANTOVA ⁽¹⁾ - DANIELA SATERIALE ⁽²⁾ - BEATRICE BOTTI ⁽¹⁾ - ELENA SCAGLIONE ⁽³⁾ - CHIARA PAGLIUCA ⁽¹⁾ - CATERINA PAGLIARULO ⁽²⁾ - MARIATERESA VITIELLO ⁽³⁾ - PAOLA SALVATORE ⁽⁴⁾ - ROBERTA COLICCHIO ⁽³⁾	327
University of Naples Federico II, Department of Molecular Medicine and Medical Biotechnology, University of Naples Federico II, Naples, Italia ⁽¹⁾ - University of Sannio, Benevento, Italy, Department of Science and Technology, University of Sannio, Via F. De Sanctis snc, 82100 Benevento, Italy, Naples, Italia ⁽²⁾ - University of Naples Federico II, Dpt. of Mol. Med. and Med. Biotec., Uni. of Naples Federico II;Dpt. of Int. Act. of Lab. Med. and Tra., C. O.U of Cli. Micro., Uni. Hosp. Fed. II, Naples, Italia ⁽³⁾ - University of Naples Federico II, Dpt. of Mol. Med. and Med.	

Biotec; Dpt. of Int. Act. of Lab. Med. and Tra., C.O.U of Cli. Micro; Task Force on Micro. Stud. ; Ceinge Franco Salvatore, Naples, Italia ⁽⁴⁾	327
In vitro antibacterial effects of Thyme essential oil against Salmonella enterica group B	327
MARTINA DI ROSARIO ¹ , GIUSEPPE MANTOVA ¹ , DANIELA SATERIALE ² , BEATRICE BOTTI ¹ , ELENA SCAGLIONE ^{1,3} , CHIARA PAGLIUCA ¹ , CATERINA PAGLIARULO ² , MARIATERESA VITIELLO ^{1,3} , PAOLA SALVATORE ^{1,3,4,5} , ROBERTA COLICCHIO ^{1,3}	327
¹ Department of Molecular Medicine and Medical Biotechnology, University of Naples Federico II, Naples, Italy; ² Department of Science and Technology, University of Sannio, Via F. De Sanctis snc, 82100 Benevento, Italy; ³ Department of Integrated Activity of Laboratory Medicine and Transfusion, Complex Operative Unit of Clinical Microbiology, University Hospital Federico II, Naples, Italy; ⁴ Task Force on Microbiome Studies, University of Naples Federico II, Naples, Italy; ⁵ CEINGE, Advanced Biotechnologies Franco Salvatore s.c.ar.l., Naples, Italy.	
206 - Antimicrobial activity of WMR-K and WMR-K+gH625 against Salmonella enterica group B	329
ELENA SCAGLIONE ⁽¹⁾ - CHIARA PAGLIUCA ⁽²⁾ - GIUSEPPE MANTOVA ⁽²⁾ - MARTINA DI ROSARIO ⁽²⁾ - CHIARA OLANDESE ⁽²⁾ - ANNARITA FALANGA ⁽³⁾ - ROBERTA COLICCHIO ⁽¹⁾ - STEFANIA GALDIERO ⁽⁴⁾ - PAOLA SALVATORE ⁽⁵⁾ - MARIATERESA VITIELLO ⁽¹⁾	329
University of Naples Federico II, Department of Molecular Medicine and Medical Biotechnology; Dep. of Int. Act. of Lab. Med. and Trans. UOC Clinical Microbiology, Naples, Italia ⁽¹⁾ - University of Naples Federico II, Department of Molecular Medicine and Medical Biotechnology, Naples, Italia ⁽²⁾ - University of Naples Federico II, Department of Agriculture, Naples, Italia ⁽³⁾ - University of Naples Federico II, Department of Pharmacy, Naples, Italia ⁽⁴⁾ - University of Naples Federico II, Dep. of Mol. Med. and Med. Biotec.; Dep. of Int. Act. of Lab. Med. and Trans. UOC Clin. Micr.; CEINGE Franco Salvatore; Task Force Microb. Studies, Naples, Italia ⁽⁵⁾	
Antimicrobial activity of WMR-K and WMR-K+gH625 against Salmonella enterica group B..	329
ELENA SCAGLIONE ^{1,2} , CHIARA PAGLIUCA ¹ , GIUSEPPE MANTOVA ¹ , MARTINA DI ROSARIO ¹ , CHIARA OLANDESE ¹ , ANNARITA FALANGA ³ , ROBERTA COLICCHIO ^{1,2} , STEFANIA GALDIERO ⁴ , PAOLA SALVATORE ^{1,2,5,6} , MARIATERESA VITIELLO ^{1,2}	329
¹ Department of Molecular Medicine and Medical Biotechnology, University of Naples Federico II, Naples, Italy; ² Department of Integrated Activity of Laboratory Medicine and Transfusion, Complex Operative Unit of Clinical Microbiology, University Hospital Federico II, Naples, Italy; ³ Department of Agriculture, University of Naples Federico II, Naples, Italy; ⁴ Department of Pharmacy, University of Naples Federico II, Naples, Italy; ⁵ CEINGE, Advanced Biotechnologies s.c.ar.l., Naples, Italy; ⁶ Task Force on Microbiome Studies, University of Naples Federico II, Naples, Italy.....	
208 - Isolation and characterization of novel bacteriophages active against clinical strains of Klebsiella pneumoniae	331
ELISA FAUSTI ⁽¹⁾ - CLAUDIA CAMPOBASSO ⁽²⁾ - MARIANNA LUONGO ⁽²⁾ - SELENIA BANCHERI ⁽²⁾ - DARIA BOTTAI ⁽²⁾ - CESIRA GIORDANO ⁽³⁾ - SIMONA BARNINI ⁽³⁾ - ARIANNA TAVANTI ⁽²⁾ - MARIAGRAZIA DI LUCA ⁽²⁾	331
Università Roma Tor Vergata, Dipartimento di Medicina Sperimentale e Chirurgia, Roma, Italia ⁽¹⁾ - Università di Pisa, Dipartimento di Biologia, Pisa, Italia ⁽²⁾ - Azienda Ospedaliera Universitaria Pisana, Ospedale di Cisanello, Unità di Microbiologia, Pisa, Italia ⁽³⁾	
Isolation and characterization of novel bacteriophages active against clinical strains of Klebsiella pneumoniae	331

ELISA FAUSTI ¹ , CLAUDIA CAMPOBASSO ^{2,3} , MARIANNA LUONGO ² , SELENIA C. BANCHERI ² , DARIA BOTTAI ² , CESIRA GIORDANO ⁴ , SIMONA BARNINI ⁴ , ARIANNA TAVANTI ^{2,3} , MARIAGRAZIA DI LUCA ²	331
¹ Department of Experimental Medicine and Surgery, University of Rome Tor Vergata, Rome, Italy; Department of Biology, ² Unit of Genetics and Microbiology, University di Pisa, Italy; ³ Department of Biosystems, Laboratory of Gene Technology, KU Leuven, Leuven, Belgium; ⁴ Microbiology Unit, Azienda Ospedaliera Universitaria Pisana, Pisa, Italy.....	
212 - Synergistic effects of carvacrol in combination with antibiotics against <i>Acinetobacter baumannii</i> , <i>Escherichia coli</i> and <i>Pseudomonas aeruginosa</i>	333
GIOVANNA GINESTRA ⁽¹⁾ - ANDREANA MARINO ⁽¹⁾ - LOREDANA FERRERI ⁽²⁾ - GIUSEPPE GRANATA ⁽²⁾ - GRAZIA M.L. CONSOLI ⁽²⁾ - ANTONIA NOSTRO ⁽¹⁾	333
Università degli Studi di Messina, Dipartimento di Scienze Chimiche, Biologiche, Farmaceutiche ed Ambientali, Messina, Italia ⁽¹⁾ - C.N.R., Istituto di Chimica Biomolecolare, Catania, Italia ⁽²⁾	
Synergistic effects of carvacrol in combination with antibiotics against <i>Acinetobacter baumannii</i> , <i>Escherichia coli</i> and <i>Pseudomonas aeruginosa</i>	333
GIOVANNA GINESTRA ¹ , ANDREANA MARINO ¹ , LOREDANA FERRERI ² , GIUSEPPE GRANATA ² , GRAZIA M.L. CONSOLI ² , ANTONIA NOSTRO ¹	333
¹ Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Italy; ² Institute of Biomolecular Chemistry (ICB-CNR) Catania, Italy	
221 - Complex Electromagnetic Fields: eco-sustainable strategy to counteract the resistant <i>Candida albicans</i> infections.....	335
SILVIA DI LODOVICO ⁽¹⁾ - PAOLA DI FERMO ⁽²⁾ - MORENA PETRINI ⁽²⁾ - EMIRA D'AMICO ⁽²⁾ - SARA D'ARCANGELO ⁽¹⁾ - FIRAS DIBAN ⁽¹⁾ - LUIGINA CELLINI ⁽¹⁾ - MARA DI GIULIO ⁽¹⁾ - SIMONETTA D'ERCOLE ⁽²⁾	335
University "G. d'Annunzio" Chieti-Pescara, Department of Pharmacy, Chieti, Italia ⁽¹⁾ - University "G. d'Annunzio" Chieti-Pescara, Department of Medical, Oral and Biotechnological Sciences, Chieti, Italia ⁽²⁾	
Complex Electromagnetic Fields: eco-sustainable strategy to counteract the resistant <i>Candida albicans</i> infections	335
SILVIA DI LODOVICO ¹ , PAOLA DI FERMO ² , MORENA PETRINI ² , EMIRA D'AMICO ² , SARA D'ARCANGELO ¹ , FIRAS DIBAN ¹ , LUIGINA CELLINI ¹ , MARA DI GIULIO ¹ , SIMONETTA D'ERCOLE ²	335
¹ Department of Pharmacy, University "G. d'Annunzio" Chieti-Pescara, 66100, Chieti, Italy. ² Department of Medical, Oral and Biotechnological Sciences, University of "G. d'Annunzio" Chieti-Pescara, 66100, Chieti, Italy.	
232 - A preliminary study on bacterial control strains to assess the synergistic effect of new heteroaryl-ethylene molecules in combination with antibiotics.	337
PAOLO GIUSEPPE BONACCI ⁽¹⁾ - CARMELO BONOMO ⁽¹⁾ - DALIDA ANGELA BIVONA ⁽¹⁾ - EMANUELE NICITRA ⁽¹⁾ - CARMELA BONACCORSO ⁽²⁾ - DAFNE SAMANTHA IRENE BONGIORNO ⁽¹⁾ - STEFANIA STEFANI ⁽¹⁾ - NICOLÒ MUSSO ⁽¹⁾	337
Università degli studi di Catania, Dipartimento di scienze biomediche e biotecnologiche (BIOMETEC), Catania, Italia ⁽¹⁾ - Università degli studi di Catania, Dipartimento di Scienze Chimiche, Catania, Italia ⁽²⁾	

A preliminary study on bacterial control strains to assess the synergistic effect of new heteroaryl-ethylene molecules in combination with antibiotics.....	337
PAOLO G. BONACCI¹, CARMELO BONOMO¹, DALIDA A. BIVONA¹, EMANUELE NICITRA¹, CARMELA BONACCORSO², DAFNE BONGIORNO¹, STEFANIA STEFANI¹ E NICOLÒ MUSSO¹.	337
¹Department of Biomedical and Biotechnological Sciences (BIOMETEC), Università degli Studi di Catania, Via S. Sofia, 97, 95123 Catania, Italy	337
²Department of Chemical Sciences, Università degli Studi di Catania, Viale Andrea Doria 6, 95125 Catania, Italy.....	337
236 - Ligand-free AgNPs: an innovative antimicrobial strategy	339
MARIA VITTORIA MORONE ⁽¹⁾ - FEDERICA DELL' ANNUNZIATA ⁽¹⁾ - ANNALISA CHIANESE ⁽¹⁾ - VERONICA FOLLIERO ⁽²⁾ - CARLA ZANNELLA ⁽¹⁾ - ANNA DE FILIPPIS ⁽¹⁾ - GIANLUIGI FRANCI ⁽²⁾ - ANTONIO MORONE ⁽³⁾ - MASSIMILIANO GALDIERO ⁽¹⁾	339
Dipartimento di Medicina Sperimentale, Università degli studi della Campania "Luigi Vanvitelli", Napoli, Italia ⁽¹⁾ - Department of Medicine, Surgery and Dentistry "Scuola Medica Salernitana", Università di Salerno, Baronissi, Italia ⁽²⁾ - Istituto struttura della materia U.O. di Tito Scalo, Consiglio Nazionale Delle Ricerche, Tito Scalo, Italia ⁽³⁾	339
Ligand-free AgNPs: an innovative antimicrobial strategy.....	339
Maria Vittoria Morone¹, Federica Dell'Annunziata^{1,2}, Annalisa Chianese¹, Veronica Folliero², Carla Zannella¹, Anna De Filippis¹, Gianluigi Franci², Antonio Morone³, Massimiliano Galdiero¹.	339
¹ Department of Experimental Medicine, University of Campania "Luigi Vanvitelli", 80138 Naples, Italy.....	339
² Department of Medicine, Surgery and Dentistry "Scuola Medica Salernitana" University of Salerno, 84081 Baronissi, Italy.....	339
³ National Research Council, Institute of Structure of Matter U.O. di Tito Scalo, 85050, Italy.....	339
237 - New imidazolium alkaloids from the marine bacterium Shewanella aquimarina as innovative antimicrobial strategies.....	341
ROSA GIUGLIANO ⁽¹⁾ - ANNALISA AMBROSINO ⁽¹⁾ - CARMINE BUONOCORE ⁽¹⁾ - GERARDO DELLA SALA ⁽²⁾ - PIETRO TEDESCO ⁽²⁾ - CARLA ZANNELLA ⁽¹⁾ - GIANLUIGI FRANCI ⁽³⁾ - ANNA DE FILIPPIS ⁽¹⁾ - DONATELLA DE PASCALE ⁽²⁾ - MASSIMILIANO GALDIERO ⁽¹⁾	341
Università degli Studi della Campania "Luigi Vanvitelli", dipartimento medicina sperimentale, Napoli, Italia ⁽¹⁾ - stazione zoologica Anton Dohrn, 2.	
Department of Marine Biotechnology, Napoli, Italia ⁽²⁾ - University of Salerno, Department of Medicine, Surgery and Dentistry, salerno, Italia ⁽³⁾	
341	
New imidazolium alkaloids from the marine bacterium Shewanella aquimarina as innovative antimicrobial strategies.....	341
Rosa Giugliano¹, Annalisa Ambrosino¹, Carmine Buonocore², Gerardo Della Sala², Pietro Tedesco², Carla Zannella¹, Gianluigi Franci³, Anna De Filippis¹, Donatella de Pascale², Massimiliano Galdiero¹.....	341

¹ Department of Experimental Medicine, University of Campania “Luigi Vanvitelli”, 80138 Naples, Italy	341
² Department of Ecosustainable Marine Biotechnology, Stazione Zoologica Anton Dohrn, Via Ammiraglio Acton, 55, 80133 Naples, Ital	341
³ Department of Medicine, Surgery and Dentistry “Scuola Medica Salernitana”, University of Salerno, 84081 Baronissi, Italy.....	341
238 - Anti-staphylococcal and anti-biofilm properties of the cationic peptide Orechromicin-1 from <i>Oreochromis niloticus</i>	343
FRANCESCA PALMA ⁽¹⁾ - MARINA ACUNZO ⁽¹⁾ - ANNALISA CHIANESE ⁽¹⁾ - ERICA PANICO ⁽²⁾ - NUNZIANNA DOTI ⁽³⁾ - ALESSANDRA MONTI ⁽³⁾ - ALESSANDRA FUSCO ⁽¹⁾ - CARLA ZANNELLA ⁽¹⁾ - ANNA DE FILIPPIS ⁽¹⁾ - GIOVANNA DONNARUMMA ⁽¹⁾ - MASSIMILIANO GALDIERO ⁽⁴⁾	343
Università degli Studi della Campania "Luigi Vanvitelli", Dipartimento di Medicina Sperimentale, Napoli, Italia ⁽¹⁾ - Università degli Studi della Campania "Luigi Vanvitelli", Azienda Ospedaliera Universitaria, Sezione di Microbiologia e Virologia, Napoli, Italia ⁽²⁾ - Istituto di Biostrutture e Bioimaging, Consiglio Nazionale delle Ricerche, Napoli, Italia ⁽³⁾ - Università degli Studi della Campania "Luigi Vanvitelli", Dipartimento di Medicina Sperimentale, Napoli, Italia ⁽⁴⁾	343
Anti-staphylococcal and anti-biofilm properties of the cationic peptide Orechromicin-1 from <i>Oreochromis niloticus</i>	343
FRANCESCA PALMA ¹ , MARINA ACUNZO ¹ , ANNALISA CHIANESE ^{1,2} , ERICA PANICO ² , NUNZIANNA DOTI ³ , ALESSANDRA MONTI ³ , ALESSANDRA FUSCO ¹ , CARLA ZANNELLA ^{1,2} , ANNA DE FILIPPIS ¹ , GIOVANNA DONNARUMMA ^{1,2} , MASSIMILIANO GALDIERO ^{1,2}	343
¹ Department of Experimental Medicine; University of Campania “Luigi Vanvitelli”; 80138 Naples; Italy.....	343
² Section of Microbiology and Virology; University Hospital "Luigi Vanvitelli" of Naples, Naples, Italy.	343
³ Institute of Biostructures and Bioimaging (IBB), National Research Council (CNR), 80134 Naples, Italy.....	343
242 - Effect of resveratrol on antibiofilm action against <i>Pseudomonas aeruginosa</i> clinical strains	345
MARIA RITA TRICOLI ⁽¹⁾ - ANNA GIAMMANCO ⁽²⁾ - VIVIANA DE CARO ⁽³⁾ - IGNAZIO ARRIGO ⁽¹⁾ - GIUSEPPE ANGELLOTTI ⁽³⁾ - TERESA FASCIANA ⁽⁴⁾	345
Laboratorio Microbiologia, A.O.U.P "P.Giaccone", Palermo, Italia ⁽¹⁾ - Università Degli Studi Di Palermo, Promise, Palermo, Italia ⁽²⁾ - Università Degli Studi Di Palermo, Scienze E Tecnologie Biologiche Chimiche E Farmaceutiche, Palermo, Italia ⁽³⁾ - Dipartimento Promise, A.O.U.P "P.Giaccone" /Universita' Di Palermo, Palermo, Italia ⁽⁴⁾	345
Effect of resveratrol on antibiofilm action against <i>Pseudomonas aeruginosa</i> clinical strains	345
Maria Rita Tricoli, Anna Giammanco, Viviana De Caro, Ignazio Arrigo, Giuseppe Angelotti, Teresa Fasciana	345
244 - Pilocarpine hydrochloride promotes <i>Candida albicans</i> cell membrane damage leading to reactive species production and apoptosis.....	346
EMERENZIANA OTTAVIANO ⁽¹⁾ - SILVIA ANCONA ⁽¹⁾ - MICHELE DEI CAS ⁽²⁾ - ELISA BORGHI ⁽¹⁾	346

Universita' Degli Studi Di Milano, Dip. Scienze Della Salute, Milano, Italia ⁽¹⁾ - Università Degli Studi Di Milano, Dip. Scienze Della Salute, Milano, Italia ⁽²⁾	346
Title: Pilocarpine hydrochloride promotes <i>Candida albicans</i> cell membrane damage leading to reactive species production and apoptosis.....	346
Authors:	346
Emerenziana Ottaviano ¹ , Silvia Ancona ¹ , Michele V. Dei Cas ¹ , Elisa Borghi ¹	346
1. Department of Health Sciences, Università degli Studi di Milano, Milan (Italy);	346
249 - Cryogel based on b-cyclodextrin and a maltol derivative for antimicrobial applications.....	348
VIRGINIA FUOCHI ⁽¹⁾ - CHIARA ZAGNI ⁽²⁾ - SALVATORE FURNARI ⁽¹⁾ - VINCENZO PATAMIA ⁽²⁾ - GIUSEPPE FLORESTA ⁽²⁾ - ANTONIO RESCIFINA ⁽²⁾ - PIO MARIA FURNERI ⁽¹⁾	348
Università di Catania, BIOMETEC, Catania, Italia ⁽¹⁾ - Università di Catania, Dip. Scienze del Farmaco e della Salute, Catania, Italia ⁽²⁾	348
Cryogel based on b-cyclodextrin and a maltol derivative for antimicrobial applications	348
VIRGINIA FUOCHI ¹ , CHIARA ZAGNI ² , SALVATORE FURNARI ¹ , VINCENZO PATAMIA ² , GIUSEPPE FLORESTA ² , ANTONIO RESCIFINA ² E PIO MARIA FURNERI ¹	348
1 Dipartimento di Scienze Biomediche e Biotecnologiche, Sez. di Microbiologia, Università di Catania, Catania, Italia; 2 Dipartimento di Scienze del Farmaco e della Salute, Università di Catania, Catania, Italia	348
258 - Antiviral activity of lipophilic extracts derived from <i>Lavandula austroapennina</i> organs	349
ANNALISA AMBROSINO ⁽¹⁾ - CLAUDIA GRAVINA ⁽²⁾ - MARIAVITTORIA MORONE ⁽¹⁾ - PRAGATI RAJENA MORE ⁽¹⁾ - FRANCESCO FOGLIA ⁽¹⁾ - SIMONA PICCOLELLA ⁽²⁾ - CARLA ZANNELLA ⁽¹⁾ - ASSUNTA ESPOSITO ⁽¹⁾ - SEVERINA PACIFICO ⁽²⁾ - ANNA DE FILIPPIS ⁽¹⁾ - MASSIMILIANO GALDIERO ⁽¹⁾	349
University of Campania "Luigi Vanvitelli", Department of Experimental Medicine, Napoli, Italia ⁽¹⁾ - University of Campania "Luigi Vanvitelli", Department of Environmental, Biological and Pharmaceutical Sciences and Technologies, Caserta, Italia ⁽²⁾	349
Antiviral activity of lipophilic extracts derived from <i>Lavandula austroapennina</i> organs.....	349
264 - Exploring the Inhibitory Potential of oreochromicin peptide against viruses with different Classes of Fusion Proteins.....	350
BIANCA MARIA NASTRI ⁽¹⁾ - ANNALISA CHIANESE ⁽¹⁾ - CARLA CAPASSO ⁽¹⁾ - NUNZIANNA DOTI ⁽²⁾ - ALESSANDRA MONTI ⁽²⁾ - VALENTINA IOVANE ⁽³⁾ - SERENA MONTAGNARO ⁽⁴⁾ - UGO PAGNINI ⁽⁴⁾ - CARLA ZANNELLA ⁽¹⁾ - ANNA DE FILIPPIS ⁽¹⁾ - MASSIMILIANO GALDIERO ⁽¹⁾	350
University of Campania "Luigi Vanvitelli", Department of Experimental Medicine, Naples, Italia ⁽¹⁾ - National Research Council (CNR), Institute of Biostructures and Bioimaging (IBB), Naples, Italia ⁽²⁾ - University of Naples "Federico II", Department of Agricultural Sciences, Naples, Italia ⁽³⁾ - University of Naples "Federico II", Department of Veterinary Medicine and Animal Productions, Naples, Italia ⁽⁴⁾	350
Exploring the Inhibitory Potential of oreochromicin peptide against viruses with different Classes of Fusion Proteins.....	350
277 - Antimicrobial and antioxidant properties of vesicles loaded with an extract obtained from olive leaves.....	352

ILENIA DELOGU ⁽¹⁾ - INES CASTANGIA ⁽²⁾ - SILVIA PUXEDDU ⁽¹⁾ - ELISA RAGA ⁽¹⁾ - MARIA LETIZIA MANCA ⁽²⁾ - ALDO MANZIN ⁽¹⁾ - FABRIZIO ANGIUS ⁽¹⁾	352
Università Degli Studi di Cagliari, Dipartimento Scienze Biomediche, Cagliari, Italia ⁽¹⁾ - Università Degli Studi di Cagliari, Dipartimento di Scienze della Vita e dell'Ambiente, Cagliari, Italia ⁽²⁾	352
Antimicrobial and antioxidant properties of vesicles loaded with an extract obtained from olive leaves	352
ILENIA DELOGU ¹, INES CASTANGIA ², SILVIA PUXEDDU ¹, ELISA RAGA ¹, MARIA LETIZIA MANCA ², ALDO MANZIN ^{1,3}, FABRIZIO ANGIUS ¹	352
¹ Department of Biomedical Sciences, Section of Microbiology and Virology, University of Cagliari, Cagliari, Italy.....	352
² Department of Life and Environmental Sciences, Section of Pharmaceutical Sciences,.....	352
University of Cagliari, Cagliari, Italy	352
AOU Policlinico "D. CASULA", APPLIED MICROBIOLOGY LAB, MONSERRATO (CA), Italy	352
280 - Ionic liquids as a new effective antimicrobial approach	354
Silvia Puxeddu (1) - Ilenia Delogu (1) - Alessandra Scano (2) - Sarah Vascellati (1) - Giuseppe Pala (1) - Guido Ennas (2) - Aldo Manzin (1) - Fabrizio Angius (1).....	354
Università degli studi di Cagliari, Dipartimento di Scienze Biomediche, Cagliari, Italia (1) - Università degli studi di Cagliari, Dipartimento di chimica e scienze geologiche, Cagliari, Italia (2)	354
Ionic liquids as a new effective antimicrobial approach	354
SILVIA PUXEDDU 1, ILENIA DELOGU 1, ALESSANDRA SCANO 3,4, SARAH VASCELLARI 1, GIUSEPPE PALA 1, GUIDO ENNAS 3,4, ALDO MANZIN 1,2, FABRIZIO ANGIUS 1.....	354
1 Department of Biomedical Sciences, Unit of Microbiology and Virology, University of Cagliari, Cagliari, Italy	354
2 AOUPoliclinico "D. Casula", Applied Microbiology Lab, Monserrato (CA), Italy	354
3 Department of Chemical and Geological Sciences, University of Cagliari, Cagliari, Italy	354
4 Cagliari Research Unit of the National Consortium of Materials Science and Technology (INSTM), Cagliari, Italy	354
282 - Screening of novel anti-HIV drugs as inhibitors against Sars-CoV-2 and HSV-2 viruses	356
SILVIA NOTTOLI ⁽¹⁾ - CARLO DI MARZO ⁽²⁾ - MARIA SIDOTI ⁽¹⁾ - PAOLA QUARANTA ⁽³⁾ - PAOLA MAZZETTI ⁽¹⁾ - GIULIA FREER ⁽²⁾ - ELENA DREASSI ⁽⁴⁾ - MAURO PISTELLO ⁽¹⁾	356
unità operativa di virologia, Azienda Ospedaliera Universitaria Pisana, Pisa, Italia ⁽¹⁾ - Centro Retrovirus, Dipartimento di ricerca traslazionale e nuove tecnologie in medicina e chirurgia, Università di Pisa, Pisa, Italia ⁽²⁾ - Istituto di Neuroscienze, Cnr, Pisa, Italia ⁽³⁾ - Dipartimento di biotecnologie, Università di Siena, Siena, Italia ⁽⁴⁾	356
Screening of novel anti-HIV drugs as inhibitors against Sars-CoV-2 and HSV-2 viruses	356
Topic: Nuovi approcci antimicrobici	356
SILVIA NOTTOLI¹, CARLO DI MARZO², MARIA SIDOTI¹, PAOLA QUARANTA⁴, PAOLA MAZZETTI¹, GIULIA FREER², ELENA DREASSI³, MAURO PISTELLO^{1,2}	356

¹ Virology Operative Unit, Pisa University Hospital, Pisa, Italy; ² Retrovirus Center, Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa, Pisa, Italy; ³ Department of Biotechnologies, University of Siena, Siena, Italy; ⁴ Istitute of Neuroscience, CNR, Pisa, Italy.....	356
286 - Resveratrol derivatives, promising strategy for innovative therapeutic schemes to tackle the Helicobacter pylori persistant infections	357
MARA DI GIULIO ⁽¹⁾ - PAOLA DI FERMO ⁽¹⁾ - FIRAS DIBAN ⁽¹⁾ - SARA D'ARCANGELO ⁽¹⁾ - BARBARA DE FILIPPIS ⁽¹⁾ - SILVIA DI LODOVICO ⁽¹⁾ - SIMONETTA D'ERCOLE ⁽²⁾ - EMANUELA DI CAMPLI ⁽¹⁾ - LUIGINA CELLINI ⁽¹⁾	357
Università "G.d'Annunzio" Chieti-Pescara, Dipartimento di Farmacia, chieti, Italia ⁽¹⁾ - Università "G.d'Annunzio" Chieti-Pescara, Dipartimento di Scienze mediche , orali e biotecnologiche, Chieti, Italia ⁽²⁾	357
Resveratrol derivatives, promising strategy for innovative therapeutic schemes to tackle the Helicobacter pylori persistant infections.....	357
MARA DI GIULIO ¹ , PAOLA DI FERMO ¹ , FIRAS DIBAN ¹ , SARA D'ARCANGELO ¹ , BARBARA DE FILIPPIS ¹ , SILVIA DI LODOVICO ¹ , SIMONETTA D'ERCOLE ² , EMANUELA DI CAMPLI ¹ , LUIGINA CELLINI ¹	357
¹ Department of Pharmacy, University of “G. d’Annunzio” Chieti-Pescara, Italy; ² Department of Medical, Oral and Biotechnological Sciences, University “G. d’Annunzio” Chieti-Pescara, Italy	357
306 - Outer membrane vesicles derived from Gram-Negative bacteria: biological vectors for innovative antiviral therapies	359
SANDRA RUFOLO ⁽¹⁾ - FEDERICA DELL'ANNUNZIATA ⁽¹⁾ - FRANCESCA MENSITIERI ⁽¹⁾ - VERONICA FOLLIERO ⁽¹⁾ - GIULIA GAUDINO ⁽¹⁾ - MASSIMILIANO GALDIERO ⁽²⁾ - FABRIZIO DAL PIAZ ⁽¹⁾ - VIVIANA IZZO ⁽¹⁾ - GIANLUIGI FRANCI ⁽¹⁾	359
Department of Medicine, Surgery and Dentistry "Scuola Medica Salernitana", University Salerno, Salerno, Italia ⁽¹⁾ - Department of Experimental Medicine, University of Campania Luigi Vanvitelli, Napoli, Italia ⁽²⁾	359
Outer membrane vesicles derived from Gram-Negative bacteria: biological vectors for innovative antiviral therapies	359
Federica Dell’Annunziata ^{1,2} , Francesca Mensitieri ¹ , Veronica Folliero ¹ , Giulia Gaudino ¹ , Sandra Rufolo ¹ , Massimiliano Galdiero ^{2,3} , Fabrizio Dal Piaz ¹ , Viviana Izzo ¹ , Gianluigi Franci ^{1,4}	359
¹ Department of Medicine, Surgery and Dentistry "Scuola Medica Salernitana", University of Salerno, 84081 Baronissi Salerno, Italy.....	359
² Department of Experimental Medicine, University of Campania Luigi Vanvitelli, 80138 Naples, Italy	359
³ Complex Operative Unity of Virology and Microbiology, University Hospital of Campania "Luigi Vanvitelli", 80138 Naples, Italy	359
⁴ Clinical Pathology and Microbiology Unit, San Giovanni di Dio e Ruggi D’Aragona University Hospital, 84126 Salerno, Italy	359
317 - PYED-1: new molecule able to re-sensitize colistin-resistant Acinetobacter baumannii strains	361
MARIA STABILE ⁽¹⁾ - ANTONELLA MIGLIACCIO ⁽²⁾ - ANNA ESPOSITO ⁽³⁾ - ANNALISA GUARAGNA ⁽³⁾ - RAFFAELE ZARRILLI ⁽²⁾ - ELIANA DE GREGORIO ⁽⁴⁾	361

Università, Complex Operational Unit of Clinical Microbiology, University Hospital “Federico II”, Napoli, Italia ⁽¹⁾ - Università, Department of Public Health, University of Naples “Federico II”, Napoli, Italia ⁽²⁾ - Università, Department of Chemical, Materials and Production Engineering, University of Naples “Federico II”, Napoli, Italia ⁽³⁾ - Università, Department of Molecular Medicine and Medical Biotechnology, University of Naples “Federico II”, Napoli, Italia ⁽⁴⁾	361
PYED-1: new molecule able to re-sensitize colistin-resistant Acinetobacter baumannii strains..	361
MARIA STABILE^{1,2}, ANTONELLA MIGLIACCIO³, ANNA ESPOSITO⁴, ANNALISA GUARAGNA⁴, RAFFAELE ZARRILLI³ AND ELIANA DE GREGORIO ^{2,5}	361
¹ Department of Chemical Sciences, University of Naples “Federico II”, Naples, Italy; ² Complex Operational Unit of Clinical Microbiology, University Hospital “Federico II”, Naples, Italy; ³ Department of Public Health, University of Naples “Federico II”, Naples, Italy; ⁴ Department of Chemical, Materials and Production Engineering, University of Naples “Federico II”, Naples, Italy; ⁵ Department of Molecular Medicine and Medical Biotechnology, University of Naples “Federico II”, Naples, Italy	361
327 - Potential of Mesenchymal Stromal Cells secretome as host-directed therapy for mycobacterial infection	363
FRANCESCO PAGLIONE ⁽¹⁾ - IVANA PALUCCI ⁽²⁾ - CATALDO MARIA MANNAVOLA ⁽¹⁾ - ALESSANDRO SALUSTRI ⁽²⁾ - FLAVIO DE MAIO ⁽³⁾ - ANTONIETTA ROSA SILINI ⁽⁴⁾ - ORNELLA PAROLINI ⁽⁵⁾ - GIOVANNI DELOGU ⁽⁶⁾	363
Istituto di Microbiologia, Fondazione Policlinico Universitario “A. Gemelli”, IRCCS, Roma, Italia ⁽¹⁾ - Department of Sciences of Basic Biotechnologies, Intensive and Perioperative Clinics, Section of Microbiology, Università Cattolica del Sacro Cuore, Roma, Italia ⁽²⁾ - Department of Laboratory and Infective Sciences, Faculty of Medicine, Fondazione Policlinico A. Gemelli IRCCS, Università Cattolica del Sacro Cuore, Roma, Italia ⁽³⁾ - Centro di Ricerca "E. Menni", Fondazione Poliambulanza - Istituto Ospedaliero, Brescia, Italia ⁽⁴⁾ - Department of Life Science and Public Health, Università Cattolica del Sacro Cuore, Roma, Italia ⁽⁵⁾ - Medicina di laboratorio, Mater Olbia Hospital, Olbia, Italia ⁽⁶⁾	363
Potential of Mesenchymal Stromal Cells secretome as host-directed therapy for mycobacterial infection.....	363
Francesco Paglione¹, Ivana Palucci^{2,3}, Cataldo M. Mannavola¹, Alessandro Salustri², Flavio De Maio³, Antonietta R. Silini⁵, Ornella Parolini^{1,4}, Giovanni Delogu^{3,6}	363
¹ Fondazione Policlinico Universitario “A. Gemelli”, IRCCS, Rome, Italy;	363
² Department of Sciences of Basic Biotechnologies, Intensive and Perioperative Clinics, Section of Microbiology, Università Cattolica del Sacro Cuore, Rome, Italy;	363
³ Department of Laboratory and Infective Sciences, Faculty of Medicine, Fondazione Policlinico A. Gemelli IRCCS, Università Cattolica del Sacro Cuore, Rome, Italy;	363
⁴ Department of Life Science and Public Health, Università Cattolica del Sacro Cuore, 00168 Rome, Italy	363
⁵ Centro di Ricerca "E. Menni", Fondazione Poliambulanza - Istituto Ospedaliero, Brescia, Italy	363
⁶ Mater Olbia Hospital, Olbia, Italy;	363
331 - The Secondary Resistome to Oxacillin and Tetracycline in MRSA ST398	365

NADER ABDELMALEK ⁽¹⁾ - MOSAED SALEH ALOBAIDALLAH ⁽²⁾ - SALLY WAHEED YOUSIEF ⁽¹⁾ - ALESSANDRO TANCA ⁽¹⁾ - JOHN EMERDAHL OLSEN ⁽²⁾ - SALVATORE RUBINO ⁽¹⁾ - BIANCA PAGLIETTI ⁽¹⁾	365
Università, Università di Sassari/Dipartimento di Scienze Biomediche, Sassari, Italia ⁽¹⁾ - University of Copenhagen, Department of Veterinary and Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Danimarca ⁽²⁾	365
The Secondary Resistome to Oxacillin and Tetracycline in MRSA ST398.....	365
Nader Abdelmalek ¹ , Mosaed S. Alobaidallah ² , Sally W. Yousief ¹ , Alessandro Tanca ¹ , John E. Olsen ² , Salvatore Rubino ¹ , Bianca Paglietti ¹	365
¹ Department of Biomedical Sciences, University of Sassari, Sassari, Italy.....	365
² Department of Veterinary and Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Frederiksberg, Denmark.....	365
07 One Health	366
2 - Zoonotic pathogenic microorganisms from the marine environment: A One Health perspective	366
MARIANNA MARANGI ⁽¹⁾ - CATERINA LANFREDI ⁽²⁾ - SABINA AIROLDI ⁽²⁾	366
Università di Foggia, Dipartimento di Medicina Clinica e Sperimentale, Foggia, Italia ⁽¹⁾ - Tethys Research Institute, Tethys Research Institute, Milano, Italia ⁽²⁾	366
Zoonotic pathogenic microorganisms from the marine environment: A One Health perspective.....	366
MARIANNA MARANGI ¹ , CATERINA LANFREDI ² , SABINA AIROLDI ²	366
¹ Department of Clinical and Experimental Medicine, University of Foggia, Viale Luigi Pinto, 71122, Foggia, Italy; ² Tethys Research Institute, Viale G.B. Gadio 2, 20121 Milano, Italy	366
162 - Echinococcus granulosus sensu lato in hunters and hunting dogs from southern Italy: a One Health approach	367
ANNA MOREA ⁽¹⁾ - GIOVANNI SGROI ⁽²⁾ - FRANCESCO BUONO ⁽³⁾ - STEFANO SCARCELLI ⁽³⁾ - ELISA CASTALDO ⁽³⁾ - FRANCESCO LOCANTORE ⁽¹⁾ - FRANCESCA INDRACCOLO ⁽¹⁾ - ANGELA STUFANO ⁽¹⁾ - VALENTINA SCHINO ⁽¹⁾ - PIERO LOVREGGIO ⁽¹⁾ - NICOLA D'ALESSIO ⁽²⁾ - VINCENZO VENEZIANO ⁽³⁾ - DOMENICO OTRANTO ⁽⁴⁾ - ROBERTA IATTA ⁽¹⁾	367
University of Bari Aldo Moro, Interdisciplinary Department of Medicine, Bari, Italia ⁽¹⁾ - Istituto Zooprofilattico Sperimentale del Mezzogiorno, Istituto Zooprofilattico Sperimentale del Mezzogiorno, Napoli, Italia ⁽²⁾ - University of Naples Federico II, Naples, Department of Veterinary Medicine, Napoli, Italia ⁽³⁾ - University of Bari Aldo Moro, Department of Veterinary Medicine, Bari, Italia ⁽⁴⁾	367
Echinococcus granulosus sensu lato in hunters and hunting dogs from southern Italy: a One Health approach.....	367
ANNA MOREA ¹ , GIOVANNI SGROI ² , FRANCESCO BUONO ³ , STEFANO SCARCELLI ³ , ELISA CASTALDO ³ , FRANCESCO LOCANTORE ¹ , FRANCESCA INDRACCOLO ¹ , ANGELA STUFANO ¹ , VALENTINA SCHINO ¹ , PIERO LOVREGGIO ¹ , NICOLA D'ALESSIO ² , VINCENZO VENEZIANO ³ , DOMENICO OTRANTO ⁴ , ROBERTA IATTA ¹	367
¹ Interdisciplinary Department of Medicine, University of Bari Aldo Moro, Bari, Italy;	
² Department of Animal Health, Istituto Zooprofilattico Sperimentale del Mezzogiorno, Portici (Naples), Italy;	367

³ Department of Veterinary Medicine, University of Naples Federico II, Naples, Italy; ⁴ Department of Veterinary Medicine, University of Bari “Aldo Moro	367
178 - Occurrence of ESKAPE bacteria in healthy cattle population and the related zoonotic risk.	369
ANNA-RITA ATTILI ⁽¹⁾ - ALESSANDRO GUERRINI ⁽¹⁾ - FRANCESCA GIGLI ⁽¹⁾ - FRANCESCA PAOLA NOCERA ⁽²⁾ - CORRADO MARIA CORRADINI ⁽¹⁾ - FILOMENA FIORITO ⁽²⁾ - CLAUDIA CERRACCHIO ⁽²⁾ - VINCENZO CUTERI ⁽¹⁾ -LUISA DE MARTINO ⁽²⁾	369
Università di Camerino, Scuola di Bioscienze e Medicina Veterinaria, Camerino, Italia ⁽¹⁾ - Università di Napoli Federico II, Dipartimento di Medicina Veterinaria e Produzioni Animali, Napoli, Italia ⁽²⁾	369
Occurrence of ESKAPE bacteria in healthy cattle population and the related zoonotic risk	369
ANNA-RITA ATTILI ¹ , ALESSANDRO GUERRINI ¹ , FRANCESCA GIGLI ¹ , FRANCESCA P. NOCERA ² , CORRADO M. CORRADINI ¹ , FILOMENA FIORITO ² , CLAUDIA CERRACCHIO ² , VINCENZO CUTERI ¹ , LUISA DE MARTINO ²	369
¹ School of Biosciences and Veterinary Medicine, Microbiology and Infectious Diseases, University of Camerino, Matelica, Italy; ² Department of Veterinary Medicine and Animal Production, Microbiology and Infectious Diseases, University of Naples “Federico II”, Naples, Italy	369
241 - Rising threat: the prevalence of K. pneumoniae Ceftazidime/Avibactam resistant strains in healthcare facilities	370
ALESSIA MIRABILE ⁽¹⁾ - DALIDA ANGELA BIVONA ⁽¹⁾ - KRISTINA NICOLE MARTIN ⁽²⁾ - DAVIDE LO PORTO ⁽³⁾ - ANDREA CONA ⁽³⁾ - FRANCESCO MONACO ⁽³⁾ - ALESSANDRA MULARONI ⁽³⁾ - STEFANIA STEFANI ⁽¹⁾ - DAFNE BONGIORNO ⁽¹⁾	370
Università di Catania, Department of Biomedical and Biotechnological Science, Catania, Italia ⁽¹⁾ - Università di Catania, U.O.C. Laboratory Analysis, University Hospital Policlinico-San Marco,, Catania, Italia ⁽²⁾ - ISMETT-IRCCS Istituto Mediterraneo per i Trapianti e Terapie ad Alta Specializzazione, Unit of Infectious Diseases, Palermo, Italia ⁽³⁾	370
Rising threat: the prevalence of K. pneumoniae Ceftazidime/Avibactam resistant strains in healthcare facilities	370
Alessia Mirabile ¹ , Dalida Angela Bivona ¹ , Kristina Nicole Martin ² , Davide Lo Porto ² , Andrea Cona ² , Francesco Monaco ² , Alessandra Mularoni ² , Stefania Stefani ¹ and Dafne Bongiorno ¹	370
Department of Biomedical and Biotechnological Science, Microbiology section University of Catania.....	370
U.O.C. Laboratory Analysis, University Hospital Policlinico-San Marco, University of Catania	370
2. Unit of Infectious Diseases, ISMETT-IRCCS Istituto Mediterraneo per i Trapianti e Terapie ad Alta Specializzazione, Via E. Tricomi, 5, 90127 Palermo, Italy.....	370
253 - Uncovering Antibiotic Resistance in Italian Wastewater: Exploring ARB, ARGs, and Microbial Diversity in WWTPs.....	372
GIUSY BONANNO FERRARO ⁽¹⁾ - CARMELO BONOMO ⁽²⁾ - PAMELA MANCINI ⁽¹⁾ - CAROLINA VENERI ⁽¹⁾ - ROSSELLA BRIANCESCO ⁽¹⁾ - ANNA MARIA COCCIA ⁽¹⁾ - ELISABETTA SUFFREDINI- DAFNE BONGIORNO ⁽²⁾ - NICOLÒ MUSSO ⁽²⁾ - STEFANIA STEFANI ⁽²⁾ - GIUSEPPINA LA ROSA ⁽¹⁾	372
Istituto Superiore di Sanità, Dipartimento Ambiente e Salute, Roma, Italia ⁽¹⁾ - Università di Catania, Dipartimento di Scienze Biomediche e Biotecnologiche, Catania, Italia ⁽²⁾	372

Uncovering Antibiotic Resistance in Italian Wastewater: Exploring ARB, ARGs, and Microbial Diversity in WWTPs.....	372
Giusy Bonanno Ferraro¹, Carmelo Bonomo², Pamela Mancini¹, Carolina Veneri¹, Rossella Briancesco¹, Anna Maria Coccia¹, Dafne Bongiorno², Nicolò. Musso², Stefania Stefani², Giuseppina La Rosa¹	372
¹ Department of Environment and Health, Istituto Superiore di Sanità, Rome, Italy	372
² Department of Biomedical and Biotechnological Science, University of Catania, Italy	372
275 - Following mcr genes: from one-health surveillance to functional genomics of bacterial mobile elements	374
ERIKA SCALTRITI ⁽¹⁾ - CHIARA BRACCHI ⁽¹⁾ - MARTINA TAMBASSI ⁽¹⁾ - ILARIA MENOZZI ⁽¹⁾ - ALESSANDRA DODI ⁽¹⁾ - MARINA MORGANTI ⁽¹⁾ - MELISSA BERNI ⁽¹⁾ - MICRO-RER CONSORTIUM ⁽²⁾ - STEFANO PONGOLINI ⁽¹⁾ - LUCA BOLZONI ⁽¹⁾	374
IZSLER, Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna, Risk Analysis and Genomic Epidemiology Unit, Parma, Italia ⁽¹⁾ - Emilia Romagna, Region, Bologna, Italia ⁽²⁾	374
296 - Ticks and tick-borne pathogens at human-domestic-wildlife interfaces in Malawi through a One Health approach	376
ALESSANDRA MISTRAL DE PASCALI ⁽¹⁾ - CARLOTTA PASETTO ⁽²⁾ - VALERIA BLANDA ⁽³⁾ - DAVID KALENZI ATUHAIRE ⁽⁴⁾ - AMANDA L SALB ⁽⁵⁾ - CHARLOTTE BENTELEY ⁽⁵⁾ - LASTON CHIMALIRO ⁽⁵⁾ - ALBERTO ALBERTI ⁽⁶⁾ - CARLA CACCIOTTO ⁽⁶⁾ - MARTINA BRANDOLINI ⁽¹⁾ - GIULIA GATTI ⁽¹⁾ - MONICA CRICCA ⁽¹⁾ - VITTORIO SAMBRI ⁽¹⁾ - ALESSANDRA TORINA ⁽³⁾ - ALESSANDRA SCAGLIARINI ⁽¹⁾	376
Università di Bologna, Dipartimento di Scienze Mediche e Chirurgiche, Bologna, Italia ⁽¹⁾ - Università di Bologna, Dipartimento di Scienze Mediche Veterinarie, Bologna, Italia ⁽²⁾ - Istituto Zooprofilattico Sperimentale della Sicilia, Istituto Zooprofilattico Sperimentale della Sicilia, Palermo, Italia ⁽³⁾ - African Union Centre for Tick and Tick Borne Diseases (CTTBD), Lilongwe, Malawi, African Union Centre for Tick and Tick Borne Diseases (CTTBD), Lilongwe, Malawi, Malawi, Malawi ⁽⁴⁾ - Lilongwe Wildlife Trust, Lilongwe, Malawi, Lilongwe Wildlife Trust, Lilongwe, Malawi, Malawi, Malawi ⁽⁵⁾ - Università di Sassari, Dipartimento di Medicina Veterinaria, Sassari, Italia ⁽⁶⁾	376
Ticks and tick-borne pathogens at human-domestic-wildlife interfaces in Malawi through a One Health approach	376
ALESSANDRA M DE PASCALI^{1,2}, CARLOTTA PASETTO³, VALERIA BLANDA⁴, DAVID KALENZI ATUHAIRE⁵, AMANDA L SALB⁶, CHARLOTTE BENTLEY⁶, LASTON CHIMALIRO⁶, ALBERTO ALBERTI⁷, CARLA CACCIOTTO⁷, MARTINA BRANDOLINI^{1,2}, GIULIA GATTI^{1,2}, MONICA CRICCA^{1,2}, VITTORIO SAMBRI^{1,2}, ALESSANDRA TORINA⁴, ALESSANDRA SCAGLIARINI²	376
¹Unit of Microbiology, The Greater Romagna Area Hub Laboratory, Cesena, Italy;	376
²Department Medical and Surgical Sciences (DIMEC)—Alma Mater Studiorum, University of Bologna, Italy;	376
³ Department of Veterinary Medical Sciences (DIMEVET)—Alma Mater Studiorum, University of Bologna, Italy;	376
⁴ Istituto Zooprofilattico della Sicilia, Palermo, Italy;	376
⁵ African Union – Centre for Tick and Tick Borne Diseases (CTTBD), Lilongwe, Malawi;	376

⁶ Lilongwe Wildlife Trust, Lilongwe, Malawi;	376
⁷ Department of Veterinary Medicine, University of Sassari, Italy	376
310 - Evaluation of Antiviral Properties of Grape Pomace Extracts: A Sustainable Approach for Broad-Spectrum Viral Inhibition.....	378
ELENA IACONO (1) - CARLO DI MARZO (2) - PAOLA QUARANTA (3) - ALESSANDRA BRACA (4) - MARINELLA DE LEO (4) - GIULIA FREER (5) - MAURO PISTELLO (5) - MICHELE LAI (5).....	378
Dipartimento di biotecnologie mediche, università di Siena, Dipartimento di biotecnologie mediche, università di Siena, Siena, Italia (1) - Università di Pisa, Università di Pisa, Centro Retrovirus, Pisa, Italia (2) - Università Sant'Anna, Università Sant'Anna, Pisa, Italia (3) - Dipartimento di Farmacia, Dipartimento di Farmacia, Pisa, Italia (4) - Università di Pisa, Centro Retrovirus, Università di Pisa, Centro Retrovirus, Pisa, Italia (5).....	378
Title: Evaluation of Antiviral Properties of Grape Pomace Extracts: A Sustainable Approach for Broad-Spectrum Viral Inhibition.....	378
ELENA IACONO^{1,2}, CARLO DI MARZO¹, PAOLA QUARANTA¹, MAURO DI STASI³, MARINELLA DE LEO^{3,5}, EMILY CIONI³, FRANCESCA GAMBINERI⁴, GIULIANA LUMINARE⁴, ALESSANDRA BRACA³, GIULIA FREER¹, MAURO PISTELLO¹, MICHELE LAI^{1,5}.	378
1Retrovirus Center, Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa, Pisa, Italy; 2Department of Medical Biotechnologies, University of Siena, Siena, Italy; 3Department of Pharmacy, University of Pisa, Pisa, Italy; 4Archa srl, Pisa, Italy; 5CISUP, University of Pisa, Pisa, Italy.....	378
312 - Distribution of Mycobacterium tuberculosis lineages and drug resistance in Campania Region	380
VERONICA FOLLIERO ⁽¹⁾ - CARLO FERRAVANTE ⁽¹⁾ - ANNAMARIA SALVATI ⁽¹⁾ - LAURA CRESCENZO ⁽¹⁾ - GIUSY CORVINO ⁽²⁾ - MARIA GRAZIA COPPOLA ⁽³⁾ - LUIGI ATRIPALDI ⁽³⁾ - MARIA TERESA DELLA ROCCA ⁽⁴⁾ - RITA GRECO ⁽⁴⁾ - VITTORIO PANETTA ⁽⁴⁾ - ALESSANDRO TRANFA ⁽⁵⁾ - ANNAMARIA D'ARGENIO ⁽⁵⁾ - ROSAMARIA ABATE ⁽²⁾ - ROSSELLA PERNA ⁽³⁾ - ANNALISA DEL GIUDICE ⁽³⁾ - GIUSEPPE GRECO ⁽⁶⁾ - TERESA BALDONI ⁽³⁾ - BRUNO SARNELLI ⁽⁷⁾ - VALENTINA IOVANE ⁽⁸⁾ - UGO PAGNINI ⁽⁹⁾ - GIUSEPPE IOVANE ⁽⁹⁾ - EMILIANA FINAMORE ⁽¹⁰⁾ - GIORGIO GIURATO ⁽¹⁾ - GIOVANNI NASSA ⁽¹⁾ - ALESSANDRO WEISZ ⁽¹⁾ - GIANLUIGI FRANCI ⁽¹⁾ - MASSIMILIANO GALDIERO ⁽¹⁰⁾.....	380
University of Salerno, Department of Medicine, Surgery and Dentistry "Scuola Medica Salernitana, Salerno, Italia ⁽¹⁾ - Ospedale Cardinale Ascalesi, UOC Microbiology, Napoli, Italia ⁽²⁾ - Ospedali dei Colli, Laboratory of Microbiology and Virology, Napoli, Italia ⁽³⁾ - AORN Sant 'Anna and San Sebastiano, UOSD Microbiology, Caserta, Italia ⁽⁴⁾ - San Giuseppe Moscati Hospital, Avellino, UOC Microbiology, Avellino, Italia ⁽⁵⁾ - San Giovanni di Dio e Ruggi D'Aragona University Hospital, Clinical Pathology and Microbiology Unit, Salerno, Italia ⁽⁶⁾ - Directorate General for Health Protection and Coordination of the Campania Regional Health System, ,, Napoli, Italia ⁽⁷⁾ - University of Naples Federico II, Department of Agricultural Sciences, Napoli, Italia ⁽⁸⁾ - University of Naples Federico II, Department of Veterinary Medicine and Animal Productions, Napoli, Italia ⁽⁹⁾ - University of Campania "Luigi Vanvitelli", Department of Experimental Medicine, Napoli, Italia ⁽¹⁰⁾	380
Distribution of Mycobacterium tuberculosis lineages and drug resistance in Campania Region	380

Veronica Folliero^{1*}, Carlo Ferravante^{1*}, Annamaria Salvati¹, Laura Crescenzo¹, Giusy Corvino², Mariagrazia Coppola³, Luigi Atripaldi³, Maria T. Della Rocca⁴, Rita Greco⁴, Vittorio Panetta⁴, Alessandro Tranfa⁵, Annamaria D'Argenio⁵, Rosamaria Abate², Annalisa Del Giudice³, Giuseppe Greco⁶, Teresa Baldoni³, Bruno Sarnelli⁷, Valentina Iovane⁸, Ugo Pagnini⁹, Giuseppe Iovane⁹, Emiliana Finamore¹⁰, Giorgio Giurato¹, Giovanni Nassa¹, Alessandro Weisz¹, Gianluigi Franci¹, Massimiliano Galdiero¹⁰ 380

1. Department of Medicine, Surgery and Dentistry "Scuola Medica Salernitana", University of Salerno, Salerno, Italy;
2. UOC Microbiology - Ospedale Cardinale Ascalesi, ASL NA1, Naples, Italy; 3. Laboratory of Microbiology and Virology, Ospedali dei Colli, Naples, Italy; 4. UOSD Microbiology - AORN Sant 'Anna and San Sebastiano, Caserta, Italy; 5. UOC Microbiology - San Giuseppe Moscati Hospital, Avellino, Italy; 6. Clinical Pathology and Microbiology Unit, San Giovanni di Dio e Ruggi D'Aragona University Hospital, Salerno, Italy 7. Directorate General for Health Protection and Coordination of the Campania Regional Health System, Naples, Italy;

380

8. Department of Agricultural Sciences, University of Naples Federico II, Portici, Naples, Italy. 9. Department of Veterinary Medicine and Animal Productions, University of Naples Federico II, Naples, Italy; 10. Department of Experimental Medicine, University of Campania "Luigi Vanvitelli", 80138 Naples, Italy..... 381

313 - Methicillin-Susceptible Staphylococcus aureus wild-type and small colony variant (SCVs) isolated from osteomyelitis: genomic and expression analysis of selected genes..... 382

DALIDA ANGELA BIVONA ⁽¹⁾ - CARMELO BONOMO ⁽¹⁾ - EMANUELE NICITRA ⁽¹⁾ - EMMANUELE VENANZI RULLO ⁽¹⁾ - GIUSEPPE NUNNARI ⁽²⁾ - NICOLÒ MUSSO ⁽¹⁾ - STEFANIA STEFANI ⁽¹⁾ - DAFNE BONGIORNO ⁽¹⁾..... 382

Università di Catania, Dipartimento di Scienze Biomediche e Biotecnologiche, Catania, Italia ⁽¹⁾ - Università di Catania, Medicina clinica e sperimentale, Catania, Italia ⁽²⁾ 382

Methicillin-Susceptible Staphylococcus aureus wildtype and small colony variant (SCVs) isolated from osteomyelitis: genomic and expression analysis of selected genes 382

Dalida A. Bivona¹, Carmelo Bonomo¹, Emanuele Nicitra¹, Emmanuele Venanzi Rullo², Giuseppe Nunnari³, Nicolò Musso¹, Stefania Stefani¹, Dafne Bongiorno¹. 382

Department of Biomedical and Biotechnological Science, Microbiology section University of Catania 382

Dipartimento di Medicina Clinica Sperimentale, UOC Malattie Infettive, University of Messina 382

3. Dipartimento di Medicina Clinica Sperimentale, Unità Malattie Infettive, University of Catania 382

08 Patogenesi e meccanismi di virulenza microbici..... 384

4 - EXPLOITING LIPID METABOLISM BY HSV-1: A CHALLENGE TO RETHINK NEW THERAPIES FOR ALZHEIMER'S DISEASE..... 384

Linda Trifirò ⁽¹⁾ - Camilla Albano ⁽¹⁾ - Selina Pasquero ⁽¹⁾ - Francesca Gugliesi ⁽¹⁾ - Greta Bajetto ⁽²⁾ - Weronika HEWELT-BELKA ⁽³⁾ - Erika Mina ⁽⁴⁾ - Paolo Porporato ⁽⁴⁾ - Dana Cairns ⁽⁵⁾ - David Kaplan ⁽⁵⁾ - Adam Mullis ⁽⁵⁾ - Santo Landolfo ⁽¹⁾ - Marco De Andrea ⁽¹⁾ - Valentina Dell'Oste ⁽¹⁾ - Matteo Biolatti ⁽¹⁾ 384

Università degli Studi di Torino, Dipartimento di Scienze della Sanità Pubblica e Pediatriche, Torino, Italia ⁽¹⁾ - Università del Piemonte Orientale, CAAD Center for Translational Research on Autoimmune and Allergic Disease, Novara, Italia ⁽²⁾ - Gdańsk University of Technology, Department of Analytical Chemistry, Gdańsk, Polonia ⁽³⁾ - Università degli Studi di Torino, Dipartimento di Biotecnologie molecolari, Torino, Italia ⁽⁴⁾ - University of Arizona, Department of Immunobiology, Tucson, Arizona, Stati Uniti D'america ⁽⁵⁾	384
Exploiting Lipid Metabolism By Hsv-1: A Challenge To Rethink New Therapies For Alzheimer's Disease.....	384
LINDA TRIFIRÒ ^a , CAMILLA ALBANO ^a , SELINA PASQUERO ^a , FRANCESCA GUGLIESI ^a , GRETA BAJETTO ^b , WERONIKA HEWELT-BELKA ^c , ERICA MINA ^d , PAOLO PORPORATO ^d , DANA CAIRNS ^e , DAVID KAPLAN ^e , ADAM MULLIS ^e , SANTO LANDOLFO ^a , MARCO DE ANDREA ^{a,b} , VALENTINA DELL'OSTE ^a , MATTEO BIOLATTI ^a	384
^a Department of Public Health and Pediatric Sciences, University of Turin, Turin, Italy; ^b CAAD Center for Translational Research on Autoimmune and Allergic Disease, Novara, Italy;	
^c Department of Analytical Chemistry, Gdańsk University of Technology, Gdańsk, Poland;	
^d Department of Molecular Biotechnology and Health Sciences, University of Turin, Turin, Italy;	
^e Department of Immunobiology, University of Arizona, Tucson, Arizona, USA.	
19 - A novel role of TMEM16A and TMEM16F in SARS-CoV-2 pathogenesis	386
MARTINA VIDMAR ⁽¹⁾ - ILARIA SECCO ⁽²⁾ - RITU GARG ⁽²⁾ - MANOLA COMAR ⁽¹⁾ - MAURO GIACCA ⁽²⁾	386
Università degli Studi di Trieste, IRCCS Burlo Garofolo, SSD Diagnostica Avanzata e Microbiologia Traslazionale, Dipartimento universitario clinico di Scienze Mediche Chirurgiche e della Salute, Trieste, Italia ⁽¹⁾ - King's College London, James Black Centre, Cardiovascular Department, London, Regno Unito ⁽²⁾	
A novel role of TMEM16A and TMEM16F in SARS-CoV-2 pathogenesis	386
Martina Vidmar ^{1,2,3} , Iliaria Secco ³ , Ritu Garg ³ , Mauro Giacca ^{1,3,4} , Manola Comar ^{1,2}	386
1) Dipartimento Universitario Clinico di Scienze Mediche Chirurgiche e della Salute, Università degli Studi di Trieste, Trieste, Italy	386
2) SSD Diagnostica Avanzata e Microbiologia Traslazionale, IRCCS Burlo Garofolo, Trieste, Italy	386
3) Cardiovascular Science Department, James Black Centre, King's College London, London, UK	386
4) Molecular Medicine department, International Centre for Genetic Engineering and Biotechnology (ICGEB), Trieste, Italy	386
22 - The protein HslJ boosts Acinetobacter baumannii survival against oxidative stress.....	388
CECILIA AMBROSI ⁽¹⁾ - MEYSAM SARSHAR ⁽²⁾ - MARTINA PASQUA ⁽³⁾ - CARLO ZAGAGLIA ⁽⁴⁾ - ANNA TERESA PALAMARA ⁽⁵⁾ - DANIELA SCRIBANO ⁽⁴⁾	388
San Raffaele Open University, IRCCS, Department of Human Sciences and Promotion of the Quality of Life, Roma, Italia ⁽¹⁾ - Bambino Gesù Children's Hospital, IRCCS, Research Laboratories, Roma, Italia ⁽²⁾ - Sapienza Università di Roma, Department of Biology and Biotechnology "C. Darwin", Roma, Italia ⁽³⁾ - Sapienza Università di Roma, Dipartimento di Sanità Pubblica e Malattie Infettive, Roma, Italia ⁽⁴⁾ - Sapienza Università di Roma, Department of Public Health and Infectious Diseases, Sapienza University of Rome, Laboratory Affiliated to Institute Pasteur Italia-Cenci Bolognetti, Roma, Italia ⁽⁵⁾	
The protein HslJ boosts Acinetobacter baumannii survival against oxidative stress	388

Cecilia Ambrosi ¹ , Meysam Sarshar ² , Martina Pasqua ³ , Carlo Zagaglia ⁴ , Anna Teresa Palamara ^{5,6} , and Daniela Scribano ⁴	388
¹ Department of Human Sciences and Promotion of the Quality of Life, San Raffaele Open University, IRCCS, Rome, Italy; ² Research Laboratories, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy; ³ Department of Biology and Biotechnology "C. Darwin", Sapienza University, Rome, Italy; ⁴ Department of Public Health and Infectious Diseases, Sapienza University, Rome, Italy; ⁵ Department of Public Health and Infectious Diseases, Sapienza University of Rome, Laboratory Affiliated to Institute Pasteur Italia-Cenci Bolognetti Foundation, Rome, Italy; ⁶ Department of Infectious Diseases, Istituto Superiore di Sanità, Rome, Italy.....	
40 - SARS-CoV-2 ORF3c impairs mitochondrial respiratory metabolism, oxidative stress, and autophagic flux.....	389
RACHELE CAGLIANI ⁽¹⁾ - ALESSANDRA MOZZI ⁽¹⁾ - MONICA OLDANI ⁽²⁾ - MATILDE E. FORCELLA ⁽²⁾ - CHIARA VANTAGGIATO ⁽¹⁾ - DIEGO FORNI ⁽¹⁾ - MANUELA SIRONI ⁽¹⁾ - PAOLA FUSI ⁽²⁾	389
Scientific Institute IRCCS E. MEDEA, Bioinformatics, Bosisio Parini, Italia ⁽¹⁾ - University of Milano-Bicocca, Department of Biotechnology and Biosciences, Milano, Italia ⁽²⁾	389
SARS-CoV-2 ORF3c impairs mitochondrial respiratory metabolism, oxidative stress, and autophagic flux.....	389
Rachele Cagliani ¹ , Alessandra Mozzi ¹ , Monica Oldani ² , Matilde E. Forcella ² , Chiara Vantaggiato ² , Diego Forni ¹ , Manuela Sironi ¹ and Paola Fusi ²	389
¹ Scientific Institute IRCCS E. MEDEA, Bioinformatics, Bosisio Parini, 23842, Italy; ² Department of Biotechnology and Biosciences, University of Milano-Bicocca, Milano, 20126, Italy.	389
61 - Identification of druggable host dependency factors shared by multiple SARS-CoV-2 variants of concern	391
ILARIA FRASSON ⁽¹⁾ - LINDA DIAMANTE ⁽¹⁾ - MANUELA ZANGROSSI ⁽¹⁾ - ELENA CARBOGNIN ⁽²⁾ - ANNA DALLA PIETÀ ⁽³⁾ - ALESSANDRO PENNA ⁽³⁾ - ANTONIO ROSATO ⁽³⁾ - RANIERI VERIN ⁽⁴⁾ - FILIPPO TORRIGIANI ⁽⁴⁾ - CRISTIANO SALATA ⁽¹⁾ - MARIA PAULA DIZANZO ⁽¹⁾ - LORENZO VACCARO ⁽⁵⁾ - DAVIDE CACCHIARELLI ⁽⁵⁾ - MARCO MONTAGNER ⁽¹⁾ - GRAZIANO MARTELLO ⁽²⁾ - SARA N. RICHTER ⁽¹⁾	391
Università di Padova, Dip. Medicina Molecolare, Padova, Italia ⁽¹⁾ - Università di Padova, Dip. Biologia, Padova, Italia ⁽²⁾ - Università di Padova, Dip. Scienze Chirurgiche Oncologiche e Gastroenterologiche, Padova, Italia ⁽³⁾ - Università di Padova, Dipartimento di Biomedicina Comparata e Alimentazione, Padova, Italia ⁽⁴⁾ - Università di Napoli, Dip. Medicina Traslazionale, Napoli, Italia ⁽⁵⁾	391
Identification of druggable host dependency factors shared by multiple SARS-CoV-2 variants of concern.....	391
Ilaria Frasson, ¹ Linda Diamante, ^{1,2} , Manuela Zangrossi, ^{1,10} Elena Carbognin, ² Anna Dalla Pietà, ³ Alessandro Penna, ³ Antonio Rosato, ^{3,4} Ranieri Verin, ⁵ Filippo Torrigiani, ⁵ Cristiano Salata, ¹ Maria Paula Dizanzo, ¹ Lorenzo Vaccaro, ^{6,7} Davide Cacchiarelli, ^{6,7,8} Marco Montagner, ¹ Graziano Martello, ² and Sara N. Richter ^{1,9}	391
1: Department of Molecular Medicine, University of Padua, Padua 35121, Italy	391
2: Department of Biology, University of Padua, Padua 35131, Italy.....	391
3: Dept. of Surgery, Oncology and Gastroenterology, University of Padua, Padua 35128, Italy .	391
4: Veneto Institute of Oncology IOV-IRCCS, Padua 35128, Italy.....	391

5: Department of Comparative Biomedicine and Food Science, University of Padua, Padua 35020, Italy	391
6: Telethon Institute of Genetics and Medicine (TIGEM), Pozzuoli 80078, Italy	391
7: Department of Translational Medicine, Federico II University, Naples 80138, Italy	391
8: School for Advanced Studies, University of Naples "Federico II", Naples 80138, Italy	391
9: Microbiology and Virology Unit, Padua University Hospital, Padua 35128, Italy	391
72 - A study on cross-link formation between fibrinogen and region A of FnBPA from Staphylococcus aureus promoted by von Willebrand Factor-binding protein (vWbp) and tissue Transglutaminase 2 (TG2)	393
CHIARA MOTTA ⁽¹⁾ - GIULIA BARBIERI ⁽²⁾ - ANGELICA PELLEGRINI ⁽¹⁾ - JOAN GEOGHEGAN ⁽³⁾ - GIAMPIERO PIETROCOLA ⁽¹⁾	393
Università di Pavia, Dipartimento di Medicina Molecolare, unità di Biochimica, Pavia, Italia ⁽¹⁾ - Università di Pavia, Dipartimento di Biologia e Biotecnologie "Lazzaro Spallanzani", Pavia, Italia ⁽²⁾ - University of Birmingham, Institute of Microbiology and Infection, Birmingham, Regno Unito ⁽³⁾	393
A study on cross-link formation between fibrinogen and region A of FnBPA from Staphylococcus aureus promoted by von Willebrand Factor-binding protein (vWbp) and tissue Transglutaminase 2 (TG2).....	393
Chiara MOTTA ¹ , Giulia BARBIERI ² , Angelica PELLEGRINI ¹ , Joan GEOGHEGAN ³ , Giampiro PIETROCOLA ¹	393
¹ Department of Molecular Medicine, unit of Biochemistry, University of Pavia - Pavia (Italy); ² Department of Biology and Biotechnology "Lazzaro Spallanzani," University of Pavia - Pavia (Italy); ³ Institute of Microbiology and Infection, University of Birmingham – Edgbaston, Birmingham (United Kingdom).....	393
85 - The SaeRS two-component system regulates the expression of group B streptococcal genes that are highly expressed in vivo	395
FRANCESCO COPPOLINO ⁽¹⁾ - GIUSEPPE V. DE GAETANO ⁽²⁾ - GERMANA LENTINI ⁽²⁾ - AGATA FAMÀ ⁽²⁾ - PASQUALINA LAGANÀ ⁽¹⁾ - CONCETTA BENINATI ⁽²⁾ - GIUSEPPE TETI ⁽³⁾	395
Università degli Studi di Messina, Dipartimento di Scienze Biomediche, Odontoiatriche e delle Immagini Morfologiche e Funzionali, Messina, Italia ⁽¹⁾ - Università degli Studi di Messina, Dipartimento di Patologia Umana, Messina, Italia ⁽²⁾ - Scylla Biotech Srl, Scylla Biotech Srl, Messina, Italia ⁽³⁾	395
The SaeRS two-component system regulates the expression	395
of group B streptococcal genes that are highly expressed in vivo	395
FRANCESCO COPPOLINO ¹ , GIUSEPPE V. DE GAETANO ² , GERMANA LENTINI ² , AGATA FAMÀ ² , PASQUALINA LAGANÀ ¹ , ARNAUD FIRON ³ , GIUSEPPE TETI ⁴ , CONCETTA BENINATI ^{2,4}	395
Departments of Biomedical, Dental and Imaging Sciences ¹ and of Human Pathology and Medicine ² , University of Messina, Messina, Italy; ³ Microbiology Department, Biology of Gram-positive pathogens, Institut Pasteur, Paris, France; ⁴ Scylla Biotech Srl, Messina, Italy.	395
91 - Investigation of Merkel Cell Polyomavirus (MCPyV) status in Merkel Cell Carcinoma (MCC)	397

SARA PASSERINI ⁽¹⁾ - CARLA PREZIOSO ⁽²⁾ - GIULIA BABINI ⁽¹⁾ - AMEDEO FERLOSIO ⁽³⁾ - TERENZIO COSIO ⁽⁴⁾ - ELENA CAMPIONE ⁽⁴⁾ - UGO MOENS ⁽⁵⁾ - MARCO CIOTTI ⁽⁶⁾ - VALERIA PIETROPAOLO ⁽¹⁾	397
“Sapienza” University of Rome, Department of Public Health and Infectious Diseases, Rome, Italia ⁽¹⁾ - IRCCS San Raffaele Roma, Laboratory of Microbiology of Chronic-Neurodegenerative Diseases, Rome, Italia ⁽²⁾ - Tor Vergata University of Rome, Anatomic Pathology, Department of Biomedicine and Prevention, Rome, Italia ⁽³⁾ - Tor Vergata University of Rome, Dermatologic Unit, Department of Systems Medicine, Rome, Italia ⁽⁴⁾ - University of Tromsø—The Arctic University of Norway, Department of Medical Biology, Faculty of Health Sciences, Tromsø, Norvegia ⁽⁵⁾ - Polyclinic Tor Vergata Foundation, Virology Unit, Rome, Italia ⁽⁶⁾	397
Investigation of Merkel Cell Polyomavirus (MCPyV) status in Merkel Cell Carcinoma (MCC)	397
SARA PASSERINI ¹ , CARLA PREZIOSO ² , GIULIA BABINI ¹ , AMEDEO FERLOSIO ³ , TERENZIO COSIO ^{4,5} , ELENA CAMPIONE ⁴ , UGO MOENS ⁶ , MARCO CIOTTI ⁷ , VALERIA PIETROPAOLO ¹	397
¹ Department of Public Health and Infectious Diseases, “Sapienza” University of Rome, 00185 Rome, Italy; ² Laboratory of Microbiology of Chronic-Neurodegenerative Diseases, IRCCS San Raffaele Roma, 00166 Rome, Italy; ³ Anatomic Pathology, Department of Biomedicine and Prevention, Tor Vergata University of Rome, 00133 Rome, Italy; ⁴ Dermatologic Unit, Department of Systems Medicine, Tor Vergata University of Rome, 00133 Rome, Italy; ⁵ Department of Experimental Medicine, Tor Vergata University of Rome, 00133 Rome, Italy; ⁶ Department of Medical Biology, Faculty of Health Sciences, University of Tromsø—The Arctic University of Norway, 9037 Tromsø, Norway; ⁷ Virology Unit, Polyclinic Tor Vergata Foundation, 00133 Rome, Italy.	397
98 - SARS-CoV-2 infection induces Tau pathological signature.....	399
PAOLA QUARANTA ⁽¹⁾ - MARIANNA MIGNANELLI ⁽²⁾ - GIACOMO SIANO ⁽²⁾ - MATTEO BIMBATI ⁽²⁾ - PAOLA PERRERA ⁽¹⁾ - CARMEN RITA PIAZZA ⁽¹⁾ - PIETRO GIORGIO SPEZIA ⁽¹⁾ - MARIO COSTA ⁽³⁾ - ANTONINO CATTANEO ⁽²⁾ - MAURO PISTELLO ⁽¹⁾ - CRISTINA DIPRIMIO ⁽³⁾	399
Università di Pisa, Dipartimento di Ricerca Traslationale e Nuove Tecnologie in Medicina, Pisa, Italia ⁽¹⁾ - Scuola Normale superiore, Laboratorio di Biologia Bio@SNS, Pisa, Italia ⁽²⁾ - Centro Nazionale di Ricerca CNR, istituto di Neuroscienze, Pisa, Italia ⁽³⁾	399
SARS-CoV-2 infection induces Tau pathological signature	399
Paola Quaranta ^{1,2} , Marianna Mignanelli ³ , Giacomo Siano ³ , Matteo Bimbati ³ , Paola Perrera ¹ , Carmen Rita Piazza ^{1,4} , Pietro Giorgio Spezia ¹ , Mario Costa ² , Antonino Cattaneo ³ , Mauro Pistello ^{1,5} Cristina Di Primio ²	399
1. Department of Translational Research and New Technologies in Medicine and Surgery, Retrovirus Center, University of Pisa, Pisa, Italy.	399
2. Institute of Neuroscience, Italian National Research Council (CNR), Pisa, Italy	399
3. Laboratorio di Biologia Bio@SNS, Scuola Normale Superiore di Pisa, Pisa, Italy	399
4. Department of Medical Biotechnologies, University of Siena, Siena, Italy.....	399
5. Azienda Ospedaliera Universitaria Pisana, Virology Unit, Pisa, Italy.....	399
107 - Transcriptome analysis of Parvovirus B19 infected erythroid progenitor cells	400
ERIKA FASANO (1) - FEDERICA BICHICCHI (1) - GLORIA BUA (1) - ELISABETTA MANARESI (1) - FRANCESCA BONVICINI (1) - GIORGIO GALLINELLA (2)	400

Università Di Bologna, Fabit, Bologna, Italia (1) - IRCCS Azienda Ospedaliero-Universitaria Di Bologna, Unità Di Microbiologia, Bologna, Italia (2)	400
Transcriptome analysis of Parvovirus B19 infected erythroid progenitor cells	400
ERIKA FASANO ¹ , FEDERICA BICHICCHI ¹ , GLORIA BUA ¹ , ELISABETTA MANARESI ¹ , FRANCESCA BONVICINI ¹ , GIORGIO GALLINELLA ²	400
¹ Dept of Pharmacy and Biotechnology, University of Bologna, Bologna, Italy; ² Microbiology Unit, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy	400
117 - Longitudinal study of Human Polyomaviruses viruria in kidney transplant recipients.....	402
MARIA DOLCI (1) - CATERINA COLICO (2) - FEDERICO AMBROGI (3) - EVALDO FAVI (4) - LUCIA SIGNORINI (1) - MARTA PEREGO (3) - EDOARDO CAMPIOLI (3) - KEVIN KAMAU MAINA (1) - PASQUALE FERRANTE (1) - MARIANO FERRARESSO (4) - SERENA DELBUE (1)	402
University of Milano, Department of Biomedical, Surgical and Dental Sciences, Milano, Italia (1) - Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Department of Emergency Surgery, Milano, Italia (2) - University of Milano, Department of Clinical Sciences and Community Health, Milano, Italia (3) - Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, General Surgery and Kidney Transplantation, Milano, Italia (4)	402
Longitudinal study of Human Polyomaviruses viruria in kidney transplant recipients	402
Maria Dolci ¹ , Caterina Colico ² , Federico Ambrogi ³ , Evaldo Favi ⁴ , Lucia Signorini ¹ , Marta Perego ³ , Edoardo Campioli ³ , Kevin Kamau Maina ¹ , Pasquale Ferrante ¹ , Mariano Ferrarezzo ⁴ , Serena Delbue ¹	402
¹ Laboratory of Molecular Virology, Department of Biomedical, Surgical and Dental Sciences, University of Milano, Via Carlo Pascal 36, 20133 Milano, Italy	402
² Department of Emergency Surgery, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy.....	402
³ Department of Clinical Sciences and Community Health, University of Milan, Milan, Italy	402
⁴ General Surgery and Kidney Transplantation, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy	402
118 - The BM 110, a hypervirulent strain of group B streptococci, exploits $\alpha_2\beta_1$ and $\alpha_3\beta_1$ integrins to invade human enterocytes	403
GIUSEPPE VALERIO DE GAETANO ⁽¹⁾ - GERMANA LENTINI ⁽¹⁾ - FRANCESCO COPPOLINO ⁽²⁾ - AGATA FAMÀ ⁽¹⁾ - GIAMPIERO PIETROCOLA ⁽³⁾ - GIUSEPPE TETI ⁽⁴⁾ - CONCETTA BENINATI ⁽¹⁾	403
Università, Dipartimento di Patologia Umana, Messina, Italia ⁽¹⁾ - Università, Dipartimento di Scienze biomediche, odontoiatriche e delle immagini morfologiche e funzionali, Messina, Italia ⁽²⁾ - Università, Dipartimento di Medicina Molecolare, Pavia, Italia ⁽³⁾ - Scylla Biotech Srl, Scylla Biotech Srl, Messina, Italia ⁽⁴⁾	403
The BM 110, a hypervirulent strain of group B streptococci, exploits $\alpha_2\beta_1$ and $\alpha_3\beta_1$ integrins to invade human enterocytes	403
Giuseppe Valerio De Gaetano ¹ , Germana Lentini ¹ , Francesco Coppolino ² , Agata Famà ¹ , Giampietro Pietrocola ³ , Giuseppe Teti ⁴ , Concetta Beninati ^{1,4}	403
¹ Department of Human Pathology, University of Messina, Messina, Italy; ² Department of Biomedical, Dental and Imaging Sciences, University of Messina, Messina, Italy; ³ Department of	

Molecular Medicine, Biochemistry Section, University of Pavia, Pavia, Italy; ⁴ Scylla Biotech Srl, Messina, Italy.....	403
120 - Pseudotyped lentiviral particles may help shed light on HIV maturation	405
GIULIA FREER ⁽¹⁾ - GIULIA LOTTINI ⁽²⁾ - ERIKA PLICANTI ⁽²⁾ - ALESSIA CALÒ ⁽¹⁾ - MATTEO MATTEUCCI ⁽¹⁾ - MICHELE LAI ⁽¹⁾	405
University of Pisa, Centro Retrovirus, Pisa, Italia ⁽¹⁾ - University of Siena, Dept. Medical Biotechnologies, Siena, Italia ⁽²⁾	405
Pseudotyped lentiviral particles may help shed light on HIV maturation	405
GIULIA FREER ¹ , GIULIA LOTTINI ^{1, 2} , ERIKA PLICANTI ^{1, 2} , ALESSIA CALO' ¹ , MATTEO MATTEUCCI ¹ , MICHELE LAI ¹	405
¹ Dept. Translational Research, Retrovirus Center, University of Pisa, Pisa, Italy	405
² Dept. Medical Biotechnologies, University of Siena, Siena, Italy.....	405
132 - Fimbriae and Adhesins in clinical, Extremely Drug-Resistant Providencia stuartii Isolates producing NDM-1.....	406
VALERIO CAPITANI ⁽¹⁾ - MIRIANA QUARANTA ⁽²⁾ - GABRIELE ARCARI ⁽³⁾ - CAROLINA MARZUILLO ⁽¹⁾ - ALESSANDRA CARATTOLI ⁽³⁾	406
Università La Sapienza, Dipartimento di Sanità Pubblica e Malattie Infettive, Roma, Italia ⁽¹⁾ - Università La Sapienza, Dipartimento di Scienze Biochimiche A. Rossi Fanelli, Roma, Italia ⁽²⁾ - Università La Sapienza, Dipartimento di Medicina Molecolare, Roma, Italia ⁽³⁾	406
Fimbriae and Adhesins in clinical, Extremely Drug-Resistant Providencia stuartii Isolates producing NDM-1.....	406
Valerio Capitani ¹ , Miriana Quaranta ² , Gabriele Arcari ³ , Carolina Marzuillo ¹ , Alessandra Crattoli ³	406
1 Dipartimento di Sanità Pubblica e Malattie Infettive; Laboratorio di Epidemiologia Molecolare; Università La Sapienza; Roma; Italia	406
2 Dipartimento di Scienze Biochimiche A. Rossi Fanelli; Università La Sapienza; Roma; Italia	406
3 Dipartimento di Medicina Molecolare; Università La Sapienza; Roma; Italia	406
144 - The serine-rich repeat glycoprotein Srr2 from Streptococcus agalactiae acts as an adhesin for the human fibronectin	408
ANGELICA PELLEGRINI ⁽¹⁾ - GIULIA BARBIERI ⁽²⁾ - CHIARA MOTTA ⁽¹⁾ - GIAMPIERO PIETROCOLA ⁽¹⁾	408
Università di Pavia, Dipartimento di Medicina Molecolare, Pavia, Italia ⁽¹⁾ - Università di Pavia, Dipartimento di Biologia e Biotechnologie "Lazzaro-Spallanzani", Pavia, Italia ⁽²⁾	408
The serine-rich repeat glycoprotein Srr2 from Streptococcus agalactiae acts as an adhesin for the human fibronectin	408
Angelica PELLEGRINI ¹ , Giulia BARBIERI ² , Chiara MOTTA ¹ , Giampiero PIETROCOLA ¹ ..	408
1, Department of Molecular Medicine, Unit of Biochemistry, University of Pavia, Pavia, (Italy); 2, Department of Biology and Biotechnology "Lazzaro-Spallanzani", University of Pavia, Pavia, (Italy).....	408
149 - Acinetobacter baumannii OmpA-like porins: functional characterization in bacterial physiology, antibiotic-resistance, and virulence.....	409

DANIELA SCRIBANO ⁽¹⁾ - ARIANNA POMPILIO ⁽²⁾ - ELENA CHERI ⁽³⁾ - MANUEL BELLI ⁽⁴⁾ - LUIGI SANSONE ⁽⁴⁾ - MARIO CRISTINA ⁽⁴⁾ - MEYSAM SARSHAR ⁽³⁾ - CARLO ZAGAGLIA ⁽¹⁾ - GIOVANNI DI BONAVENTURA ⁽²⁾ - ANNA TERESA PALAMARA ⁽⁵⁾ - CECILIA AMBROSI ⁽⁶⁾	409
Sapienza University of Rome, Department of Public Health and Infectious Diseases,, Roma, Italia	
⁽¹⁾ - “G. d’Annunzio” University of Chieti-Pescara, Center for Advanced Studies and Technology (CAST), Department of Medical, Oral and Biotechnological Sciences, Chieti, Italia ⁽²⁾ - Bambino Gesù Children’s Hospital, IRCCS, Research Laboratories, Roma, Italia ⁽³⁾ - IRCCS San Raffaele Roma, MEBIC Consortium, Roma, Italia ⁽⁴⁾ - Sapienza University of Rome, Department of Public Health and Infectious Diseases, Sapienza University of Rome, Laboratory Affiliated to Institute Pasteur Italia-Cenci Bolognetti, Roma, Italia ⁽⁵⁾ - San Raffaele Open University, IRCCS, Department of Human Sciences and Promotion of the Quality of Life, Roma, Italia ⁽⁶⁾	409
Acinetobacter baumannii OmpA-like porins: functional characterization in bacterial physiology, antibiotic-resistance, and virulence.....	409
Daniela Scribano¹, Arianna Pompilio², Elena Cheri³, Manuel Belli⁴, Luigi Sansone⁴, Mario Cristina⁴, Meysam Sarshar³, Carlo Zagaglia¹, Giovanni Di Bonaventura², Anna Teresa Palamara^{5,6}, Cecilia Ambrosi⁷	409
¹ Department of Public Health and Infectious Diseases, Sapienza University of Rome, Rome, Italy;	
² Center for Advanced Studies and Technology (CAST), Department of Medical, Oral and Biotechnological Sciences, “G. d’Annunzio” University of Chieti-Pescara, Chieti, Italy; ³ Research Laboratories, Bambino Gesù Children’s Hospital, IRCCS, Rome, Italy; ⁴ MEBIC Consortium, IRCCS San Raffaele Roma, Rome, Italy; ⁵ Department of Public Health and Infectious Diseases, Sapienza University of Rome, Laboratory Affiliated to Institute Pasteur Italia-Cenci Bolognetti Foundation, Rome, Italy; ⁶ Department of Infectious Diseases, Istituto Superiore di Sanità, Rome, Italy; ⁷ Department of Human Sciences and Promotion of the Quality of Life, San Raffaele Open University, IRCCS, Rome, Italy.	
168 - Invasive meningococcal disease caused by rare capsular serogroups in Italy: genomic investigation of virulence genes associated with pathogenesis	411
PAOLA VACCA ⁽¹⁾ - CECILIA FAZIO ⁽¹⁾ - ARIANNA NERI ⁽¹⁾ - LUIGINA AMBROSIO ⁽¹⁾ - ANNA CARANNANTE ⁽¹⁾ - FLORIGIO ROMANO LISTA ⁽²⁾ - SILVIA FILLO ⁽²⁾ - ANDREA CIAMMARUCONI ⁽²⁾ - ANTONELLA FORTUNATO ⁽²⁾ - PAOLA STEFANELLI ⁽¹⁾	411
ISTITUTO SUPERIORE DI SANITA', DIPARTIMENTO MALATTIE INFETTIVE, ROME, Italia ⁽¹⁾ - ARMY MEDICAL RESEARCH CENTRE, SCIENTIFIC DEPARTMENT, ROME, Italia ⁽²⁾	
Invasive meningococcal disease caused by rare capsular serogroups in Italy: genomic investigation of virulence genes associated with pathogenesis	411
Paola Vacca ^a, Cecilia Fazio ^a, Neri Arianna ^a, Luigina Ambrosio ^a, Anna Carannante ^a, Florigio R. Lista ^b, Silvia Fillo ^b, Andrea Ciammaruconi ^b, Antonella Fortunato ^b, and Paola Stefanelli ^a. ...	411
^a Department Infectious Diseases, Vaccine-Preventable Diseases (VPD) - Reference Lab Unit, Istituto Superiore di Sanità, Rome Italy; ^b Scientific Department-Army Medical Research Centre, Rome, Italy	
170 - The study of the Unfolded Protein Response and Herpes Simplex Virus infection in neuronal cell line	413
PAOLA PERRERA ⁽¹⁾	413
Università di Pisa, Dipartimento di Ricerca Traslazionale, Pisa, Italia ⁽¹⁾	413

The study of the Unfolded Protein Response and Herpes Simplex Virus infection in neuronal cell line.....	413
PAOLA PERRERA ^{1,2}, CARMEN RITA PIAZZA ^{2,3}, PAOLA QUARANTA ², MICHELE LAI ², GIULIA FREER ², MAURO PISTELLO ^{2,4}	413
Affiliations: ¹Department of Translational Research, Retrovirus Center and Virology Section, University of Pisa, Pisa, Italy; ²Centro Retrovirus, University of Pisa, Pisa, Italy; ³Department of Medical Biotechnologies, University of Siena, Siena, Italy; ⁴Virology Operative Unit, University Hospital of Pisa, Pisa, Italy.	
175 - Unveiling the role of NAAA enzyme and peroxisome regulation in HSV-1 infection	414
CAROLINA FILIPPONI ⁽¹⁾ - ELENA IACONO ⁽²⁾ - VERONICA LA ROCCA ⁽³⁾ - GIULIA SCIANDRONE ⁽¹⁾ - ALESSANDRO DE CARLI ⁽²⁾ - GIULIA FREER ⁽¹⁾ - MAURO PISTELLO ⁽¹⁾ - MICHELE LAI ⁽¹⁾	414
Università di Pisa, Dipartimento di Ricerca Traslazionale e delle Nuove Tecnologie in Medicina e Chirurgia, Pisa, Italia ⁽¹⁾ - Università di Siena, Dipartimento di Biotecnologie Mediche, Siena, Italia ⁽²⁾ - Institute of Life Sciences, Scuola Superiore Sant'Anna, Pisa, Italia ⁽³⁾	
Unveiling the role of NAAA enzyme and peroxisome regulation in HSV-1 infection	414
CAROLINA FILIPPONI¹, ELENA IACONO¹⁻², VERONICA LA ROCCA¹⁻³, GIULIA SCIANDRONE¹, ALESSANDRO DE CARLI¹⁻², GIULIA FREER¹, MAURO PISTELLO¹⁻⁴, MICHELE LAI¹	414
¹Retrovirus Center, Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa, Pisa, Italy; ²Department of Medical Biotechnologies, University of Siena, Siena, Italy; ³Institute of Life Sciences, Sant'Anna School of Advanced Studies, Pisa, Italy; ⁴Department of Clinical and Experimental Medicine, University of Pisa, Pisa, Italy.....	
181 - In vivo virulence study of Listeria monocytogenes with truncated Internalin A in Galleria mellonella larvae.....	416
MARIA GORI ⁽¹⁾ - EMERENZIANA OTTAVIANO ⁽¹⁾ - MATTEO MARSIGLIA ⁽²⁾ - SILVIA ANCONA ⁽¹⁾ - SILVIA BIANCHI ⁽¹⁾ - ELISABETTA TANZI ⁽¹⁾ - ELISA BORGHI ⁽¹⁾	416
Università degli Studi di Milano, Dipartimento di Scienze della Salute, Milano, Italia ⁽¹⁾ - Università degli Studi di Milano, Department of Biomedical and Clinical Sciences, Milano, Italia ⁽²⁾	
In vivo virulence study of Listeria monocytogenes with truncated Internalin A in Galleria mellonella larvae.....	416
MARIA GORI¹, EMERENZIANA OTTAVIANO¹, MATTEO D. MARSIGLIA², SILVIA ANCONA¹, SILVIA BIANCHI¹, ELISABETTA TANZI¹, ELISA BORGHI¹	416
1. Department of Health Sciences, Università degli Studi di Milano, Milan (Italy);	416
2. Department of Biomedical and Clinical Sciences, Università degli Studi di Milano, Milan (Italy)	416
194 - Analysis of viral variants associated with high-risk Human Papillomavirus (HPV) persisting infection	418
MARIANNA MARTINELLI ⁽¹⁾ - CHIARA GIUBBI ⁽¹⁾ - CHIARA VILLA ⁽¹⁾ - RUTH NJOKU ⁽²⁾ - FEDERICA PERDONI ⁽¹⁾ - MARIA LETIZIA DI MEO ⁽³⁾ - ROSARIO MUSUMECI ⁽¹⁾ - ILLARI SECHI ⁽²⁾ - NARCISA MURESU ⁽²⁾ - ANDREA PIANA ⁽²⁾ - ROBERT FRUSCIO ⁽¹⁾ - FABIO LANDONI ⁽¹⁾ - CLEMENTINA COCUZZA ⁽¹⁾	418

University of Milano-Bicocca, Department of Medicine and Surgery, Monza, Italia ⁽¹⁾ - University of Sassari, Department of Biomedical Sciences, Sassari, Italia ⁽²⁾ - ASST Monza, San Gerardo Hospital, Monza, Italia ⁽³⁾	418
Analysis of viral variants associated with high-risk Human Papillomavirus (HPV) persisting infection.....	418
MARIANNA MARTINELLI ¹ , CHIARA GIUBBI ¹ , CHIARA VILLA ¹ , RUTH C. NJOKU ^{1,2} , FEDERICA PERDONI ¹ , MARIA LETIZIA DI MEO ³ , ROSARIO MUSUMECI ¹ , ILLARI SECHI ² , NARCISA MURESU ² , ANDREA F. PIANA ² , ROBERT FRUSCIO ^{1,3} , FABIO LANDONI ^{1,3} , CLEMENTINA E. COCUZZA ¹	418
¹ Department of Medicine and Surgery, University of Milano-Bicocca, Monza, Italy;.....	418
² Department of Biomedical Sciences, University of Sassari, Sassari, Italy;.....	418
³ San Gerardo Hospital, ASST Monza, Monza, Italy;.....	418
202 - Sudden and unexplained death of an 18-month-old healthy boy due to interstitial pneumonia with multiple superinfections	420
ANNA-LISA CAPRIA ⁽¹⁾ - MARIA ELENA FILICE ⁽²⁾ - PIETRO GIORGIO SPEZIA ⁽³⁾ - MICHELE LAI ⁽³⁾ - VINCENZO NARDINI ⁽²⁾ - PAOLA MAZZETTI ⁽¹⁾ - ARIANNA PRECISI O PROCISSI ⁽⁴⁾ - MAURO PISTELLO ⁽¹⁾	420
Pisa University Hospital, Virology Division, Pisa, Italia ⁽¹⁾ - Pisa University Hospital, Pathological Anatomy Division 2, Pisa, Italia ⁽²⁾ - University of Pisa, Department of Translation, Retrovirus Center and Virology Section, Pisa, Italia ⁽³⁾ - Pisa University Hospital, Transplant Laboratory, Pisa, Italia ⁽⁴⁾	420
Sudden and unexplained death of an 18-month-old healthy boy due to interstitial pneumonia with multiple superinfections.....	420
Anna-Lisa Capria ¹ , Maria Elena Filice ³ , Pietro Giorgio Spezia ² , Michele Lai ² , Vincenzo Nardini ³ , Paola Mazzetti ¹ , Arianna Precisi o ProciSSI ⁴ , Mauro Pistello ^{1,2}	420
¹ Virology Division, Pisa University Hospital, Pisa, Italy;.....	420
² Department of Translation, Retrovirus Center and Virology Section, University of Pisa, Pisa, Italy; ³ Pathological Anatomy Division 2, Pisa University Hospital, Pisa, Italy;	420
⁴ Transplant Laboratory, Pisa University Hospital, Pisa, Italy.	420
213 - Neisseria meningitidis activates pyroptotic pathways in a mouse model of meningitis: role of a two-partner secretion system.....	422
CHIARA PAGLIUCA ⁽¹⁾ - ELENA SCAGLIONE ⁽²⁾ - GIUSEPPE MANTOVA ⁽¹⁾ - SILVIA CATERINA RESTA ⁽³⁾ - CATERINA PAGLIARULO ⁽⁴⁾ - CECILIA BUCCI ⁽³⁾ - PIETRO ALIFANO ⁽³⁾ - PAOLA SALVATORE ⁽⁵⁾ - ROBERTA COLICCHIO ⁽²⁾	422
University of Naples Federico II, Department of Molecular Medicine and Medical Biotechnology, Naples, Italia ⁽¹⁾ - University of Naples Federico II, Department of Molecular Medicine and Medical Biotechnology; Dep. of Int. Activity of Laboratory Medicine and Trans. UOC Clinical Microbiology, Naples, Italia ⁽²⁾ - University of Salento, Department of Biological and Environmental Sciences and Technologies, Lecce, Italia ⁽³⁾ - University of Sannio, Department of Science and Technology, Benevento, Italia ⁽⁴⁾ - University of Naples Federico II, Department of Molecular Medicine and Medical Biotechnology; Dep. of Int. Act. of Lab. Med. and Trans. UOC Clin. Micr.; CEINGE Franco Salvatore, Naples, Italia ⁽⁵⁾	422
Neisseria meningitidis activates pyroptotic pathways in a mouse model of meningitis: role of a two-partner secretion system.	422

CHIARA PAGLIUCA ¹ , ELENA SCAGLIONE ^{1,2} , GIUSEPPE MANTOVA ¹ , SILVIA C. RESTA ³ , CATERINA PAGLIARULO ⁴ , CECILIA BUCCI ³ , PIETRO ALIFANO ³ , PAOLA SALVATORE ^{1,2,5} , ROBERTA COLICCHIO ^{1,2}	422
¹ Department of Molecular Medicine and Medical Biotechnology, University of Naples Federico II, Naples, Italy; ² Department of Integrated Activity of Laboratory Medicine and Transfusion, Complex Operative Unit of Clinical Microbiology, University Hospital Federico II, Naples, Italy; ³ Department of Biological and Environmental Sciences and Technologies, University of Salento, Lecce, Italy; ⁴ Department of Science and Technology, University of Sannio, Benevento, Italy; ⁵ CEINGE, Advanced Biotechnologies s.c.ar.l., Naples, Italy.....	422
222 - Parvovirus B19 infection in a co-culture model of erythroid progenitor cells and mesenchymal stromal cells.....	424
GLORIA BUA ⁽¹⁾ - CHIARA GAMBERINI ⁽²⁾ - PASQUALE MARRAZZO ⁽²⁾ - LAURA BONSI ⁽²⁾ - FRANCESCO ALVIANO ⁽³⁾ - GIORGIO GALLINELLA ⁽¹⁾	424
Università di Bologna, Dipartimento di Farmacia e Biotecnologie, Bologna, Italia ⁽¹⁾ - Università di Bologna, Dipartimento di Scienze Mediche e Chirurgiche, Bologna, Italia ⁽²⁾ - Università di Bologna, Dipartimento di Scienze Biomediche e Neuromotorie, Bologna, Italia ⁽³⁾	424
Parvovirus B19 infection in a co-culture model of erythroid progenitor cells and mesenchymal stromal cells.....	424
GLORIA BUA ¹ , CHIARA GAMBERINI ² , PASQUALE MARRAZZO ² , LAURA BONSI ² , FRANCESCO ALVIANO ³ , GIORGIO GALLINELLA ¹	424
¹ Department of Pharmacy and Biotechnology, University of Bologna, Bologna, Italy; ² Department of Medical and Surgical Sciences, University of Bologna, Bologna, Italy; ³ Department of Biomedical and Neuromotor Sciences, University of Bologna, 40126 Bologna, Italy	424
229 - Cervical lesions and HPV oral infection: is there a relation?.....	425
ARIANNA SUCATO ⁽¹⁾ - MICHELA BUTTÀ ⁽¹⁾ - NICOLA SERRA ⁽²⁾ - RITA IMMORDINO ⁽³⁾ - GIUSEPPINA CAPRA ⁽¹⁾	425
Università degli studi di Palermo, Dipartimento di Promozione della Salute, Materno-Infantile, di Medicina Interna e Specialistica di Eccellenza “G. D’Alessandro”, Palermo, Italia ⁽¹⁾ - Università Federico II di Napoli, Dipartimento della salute pubblica, Napoli, Italia ⁽²⁾ - Policlinico, Paolo Giaccone, Palermo, Unità di Microbiologia e Virologia, Ospedale Policlinico P. Giaccone, Palermo, Italia ⁽³⁾	425
Cervical lesions and HPV oral infection: is there a relation?	425
ARIANNA SUCATO ¹ , MICHELA BUTTÀ ¹ , NICOLA SERRA ² , RITA IMMORDINO ³ AND GIUSEPPINA CAPRA ^{1,3}	425
¹ Department of Health Promotion, Mother and Child Care, Internal Medicine and Medical Specialties (ProMISE) “G. D’Alessandro”, University of Palermo, Palermo, Italy;	425
² Department of Public Health, University Federico II of Naples, Naples, Italy;	425
³ Microbiology and Virology Unit. Polyclinic Hospital “P. Giaccone”, Palermo, Italy.	425
231 - Eating the enemy: Mycoplasma strategies to evade Neutrophil Extracellular Traps (NETs) promoting bacterial nucleotides uptake and inflammatory damage	427
CARLA CACCIOTTO ⁽¹⁾ - ROSANNA ZOBBA ⁽¹⁾ - ALBERTO ALBERTI ⁽¹⁾	427
Università degli Studi di Sassari, Dipartimento di Medicina Veterinaria, Sassari, Italia ⁽¹⁾	427

Eating the enemy: Mycoplasma strategies to evade Neutrophil Extracellular Traps (NETs) promoting bacterial nucleotides uptake and inflammatory damage.....	427
CARLA CACCIOTTO^{1,2}, ROSANNA ZOBBA¹, AND ALBERTO ALBERTI^{1,2}	427
¹Dipartimento di Medicina Veterinaria, Università degli Studi di Sassari, Sassari, Italy.....	427
²Mediterranean Center for Disease Control, Università degli Studi di Sassari, Sassari, Italy.....	427
247 - Development and characterisation of a mouse model of invasive Non-Typhoidal Salmonella (iNTS) disease.....	428
ELENA PETTINI ⁽¹⁾ - FABIO FIORINO ⁽²⁾ - VALENTINA MOCCI ⁽¹⁾ - STEFANO LAZZI ⁽³⁾ - SUSANNA RICCI ⁽¹⁾ - GIANNI POZZI ⁽¹⁾ - DONATA MEDAGLINI ⁽¹⁾	428
University of Siena, Department of Medical Biotechnologies, Laboratory of Molecular Microbiology and Biotechnology (LA.M.M.B.), Siena, Italia ⁽¹⁾ - LUM University "Giuseppe Degennaro", Department of Medicine and Surgery, Bari, Italia ⁽²⁾ - University of Siena, Department of Medical Biotechnologies, Siena, Italia ⁽³⁾	428
Development and characterisation of a mouse model of invasive Non-Typhoidal Salmonella (iNTS) disease.....	428
ELENA PETTINI¹, FABIO FIORINO^{1,2}, VALENTINA MOCCI¹, STEFANO LAZZI³, SUSANNA RICCI¹, GIANNI POZZI¹, DONATA MEDAGLINI¹	428
¹ Laboratory of Molecular Microbiology and Biotechnology (LA.M.M.B.), Department of Medical Biotechnologies, University of Siena, Siena, Italy; ² Department of Medicine and Surgery, LUM University "Giuseppe Degennaro", Bari, Italy; ³ Department of Medical Biotechnologies, University of Siena, Siena, Italy	428
268 - HSV-1 exploits extracellular vesicles to disseminate neurotoxic proteins within the brain.....	430
Virginia Protto ⁽¹⁾ - Mariya T. Miteva ⁽²⁾ - Filomena Iannuzzi ⁽³⁾ - Domenica D. Li Puma ⁽⁴⁾ - Maria E. Marocchi ⁽²⁾ - Anna T. Palamara ⁽¹⁾ - Giovanna De Chiara ⁽³⁾.....	430
Istituto Superiore di Sanità, Dipartimento di Malattie Infettive, Roma, Italia ⁽¹⁾ - Sapienza Università di Roma, Dipartimento di Sanità Pubblica e Malattie Infettive Lab Affiliato a Istituto Pasteur Italia-Fondazione Cenci Bolognetti, Roma, Italia ⁽²⁾ - Centro Nazionale delle Ricerche, Istituto di Farmacologia Traslazionale, Roma, Italia ⁽³⁾ - Università Cattolica del Sacro Cuore, Dipartimento di Neuroscienze, Roma, Italia ⁽⁴⁾.....	430
HSV-1 exploits extracellular vesicles to disseminate neurotoxic proteins within the brain	430
VIRGINIA PROTTO¹, MARIYA T. MITEVA², FILOMENA IANNUZZI³, DOMENICA D. LI PUMA⁴, MARIA E. MARCOCCI², ANNA T. PALAMARA¹, GIOVANNA DE CHIARA³.....	430
1 Department of Infectious Diseases, Istituto Superiore di Sanità, Rome, Italy; 2 Department of Public Health and Infectious Diseases, Sapienza University of Rome, Laboratory affiliated to Istituto Pasteur Italia-Fondazione Cenci Bolognetti, Rome, Italy; 3 Institute of Translational Pharmacology, CNR, Rome, Italy; 4 Department of Neuroscience, Università Cattolica del Sacro Cuore, Rome, Italy, Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy;.....	430
271 - Phenotypic and genotypic characterization of Acinetobacter baumannii clinical isolates: yesterday and today.....	432
ASTRI DWYANTI TAGUEHA ⁽¹⁾ - DANIELA SCRIBANO ⁽¹⁾ - CARTESIO D'AGOSTINI ⁽²⁾ - CARLOTTA FIORILLA ⁽³⁾ - MEYSAM SARSHAR ⁽⁴⁾ - DOLORES LIMONGI ⁽⁵⁾ - SILVIA IANNARELLI ⁽¹⁾ - ZUZANA MICIÁKOVA ⁽⁶⁾ - ANNA TERESA PALAMARA ⁽⁷⁾ - CECILIA AMBROSI ⁽⁵⁾	432

Sapienza University of Rome, Department of Public Health and Infectious Diseases, Rome, Italia (¹) - University of Rome Tor Vergata, Department of Experimental Medicine, Rome, Italia (²) - Policlinico Tor Vergata, Laboratory of Clinical Microbiology, Rome, Italia (³) - Bambino Gesù Children's Hospital, IRCCS, Research Laboratories, Rome, Italia (⁴) - San Raffaele Open University, IRCCS, Department of Human Sciences and Promotion of the Quality of Life, Rome, Italia (⁵) - Sapienza University of Rome, Department of Molecular Medicine, Rome, Italia (⁶) - Sapienza University of Rome, Department of Public Health and Infectious Diseases, Laboratory Affiliated to Institute Pasteur Italia-Cenci Bolognetti, Rome, Italia (⁷)	432
Phenotypic and genotypic characterization of <i>Acinetobacter baumannii</i> clinical isolates: yesterday and today.....	432
Astri D. Tagueha ¹ , Daniela Scribano ¹ , Cartesio D'Agostini ^{2,3} , Carlotta Fiorilla ³ , Meysam Sarshar ⁴ , Dolores Limongi ⁵ , Silvia Iannarelli ¹ , Zuzana Miciakova ⁶ , Anna Teresa Palamara ^{7,8} , Cecilia Ambrosi ⁵	432
¹ Department of Public Health and Infectious Diseases, Sapienza University of Rome, Rome, Italy; ² Department of Experimental Medicine, University of Rome Tor Vergata, via Montpellier 1, Rome, Italy; ³ Laboratory of Clinical Microbiology, Policlinico Tor Vergata, viale Oxford 81, Rome, Italy; ⁴ Research Laboratories, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy; ⁵ Department of Human Sciences and Promotion of the Quality of Life, San Raffaele Open University, IRCCS, Rome, Italy; ⁶ Department of Molecular Medicine, Sapienza University of Rome; ⁷ Department of Public Health and Infectious Diseases, Sapienza University of Rome, Laboratory Affiliated to Institute Pasteur Italia-Cenci Bolognetti Foundation, Rome, Italy; ⁸ Department of Infectious Diseases, Istituto Superiore di Sanità, Rome, Italy.....	432
292 - <i>Klebsiella pneumoniae</i> -OMVs activate death-signaling pathways in human bronchial epithelial host cells (BEAS-2B).....	434
FEDERICA DELL'ANNUNZIATA (¹) - ELENA CIAGLIA (¹) - VERONICA FOLLIERO (¹) - VALENTINA LOPARDO (¹) - ANNA MACIAG (²) - ILARIA COSIMATO (³) - MASSIMILIANO GALDIERO (⁴) - ANNIBALE ALESSANDRO PUCA (¹) - GIANLUIGI FRANCI (¹)	434
Department of Medicine, Surgery and Dentistry, University Salerno, Salerno, Italia (¹) - Cardiovascular Research Unit, MultiMedica, Milan, Italia (²) - Clinical Pathology and Microbiology Unit, San Giovanni di Dio e Ruggi D'Aragona University Hospital, Salerno, Italia (³) - University of Campania Luigi Vanvitelli, Department of Experimental Medicine, Napoli, Italia (⁴)	434
<i>Klebsiella pneumoniae</i> -OMVs activate death-signaling pathways in human bronchial epithelial host cells (BEAS-2B).....	434
Federica Dell'Annunziata ^{1,2} , Elena Ciaglia ¹ , Veronica Folliero ¹ , Valentina Lopardo ¹ , Anna Maciag ³ , Iliara Cosimato ^{4,5} , Massimiliano Galdiero ^{2,5} , Annibale Alessandro Puca ^{1,3} , Gianluigi Franci ^{1,5}	434
¹ Department of Medicine, Surgery and Dentistry "Scuola Medica Salernitana", University of Salerno, 84081 Baronissi Salerno, Italy	434
² Department of Experimental Medicine, University of Campania Luigi Vanvitelli, 80138 Naples, Italy	434
³ Cardiovascular Research Unit, IRCCS MultiMedica, 20138 Milan, Italy	434
⁴ Clinical Pathology and Microbiology Unit, San Giovanni di Dio e Ruggi D'Aragona University Hospital, 84126 Salerno, Italy	434
⁵ Complex Operative Unity of Virology and Microbiology, University Hospital of Campania "Luigi Vanvitelli", 80138 Naples, Italy	434

309 - The role of human DDX3 helicase in HSV-2 infection	436
CARMEN RITA PIAZZA ⁽¹⁾ - GIULIA LOTTINI ⁽¹⁾ - PAOLA PERRERA ⁽²⁾ - CRISTINA DI PRIMIO ⁽³⁾ - MARIANNA MIGNANELLI ⁽⁴⁾ - PIETRO GIORGIO SPEZIA ⁽²⁾ - MICHELE LAI ⁽⁵⁾ - GIULIA FREER ⁽⁵⁾ - PAOLA QUARANTA ⁽⁵⁾ - MAURO PISTELLO ⁽⁶⁾	
Università di Siena, Dipartimento di Biotecnologie Mediche, Siena, Italia ⁽¹⁾ - Università di Pisa, Dipartimento di Ricerca Traslazionale, Pisa, Italia ⁽²⁾ - CNR di Pisa, Istituto di Neuroscienze, Pisa, Italia ⁽³⁾ - Scuola Normale Superiore, Laboratorio di Biologia Bio@SNS, Pisa, Italia ⁽⁴⁾ - Università di Pisa, Centro Retrovirus, Pisa, Italia ⁽⁵⁾ - Università Ospedale di Pisa, Unità Operativa di Virologia, Pisa, Italia ⁽⁶⁾	
328 - Inhibiting immunoregulatory amidase NAAA blocks ZIKV maturation in Human Neural Stem Cells.....	437
MICHELE LAI ⁽¹⁾ - VERONICA LA ROCCA ⁽¹⁾ - ELENA IACONO ⁽¹⁾ - ALESSANDRO DE CARLI ⁽¹⁾ - DOMENICO FAVARO ⁽¹⁾ - FABIO FILIPPINI ⁽¹⁾ - PIETRO GIORGIO SPEZIA ⁽¹⁾ - NICOLA CLEMENTI ⁽²⁾ - GIULIA FREER ⁽¹⁾ - MAURO PISTELLO ⁽¹⁾	
Università di Pisa, Nuove tecnologie in Medicina e Chirurgia, Pisa, Italia ⁽¹⁾ - San Raffaele, Laboratory of medical microbiology and virology, milano, Italia ⁽²⁾	
Inhibiting immunoregulatory amidase NAAA blocks ZIKV maturation in Human Neural Stem Cells.....	437
Michele Lai, Veronica La Rocca, Elena Iacono, Carolina Filippini, Alessandro De Carli, Domenico Favaro, Giulia Lottini, Nicola Clementi, Giulia Freer, Mauro Pistello.....	
09 Patogeni emergenti e riemergenti	439
3 - Prevalence of gastrointestinal pathogens and COVID-19 in patients with diarrhoea referring to a University Hospital	439
MARIANNA MARANGI ⁽¹⁾ - ROSELLA DE NITTIS ⁽²⁾ - MAURIZIO MARGAGLIONE ⁽¹⁾ - FABIO ARENA ⁽¹⁾	
Università di Foggia, Dipartimento di Medicina Clinica e Sperimentale, Foggia, Italia ⁽¹⁾ - Azienda Ospedaliero-Universitaria "Policlinico Riuniti", SSVD Microbiologia e Virologia, Foggia, Italia ⁽²⁾	
Prevalence of gastrointestinal pathogens and COVID-19 in patients with diarrhoea referring to a University Hospital.....	439
MARIANNA MARANGI¹, ROSELLA DE NITTIS², MAURIZIO MARGAGLIONE¹, FABIO ARENA^{1,2}	
¹Department of Clinical and Experimental Medicine, University of Foggia, Viale Luigi Pinto, 71122, Foggia, Italy; ²Microbiology and Virology Unit, Ospedali Riuniti, Viale Luigi Pinto, 71122, Foggia, Italy.....	
14 - Dirofilaria in humans: a new insight into an old knowledge.....	441
ALICE NICOLOSI ⁽¹⁾ - STEFANO REALE ⁽²⁾ - ANDREA RUSSO ⁽³⁾ - ILDEBRANDO PATAMIA ⁽¹⁾	
Unità Operativa Complessa Laboratorio Analisi, Azienda Ospedaliera Universitaria Policlinico-San Marco, Catania, Italia ⁽¹⁾ - Istituto Zooprofilattico Sperimentale della Sicilia, Istituto Zooprofilattico Sperimentale della Sicilia, Palermo, Italia ⁽²⁾ - Dipartimento di Oftalmologia, Azienda Ospedaliera Universitaria Policlinico-San Marco, Catania, Italia ⁽³⁾	
Dirofilaria in humans: a new insight into an old knowledge	441

ALICE NICOLOSI ¹ , STEFANO REALE ² , ANDREA RUSSO ³ , ILDEBRANDO PATAMIA ¹	441
¹ U.O.C. Laboratory Analysis, University Hospital Policlinico-San Marco, Catania, Italy;	
² Experimental Zooprophyllactic Institute of Sicily, Palermo, Italy; ³ Department of Ophthalmology, University of Catania, Catania, Italy	441
18 - Screening for tick-borne and tick-associated viruses in ticks collected in Sardinia, Italy	443
SILVIA FABI ⁽¹⁾ - SARAH DI SOPRA ⁽²⁾ - MICHELE PACCAGNELLA ⁽²⁾ - VALENTINA CHISU ⁽³⁾ - CIPRIANO FOXI ⁽³⁾ - CLAUDIA DEL VECCHIO ⁽²⁾ - GIOVANNA MASALA ⁽³⁾ - CRISTIANO SALATA ⁽²⁾	443
Università di Padova (coordinata da IUSS Pavia), Dipartimento di Medicina Molecolare, Padova, Italia ⁽¹⁾ - Università di Padova, Dipartimento di Medicina Molecolare, Padova, Italia ⁽²⁾ - Istituto Zooprofilattico Sperimentale della Sardegna, Istituto Zooprofilattico Sperimentale della Sardegna, Sassari, Italia ⁽³⁾	443
Screening for tick-borne and tick-associated viruses in ticks collected in Sardinia, Italy	443
SILVIA FABI ^{1,2} , SARAH DI SOPRA ¹ , MICHELE PACCAGNELLA ¹ , VALENTINA CHISU ³ , CIPRIANO FOXI ³ , CLAUDIA DEL VECCHIO ³ , GIOVANNA MASALA ³ , CRISTIANO SALATA ¹	443
1. Dipartimento di Medicina Molecolare, Università degli studi di Padova, Via A. Gabelli, 63 – 35121 Padova, Italy; 2. Scuola Universitaria Superiore (IUSS), Palazzo del Broletto, Piazza della Vittoria, 15 – 27100, Pavia, Italy; 3. Istituto Zooprofilattico Sperimentale della Sardegna, Via Vienna, 2 - 07100 Sassari, Italy	443
23 - Amino acid variability of small-molecule inhibitors and mAbs target sites in globally circulating RSV strains.....	444
VALERIA FOX ⁽¹⁾ - ROSSANA SCUTARI ⁽¹⁾ - ROMINA SALPINI ⁽²⁾ - SIMONE LA FRAZIA ⁽³⁾ - ALESSANDRA BANDERA ⁽⁴⁾ - ANTONIO PIRALLA ⁽⁵⁾ - ELENA PARIANI ⁽⁶⁾ - ANDREA GORI ⁽⁷⁾ - VALENTINA SVICHER ⁽³⁾ - CLAUDIA ALTERI ⁽¹⁾	444
Department Of Oncology And Hemato-oncology, University Of Milan, Milan, Italia ⁽¹⁾ - Department of Experimental Medicine, University of Rome Tor Vergata, Rome, Italia ⁽²⁾ - Department of Biology, University of Rome Tor Vergata, Rome, Italia ⁽³⁾ - Infectious Diseases Unit, Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico,, &Department of Pathophysiology and Transplantation, University of Milan, Milan, Italia ⁽⁴⁾ - Microbiology and Virology Department, Fondazione IRCCS Policlinico San Matteo, Pavia, Italia ⁽⁵⁾ - Department of Biomedical Sciences for Health, University of Milan, Milan, Italia ⁽⁶⁾ - Department of Pathophysiology and Transplantation, University of Milan, &Division of Infectious Diseases, L. Sacco University Hospital, Milan, Italia ⁽⁷⁾	444
Category: Patogeni emergenti e riemergenti.....	444
Title: Amino acid variability of small-molecule inhibitors and mAbs target sites in globally circulating RSV strains.....	444
Valeria Fox ¹ , Rossana Scutari ¹ , Romina Salpini ² , Simone La Frazia ³ , Alessandra Bandera ^{4,5} , Antonio Piralla ⁶ , Elena Pariani ⁷ , Andrea Gori ^{5,8} , Valentina Svicher ³ , Claudia Alteri ¹	444
1 Department Of Oncology And Hemato-oncology, University Of Milan, Milan, Italy	444
2 Department of Experimental Medicine, University of Rome Tor Vergata, Rome, Italy	444
3 Department of Biology, University of Rome Tor Vergata, Rome, Italy	444
4 Infectious Diseases Unit, Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy	444

5 Department of Pathophysiology and Transplantation, University of Milan, Milan, Italy	444
6 Microbiology and Virology Department, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy	444
7 Department of Biomedical Sciences for Health, University of Milan, Milan, Italy	444
8 Division of Infectious Diseases, L. Sacco University Hospital, Milan, Italy.....	444
29 - First case of <i>Chryseobacterium gallinarum</i> bloodstream infection: a diagnostic challenge for an emerging pathogen	446
Mattia Genco ⁽¹⁾ - Antonio Curtoni ⁽¹⁾ - Lorenza Cavallo ⁽²⁾ - Silvia Scabini ⁽³⁾ - Luisa Guarrasi ⁽¹⁾ - Alessandro Bondi ⁽¹⁾ - Silvia Corcione ⁽³⁾ - Paolo Bottino ⁽¹⁾ - Narcisa Mandras ⁽²⁾ - Rossana Cavallo ⁽¹⁾ - Cristina Costa ⁽¹⁾	446
SC Microbiologia Virologia U, Università di Torino, Torino, Italia ⁽¹⁾ - Dipartimento di Scienze della Sanità Pubblica e Pediatriche, Università di Torino, Torino, Italia ⁽²⁾ - SC Malattie Infettive U, Università di Torino, Torino, Italia ⁽³⁾	446
First case of <i>Chryseobacterium gallinarum</i> bloodstream infection: a diagnostic challenge for an emerging pathogen	446
MATTIA GENCO ¹ , ANTONIO CURTONI ^{1,2} , LORENZA CAVALLO ¹ , SILVIA SCABINI ³ , LUISA GUARRASI ¹ , ALESSANDRO BONDI ^{1,2} , SILVIA CORCIONE ³ , PAOLO BOTTINO ¹ , NARCISA MANDRAS ¹ , ROSSANA CAVALLO ^{1,2} , CRISTINA COSTA ^{1,2}	446
¹ Department of Sciences of Public Health and Pediatrics, University of Turin, Turin, Italy;.....	446
² Department of Laboratory Medicine, Microbiology and Virology Unit, University Hospital Città della Salute e della Scienza di Torino, Turin, Italy;	446
³ Department of Medical Sciences, Infectious Disease Unit, University of Turin, Turin, Italy.....	446
47 - New putative <i>Pannonibacter</i> species isolated from nosocomial bacteraemia in two oncological pediatric patients	448
FRANCESCA INDRACCOLO ⁽¹⁾ - VITTORIANA DE LAURENTIIS ⁽¹⁾ - LOREDANA CAPOZZI ⁽²⁾ - STEFANO CASTELLANA ⁽²⁾ - ANTONIO PARISI ⁽²⁾ - MICHELA PARISI ⁽³⁾ - FRANCESCO DE LEONARDIS ⁽⁴⁾ - MASSIMO GRASSI ⁽⁴⁾ - ENRICA M. RANIERI ⁽¹⁾ - RAFFAELE DEL PRETE ⁽⁵⁾ - CARMELA DE CARLO ⁽¹⁾ - LUIGI RONGA ⁽¹⁾ - STEFANIA STOLFA ⁽¹⁾ - FEDERICA ROMANELLI ⁽¹⁾ - ELEONORA SPARAPANO ⁽¹⁾ - LUIGI SANTACROCE ⁽⁵⁾ - MARIA TERESA MONTAGNA ⁽⁵⁾ - ADRIANA MOSCA ⁽⁵⁾	448
UOC Microbiology and Virology, Università di Bari "Aldo Moro", Bari, Italia ⁽¹⁾ - Experimental Zooprophyllactic Institute of Apulia and Basilicata, Laboratorio di Biologia Molecolare, Foggia, Italia ⁽²⁾ - Pediatria, Università degli Studi di Tor Vergata, Roma, Italia ⁽³⁾ - Pediatric Hematology and Oncology, University Hospital of Policlinico - Bari, Bari, Italia ⁽⁴⁾ - Section of Microbiology, Interdisciplinary Department of Medicine (DIM), School of Medicine, University of Bari " Aldo Moro", Bari, Italia ⁽⁵⁾	448
New putative <i>Pannonibacter</i> species isolated from nosocomial bacteraemia in two oncological pediatric patients.....	448
Francesca Indraccolo ¹ , Vittoriana De Laurentiis ¹ , Loredana Capozzi ² , Stefano Castellana ² , Antonio Parisi ² , Michela Parisi ⁵ , Francesco De Leonardis ³ , Massimo Grassi ³ , Enrica M. Ranieri ¹ , Raffaele Del Prete ⁴ , Carmela De Carlo ¹ , Luigi Ronga ¹ , Stefania Stolfa ¹ , Federica Romanelli ¹ , Eleonora Sparapano ¹ , Luigi Santacroce ⁴ , Maria TeresaMontagna ⁴ , Adriana Mosca ⁴	448
1 UOC Microbiology and Virology, University "Aldo Moro", Bari, Italy.....	448

2 Experimental Zooprophyllactic Institute of Apulia and Basilicata - Via Manfredonia, 20, Foggia, Italy	448
3 Pediatric Hematology and Oncology - University Hospital of Policlinico - Bari Piazza G. Cesare,11, Bari, Italy	448
4 Section of Microbiology, Interdisciplinary Department of Medicine (DIM), School of Medicine, University of Bari " Aldo Moro", Bari, Italy	448
5 Pediatria-Università degli Studi di Roma Tor Vergata, Roma, Italy	448
68 - Cutibacterium acnes in shoulder arthroplasty: preliminary results on the efficacy of an intraoperative iodopovidone irrigation in reducing its contamination.....	450
FABIO LONGO ⁽¹⁾ - ELEONORA MANISCALCO ⁽¹⁾ - FRANCESCA MENOTTI ⁽¹⁾ - ALESSANDRO BONDI ⁽²⁾ - CRISTINA COSTA ⁽²⁾ - ANTONIO CURTONI ⁽²⁾ - LORENZA CAVALLO ⁽¹⁾ - CLAUDIA PAGANO ⁽¹⁾ - ANNA MARIA CUFFINI ⁽¹⁾ - ENRICO BELLATO ⁽³⁾ - FILIPPO CASTOLDI ⁽³⁾ - DAVIDE BLONNA ⁽⁴⁾ - GIULIANA BANCHE ⁽¹⁾ - VALERIA ALLIZOND ⁽¹⁾	450
Università degli Studi di Torino, Dipartimento di scienze della sanità pubbliche e pediatriche, Torino, Italia ⁽¹⁾ - Città della Salute e della Scienza di Torino, Unità di Microbiologia e Virologia, Torino, Italia ⁽²⁾ - A.O.U San Luigi Gonzaga, Orbassano, Unità di Ortopedia e Traumatologia, Torino, Italia ⁽³⁾ - A.O. Ordine Mauriziano di Torino, Unità di Ortopedia e Traumatologia, Torino, Italia ⁽⁴⁾	450
Cutibacterium acnes in shoulder arthroplasty: preliminary results on the efficacy of an intraoperative iodopovidone irrigation in reducing its contamination	450
FABIO LONGO ^{1,2} , ELEONORA MANISCALCO ¹ , FRANCESCA MENOTTI ¹ , ALESSANDRO BONDI ² , CRISTINA COSTA ² , ANTONIO CURTONI ² , LORENZA CAVALLO ¹ , PAGANO CLAUDIA ¹ , ANNA MARIA CUFFINI ¹ , ENRICO BELLATO ³ , FILIPPO CASTOLDI ³ , DAVIDE BLONNA ⁴ , GIULIANA BANCHE ¹ , VALERIA ALLIZOND ¹	450
¹ Department of Public Health and Pediatrics, University of Torino, Turin, Italy; ² Microbiology and Virology Unit, Città della Salute e della Scienza di Torino, Turin, Italy; ³ Orthopedics and Traumatology Unit, AOU San Luigi Gonzaga, Orbassano, Turin, Italy; ⁴ Orthopedics and Traumatology Unit, A.O. Ordine Mauriziano di Torino, Turin, Italy.	450
96 - Genomic dissection of SARS-CoV-2 evolution in immunocompromised patient with prolonged viral shedding.....	452
BEATRICE MOLA ⁽¹⁾ - SILVIA SILENZI ⁽²⁾ - LUCA RUOTOLO ⁽¹⁾ - TIZIANA LAZZAROTTO ⁽¹⁾ - GIADA ROSSINI ⁽²⁾	452
Alma Mater Studiorum - Università di Bologna, Microbiologia, Dipartimento di Scienze Mediche e Chirurgiche – DIMEC Alma Mater Studiorum - Università di Bologna, Bologna, Italia ⁽¹⁾ - Azienda Ospedaliero-Universitaria of Bologna, Bologna, Italy, UO Microbiologia, IRCCS Azienda Ospedaliero-Universitaria of Bologna, Bologna, Italy, Bologna, Italia ⁽²⁾	452
Patogeni emergenti e riemergenti	452
Genomic dissection of SARS-CoV-2 evolution in immunocompromised patient with prolonged viral shedding	452
BEATRICE MOLA ¹ , SILVIA SILENZI ² , LUCA RUOTOLO ¹ , TIZIANA LAZZAROTTO ^{1,2} , GIADA ROSSINI ²	452
¹ Microbiologia, Dipartimento di Scienze Mediche e Chirurgiche – DIMEC Alma Mater Studiorum - Università di Bologna	452

² UO Microbiologia, IRCCS Azienda Ospedaliero-Universitaria of Bologna, Bologna, Italy	452
104 - The infection rate of respiratory viruses in pandemic SARS-CoV2 period (2019-2023): an observational study.....	454
GAETANA COSTANZA ⁽¹⁾ - PIERPAOLO PABA ⁽¹⁾ - MARCO CIOTTI ⁽¹⁾ - DOMENICO OMBRES ⁽¹⁾ - ADA BERTOLI ⁽¹⁾ - FABBIO MARCUCCILLI ⁽¹⁾ - STEFANIA LOSCHI ⁽¹⁾ - CLAUDIO CARAPELLESE ⁽¹⁾ - NICOLA BOTTALICO ⁽¹⁾ - ROSALBA PETRUCCELLI ⁽¹⁾ - VITA PETRONE ⁽²⁾ - MARIA LAURA FANELLI ⁽²⁾ - LUCIA PIREDDA ⁽³⁾ - SERGIO BERNARDINI ⁽²⁾ - EMANUELA BALESTRIERI ⁽²⁾ - CLAUDIA MATTEUCCI ⁽²⁾ - ANTONELLA MINUTOLO ⁽²⁾ - SANDRO GRELLI ⁽¹⁾.....	454
Unit of Virology, Policlinic of Tor Vergata, ROMA, Italia ⁽¹⁾ - Università degli studi di Roma "Tor Vergata", Dipartimento di Medicina Sperimentale, Roma, Italia ⁽²⁾ - Università degli studi di Roma "Tor Vergata", Dipartimento di Biologia, Roma, Italia ⁽³⁾	454
The infection rate of respiratory viruses in pandemic SARS-CoV2 period (2019-2023): an observational study.	454
GAETANA COSTANZA¹, PIERPAOLO PABA¹, MARCO CIOTTI¹, DOMENICO OMBRES¹, ADA BERTOLI^{1,2}, FABBIO MARCUCCILLI¹, STEFANIA LOSCHI¹, CLAUDIO CARAPELLESE¹, NICOLA BOTTALICO¹, ROSALBA PETRUCCELLI¹, VITA PETRONE², MARIA LAURA FANELLI², LUCIA PIREDDA³, SERGIO BERNARDINI^{1,4}, EMANUELA BALESTRIERI², CLAUDIA MATTEUCCI², ANTONELLA MINUTOLO², SANDRO GRELLI^{1,2}	454
1. Unit of Virology, Policlinic of Tor Vergata, Rome, Italy; 2. Department of Experimental Medicine, University of Rome Tor Vergata, Rome, 00133, Italy; 3. Department of Biology, University of Rome Tor Vergata, Rome, 00133, Italy; 4. Laboratory Medicine, Policlinic of Tor Vergata, Rome, Italy.	454
105 - Prevalence of Schistosoma mansoni monoinfection and co-infections in the Romagna region	456
MARTINA BRANDOLINI ⁽¹⁾ - LAURA GRUMIRO ⁽¹⁾ - ALESSANDRA MISTRAL DE PASCALI ⁽¹⁾ - IRENE ZAGHI ⁽¹⁾ - GIORGIO DIRANI ⁽¹⁾ - SILVIA ZANNOLI ⁽¹⁾ - FRANCESCA TADDEI ⁽¹⁾ - GIULIA GATTI ⁽¹⁾ - ANNA MARZUCCO ⁽¹⁾ - MARIA SOFIA MONTANARI ⁽¹⁾ - SIMONA SEMPRINI ⁽¹⁾ - MONICA CRICCA ⁽¹⁾ - VITTORIO SAMBRI ⁽¹⁾	456
Unit of Microbiology, AUSL Romagna, Department of Laboratory and Transfusion Medicine, Cesena, Italia ⁽¹⁾	456
Prevalence of Schistosoma mansoni monoinfection and co-infections in the Romagna region ...	456
MARTINA BRANDOLINI^{1,2}, LAURA GRUMIRO^{1,2}, ALESSANDRA MISTRAL DE PASCALI^{1,2}, IRENE ZAGHI^{1,3}, GIORGIO DIRANI¹, SILVIA ZANNOLI¹, FRANCESCA TADDEI^{1,2}, GIULIA GATTI¹, ANNA MARZUCCO¹, MARIA SOFIA MONTANARI¹, SIMONA SEMPRINI¹, MONICA CRICCA^{1,2}, VITTORIO SAMBRI^{1,2}.	456
Department of Laboratory and Transfusion Medicine (DiMeLabeT), Unit of Microbiology, The Greater Romagna Area Hub Laboratory, 47522 Cesena, Italy;	456
Department of Medical and Surgical Sciences (DIMEC), Unit of Microbiology, Alma Mater Studiorum University of Bologna, 47522 Cesena, Italy;	456
Department of Specialty Medicine, Unit of Infectious Diseases, Santa Maria delle Croci Hospital, 48100 Ravenna, Italy	456
108 - Presence of alive Ascaris lumbricoides in a symptomatic patient repeatedly negative for worm eggs in fecal samples	458

SIMONA GIGLIOTTI ⁽¹⁾ - ELETTRA MANCUSO ⁽¹⁾ - MARTA PANTANELLA ⁽¹⁾ - MICHELE MANNO ⁽¹⁾ - CRISTINA COSCO ⁽²⁾ - FRANCESCA DIVENUTO ⁽¹⁾ - MORENA CAMPOLO ⁽¹⁾ - NORA NOEMI RASO ⁽¹⁾ - ERIKA GAMBARDELLA ⁽¹⁾ - CHIARA MAIDA ⁽¹⁾ - NADIA MARASCIO ⁽¹⁾ - PATRIZIA DOLDO ⁽³⁾ - ANGELA QUIRINO ⁽¹⁾ - GIOVANNI MATERA ⁽¹⁾ ...	458
UOC Microbiologia Clinica, Università Magna Graecia, Catanzaro, Italia ⁽¹⁾ - Uo gastroenterologia ed endoscopia operativa, università magna graecia, catanzaro, Italia ⁽²⁾ - UO gastroenterologia ed endoscopia digestiva, università magna graecia, catanzaro, Italia ⁽³⁾	458
Presence of alive <i>Ascaris lumbricoides</i> in a symptomatic patient repeatedly negative for worm eggs in fecal samples.	458
SIMONA GIGLIOTTI ¹ , ELETTRA MANCUSO ¹ , MARTA PANTANELLA ¹ , MICHELE MANNO ¹ , CRISTINA COSCO ² , FRANCESCA DIVENUTO ¹ , MORENA CAMPOLO ¹ , NORA N. RASO ¹ , ERIKA GAMBARDELLA ¹ , CHIARA MAIDA ¹ , NADIA MARASCIO ¹ , PATRIZIA DOLDO ² , ANGELA QUIRINO ¹ , GIOVANNI MATERA ¹	458
¹ Department of Health Sciences, Unit of Clinical Microbiology, "Magna Graecia" University of Catanzaro-"Mater Domini" Teaching Hospital, Catanzaro, Italy; ² Department of Experimental and Clinical Medicine, Magna Graecia University of Catanzaro, 88100 Catanzaro, Italy.....	458
129 - The cranberry (<i>Vaccinium macrocarpon</i>) extract blocks Ebola virus and Crimean-Congo hemorrhagic fever virus infection by interfering with viral entry into target cells.....	460
DENIS PASQUAL ⁽¹⁾ - MATTIA MIRANDOLA ⁽¹⁾ - SOFIA APPELBERG ⁽²⁾ - MASSIMO EMILIO MAFFEI ⁽³⁾ - GIORGIO GRIBAUDO ⁽⁴⁾ - ALI MIRAZIMI ⁽⁵⁾ - CRISTIANO SALATA ⁽⁶⁾ - GIULIA SIBILLE ⁽³⁾ - ANNA LUGANINI ⁽³⁾	460
University of Padua, Dip.Medicina Molecolare, Padova, Italia ⁽¹⁾ - Public Health Agency of Sweden, Departement of Microbiology, Solna, Svezia ⁽²⁾ - University of Turin, Dip. di Scienze della vita e biologia dei sistemi, Torino, Italia ⁽³⁾ - University of Turin, Dip. Scienze della vita e biologia dei sistemi, Torino, Italia ⁽⁴⁾ - Karolinska Institute, Departement of Laboratory medicine, Solna, Svezia ⁽⁵⁾ - University of Padua, Dip. Medicina Molecolare, Padova, Italia ⁽⁶⁾	460
The cranberry (<i>Vaccinium macrocarpon</i>) extract blocks Ebola virus and Crimean-Congo hemorrhagic fever virus infection by interfering with viral entry into target cells.	460
Autors.....	460
Denis PASQUAL ¹ , Mattia MIRANDOLA ¹ , Sofia APPELBERG ² , Giulia SIBILLE ³ , Anna LUGANINI ³ , Massimo E. MAFFEI ³ , Ali MIRAZIMI ^{2,4,5} , Giorgio GRIBAUDO ³ , Cristiano SALATA ¹	460
¹ Department of Molecular Medicine, University of Padova, Padova, Italy;	460
² Department of Microbiology, Public Health Agency of Sweden, Solna, Sweden;	460
³ Department of Life Sciences and Systems Biology, University of Turin, Turin, Italy;	460
⁴ Department of Laboratory Medicine, Karolinska Institutet, Stockholm, Sweden;.....	460
⁵ National Veterinary Institute, 75189 Uppsala, Sweden.	460
130 - Hepatitis E virus acute infection in an immunocompetent subject with non-alcoholic fatty liver disease.	462
NADIA MARASCIO ⁽¹⁾ - GRAZIA PAVIA ⁽¹⁾ - MARTA PANTANELLA ⁽¹⁾ - CLAUDIA CICINO ⁽¹⁾ - AIDA GIANCOTTI ⁽¹⁾ - FRANCESCA TRIMBOLI ⁽¹⁾ - GIORGIO S. BARRECA ⁽¹⁾ - ANGELO G. LAMBERTI ⁽¹⁾ - FABIO MONGIARDO ⁽¹⁾ - MASSIMO DE SIENA ⁽²⁾ - SEBASTIANO DI SALVO ⁽²⁾ - TIZIANA GRAVINA ⁽²⁾ - GIOVANNI MATERA ⁽¹⁾ - ANGELA QUIRINO ⁽¹⁾	462

UOC Microbiologia Clinica, Magna Graecia University, Catanzaro, Italia ⁽¹⁾ - UO Epatologia, Mater Domini University Hospital, Catanzaro, Italia ⁽²⁾	462
Hepatitis E virus acute infection in an immunocompetent subject with non-alcoholic fatty liver disease.	462
NADIA MARASCIO ¹ , GRAZIA PAVIA ¹ , MARTA PANTANELLA ¹ , CLAUDIA CICINO ¹ , AIDA GIANCOTTI ¹ , FRANCESCA TRIMBOLI ¹ , GIORGIO S. BARRECA ¹ , ANGELO G. LAMBERTI ¹ , FABIO MONGIARDO ¹ , MASSIMO DE SIENA ² , SEBASTIANO DI SALVO ² , TIZIANA GRAVINA ² , GIOVANNI MATERA ¹ AND ANGELA QUIRINO ¹	462
¹ Department of Health Sciences, Clinical Microbiology Unit, “Mater Domini” University Hospital, Catanzaro, Italy; ² Unit of Hepatology, “Mater Domini” University Hospital, Catanzaro, Italy..	462
164 - Case report of pulmonary echinococcosis in a young patient from southern Italy.	464
ANNA MOREA ⁽¹⁾ - ANGELA DE PALMA ⁽²⁾ - GIULIA DE IACO ⁽²⁾ - RICCARDO P. LIA ⁽³⁾ - FRANCESCO DI GENNARO ⁽⁴⁾ - ANNALISA SARACINO ⁽⁴⁾ - DOMENICO OTRANTO ⁽³⁾ - GIUSEPPE MARULLI ⁽²⁾ - ROBERTA IATTA ⁽¹⁾	464
Sezione di Parassitologia, Università degli Studi di Bari/Dipartimento Interdisciplinare di Medicina, BARI, Italia ⁽¹⁾ - Unità operativa di Chirurgia Toracica, Policlinico/Università degli Studi di Bari/Dipartimento di Medicina di Precisione e Rigenerativa e Area Ionica, BARI, Italia ⁽²⁾ - Medicina Veterinaria, Università degli Studi di Bari/Dipartimento di Medicina Veterinaria, BARI, Italia ⁽³⁾ - Clinica di Malattie Infettive, Policlinico/Università degli Studi di Bari/Dipartimento di Medicina di Precisione e Rigenerativa e Area Ionica, BARI, Italia ⁽⁴⁾	464
Case report of pulmonary echinococcosis in a young patient from southern Italy	464
ANNA MOREA ¹ , ANGELA DE PALMA ² , GIULIA DE IACO ² , RICCARDO P. LIA ³ , FRANCESCO DI GENNARO ⁴ , ANNALISA SARACINO ⁴ , DOMENICO OTRANTO ³ , GIUSEPPE MARULLI ² , ROBERTA IATTA ¹	464
¹ Interdisciplinary Department of Medicine, University of Bari Aldo Moro, Bari, Italy;.....	464
² Department of Precision and Regenerative Medicine and Ionian Area, Thoracic Surgery Unit, University of Bari Aldo Moro, Bari, Italy;	464
³ Department of Veterinary Medicine, University of Bari Aldo Moro, Bari, Italy;	464
⁴ Department of Precision and Regenerative Medicine and Ionian Area, Clinic of Infectious diseases, University of Bari Aldo Moro, Bari, Italy	464
171 - The role of whole-genome sequencing in cluster investigation of Carbapenem-Resistant K. pneumoniae isolated in a northern Italy hospital	466
GABRIELLA TOCCI ⁽¹⁾ - VALENTINA LEPERA ⁽¹⁾ - CAMILLA REBOLI ⁽¹⁾ - ANDREA ZAPPAVIGNA ⁽¹⁾ - CHIARA GORRINI ⁽¹⁾ - CLAUDIA CORDINI ⁽¹⁾ - ROBERTA SCHIAVO ⁽¹⁾ - LORENA LA VERGATA ⁽¹⁾ - BEATRICE ZERBI ⁽²⁾ - MARIA GRAZIA SILVOTTI ⁽²⁾ - STEFANO PONGOLINI ⁽³⁾ - ERIKA SCALTRITI ⁽³⁾ - GIULIANA LO CASCIO ⁽¹⁾	466
Microbiology Unit, Clinical Pathology Department, AUSL Piacenza, Piacenza, Italia ⁽¹⁾ - Hospital Hygiene Unit, Medical management Department, AUSL Piacenza, Piacenza, Italia ⁽²⁾ - Risk Analysis and Genomic Epidemiology Unit, Istituto Zooprofilattico Sperimentale della Lombardia dell'Emilia Romagna (IZSLER), Parma, Italia ⁽³⁾	466
The role of whole-genome sequencing in cluster investigation of Carbapenem-Resistant K. pneumoniae isolated in a northern Italy hospital	466
GABRIELLA TOCCI ¹ , VALENTINA LEPERA ¹ , CAMILLA REBOLI ¹ , ANDREA ZAPPAVIGNA ¹ , CHIARA GORRINI ¹ , CLAUDIA CORDINI ¹ , ROBERTA SCHIAVO ¹ , LORENA	

LAVERGATA ¹ , BEATRICE ZERBI ³ , MARIA GRAZIA SILVOTTI ³ , STEFANO PONGOLINI ² , ERIKA SCALTRITI ² , GIULIANA LO CASCIO ¹	466
¹ Microbiology Unit, Clinical Pathology Department, AUSL Piacenza, Italy; ² Risk Analysis and Genomic Epidemiology Unit, Istituto Zooprofilattico Sperimentale della Lombardia dell'Emilia Romagna (IZSLER), Parma, Italy; ³ Hospital Hygiene Unit, Medical management Department, AUSL Piacenza, Italy	466
218 - Novel benzimidazole derivatives as potent and selective anti Poxvirus agents.....	468
VALERIA MANCA ⁽¹⁾ - GIUSEPPINA SANNA ⁽¹⁾ - ROBERTA IBBA ⁽²⁾ - VANESSA PALMAS ⁽¹⁾ - VERONICA FOLLIERO ⁽³⁾ - SANDRA PIRAS ⁽²⁾ - LAURA SANNA ⁽²⁾ - ANTONIO CARTA ⁽²⁾ - ALDO MANZIN ⁽¹⁾	468
Dipartimento di Scienze Biomediche, Università degli Studi di Cagliari, Monserrato, Cagliari, Italia ⁽¹⁾ - Dipartimento di Medicina, Chirurgia e Farmacia, Università di Sassari, Sassari, Italia ⁽²⁾ - Dipartimento di Medicina, Chirurgia e Odontoiatria, Scuola Medica Salernitana, Salerno, Italia ⁽³⁾	468
Novel benzimidazole derivatives as potent and selective anti Poxvirus agents	468
VALERIA MANCA ¹ , GIUSEPPINA SANNA ¹ , ROBERTA IBBA ² , VANESSA PALMAS ¹ , VERONICA FOLLIERO ³ , SANDRA PIRAS ² , LAURA SANNA ² , ANTONIO CARTA ² , ALDO MANZIN ¹	468
¹ Department of Biomedical Sciences, University of Cagliari, Cittadella Universitaria, 09042, Monserrato, Cagliari, Italy;	468
² Department of Medicine, Surgery and Pharmacy, University of Sassari, via Muroni 23A, 07100 Sassari, Italy;	468
³ Department of Medicine, Surgery and Dentistry "Scuola Medica Salernitana", Via Salvatore Allende, 84081, Salerno, Italy	468
219 - Efficacy of dispirotripiperazine PDSTP in a golden Syrian hamster model of SARS-CoV-2 pneumonia	469
GIUSEPPINA SANNA ⁽¹⁾ - OLGA RIABOVA ⁽²⁾ - ANNA EGOROVA ⁽²⁾ - ELENA KAZAKOVA ⁽²⁾ - ALEXANDER LEPIOSHKIN ⁽²⁾ - NATALIA MONAKHOVA ⁽²⁾ - ALESSANDRA MARONGIU ⁽¹⁾ - VANESSA PALMAS ⁽¹⁾ - GIANLUIGI FRANCI ⁽³⁾ - VADIM MAKAROV ⁽²⁾ - ALDO MANZIN ⁽¹⁾	469
Dipartimento di Scienze Biomediche, Università degli Studi di Cagliari, Monserrato, Cagliari, Italia ⁽¹⁾ - Federal Research Centre "Fundamentals of Biotechnology", Russian Academy of Sciences, Moscow, Russia ⁽²⁾ - Dipartimento di Medicina, Chirurgia e Odontoiatria, Scuola Medica Salernitana, Università di Salerno, Salerno, Baronissi, Italia ⁽³⁾	469
Efficacy of dispirotripiperazine PDSTP in a golden Syrian hamster model of SARS-CoV-2 pneumonia	469
GIUSEPPINA SANNA ¹ , OLGA RIABOVA ² , ANNA EGOROVA ² , ELENA KAZAKOVA ² , ALEXANDER LEPIOSHKIN ² , NATALIA MONAKHOVA ² , ALESSANDRA MARONGIU ¹ , VANESSA PALMAS ¹ , GIANLUIGI FRANCI ³ , VADIM MAKAROV ² , ALDO MANZIN ¹	469
¹ Department of Biomedical Sciences, Microbiology and Virology Unit, University of Cagliari, Cittadella Universitaria, Sp. 8, 09042 Monserrato (CA), Italy;	469
² Federal Research Centre "Fundamentals of Biotechnology" of the Russian Academy of Sciences (Research Centre of Biotechnology RAS), Leninsky prospect, 33, build. 2, 119071 Moscow, Russia;	469

³ Department of Medicine, Surgery and Dentistry "Scuola Medica Salernitana", University of Salerno, 84081 Baronissi, Italy.....	469
220 - Elevated plasma level of IP-10 /CXCL10 is correlated with severity of human West Nile virus (WNV) infection.....	471
GIADA ROSSINI ⁽¹⁾ - STEFANIA VARANI ⁽²⁾ - SILVIA SILENZI ⁽¹⁾ - LUCA RUOTOLO ⁽³⁾ - BEATRICE MOLA ⁽¹⁾ - TIZIANA LAZZAROTTO ⁽²⁾	471
UO Microbiologia, IRCCS Azienda Ospedaliero-Universitaria of Bologna, Bologna, Italia ⁽¹⁾ - UO Microbiologia, IRCCS Azienda Ospedaliero-Universitaria of Bologna, Microbiologia, Dipartimento di Scienze Mediche e Chirurgiche – DIMEC Alma Mater Studiorum - Università di Bologna, Bologna, Italia ⁽²⁾ - Microbiologia, Dipartimento di Scienze Mediche e Chirurgiche – DIMEC Alma Mater Studiorum - Università di Bologna, Bologna, Italia ⁽³⁾	471
Elevated plasma level of IP-10 /CXCL10 is correlated with severity of human West Nile virus (WNV) infection	471
GIADA ROSSINI ¹ , STEFANIA VARANI ^{1,2} , SILVIA SILENZI ¹ , LUCA RUOTOLO ² , BEATRICE MOLA ¹ , TIZIANA LAZZAROTTO ^{1,2}	471
¹ UO Microbiologia, IRCCS Azienda Ospedaliero-Universitaria of Bologna, Bologna, Italy	471
² Microbiologia, Dipartimento di Scienze Mediche e Chirurgiche – DIMEC Alma Mater Studiorum - Università di Bologna, Italy	471
233 - Asymptomatic Mpox infection in subjects presenting for MVA-BN vaccine	473
GIULIA MATUSALI ⁽¹⁾ - VALENTINA MAZZOTTA ⁽²⁾ - PIERLUCA PISELLI ⁽³⁾ - AURORA BETTINI ⁽¹⁾ - FRANCESCA COLAVITA ⁽¹⁾ - SABRINA COEN ⁽¹⁾ - FRANCESCO VAIA ⁽⁴⁾ - ENRICO GIRARDI ⁽⁵⁾ - ANDREA ANTINORI ⁽²⁾ - FABRIZIO MAGGI ⁽¹⁾	473
Istituto Nazionale per le Malattie Infettive "Lazzaro Spallanzani", Laboratorio di Virologia, Roma, Italia ⁽¹⁾ - Istituto Nazionale per le Malattie Infettive "Lazzaro Spallanzani", HIV/AIDS Unit, Roma, Italia ⁽²⁾ - Istituto Nazionale per le Malattie Infettive "Lazzaro Spallanzani", Epidemiology Unit, Roma, Italia ⁽³⁾ - Istituto Nazionale per le Malattie Infettive "Lazzaro Spallanzani", General Direction, Roma, Italia ⁽⁴⁾ - Istituto Nazionale per le Malattie Infettive "Lazzaro Spallanzani", Scientific Direction, Roma, Italia ⁽⁵⁾	473
Asymptomatic Mpox infection in subjects presenting for MVA-BN vaccine	473
GIULIA MATUSALI ¹ , VALENTINA MAZZOTTA ² , PIERLUCA PISELLI ³ , AURORA BETTINI ¹ , FRANCESCA COLAVITA ¹ , SABRINA COEN ¹ , FRANCESCO VAIA ⁴ , ENRICO GIRARDI ⁵ , ANDREA ANTINORI ² , FABRIZIO MAGGI ¹	473
1Laboratory of Virology, National Institute for Infectious Diseases Lazzaro Spallanzani IRCCS, Rome, Italy; 2 HIV/AIDS Unit, National Institute for Infectious Diseases Lazzaro Spallanzani IRCCS, Rome, Italy; 3 Epidemiology Unit, National Institute for Infectious Diseases Lazzaro Spallanzani IRCCS, Rome, Italy; 4 General Direction, National Institute for Infectious Diseases Lazzaro Spallanzani IRCCS Rome, Italy; 5 Scientific Direction, National Institute for Infectious Diseases Lazzaro Spallanzani IRCCS, Rome, Italy.	473
260 - Prevalence of respiratory viruses during the late pandemic and early post-pandemic months in an Italian tertiary university hospital.	475
FEDERICA NOVAZZI ⁽¹⁾ - FRANCESCA DRAGO FERRANTE ⁽²⁾ - ANGELO PAOLO GENONI ⁽¹⁾ - ANDREINA BAJ ⁽¹⁾ - RICCARDO CAPUANO ⁽²⁾ - GIANLUCA CASSANI ⁽²⁾ - ALBERTO COLOMBO ⁽²⁾ - PAOLO GIGANTE ⁽²⁾ - RENÈE PASCIUTA ⁽²⁾ - AGOSTINO ROSSI ⁽²⁾ - NICASIO MANCINI ⁽¹⁾	475

University of Insubria, Varese, Italy, Laboratory of Medical Microbiology and Virology, Department of Medicine and Technological Innovation,, Varese, Italia ⁽¹⁾ - Laboratory of Medical Microbiology and Virology,, University Hospital of Varese, Italy, Varese, Italia ⁽²⁾	475
Prevalence of respiratory viruses during the late pandemic and early post-pandemic months in an Italian tertiary university hospital.	475
Federica Novazzi ^{a,b} , Francesca Drago Ferrante ^b , Angelo Paolo Genoni ^{a,b} , Andreina Baj ^{a,b} , Riccardo Capuano ^b , Gianluca Cassani ^b , Alberto Colombo ^b , Paolo Gigante ^b , Renè Pasciuta ^b , Agostino Rossi ^b , Antonio Tamborini ^b and Nicasio Mancini ^{a,b}	475
^a Laboratory of Medical Microbiology and Virology, Department of Medicine and Technological Innovation, University of Insubria, Varese, Italy; ^b Laboratory of Medical Microbiology and Virology, University Hospital of Varese, Italy	475
267 - Frog-derived antiviral peptides (AVPs): pan-inhibitors against respiratory viruses.....	477
CARLA ZANNELLA ⁽¹⁾ - ANNALISA CHIANESE ⁽¹⁾ - ROSA GIUGLIANO ⁽¹⁾ - ANNALISA AMBROSINO ⁽¹⁾ - ALESSANDRA MONTI ⁽²⁾ - NUNZIANNA DOTI ⁽²⁾ - ANNA DE FILIPPIS ⁽¹⁾ - MASSIMILIANO GALDIERO ⁽¹⁾	477
Università degli Studi della Campania "Luigi Vanvitelli", Dipartimento di Medicina Sperimentale-Sezione Microbiologia e Virologia, Napoli, Italia ⁽¹⁾ - Institute of Biostructures and Bioimaging (IBB), National Research Council (CNR), Napoli, Italia ⁽²⁾	477
Frog-derived antiviral peptides (AVPs): pan-inhibitors against respiratory viruses	477
Carla Zannella ^{1,2} , Annalisa Chianese ^{1,2} , Rosa Giugliano ¹ , Annalisa Ambrosino ^{1,2} , Alessandra Monti ³ , Nunzianna Doti ³ , Anna De Filippis ¹ , Massimiliano Galdiero ^{1,2}	477
1 Department of Experimental Medicine, University of Campania "Luigi Vanvitelli", 80138 Naples, Italy.....	477
2 Section of Microbiology and Virology; University Hospital "Luigi Vanvitelli" of Naples, 80138 Naples, Italy.....	477
3 Institute of Biostructures and Bioimaging (IBB), National Research Council (CNR), 80131 Naples, Italy.....	477
272 - Design of Mopeia virus fusion inhibitors on GP2 glycoprotein	478
ANNALISA CHIANESE ⁽¹⁾ - CARLA ZANNELLA ⁽¹⁾ - FRANCESCA PALMA ⁽¹⁾ - ROSA GIUGLIANO ⁽¹⁾ - ALESSANDRA MONTI ⁽²⁾ - NUNZIANNA DOTI ⁽²⁾ - ANNA DE FILIPPIS ⁽¹⁾ - MASSIMILIANO GALDIERO ⁽¹⁾	478
Università, Dipartimento di Medicina Sperimentale, Università degli studi della Campania "Luigi Vanvitelli", Napoli, Italia ⁽¹⁾ - Istituto di Biostructures e Bioimaging, Consiglio Nazionale di Ricerca, Napoli, Italia ⁽²⁾	478
Design of Mopeia virus fusion inhibitors on GP2 glycoprotein	478
Annalisa Chianese ^{1,2} , Carla Zannella ^{1,2} , Francesca Palma ¹ , Rosa Giugliano ¹ , Alessandra Monti ³ , Nunzianna Doti ³ , Anna De Filippis ¹ , Massimiliano Galdiero ^{1,2}	478
1 Department of Experimental Medicine, University of Campania "Luigi Vanvitelli", 80138 Naples, Italy.....	478
2 Section of Microbiology and Virology; University Hospital "Luigi Vanvitelli" of Naples, 80138 Naples, Italy.....	478
3 Institute of Biostructures and Bioimaging (IBB), National Research Council (CNR), 80131 Naples, Italy.....	478

EMIL TONON ⁽¹⁾ - RICCARDO CECCHETTO ⁽¹⁾ - ERICA DIANI ⁽¹⁾ - ANNA LAGNI ⁽¹⁾ - VIRGINIA LOTTI ⁽¹⁾ - NICOLETTA MEDAINA ⁽²⁾ - GIONA TURRI ⁽³⁾ - MOSÈ FAVARATO ⁽⁴⁾ - DAVIDE GIBELLINI ⁽¹⁾ 479

UNIVERSITÀ DI VERONA, DIP.DIAGNOSTICA E SANITÀ PUBBLICA, VERONA, Italia ⁽¹⁾ - OSPEDALE G. B. RIOSI, UOC MICROBIOLOGIA, VERONA, Italia ⁽²⁾ - OSPEDALE, UOC MICROBIOLOGIA, ERONA, Italia ⁽³⁾ - AULSS 3, UOSD of Molecular Diagnostics and Genetics, VENEZIA, Italia ⁽⁴⁾ 479

Detection of SARS-CoV-2 large ORF8 deletion cluster in routine NGS screening for epidemiological surveillance 479

EMIL TONON^{1,2}, RICCARDO CECCHETTO^{1,2}, ERICA DIANI¹, ANNA LAGNI¹, VIRGINIA LOTTI¹, NICOLETTA MEDAINA², GIONA TURRI², MOSE' FAVARATO³, DAVIDE GIBELLINI^{1,2} 479

¹ Department of Diagnostic and Public Health, Verona University, 37134 Verona, Italy; ² UOC Microbiology, AOUI Verona, 37134 Verona, Italy; UOSD OFMolecular Diagnostics and Genetics, AULSS 3 Serenissima, Venice, Italy. 479

135 - Exploring the antibacterial effect of Lacticaseibacillus rhamnosus cell-free culture supernatants against Pseudomonas aeruginosa in an air-liquid interface lung infection model 482

MARTA BIANCHI ⁽¹⁾ - GIUSEPPANTONIO MAISETTA ⁽¹⁾ - BRUNELLA GRASSIRI ⁽²⁾ - ESINGUL KAYA ⁽¹⁾ - MICHAL MALINOWSKI ⁽³⁾ - ANNA MARIA PIRAS ⁽²⁾ - GIOVANNA BATONI ⁽¹⁾ - SEMIH ESIN ⁽¹⁾ 482

Università di Pisa, Dip. di Ricerca Trasazionale e delle nuove Tecnologie in Medicina e Chirurgia, Pisa, Italia ⁽¹⁾ - Università di Pisa, Dip. di Farmacia, Università di Pisa, Pisa, Italia ⁽²⁾ - University of Wroclaw, Medical University of Wroclaw, Wroclaw, Polonia ⁽³⁾ 482

Exploring the antibacterial effect of Lacticaseibacillus rhamnosus cell-free culture supernatants against Pseudomonas aeruginosa in an air-liquid interface lung infection model 482

146 - Different activity of Lactobacillus spp. against two Proteus mirabilis isolated clinical strains in different anatomical sites: an explorative study to improve the therapeutic approach 483

ALESSANDRA FUSCO ⁽¹⁾ - VITTORIA SAVIO ⁽¹⁾ - BRUNELLA PERFETTO ⁽¹⁾ - ADRIANA CHIAROMONTE ⁽¹⁾ - GIOVANNA TORELLI ⁽¹⁾ - GIUSY MAVILIO ⁽¹⁾ - ALBERTO ALFANO ⁽²⁾ - GIOVANNA DONNARUMMA ⁽¹⁾ 483

Università della Campania Luigi Vanvitelli, Dipartimento di Medicina Sperimentale sezione Microbiologia e Microbiologia clinica, Napoli, Italia ⁽¹⁾ - Università della Campania Luigi Vanvitelli, Dipartimento di Medicina Sperimentale sezione Biotecnologie e Biologia Molecolare, Napoli, Italia ⁽²⁾ 483

Different activity of Lactobacillus spp. against two Proteus mirabilis isolated clinical strains in different anatomical sites: an explorative study to improve the therapeutic approach 483

ALESSANDRA FUSCO¹, VITTORIA SAVIO¹, BRUNELLA PERFETTO¹, ADRIANA CHIAROMONTE¹, GIOVANNA TORELLI¹, GIUSY MAVILIO¹, ALBERTO ALFANO², GIOVANNA DONNARUMMA¹ 483

¹Department of Experimental Medicine, Section of Microbiology and Clinical Microbiology, University of Campania "Luigi Vanvitelli", Naples, Italy 483

²Department of Experimental Medicine, Section of Biotechnology and Molecular Biology, University of Campania "Luigi Vanvitelli", Naples, Italy 483

169 - Activity of cell-free supernatants from Lacticaseibacillus rhamnosus against mono and dual-species biofilms of wound pathogens in a 3D in vitro collagen wound infection model 485

ESINGUL KAYA ⁽¹⁾ - MARTA BIANCHI ⁽¹⁾ - GIUSEPPANTONIO MAISETTA ⁽¹⁾ - SEMIH ESIN ⁽¹⁾ - GIOVANNA BATONI ⁽¹⁾	485
Università Di Pisa, Dipartimento Di Ricerca Traslazionale E Nuove Tecnologie In Medicina E Chirurgia, Pisa, Italia ⁽¹⁾	485
Activity of cell-free supernatants from <i>Lactocaseibacillus rhamnosus</i> against mono and dual-species biofilms of wound pathogens in a 3D in vitro collagen wound infection model.....	485
ESINGÜL KAYA, MARTA BIANCHI, GIUSEPPANTONIO MAISETTA, SEMIH ESIN AND GIOVANNA BATONI.....	485
Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa, Pisa, Italy	485
211 - Probiotic pretreatment protects corneal epithelial cells against <i>Staphylococcus aureus</i> infection	486
IRENE PATERNITI ⁽¹⁾ - SARAH ADRIANA SCUDERI ⁽¹⁾ - LUCIA CAMBRIA ⁽¹⁾ - ANTONIA NOSTRO ⁽¹⁾ - EMANUELA ESPOSITO ⁽¹⁾ - ANDREANA MARINO ⁽¹⁾	486
Università, Università di Messina/Dipartimento di Scienze Chimiche, Biologiche, Farmaceutiche e Ambientali, Messina, Italia ⁽¹⁾	486
Probiotic pretreatment protects corneal epithelial cells against <i>Staphylococcus aureus</i> infection	486
IRENE PATERNITI, SARA A. SCUDERI, LUCIA CAMBRIA, ANTONIA NOSTRO, EMANUELA ESPOSITO, ANDREANA MARINO	486
Department of Chemical, Biological, Pharmaceutical and Environmental Sciences. University of Messina, Messina, Italy.....	486
224 - In vitro evaluation of the most active probiotic strains able to improve the intestinal barrier functions and to prevent inflammatory diseases of the gastrointestinal system	487
VITTORIA SAVIO ⁽¹⁾ - ALESSANDRA FUSCO ⁽¹⁾ - BRUNELLA PERFETTO ⁽¹⁾ - ADRIANA CHIAROMONTE ⁽¹⁾ - GIOVANNA TORELLI ⁽¹⁾ - GIUSY MAVILIO ⁽¹⁾ - MARINA ACUNZO ⁽¹⁾ - SERGIO D'AMBROSIO ⁽²⁾ - DONATELLA CIMINI ⁽²⁾ - GIOVANNA DONNARUMMA ⁽¹⁾	487
Università degli Studi della Campania 'Luigi Vanvitelli', Dipartimento di Medicina Sperimentale - Sezione Microbiologia e Microbiologia clinica, Napoli, Italia ⁽¹⁾ - Università degli Studi della Campania 'Luigi Vanvitelli', Dipartimento di Medicina Sperimentale - Sezione Biotecnologie e Biologia Molecolare, Napoli, Italia ⁽²⁾	487
In vitro evaluation of the most active probiotic strains able to improve the intestinal barrier functions and to prevent inflammatory diseases of the gastrointestinal system	487
VITTORIA SAVIO ¹ , ALESSANDRA FUSCO ¹ , BRUNELLA PERFETTO ¹ , ADRIANA CHIAROMONTE ¹ , GIOVANNA TORELLI ¹ , GIUSY MAVILIO ¹ , MARINA ACUNZO ¹ , SERGIO D'AMBROSIO ² , DONATELLA CIMINI ² , GIOVANNA DONNARUMMA ¹	487
¹ Department of Experimental Medicine, Section of Microbiology and Clinical Microbiology, University of Campania "Luigi Vanvitelli", Naples, Italy	487
² Department of Experimental Medicine, Section of Biotechnology and Molecular Biology, University of Campania "Luigi Vanvitelli", Naples, Italy	487
239 - Regulatory effects of <i>Lactiplantibacillus plantarum</i> on human skin health by counteract <i>Malassezia furfur</i> effects.	488

BRUNELLA PERFETTO ⁽¹⁾ - ALESSANDRA FUSCO ⁽¹⁾ - VITTORIA SAVIO ⁽¹⁾ - ADRIANA CHIAROMONTE ⁽¹⁾ - GIUSY MAVILIO ⁽¹⁾ - GIOVANNA TORELLI ⁽¹⁾ - CARLA CAPASSO ⁽¹⁾ - GIOVANNA DONNARUMMA ⁽¹⁾	488
Università degli Studi della Campania "Luigi Vanvitelli", Dipartimento di Medicina Sperimentale; Sezione di Microbiologia, Napoli, Italia ⁽¹⁾	488
Regulatory effects of Lactiplantibacillus plantarum on human skin health by counteract.....	488
Malassezia furfur effects.....	488
BRUNELLA PERFETTO, ALESSANDRA FUSCO, VITTORIA SAVIO, ADRIANA CHIAROMONTE, GIOVANNA TORELLI, GIUSY MAVILIO, CARLA CAPASSO, GIOVANNA DONNARUMMA	488
Department of Experimental Medicine, Section of Microbiology and Clinical Microbiology, University of Campania "Luigi Vanvitelli", Naples, Italy	488
319 - Anti-virulence effect of Lactobacillus rhamnosus and Lactobacillus plantarum cell-free supernatants on P. aeruginosa from cystic fibrosis patients	489
VERONICA LUPETTI ⁽¹⁾ - GIOVANNI DI BONAVENTURA ⁽¹⁾ - GIUSEPPANTONIO MAISETTA ⁽²⁾ - ESINGÜL KAYA ⁽²⁾ - SEMIH ESIN ⁽²⁾ - GIOVANNA BATONI ⁽²⁾ - ARIANNA POMPILIO ⁽¹⁾	489
Department of Medical, Oral and Biotechnological Sciences, "G. d'Annunzio" University of Chieti-Pescara, Chieti, Italia ⁽¹⁾ - Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa, Pisa, Italia ⁽²⁾	489
Anti-virulence effect of Lactobacillus rhamnosus and Lactobacillus plantarum cell-free supernatants on P. aeruginosa from cystic fibrosis patients	489
VERONICA LUPETTI,¹ GIOVANNI DI BONAVENTURA,¹ GIUSEPPANTONIO MAISETTA,² ESINGÜL KAYA,² SEMIH ESIN,² GIOVANNA BATONI,² ARIANNA POMPILIO¹	489
¹Department of Medical, Oral and Biotechnological Sciences, and Center of Advanced Studies and Technologies (CAST), "G. d'Annunzio" University of Chieti-Pescara, Chieti, Italy. ²Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa, Pisa, Italy.....	489
11 Resistenza ai farmaci antimicrobici/Terapia antimicrobica/Sensibilità agli antimicrobici e risvolti terapeutici.....	490
10 - Synergistic effects of antivirals and monoclonal antibodies in vitro against SARS-CoV-2 Wild Type B.1 strain and BQ.1.1 Omicron sublineage.....	490
LIA FIASCHI ⁽¹⁾ - CAMILLA BIBA ⁽¹⁾ - ILENIA VARASI ⁽¹⁾ - NICCOLÒ BARTOLINI ⁽¹⁾ - CHIARA PALETTI ⁽¹⁾ - FEDERICA GIAMMARINO ⁽¹⁾ - FRANCESCO SALADINI ⁽¹⁾ - MAURIZIO ZAZZI ⁽¹⁾ - ILARIA VICENTI ⁽¹⁾	490
Dipartimento di Biotechnologie Mediche, Università di Siena, Siena, Italia ⁽¹⁾	490
Synergistic effects of antivirals and monoclonal antibodies in vitro against SARS-CoV-2 Wild Type B.1 strain and BQ.1.1 Omicron sublineage.....	490
LIA FIASCHI¹, CAMILLA BIBA¹, ILENIA VARASI¹, NICCOLÒ BARTOLINI¹, CHIARA PALETTI¹, FEDERICA GIAMMARINO¹, FRANCESCO SALADINI¹, MAURIZIO ZAZZI¹, ILARIA VICENTI¹.....	490
¹Department of Medical Biotechnologies, University of Siena, Siena, Italy	490

13 - SigH stress response mediates killing of Mycobacterium tuberculosis by activating nitronaphthofuran prodrugs	492
FRANCESCA BOLDRIN ⁽¹⁾	492
UNIVERSITA' DEGLI STUDI DI PADOVA, DIP. DI MEDICINA MOLECOLARE, PADOVA, Italia ⁽¹⁾	492
SigH stress response mediates killing of Mycobacterium tuberculosis by activating nitronaphthofuran prodrugs.....	492
FRANCESCA BOLDRIN ¹ , LAURA CIOETTO-MAZZABÒ ¹ CLAIRE BEAUVINEAU ² , MARTIN SPETH ³ , ALBERTO MARINA ⁴ , AMINE NAMOUCHI ^{5,6} , GRETA SEGAFREDDO ¹ , MENA CIMINO ⁵ , SANDRINE FAVRE-ROCHEX ⁵ , SEETHA BALASINGHAM ⁷ , BEATRIZ TRASTOY ^{4,8} , HÉLÈNE MUNIER-LEHMANN ⁹ , GARETH GRIFFITHS ³ , BRIGITTE GICQUEL ^{5,10} , MARCELO E. GUERIN ^{4,8,11} , RICCARDO MANGANELLI ¹ , NOELIA ALONSO-RODRÍGUEZ.....	492
¹ Department of Molecular Medicine, University of Padova, Padova 35122, Italy; ² Chemical Library Institut Curie/CNRS, CNRS UMR9187, INSERM U1196 and CNRS UMR3666, INSERM U1193, Université Paris-Saclay, Orsay 91405, France; ³ Department Biosciences, Faculty of Mathematics and Natural Sciences, University of Oslo, Oslo 0371, Norway; ⁴ Center for Cooperative Research in Biosciences (CIC bioGUNE), Basque Research and Technology Alliance (BRTA), Bizkaia Technology Park, Derio 48160 Spain; ⁵ Génétique Mycobactérienne, Institut Pasteur, Paris 75015, France; ⁶ Centre for Ecological and Evolutionary Synthesis (CEES), Department of Biosciences, University of Oslo, Oslo 0371, Norway; ⁷ Department of Microbiology, Oslo University Hospital, Oslo 0450, Norway; ⁸ Structural Glycobiology Laboratory, Biocruces Bizkaia Health Research Institute, Cruces University Hospital, Bizkaia 48903, Spain; ⁹ Département de Biologie Structurale et Chimie, Institut Pasteur, CNRS UMR3523, Université de Paris, Paris 75015, France; ¹⁰ Department of Tuberculosis Control and Prevention, Shenzhen Nanshan Centre for Chronic Disease Control, Shenzhen 518054, China; ¹¹ IKERBASQUE, Basque Foundation for Science, Bilbao 48009, Spain.	
17 - abcd	494
SARAH TORELLI ⁽¹⁾	494
Ciao, Ciao, Roma, Italia ⁽¹⁾	494
24 - Strepto Killer, qu'est-ce que c'est?: Identification of compounds inhibiting streptococcal cell division by targeting FtsZ as a novel strategy to fight Gram-positive ovococcal pathogens	496
BERENICE FURLAN ⁽¹⁾ - VALENTINA STRANIERO ⁽²⁾ - LORENZO SUIGO ⁽²⁾ - ERMANNO VALOTI ⁽²⁾ - ORIETTA MASSIDDA ⁽¹⁾	496
Dipartimento di Biologia Computazionale, Cellulare e Integrata, Università di Trento, Trento, Italia ⁽¹⁾ - Department of Pharmaceutical Sciences, Università di Milano, Milano, Italia ⁽²⁾	496
Strepto Killer, qu'est-ce que c'est?: Identification of compounds inhibiting streptococcal cell division by targeting FtsZ, as a novel strategy to fight Gram-positive ovococcal pathogens.....	496
Berenice Furlan ^{1*} , Valentina Straniero ^{2*} , Lorenzo Suigo ² , Ermanno Valoti ² , Orietta Massidda ¹	496
¹ Department of Cellular, Computational and Integrative Biology (DiCIBIO), University of Trento, Italy; ² Department of Pharmaceutical Sciences (DiSFarm), University of Milan, Milano, Italy .	
33 - Role of transcriptomic and genomic analyses in a step ahead in the comprehension of cefiderocol activity in A. baumannii.....	498

STEFANO STRACQUADANIO ⁽¹⁾ - ALICE NICOLOSI ⁽¹⁾ - GRETE PRIVITERA ⁽²⁾ - MARIACRISTINA MASSIMINO ⁽³⁾ - ANDREA MARINO ⁽⁴⁾ - DAFNE BONGIORNO ⁽¹⁾ - STEFANIA STEFANI ⁽¹⁾	498
Università degli Studi di Catania, Dipartimento di Scienze Biomediche e Biotecnologiche, Catania, Italia ⁽¹⁾ - Università degli Studi di Catania, Dipartimento di Medicina Clinica e Sperimentale, Catania, Italia ⁽²⁾ - Università di Pisa, Dipartimento di ricerca traslazionale e nuove tecnologie in medicina e chirurgia, Pisa, Italia ⁽³⁾ - Università degli Studi di Catania, ARNAS Garibaldi - Unità di malattie infettive, Catania, Italia ⁽⁴⁾	498
Role of transcriptomic and genomic analyses in a step ahead in the comprehension of cefiderocol activity in <i>A. baumannii</i>	498
STEFANO STRACQUADANIO ¹ , ALICE NICOLOSI ¹ , GRETE F. PRIVITERA ² , MARIACRISTINA MASSIMINO ³ , ANDREA MARINO ⁴ , DAFNE BONGIORNO ¹ , STEFANIA STEFANI ¹	498
1. Dipartimento di Scienze Biomediche e Biotecnologiche (BIOMETEC), laboratorio di Microbiologia Medica Molecolare e Antibiotico Resistenza – Università degli Studi di Catania – Catania, Italia; 2. Dipartimento di Medicina Clinica e Sperimentale, Unità di Bioinformatica – Università degli Studi di Catania – Catania, Italia; 3. Dipartimento di ricerca traslazionale e nuove tecnologie in medicina e chirurgia – Università di Pisa – Pisa, Italia; 4. Dipartimento di Medicina Clinica e Sperimentale, Unità di Malattie Infettive – ARNAS Garibaldi – Catania, Italia.	498
37 - Study of the mechanism of action of the novel antitubercular compound 11826350	500
DEBORAH RECCHIA ⁽¹⁾ - GHERARD BATISTI BIFFIGNANDI ⁽²⁾ - ANNA EGOROVA ⁽³⁾ - ANTONIO MARINO CERRATO ⁽²⁾ - ELENA FRANCHI ⁽²⁾ - LAURENT R. CHIARELLI ⁽²⁾ - DAVIDE SASSERA ⁽²⁾ - VADIM MAKAROV ⁽³⁾ - MARIA ROSALIA PASCA ⁽²⁾ - GIULIA DEGIACOMI ⁽²⁾	500
Università di Pavia, Dipartimento di Biologia e Biotecnologie "Lazzaro Spallanzani", Pavia, Italia ⁽¹⁾ - University of Pavia, Department of Biology and Biotechnology "Lazzaro Spallanzani", Pavia, Italia ⁽²⁾ - Research Center of Biotechnology Russian Academy of Science, a, Mosca, Russia ⁽³⁾ .	500
Study of the mechanism of action of the novel antitubercular compound 11826350.....	500
DEBORAH RECCHIA ¹ , GHERARD BATISTI BIFFIGNANDI ¹ , ANNA EGOROVA ² , ANTONIO MARINO CERRATO ¹ , ELENA FRANCHI ¹ , LAURENT R. CHIARELLI ¹ , DAVIDE SASSERA ^{1,3} , VADIM MAKAROV ² , MARIA ROSALIA PASCA ^{1,3,*} , GIULIA DEGIACOMI ^{1,*} .	500
1 Department of Biology and Biotechnology "Lazzaro Spallanzani", University of Pavia, Pavia, Italy.	500
2 Research Center of Biotechnology Russian Academy of Science, Moscow, Russia.....	500
3 Fondazione IRCCS Policlinico San Matteo, Pavia, Italy.	500
* Co-corresponding authors	500
38 - In vivo cefiderocol resistance escalation and intra-host evolution of KPC-3 and VIM-1 co- producing <i>Klebsiella pneumoniae</i> Sequence Type 512.....	502
GABRIELE ARCARI ⁽¹⁾ - FEDERICO CECILIA ⁽¹⁾ - ALESSANDRA OLIVA ⁽²⁾ - RICCARDO POLANI ⁽¹⁾ - GIAMMARCO RAPONI ⁽²⁾ - ALICE DE FRANCESCO ⁽¹⁾ - FEDERICA SACCO ⁽²⁾ - GUIDO ANTONELLI ⁽¹⁾ - ALESSANDRA CARATTOLI ⁽¹⁾	502
University, Department of Molecular Medicine, Sapienza University of Rome, Rome, Italy, Roma, Italia ⁽¹⁾ - University, Department of Public Health and Infectious Diseases, Sapienza University of Rome, Rome, Italy, Roma, Italia ⁽²⁾	502

In vivo cefiderocol resistance escalation and intra-host evolution of KPC-3 and VIM-1 co-producing <i>Klebsiella pneumoniae</i> Sequence Type 512	502
GABRIELE ARCARI¹, FEDERICO CECILIA¹, ALESSANDRA OLIVA², RICCARDO POLANI¹, GIAMMARCO RAPONI², FEDERICA SACCO², ALICE DE FRANCESCO¹, GUIDO ANTONELLI¹ and ALESSANDRA CARATTOLI¹	502
1 Department of Molecular Medicine, Sapienza University of Rome, Rome, Italy; 2 Department of Public Health and Infectious Diseases, Sapienza University of Rome, Rome, Italy	502
39 - The Fitness Omic burden associated to new Adaptive Reduced-Glycopeptide-Susceptibility Network in CA-MRSA	504
ELEONORA CHINES ⁽¹⁾ - MARTINA PAPPALARDO ⁽¹⁾ - CARLOTTA ZIMBONE ⁽¹⁾ - ELISA BELLA ⁽¹⁾ - STEFANIA STEFANI ⁽¹⁾ - VIVIANA CAFISO ⁽¹⁾	504
Università degli Studi di Catania, Università di Catania/ BIOMETEC, Catania, Italia ⁽¹⁾	504
The Fitness Omic burden associated to new Adaptive Reduced-Glycopeptide-Susceptibility Network in CA-MRSA	504
ELEONORA CHINES ¹, MARTINA PAPPALARDO¹, CARLOTTA ZIMBONE¹, ELISA BELLA¹, STEFANIA STEFANI ¹, VIVIANA CAFISO ¹	504
¹ BIOMETEC Dept - University of Catania - Catania (Italy).....	504
42 - Shift between echinocandins through computerized prescribing software and antifungal resistance profiles	505
GIACOMO BERTOLINO ⁽¹⁾ - RAFFAELE DEIDDA ⁽¹⁾ - ROSSELLA M. GHIANI ⁽²⁾ - ROBERTO MURA ⁽¹⁾ - SILVIA VARGIU ⁽¹⁾ - RAFFAELLO POMPEI ⁽³⁾ - ELENA POMA ⁽⁴⁾ - ELISA CASULA ⁽⁴⁾ - PATRIZIA FERRAGUTI ⁽⁴⁾ - FERDINANDO COGHE ⁽⁴⁾ - ARIANNA CADEDU ⁽¹⁾	505
Servizio di Farmacia, Azienda Ospedaliero Universitaria di Cagliari, Cagliari, Italia ⁽¹⁾ - SOL Group, SOL, Cagliari, Italia ⁽²⁾ - RES-S, APE (No Profit Organization), Cagliari, Italia ⁽³⁾ - Clinical Analysis Laboratory, Azienda Ospedaliero Universitaria di Cagliari, Cagliari, Italia ⁽⁴⁾	505
Shift between echinocandins through computerized prescribing software and antifungal resistance profiles	505
GIACOMO BERTOLINO⁽¹⁾, RAFFAELE DEIDDA⁽¹⁾, ROSSELLA M. GHIANI⁽²⁾, ROBERTO MURA⁽¹⁾, SILVIA VARGIU⁽¹⁾, RAFFAELLO POMPEI⁽³⁾, ELENA POMA⁽⁴⁾, CASULA ELISA⁽⁴⁾, PATRIZIA FERRAGUTI⁽⁴⁾, FERDINANDO COGHE⁽⁴⁾, ARIANNA CADEDU⁽¹⁾.....	505
1) Pharmaceutical Department, Azienda Ospedaliero Universitaria di Cagliari, Cagliari, Italy	505
2) Pharmacist - SOL Group, Cagliari, Italy	505
3) RES-R, APS No Profit Organization, Capoterra, Italy	505
4) Clinical Analysis Laboratory – Azienda Ospedaliero Universitaria di Cagliari, Cagliari, Italy	505
45 - Antimicrobial resistance prediction of <i>Escherichia coli</i> strains to Ampicillin via machine learning models on MALDI-TOF mass spectra.....	507
ETTORE ROCCHI ⁽¹⁾ - MAURO FACCIN ⁽¹⁾ - CLAUDIA SALA ⁽¹⁾ - DONATELLA LOMBARDO ⁽²⁾ - SIMONE AMBRETTI ⁽²⁾ - GASTONE CASTELLANI ⁽¹⁾	507
Università di Bologna, Dipartimento di Scienze Mediche e Chirurgiche, Bologna, Italia ⁽¹⁾ - IRCCS Azienda Ospedaliero-Universitaria di Bologna, UO Microbiologia, Bologna, Italia ⁽²⁾ ...	507

Antimicrobial resistance prediction of <i>Escherichia coli</i> strains to Ampicillin via machine learning models on MALDI-TOF mass spectra	507
ETTORE ROCCHI ¹ , MAURO FACCIN ¹ , CLAUDIA SALA ¹ , DONATELLA LOMBARDO ² , SIMONE AMBRETTI ² , GASTONE CASTELLANI ¹	507
¹ Department of Medical and Surgical Sciences, Università di Bologna, Bologna, Italy;	
² Microbiology Unit, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy	507
48 - <i>Campylobacter</i> spp. infections in Italy: a 5-year multicenter retrospective study	509
VERENA ZERBATO ⁽¹⁾ - STEFANO DI BELLA ⁽¹⁾ - RICCARDO POL ⁽¹⁾ - SIMONE AMBRETTI ⁽²⁾ - STEFANO ANDREONI ⁽³⁾ - RICHARD ASCHBACHER ⁽⁴⁾ - MARIANO BERNARDO ⁽⁵⁾ - ALESSANDRA BIELLI ⁽⁶⁾ - GIOCONDA BRIGANTE ⁽⁷⁾ - MARINA Busetti ⁽⁸⁾ - GIULIO CAMARLINGHI ⁽⁹⁾ - DAVIDE CARCIONE ⁽⁷⁾ - ANTONELLA CARDUCCI ⁽⁷⁾ - EDOARDO CARRETTO ⁽¹⁰⁾ - CHIARA CHILLERI ⁽¹¹⁾ - NICOLA CLEMENTI ⁽¹²⁾ - ALESSANDRA CONSONNI ⁽¹³⁾ - VENERE CORTAZZO ⁽¹⁴⁾ - SAVERIA DODARO ⁽¹⁵⁾ - BARBARA FIORI ⁽¹⁴⁾ - AURORA GARCIA FERNANDEZ ⁽¹⁶⁾ - ELISA GOBBATO ⁽³⁾ - FRANCESCA GRECO ⁽¹⁵⁾ - NICASIO MANCINI ⁽¹⁷⁾ - ALBERTO ENRICO MARAOLO ⁽¹⁸⁾ - ROBERTA MARROLLO ⁽¹⁰⁾ - CAROLA MAURI ⁽¹³⁾ - ANNARITA MAZZARIOL ⁽¹⁹⁾ - GIANLUCA MORRONI ⁽²⁰⁾ - ADRIANA MOSCA ⁽²¹⁾ - GIACOMO NIGRISOLI ⁽²⁾ - ELISABETTA PAGANI ⁽⁴⁾ - EVA MARIA PARISIO ⁽⁹⁾ - SIMONA POLLINI ⁽¹¹⁾ - MARIO SARTI ⁽²²⁾ - ANNA MARCHESE ⁽²³⁾ - GIULIA CODDA ⁽²³⁾ - ROBERTO LA RAGIONE ⁽²⁴⁾ - ANNARITA SORRENTINO ⁽¹⁹⁾ - DOMENICO TROTTA ⁽²⁰⁾ - LAURA VILLA ⁽¹⁶⁾ - CHIARA VISMARA ⁽⁶⁾ - GIANFRANCO SANSON ⁽¹⁾ - ROBERTO LUZZATI ⁽¹⁾ - LUIGI PRINCIPE ⁽²⁵⁾	509
Università di Trieste, Dipartimento di Scienze Mediche, Chirurgiche e della Salute, UOC Malattie Infettive, Trieste, Italia ⁽¹⁾ - Policlinico Universitario Sant'Orsola-Malpighi di Bologna, UOC Microbiologia e Virologia, Bologna, Italia ⁽²⁾ - Azienda Ospedaliero Universitaria Maggiore della Carità di Novara, UOC Microbiologia e Virologia, Novara, Italia ⁽³⁾ - Comprensorio Sanitario di Bolzano, Azienda Sanitaria dell'Alto Adige, Laboratorio Aziendale di Microbiologia e Virologia, Bolzano, Italia ⁽⁴⁾ - Ospedale Monaldi, AORN dei Colli, UOC Microbiologia e Virologia, Napoli, Italia ⁽⁵⁾ - ASST Grande Ospedale Metropolitano Niguarda, UOC Microbiologia e Virologia, Milano, Italia ⁽⁶⁾ - ASST Valle Olona, UOC Medicina di Laboratorio, Busto Arsizio, Italia ⁽⁷⁾ - Ospedale Universitario di Trieste, UOC Microbiologia e Virologia, Trieste, Italia ⁽⁸⁾ - Azienda USL Toscana sud Est, Arezzo, Dipartimento di Medicina di Laboratorio e Trasfusionale, UOSD Microbiologia, Arezzo, Italia ⁽⁹⁾ - IRCCS Arcispedale Santa Maria Nuova, Laboratorio di Microbiologia Clinica, Reggio Emilia, Italia ⁽¹⁰⁾ - Policlinico Universitario Careggi, UOC Microbiologia e Virologia, Firenze, Italia ⁽¹¹⁾ - IRCCS Policlinico Universitario "San Raffaele", Servizio di Medicina di Laboratorio, Unità di Microbiologia e Virologia, Milano, Italia ⁽¹²⁾ - Ospedale "A. Manzoni", UOC Microbiologia e Virologia, Lecco, Italia ⁽¹³⁾ - Policlinico Universitario "A. Gemelli", UOC Microbiologia, Roma, Italia ⁽¹⁴⁾ - Ospedale "Annunziata", UOC Microbiologia e Virologia, Cosenza, Italia ⁽¹⁵⁾ - Istituto Superiore di Sanità, Dipartimento di Malattie Infettive, Roma, Italia ⁽¹⁶⁾ - Azienda Ospedaliero Universitaria Fondazione Macchi, Dipartimento di Medicina ed Innovazione Tecnologica; Laboratorio di Microbiologia e Virologia, Varese, Italia ⁽¹⁷⁾ - Ospedale Cotugno, AORN dei Colli, Prima Divisione di Malattie Infettive, Napoli, Italia ⁽¹⁸⁾ - Azienda Ospedaliero Universitaria Integrata di Verona, UOC Microbiologia e Virologia, Verona, Italia ⁽¹⁹⁾ - Università Politecnica delle Marche, Dipartimento di Scienze Biomediche e Salute Pubblica, Unità di Microbiologia, Ancona, Italia ⁽²⁰⁾ - Policlinico Universitario "Aldo Moro", Dipartimento Interdisciplinare di Medicina, Bari, Italia ⁽²¹⁾ - Azienda Ospedaliero Universitaria Policlinico, UOC Microbiologia e Virologia, Modena, Italia ⁽²²⁾ - Policlinico Universitario San Martino, UOC Microbiologia e Virologia, Genova, Italia ⁽²³⁾ - University of Surrey, Department of Pathology and Infectious Diseases, School of Veterinary	

Medicine, Guildford, Regno Unito ⁽²⁴⁾ - Azienda Sanitaria Provinciale di Crotone, Ospedale "S. Giovanni di Dio" - UOSD Patologia Clinica e Microbiologia, Crotone, Italia ⁽²⁵⁾	509
Section: Resistenza ai farmaci antimicrobici/Terapia antimicrobica/Sensibilità agli antimicrobici e risvolti terapeutici.....	510
Title - Campylobacter spp. infections in Italy: a 5-year multicenter retrospective study	510
Authors: Verena Zerbato ¹ , Stefano Di Bella ¹ , Riccardo Pol ¹ , Simone Ambretti ² , Stefano Andreoni ³ , Richard Aschbacher ⁴ , Mariano Bernardo ⁵ , Alessandra Bielli ⁶ , Gioconda Brigante ⁷ , Marina Busetti ⁸ , Giulio Camarlinghi ⁹ , Davide Carcione ⁷ , Antonella Carducci ⁷ , Edoardo Carretto ¹⁰ , Chiara Chilleri ¹¹ , Clementi Nicola ¹² , Alessandra Consonni ¹³ , Venere Cortazzo ¹⁴ , Saveria Dodaro ¹⁵ , Barbara Fiori ¹⁴ , Aurora Garcia Fernandez ¹⁶ , Elisa Gobbato ³ , Francesca Greco ¹⁵ , Nicasio Mancini ¹⁷ , Alberto Enrico Maraolo ¹⁸ , Roberta Marrollo ¹⁰ , Carola Mauri ¹³ , Annarita Mazzariol ¹⁹ , Gianluca Morroni ²⁰ , Adriana Mosca ²¹ , Giacomo Nigrisoli ² , Elisabetta Pagani ⁴ , Eva Maria Parisio ⁹ , Simona Pollini ¹¹ , Mario Sarti ²² , Anna Marchese ²³ , Giulia Codda ²³ , Roberto La Ragione ²⁴ , Annarita Sorrentino ¹⁹ , Domenico Trotta ²⁰ , Laura Villa ¹⁶ , Chiara Vismara ⁶ , Gianfranco Sanson ¹ , Roberto Luzzati ¹ , Luigi Principe ²⁵	510
Affiliations:.....	510
¹ Dipartimento di Scienze Mediche, Chirurgiche e della Salute, UOC Malattie Infettive, Università di Trieste, Italia;	510
² UOC Microbiologia e Virologia, Policlinico Universitario Sant'Orsola-Malpighi di Bologna, Italia;.....	510
³ UOC Microbiologia e Virologia, Azienda Ospedaliero Universitaria Maggiore della Carità di Novara, Italia;	510
⁴ Laboratorio Aziendale di Microbiologia e Virologia, Comprensorio Sanitario di Bolzano, Azienda Sanitaria dell'Alto Adige, Bolzano, Italia;	510
⁵ UOC Microbiologia e Virologia, Ospedale Monaldi, AORN dei Colli, Napoli, Italia;	510
⁶ UOC Microbiologia e Virologia, ASST Grande Ospedale Metropolitano Niguarda, Milano, Italia;.....	510
⁷ UOC Medicina di Laboratorio, ASST Valle Olona, Busto Arsizio, Italia;	510
⁸ UOC Microbiologia e Virologia, Ospedale Universitario, Trieste, Italia;	510
⁹ Dipartimento di Medicina di Laboratorio e Trasfusionale, UOSD Microbiologia, Azienda USL Toscana sud Est, Arezzo, Italia;	510
¹⁰ Laboratorio di Microbiologia Clinica, IRCCS Arcispedale Santa Maria Nuova, Reggio Emilia, Italia;.....	510
¹¹ UOC Microbiologia e Virologia, Policlinico Universitario Careggi, Firenze, Italia;	510
¹² Servizio di Medicina di Laboratorio, Unità di Microbiologia e Virologia, IRCCS Policlinico Universitario "San Raffaele", Milano, Italia;.....	510
¹³ UOC Microbiologia e Virologia, Ospedale "A. Manzoni", Lecco, Italia;.....	510
¹⁴ UOC Microbiologia, Policlinico Universitario "A. Gemelli", Roma, Italia;	510
¹⁵ UOC Microbiologia e Virologia, Ospedale "Annunziata", Cosenza, Italia;	510
¹⁶ Dipartimento di Malattie Infettive, Istituto Superiore di Sanità, Roma, Italia;.....	510
¹⁷ Dipartimento di Medicina ed Innovazione Tecnologica; Laboratorio di Microbiologia e Virologia, Azienda Ospedaliero Universitaria Fondazione Macchi, Varese, Italia;.....	510

¹⁸ Prima Divisione di Malattie Infettive, Ospedale Cotugno, AORN dei Colli, Napoli, Italia;	510
¹⁹ UOC Microbiologia e Virologia, Azienda Ospedaliero Universitaria Integrata di Verona, Verona, Italia;.....	510
²⁰ Dipartimento di Scienze Biomediche e Salute Pubblica, Unità di Microbiologia, Università Politecnica delle Marche, Ancona, Italia;.....	511
²¹ Dipartimento Interdisciplinare di Medicina, Policlinico Universitario “Aldo Moro”, Bari, Italia;	511
²² UOC Microbiologia e Virologia, Azienda Ospedaliero Universitaria Policlinico, Modena, Italia;	511
²³ UOC Microbiologia e Virologia, Policlinico Universitario San Martino, Genova, Italia;.....	511
²⁴ Department of Pathology and Infectious Diseases, School of Veterinary Medicine, University of Surrey, Guildford, UK;.....	511
²⁵ UOSD Patologia Clinica e Microbiologia, Ospedale “S. Giovanni di Dio”, Crotone, Italia.....	511
53 - Genome sequence analysis of infertility-associated Enterococcus faecalis strains revealed the presence of the novel Tn7086 carrying aminoglycoside resistance genes	513
LORENZO COLOMBINI ⁽¹⁾ - SUSANNA RICCI ⁽¹⁾ - MARIANA TIRZIU ⁽¹⁾ - ELISA LAZZERI ⁽¹⁾ - ANNA MARIA CUPPONE ⁽¹⁾ - STEFANO DE GIORGI ⁽¹⁾ - DAVID PINZAUTI ⁽¹⁾ - FRANCESCO SANTORO ⁽¹⁾ - FRANCESCO IANNELLI ⁽¹⁾ - GIANNI POZZI ⁽¹⁾.....	513
Laboratorio di Microbiologia Molecolare e Biotecnologia (LAMMB), Università degli Studi di Siena/ Dipartimento di Biotecnologie Mediche, Siena, Italia ⁽¹⁾	513
Genome sequence analysis of infertility-associated Enterococcus faecalis strains revealed the presence of the novel Tn7086 carrying aminoglycoside resistance genes	513
LORENZO COLOMBINI, SUSANNA RICCI, MARIANA TIRZIU, ELISA LAZZERI, ANNA M. CUPPONE, STEFANO DE GIORGI, DAVID PINZAUTI, FRANCESCO SANTORO, FRANCESCO IANNELLI, GIANNI POZZI.....	513
Department of Medical Biotechnologies, Laboratory of Molecular Microbiology and Biotechnology (LA.M.M.B.), University of Siena, Siena, Italy	513
58 - Multiclonal presence of convergent ceftazidime/avibactam resistant and hypervirulent Klebsiella pneumoniae clinical isolates: when resistance meets virulence	515
AURORA PIAZZA ⁽¹⁾ - DAVIDE CARCIONE ⁽²⁾ - ILARIA PETRIZZI ⁽¹⁾ - GHERARD BATISTI BIFFIGNANDI ⁽³⁾ - VITTORIA MATTIONI MARCHETTI ⁽¹⁾ - GIOCONDA BRIGANTE ⁽²⁾ - ROBERTA MIGLIAVACCA ⁽¹⁾	515
Università di Pavia, Dipartimento di Scienze Clinico Chirurgiche Diagnostiche e Pediatriche, Pavia, Italia ⁽¹⁾ - ASST Valle Olona, Clinical Pathology Laboratory, Busto Arsizio, Italia ⁽²⁾ - Università di Pavia, Dipartimento di Biologia e Biotecnologie "L. Spallanzani", Pavia, Italia ⁽³⁾.....	515
Multiclonal presence of convergent ceftazidime/avibactam resistant and hypervirulent Klebsiella pneumoniae clinical isolates: when resistance meets virulence	515
Aurora Piazza¹, Davide Carcione², Iliara Petrizzi¹, Gherard Batisti Biffignandi³, Vittoria Mattioni Marchetti¹, Gioconda Brigante², Roberta Migliavacca¹	515
¹Department of Clinical-Surgical Diagnostic and Pediatric Sciences, Unit of Microbiology and Clinical Microbiology, University of Pavia, Pavia, Italy; ²Clinical Pathology Laboratory, ASST Valle Olona, Busto Arsizio, Italy; ³Department of Biology and Biotechnology "L. Spallanzani", University of Pavia, Pavia, Italy.....	515

62 - KPC-166, a novel KPC variant involved in ceftazidime/avibactam resistance	517
FRANCESCA PISCOPIELLO ⁽¹⁾ - ALESSANDRA BIELLI ⁽²⁾ - VITTORIA MATTIONI MARCHETTI ⁽¹⁾ - RICCARDO GIUDICI ⁽³⁾ - AURORA PIAZZA ⁽¹⁾ - LIVIA TARTAGLIONE ⁽²⁾ - GHERARD BATISTI BIFFIGNANDI ⁽⁴⁾ - MARCO MERLI ⁽⁵⁾ - ROBERTA MIGLIAVACCA ⁽¹⁾ - CHIARA VISMARA ⁽²⁾.....	
517	
Università di Pavia, Dipartimento di Scienze Clinico Chirurgiche Diagnostiche e Pediatriche, Pavia, Italia ⁽¹⁾ - ASST Grande Ospedale Metropolitano Niguarda, Chemical-clinical and Microbiological Analysis, Milano, Italia ⁽²⁾ - ASST Grande Ospedale Metropolitano Niguarda, Department of Anesthesia and Critical Care, Milano, Italia ⁽³⁾ - Università di Pavia, Dipartimento di Biologia e Biotecnologie "L. Spallanzani", Pavia, Italia ⁽⁴⁾ - ASST Grande Ospedale Metropolitano Niguarda, Infectious Diseases Clinic, Milano, Italia ⁽⁵⁾	
517	
KPC-166, a novel KPC variant involved in ceftazidime/avibactam resistance	517
Francesca Piscopiello ¹ , Alessandra Bielli ² , Vittoria Mattioni Marchetti ¹ , Riccardo Giudici ³ , Aurora Piazza ¹ , Livia Tartaglione ² , Gherard Batisti Biffignandi ^{1,4} , Marco Merli ⁵ , Roberta Migliavacca ¹ , Chiara Vismara ²	
517	
Department of Clinical-Surgical, Diagnostic and Pediatric Sciences, Unit of Microbiology and Clinical Microbiology, University of Pavia, Pavia, Italy; Chemical-clinical and Microbiological Analysis, ASST Grande Ospedale Metropolitano Niguarda, Milan, Italy; Department of Anesthesia and Critical Care, ASST Grande Ospedale Metropolitano Niguarda, Milan, Italy; Infectious Diseases Clinic, ASST Grande Ospedale Metropolitano Niguarda, Milan, Italy; Department of Biology and Biotechnology "L. Spallanzani", University of Pavia, Pavia, Italy..	
517	
64 - Surveillance of Clostridioides difficile infection in the Pisa University Hospital: a correlation between clinical, environmental and pharmaceutical features.....	519
MICHELE TOTARO ⁽¹⁾ - ANDREA PORRETTA ⁽²⁾ - GIULIA GEMIGNANI ⁽³⁾ - ANGELO BAGGIANI ⁽²⁾ - IELIZZA DESIDERI ⁽⁴⁾ - CLAUDIA CARMIGNANI ⁽⁴⁾ - EMILIA GHELARDI ⁽²⁾.....	
519	
Università di Pisa, Scuola di Specializzazione in Microbiologia e Virologia, Pisa, Italia ⁽¹⁾ - Università di Pisa, Dipartimento di Ricerca Traslazionale NTMC, Pisa, Italia ⁽²⁾ - Azienda Ospedaliera Universitaria Pisana, Organizzazione Servizi Ospedalieri (OSO), Pisa, Italia ⁽³⁾ - Azienda Ospedaliera Universitaria Pisana, Farmacia Ospedaliera, Pisa, Italia ⁽⁴⁾	
519	
Title:.....	519
Surveillance of Clostridioides difficile infection in the Pisa University Hospital: a correlation between clinical, environmental and pharmaceutical features.	
519	
Authors:	519
Michele Totaro ^{1,2} , Andrea Porretta ^{2,3} , Giulia Gemignani ³ , Angelo Baggiani ^{2,3} , Ielizza Desideri ³ , Claudia Carmignani ³ , Emilia Ghelardi ^{2,3}	
519	
Affiliations:.....	519
¹ Specialization School in Microbiology and Virology, University of Pisa, Italy.....	
519	
² Department of Translational Research in New Technologies in Medicine and Surgery, University of Pisa, Italy.....	
519	
³ The Azienda Ospedaliero-Universitaria Pisana, Pisa, Italy	
519	
65 - Study of the role of wild boar (Sus Scrofa) as a reservoir of antibiotic resistance	521

RUBINA PARADISO ⁽¹⁾ - GIORGIA BORRIELLO ⁽¹⁾ - NICOLA D'ALESSIO ⁽¹⁾ - MARCO PAONE ⁽¹⁾ - GIOVANNA DE LUCA ⁽¹⁾ - NUNZIA RICCONI ⁽¹⁾ - FRANCESCA DEL PIANO ⁽¹⁾ - ESTERINA DE CARLO ⁽¹⁾ - GIOVANNA FUSCO ⁽¹⁾	521
Istituto Zooprofilattico Sperimentale del Mezzogiorno, Dipartimento Coordinamento Sanità Animale, Portici, Italia ⁽¹⁾	521
Study of the role of wild boar (<i>Sus Scrofa</i>) as a reservoir of antibiotic resistance.....	521
RUBINA PARADISO, GIORGIA BORRIELLO, NICOLA D'ALESSIO, MARCO PAONE, GIOVANNA DE LUCA, NUNZIA RICCONI, FRANCESCA DEL PIANO, ESTERINA DE CARLO, GIOVANNA FUSCO	521
Dipartimento Coordinamento Sanità Animale, Istituto Zooprofilattico Sperimentale del Mezzogiorno, Portici (NA), Italy	521
76 - Endosymbiosis of <i>Mycoplasma hominis</i> in <i>Trichomonas vaginalis</i>: influence of intracellular localization on its resistance to antibiotics.....	522
VALENTINA MARGARITA ⁽¹⁾ - GAVINO CARBONI ⁽¹⁾ - NICIA DIAZ ⁽¹⁾ - PAOLA RAPPELLI ⁽¹⁾ - PIER LUIGI FIORI ⁽¹⁾	522
Universita' di Sassari, Dipartimento di Scienze Biomediche, Sassari, Italia ⁽¹⁾	522
Endosymbiosis of <i>Mycoplasma hominis</i> in <i>Trichomonas vaginalis</i>: influence of intracellular localization on its resistance to antibiotics.....	522
Valentina Margarita¹, Gavino Carboni¹, Nicia Diaz¹, Paola Rappelli^{1;2}, Pier Luigi Fiori^{1;2}	522
1. Department of Biomedical Sciences, University of Sassari, Viale San Pietro 43/B, 07100 Sassari, Italy	522
2. Azienda Universitaria Ospedaliera AOU Sassari, 07110 Sassari, Italy	522
81 - A strategy to screen compounds against flaviviral infection helps identify a novel compound against Zika virus	523
ERIKA PLICANTI ⁽¹⁾ - GIULIA LOTTINI ⁽¹⁾ - SILVIA VEGNI ⁽²⁾ - SILVIA NOTTOLI ⁽³⁾ - MICHELE LAI ⁽²⁾ - ROBERTA IBBA ⁽⁴⁾ - SANDRA PIRAS ⁽⁴⁾ - ANTONIO CARTA ⁽⁴⁾ - GIULIA FREER ⁽²⁾	523
Università di Siena, Dipartimento di Biotecnologie mediche, Siena, Italia ⁽¹⁾ - Università di Pisa, Dipartimento di ricerca traslazionale, Pisa, Italia ⁽²⁾ - Ospedale Cisanello, Unità di virologia, Pisa, Italia ⁽³⁾ - Università di Sassari, Dipartimento di medicina, chirurgia e farmacia, Sassari, Italia ⁽⁴⁾	523
A strategy to screen compounds against flaviviral infection helps identify a novel compound against Zika virus.	523
ERIKA PLICANTI^{a,c}, GIULIA LOTTINI^{a,c}, SILVIA VEGNI^a, SILVIA NOTTOLI^b, MICHELE LAI^a, ROBERTA IBBA^d, SANDRA PIRAS^d, ANTONIO CARTA^d, GIULIA FREER^a	523
a: Retrovirus Centre, Department of Translational Research, University of Pisa, Pisa, Italy; b: Virology Unit, Pisa University Hospital, Pisa, Italy; c: Department of Medical Biotechnologies, University of Siena, Siena, Italy; d: Department of Medicine, Surgery and Pharmacy, University of Sassari, Sassari, Italy	523
84 - Minimum inhibitory concentration distribution of <i>Candida auris</i> strains: preliminary data from a large tertiary hospital in Piedmont	525
LISA PASTRONE (1) - ANTONIO CURTONI (1) - ALESSANDRO BONDI (1) - TERESA ZACCARIA (2) - ROBERTO CASALE (1) - FEDERICA ZULLO (1) - LORENZA CAVALLO (1)	

- GIORGIA MONTRUCCHIO (3) - SILVIA CORCIONE (4) - ROSSANA CAVALLO (1) - CRISTINA COSTA (1)	525
SC Microbiologia e Virologia, Dipartimento di Scienze della Sanità Pubblica e Pediatrica, Università di Torino, Torino, Italia (1) - SC Microbiologia e Virologia, Dipartimento di Medicina di Laboratorio, Ospedale Universitario Città della Salute e della Scienza di Torino, Torino, Italia (2) - Anestesia e Rianimazione 1U, Dipartimento di Anestesia, Terapia Intensiva ed Emergenza, Ospedale Universitario Città della Salute e della Scienza di Torino, Torino, Italia (3) - Unità di Malattie Infettive, Dipartimento di Scienze Mediche, Università di Torino, Torino, Italia (4)	525
Minimum inhibitory concentration distribution of <i>Candida auris</i> strains: preliminary data from a large tertiary hospital in Piedmont	525
LISA PASTRONE ¹ , ANTONIO CURTONI ^{1,2} , ALESSANDRO BONDI ^{1,2} , TERESA ZACCARIA ² , ROBERTO CASALE ¹ , FEDERICA ZULLO ¹ , LORENZA CAVALLO ¹ , GIORGIA MONTRUCCHIO ³ , SILVIA CORCIONE ⁴ , ROSSANA CAVALLO ^{1,2} , CRISTINA COSTA ^{1,2}	525
1Department of Sciences of Public Health and Pediatrics, University of Turin, Turin, Italy;	525
2Department of Laboratory Medicine, Microbiology and Virology Unit, University Hospital Città della Salute e della Scienza di Torino, Turin, Italy;	525
3Department of Anesthesia, Intensive Care and Emergency, Anestesia e Rianimazione 1U, University Hospital Città della Salute e della Scienza di Torino, Turin, Italy;	525
4Department of Medical Sciences, Infectious Disease Unit, University of Turin, Turin, Italy	525
86 - Characterization of a prophage and a defective integrative conjugative element carrying the <i>optrA</i> gene in linezolid-resistant <i>Streptococcus dysgalactiae</i> subsp. <i>equisimilis</i> isolates from pigs, Italy	527
SONIA NINA COCCITTO ⁽¹⁾ - MARZIA CINTHI ⁽²⁾ - FRANCESCA ROMANA MASSACCI ⁽³⁾ - ELISA ALBINI ⁽³⁾ - LUCILLA CUCCO ⁽³⁾ - MASSIMILIANO ORSINI ⁽⁴⁾ - SERENA SIMONI ⁽²⁾ - ELEONORA GIOVANETTI ⁽²⁾ - CHIARA FRANCESCA MAGISTRALI ⁽³⁾ - ANDREA BRENCIANI ⁽¹⁾	527
Università Politecnica delle Marche, Dipartimento Scienze Biomediche e sanità Pubblica, Ancona, Italia ⁽¹⁾ - Università Politecnica delle Marche, Dipartimento Scienze della Vita e dell'Ambiente, Ancona, Italia ⁽²⁾ - Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche, Togo Rosati, Dipartimento di Ricerca e Sviluppo, Perugia, Italia ⁽³⁾ - Istituto Zooprofilattico Sperimentale delle Venezie, Dipartimento di Microbiologia, Padova, Italia ⁽⁴⁾	527
Characterization of a prophage and a defective integrative conjugative element carrying the <i>optrA</i> gene in linezolid-resistant <i>Streptococcus dysgalactiae</i> subsp. <i>equisimilis</i> isolates from pigs, Italy	527
SONIA N. COCCITTO ³ , MARZIA CINTHI ¹ , FRANCESCA R. MASSACCI ² , ELISA ALBINI ² , LUCILLA CUCCO ² , MASSIMILIANO ORSINI ⁴ , SERENA SIMONI ¹ , ELEONORA GIOVANETTI ¹ , CHIARA F. MAGISTRALI ² , ANDREA BRENCIANI ³	527
¹ Unit of Microbiology, Department of Life and Environmental Sciences, Polytechnic University of Marche, Ancona, Italy; ² Department of Research and Development, Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche 'Togo Rosati', Perugia, Italy; ³ Unit of Microbiology, Department of Biomedical Sciences and Public Health, Polytechnic University of Marche Medical School, Ancona, Italy; ⁴ Department of Microbiology, Istituto Zooprofilattico Sperimentale delle Venezie, Padova, Italy	527

87 - Outbreak of multi drug-resistant OXA-48 and NDM-5 producing Klebsiella pneumoniae in a central Italy regional hospital.....	529
GLORIA D'ACHILLE ⁽¹⁾ - ILARIA NUNZI ⁽¹⁾ - SONIA NINA COCCITTO ⁽¹⁾ - MARZIA CINTHI ⁽²⁾ - SIMONA FIORITI ⁽¹⁾ - ANDREA BRENCIANI ⁽¹⁾ - LUCIA TEODORI ⁽³⁾ - MARINA MINGOIA ⁽¹⁾ - GIANLUCA MORRONI ⁽¹⁾.....	529
Università Politecnica delle Marche, Dipartimento di Scienze Biomediche e Sanità Pubblica, Ancona, Italia ⁽¹⁾ - Università Politecnica delle Marche, Dipartimento di Scienze della Vita e dell'Ambiente, Ancona, Italia ⁽²⁾ - Ospedali Riuniti di Ancona, Laboratorio di Microbiologia, Ancona, Italia ⁽³⁾	529
Outbreak of multi drug-resistant OXA-48 and NDM-5 producing Klebsiella pneumoniae in a central Italy regional hospital.	529
GLORIA D'ACHILLE¹, ILARIA NUNZI¹, SONIA N. COCCITTO¹, MARZIA CINTHI², SIMONA FIORITI¹, ANDREA BRENCIANI¹, LUCIA TEODORI³, MARINA MINGOIA¹, GIANLUCA MORRONI¹.....	529
¹Department of Biomedical Sciences and Public Health, Polytechnic University of Marche, Ancona, Italy; ²Department of Life and Environmental Sciences, Unit of Microbiology, Polytechnic University of Marche, Ancona, Italy; ³Microbiology Laboratory, Azienda Ospedaliero Universitaria “Ospedali Riuniti”, Ancona, Italy.....	529
88 - Fosfomycin-meropenem synergistic combination against NDM carbapenemase-producing Klebsiella pneumoniae strains.....	531
Maria Teresa Della Rocca ⁽¹⁾ - Vittorio Panetta ⁽¹⁾ - Filomena Merola ⁽¹⁾ - Giusy Natale ⁽¹⁾ - Giovanni Di Caprio ⁽²⁾ - Francesca Colucci ⁽²⁾ - Emanuele Cordua ⁽²⁾ - Adriana Durante ⁽¹⁾ - Rita Greco ⁽¹⁾	531
UOSD Microbiology, AORN Sant'Anna and San Sebastiano, Caserta, Italia ⁽¹⁾ - Infectious and Tropical Diseases Clinic, AORN Sant 'Anna and San Sebastiano, caserta, Italia ⁽²⁾	531
Fosfomycin-meropenem synergistic combination against NDM carbapenemase-producing Klebsiella pneumoniae strains	531
MARIA T. DELLA ROCCA.¹, VITTORIO PANETTA¹, FILOMENA MEROLA¹, GIUSY NATALE¹, GIOVANNI DI CAPRIO², FRANCESCA COLUCCI ², EMANUELE CORDUA², ADRIANA DURANTE¹, RITA GRECO¹	531
¹UOSD Microbiology - AORN Sant 'Anna and San Sebastiano, Caserta, Italy;.....	531
² Infectious and Tropical Diseases Clinic, AORN Sant 'Anna and San Sebastiano, Caserta, Italy	531
92 - The N-acylethanolamine acid amidase is a SARS-CoV-2 host factor.....	533
VERONICA LA ROCCA ⁽¹⁾	533
Scuola Superiore Sant'Anna Pisa, Scuola Superiore Sant'Anna Pisa, Pisa, Italia ⁽¹⁾	533
The N-acylethanolamine acid amidase is a SARS-CoV-2 host factor	533
VERONICA LA ROCCA^{1,2}, ROSSELLA FONNESU², CAROLINA FILIPPONI², ELENA IACONO², VIKTORIA DIESENDORF³, KATHERINA SEWALD⁴, HELENA OBERNOLTE⁴, GIULIA FREER², JOCHEN BODEM³, MAURO PISTELLO^{2,5}, MICHELE LAI²	533
¹Institute of Life Sciences, Sant'Anna School of Advanced Studies, Pisa, Italy; ² Retrovirus Center, Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa, Pisa, Italy; ³ Institute of Virology and Immunobiology, Julius-Maximilians-Universität Würzburg, Würzburg, Germany. ⁴Fraunhofer Institute for Toxicology and Experimental	

Medicine ITEM, Member of Fraunhofer International Consortium for Anti-Infective Research (iCAIR), Member of the German Center for Lung Research (DZL), Biomedical Research in Endstage and Obstructive Lung Disease (BREATH), Hannover, Germany ⁵ Virology Unit, Pisa University Hospital, Pisa, Italy.....	533
103 - Time to positivity of blood cultures (Tpos) as surrogate biomarker of in vivo antimicrobial efficacy: in vitro simulations with bacteria and yeasts.....	535
IRENE ZAGHI ⁽¹⁾ - MARTINA BRANDOLINI ⁽¹⁾ - ANNA MARZUCCO ⁽²⁾ - SOFIA MONTANARI ⁽²⁾ - GIULIA GATTI ⁽²⁾ - FRANCESCA TADDEI ⁽²⁾ - CLAUDIA COLOSIMO ⁽²⁾ - MONICA CRICCA ⁽¹⁾ - VITTORIO SAMBRI ⁽¹⁾ - JASON ROBERTS ⁽³⁾ - RUSSELL E. LEWIS ⁽⁴⁾	535
Department of Medical and Surgical Sciences, University of Bologna, Italy, Department of Medical and Surgical Sciences, University of Bologna, Italy, Bologna, Italia ⁽¹⁾ - Laboratorio Unico della AUSL della Romagna, in Pievesestina di Cesena, Italy, Laboratorio Unico della AUSL della Romagna, in Pievesestina di Cesena, Italy, Cesena, Italia ⁽²⁾ - The University of Queensland Centre for Clinical Research, Brisbane, Queensland, Australia, The University of Queensland Centre for Clinical Research, Brisbane, Queensland, Australia, Brisbane, Australia ⁽³⁾ - Department of Molecular Medicine, University of Padua, Padua, Italy, Department of Molecular Medicine, University of Padua, Padua, Italy, Padova, Italia ⁽⁴⁾	535
Time to positivity of blood cultures (Tpos) as surrogate biomarker of in vivo antimicrobial efficacy: in vitro simulations with bacteria and yeasts	535
IRENE ZAGHI ^{1,2} , MARTINA BRANDOLINI ^{1,2} , ANNA MARZUCCO ¹ , SOFIA MONTANARI ¹ , GIULIA GATTI ¹ , FRANCESCA TADDEI ¹ , CLAUDIA COLOSIMO ¹ , MONICA CRICCA ^{1,2} , VITTORIO SAMBRI ^{1,2} JASON ROBERTS ³ , RUSSELL E. LEWIS ⁴	535
¹ Laboratorio Unico della AUSL della Romagna, in Pievesestina di Cesena, Italy;.....	535
² Department of Medical and Surgical Sciences, University of Bologna, Italy;.....	535
³ The University of Queensland Centre for Clinical Research, Brisbane, Queensland, Australia;	535
⁴ Department of Molecular Medicine, University of Padua, Padua, Italy	535
126 - In vitro activity of cefiderocol, alone and in combination with imipenem or bacteriophages, against biofilm-embedded Pseudomonas aeruginosa	537
CATERINA FERRETTI ⁽¹⁾ - MARIANO BERNARDO ⁽²⁾ - CLAUDIA CAMPOBASSO ⁽¹⁾ - SARA BOLOGNINI ⁽¹⁾ - LAURA RINDI ⁽³⁾ - ARIANNA TAVANTI ⁽¹⁾ - CARLO TASCINI ⁽⁴⁾ - MARIAGRAZIA DI LUCA ⁽¹⁾	537
Università di Pisa, Dipartimento di Biologia, Pisa, Italia ⁽¹⁾ - AORN Ospedali dei Colli-Monaldi, Unità di Microbiologia, Napoli, Italia ⁽²⁾ - Università di Pisa, Dipartimento di Ricerca Traslazionale e delle Nuove Tecnologie in Medicina e Chirurgia, Pisa, Italia ⁽³⁾ - Università di Udine, Dipartimento di Medicina, Udine, Italia ⁽⁴⁾	537
In vitro activity of cefiderocol, alone and in combination with imipenem or bacteriophages, against biofilm-embedded Pseudomonas aeruginosa	537
CATERINA FERRETTI ¹ , MARIANO BERNARDO ² , CLAUDIA CAMPOBASSO ^{1,3} , SARA BOLOGNINI ¹ , LAURA RINDI ⁴ , ARIANNA TAVANTI ¹ , CARLO TASCINI ⁵ , MARIAGRAZIA DI LUCA ¹	537
¹ Department of Biology, University of Pisa, Pisa, Italy; ² Microbiology Unit, AORN Ospedali dei Colli-Monaldi Hospital, Naples, Italy; ³ Department of Biosystems, KU Leuven, Leuven, Belgium;	

⁴ Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa, Pisa, Italy; ⁵ Department of Medicine, University of Udine, Udine, Italy.....	537
127 - K. pneumoniae-KPC: Molecular tools to improve epidemiological surveillance of resistance genes.....	539
CLAUDIA CICINO ⁽¹⁾ - GRAZIA PAVIA ⁽¹⁾ - GIUSEPPE GUIDO MARIA SCARLATA ⁽¹⁾ - LICIA ELVIRA PRESTAGIACOMO ⁽¹⁾ - LUIGIA GALLO ⁽¹⁾ - CAROLINA DI CELLO ⁽¹⁾ - VALENTINA ESPOSITO ⁽¹⁾ - NADIA MARASCIO ⁽¹⁾ - GIOVANNI MATERA ⁽¹⁾ - ANGELA QUIRINO ⁽¹⁾	539
Università degli Studi "Magna Graecia" di Catanzaro, Azienda Ospedaliero-Universitaria "R. Dulbecco" Presidio "Mater Domini", Catanzaro, Dipartimento Scienze della Salute, Catanzaro, Italia ⁽¹⁾	539
K. pneumoniae-KPC: Molecular tools to improve epidemiological surveillance of resistance genes.	539
CLAUDIA CICINO ¹ , GRAZIA PAVIA ¹ , GIUSEPPE G.M. SCARLATA ¹ , LICIA E. PRESTAGIACOMO ¹ , LUIGIA GALLO ¹ , CAROLINA DI CELLO ¹ , VALENTINA ESPOSITO ¹ , NADIA MARASCIO ¹ , GIOVANNI MATERA ¹ AND ANGELA QUIRINO ¹	539
¹ Department of Health Sciences, Institute of Clinical Microbiology, "Magna Graecia" University of Catanzaro, Italy.	539
142 - Linezolid-induced mitochondrial damage impairs wound closure after MRSA infection	541
GIANLUCA MORRONI ⁽¹⁾ - CATERINA LICINI ⁽²⁾ - GUENDALINA LUCARINI ⁽²⁾ - GLORIA D'ACHILLE ⁽¹⁾ - FIORENZA ORLANDO ⁽³⁾ - MAURO PROVINCIALI ⁽³⁾ - SIMONA FIORITI ⁽¹⁾ - ILARIA NUNZI ⁽²⁾ - ANDREA GIACOMETTI ⁽¹⁾ - ORIANA SIMONETTI ⁽²⁾ - OSCAR CIRIONI ⁽¹⁾ - SAVERIO MARCHI ⁽²⁾	541
Università Politecnica delle Marche, Dipartimento di Scienze Biomediche e Sanità Pubblica, Ancona, Italia ⁽¹⁾ - Università Politecnica delle Marche, Dipartimento di Scienze Cliniche e Molecolari, Ancona, Italia ⁽²⁾ - IRCCS - INRCA, Experimental Animal Models for Aging Unit, Ancona, Italia ⁽³⁾	541
Linezolid-induced mitochondrial damage impairs wound closure after MRSA infection.....	541
GIANLUCA MORRONI ¹ , CATERINA LICINI ² , GUENDALINA LUCARINI ² , GLORIA D'ACHILLE ¹ , FIORENZA ORLANDO ³ , MAURO PROVINCIALI ³ , SIMONA FIORITI ⁴ , ILARIA NUNZI ² , ANDREA GIACOMETTI ⁴ , ORIANA SIMONETTI ⁵ , OSCAR CIRIONI ⁴ , SAVERIO MARCHI ²	541
1 Dipartimento di Scienze Biomediche e Sanità Pubblica, sezione Microbiologia, Università Politecnica delle Marche, Ancona, Italia; 2 Dipartimento di Scienze Cliniche e Molecolari, sezione Istologia, Università Politecnica delle Marche, Ancona, Italia; 3 Experimental Animal Models for Aging Unit, IRCCS INRCA, Ancona, Italia; 4 Dipartimento di Scienze Biomediche e Sanità Pubblica, sezione Malattie Infettive, Università Politecnica delle Marche, Ancona, Italia; 5 Dipartimento di Scienze Cliniche e Molecolari, sezione Dermatologia, Università Politecnica delle Marche, Ancona, Italia	541
152 - Molecular epidemiology of carbapenemase-producing Enterobacteriaceae in the Piacenza area	543
MELISSA SPALLA ⁽¹⁾ - FRANCESCA PISCOPIELLO ⁽¹⁾ - AURORA PIAZZA ⁽¹⁾ - GABRIELLA TOCCI ⁽²⁾ - ANDREA ZAPPAVIGNA ⁽²⁾ - VALENTINA LEPERA ⁽²⁾ - VITTORIA MATTIONI MARCHETTI ⁽¹⁾ - GIULIANA LO CASCIO ⁽²⁾ - ROBERTA MIGLIAVACCA ⁽¹⁾	543

Università di Pavia, Dipartimento di Scienze Clinico Chirurgiche Diagnostiche e Pediatriche, Pavia, Italia ⁽¹⁾ - AUSL “Guglielmo da Saliceto” ospedale di Piacenza, Dipartimento di Patologia Clinica, Unità di Microbiologia, Piacenza, Italia ⁽²⁾	543
Molecular epidemiology of carbapenemase-producing Enterobacteriaceae in the Piacenza area	543
Melissa Spalla ¹ , Francesca Piscopiello ¹ , Aurora Piazza ¹ , Gabriella Tocci ² , Andrea Zappavigna ² , Valentina Lepera ² , Vittoria Mattioni Marchetti ¹ , Giuliana Lo Cascio ² , Roberta Migliavacca ¹ ...	543
¹ University of Pavia, Dep. SCCDP, Microbiology and Clinical Microbiology Unit, Pavia (PV); ² AUSL “Guglielmo da Saliceto” hospital of Piacenza, Dep. Clinical Pathology, Microbiology Unit, Piacenza (PC)	543
153 - Next Generation Sequencing assay for HIV genotyping and resistance testing: the sicilian experience.....	545
CELESTINO BONURA ⁽¹⁾ - CHIARA MASCARELLA ⁽¹⁾ - SARA CANNELLA ⁽¹⁾ - GIUSEPPA SCIORTINO ⁽¹⁾ - GIOVANNI GIAMMANCO ⁽¹⁾	545
Università degli Studi di Palermo, Dipartimento di Promozione della Salute, Materno Infantile, Medicina Interna e Specialistica di eccellenza (PROMISE) “G. D’Alessandro”, Palermo, Italia ⁽¹⁾	545
Next Generation Sequencing assay for HIV genotyping and resistance testing: the sicilian experience.....	545
*Chiara Mascarella ^{1,2} , *Sara Cannella ^{1,2} , Celestino Bonura ^{1,2} , Giuseppa Sciortino ^{1,2} and Giovanni Giammanco ^{1,2} , on behalf of the Sicilian GRT-HIV Network	545
*These authors contributed equally	545
¹ Dipartimento di Promozione della Salute, Materno Infantile, Medicina Interna e Specialistica di eccellenza (PROMISE) “G. D’Alessandro” – Università degli Studi di Palermo.....	545
² Azienda Ospedaliera Universitaria Policlinico Paolo Giaccone di Palermo - U.O.C Microbiologia e Virologia	545
³ The Sicilian GRT-HIV Network: Antonio Cascio (Azienda Ospedaliera Universitaria Policlinico “Paolo Giaccone” di Palermo); Chiara Iaria (ARNAS Ospedale Civico Di Cristina di Palermo); Pietro Colletti (Ospedale Paolo Borsellino di Marsala, TP); Giovanni Mazzola (Presidio Ospedaliero Sant’Elia, Caltanissetta); Maria Antonietta Di Rosolini (Ospedale Giovanni Paolo II, Ragusa); Antonella Franco (Presidio Ospedaliero Umberto I, Siracusa); Rosa Boncoraglio (Ospedale Maggiore di Modica, RG); Luigi Guarneri (Ospedale Umberto I di Enna); Rosa Fontana Del Vecchio (Ospedale Umberto I di Enna); Giovanni Francesco Pellicanò (Università degli Studi di Messina, AOU Policlinico “G. Martino” di Messina); Antonio Albanese (Azienda Ospedaliera Papardo di Messina)	545
155 - ST2502 KPC-Klebsiella pneumoniae and OXA-23-Acinetobacter baumannii from LTCFs in Piacenza area: results of a Point Prevalence Study of colonization	547
GABRIELLA TOCCI ⁽¹⁾ - FRANCESCA PISCOPIELLO ⁽²⁾ - MELISSA SPALLA ⁽²⁾ - ROBERTA SCHIAVO ⁽¹⁾ - MARIA G. SILVOTTI ⁽³⁾ - FRANCO FEDERICI ⁽³⁾ - AURORA PIAZZA ⁽²⁾ - GIULIANA LO CASCIO ⁽¹⁾ - ROBERTA MIGLIAVACCA ⁽²⁾	547
AUSL “Guglielmo da Saliceto” ospedale di Piacenza, Dipartimento di Patologia Clinica, Unità di Microbiologia, Piacenza, Italia ⁽¹⁾ - Università di Pavia, Dipartimento di Scienze Clinico Chirurgiche Diagnostiche e Pediatriche, Pavia, Italia ⁽²⁾ - AUSL “Guglielmo da Saliceto” ospedale di Piacenza, Dipartimento di Igiene, Piacenza, Italia ⁽³⁾	547
ST2502 KPC-Klebsiella pneumoniae and OXA-23-Acinetobacter baumannii from LTCFs in Piacenza area: results of a Point Prevalence Study of colonization	547

Gabriella Tocci ¹ , Francesca Piscopiello ² , Melissa Spalla ² , Roberta Schiavo ¹ , Maria G. Silvotti ³ , Franco Federici ³ , Aurora Piazza ² , Giuliana Lo Cascio ¹ , Roberta Migliavacca ²	547
AUSL “Guglielmo da Saliceto” hospital of Piacenza, Dep. Clinical Pathology, Microbiology Unit, Piacenza (PC); Department of Clinical-Surgical, Diagnostic and Pediatric Sciences, Unit of Microbiology and Clinical Microbiology, University of Pavia, Pavia, Italy; AUSL of Piacenza, Hospital Hygiene Piacenza (PC)	547
166 - Antibiotic resistance of <i>Streptococcus pneumoniae</i> causing invasive diseases during the 13-valent conjugate vaccine era in Italy.....	548
SARA GIANCRISTOFARO (1) - FABIO D'AMBROSIO (1) - GIULIA ERRICO (1) - MICHELA PAGNOTTA (1) - MONICA MONACO (1) - MARIA DEL GROSSO (1) - ROMINA CAMILLI (1)	548
Istituto Superiore di Sanità, Malattie Infettive, Roma, Italia (1)	548
Antibiotic resistance of <i>Streptococcus pneumoniae</i> causing invasive diseases during the 13-valent conjugate vaccine era in Italy	548
SARA GIANCRISTOFARO, FABIO D'AMBROSIO , GIULIA ERRICO, MICHELA PAGNOTTA , MONICA MONACO, MARIA DEL GROSSO, ROMINA CAMILLI	548
Department of Infectious Diseases, Antibiotic-Resistance and Special Pathogens Unit, Istituto Superiore di Sanità, Rome, Italy	548
173 - Molecular investigation to monitor the spreading of fluoroquinolone and tetracycline resistance among <i>Campylobacter jejuni</i> clinical isolates	549
ALICE TOSADORI ⁽¹⁾ - ANNARITA SORRENTINO ⁽²⁾ - ELENA ADDIS ⁽¹⁾ - MICHELE BORDINI ⁽¹⁾ - DAVIDE GIBELLINI ⁽¹⁾ - ANNARITA MAZZARIOL ⁽¹⁾	549
Università di Verona, Dipartimento di Diagnostica e Sanità Pubblica, Verona, Italia ⁽¹⁾ - Azienda Ospedaliera Universitaria Integrata Verona, UOC Microbiologia e Virologia, Verona, Italia ⁽²⁾	549
Molecular investigation to monitor the spreading of fluoroquinolone and tetracycline resistance among <i>Campylobacter jejuni</i> clinical isolates	549
ALICE TOSADORI ¹ , ANNARITA SORRENTINO ² , ELENA ADDIS ¹ , MICHELE BORDINI ¹ , DAVIDE GIBELLINI ¹ , ANNARITA MAZZARIOL ¹	549
¹ Department of Diagnostics and Public Health, University of Verona, Verona, Italy	549
² UOC Microbiologia e Virologia, Azienda Ospedaliera Universitaria Integrata di Verona, Verona, Italy	549
176 - <i>Clostridioides difficile</i> over the years: evaluation of resistance profile in clinical isolates at Padua University Hospital	551
SHIRIN ASA'AD ⁽¹⁾ - GIULIA BERNABÈ ⁽¹⁾ - IOANNIS BEKAS ⁽¹⁾ - CLAUDIA DEL VECCHIO ⁽¹⁾ - IGNAZIO CASTAGLIUOLO ⁽¹⁾ - VALERIA BESUTTI ⁽²⁾	551
Università di Padova, Dipartimento di Medicina Molecolare, Padova, Italia ⁽¹⁾ - Unità di Microbiologia, Ospedale Università di Padova, Padova, Italia ⁽²⁾	551
<i>Clostridioides difficile</i> over the years: evaluation of resistance profile in clinical isolates at Padua University Hospital.....	551
SHIRIN ASA'AD 1, GIULIA BERNABÈ 1, IOANNIS BEKAS 1, CLAUDIA DEL VECCHIO 1;2, IGNAZIO CASTAGLIUOLO 1;2, VALERIA BESUTTI 2	551
1 Department of Molecular Medicine, University of Padua, Padua, Italy; 2 Microbiology Unit of Padua University Hospital, Padua, Italy	551

182 - In vitro antimicrobial susceptibility testing and biofilm characterization of <i>Borrelia burgdorferi</i> sensu lato isolated from patients with erythema migrans.....	553
GIORGIA FABRIZIO ⁽¹⁾ - ILARIA CAVALLO ⁽¹⁾ - FRANCESCA SIVORI ⁽¹⁾ - MARTINA PONTONE ⁽¹⁾ - MAURO TRUGLIO ⁽¹⁾ - FULVIA PIMPINELLI ⁽¹⁾ - ENEA GINO DI DOMENICO ⁽²⁾	553
Microbiology and Virology, San Gallicano Dermatological Institute, IRCCS, ROMA, Italia ⁽¹⁾ - Department of Biology and Biotechnology "C. Darwin", Sapienza University of Rome, ROMA, Italia ⁽²⁾	553
In vitro antimicrobial susceptibility testing and biofilm characterization of <i>Borrelia burgdorferi</i> sensu lato isolated from patients with erythema migrans.....	553
Giorgia FABRIZIO¹, Iliaria CAVALLO¹, Francesca SIVORI¹, Martina PONTONE¹, Mauro TRUGLIO¹, Fulvia PIMPINELLI¹, Enea G. DI DOMENICO²	553
¹ Microbiology and Virology, San Gallicano Dermatological Institute, IRCCS, Rome, Italy;	553
² Department of Biology and Biotechnology "C. Darwin" Sapienza University of Rome, 00185 Rome, Italy.	553
183 - Prevalence and drug resistance for invasive candidiasis in Pediatric and adult Hospitalized Patients	555
SILVIA MENEGHELLO ⁽¹⁾ - GIUSEPPE DI PIETRA ⁽¹⁾ - IGNAZIO CASTAGLIUOLO ⁽¹⁾ - PAOLA BRUN ⁽²⁾	555
Ospedale di Padova, Unità di Microbiologia, Padova, Italia ⁽¹⁾ - Università di Padova, Dip Medicina Molecolare, Padova, Italia ⁽²⁾	555
Prevalence and drug resistance for invasive candidiasis in Pediatric and adult Hospitalized Patients.....	555
Silvia Meneghello, Giuseppe Di Pietra, Ignazio Castagliuolo, Paola Brun	555
185 - Insight into <i>Acinetobacter baumannii</i>'s phenotypic and genotypic traits in oncological patients	556
ILARIA CAVALLO ⁽¹⁾ - FRANCESCA SIVORI ⁽¹⁾ - MAURO TRUGLIO ⁽¹⁾ - REBECCA PAGES ⁽¹⁾ - FRANCESCO MARCHESI ⁽²⁾ - LUIGI TOMA ⁽³⁾ - ELVA ABRIL ⁽¹⁾ - ANDREA MENGARELLI ⁽²⁾ - FULVIA PIMPINELLI ⁽¹⁾ - ENEA GINO DI DOMENICO ⁽⁴⁾	556
Microbiology and Virology, San Gallicano Dermatological Institute, IRCCS, ROMA, Italia ⁽¹⁾ - Hematology and Stem Cell Transplant Unit, IRCCS Regina Elena National Cancer Institute, Roma, Italia ⁽²⁾ - Department of Research, Advanced Diagnostics, and Technological Innovation, Translational Research Area, IRCCS Regina Elena National Cancer Institute, Roma, Italia ⁽³⁾ - Department of Biology and Biotechnology, Sapienza University of Rome, ROMA, Italia ⁽⁴⁾.....	556
Insight into <i>Acinetobacter baumannii</i>'s phenotypic and genotypic traits in oncological patients	556
Iliaria CAVALLO^a, Francesca SIVORI^a, Mauro TRUGLIO^a, Rebecca PAGES^a, Francesco MARCHESI^b, Luigi TOMA^c, Elva ABRIL^a, Andrea MENGARELLI^b, Fulvia PIMPINELLI^a, Enea G. DI DOMENICO^c	556
^a Microbiology and Virology, San Gallicano Dermatological Institute, IRCCS, Rome, Italy; ^b Hematology and Stem Cell Transplant Unit, IRCCS Regina Elena National Cancer Institute, Rome, Italy; ^c Department of Research, Advanced Diagnostics, and Technological Innovation, Translational Research Area, IRCCS Regina Elena National Cancer Institute, Rome, Italy; ^d Scientific Direction, IRCCS San Gallicano Institute, Rome, Italy; ^e Department of Biology and Biotechnology "C. Darwin" Sapienza University of Rome, Rome, Italy.....	556

187 - Exploring the Interaction between Ceftobiprole and High Molecular Mass (HMM) PBPs ...	558
PAOLA CONTI ⁽¹⁾ - LORENZO MATTIA LAZZARO ⁽¹⁾ - FEDERICA LENZO ⁽¹⁾ - SEBASTIANO ALBERTO FORTUNA ⁽¹⁾ - FLORIANA CAMPANILE ⁽¹⁾.....	558
Università degli Studi di Catania, BIOMETEC, Catania, Italia ⁽¹⁾.....	558
Exploring the Interaction between Ceftobiprole and High Molecular Mass (HMM) PBPs	558
PAOLA CONTI, LORENZO M. LAZZARO, FEDERICA LENZO, SEBASTIANO A. FORTUNA, FLORIANA CAMPANILE.	558
Department of Biomedical and Biotechnological Sciences (BIOMETEC), University of Catania, Catania, Italy.	558
223 - Impact of Interdisciplinary Antimicrobial Diagnostic Stewardship on Bloodstream infections and antibiotic resistance patterns.	559
FRANCESCO FOGLIA (1) - GIUSEPPE GRECO (2) - MARIA TERESA DELLA ROCCA (2) - COLOMBINA MELARDO (2) - MARIA VASCO (2) - ELENA GRIMALDI (2) - CONCETTA BENTIVOGLIO (2) - FORTUNATO MONTELLA (2) - VALERIA CRUDELE (2) - EMILIANA FINAMORE (2) - ANNA DE FILIPPIS (3) - MASSIMILIANO GALDIERO (1)	559
Dipartimento di Medicina Sperimentale, Università degli Studi della Campania "Luigi Vanvitelli", Sezione di Microbiologia e Microbiologia Clinica, Azienda Ospedaliera Universitaria "Luigi Vanvitelli" di Napoli, U.O.C. di Virologia e Microbiologia, Sant'Andrea delle Dame, 80138 Napoli, Italia., Napoli, Italia (1) - Azienda Ospedaliera Universitaria "Luigi Vanvitelli" di Napoli, U.O.C. di Virologia e Microbiologia, Sant'Andrea delle Dame, 80138 Napoli, Italia., Napoli, Italia (2) - Dipartimento di Medicina Sperimentale, Università degli Studi della Campania "Luigi Vanvitelli", Sezione di Microbiologia e Microbiologia Clinica, Via Santa Maria di Costantinopoli 16, 80138 Napoli, Italia., Napoli, Italia (3).....	559
Impact of Interdisciplinary Antimicrobial Diagnostic Stewardship on Bloodstream infections and antibiotic resistance patterns.	559
Foglia Francesco1,2, Giuseppe Greco2, Maria Teresa Della Rocca2, Colombina Melardo2, Maria Vasco2, Elena Grimaldi2, Concetta Bentivoglio2, Fortunato Montella2, Valeria Crudele2, Emiliana Finamore2, Anna De Filippis1, Massimiliano Galdiero1,2	559
1Dipartimento di Medicina Sperimentale, Università degli Studi della Campania "Luigi Vanvitelli", Sezione di Microbiologia e Microbiologia Clinica, Via Santa Maria di Costantinopoli 16, 80138 Napoli, Italia.	559
2Azienda Ospedaliera Universitaria "Luigi Vanvitelli" di Napoli, U.O.C. di Virologia e Microbiologia, Sant'Andrea delle Dame, 80138 Napoli, Italia.....	559
252 - In vivo transfer of linezolid resistance among enterococci in Mytilus galloprovincialis.....	561
SERENA SIMONI ⁽¹⁾ - CARLA VIGNAROLI ⁽²⁾ - ANDREA BRENCIANI ⁽³⁾ - ELEONORA GIOVANETTI ⁽²⁾ - LUIGI VEZZULLI ⁽⁴⁾ - ELISA TAVIANI ⁽⁵⁾ - MANON AUGUSTE ⁽⁵⁾ - ALESSANDRA DI GREGORIO ⁽²⁾.....	561
UNIVERSITA POLITECNICA DELLE MARCHE, Department of Life and Environmental Sciences, ANCONA, Italia ⁽¹⁾ - UNIVERSITA POLITECNICA DELLE MARCHE, DEPARTMENT OF LIFE AND ENVIRONMENTAL SCIENCES, ANCONA, Italia ⁽²⁾ - UNIVERSITA POLITECNICA DELLE MARCHE, Department of Biomedical Sciences and Public Health, ANCONA, Italia ⁽³⁾ - UNIVERSITA DI GENOVA, Department of Earth, Environment, and Life Sciences, ANCONA, Italia ⁽⁴⁾ - UNIVERSITA DI GENOVA, Department of Earth, Environment, and Life Sciences, GENOVA, Italia ⁽⁵⁾	561
In vivo transfer of linezolid resistance among enterococci in Mytilus galloprovincialis.....	561

SERENA SIMONI ¹ , ALESSANDRA DI GREGORIO ¹ , MANON AUGUSTE ² , ELISA TAVIANI ² , LUIGI VEZZULLI ² , ANDREA BRENCIANI ³ , ELEONORA GIOVANETTI ² , CARLA VIGNAROLI ¹	561
¹ Department of Life and Environmental Sciences, Polytechnic University of Marche, Ancona, Italy; ² DISTAV, Department of Earth, Environment, and Life Sciences, University of Genoa, Genoa, Italy; ³ Department of Biomedical Sciences and Public Health, Polytechnic University of Marche, Ancona, Italy	561
254 - Evaluation of Ceftazidime/Avibactam resistance in clinical isolates of Enterobacterales from a large tertiary-care hospital, Italy, 2019-2023	563
LUCA CALABRESE ⁽¹⁾ - EDWARD WILLISON ⁽¹⁾ - GIULIA CODDA ⁽¹⁾ - ELISA COSTA ⁽¹⁾ - VINCENZO DI PILATO ⁽¹⁾ - ANNA MARCHESE ⁽¹⁾	563
DISC, Università di Genova, Genova, Italia ⁽¹⁾	563
Evaluation of Ceftazidime/Avibactam resistance in clinical isolates of Enterobacterales from a large tertiary-care hospital, Italy, 2019-2023.....	563
LUCA CALABRESE ^a , EDWARD WILLISON ^a , GIULIA CODDA ^a , ELISA COSTA ^a , VINCENZO DI PILATO ^a , ANNA MARCHESE ^a	563
^a DISC, Microbiology Unit, University of Genoa, Genoa, Italy	563
262 - ESKAPE pathogens in the wounds of stray dogs and cats and their antimicrobial resistance profile.....	565
FRANCESCA PIZZANO ⁽¹⁾ - ANGELO MASULLO ⁽¹⁾ - CRISTINA DI PALMA ⁽¹⁾ - SILVIA CAPPIELLO ⁽²⁾ - MARINA POMPAMEO ⁽²⁾ - BARBARA LAMAGNA ⁽¹⁾ - LUISA DE MARTINO ⁽¹⁾ - FRANCESCA PAOLA NOCERA ⁽¹⁾	565
Università degli Studi di Napoli "Federico II", Dipartimento di Medicina Veterinaria e Produzioni Animali, Napoli, Italia ⁽¹⁾ - ASL Napoli 1, Ospedale Veterinario Frullone, Napoli, Italia ⁽²⁾	565
ESKAPE pathogens in the wounds of stray dogs and cats and their antimicrobial resistance profile	565
FRANCESCA PIZZANO ¹ , ANGELO MASULLO ¹ , CRISTINA DI PALMA ¹ , SILVIA CAPPIELLO ² , MARINA POMPAMEO ² , BARBARA LAMAGNA ¹ , LUISA DE MARTINO ^{1,3} AND FRANCESCA PAOLA NOCERA ¹	565
¹ Department of Veterinary Medicine and Animal Production, University of Naples Federico II, Via F. Delpino 1, 80137 Naples, Italy; ² Veterinary Hospital, ASL Napoli 1 Centro, via M. Rocco di Torrepadula 13, 80145 Naples, Italy; ³ Task Force on Microbiome Studies, University of Naples “Federico II”, Naples, Italy.....	565
263 - Strains Of Staphylococcus Epidermidis Isolated In The Milking Parlor Of Buffalo Farms In The Campania Region.....	567
ANGELO MASULLO (1) - FRANCESCA PAOLA NOCERA (1) - VALENTINA IOVANE (2) - FRANCESCA PIZZANO (1) - LUISA DE MARTINO (1).....	567
Università degli Studi di Napoli "Federico II", Dipartimento di Medicina Veterinaria e Produzioni Animali, Napoli, Italia (1) - Università degli Studi di Napoli "Federico II", Dipartimento di Agraria, Portici (Napoli), Italia (2)	567
STRAINS OF STAPHYLOCOCCUS EPIDERMIDIS ISOLATED IN THE MILKING PARLOR OF BUFFALO FARMS IN THE CAMPANIA REGION	567

ANGELO MASULLO ¹ , FRANCESCA PAOLA NOCERA ¹ , VALENTINA IOVANE ² , FRANCESCA PIZZANO ¹ , LUISA DE MARTINO ^{1,3}	567
1Department of Veterinary Medicine and Animal Production, University of Naples “Federico II”, Naples, Italy; 2Department of Agricultural Sciences, University of Naples Federico II, 80055 Naples, Italy; 3Task Force on Microbiome Studies, University of Naples “Federico II”, Naples, Italy.	
270 - Antimicrobial activity of gemini quaternary ammonium compounds against multidrug- resistant bacteria isolated from central venous catheters.	569
MARIANGELA PIZZO ⁽¹⁾ - ANTONIO PALUMBO PICCIONELLO ⁽²⁾ - SARA AMATA ⁽²⁾ - RITA IMMORDINO ⁽³⁾ - CARLA RIZZO ⁽²⁾ - IVANA PIBIRI ⁽²⁾ - GIUSEPPA SCIORTINO ⁽³⁾ - GIOVANNI M. GIAMMANCO ⁽¹⁾ - CINZIA CALÀ ⁽¹⁾	569
Università degli studi di Palermo, Promozione della Salute, Materno-Infantile, di Medicina Interna e Specialistica di Eccellenza “G. D’Alessandro” (PROMISE), Palermo, Italia ⁽¹⁾ - Università degli studi di Palermo, Dipartimento di Scienze e Tecnologie Biologiche Chimiche e Farmaceutiche (STEBICEF), Palermo, Italia ⁽²⁾ - A.O.U.P. "Paolo Giaccone", Palermo, U.O.C di Microbiologia, Virologia e Parassitologia, Palermo, Italia ⁽³⁾	
Antimicrobial activity of gemini quaternary ammonium compounds against multidrug-resistant bacteria isolated from central venous catheters.	569
MARIANGELA PIZZO*, ANTONIO PALUMBO PICCIONELLO**, SARA AMATA**, RITA IMMORDINO***, CARLA RIZZO**, IVANA PIBIRI**, GIUSEPPA SCIORTINO***, GIOVANNI M. GIAMMANCO*, CINZIA CALÀ*	569
*Department of Health Promotion, Mother and Child Care, Internal Medicine and Medical Specialties (PROMISE), University of Palermo.....	
** Department of Biological, Chemical and Pharmaceutical Sciences and Technologies (STEBICEF), University of Palermo	
*** U.O.C. of Microbiology, Virology and Parassitology, A.O.U.P. "Paolo Giaccone", Palermo, Italy	
274 - Molnupiravir imprinting of SARS-CoV-2 genome: a case-control study	571
EMANUELA GIOMBINI ⁽¹⁾ - FABIO TUCCI ⁽¹⁾ - MARTINA RUECA ⁽¹⁾ - LAVINIA FABENI ⁽¹⁾ - CESARE GRUBER ⁽¹⁾ - VALENTINA MAZZOTTA ⁽²⁾ - GIULIA GRAMIGNA ⁽¹⁾ - ALESSANDRA VERGORI ⁽²⁾ - ORNELLA BUTERA ⁽³⁾ - PIETRO SPEZIA ⁽⁴⁾ - DANIELE FOCOSI ⁽⁵⁾ - EMANUELE NICASTRI ⁽²⁾ - ENRICO GIRARDI ⁽⁶⁾ - FRANCESCO VAIA ⁽⁷⁾ - ANDREA ANTINORI ⁽²⁾ - FABRIZIO MAGGI ⁽¹⁾	571
INMI Lazzaro Spallanzani IRCCS, Laboratorio di Virologia, Roma, Italia ⁽¹⁾ - INMI Lazzaro Spallanzani IRCCS, Clinical and Research Infectious Diseases Department, Roma, Italia ⁽²⁾ - INMI Lazzaro Spallanzani IRCCS, Laboratorio di Microbiologia, Roma, Italia ⁽³⁾ - University of Pisa, Department of Translational Research, Retrovirus Center, Pisa, Italia ⁽⁴⁾ - Pisa University Hospital, North-Western Tuscany Blood Bank, Pisa, Italia ⁽⁵⁾ - INMI Lazzaro Spallanzani IRCCS, Direzione Scientifica, Roma, Italia ⁽⁶⁾ - INMI Lazzaro Spallanzani IRCCS, Direzione Generale, Roma, Italia ⁽⁷⁾	
Molnupiravir imprinting of SARS-CoV-2 genome: a case-control study.....	571
Emanuela Giombini ¹ , Fabio G. Tucci ¹ , Martina Rueca ¹ , Lavinia Fabeni ¹ , Cesare E.M. Gruber ¹ , Valentina Mazzotta ² , Giulia Gramigna ¹ , Alessandra Vergori ² , Ornella Butera ³ , Piergiorgio Spezia ⁴ , Daniele Focosi ⁵ , Emanuele Nicastrì ² , Enrico Girardi ⁶ , Francesco Vaia ⁷ , Andrea Antinori ² , Fabrizio Maggi ¹	571

¹ Laboratory of Virology, National Institute for Infectious Diseases “L. Spallanzani” - IRCCS, Rome, Italy	571
² Clinical and Research Infectious Diseases Department, National Institute for Infectious Diseases “L. Spallanzani” - IRCCS, Rome, Italy	571
³ Laboratory of Microbiology, National Institute for Infectious Diseases “L. Spallanzani” - IRCCS, Rome, Italy	571
⁴ Department of Translational Research, Retrovirus Center - University of Pisa, Pisa, Italy.....	571
⁵ North-Western Tuscany Blood Bank, Pisa University Hospital, Pisa, Italy	571
⁶ Scientific Direction, National Institute for Infectious Diseases “L. Spallanzani” - IRCCS, Rome, Italy	571
⁷ General Direction, National Institute for Infectious Diseases “L. Spallanzani” - IRCCS, Rome, Italy	571
281 - Drugs porins-permeability coefficient correlate with the antimicrobial effectiveness	573
SILVIA PUXEDDU ⁽¹⁾ - SARA BOI ⁽²⁾ - M. ANDREA SCORCIAPINO ⁽²⁾ - MATTEO CECCARELLI ⁽³⁾ - STEFAN MILENKOVIC ⁽³⁾ - ALDO MANZIN ⁽¹⁾ - FABRIZIO ANGIUS ⁽¹⁾	573
Università degli studi di Cagliari, Dipartimento di Scienze Biomediche, Cagliari, Italia ⁽¹⁾ -	
Università degli studi di Cagliari, Dipartimenti di Scienze Chimiche e Geologiche, Cagliari, Italia	
⁽²⁾ - Università degli studi di Cagliari, Dipartimento di fisica, Cagliari, Italia ⁽³⁾	573
Drugs porins-permeability coefficient correlate with the antimicrobial effectiveness.....	573
SILVIA PUXEDDU ¹, SARA BOI ², M. ANDREA SCORCIAPINO ², MATTEO CECCARELLI ³, STEFAN MILENKOVIC ³, ALDO MANZIN ¹, FABRIZIO ANGIUS ¹	573
¹ Department of Biomedical Sciences, Unit of Microbiology and Virology, University of Cagliari, Cagliari, Italy	573
² Department of Chemical and Geological Sciences, University of Cagliari, Cagliari, Italy	573
³ Department of Physics, University of Cagliari, Cagliari, Italy	573
283 - Sulfonamide treatments inhibit gamma-Herpesvirus persistent infection in vivo	575
FABRIZIO ANGIUS ⁽¹⁾ - TANIA RODRIGUES ⁽²⁾ - SILVIA PUXEDDU ⁽¹⁾ - ILENIA DELOGU ⁽¹⁾ - RAFFAELLO POMPEI ⁽¹⁾ - J. PEDRO SIMAS ⁽²⁾ - ALDO MANZIN ⁽¹⁾.....	575
Università di Cagliari, Dipartimento di Scienze Biomediche, Cagliari, Italia ⁽¹⁾ - Universidade de Lisboa, Instituto de Medicina Molecular, Faculdade de Medicina, Lisboa, Portugal ⁽²⁾	575
Sulfonamide treatments inhibit gamma-Herpesvirus persistent infection in vivo	575
FABRIZIO ANGIUS ¹, TÂNIA RODRIGUES ², SILVIA PUXEDDU ¹, ILENIA DELOGU ¹, RAFFAELLO POMPEI ¹, J. PEDRO SIMAS ^{2,3}, ALDO MANZIN ¹	575
¹ Department of Biomedical Sciences, Unit of Microbiology and Virology, University of Cagliari, Cagliari, Italy;	575
² Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Lisboa, Portugal;	575
³ Católica Biomedical Research and Católica Medical School, Universidade Católica Portuguesa, Lisboa, Portugal.	575
297 - Antiviral activity of three residues peptides against SARS-CoV-2 Infection	577

GIANLUIGI FRANCI⁽¹⁾ - AVINASH MALI⁽²⁾ - CARLA ZANNELLA⁽²⁾ - ANNALISA CHIANESE⁽²⁾ - GIUSEPPINA SANNA⁽³⁾ - SHUBAASH ANTHIYA⁽⁴⁾ - ANA M. LÓPEZ-ESTÉVEZ⁽⁴⁾ - ALESSANDRA MONTI⁽⁵⁾ - GIUSEPPE SQUILLACI⁽⁶⁾ - BIAGIO SANTELLA⁽²⁾ - GIUSEPPE GRECO⁽²⁾ - ANNA DE FILIPPIS⁽²⁾ - FRANCESCO PALMIERI⁽¹⁾ - GIANNI D'ANGELO⁽⁷⁾ - ALESSANDRA MORANA⁽⁶⁾ - NUNZIANNA DOTI⁽⁵⁾ - MARÍA JOSÉ ALONSO⁽⁴⁾ - ALDO MANZIN⁽³⁾ - MASSIMILIANO GALDIERO⁽⁸⁾ 577

University of Salerno, Department of Medicine, Surgery and Dentistry, Scuola Medica Salernitana, Salerno, Italia⁽¹⁾ - University of Campania "Luigi Vanvitelli", Department of Experimental Medicine, Napoli, Italia⁽²⁾ - University of Cagliari, Cittadella Universitaria, Department of Biomedical Sciences, Cagliari, Italia⁽³⁾ - University of Santiago de Compostela, Department of Pharmacy and Pharmaceutical Technology, School of Pharmacy, Santiago de Compostela, Spagna⁽⁴⁾ - National Research Council (CNR), Institute of Biostructures and Bioimaging (IBB), Napoli, Italia⁽⁵⁾ - National Research Council (CNR), Research Institute on Terrestrial Ecosystems, Napoli, Italia⁽⁶⁾ - University of Salerno, Department of Computer Science, Salerno, Italia⁽⁷⁾ - University of Campania "Luigi Vanvitelli", Department of Experimental Medicine, Napoli, Italia⁽⁸⁾ 577

Antiviral activity of three residues peptides against SARS-CoV-2 Infection 577

Gianluigi Franci¹, Avinash Mali², Carla Zannella², Annalisa Chianese², Giuseppina Sanna³, Shubaash Anthiya⁴, Ana M. López-Estévez⁴, Alessandra Monti⁵, Giuseppe Squillaci⁶, Biagio Santella², Giuseppe Greco², Anna De Filippis², Francesco Palmieri¹, Gianni D'Angelo⁷, Alessandra Morana⁶, Nunzianna Doti⁵, María José Alonso⁴, Aldo Manzin³, Massimiliano Galdiero²..... 577

1. Department of Medicine, Surgery and Dentistry, "Scuola Medica Salernitana", University of Salerno, Salerno, Italy;
2. Department of Experimental Medicine, University of Campania "Luigi Vanvitelli", Naples, Italy; 3. Department of Biomedical Sciences, University of Cagliari, Cittadella Universitaria, Cagliari, Italy; 4. Center for Research in Molecular Medicine and Chronic Diseases (CIMUS), Health Research Institute of Santiago de Compostela (IDIS), Department of Pharmacy and Pharmaceutical Technology, School of Pharmacy, University of Santiago de Compostela, Santiago de Compostela, Spain; 5. Institute of Biostructures and Bioimaging (IBB), National Research Council (CNR), Naples, Italy; 6. Research Institute on Terrestrial Ecosystems, National Research Council (CNR), Naples, Italy; 7. Department of Computer Science, University of Salerno, Salerno, Italy;

577

298 - Genome-wide DNA methylome and transcriptome identify epigenetically based drug-resistance mechanisms in *Acinetobacter baumannii* 579

VERONICA FOLLIERO⁽¹⁾ - ROSARIO BRANCACCIO⁽¹⁾ - DOMENICO DI ROSA⁽¹⁾ - FEDERICA DELL'ANNUNZIATA⁽¹⁾ - RITA GRECO⁽²⁾ - ALESSANDRO WEISZ⁽¹⁾ - FRANCESCA RIZZO⁽¹⁾ - GIANLUIGI FRANCI⁽¹⁾ 579

University of Salerno, Department of Medicine, Surgery and Dentistry "Scuola Medica Salernitana, Salerno, Italia⁽¹⁾ - AORN Sant 'Anna and San Sebastiano, UOSD Microbiology, Caserta, Italia⁽²⁾ 579

Genome-wide DNA methylome and transcriptome identify epigenetically based drug-resistance mechanisms in *Acinetobacter baumannii*..... 579

Veronica Folliero¹, Rosario Brancaccio¹, Domenico Di Rosa¹, Federica Dell'Annunziata¹, Rita Greco², Alessandro Weisz¹, Francesca Rizzo¹, Gianluigi Franci¹ 579

1. Department of Medicine, Surgery and Dentistry "Scuola Medica Salernitana", University of Salerno, Salerno, Italy;	579
2. UOSD Microbiology - AORN Sant 'Anna and San Sebastiano, Caserta, Italy	579
299 - Broad-spectrum antimicrobial potential of Oftasecur and Visuprime ophthalmic solutions .	580
ILARIA COSIMATO ⁽¹⁾ - FEDERICA DELL'ANNUNZIATA ⁽¹⁾ - MARIA VITTORIA MORONE ⁽²⁾ - MARCO GIOIA ⁽¹⁾ - FERDINANDO CIONE ⁽¹⁾ - MASSIMILIANO GALDIERO ⁽²⁾ - NICOLA ROSA ⁽¹⁾ - MADDALENA DE BERNARDO ⁽¹⁾ - VERONICA FOLLIERO ⁽¹⁾ - GIANLUIGI FRANCI ⁽¹⁾	580
Department of Medicine, Surgery and Dentistry "Scuola Medica Salernitana", University Salerno, Salerno, Italia ⁽¹⁾ - University of Campania Luigi Vanvitelli, Department of Experimental Medicine, Napoli, Italia ⁽²⁾	580
Broad-spectrum antimicrobial potential of Oftasecur and Visuprime ophthalmic solutions	580
Federica Dell'Annunziata ^{1,2} , Maria Vittoria Morone ² , Marco Gioia ¹ , Ferdinando Cione ¹ , Ilaria Cosimato ^{1,2} , Massimiliano Galdiero ^{2,3} , Nicola Rosa ¹ , Maddalena De Bernardo ¹ , Veronica Folliero ¹ , Gianluigi Franci ^{1,4}	580
¹ Department of Medicine, Surgery and Dentistry "Scuola Medica Salernitana", University of Salerno, 84081 Baronissi Salerno, Italy	580
² Department of Experimental Medicine, University of Campania Luigi Vanvitelli, 80138 Naples, Italy	580
³ Complex Operative Unity of Virology and Microbiology, University Hospital of Campania "Luigi Vanvitelli", 80138 Naples, Italy	580
⁴ Clinical Pathology and Microbiology Unit, San Giovanni di Dio e Ruggi D'Aragona University Hospital, 84126 Salerno, Italy	580
308 - Genotypic characterization of linezolid-resistant Staphylococcus epidermidis strains isolated from invasive infections collected in the Careggi University Hospital of Florence.....	582
ILARIA BACCANI ⁽¹⁾ - MARCO COPPI ⁽¹⁾ - LORENZO PACI ⁽²⁾ - NICLA GIOVACCHINI ⁽²⁾ - ALBERTO ANTONELLI ⁽¹⁾ - TOMMASO GIANI ⁽¹⁾ - ORIETTA MASSIDDA ⁽³⁾ - GIAN MARIA ROSSOLINI ⁽¹⁾	582
University of Florence, Department of Experimental and Clinical Medicine, University of Florence; Microbiology and Virology Unit, Careggi University Hospital, Florence, Italia ⁽¹⁾ - University of Florence, Department of Experimental and Clinical Medicine, University of Florence, Florence, Italia ⁽²⁾ - University of Trento, Department of Cellular, Computational and Integrative Biology (CIBIO), Trento, Italia ⁽³⁾	582
Genotypic characterization of linezolid-resistant Staphylococcus epidermidis strains isolated from invasive infections collected in the Careggi University Hospital of Florence	582
ILARIA BACCANI ^{1,2} , MARCO COPPI ^{1,2} , LORENZO PACI ¹ , NICLA GIOVACCHINI ¹ , ALBERTO ANTONELLI ^{1,2} , TOMMASO GIANI ^{1,2} , ORIETTA MASSIDDA ³ , GIAN MARIA ROSSOLINI ^{1,2}	582
1 Department of Experimental and Clinical Medicine, University of Florence, Florence, Italy; 2 Microbiology and Virology Unit, Careggi University Hospital, Florence, Italy; 3 Department of Cellular, Computational and Integrative Biology (CIBIO), University of Trento, Trento, Italy..	582
311 - Phenotypic and genotypic characterization of a clinical isolate of IMP-19 producing Enterobacter hormaechei.....	584

CHIARA CHILLERI ⁽¹⁾ - ALBERTO ANTONELLI ⁽¹⁾ - NOEMI AIEZZA ⁽¹⁾ - MARCO COPPI ⁽¹⁾ - GUENDALINA VAGGELLI ⁽²⁾ - TOMMASO GIANI ⁽¹⁾ - GIAN MARIA ROSSOLINI ⁽¹⁾	584
University of Florence, Department of Experimental and Clinical Medicine; Clinical Microbiology and Virology Unit, Careggi University Hospital, Firenze, Italia ⁽¹⁾ - Clinical Microbiology and Virology Unit, Careggi University Hospital, Firenze, Italia ⁽²⁾	584
Phenotypic and genotypic characterization of a clinical isolate of IMP-19 producing <i>Enterobacter hormaechei</i>	584
CHIARA CHILLERI^{1,2}, ALBERTO ANTONELLI^{1,2}, NOEMI AIEZZA^{1,2}, MARCO COPPI^{1,2}, GUENDALINA VAGGELLI², TOMMASO GIANI^{1,2}, GIAN MARIA ROSSOLINI^{1,2}	584
¹ Department of Experimental and Clinical Medicine, University of Florence, Italy	584
² Clinical Microbiology and Virology Unit, Careggi University Hospital, Florence, Italy	584
320 - IS26-mediated chromosomal integration of a blaKPC-3-harboring InFII-derived plasmid in a clinical isolate of <i>Pseudomonas aeruginosa</i>	585
ALBERTO ANTONELLI ⁽¹⁾ - BENEDETTA CASCIATO ⁽¹⁾ - MARIA NARDONE ⁽²⁾ - GRAZIA LOMBARDI ⁽²⁾ - CHIARA BIANCHIMANI ⁽²⁾ - FEDERICO POSTERARO ⁽³⁾ - STEFANO RAPI ⁽²⁾ - TOMMASO GIANI ⁽¹⁾ - GIAN MARIA ROSSOLINI ⁽¹⁾	585
Department of Experimental and Clinical Medicine; Microbiology and Virology Unit, University of Florence; Careggi University Hospital, Firenze, Italia ⁽¹⁾ - Clinical Chemistry and Microbiology Analysis Unit, San Luca Hospital, AUSL Toscana Nord Ovest, Lucca, Italia ⁽²⁾ - Rehabilitation Department, Versilia Hospital, AUSL Toscana Nord Overst, Camaiore, Italia ⁽³⁾	585
IS26-mediated chromosomal integration of a bla _{KPC-3} -harboring InFII-derived plasmid in a clinical isolate of <i>Pseudomonas aeruginosa</i>	585
Alberto Antonelli ^{1,2} , Benedetta Casciato ^{1,2} , Maria Nardone ³ , Grazia Lombardi ³ , Chiara Bianchimani ³ , Federico Posteraro ⁴ , Stefano Rapi ³ , Tommaso Giani ^{1,2} , Gian Maria Rossolini ^{1,2} .	585
¹ Department of Experimental and Clinical Medicine, University of Florence, Florence, Italy...	585
² Microbiology and Virology Unit, Careggi University Hospital, Florence, Italy	585
³ Clinical Chemistry and Microbiology Analysis Unit, San Luca Hospital, AUSL Toscana Nord Ovest, Lucca, Italy	585
⁴ Rehabilitation Department, Versilia Hospital, AUSL Toscana Nord Overst, Camaiore, Lucca, Italy.	585
12 Risposta dell'ospite alle infezioni/Correlati immunologici	587
6 - Role of Epstein-Barr virus and Mycobacterium avium paratuberculosis in CNS demyelinating disorders	587
DAVIDE COSSU ⁽¹⁾ - NOBUTAKA HATTORI ⁽²⁾ - LEONARDO ANTONIO SECHI ⁽¹⁾	587
Universita di Sassari, universita, dipartimento di scienze biomediche, Sassari, Italia ⁽¹⁾ - Juntendo University, dipartimento neurologia, Tokyo, Giappone ⁽²⁾	587
Role of Epstein-Barr virus and Mycobacterium avium paratuberculosis in CNS demyelinating disorders	587
Davide Cossu ^{a,b} , Nobutaka Hattori ^b , Leonardo Antonio Sechi ^a	587
^a Sassari University, Department of Biomedical Sciences, Sassari, Italy	587
^b Juntendo University, Department of Neurology, Tokyo, Japan	587

8 - Neutralizing antibodies response to novel SARS-CoV-2 omicron sublineages in long-term care facility residents after the fourth dose of monovalent BNT162b2 Covid-19 vaccination..... 588

ILENIA VARASI ⁽¹⁾ - ALESSIA LAI ⁽²⁾ - ANNALISA BERGNA ⁽²⁾ - LIA FIASCHI ⁽¹⁾ - ANTONELLA GATTI ⁽³⁾ - BARBARA CAIMI ⁽³⁾ - CLAUDIA BALOTTA ⁽³⁾ - CAMILLA BIBA ⁽¹⁾ - AGOSTINO RIVA ⁽²⁾ - GIANGUGLIELMO ZEHENDER ⁽²⁾ - MAURIZIO ZAZZI ⁽¹⁾ - ILARIA VICENTI ⁽¹⁾ 588

Università degli Studi di Siena, Dipartimento Biotecnologie Mediche/AOUS Siena, Siena, Italia ⁽¹⁾ - University of Milan, Department of Biomedical and Clinical Sciences, Milano, Italia ⁽²⁾ - Istituto Milanese Martinit e Stelline e Pio Albergo Trivulzio, Azienda di Servizi alla Persona, Milano, Italia ⁽³⁾ 588

Neutralizing antibodies response to novel SARS-CoV-2 omicron sublineages in long-term care facility residents after the fourth dose of monovalent BNT162b2 Covid-19 vaccination..... 588

ILENIA VARASI¹, ALESSIA LAI², ANNALISA BERGNA², LIA FIASCHI¹, ANTONELLA GATTI³, BARBARA CAIMI³, CLAUDIA BALOTTA³, CAMILLA BIBA¹, AGOSTINO RIVA², GIANGUGLIELMO ZEHENDER², MAURIZIO ZAZZI¹, ILARIA VICENTI¹ 588

¹ Department of Medical Biotechnologies, University of Siena, Siena, Italy; ² Department of Biomedical and Clinical Sciences, University of Milan, Milan, Italy; ³ Azienda di Servizi alla Persona, Istituto Milanese Martinit e Stelline e Pio Albergo Trivulzio, Milan, Italy 588

12 - Prevalence of polyomaviruses and papillomaviruses related to lymphocyte immunophenotyping in Actinic Keratoses and Squamous Cell Carcinoma: one-year data from clinical practice 590

TERENZIO COSIO ⁽¹⁾ - MARCO CIOTTI ⁽²⁾ - CARLA PREZIOSO ⁽³⁾ - GABRIELE BRAZZINI ⁽³⁾ - SARA PASSERINI ⁽³⁾ - SERGIO BERNARDINI ⁽¹⁾ - UGO MOENS ⁽⁴⁾ - VALERIA PIETROPAOLO ⁽³⁾ - ROBERTA GAZIANO ⁽¹⁾ - ELENA CAMPIONE ⁽⁵⁾ 590

Università di Roma Tor Vergata, Department of Experimental Medicine, Roma, Italia ⁽¹⁾ - University of Rome Tor Vergata, Virology Unit, Polyclinic Tor Vergata Foundation, Viale Oxford 81, 00133 Rome, Italy., Roma, Italia ⁽²⁾ - "Sapienza" University of Rome, 00185 Rome, Italy, Department of Public Health and Infectious Diseases, "Sapienza" University of Rome, 00185 Rome, Italy; , Roma, Italia ⁽³⁾ - University of Tromsø-The Arctic University of Norway, Department of Medical Biology, Faculty of Health Sciences, University of Tromsø-The Arctic University of Norway, 9037 Tromsø, Norway, Tromsø, Norvegia ⁽⁴⁾ - Tor Vergata University of Rome, Department of Systems Medicine, Dermatology Unit, Tor Vergata University Hospital, 00133 Rome, Italy, Roma, Italia ⁽⁵⁾ 590

Prevalence of polyomaviruses and papillomaviruses related to lymphocyte immunophenotyping in Actinic Keratoses and Squamous Cell Carcinoma: one-year data from clinical practice 590

Terenzio COSIO^{1,2}, Marco CIOTTI³, Carla PREZIOSO^{4,5}, Gabriele BRAZZINI⁵, Sara PASSERINI⁵, Sergio BERNARDINI¹, Ugo MOENS⁶, Valeria PIETROPAOLO⁵, Roberta GAZIANO¹, Elena CAMPIONE² 590

¹Department of Experimental Medicine, Tor Vergata University of Rome, 00133 Rome, Italy; . 590

²Department of Systems Medicine, Dermatology Unit, Tor Vergata University Hospital, 00133 Rome, Italy; 590

³Virology Unit, Polyclinic Tor Vergata Foundation, Viale Oxford 81, 00133 Rome, Italy 590

⁴IRCSS San Raffaele Roma, Microbiology of Chronic Neuro-Degenerative Pathologies, 00163 Rome, Italy; 590

⁵Department of Public Health and Infectious Diseases, "Sapienza" University of Rome, 00185 Rome, Italy; 590

⁶ Department of Medical Biology, Faculty of Health Sciences, University of Tromsø-The Arctic University of Norway, 9037 Tromsø, Norway;.....	590
30 - Caspase-8 controls a spontaneous pro-inflammatory program that can be exploited therapeutically to control bacterial infections	592
GERMANA LENTINI ⁽¹⁾ - AGATA FAMA' ⁽¹⁾ - GIUSEPPE VALERIO DE GAETANO ⁽¹⁾ - FRANCESCO COPPOLINO ⁽²⁾ - AHLEM MAHJOUB ⁽¹⁾ - LIV RYAN ⁽³⁾ - EGIL LIEN ⁽⁴⁾ - TERJE ESPEVIK ⁽³⁾ - CONCETTA BENINATI ⁽¹⁾ - GIUSEPPE TETI ⁽⁵⁾	592
Universita' Degli Studi Di Messina, Dipartimento Di Patologia Umana E Dell'eta' Evolutiva G. Barresi, Messina, Italia ⁽¹⁾ - Universita' Degli Studi Di Messina, Dipartimento Di Scienze Biomediche, Odontoiatriche E Delle Immagini Morfologiche E Funzionali, Messina, Italia ⁽²⁾ - Centre Of Molecular Inflammation Research(Cemir), Department Of Cancer Research And Molecular Medicine, Norwegian University Of Science And Technology, Trondheim, Norvegia ⁽³⁾ - Division Of Infectious Diseases And Immunology Program In Innate Immunity, Department Of Medicine, University Of Massachusetts Chan Medical School, Worcester, Stati Uniti D'america ⁽⁴⁾ - Scylla Biotech Srl, Messina, Italy, Scylla Biotech Srl, Messina, Italy, Messina, Italia ⁽⁵⁾	592
Caspase-8 controls a spontaneous pro-inflammatory program that can be exploited therapeutically to control bacterial infections	592
GERMANA LENTINI ¹ , AGATA FAMÀ ¹ , GIUSEPPE V. DE GAETANO ¹ , FRANCESCO COPPOLINO ² , AHLEM KHACHROUB MAHJOUB ¹ , LIV RYAN ³ , EGIL LIEN ^{3,4} , TERJE ESPEVIK ³ , CONCETTA BENINATI ^{1,5} AND GIUSEPPE TETI ⁵	592
¹ Department of Human Pathology of Adult and Developmental Age "Gaetano Barresi", University of Messina, Messina, Italy; ² Department of Biomedical, Dental and Imaging Sciences, University of Messina, Messina, Italy; ³ Centre of Molecular Inflammation Research, Department of Cancer Research and Molecular Medicine, Norwegian University of Science and Technology, Trondheim, Norway; ⁴ Division of Infectious Diseases and Immunology, Program in Innate Immunity, Department of Medicine, University of Massachusetts Chan Medical School, Worcester, MA, USA;	592
⁵ Scylla Biotech Srl, Messina, Italy.....	592
46 - SARS-CoV-2 evolution during persistent infection in a CAR-T recipient shows an escape to both sotrovimab and T cell response	594
MARIA SIDOTI ⁽¹⁾ - PAOLA MAZZETTI ⁽¹⁾ - PIETRO GIORGIO SPEZIA ⁽²⁾ - ANNALISA CAPRIA ⁽¹⁾ - GIULIA FREER ⁽²⁾ - SILVIA COSTARELLI ⁽³⁾ - ALICE CARA ⁽¹⁾ - ALFREDO ROSELLINI ⁽¹⁾ - SUSI FRATESCHI ⁽¹⁾ - GIOVANNA MOSCATO ⁽¹⁾ - IACOPO FRANCONI ⁽¹⁾ - MICHELE CURCIO ⁽⁴⁾ - MARIA LINDA VATTERONI ⁽¹⁾ - MICHELE LAI ⁽²⁾ - PAOLA QUARANTA ⁽²⁾ - DANIELE FOCOSI ⁽⁵⁾ - SPARTACO SANI ⁽³⁾ - ALESSIA CALÒ ⁽²⁾ - MAURO PISTELLO ⁽¹⁾	594
Azienda ospedaliera universitaria pisana, U.O. Virologia, Ospedale Cisanello, Pisa, Italia ⁽¹⁾ - Centro Retrovirus, Università di Pisa, Pisa, Italia ⁽²⁾ - Azienda Usl Toscana Nord Ovest, Dipartimento di malattie infettive e tropicali, ospedale di Livorno, Livorno, Italia ⁽³⁾ - Azienda ospedaliera universitaria pisana, U.O. Immunogenetica, Ospedale di Cisanello, Pisa, Italia ⁽⁴⁾ - Azienda Ospedaliera Universitaria Pisana, Banca del Sangue, Ospedale di Cisanello, Pisa, Italia ⁽⁵⁾	594
SARS-CoV-2 evolution during persistent infection in a CAR-T recipient shows an escape to both sotrovimab and T-cell response.....	594
MARIA SIDOTI ^{a,b} , PAOLA MAZZETTI ^a , PIETRO G. SPEZIA ^b , ANNALISA CAPRIA ^a , GIULIA FREER ^b , SILVIA COSTARELLI ^c , ALICE CARA ^a , ALFREDO ROSELLINI ^a , SUSI	

FRATESCHI ^a , GIOVANNA MOSCATO ^a , IACOPO FRANCONI ^a , MICHELE CURCIO ^d , MARIA L. VATTERONI ^a , MICHELE LAI ^b , PAOLA QUARANTA ^b , DANIELE FOCOSI ^e , SPARTACO SANI ^c , ALESSIA CALÓ ^b , MAURO PISTELLO ^{a,b}	594
a) Department of Laboratory Medicine, Virology Unit, Pisa University Hospital, Pisa, Italy; b) Department of Translational Research, Retrovirus Center and Virology Section, University of Pisa, Pisa, Italy; c)Department of Infectious and Tropical Diseases, Infectious Diseases Unit, Leghorn Hospital, Azienda USL Toscana Nord-Ovest, Leghorn, Italy; d)Department of Laboratory Medicine, Immunogenetic Unit, Pisa University Hospital, Pisa, Italy; e)Department of Laboratory Medicine, North-Western Tuscany Blood Bank, Pisa University Hospital, Pisa, Italy.	594
59 - The Propionibacterium spp. extract reduces Candida albicans-induced damage to vaginal epithelial cells and increases mitochondrial response to Candida albicans infection in vitro	596
FRANCESCO RICCHI ⁽¹⁾ - ANDREA ARDIZZONI ⁽²⁾ - ELISABETTA BLASI ⁽²⁾ - FRANCESCO DE SETA ⁽³⁾ - EVA PERICOLINI ⁽²⁾	596
Università degli studi di Modena e Reggio Emilia, Dipartimento di Scienze Biomediche, Metaboliche e Neuroscienze, Modena, Italia ⁽¹⁾ - Università degli studi di Modena e Reggio Emilia, Dipartimento Chirurgico, Medico, Odontoiatrico e di Scienze Morfologiche con Interesse Trapiantologico, Oncologico e di Medicina Rigenerativa, Modena, Italia ⁽²⁾ - Università degli studi di Trieste, Dipartimento di Scienze Mediche, Trieste, Italia ⁽³⁾	596
The Propionibacterium spp. extract reduces Candida albicans-induced damage to vaginal epithelial cells and increases mitochondrial response to Candida albicans infection in vitro.....	596
FRANCESCO RICCHI ¹ , ANDREA ARDIZZONI ² , ELISABETTA BLASI ² , FRANCESCO DE SETA ^{3,4} , EVA PERICOLINI ²	596
¹ Clinical and Experimental Medicine Ph.D. Program, University of Modena and Reggio Emilia, Modena, Italy;	596
² Department of Surgical, Medical, Dental and Morphological Sciences with interest in Transplant, Oncological and Regenerative Medicine, University of Modena and Reggio Emilia, Modena, Italy	596
³ Department of Medical Sciences, University of Trieste, Trieste, Italy;	596
⁴ Institute for Maternal and Child Health IRCCS "Burlo Garofolo", Trieste, Italy.	596
83 - The interferon-beta/MLKL axis promotes anti-bacterial defenses by enhancing neutrophil- mediated pathogen killing.....	598
Agata Famà ⁽¹⁾ - GERMANA LENTINI ⁽¹⁾ - GIUSEPPE VALERIO DE GAETANO ⁽¹⁾ - FRANCESCO COPPOLINO ⁽²⁾ - GIUSEPPE TETI ⁽³⁾ - CONCETTA BENINATI ⁽¹⁾	598
Università degli Studi di Messina, Dipartimento di Patologia Umana, Messina, Italia ⁽¹⁾ - UNIVERSITA' DEGLI STUDI DI MESSINA, DIPARTIMENTO DELLE SCIENZE BIOMEDICHE , ODONTOIATRICHE E DELLE IMMAGINI MORFOLOGICHE E FUNZIONALI, MESSINA, Italia ⁽²⁾ - Scylla Biotech, srl, Scylla Biotech, srl, MESSINA, Italia ⁽³⁾	598
The interferon-beta/MLKL axis promotes anti-bacterial defenses by enhancing neutrophil- mediated pathogen killing.....	598
AGATA FAMA ¹ , GERMANA LENTINI ¹ , GIUSEPPE V. DE GAETANO ¹ , FRANCESCO COPPOLINO ² , GIUSEPPE TETI ³ AND CONCETTA BENINATI ^{1,3}	598
1. Department of Human Pathology, University of Messina, Messina, Italy;.....	598

2. Department of Biomedical, Dental and Imaging Sciences, University of Messina, Messina, Italy;	598
3. Scylla Biotech Srl, Messina, Italy	598
95 - Kinetics and quality of humoral immune response against SARS-CoV-2 after vaccination and natural infection.....	600
SARA CAUCCI ⁽¹⁾ - ROBERTA LONGO ⁽¹⁾ - IRENE CORI ⁽¹⁾ - BENEDETTA CORVARO ⁽¹⁾ - ANNA VALENZA ⁽²⁾ - KATIA MARINELLI ⁽²⁾ - STEFANO MENZO ⁽¹⁾	600
Dipartimento di scienze biomediche e sanità pubblica, Università politecnica delle Marche, Ancona, Italia ⁽¹⁾ - Laboratorio di virologia, Azienda ospedaliero universitaria delle Marche, Ancona, Italia ⁽²⁾	600
Kinetics and quality of humoral immune response against SARS-CoV-2 after vaccination and natural infection.	600
SARA CAUCCI ¹ , ROBERTA LONGO ¹ , CORI IRENE ¹ , BENEDETTA CORVARO ¹ , ANNA VALENZA ² , KATIA MARINELLI ² , AND STEFANO MENZO ^{1,2}	600
¹ Department of Biomedical Sciences and Public Health, Università Politecnica delle Marche, Ancona, Italy;.....	600
² Virology Laboratory, Azienda Ospedaliero Universitaria delle Marche, Ancona, Italy;	600
101 - A novel subset of IFNγ-producing Th17 cells is selectively associated with intestinal inflammation in Crohn's Disease and specifically activated by adherent-invasive Escherichia coli (AIEC)	602
MOIRA PARONI ⁽¹⁾ - GABRIELLA LECCESE ⁽¹⁾ - VALERIA RANZANI ⁽²⁾ - DANIELE NOVIELLO ⁽³⁾ - MAURIZIO VECCHI ⁽³⁾ - FEDERICA FACCIOTTI ⁽⁴⁾ - JENS GEGINAT ⁽²⁾ - FLAVIO CAPRIOLI ⁽³⁾	602
Università degli studi di Milano, Dipartimento di Bioscienze, Milano, Italia ⁽¹⁾ - INGM, Istituto Nazionale di Genetica Molecolare, milano, Italia ⁽²⁾ - Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Gastroenterology and Endoscopy Unit, Milano, Italia ⁽³⁾ - University of Milano-Bicocca, Italy, Department of Biotechnology and Bioscience, Milano, Italia ⁽⁴⁾	602
Moira Paroni ¹ *, Gabriella Leccese ¹ , Valeria Ranzani ² , Daniele Noviello ³ , Maurizio Vecchi ³ , Federica Facciotti ⁴ , Jens Geginat ² and Flavio Caprioli ³	602
¹ Department of Biosciences, Università degli Studi di Milano, Milan, Italy.....	602
² INGM-National Institute of Molecular Genetics "Romeo ed Enrica Invernizzi", Milan, Italy .	602
³ Gastroenterology and Endoscopy Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy Department of Experimental Oncology, European Institute of Oncology, Milan, Italy.....	602
⁴ Department of Biotechnology and Bioscience, University of Milano-Bicocca, Milan,Italy	602
A novel subset of IFNγ-producing Th17 cells is selectively associated with intestinal inflammation in Crohn's Disease and specifically activated by adherent-invasive Escherichia coli (AIEC)	602
109 - Anti-HBc loss in multiple sclerosis patients treated with ocrelizumab.....	604
PATRIZIA PASCULLI ⁽¹⁾ - MARIA ANTONELLA ZINGAROPOLI ⁽¹⁾ - FEDERICA DOMINELLI ⁽¹⁾ - EEVA TORTELLINI ⁽¹⁾ - MATTEO TARTAGLIA ⁽²⁾ - GINA FERRAZZANO ⁽³⁾ - ANTONELLA CONTE ⁽³⁾ - CLAUDIO MARIA MASTROIANNI ⁽¹⁾ - MARIA ROSA CIARDI ⁽¹⁾	604
Policlinico Umberto I, Department of Public Health and Infectious Diseases, Sapienza University of Rome, Roma, Italia ⁽¹⁾ - Policlinico Umberto I, Department of Human Neuroscience, Sapienza University	

of Rome, Viale dell'Università, Italia, Italia ⁽²⁾ - Policlinico Umberto I, Department of Human Neuroscience, Sapienza University of Rome, Viale dell'Università, Roma, Italia ⁽³⁾	604
Anti-HBc loss in multiple sclerosis patients treated with ocrelizumab	604
Patrizia Pasculli ¹ , Maria Antonella Zingaropoli ¹ , Federica Dominelli ¹ ,Eeva Tortellini ¹ , Matteo Tartaglia ² , Gina Ferrazzano ² , Antonella Conte ² , Claudio Maria Mastroianni ¹ , Maria Rosa Ciardi ¹	604
¹ Department of Public Health and Infectious Diseases, Sapienza University of Rome, Viale del Policlinico 155, 00185, Rome, Italy.	604
² Department of Human Neuroscience, Sapienza University of Rome, Viale dell'Università 30, 00185, Rome, Italy.	604
119 - Infection of macrophages by SARS-CoV-2: implications for viral spreading and immune response	606
LUCIA SIGNORINI ⁽¹⁾ - STEFANO GIANOLI ⁽²⁾ - MARIA DOLCI ⁽¹⁾ - KELVIN KAMAU MAINA ⁽¹⁾ - MASSIMO LOCATI ⁽²⁾ - SERENA DELBUE ⁽¹⁾	606
University of Milan, Department of Biomedical, Surgical and Dental Sciences, University of Milan, Milan, Italy, Milano, Italia ⁽¹⁾ - University of Milan, Milan, Italy, Department of Medical Biotechnologies and Translational Medicine, Milano, Italia ⁽²⁾	606
Infection of macrophages by SARS-CoV-2: implications for viral spreading and immune response	606
LUCIA SIGNORINI¹, STEFANO GIANOLI² MARIA DOLCI¹, KELVIN KAMAU MAINA¹, MASSIMO LOCATI^{2,3}, SERENA DELBUE¹	606
¹ Department of Biomedical, Surgical and Dental Sciences, University of Milan, Milan, Italy;	
² Department of Medical Biotechnologies and Translational Medicine, University of Milan, Milan, Italy;.....	606
³ IRCCS Human Research Hospital, Rozzano, Italy.	606
122 - Questioning the role of fibroblast growth factor-2 during Zika virus infection and infection by other viruses.	608
GIULIA LOTTINI ⁽¹⁾ - ERIKA PLICANTI ⁽¹⁾ - MATTEO MATTEUCCI ⁽²⁾ - MICHELE LAI ⁽²⁾ - MAURO PISTELLO ⁽²⁾ - GIULIA FREER ⁽²⁾	608
University of Siena, Department of Medical biotechnologies, Siena, Italia ⁽¹⁾ - University of Pisa, Department of Translational Research, Pisa, Italia ⁽²⁾	608
Questioning the role of fibroblast growth factor-2 during Zika virus infection and infection by other viruses.	608
GIULIA LOTTINI^{1, 2}, ERIKA PLICANTI^{1, 2}, MATTEO MATTEUCCI¹, MICHELE LAI¹, MAURO PISTELLO ¹, GIULIA FREER¹,.....	608
¹ Dept. Translational Research, Retrovirus Center, University of Pisa, Pisa, Italy;	608
² Dept. Medical Biotechnologies, University of Siena, Siena, Italy.	608
134 - HERV-K Antibodies and their Potential Protective Effect in Amyotrophic Lateral Sclerosis	610
ELENA RITA SIMULA ⁽¹⁾ - MARTA GARCIA-MONTOJO ⁽²⁾ - AVINDRA NATH ⁽²⁾ - LEONARDO ANTONIO SECHI ⁽¹⁾	610
Università degli Studi di Sassari, Dipartimento di Scienze Biomediche, Sassari, Italia ⁽¹⁾ - National Institutes of Health, National Institute of Neurological Disorders and Stroke, Bethesda, Stati Uniti D'america ⁽²⁾	610

HERV-K Antibodies and their Potential Protective Effect in Amyotrophic Lateral Sclerosis.....	610
ELENA R. SIMULA¹, MARTA GARCIA-MONTOJO³, AVINDRA NATH³, LEONARDO A. SECHI^{1,2}	610
¹Department of Biomedical Sciences, Division of Microbiology and Virology, University of Sassari, Sassari, Italy;.....	610
²Struttura Complessa Microbiologia e Virologia, Azienda Ospedaliera Universitaria Sassari, Sassari, Italy;.....	610
³National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, Maryland, USA.....	610
136 - Cerebrospinal fluid and plasma biomarkers in patients with central nervous system infections: a retrospective study	612
MARIA ANTONELLA ZINGAROPOLI ⁽¹⁾ - PATRIZIA PASCULLI ⁽¹⁾ - FEDERICA DOMINELLI ⁽¹⁾ - FEDERICA CICCONE ⁽¹⁾ - MATTEO TARTAGLIA ⁽²⁾ - GINA FERRAZZANO ⁽²⁾ - TIZIANA LATRONICO ⁽³⁾ - MIRIAM LICHTNER ⁽⁴⁾ - ANTONELLA CONTE ⁽²⁾ - GIOACCHINO GALARDO ⁽⁵⁾ - GRAZIA MARIA LIUZZI ⁽³⁾ - CLAUDIO MARIA MASTROIANNI ⁽¹⁾ - GUIDO ANTONELLI ⁽⁶⁾ - OMBRETTA TURRIZIANI ⁽⁶⁾ - MARIA ROSA CIARDI ⁽¹⁾	612
Rome University of Rome, Department of Public Health and Infectious Diseases, Rome, Italia ⁽¹⁾ - Sapienza University of Rome, Department of Human Neurosciences, Rome, Italia ⁽²⁾ - University of Bari Aldo Moro, Department of Biosciences, Biotechnologies and Biopharmaceutics, Bari, Italia ⁽³⁾ - Sapienza University of Rome, Department of Neurosciences Mental Health and Sensory Organs, Rome, Italia ⁽⁴⁾ - Sapienza University of Rome, Medical Emergency Unit, Policlinico Umberto I, Rome, Italia ⁽⁵⁾ - Sapienza University of Rome, Department of Molecular Medicine, Rome, Italia ⁽⁶⁾	612
Cerebrospinal fluid and plasma biomarkers in patients with central nervous system infections: a retrospective study.....	612
Maria Antonella Zingaropoli¹, Patrizia Pasculli¹, Federica Dominelli¹, Federica Ciccone¹, Matteo Tartaglia², Gina Ferrazzano², Tiziana Latronico³, Miriam Lichtner⁴, Antonella Conte², Gioacchino Galardo⁵, Grazia Maria Liuzzi³, Claudio Maria Mastroianni¹, Guido Antonelli⁶, Ombretta Turriziani⁶, Maria Rosa Ciardi¹	612
¹Department of Public Health and Infectious Diseases, Sapienza University of Rome – Rome... 612	
²Department of Human Neurosciences, Sapienza University of Rome - Rome	612
³Department of Biosciences, Biotechnologies and Biopharmaceutics, University of Bari Aldo Moro - Bari	612
⁴Department of Neurosciences Mental Health and Sensory Organs, Sapienza University of Rome - Rome	612
⁵Medical Emergency Unit, Policlinico Umberto I - Sapienza University of Rome- Rome.....	612
⁶Department of Molecular Medicine - Sapienza University of Rome - Rome	612
139 - Evaluation of LIAISON® MeMed BV® to discriminate bacterial and viral infections in patients with Chronic Obstructive Pulmonary Disease	614
ALESSANDRO GRAZIANI ⁽¹⁾ - MARIA CRISTINA BITONTE ⁽¹⁾ - ERICA GIOMBETTI ⁽²⁾ - ALFREDO VILLA ⁽²⁾ - ANTONELLA MENCACCI ⁽¹⁾ - BARBARA CAMILLONI ⁽¹⁾	614
Università degli Studi di Perugia, Dipartimento di Medicina e Chirurgia, Perugia, Italia ⁽¹⁾ - Azienda Ospedaliera di Perugia, Laboratorio di Patologia Clinica, Perugia, Italia ⁽²⁾	614

Evaluation of LIAISON® MeMed BV® to discriminate bacterial and viral infections in patients with Chronic Obstructive Pulmonary Disease	614
ALESSANDRO GRAZIANI ¹ , MARIA C. BITONTE ¹ , ERICA GIOMBETTI ² , ALFREDO VILLA ² , ANTONELLA MENCACCI ¹ , BARBARA CAMILLONI ¹	614
¹ Department of Medicine and Surgery, Microbiology and Clinical Microbiology Section, "Santa Maria Della Misericordia" Hospital, University of Perugia, 06129 Perugia, Italy;.....	614
² Clinical Pathology and Haematology Laboratory, "Santa Maria Della Misericordia" Hospital, 06129 Perugia, Italy	614
143 - Association of human endogenous retroviruses with immune dysfunction and development, progression and long-term sequelae of COVID-19.....	616
VITA PETRONE ⁽¹⁾ - ANTONELLA MINUTOLO ⁽¹⁾ - MARIALAURA FANELLI ⁽¹⁾ - CHRISTIAN MARACCHIONI ⁽¹⁾ - MARTINA GIUDICE ⁽¹⁾ - ROSSELLA CHIRICO ⁽¹⁾ - CHIARA CIPRIANI ⁽¹⁾ - LUIGI COPPOLA ⁽²⁾ - ELISABETTA TETI ⁽²⁾ - CHIARA SORACE ⁽²⁾ - MARTA ZORDAN ⁽²⁾ - PIETRO VITALE ⁽¹⁾ - VINCENZO MALAGNINO ⁽²⁾ - MARCO IANNETTA ⁽²⁾ - HERVE PERRON ⁽³⁾ - SANDRO GRELLI ⁽⁴⁾ - EMANUELA BALESTRIERI ⁽¹⁾ - LOREDANA SARMAI ⁽²⁾ - CLAUDIA MATTEUCCI ⁽¹⁾	616
Università degli studi di Roma "Tor Vergata", Dipartimento di Medicina Sperimentale, ROMA, Italia ⁽¹⁾ - POLICLINO TOR VERGATA, Malattie Infettive, Roma, Italia ⁽²⁾ - GENEURO, Geneuro Innovation, Lyon, Francia ⁽³⁾ - POLICLINO TOR VERGATA, Unità di Virologia, Roma, Italia ⁽⁴⁾	616
Association of human endogenous retroviruses with immune dysfunction and development, progression and long-term sequelae of COVID-19.....	616
VITA PETRONE ¹ , ANTONELLA MINUTOLO ¹ , MARIALAURA FANELLI ¹ , CHRISTIAN MARACCHIONI ¹ , MARTINA GIUDICE ¹ , ROSSELLA CHIRICO ¹ , CHIARA CIPRIANI ¹ , LUIGI COPPOLA ² , ELISABETTA TETI ² , CHIARA SORACE ² , MARTA ZORDAN ^{2,3} , PIETRO VITALE ^{2,3} , VINCENZO MALAGNINO ^{2,3} , MARCO IANNETTA ^{2,3} , HERVÈ PERRON ⁴ , SANDRO GRELLI ^{1,5} , EMANUELA BALESTRIERI ¹ , LOREDANA SARMAI ^{2,3} , CLAUDIA MATTEUCCI ¹	616
1. Department of Experimental Medicine, University of Rome Tor Vergata, Rome, 00133, Italy; 2. Infectious Diseases Clinic, Policlinic of Tor Vergata, Rome, 00133, Italy; 3. Department of Systems Medicine, University of Rome Tor Vergata, Rome, 00133, Italy; 4. Geneuro – Innovation, Lyon-France 69008;5. Virology Unit, Policlinic of Tor Vergata, Rome, 00133, Italy.	616
156 - Differential type I interferon expression between children and adolescents with long covid..	618
MATTEO FRACELLA ⁽¹⁾ - FEDERICA FRASCA ⁽¹⁾ - LEONARDO SORRENTINO ⁽¹⁾ - ALESSANDRA D'AURIA ⁽¹⁾ - LETIZIA SANTINELLI ⁽²⁾ - LUCA MADDALONI ⁽²⁾ - GINEVRA BUGANI ⁽²⁾ - CHIARA VIRIGILLITO ⁽²⁾ - ENRICA MANCINO ⁽³⁾ - GRETA DI MATTIA ⁽³⁾ - MARIA GIULIA CONTI ⁽³⁾ - LUIGI MATERA ⁽³⁾ - BENIAMINO CAPUTO ⁽²⁾ - GABRIELLA D'ETTORRE ⁽²⁾ - FABIO MIDULLA ⁽³⁾ - GUIDO ANTONELLI ⁽¹⁾ - ALESSANDRA PIERANGELI ⁽¹⁾ - CAROLINA SCAGNOLARI ⁽¹⁾	618
Sapienza - University Of Rome, Virology Laboratory, Department Of Molecular Medicine, Rome, Italia ⁽¹⁾ - Sapienza - University Of Rome, Department Of Public Health And Infectious Diseases, Rome, Italia ⁽²⁾ - Sapienza - University Of Rome, Department Of Pediatrics And Infantile Neuropsychiatry, Rome, Italia ⁽³⁾	618
Differential Type I Interferon Expression Between Children And Adolescents With Long Covid	618

MATTEO FRACELLA ¹ , FEDERICA FRASCA ¹ , LEONARDO SORRENTINO ¹ , ALESSANDRA D'AURIA ¹ , LETIZIA SANTINELLI ² , LUCA MADDALONI ² , GINEVRA BUGANI ² , CHIARA VIRGILLITO ² , ENRICA MANCINO ³ , GRETA DI MATTIA ³ , MARIA GIULIA CONTI ³ , LUIGI MATERA ³ , BENIAMINO CAPUTO ² , GABRIELLA D'ETTORRE ² , FABIO MIDULLA ³ , GUIDO ANTONELLI ¹ , ALESSANDRA PIERANGELI ¹ , CAROLINA SCAGNOLARI ¹	618
1department Of Molecular Medicine, Virology Laboratory, "Sapienza" University, Rome, Italy;	
2department Of Public Health And Infectious Diseases, "Sapienza University", Rome, Italy;	
3department Of Pediatrics And Infantile Neuropsychiatry, "Sapienza" University, Rome, Italy.	618
163 - Phosphatidylserine liposomes as a possible candidate for the therapeutic management of Mycobacterium abscessus infection	620
NOEMI POERIO ⁽¹⁾ - TOMMASO OLIMPIERI ⁽¹⁾ - GRETA PONSECCHI ⁽¹⁾ - FABIANA CICIRIELLO ⁽²⁾ - VINCENZINA LUCIDI ⁽²⁾ - MARCO MARIA D'ANDREA ⁽¹⁾ - MAURIZIO FRAZIANO ⁽¹⁾	620
University of Rome "Tor Vergata", Department of Biology, Rome, Italia ⁽¹⁾ - Bambino Gesù Pediatric Hospital, Department of Pediatric Medicine, Cystic Fibrosis Complex Operating Unit, Rome, Italia ⁽²⁾	620
Phosphatidylserine liposomes as a possible candidate for the therapeutic management of Mycobacterium abscessus infection	620
NOEMI POERIO ¹ , TOMMASO OLIMPIERI ¹ , GRETA PONSECCHI ^{1,3} , FABIANA CICIRIELLO ² , VINCENZINA LUCIDI ² , MARCO M. D'ANDREA ¹ , MAURIZIO FRAZIANO ¹	620
¹ Dept. of Biology, University of Rome "Tor Vergata", Rome, Italy;	620
² Department of Pediatric Medicine, Cystic Fibrosis Complex Operating Unit, Bambino Gesù Pediatric Hospital, Rome, Italy.	620
³ PhD Program in Evolutionary Biology and Ecology, Dept. of Biology, University of Rome "Tor Vergata", Rome, Italy.	620
184 - Yet another potential microbial player in autoimmunity: multicentric analysis of the association between Mycoplasma hominis serostatus and rheumatoid arthritis	621
ANTONELLA CONGIARGIU (1) - NICIA DIAZ (1) - NGOC DAN THANH PHAN (2) - CIRIACO CARRU (1) - LEONARDO ANTONIO SECHI (1) - PAOLA RAPPELLI (1) - PIER LUIGI FIORI (1) - ALBERTO ALBERTI (3) - GIANLUCA ERRE (4) - DANIELE DESSI (1)	621
Università degli Studi di Sassari, Dipartimento di Scienze Biomediche, Sassari, Italia (1) - Hue University of Medicine and Pharmacy, Department of Parasitology Hue University of Medicine and Pharmacy, Hue, Vietnam (2) - Università degli Studi di Sassari, Dipartimento di Medicina Veterinaria, Sassari, Italia (3) - Università degli Studi di Sassari, Dipartimento di Medicina, Chirurgia e Farmacia, Sassari, Italia (4).....	621
Yet another potential microbial player in autoimmunity: multicentric analysis of the association between Mycoplasma hominis serostatus and rheumatoid arthritis.....	621
ANTONELLA CONGIARGIU 1, NICIA DIAZ 1, NGOC DAN THANH PHAN 2, CIRIACO CARRU 1, LEONARDO A. SECHI 1, PAOLA RAPPELLI 1, PIER L. FIORI 1, ALBERTO ALBERTI 3, GIANLUCA ERRE 4, DANIELE DESSI 1	621
1 Department of Biomedical Sciences, University of Sassari, Sassari, Italy;.....	621
2 Department of Parasitology Hue University of Medicine and Pharmacy, Hue, Vietnam;	621

3 Department of Veterinary Medicine, University of Sassari, Sassari, Italy;	621
4 Department of Medicine, Surgery and Pharmacy, School of Medicine, University of Sassari, Sassari, Italy	621
199 - Evaluation of the immunomodulatory activity of Cellulophaga species LPSs.	623
GIUSEPPE MANTOVA ⁽¹⁾ - ELENA SCAGLIONE ⁽²⁾ - MARTINA DI ROSARIO ⁽¹⁾ - CIRO VECE ⁽¹⁾ - ROBERTA CIRELLA ⁽³⁾ - MOLLY D. PITHER ⁽³⁾ - CHIARA PAGLIUCA ⁽¹⁾ - FLAVIANA DI LORENZO ⁽⁴⁾ - ALBA SILIPO ⁽⁴⁾ - ANTONIO MOLINARO ⁽⁴⁾ - MARIA TERESA VITILIELLO ⁽²⁾ - ROBERTA COLICCHIO ⁽²⁾ - PAOLA SALVATORE ⁽⁵⁾	623
University of Naples Federico II, Department of Molecular Medicine and Medical Biotechnology, Naples, Italia ⁽¹⁾ - University of Naples Federico II, Dpt of Mol Med and Med Bio; Dpt of Int Act of Lab Med and Transf, Complex Oper Unit of Clinical Microbi, Naples, Italia ⁽²⁾ - University of Naples Federico II, Department of Chemical Sciences, Naples, Italia ⁽³⁾ - University of Naples Federico II, Department of Chemical Sciences; Task Force on Microbiome Studies, Naples, Italia ⁽⁴⁾ - University of Naples Federico II, Dpt of Mol Med and Med Bio; Dpt of Int Act of Lab Med and Transf, Unit of Clinical Microbi; Task Force Microbiome; CEINGE Franco Salvatore, Naples, Italia ⁽⁵⁾	623
Evaluation of the immunomodulatory activity of Cellulophaga species LPSs.	623
GIUSEPPE MANTOVA ¹ , ELENA SCAGLIONE ^{1,2} , MARTINA DI ROSARIO ¹ , CIRO VECE ¹ , ROBERTA CIRELLA ³ MOLLY D. PITHER ³ , CHIARA PAGLIUCA ¹ , FLAVIANA DI LORENZO ^{3,4} , ALBA SILIPO ^{3,4} , ANTONIO MOLINARO ^{3,4} , MARIA TERESA VITIELLO ^{1,2} , ROBERTA COLICCHIO ^{1,2} , PAOLA SALVATORE ^{1,2,4,5}	623
¹ Department of Molecular Medicine and Medical Biotechnology, University of Naples Federico II, Naples, Italy; ² Department of Integrated Activity of Laboratory Medicine and Transfusion, Complex Operative Unit of Clinical Microbiology, University Hospital Federico II, Naples, Italy; ³ Department of Chemical Sciences, University of Naples Federico II, Naples, Italy; ⁴ Task Force on Microbiome Studies, University of Naples Federico II, Naples, Italy; ⁵ CEINGE, Advanced Biotechnologies Franco Salvatore s.c.ar.l., Naples, Italy.....	623
245 - Regulation of permissiveness/restriction to herpes simplex virus 1 infection in different cell types by cellular microRNAs	625
AGNESE RAGNO (1) - DOMENICO GIOSA (1) - VALERIA STEFANIZZI (2) - ANTONIO MASTINO (3) - FRANCESCA MARINO-MERLO (1).....	625
Department of Chemical, Biological, Pharmaceutical, and Environmental Sciences,, University of Messina, MESSINA, Italia (1) - Department of Chemical Science and Technology, University of i Rome “Tor Vergata”, ROMA, Italia (2) - The Institute of Translational Pharmacology, CNR, ROMA, Italia (3)	625
Regulation of permissiveness/restriction to herpes simplex virus 1 infection in different cell types by cellular microRNAs.....	625
AGNESE RAGNO ¹ , DOMENICO GIOSA ¹ , VALERIA STEFANIZZI ² , ANTONIO MASTINO ³ , FRANCESCA MARINO-MERLO ¹	625
¹ Department of Chemical, Biological, Pharmaceutical, and Environmental Sciences, University of Messina, Messina, Italy; ² Department of Chemical Science and Technology, University of Rome “Tor Vergata”, Rome, Italy; ³ The Institute of Translational Pharmacology, CNR, Rome, Italy.	625
250 - The viral sensor IFI16 at the crossroads between damage and tolerance during human Coronavirus infection.....	627

CINZIA BORGOGNA ⁽¹⁾ - ILARIA CISLAGHI ⁽¹⁾ - SARAH TURATI ⁽¹⁾ - GLORIA GRIFFANTE ⁽¹⁾ - SHIKA CHANDEL ⁽¹⁾ - IRENE LO CIGNO ⁽¹⁾ - MARCO DE ANDREA ⁽²⁾ - LUCIA SIGNORINI ⁽³⁾ - SERENA DELBUE ⁽³⁾ - MARISA GARIGLIO ⁽¹⁾	627
Università Piemonte Orientale, Dip. Medicina Traslazionale, Novara, Italia ⁽¹⁾ - Università Studi di Torino, Dip. Salute Pubblica e Scienze Pediatriche, Torino, Italia ⁽²⁾ - Università di Milano, Dip. Scienze Biomediche, Chirurgiche ed Odontoiatriche, Milano, Italia ⁽³⁾	627
The viral sensor IFI16 at the crossroads between damage and tolerance during human Coronavirus infection	627
Cinzia BORGOGNA ¹ , Iliaria CISLAGHI ¹ , Sarah TURATI ¹ , Gloria GRIFFANTE ¹ , Shika CHANDEL ¹ , Irene LO CIGNO ¹ , Marco DE ANDREA ^{2,3} , Lucia SIGNORINI ⁴ , Serena DELBUE ⁴ , Marisa GARIGLIO ^{1,2}	627
¹ Virology Unit, Department of Translational Medicine, University of Piemonte Orientale, Novara, Italy.	627
² CAAD Center for Translational Research on Autoimmune and Allergic Disease, Novara, Italy.	627
³ Viral Pathogenesis Unit, Department of Public Health and Pediatric Sciences, University of Turin, Turin, Italy.	627
⁴ Biomedical, Surgical and Dental Sciences, University of Milan, Milano, Italy.	627
255 - Effects of Ropivacaine and Propofol on Human endogenous retroviruses activity, embryonic genes, CD-133 and PI3K-AKT-mTOR signaling in head-neck carcinoma cell lines: a proof-of-concept study providing	629
MARTINA GIUDICE ⁽¹⁾ - VITA PETRONE ⁽¹⁾ - MARIALAURA FANELLI ⁽¹⁾ - ERMAL PASHAJ ⁽²⁾ - ROSSELLA CHIRICO ⁽¹⁾ - FILADELFO CONIGLIONE ⁽³⁾ - ANTONELLA MINUTOLO ⁽¹⁾ - PAOLA SINIBALDI-VALLEBONA ⁽¹⁾ - CLAUDIA MATTEUCCI ⁽¹⁾ - CHIARA CIPRIANI ⁽¹⁾ - EMANUELA BALESTRIERI ⁽¹⁾	629
University of Rome Tor Vergata, Department of Experimental Medicine, Rome, Italia ⁽¹⁾ - Catholic University'Our Lady of Good Council', Department of Surgery, Tirana, Albania ⁽²⁾ - Catholic University'Our Lady of Good Council', Department of Surgical Science, Tirana, Albania ⁽³⁾	629
Effects of Ropivacaine and Propofol on Human endogenous retroviruses activity, embryonic genes, CD-133 and PI3K-AKT-mTOR signaling in head-neck carcinoma cell lines: a proof-of-concept study providing potential implications in the clinical setting.....	629
Martina Giudice ¹ , Vita Petrone ¹ , Marialaura Fanelli ¹ , Ermal Pashaj ² , Rossella Chirico ¹ , Filadelfo Coniglione ^{3,4} , Antonella Minutolo ¹ , Paola Sinibaldi-Vallebona ¹ , Claudia Matteucci ¹ , Chiara Cipriani ¹ , Emanuela Balestrieri ¹	629
1.Department of Experimental Medicine, University of Rome Tor Vergata, Rome, 00133, Italy.	629
2. Department of Surgery at Catholic University'Our Lady of Good Council', Tirana, 1000, Albania.....	629
3. Department of Surgical Science, Catholic University'Our Lady of Good Council', Tirana, 1000, Albania.....	629
4. Department of Clinical Science and Translational Medicine, University of Rome Tor Vergata, Rome, 00133, Italy.	629
256 - Preliminary evidence of the differential expression of Human endogenous retroviruses in Kawasaki disease and SARS-CoV-2-associated multisystem inflammatory syndrome.	631

CHIARA CIPRIANI ⁽¹⁾ - MARTINA GIUDICE ⁽¹⁾ - ELENA CORINALDESI ⁽²⁾ - VITA PETRONE ⁽¹⁾ - MARIALAURA FANELLI ⁽¹⁾ - MARIANNA FABI ⁽³⁾ - MARCELLO LANARI ⁽³⁾ - ANTONELLA MINUTOLO ⁽¹⁾ - SANDRO GRELLI ⁽¹⁾ - PAOLA SINIBALDI-VALLEBONA ⁽¹⁾ - CLAUDIA MATTEUCCI ⁽¹⁾ - EMANUELA BALESTRIERI ⁽¹⁾	631
University of Rome Tor Vergata, Department of Experimental Medicine, Rome, Italia ⁽¹⁾ - Ramazzini Hospital, Pediatric Unit, Carpi, Italia ⁽²⁾ - IRCCS University Hospital of Bologna, Pediatric Emergency Unit, Bologna, Italia ⁽³⁾	631
Preliminary evidence of the differential expression of Human endogenous retroviruses in Kawasaki disease and SARS-CoV-2-associated multisystem inflammatory syndrome.....	631
Chiara Cipriani ¹ , Martina Giudice ¹ , Elena Corinaldesi ² , Vita Petrone ¹ , Marialaura Fanelli ¹ , Marianna Fabi ³ , Marcello Lanari ³ , Antonella Minutolo ¹ , Sandro Grelli ^{1,4} , Paola Sinibaldi-Vallebona ¹ , Claudia Matteucci ¹ , Emanuela Balestrieri ¹	631
1. Department of Experimental Medicine, University of Rome Tor Vergata, Rome, 00133 Italy. 2. Pediatric Unit, Ramazzini Hospital, Carpi, Mo, 41121 Italy. 3. Pediatric Emergency Unit, IRCCS University Hospital of Bologna, Bologna, 40138 Italy. 4. Virology Unit, Policlinic of Tor Vergata, Rome, 00133, Italy;.....	631
257 - "REDUCED CLEARANCE OF SARS-COV-2 INFECTION IN IMMUNOCOMPROMISED INDIVIDUALS DESPITE VACCINATION-INDUCED RESPONSE"	633
PAOLA MAZZETTI ⁽¹⁾ - MARIA SIDOTI ⁽¹⁾	633
Università di Pisa, Azienda Ospedaliero-Universitaria Pisana, pisa, Italia ⁽¹⁾	633
Reduced Clearance of SARS-CoV-2 Infection in Immunocompromised Individuals Despite Vaccination-Induced Response	633
MARIA SIDOTI ^{a,b} , PAOLA MAZZETTI ^a , PIETRO G. SPEZIA ^b , ALESSIA CALÓ ^b , ANNALISA CAPRIA ^a , GIULIA FREER ^b , SILVIA COSTARELLI ^c , ALICE CARA ^a , ALFREDO ROSELLINI ^a , SUSI FRATESCHI ^a , GIOVANNA MOSCATO ^a , IACOPO FRANCONI ^a , MARIA L. VATTERONI ^a , MICHELE LAI ^b , PAOLA QUARANTA ^b , , SPARTACO SANI ^c ENRICO TAGLIAFERRI ^d , MAURO PISTELLO ^{a,b}	633
^a Department of Laboratory Medicine, Virology Unit, Pisa University Hospital, Pisa, Italy;.....	633
^b Department of Translational Research, Retrovirus Center and Virology Section, University of Pisa, Pisa, Italy;	633
^c Department of Infectious and Tropical Diseases, Infectious Diseases Unit, Leghorn Hospital, Azienda USL Toscana Nord-Ovest, Leghorn, Italy;	633
^d Infectious Diseases Unit, Department of Clinical and Experimental Medicine, Infectious Disease Unit, Pisa University Hospital, Pisa, Italy	633
273 - Type I and III IFN signature in cells from skin lesions, anal canal brushing and nasopharyngeal swabs of MPXV infected patients.....	635
ALESSANDRA D'AURIA ⁽¹⁾ - MATTEO FRACELLA ⁽¹⁾ - FEDERICA FRASCA ⁽¹⁾ - LEONARDO SORRENTINO ⁽¹⁾ - LAURA RAIMONDO ⁽¹⁾ - LUCA MADDALONI ⁽²⁾ - GINEVRA BUGANI ⁽²⁾ - LETIZIA SANTINELLI ⁽²⁾ - ALESSANDRA PIERANGELI ⁽¹⁾ - GABRIELLA D'ETTORRE ⁽²⁾ - GUIDO ANTONELLI ⁽¹⁾ - MAURO PISTELLO ⁽³⁾ - CAROLINA SCAGNOLARI ⁽¹⁾	635
Sapienza University of Rome, Department of Molecular Medicine, Roma, Italia ⁽¹⁾ - Sapienza University of Rome, Department of Public Health and Infectious Diseases, Roma, Italia ⁽²⁾ - University of Pisa, Department of Biomedicine, Pisa, Italia ⁽³⁾	635

Type I and III IFN signature in cells from skin lesions, anal canal brushing and nasopharyngeal swabs of MPXV infected patients	635
Alessandra D'Auria ¹ , Matteo Fracella ¹ , Federica Federica ¹ , Leonardo Sorrentino ¹ , Laura Raimondo ¹ , Luca Maddaloni ² , Ginevra Bugani ² , Letizia Santinelli ² , Alessandra Pierangeli ¹ , Gabriella D'Ettore ² , Guido Antonelli ¹ , Mauro Pistello ³ , Carolina Scagnolari ¹	635
¹ Department of Molecular Medicine, Laboratory of Virology, Sapienza University of Rome, Rome, Italy; ² Department of Public Health and Infectious Diseases, Sapienza University of Rome, Rome, Italy; ³ Department of Biomedicine, Virology Section and Retrovirus Centre, University of Pisa, Pisa, Italy	635
284 - IL-6-mediated paracrine senescence drives the intercellular communication within the HCMV microenvironment in renal epithelial cells	637
STEFANO RAVIOLA ⁽¹⁾ - ANDREA IANNUCI ⁽¹⁾ - VALERIA CANEPARO ⁽¹⁾ - DAVIDE LACARBONARA ⁽¹⁾ - FEDERICA CASTIGLIONI ⁽¹⁾ - IRENE LO CIGNO ⁽²⁾ - FRANCESCO FAVERO ⁽³⁾ - DAVIDE CORÀ ⁽³⁾ - VINCENZO CANTALUPPI ⁽⁴⁾ - SANTO LANDOLFO ⁽⁵⁾ - MARISA GARIGLIO ⁽²⁾ - MARCO DE ANDREA ⁽⁵⁾	637
Università del Piemonte orientale, CAAD - Center for Translational Research on Autoimmune and Allergic Disease, Intrinsic Immunity Unit, University of Piemonte Orientale, Novara, Italia ⁽¹⁾ - Università del Piemonte orientale, Department of Translational Medicine, Virology Unit, University of Piemonte Orientale, Novara, Italia ⁽²⁾ - Università del Piemonte orientale, CAAD - Center for Translational Research on Autoimmune and Allergic Disease, Bioinformatics Unit, University of Piemonte Orientale, Novara, Italia ⁽³⁾ - Università del Piemonte orientale, Department of Translational Medicine, Nephrology and Transplantation Unit, University of Piemonte Orientale, Novara, Italia ⁽⁴⁾ - Università degli Studi di Torino, Department of Public Health and Pediatric Sciences, Viral Pathogenesis Unit, University of Turin, Torino, Italia ⁽⁵⁾ ...	637
IL-6-mediated paracrine senescence drives the intercellular communication within the HCMV microenvironment in renal epithelial cells	637
STEFANO RAVIOLA ¹ , ANDREA IANNUCCI ¹ , VALERIA CANEPARO ¹ , DAVIDE LACARBONARA ¹ , FEDERICA CASTIGLIONI ¹ , IRENE LO CIGNO ² , FRANCESCO FAVERO ⁴ , DAVIDE CORÀ ⁴ , VINCENZO CANTALUPPI ³ , SANTO LANDOLFO ⁵ , MARISA GARIGLIO ^{1,2} AND MARCO DE ANDREA ^{1,5}	637
¹ CAAD - Center for Translational Research on Autoimmune and Allergic Disease, Intrinsic Immunity Unit, University of Piemonte Orientale, Novara, Italy;	637
² Department of Translational Medicine, Virology Unit, University of Piemonte Orientale, Novara, Italy;	637
³ Department of Translational Medicine, Nephrology and Transplantation Unit, University of Piemonte Orientale, Novara, Italy;	637
⁴ CAAD - Center for Translational Research on Autoimmune and Allergic Disease, Bioinformatics Unit, University of Piemonte Orientale, Novara, Italy;	637
⁵ Department of Public Health and Pediatric Sciences, Viral Pathogenesis Unit, University of Turin, Turin, Italy.	637
285 - The PYRIN domain of IFI16 drives LPS-induced and sterile toll-like receptor 4-mediated inflammation	639
DAVIDE LACARBONARA ⁽¹⁾ - ANDREA IANNUCCI ⁽¹⁾ - VALERIA CANEPARO ⁽¹⁾ - STEFANO RAVIOLA ⁽¹⁾ - GIORGIA MARTINIELLO ⁽¹⁾ - RICCARDO MIGGIANO ⁽²⁾ - MARISA GARIGLIO ⁽³⁾ - MARCO DE ANDREA ⁽⁴⁾	639

Università del Piemonte Orientale, CAAD - Center for Translational Research on Autoimmune and Allergic Disease, Intrinsic Immunity Unit, University of Piemonte Orientale, Novara, Italia ⁽¹⁾ - Università del Piemonte Orientale, Department of Pharmaceutical Sciences, University of Piemonte Orientale, Novara, Italia ⁽²⁾ - Università del Piemonte Orientale, Department of Translational Medicine, Virology Unit, University of Piemonte Orientale, Novara, Italia ⁽³⁾ - Università di Torino, Department of Public Health and Pediatric Sciences, Viral Pathogenesis Unit, University of Turin, Torino, Italia ⁽⁴⁾	639
The PYRIN domain of IFI16 drives LPS-induced and sterile toll-like receptor 4-mediated inflammation	639
DAVIDE LACARBONARA ¹ , ANDREA IANNUCCI ¹ , VALERIA CANEPARO ¹ , STEFANO RAVIOLA ¹ , GIORGIA MARTINIELLO ¹ RICCARDO MIGGIANO ² , MARISA GARIGLIO ^{1,3} AND MARCO DE ANDREA ^{1,4}	639
¹ CAAD - Center for Translational Research on Autoimmune and Allergic Disease, Intrinsic Immunity Unit, University of Piemonte Orientale, Novara, Italy;	639
² Department of Pharmaceutical Sciences, University of Piemonte Orientale, Novara, Italy	
³ Department of Translational Medicine, Virology Unit, University of Piemonte Orientale, Novara, Italy;.....	639
⁴ Department of Public Health and Pediatric Sciences, Viral Pathogenesis Unit, University of Turin, Turin, Italy.	639
302 - Comorbidities and clinical complications associated with SARS-CoV-2 variants.	641
ROBERTA MANENTE ⁽¹⁾ - VERONICA FOLLIERO ⁽¹⁾ - AMALIA MATTIELLO ⁽²⁾ - BIAGIO SANTELLA ⁽¹⁾ - CAROLINA VITALE ⁽¹⁾ - ANGELANTONIO MAGLIO ⁽¹⁾ - ALESSANDRO VATRELLA ⁽¹⁾ - GIANLUIGI FRANCI ⁽¹⁾ - ROBERTO PARRELLA ⁽³⁾ - ALBERTO MARRA ⁽³⁾	641
Dipartimento di Medicina, Chirurgia e Odontoiatria "Scuola Medica Salernitana", Università degli studi di Salerno, Salerno, Italia ⁽¹⁾ - Dipartimento di Medicina e Chirurgia, Università di Napoli Federico II, Napoli, Italia ⁽²⁾ - UOC Malattie infettive ad indirizzo respiratorio, AORN Ospedali dei Colli-Monaldi-Cotugno-CTO, Napoli, Italia ⁽³⁾	641
Comorbidities and clinical complications associated with SARS-CoV-2 variants.....	641
Roberta Manente ¹ , Veronica Folliero ¹ , Amalia Mattiello ² , Biagio Santella ¹ , Carolina Vitale ¹ , Angelantonio Maglio ¹ , Alessandro Vatrella ¹ , Gianluigi Franci ¹ , Roberto Parrella ³ , Alberto Marra ³	641
1. Department of Medicine, Surgery and Dentistry "Scuola Medica Salernitana", University of Salerno, Salerno, Italy	641
2. Department of Medicine and Surgery, University "Federico II", Naples, Italy.....	641
3. UOC Respiratory Infectious Diseases, AORN Ospedali dei Colli-Monaldi-Cotugno-CTO, Naples, Italy.....	641
13 Vaccini.....	643
125 - Heterologous boost immunisation with Spike protein after mucosal priming with recombinant Streptococcus gordonii expressing the receptor binding domain of SARS-CoV-2 spike protein on its surface.....	643
MARIANA TIRZIU ⁽¹⁾ - ELENA PETTINI ⁽¹⁾ - FABIO FIORINO ⁽¹⁾ - LORENZO COLOMBINI ⁽¹⁾ - ELISA LAZZERI ⁽¹⁾ - ANNA MARIA CUPPONE ⁽¹⁾ - FRANCESCO SANTORO ⁽¹⁾ - FRANCESCO IANNELLI ⁽¹⁾ - GIANNI POZZI ⁽¹⁾	643

Laboratorio di Microbiologia Molecolare e Biotecnologia (LAMMB), Università degli Studi di Siena/ Dipartimento di Biotecnologie Mediche, Siena, Italia ⁽¹⁾	643
Heterologous boost immunisation with Spike protein after mucosal priming with recombinant Streptococcus gordonii expressing the receptor binding domain of SARS-CoV-2 spike protein on its surface	643
MARIANA TIRZIU, ELENA PETTINI, FABIO FIORINO, LORENZO COLOMBINI, ELISA LAZZERI, ANNA MARIA CUPPONE, FRANCESCO SANTORO, FRANCESCO IANNELLI, GIANNI POZZI	643
Department of Medical Biotechnologies, Laboratory of Molecular Microbiology and Biotechnology (LAMMB), University of Siena, Siena, Italy	643
154 - Humoral and T-cell response to SARS-CoV-2 vaccination one year after the booster dose in oncology patients	645
ROBERTA CAMPAGNA ⁽¹⁾ - FEDERICA DOMINELLI ⁽²⁾ - MARIA ANTONELLA ZINGAROPOLI ⁽²⁾ - FABIO CIURLUINI ⁽³⁾ - VINCENZO PICONE ⁽⁴⁾ - ENRICO CORTESI ⁽⁴⁾ - GUIDO ANTONELLI ⁽¹⁾ - OMBRETTA TURRIZIANI ⁽¹⁾	645
Università La Sapienza, Dipartimento di Medicina Molecolare, Roma, Italia ⁽¹⁾ - Università La Sapienza, Dipartimento di Sanità Pubblica e Malattie Infettive, Roma, Italia ⁽²⁾ - Università La Sapienza, Dipartimento di Scienze Radiologiche, Oncologiche e Anatomo-patologiche, Roma, Italia ⁽³⁾ - Policlinico Umberto I, Dipartimento di Oncologia Medica, Roma, Italia ⁽⁴⁾	645
Humoral and T-cell response to SARS-CoV-2 vaccination one year after the booster dose in oncology patients	645
Authors	645
ROBERTA CAMPAGNA 1, FEDERICA DOMINELLI 2, MARIA A. ZINGAROPOLI 2, FABIO CIURLUINI 3, VINCENZO PICONE 4, ENRICO CORTESI 4, GUIDO ANTONELLI 1, OMBRETTA TURRIZIANI 1	645
161 - Enhancing influenza prevention in the paediatric population: pre-school located vaccination program and saliva-based molecular surveillance in hospitalized children in Milan municipality.	647
ANTONELLA AMENDOLA ⁽¹⁾ - CLARA FAPPANI ⁽¹⁾ - MARIA GORI ⁽¹⁾ - SILVIA BIANCHI ⁽¹⁾ - ELISA BORGHI ⁽¹⁾ - EMERENZIANA OTTAVIANO ⁽¹⁾ - MIRIANA MORELLI ⁽¹⁾ - ERIKA CHIERA ⁽¹⁾ - CHIARA GASPARINI ⁽²⁾ - PAOLO BALDASSARRE ⁽²⁾ - FRANCESCA BARILLI ⁽²⁾ - ALICE BIANCHI ⁽²⁾ - ANDREA BIUSO ⁽²⁾ - CLAUDIA CHILLEMI ⁽²⁾ - GABRIELLA GUIDA ⁽²⁾ - FRANCESCA RIPOLI ⁽²⁾ - STEFANIA RONCAGLIA ⁽²⁾ - LAURA SGAMBETTERRA ⁽²⁾ - DANIELA COLZANI ⁽¹⁾ - ELISABETTA TANZI ⁽¹⁾ - GIANVINCENZO ZUCCOTTI ⁽²⁾	647
Università degli Studi di Milano, Dipartimento di Scienze della Salute, Milano, Italia ⁽¹⁾ - Ospedale Buzzi, Dipartimento di Pediatria, Milano, Italia ⁽²⁾	647
Enhancing influenza prevention in the paediatric population: pre-school located vaccination program and saliva-based molecular surveillance in hospitalized children in Milan municipality	647
ANTONELLA AMENDOLA ¹ , CLARA FAPPANI ¹ , MARIA GORI ¹ , SILVIA BIANCHI ¹ , ELISA BORGHI ¹ , EMERENZIANA OTTAVIANO ¹ , MIRIANA MORELLI ¹ , ERIKA CHIERA ² , CHIARA GASPARINI ³ , PAOLO BALDASSARRE ³ , FRANCESCA BARILLI ³ , ALICE BIANCHI ³ , ANDREA BIUSO ³ , CLAUDIA CHILLEMI ³ , GABRIELLA GUIDA ³ , FRANCESCA RIPOLI ³ , STEFANIA RONCAGLIA ³ , LAURA SGAMBETTERRA ³ , DANIELA COLZANI ¹ , ELISABETTA TANZI ¹ , GIANVINCENZO ZUCCOTTI ³	647

¹ Department of Health Sciences, Università degli Studi di Milano, Milan, Italy; ² Università degli Studi di Milano, Milan, Italy; ³ Department of Pediatrics, V. Buzzi Children's Hospital, Università degli Studi di Milano, Milan, Italy	647
210 - Oral potentially malignant disorders (OPMDs): HPV prevalence and vaccines	649
MICHELA BUTTÀ ⁽¹⁾ - NICOLA SERRA ⁽²⁾ - ARIANNA SUCATO ⁽¹⁾ - VERA PANZARELLA ⁽³⁾ - ERIKA MANNINO ⁽¹⁾ - DANIELA CABIBI ⁽¹⁾ - DANIELA PISTOIA ⁽⁴⁾ - GIUSEPPINA CAPRA ⁽¹⁾	649
Università degli Studi di Palermo, Dipartimento di Promozione della Salute, Materno-Infantile, di Medicina Interna e Specialistica di Eccellenza “G. D’Alessandro”, Palermo, Italia ⁽¹⁾ - Università Federico II di Napoli, Dipartimento di Sanità Pubblica, Napoli, Italia ⁽²⁾ - Università degli Studi di Palermo, Dipartimento di Discipline Chirurgiche, Oncologiche e Stomatologiche, Palermo, Italia ⁽³⁾ - Azienda Ospedaliera Universitaria Policlinico P. Giaccone, Unità di Microbiologia e Virologia, Palermo, Italia ⁽⁴⁾	649
Oral potentially malignant disorders (OPMDs): HPV prevalence and vaccines.....	649
MICHELA BUTTÀ ¹ , NICOLA SERRA ² , ARIANNA SUCATO ¹ , VERA PANZARELLA ³ , ERIKA MANNINO ¹ , DANIELA CABIBI ^{1,4} , DANIELA PISTOIA ⁵ , AND GIUSEPPINA CAPRA ^{1,5}	649
¹ Department of Health Promotion, Mother and Child Care, Internal Medicine and Medical Specialties (PROMISE) “G. D’Alessandro”, University of Palermo, Palermo, Italy;	649
² Department of Public Health, University Federico II of Naples, Naples, Italy;	649
³ Department of Surgical, Oncological, and Oral Sciences (DICHIRONS), University of Palermo, Palermo, Italy;.....	649
⁴ Anatomic Pathology Unit, Polyclinic Hospital “P. Giaccone”, Palermo, Italy;	649
⁵ Microbiology and Virology Unit, Polyclinic Hospital “P. Giaccone”, Palermo, Italy	649
227 - Characterization of T-cell response to recombinant VZV vaccine in pwMS under DMTs.....	651
FEDERICA DOMINELLI ⁽¹⁾ - MARIA ANTONELLA ZINGAROPOLI ⁽¹⁾ - PATRIZIA PASCULLI ⁽¹⁾ - MATTEO TARTAGLIA ⁽²⁾ - FEDERICA CICCONE ⁽¹⁾ - LEONARDO MALIMPENSA ⁽²⁾ - GINA FERRAZZANO ⁽²⁾ - EEVA TORTELLINI ⁽¹⁾ - MARIASILVIA GUARDIANI ⁽¹⁾ - MIRIAM LICHTNER ⁽³⁾ - CLAUDIO MARIA MASTROIANNI ⁽¹⁾ - ANTONELLA CONTE ⁽²⁾ - MARIA ROSA CIARDI ⁽¹⁾	651
Sapienza, University of Rome, Department of Public health and Infectious diseases, Roma, Italia ⁽¹⁾ - Sapienza, University of Rome, Department of Human Neurosciences, Roma, Italia ⁽²⁾ - Sapienza, University of Rome, Infectious Diseases Unit, Santa Maria Goretti Hospital, Latina, Italia ⁽³⁾	651
ABSTRACT SIM 2023	651
Title: Characterization of T-cell response to recombinant VZV vaccine in pwMS under DMTs. 651	
FEDERICA DOMINELLI ¹ , MARIA A. ZINGAROPOLI ¹ , PATRIZIA PASCULLI ¹ , MATTEO TARTAGLIA ² , FEDERICA CICCONE ¹ , LEONARDO MALIMPENSA ² , GINA FERRAZZANO ² , EEVA TORTELLINI ¹ , MARIASILVIA GUARDIANI ¹ , MIRIAM LICHTNER ^{3,4} , CLAUDIO M. MASTROIANNI ¹ , ANTONELLA CONTE ^{2,5} , MARIA R. CIARDI ¹	651
¹ Department of Public Health and Infectious Diseases, Sapienza University of Rome, Rome, Italy;	
² Department of Human Neurosciences, Sapienza University of Rome, Rome, Italy; ³ Infectious Diseases Unit, Santa Maria Goretti Hospital, Sapienza, University of Rome, Latina, Italy;	

⁴ Department of Neurosciences Mental Health and Sensory Organs, Sapienza University of Rome, Rome, Italy; ⁵ IRCCS Neuromed, Pozzilli, Italy.....	651
234 - Transcriptomic analysis after mRNA BNT162b2 vaccination revealed a specific gene expression profile in low-responders hemodialysis patients	652
SIMONE LUCCHESI ⁽¹⁾ - GIORGIO MONTESI ⁽¹⁾ - ANNALISA CIABATTINI ⁽¹⁾ - JACOPO POLVERE ⁽¹⁾ - GABIRIA PASTORE ⁽¹⁾ - FRANCESCA MONTAGNANI ⁽²⁾ - GUIDO GAROSI ⁽³⁾ - FRANCESCO SANTORO ⁽¹⁾ - DONATA MEDAGLINI ⁽¹⁾	652
Università di Siena, Dipartimento di Biotecnologie mediche, Siena, Italia ⁽¹⁾ - Azienda ospedaliero-universitaria Senese, Malattie infettive e tropicali, Siena, Italia ⁽²⁾ - Azienda ospedaliero-universitaria Senese, Nefrologia, Siena, Italia ⁽³⁾	652
Transcriptomic analysis after mRNA BNT162b2 vaccination revealed a specific gene expression profile in low-responders hemodialysis patients.....	652
Simone Lucchesi ¹ , Giorgio Montesi ¹ , Jacopo Polvere ¹ , Elena Pettini ¹ , Francesca Montagnani ^{2,3} , Guido Garosi ⁴ , Annalisa Ciabattini ¹ , Francesco Santoro ¹ , Donata Medaglini ¹	652
¹ Laboratory of Molecular Microbiology and Biotechnology, Department of Medical Biotechnologies, University of Siena, Italy	652
² Department of Medical Sciences, Infectious and Tropical Diseases Unit, Siena University Hospital, Italy.....	652
³ Department of Medical Biotechnologies, University of Siena, Italy.....	652
⁴ Nephrology, Dialysis, and Transplantation Unit, Siena University Hospital, Italy.....	652
235 - Two years after COVID-19 vaccination: persistence of spike-specific memory B cells and antibodies after homologous and heterologous vaccine combinations.....	654
ANNALISA CIABATTINI ⁽¹⁾ - CHIARA COPPOLA ⁽¹⁾ - SIMONE COSTAGLI ⁽¹⁾ - GABIRIA PASTORE ⁽¹⁾ - FABIO FIORINO ⁽¹⁾ - JACOPO POLVERE ⁽¹⁾ - SIMONE LUCCHESI ⁽¹⁾ - MASSIMILIANO FABBIANI ⁽²⁾ - FRANCESCA MONTAGNANI ⁽²⁾ - MARIO TUMBARELLO ⁽²⁾ - DONATA MEDAGLINI ⁽¹⁾	654
University of Siena, Department of of Medical Biotechnology, Siena, Italia ⁽¹⁾ - University Hospital of Siena, Department of Medical Sciences, Infectious and Tropical Diseases Unit, Siena, Italia ⁽²⁾	654
Two years after COVID-19 vaccination: persistence of spike-specific memory B cells and antibodies after homologous and heterologous vaccine combinations	654
ANNALISA CIABATTINI ¹ , CHIARA COPPOLA ¹ , SIMONE COSTAGLI ¹ , GABIRIA PASTORE ¹ , FABIO FIORINO ¹ , JACOPO POLVERE ¹ , SIMONE LUCCHESI ¹ , MASSIMILIANO FABBIANI ³ , FRANCESCA MONTAGNANI ^{2,3} , MARIO TUMBARELLO ^{2,3} , DONATA MEDAGLINI ¹	654
¹ Laboratory of Molecular Microbiology and Biotechnology, Department of Medical Biotechnologies, University of Siena; Siena, Italy; ² Department of Medical Biotechnologies, University of Siena; Siena, Italy ; ³ Department of Medical Sciences, Infectious and Tropical Diseases Unit, University Hospital of Siena; Siena, Italy	654
240 - Characterization of the Immune Responsiveness to COVID-19 Vaccination in Rett Syndrome Patients	656
ANNALISA CIABATTINI ⁽¹⁾ - FABIO FIORINO ⁽¹⁾ - SILVIA LEONCINI ⁽²⁾ - LIDIA BOASLAKO ⁽²⁾ - LUDOVICA SOLDATESCHI ⁽¹⁾ - GABIRIA PASTORE ⁽¹⁾ - JACOPO POLVERE ⁽¹⁾ - CLAUDIO DE FELICE ⁽²⁾ - DONATA MEADGLINI ⁽¹⁾	656

University of Siena, Department of of Medical Biotechnology, Siena, Italia ⁽¹⁾ - Azienda Ospedaliera Universitaria Senese, Neonatal Intensive Care Unit, Department of Women& Children Health, Siena, Italia ⁽²⁾	656
Characterization of the Immune Responsiveness to COVID-19 Vaccination in Rett Syndrome Patients.....	656
ANNALISA CIABATTINI ¹ , FABIO FIORINO ¹ , SILVIA LEONCINI ^{2,3} , LIDIA BOASIAKO ^{2,3} , LUDOVICA SOLDATESCHI ¹ , GABIRIA PASTORE ¹ , JACOPO POLVERE ¹ , CLAUDIO DE FELICE, DONATA MEDAGLINI ¹	656
¹ Laboratory of Molecular Microbiology and Biotechnology, Department of Medical Biotechnologies, University of Siena; Siena, Italy; ² Neonatal Intensive Care Unit, Department of Women& Children Health, Policlinico “S. Maria alle Scotte” Azienda Ospedaliera Universitaria Senese, Siena, Italy; ³ Rett Syndrome Trial Center, Child Neuropsychiatry Unit, Department of Mental Health, Policlinico “S. Maria alle Scotte” AOUS, Siena, Italy.	
259 - B cell response after SARS-CoV-2 mRNA vaccination in people living with HIV	658
JACOPO POLVERE ⁽¹⁾ - MASSIMILIANO FABBIANI ⁽²⁾ - GABIRIA PASTORE ⁽¹⁾ - ILARIA RANCAN ⁽²⁾ - BARBARA ROSSETTI ⁽²⁾ - MIRIAM DURANTE ⁽³⁾ - ELENA PETTINI ⁽¹⁾ - SIMONE LUCCHESI ⁽¹⁾ - SARA ZIRPOLI ⁽¹⁾ - ENRICO MORELLI ⁽³⁾ - GIORGIO MONTESI ⁽¹⁾ - FABIO FIORINO ⁽¹⁾ - MARIO TUMBARELLO ⁽²⁾ - ANNALISA CIABATTINI ⁽¹⁾ - FRANCESCA MONTAGNANI ⁽²⁾ - DONATA MEDAGLINI ⁽¹⁾	658
Laboratorio di Microbiologia Molecolare e Biotecnologia, Dipartimento di Biotecnologie Mediche, Università degli studi di Siena, Siena, Italia ⁽¹⁾ - UOC Malattie infettive e tropicali, Dipartimento di Scienze mediche, chirurgiche e neuroscienze, Azienda Ospedaliera Universitaria Senese, Siena, Italia ⁽²⁾ - Dipartimento di Biotecnologie Mediche, Università degli studi di Siena, Siena, Italia ⁽³⁾	
B cell response after SARS-CoV-2 mRNA vaccination in people living with HIV	658
JACOPO POLVERE ¹ , MASSIMILIANO FABBIANI ² , GABIRIA PASTORE ¹ , ILARIA RANCAN ^{2,3} , BARBARA ROSSETTI ² , MIRIAM DURANTE ³ , ELENA PETTINI ¹ , SIMONE LUCCHESI ¹ , SARA ZIRPOLI ¹ , ENRICO MORELLI ² , GIORGIO MONTESI ¹ , FABIO FIORINO ¹ , MARIO TUMBARELLO ^{2,3} , ANNALISA CIABATTINI ¹ , FRANCESCA MONTAGNANI ^{2,3} AND DONATA MEDAGLINI ¹	658
1 Laboratory of Molecular Microbiology and Biotechnology, Department of Medical Biotechnologies, University of Siena; Siena, Italy; 2 Department of Medical Sciences, Infectious and Tropical Diseases Unit, University Hospital of Siena; Siena, Italy; 3 Department of Medical Biotechnologies, University of Siena; Siena, Italy	
265 - Persistence of spike-specific immune responses in solid organ transplant recipients up to two years following mRNA vaccination for SARS-CoV-2.....	660
SIMONE COSTAGLI ⁽¹⁾ - CHIARA COPPOLA ⁽¹⁾ - JACOPO POLVERE ⁽¹⁾ - MASSIMILIANO FABBIANI ⁽²⁾ - MARIO TUMBARELLO ⁽³⁾ - FRANCESCA MONTAGNANI ⁽³⁾ - SONIA BERNAZZALI ⁽⁴⁾ - DAVID BENNETT ⁽⁵⁾ - ANNALISA CIABATTINI ⁽¹⁾ - DONATA MEDAGLINI ⁽¹⁾	660
Università degli Studi di Siena, Dipartimento di Biotecnologie Mediche, Laboratorio di Microbiologia Molecolare e Biotecnologie, Siena, Italia ⁽¹⁾ - Ospedale Universitario di Siena, Dipartimento di Scienze Mediche, Unità di Malattie Infettive e Tropicali, Siena, Italia ⁽²⁾ - Università degli Studi di Siena; Ospedale Universitario di Siena, Dipartimento di Biotecnologie Mediche; Dipartimento di Scienze Mediche, Unità di Malattie Infettive e Tropicali, Siena, Italia ⁽³⁾ - Ospedale Universitario di Siena, Dipartimento Cardio-Toraco-Vascolare, Unità di Chirurgia	

Cardiaca, Siena, Italia ⁽⁴⁾ - Ospedale Universitario di Siena, Dipartimento di Scienze Mediche, Unità di Malattie Respiratorie, Siena, Italia ⁽⁵⁾	660
Persistence of spike-specific immune responses in solid organ transplant recipients up to two years following mRNA vaccination for SARS-CoV-2	660
SIMONE COSTAGLI ¹ , CHIARA COPPOLA ¹ , JACOPO POLVERE ¹ , MASSIMILIANO FABBIANI ³ , MARIO TUMBARELLO ^{2,3} , FRANCESCA MONTAGNANI ^{2,3} , SONIA BERNAZZALI ⁴ , DAVID BENNETT ⁵ , DONATA MEDAGLINI ¹ , ANNALISA CIABATTINI ¹ ..	660
¹ Laboratory of Molecular Microbiology and Biotechnology, Department of Medical Biotechnologies, University of Siena; Siena, Italy; ² Department of Medical Biotechnologies, University of Siena; Siena, Italy; ³ Department of Medical Sciences, Infectious and Tropical Diseases Unit, University Hospital of Siena; Siena, Italy; ⁴ Cardio-Thoracic and Vascular Department, Cardiac Surgery Unit, University Hospital of Siena; Siena, Italy; ⁵ Department of Medical Sciences, Respiratory Disease Unit, University Hospital of Siena; Siena, Italy	660
278 - Recombinant BCG strains as innovative treatment of non-muscle invasive bladder cancer ..	662
Alessandro Salustri ⁽¹⁾ - Flavio De Maio ⁽²⁾ - Ivana Palucci ⁽²⁾ - Giulia Santarelli ⁽¹⁾ - Valentina Palmieri ⁽³⁾ - Massimiliano Papi ⁽⁴⁾ - Michela Sali ⁽¹⁾ - Giovanni Delogu ⁽⁵⁾	662
Dipartimento di Scienze biotecnologiche di base, cliniche intensivologiche e perioperatorie., Università Cattolica del Sacro Cuore, Roma, Italia ⁽¹⁾ - Dipartimento di Scienze di Laboratorio e Infettivologiche, Facoltà di Medicina e Chirurgia., Fondazione Policlinico A. Gemelli IRCCS, Università Cattolica del Sacro Cuore., Roma, Italia ⁽²⁾ - Istituto dei Sistemi Complessi, Cento Nazionale delle Ricerche (CNR), Roma, Italia ⁽³⁾ - Dipartimento di Neuroscienze, Università Cattolica del Sacro Cuore, Roma, Italia ⁽⁴⁾ - Dipartimento di Medicina di Laboratorio, Mater Olbia Hospital, Olbia, Italia ⁽⁵⁾	662
Recombinant BCG strains as innovative treatment of non-muscle invasive bladder cancer	662
Alessandro Salustri ¹ , Flavio De Maio ² , Ivana Palucci ^{1,2} , Giulia Santarelli ¹ , Valentina Palmieri ^{4,5} , Massimiliano Papi ^{3,4} , Michela Sali ^{1,2} , Giovanni Delogu ^{2,6}	662
¹ Department of Sciences of Basic Biotechnologies, Intensive and Perioperative Clinics, Section of Microbiology, Università Cattolica del Sacro Cuore, Rome, Italy;	662
² Department of Laboratory and Infective Sciences, Faculty of Medicine, Fondazione Policlinico A. Gemelli IRCCS, Università Cattolica del Sacro Cuore, Rome, Italy;	662
³ Fondazione Policlinico Universitario "A. Gemelli", IRCSS, Rome, Italy;	662
⁴ Dipartimento di Neuroscienze, Università Cattolica del Sacro Cuore, 00168 Rome, Italy;	662
⁵ Istituto dei Sistemi Complessi, CNR, Rome, Italy;	662
⁶ Mater Olbia Hospital, Olbia, Italy;	662
304 - Moving beyond the titer paradigm: rational design of a next-generation glycoconjugate vaccine inducing highly functional antibodies	664
GIUSEPPE STEFANETTI ⁽¹⁾ - DENNIS LEE KASPER ⁽²⁾	664
Universita' degli Studi di Urbino "Carlo Bo", Dipartimento di Scienze Biomolecolari (DISB), Urbino, Italia ⁽¹⁾ - Harvard Medical School, Immunology, Boston, Stati Uniti D'america ⁽²⁾	664
Moving beyond the titer paradigm: rational design of a next-generation glycoconjugate vaccine inducing highly functional antibodies	664
GIUSEPPE STEFANETTI ¹ , DENNIS L. KASPER ²	664
¹ Department of Biomolecular Sciences, University of Urbino Carlo Bo, Urbino, Italy	664

315 - Humoral And Cellular Immune Response after breakthrough infection in Orthotopic Liver Transplant patients who received 3 or 4 doses of SARS-CoV-2 mRNA BNT162b2 Vaccine..... 666

GIUSEPPINA SANNA⁽¹⁾ - DAVIDE FIRINU⁽²⁾ - PAOLA CARIA⁽¹⁾ - VALERIA MANCA⁽¹⁾ - DANIELA VIRGINIA FRAU⁽¹⁾ - VANESSA PALMAS⁽¹⁾ - MASSIMILIANO GALDIERO⁽³⁾ - CARLA MARIA DEIANA⁽²⁾ - FRANCESCO PES⁽²⁾ - LUCHINO CHESSA⁽²⁾ - ALDO MANZIN⁽¹⁾..... 666

Università di Cagliari, Scienze Biomediche, Cagliari, Italia⁽¹⁾ - Università di Cagliari, Scienze Mediche e sanità pubblica, Cagliari, Italia⁽²⁾ - Università Vanvitelli Napoli, Medicina sperimentale, Napoli, Italia⁽³⁾..... 666

Humoral And Cellular Immune Response after breakthrough infection in Orthotopic Liver Transplant patients who received 3 or 4 doses of SARS-CoV-2 mRNA BNT162b2 Vaccine. 666

GIUSEPPINA SANNA¹, DAVIDE FIRINU², PAOLA CARIA¹, VALERIA MANCA¹, DANIELA VIRGINIA FRAU¹, VANESSA PALMAS¹, MASSIMILIANO GALDIERO³, CARLA MARIA DEIANA², FRANCESCO PES², LUCHINO CHESSA², ALDO MANZIN¹..... 666

¹Department of Biomedical Sciences, Microbiology and Virology Unit, University of Cagliari, Cittadella Universitaria, Sp. 8, 09042 Monserrato (CA), Italy; 666

²Department of Medical Sciences and Public Health, University of Cagliari, Cittadella Universitaria, 09042, Monserrato, Cagliari, Italy; 666

³Department of Experimental Medicine, University of Campania Luigi Vanvitelli, 80138 Naples, Italy. 666

01 Approcci diagnostici

7 - The application of liquid colony in the sepsis diagnostic workflow: a preliminary overview on paediatric patients

Maddalena Calvo⁽¹⁾ - Giuseppe Migliorisi⁽¹⁾ - Guido Scalia⁽¹⁾ - Stefania Stefani⁽²⁾

Azienda Ospedaliera, Azienda Ospedaliera Universitaria Policlinico - San Marco, CATANIA, Italia

⁽¹⁾ - Università Di Catania, Dipartimento Di Scienze Biomediche E Biotecnologiche, CATANIA, Italia ⁽²⁾

The application of liquid colony in the sepsis diagnostic workflow: a preliminary overview on paediatric patients

MADDALENA CALVO¹, GIUSEPPE MIGLIORISI¹, GUIDO SCALIA^{1,2}, STEFANIA STEFANI²

¹U.O. Laboratory Analysis, University Hospital Policlinico, Catania, Italy; ²Department of Biomedical and Biotechnological Sciences, University of Catania, Catania, Italy.

Uncertainties in the microbiological diagnostic approach compromise the management of septic paediatric patients, due to problematic blood sample collection, low volumes and frequent uncommon aetiologies. Several studies encouraged the application of advanced technologies for the timely detection of sepsis aetiology. The FASTTM system (Qvella, ON, Canada) is an experimental technology providing a purified microbial suspension, known as a Liquid ColonyTM (LC), directly from positive blood samples (PBCs) in 30-40 minutes. Our study illustrates rapid identification (ID) and antimicrobial susceptibility testing (AST) results from liquid colonies, showing consistent time-saving in critical paediatric patients.

Qvella's FAST™ System and FAST PBC Prep cartridges were tested on 30 paediatric PBCs from the Policlinico Hospital of Catania during a six-month study. After a Gram stain, 2 millilitres of blood were converted into an LC for rapid ID (1 hour) and AST (24 hours) using Bruker Biotyper Sirius MALDI-TOF and BD Phoenix systems. The standard culture-based methods were simultaneously conducted, statistically comparing both procedures.

A 22 hours average positivity time was notified. 66.6% of samples reported a Gram-positive result (5 *Staphylococcus haemolyticus*, 5 *Staphylococcus epidermidis*, 3 *Staphylococcus aureus*, 2 *Staphylococcus hominis*, 2 *Enterococcus faecium*, 1 *Streptococcus pneumoniae*, 1 *Streptococcus agalactiae*, and 1 *Streptococcus oralis*). 33.4% of samples revealed Gram-negatives (6 *Klebsiella pneumoniae*, 1 *Klebsiella aerogenes*, 1 *Citrobacter freundii*, 1 *Pseudomonas aeruginosa* and 1 *Capnocytophaga sputigena*). Among all the samples, 96.6% successfully produced ID from the liquid colony, perfectly matching the standard methods. Each identified microorganism showed a high MALDI medium score (2.10). Kirby-Bauer and Gradient test methods were applied only for *C. sputigena*. For the other bacteria, automated AST reported no categorical agreement. Only 3.4% of samples (1 *S. pneumoniae*) failed ID and AST from the liquid colony.

Qvella's FAST™ System guaranteed optimal performances, failing only 1 pneumococcal sample, probably due to its capsular structure and granular components. Additionally, the instrument only needs a few blood quantities, bypassing a possible poor sample collection. The MALDI ID ensures a brief interval for the broadest identification spectrum, including uncommon strains such as *C. sputigena*. Moreover, the system furnishes a complete AST report within 24 hours. Although ultimate technologies require conventional confirmation, they allow for shortening diagnostic delays for critical paediatric patients. Our preliminary results encourage introducing a blood samples concentration system within the sepsis standardized diagnostic workflow.

9 - Extensive virologic characterization in an HIV-infected individual with apparent HIV remission for 2 years after allogeneic stem cell transplantation with CCR5 wild-type cells: a case study.

Ilaria Vicenti⁽¹⁾ - Simona Piemontese⁽²⁾ - Lia Fiaschi⁽¹⁾ - Camilla Biba⁽¹⁾ - Adriana Cervo⁽³⁾ - Ilenia Varasi⁽¹⁾ - Francesco Saladini⁽¹⁾ - Giovanna Leonardi⁽⁴⁾ - Carlo Federico Perno⁽⁵⁾ - Cristina Mussini⁽⁶⁾ - Maurizio Zazzi⁽¹⁾ - Vanni Borghi⁽⁶⁾

University of Siena, Department of Medical Biotechnologies, Siena, Italia⁽¹⁾ - Hematology and Bone Marrow Transplantation Unit, IRCCS San Raffaele Hospital, Milano, Italia⁽²⁾ - Infectious Diseases Unit, Department of Health Promotion Sciences and Mother and Child Care "Giuseppe D'Alessandro" (PROMISE), University of Palermo, Palermo, Italia⁽³⁾ - Hematology Unit and Chair, Department of Medical and Surgical Sciences, Azienda Ospedaliera Universitaria Di Modena, University of Modena and Reggio Emilia, Modena, Italia⁽⁴⁾ - Multimodal Research Area, Unit of Microbiology and Diagnostics in Immunology, Bambino Gesù Children's Hospital, IRCCS, Rome, Rome, Italia⁽⁵⁾ - UO of infectious Diseases, University of Modena and Reggio Emilia, Modena, Italia⁽⁶⁾

Extensive virologic characterization in an HIV-infected individual with apparent HIV remission for 2 years after allogeneic stem cell transplantation with CCR5 wild-type cells: a case study.

ILARIA VICENTI¹, SIMONA PIEMONTESE², LIA FIASCHI¹, CAMILLA BIBA¹, ADRIANA CERVO³, ILENIA VARASI¹, FRANCESCO SALADINI¹, GIOVANNA LEONARDI⁴, CARLO F. PERNO⁵, CRISTINA MUSSINI⁶, MAURIZIO ZAZZI¹, VANNI BORGHI⁶

¹Department of Medical Biotechnologies, University of Siena, Siena; ²Hematology and Bone Marrow Transplantation Unit, IRCCS San Raffaele Hospital, Milan; ³Infectious Diseases Unit, Department of Health Promotion Sciences and Mother and Child Care "Giuseppe D'Alessandro" (PROMISE), University of Palermo, Palermo; ⁴Hematology Unit and Chair, Department of Medical and Surgical Sciences, Azienda Ospedaliera Universitaria Di Modena, University of Modena and Reggio Emilia, Modena; ⁵Multimodal Research Area, Unit of Microbiology and Diagnostics in Immunology, Bambino Gesù Children's Hospital, IRCCS, Rome; ⁶UO of infectious Diseases, University of Modena and Reggio Emilia, Modena.

Introduction. Allogeneic hematopoietic stem cell transplantation (allo-HSCT) with homozygous CCR5 Δ 32 donor cells is the only potentially HIV-1 curative intervention. However, allo-HSCT has been associated with a drastic reduction in the HIV reservoir independently of engraftment with CCR5 Δ 32 or CCR5 wild-type cells. In this study, we investigated ex-vivo the reservoir of an HIV-1 infected individual with no evidence of infection at 2 years after allo-HSCT with wild-type CCR5 genotype. **Materials and Methods.** The study participant was an adult male, HIV-1 infected since 1994 with 5 antiretroviral class multidrug resistance strain and under treatment with ART. On January 2020, at the age of 58, he received an allo-HSCT from an HLA-identical sibling donor for a Hodgkin lymphoma in second complete remission after 24 cycles of therapy with the anti- PD1 pembrolizumab. The patient was discharge on day 47 after transplant without major complications. Despite positive HIV-1 serology, he maintained undetectable viremia and he was negative for HIV-1 DNA by routine diagnostic analysis. In March 2022 CD4⁺ T cells were isolated after leukapheresis and maintained in culture to determine: (i) the CCR5 genotype by Sanger sequencing, (ii) the

amount of cell-associated HIV-1 DNA (CAD) and cell-associated HIV-1 RNA (CAR) by digital droplet PCR at baseline and after ex-vivo stimulation with ionomycin (ION, 1 µg/ml) plus phorbol-myristate-acetate (PMA, 50 ng/ml). Briefly, 2-days post induction (dpi) 4×10^7 patient derived CD4⁺ were co-cultivated with MOLT-4 CCR5, a cell line permissive for HIV-1 infection. CAR and CAD were quantified at baseline (T₀) and 2-7-14-21 dpi (T₂, T₇, T₁₄, T₂₁). At T₇, T₁₄ and T₂₁, the infectivity of the CD4⁺/MOLT-4 CCR5 co-cultures was evaluated using a modified TZM-bl based assay (TZA) protocol. In addition, a positive control of 1.2×10^6 CD4⁺ T cells, deriving from an HIV-1 positive, virologically suppressed patient was analysed in parallel. Results. DNA sequencing confirmed a wild type homozygous CCR5. CAR and CAD were negative at baseline and at all time points analysed. The CD4⁺/MOLT-4 CCR5 co-cultures were not infectious in TZA at T₇, T₁₄ and T₂₁. The HIV-1 positive control was quantifiable in CAD at T₇, T₁₄ and T₂₁ with 931.2 ± 400.1 , 68.9 ± 10.1 , 128.6 ± 15.3 HIV-1 copies per 10^6 CD4⁺ T cells respectively and in TZA at T₂₁ with 16.1 [5.4-47.8] IUPM/cells. Discussion and Conclusions. Two years after wild-type CCR5 allo-HSCT, the patient remained without measurable or inducible HIV-1 DNA and RNA in the blood compartment by multiple ultrasensitive assays on a total of 40 million CD4⁺ T cells. However, the presence of the virus in other tissues was unexplored. Ultimately, only analytical treatment

16 - Characterization and classification of diagnostically unidentified mycobacteria by de novo sequencing

Giulia Gatti ⁽¹⁾ - Francesca Taddei ⁽²⁾ - Silvia Zannoli ⁽²⁾ - Giorgio Dirani ⁽²⁾ - Anna Marzucco ⁽²⁾ - Maria Sofia Montanari ⁽²⁾ - Irene Zaghi ⁽²⁾ - Martina Brandolini ⁽¹⁾ - Laura Grumiro ⁽²⁾ - Massimiliano Guerra ⁽²⁾ - Alessandra Mistral De Pascali ⁽¹⁾ - Monica Cricca ⁽¹⁾ - Vittorio Sambri ⁽¹⁾
Alma Mater Studiorum - University of Bologna, DIMEC - Department of Medical and Surgical Sciences, Bologna, Italia ⁽¹⁾ - The Great Romagna Hub Laboratory, O.U. Microbiology, Cesena, Italia ⁽²⁾

Characterization and classification of diagnostically unidentified mycobacteria by de novo sequencing

GIULIA GATTI, FRANCESCA TADDEI, SILVIA ZANNOLI, GIORGIO DIRANI, ANNA MARZUCCO, MARIA S. MONTANARI, IRENE ZAGHI, MARTINA BRANDOLINI, LAURA GRUMIRO, MASSIMILIANO GUERRA, ALESSANDRA M. DE PASCALI, MONICA CRICCA, VITTORIO SAMBRI

Department of Medical and Surgical Sciences (DIMEC), O.U. Microbiology, University of Bologna, Cesena, Italy; Local Health Authority, O.U. Microbiology, the Greater Romagna Area Hub Laboratory, Cesena, Italy; Local Health Authority, O.U. Microbiology, the Greater Romagna Area Hub Laboratory, Cesena, Italy; Local Health Authority, O.U. Microbiology, the Greater Romagna Area Hub Laboratory, Cesena, Italy; Local Health Authority, O.U. Microbiology, the Greater Romagna Area Hub Laboratory, Cesena, Italy; Local Health Authority, O.U. Microbiology, the Greater Romagna Area Hub Laboratory, Cesena, Italy; Local Health Authority, O.U. Microbiology, the Greater Romagna Area Hub Laboratory, Cesena, Italy; Department of Medical and Surgical Sciences (DIMEC), O.U. Microbiology, University of Bologna, Cesena, Italy; ; Local Health Authority, O.U. Microbiology, the Greater Romagna Area Hub Laboratory, Cesena, Italy; ; Local Health Authority, O.U. Microbiology, the Greater Romagna Area Hub Laboratory, Cesena, Italy; Department of Medical and Surgical Sciences (DIMEC), O.U. Microbiology, University of Bologna, Cesena, Italy; Department of Medical and Surgical Sciences (DIMEC), O.U. Microbiology, University of Bologna, Cesena, Italy; Department of Medical and Surgical Sciences (DIMEC), O.U. Microbiology, University of Bologna, Cesena, Italy.

Introduction: In the past thirty years, two non-tubercular mycobacteria species, *Mycobacterium saskatchewanense* and *Mycobacterium branderi*, were discovered and described associated to human infections. Since their low frequency, most of the commercial diagnostic kits does not include those microorganisms in the identification panels, thereby the absence of a proper classification can impact on the selection of a subsequent treatment. In these circumstances, new high-throughput technologies, as the Whole Genome Next Generation Sequencing (WG-NGS), represents not only a strong contribution for scientific research but also an implementation and completion for the laboratory diagnostic routine. **Material and Methods:** both analysed samples were cultured on liquid MGIT™ medium (*Mycobacterium Growth Indicator Tube* by Becton Dickinson, Franklin Lakes, United States) and solid Löwenstein-Jensens medium. After a microscopic observation and when the growth was detected, the identification was performed by hybridization through the GenoType® *Mycobacterium* CM/AS (Hain Lifescience, Nehren, Germany). Given the inability of the kits to associate the sample with a precise species, the whole genome was extracted from colonies and sequenced by the DNA Prep kit by Illumina (Illumina Inc., San Diego, United States). The reads were controlled and trimmed, the genomes reconstructed de novo and the 16S and hsp65 genes were investigated. The 16 gene was identified and blasted against the NCBI database for a first identification. Then, the hsp65 gene of *Mycobacterium*

tuberculosis H37Rv (Accession number: NC_000962.3) was used as reference to pinpoint the sequence in the tested genomes. Results: The identification performed by GenoType® Mycobacterium CM/AS resulted in a peculiar patterns of hybridization bands that were not compatible with any other species predicted by the assay. On the contrary, the WG-NGS allowed the characterization of the complete genome sequence and the precise classification of the two unknown mycobacteria in *Mycobacterium saskatchewanense* and *Mycobacterium branderi*. Discussion and conclusions: the use of commercial kits has simplified and sped up the diagnostic workflow, by also standardizing the error rate associate to the procedures. On the other hand, this implies that there are some detectable clinical pictures or pathogens restricted to a panel. Regarding the infectious diseases, this limitation can be overstepped by the de novo WG-NGS that allows to query international databases and achieve a precise identification and treatment.

34 - *Nocardia otitidiscavarium* infection in an immunocompromised host: Importance of proper microbiological testing

SONIA DONEDDU ⁽¹⁾ - MARIA FRANCESCA SECCHI ⁽²⁾ - MARCO FOIS ⁽³⁾ - MAURA FIAMMA ⁽⁴⁾ - MARIA TERESA MURGIONI ⁽¹⁾ - CARLA BOI ⁽¹⁾ - FRANCO CARTA ⁽⁴⁾ - MARIA CRISTINA GARAU ⁽⁴⁾ - ANTONELLO PADDEU ⁽⁵⁾ - SILVIA PILLONI ⁽⁵⁾ - GIACOMO OLLA ⁽⁵⁾ - ALEXANDRA USAI ⁽⁵⁾ - MARINA CUBEDDU ⁽⁴⁾ - FRANCESCO MARCO LODDO ⁽⁵⁾

Sc Laboratorio Analisi, Nostra Signora Della Mercedes Asl Ogliastra, Lanusei, Italia ⁽¹⁾ - Sds Microbiologia Clinica Cagliari, Universita' Di Cagliari, Nuoro, Italia ⁽²⁾ - Sds Malattie Infettive E Tropicali S.C. Malattie Infettive, Universita' Degli Studi Di Sassari Dipartimento Di Medicina, Chirurgia E Farmacia, Sassari, Italia ⁽³⁾ - U.C. Laboratorio Analisi/Microbiologia, Ospedale San Francesco, Nuoro, Italia ⁽⁴⁾ - Sc Anestesia E Rianimazione, Nostra Signora Della Mercedes Asl Ogliastra, Lanusei, Italia ⁽⁵⁾

Nocardia otitidiscavarium infection in an immunocompromised host: Importance of proper microbiological testing.

Introduction

Nocardia spp. can be a pathogen responsible for invasive infection in both immunocompetent and immunocompromised hosts. It is usually transmitted by inhalation and lead to pulmonary manifestation like lung abscesses, which frequently evolve into a disseminating infections. Of all species of *Nocardia*, *N. otitidiscavarium* is responsible only for the 0.3 - 2.9% of infections reported, with high mortality, especially in disseminated infections. It is usually sensible to trimethoprim/sulfamethoxazole (T/S) and linezolid, and it is often reported resistant to carbapenems (eg. imipenem, meropenem) unlike other species. In this case report we describe a case of *N. otitidiscavarium* lung infections associated with meningoencephalitis due to *L. monocytogenes* in an immunocompromised subject and the microbiological procedure to identify the microorganism and to produce a susceptibility test.

Materials and methods

A 50 years old man with a history of treatment with a high dose of corticosteroids for an autoimmune disorder is admitted to the intensive care unit subsequent to the onset of a comatous state with fever and mild respiratory failure. At the computed tomography scan of the thorax, it was visible a consolidation with a cavity containing air-fluid level in the upper section of the left lung, while the brain magnetic resonance showed inflammation signs and multiple ring-enhancing lesions. Blood cultures showed positivity for *L. monocytogenes*. The bronchoalveolar lavage culture of the lesion was positive for *N. otitidiscavarium* and identified via mass spectrometry MALDI Biotyper sirius (Bruker Daltonics S.r.l.). Susceptibility testing was performed with a 0.5 MacFarland suspension in a blood broth using MicroScan WalkAway Plus panels (Beckman Coulter, Inc.). The results were interpreted using the PK-PD EUCAST breakpoints for linezolid, imipenem, and meropenem, and the CLSI m24 3rd edition breakpoint for T/S.

Results

The isolate took 48h hours to show visible growth on the plates. The susceptibility test showed sensibility for T/S, linezolid and imipenem (MIC 0.5/9.5 mcg/mL, 2 mcg/mL and 4 mcg/mL respectively), and susceptibility at high dosage for meropenem (MIC 4 mcg/mL). To date the patient showed a gradual improvement of clinical and radiological conditions after an empiric treatment with meropenem and T/S.

Discussion and conclusions

Nocardia spp. infections are rare conditions, that need an accurate diagnosis and appropriate treatment, indeed susceptibility to antibiotics can be variable from species to species, thus an appropriate susceptibility test is mandatory to drive antimicrobial therapy adjusting the initial empiric treatment.

63 - Molecular surveillance of Candida auris colonization: our experience.

ALESSANDRO BONDI ⁽¹⁾ - ALESSIO LEONE ⁽²⁾ - FEDERICA ZULLO ⁽²⁾ - ANTONIO CURTONI ⁽¹⁾ - TERESA ZACCARIA ⁽²⁾ - TERESA ZACCARIA ⁽²⁾ - ROSSANA CAVALLO ⁽¹⁾ - CRISTINA COSTA ⁽¹⁾

Dipartimento di Scienze della Sanità Pubblica e Pediatriche, Università di Torino, Torino, Italia ⁽¹⁾ - SC Microbiologia e Virologia U, AOU Città della Salute e della Scienza, Torino, Italia ⁽²⁾

Molecular surveillance of Candida auris colonization: our experience.

Introduction

Candida auris is an emerging pathogen associated with hospital outbreaks, presenting a high mortality in patients with invasive infections. In order to prevent its spread within hospital, the identification of colonized patients and an infection control policy are mandatory. International guidelines suggest to collect an axillary and groin skin swab and to process it using standard culture methods. However, traditional methods have prolonged incubation times and can fail to correctly identify the fungal species. For this reason, it is necessary to implement diagnostic technologies, in particular through the use of molecular biology methods. The purpose of this study is to evaluate the performance of the Eazyplex ® Candida auris test (Amplex Diagnostic, Germany) for the detection of C.auris colonization.

Materials and Methods

According to Center for Disease Control one skin swab was collected for each patient. An amount of 50µl of transport medium was inoculated on Sabouraud Agar and CHROMAgar Candida. Simultaneously, 25µl were processed using Eazyplex ® Candida auris. After 72h of incubation, Candida species identification was performed with MALDI-ToF technology.

Results

From September 2022 to May 2023 a total of 338 skin swab were collected. C.auris was detected in n=32 samples (9.46%). Eazyplex ® Candida auris showed high specificity (99.7%, IC95% 98.20-99.99%) but low sensitivity (58.8%, IC95% 36.03-72.68%). The culture detected n=31 C.auris vs n=32 with culture in association with molecular test (p=0.89). A total of n=5 patients show the presence of C.auris in other body district. Five patients were colonized with C.auris (n=4 positive with both methods vs n=1 cultural test only)

Discussion and Conclusions

Eazyplex ® Candida auris is a rapid molecular method for the detection of C.auris from skin swab. The high specificity allows a rapid identification and isolation of the colonized patient. We noted that the test fails to detect low grade of colonization. Despite this, a low grade colonization may be associated to a lower risk of invasive infection.

74 - Evaluation of STANDARD M10 MDR-TB assay for rapid diagnosis of tuberculosis

LAURA RINDI ⁽¹⁾ - VINCENZO PUGLISI ⁽²⁾ - IACOPO FRANCONI ⁽¹⁾ - ANTONELLA LUPETTI ⁽¹⁾

Università di Pisa, Dipartimento di Ricerca Traslationale e Nuove Tecnologie in Medicina e Chirurgia, Pisa, Italia ⁽¹⁾ - Azienda Ospedaliero-Universitaria Pisana, S.D. Microbiologia Micologica, Pisa, Italia ⁽²⁾

Evaluation of STANDARD M10 MDR-TB assay for rapid diagnosis of tuberculosis

Laura Rindi, Vincenzo Puglisi, Iacopo Franconi, Antonella Lupetti

Dipartimento di Ricerca Traslationale e delle Nuove Tecnologie in Medicina e Chirurgia, S.D. Microbiologia Micologica, Azienda Ospedaliero-Universitaria Pisana, Pisa, Italy.

Introduction

Tuberculosis (TB) is a major infectious disease worldwide. Rapid detection of *Mycobacterium tuberculosis* and determination of resistance, based on nucleic acid amplification techniques carried out directly on clinical samples, are essential for early diagnosis and treatment. The aim of the present study is to evaluate the performance of the newly developed STANDARD M10 MDR-TB test (SD Biosensor) and to compare its efficiency with the conventional Xpert MTB/RIF Ultra assay (Cepheid) for pulmonary and extra-pulmonary samples.

Materials and Methods

Clinical samples sent to the Mycology Unit of the University Hospital of Pisa, Italy, were included in this study. STANDARD M10 MDR-TB (SD Biosensor) and Xpert MTB/RIF Ultra testing (Cepheid) were performed on the same specimen, according to the manufacturer's instructions. Clinical specimens were stained by Ziehl-Neelsen for the microscopic evaluation of acid-fast bacilli and cultured in BACTEC MGIT 960 liquid medium (Becton Dickinson). Phenotypic drug susceptibility testing was done using the BACTEC MGIT 960 system.

Results

So far, a total of 22 positive clinical samples has been recovered, 15/22 (68.2%) were respiratory samples, 4 of which were bronchoalveolar lavage fluids and 11 sputum, 3/22 (13.6%) drainage fluids, 3/22 (13.6%) wound/biopsy samples, 1 ascitic fluid. At Xpert MTB/RIF test 3 samples resulted positive with a "Very Low" score, 7 as "Low", 5 "Medium", 7 "High". Microscopic investigation highlighted the presence of mycobacteria in 11 samples, while cultures resulted positive in 17 samples. STANDARD M10 MDR-TB was performed on all the collected clinical samples and concordance diagnostic rates with Xpert MTB/RIF, culture method and microscopic examination will be evaluated according to Cohen's kappa coefficient.

Discussion and conclusions

This study will highlight the diagnostic performance of the STANDARD M10 MDR-TB compared with PCR-based and non-molecular techniques. Molecular methods have revolutionized traditional mycobacterial diagnostic processes. Despite their feasibility and rapid turn-around time, newer techniques need to be fully investigated in clinical practice comparing them with reference methods. Complete results of ongoing investigation and further data will be disclosed at the Congress event.

89 - "Fast" viral respiratory diagnosis in Pediatric Patient

VITTORIO PANETTA ⁽¹⁾ - MARIA TERESA DELLA ROCCA ⁽¹⁾ - ADRIANA DURANTE ⁽¹⁾ -
GIUSEPPINA TUCCI ⁽¹⁾ - STEFANO LABELLA ⁽¹⁾ - RITA GRECO ⁽¹⁾

UOSD Microbiology, AORN Sant'Anna and San Sebastiano, caserta, Italia ⁽¹⁾

"Fast" viral respiratory diagnosis in pediatric patients

VITTORIO PANETTA¹, MARIA T. DELLA ROCCA¹, ADRIANA DURANTE¹, GIUSEPPINA
TUCCI¹, STEFANO LABELLA¹, RITA GRECO¹

¹ UOSD MICROBIOLOGY – AORN SANT 'ANNA AND SAN SEBASTIANO, CASERTA,
ITALY.

Introduction

Respiratory viral infections are a leading cause of pediatric disease. The rapid identification of pathogens, epidemiologic tracing, description of symptoms, and development of preventive and therapeutic measures are crucial to limiting the spread of these viruses. This study aimed to compare two rapid RT-PCR M10 Flu/RSV/SARS-CoV-2 and BioFire Respiratory Panel in upper respiratory specimens' pediatric population.

Material and Methods

M10 is an all-in-one cartridge-based ready-to-use multiplex RT-PCR kit intended for the qualitative detection of influenza A, influenza B, RSV and SARS-CoV-2 RNAs. The time-to-result is 60 min. Testing was performed on the STANDARD M10 analyzer (SD Biosensor Inc., Seoul, Korea) and BioFire Respiratory Panel 2.1 plus (RP2) (BioFire, Salt Lake City, Utah, USA) according to the manufacturer's instructions.

Results

A total of 25 samples were tested in both M10 and RP2. Of these, 17 samples had negative results. The overall agreement between the M10 and RP2 test was 96.0% (24/25). Discordant results were observed for the remaining 1 sample, which were then tested with SARS-COV-2 specific PCR kit.

For what concerns final reconciled diagnoses, 8 (32%) samples were positive for at least one of four viruses and presented a wide range of Ct values (22.19-25.73). In particular, positive samples were distributed as follows: influenza A (n=2), RSV (n=2), SARS-CoV-2 (n=1), influenza A+RSV (n=1) and rhinovirus/enterovirus (n=2). The remaining 17 (68%) specimens were negative for all these viruses. In the M10 test, 97% of positive samples were displayed as such in <60 minutes than RP2 revealed positive or negative results in 80 minutes. In our study population RSV, influenza A and rhinovirus/enterovirus were the most commonly detected pathogens. RSV season last from January to March in Italy.

Discussion and conclusion

A specific, ready-to-use all-in-one cartridge like M10, in line with seasonal virus circulation and epidemiology, could potentially reduce the risk of antibacterial co-infections with more effective treatment and improvement of cost-benefit ratio. In conclusion, this study demonstrates that M10 assay reliably detects influenza A/B, RSV and SARS-CoV-2 in less than one hour and is therefore suitable for a rapid differential diagnosis of the pediatric common respiratory viruses. We therefore

suggest that M10 assay may be best allocated to the emergency and critical pediatric care departments. In this way, in pediatric population we could reduce hospital admissions, avoid further unnecessary procedures and promote the use of specific antibiotic and antiviral treatments to improve clinical outcomes.

100 - Preliminary results of valacyclovir efficacy in HCMV positive pregnant women

ENRICA MADDALENA RANIERI⁽¹⁾ - PASQUALE D'AMBROSIO⁽¹⁾ - RAFFAELE DEL PRETE⁽²⁾ - MARIALUISA SCARASCIULLI⁽²⁾ - ANNAMARIA COLACICCO⁽²⁾ - ANGELA TRENTADUE⁽¹⁾ - LUIGI SANTACROCE⁽²⁾ - MARICA COLELLA⁽¹⁾

Dipartimento Interdisciplinare di Medicina (DIM), Sezione. Microbiologia e Virologia, Università "Aldo Moro", Bari, Italia⁽¹⁾ - Dipartimento Interdisciplinare di Medicina (DIM), Sezione. Microbiologia e Virologia, Università "Aldo Moro", Bari, Italia., UOC Microbiologia e Virologia, Azienda Ospedaliero-Universitaria Policlinico di Bari, Bari, Italia⁽²⁾

Preliminary results of valacyclovir efficacy in HCMV positive pregnant women

ENRICA M. RANIERI¹, PASQUALE D'AMBROSIO¹, RAFFAELE DEL PRETE^{1,2}, MARIALUISA SCARASCIULLI^{1,2}, ANNAMARIA COLACICCO^{1,2}, ANGELA TRENTADUE¹, LUIGI SANTACROCE^{1,2}, MARICA COLELLA¹

¹ Interdisciplinary Department of Medicine (DIM), Sect. Microbiology and Virology, University "Aldo Moro", Bari, Italy;

² UOC Microbiology and Virology, Azienda Ospedaliero-Universitaria Policlinico of Bari, Bari, Italy.

Background

Human Cytomegalovirus (HCMV) is widely distributed in human population. Transmission occurs through direct contact with body fluids from an infected person to another. It can be transmitted vertically to fetus and accounts for the most common congenital viral infection. If the infection is acquired in the first trimester of pregnancy, a lot of cases of congenital hearing loss and blindness have been reported. The aim of this pilot study is to assess the role and efficacy of valacyclovir on HCMV infection in pregnancy.

Materials and methods

A total of 126 women, mean age 30±6 years, with a suspect of HCMV infection in the first trimester of pregnancy have been enrolled from Obstetrics and Gynecology ward of Azienda Ospedaliero Universitaria Policlinico, Bari, Italy, from January 2022 to June 2023. Blood samples were analyzed at Microbiology and Virology laboratory for HCMV DNA detection by serological screening and Real Time PCR (RT-PCR). All of them, with confirmed diagnosis of HCMV primary infection by RT-PCR, were eligible for valacyclovir treatment (8gr/die by oral administration until amniocentesis) and included in the MEGAL-ITALI trial.

Results

Among 126 suspected infections, 104 (83%) were HCMV DNA negative, and 22 (17%) positive. Of the 22 positives, only one (5%) received hyperimmune serum therapy, 15 (68%) had no therapy, and 6 (27%) have been treated with valacyclovir therapy. A total of 19/22 HCMV+ patients resulted negative for HCMV DNA in amniotic fluid. One out of 6 women subsequently treated with valacyclovir refused to undergo amniocentesis. In this group, 2 delivered non-HCMV newborn (one had a couple of twins), 4/5 are still ongoing. Moreover, we measured the viral load on blood samples at time T0, T7, T14, T21 till amniocentesis. At T14 blood samples from all 6 pregnant women showed no viral load.

Discussion and Conclusions

Valacyclovir is indicated for prophylaxis of CMV infection and, safe and well tolerated by pregnant women, can reduce the transmission rate of the virus to the fetus in women with primary infection acquired in the first trimester of pregnancy. Since December 2020, the Italian regulatory body for drugs (AIFA), has authorized the administration of this drug to pregnant women to limit fetal maternal

transmission of CMV. The preliminary results of this pilot study demonstrate the effectiveness of valacyclovir in reducing the vertical transmission of HCMV and preventing its consequences on the fetuses and newborns. However, further evidence to corroborate these results will be available in the future by a larger population enrolled for this clinical trial.

102 - Analytical evaluation of a novel Point Of Care (POCT) Loop Mediated Isothermal Amplification (LAMP) test for the detection of Sexually Transmitted Infections

MARTINA BRANDOLINI⁽¹⁾ - LAURA GRUMIRO⁽¹⁾ - PATRIZIA FARABEGOLI⁽¹⁾ - GIORGIO DIRANI⁽¹⁾ - IRENE ZAGHI⁽¹⁾ - FRANCESCA TADDEI⁽¹⁾ - GIULIA GATTI⁽¹⁾ - ALESSANDRA MISTRAL DE PASCALI⁽¹⁾ - ANNA MARZUCCO⁽¹⁾ - MARIA SOFIA MONTANARI⁽¹⁾ - SIMONA SEMPRINI⁽¹⁾ - MONICA CRICCA⁽¹⁾ - VITTORIO SAMBRI⁽¹⁾

Unit of Microbiology, AUSL Romagna, Department of Laboratory and Transfusion Medicine, Cesena, Italia⁽¹⁾

Analytical evaluation of a novel Point Of Care (POCT) Loop Mediated Isothermal Amplification (LAMP) test for the detection of Sexually Transmitted Infections

MARTINA BRANDOLINI^{1,2}, LAURA GRUMIRO^{1,2}, PATRIZIA FARABEGOLI¹, GIORGIO DIRANI¹, IRENE ZAGHI^{1,3}, FRANCESCA TADDEI^{1,2}, GIULIA GATTI¹, ALESSANDRA MISTRAL DE PASCALI^{1,2}, ANNA MARZUCCO¹, MARIA SOFIA MONTANARI¹, SIMONA SEMPRINI¹, MONICA CRICCA^{1,2}, VITTORIO SAMBRI^{1,2}.

Department of Laboratory and Transfusion Medicine (DiMeLabeT), Unit of Microbiology, The Greater Romagna Area Hub Laboratory, 47522 Cesena, Italy;

Department of Medical and Surgical Sciences (DIMEC), Unit of Microbiology, Alma Mater Studiorum University of Bologna, 47522 Cesena, Italy;

Department of Specialty Medicine, Unit of Infectious Diseases, Santa Maria delle Croci Hospital, 48100 Ravenna, Italy

Introduction

Sexually transmitted infections (STIs) are a major public health concern worldwide and have a profound impact on sexual and reproductive health. These perspectives and necessities call for the development of cost-effective, user-friendly and broadly available molecular assays easily deployed as POCT (Point Of Care Testing) assay in obstetrics and gynaecology clinic, emphasising the importance of improved access to early and accurate detection aimed at population surveillance and pathogen circulation mapping.

Materials and Methods

The aim of this study was to evaluate the analytical performance of a new POCT real-time LAMP (Loop Mediated Isothermal Amplification) method for the rapid (around 1 hour) detection and differentiation of 7 major sexually transmissible pathogens (*Chlamydia trachomatis*, *Trichomonas vaginalis*, *Neisseria gonorrhoeae*, *Mycoplasma genitalium*, *Mycoplasma hominis*, *Ureaplasma urealyticum* and *Ureaplasma parvum*) from urine, semen, vaginal swabs, anal swabs, urethral swabs, and oropharyngeal swabs. LAMP was performed both on automatedly extracted samples (STARMag 96 X 4 Universal Cartridge Kit, Seegene, Seoul, South Korea) and on manually extracted DNA, the latter representing a more suitable extraction method for POCT deployment. Enbiotech LAMP STI Panel (Enbiotech, Palermo, Italy) results were compared with Allplex™ STI Essential Assay (Seegene) performed on automatedly extracted samples (gold standard diagnostic workflow). A total of 1084 samples were tested.

Results

Sensitivity ranged from 69.2% for *M. genitalium* to 92.6% with *U. parvum*, and specificity exceeded 96% for all pathogens. Positive predictive value was above 96%, while negative predictive value ranged from 64.3% for *M. hominis* to 93.9% for *C. trachomatis*, respectively. Test accuracy was above 89% for all pathogens. Calculated Cohen's Kappa highlighted a good agreement between LAMP and gold standard PCR ($k > 0.7$).

Discussion and Conclusion

Overall, these data showed a good diagnostic performance of the LAMP assay and highlighted its potential to be deployed as a POCT test in clinical settings, allowing for a timely and accurate aetiological diagnosis of STIs. During the phase of POCT workflow setup some criticalities also emerged, mainly derived from technical limitations of the simplified extraction method to deal with complex matrices, such as anal swabs and urine, from which amplifiable DNA can be extracted and purified with difficulty. These results, although preliminary, suggest a great potential of this LAMP-based POCT assay which needs further development and implementation, but certainly may meet the needs of speed required by clinicians, representing a competitive alternative to other gold standard PCR-based (and laboratory-centric) assays.

123 - Dynamic expression of CD169 in blood cells and circulating microvesicles as a potential diagnostic marker of immune response in COVID-19 and PASC individuals

MARIALAURA FANELLI⁽¹⁾ - VITA PETRONE⁽¹⁾ - ROSSELLA CHIRICO⁽¹⁾ - CHRISTIAN MARACCHIONI⁽¹⁾ - MARTINA GIUDICE⁽¹⁾ - ELISABETTA TETI⁽²⁾ - LUIGI COPPOLA⁽²⁾ - CHIARA SORACE⁽³⁾ - MARCO IANNETTA⁽²⁾ - MARTA ZORDAN⁽²⁾ - PIETRO VITALE⁽³⁾ - ALEXANDRE LUCAS⁽⁴⁾ - CLAUDIA MARIA RADU⁽⁵⁾ - LOREDANA SARMAI⁽²⁾ - EMANUELA BALESTRIERI⁽¹⁾ - SANDRO GRELLI⁽⁶⁾ - CLAUDIA MATTEUCCI⁽¹⁾ - ANTONELLA MINUTOLO⁽¹⁾

University of Rome Tor Vergata, Department of Experimental Medicine, Roma, Italia⁽¹⁾ - Tor Vergata Hospital, Infectious Diseases Clinic, Roma, Italia⁽²⁾ - University of Rome Tor Vergata, Department of Systems Medicine, Roma, Italia⁽³⁾ - Inserm UMR1297 and Université Paul Sabatier, We-Met platform, Institut des Maladies Métaboliques et Cardiovasculaires (I2MC), Toulouse, Italia⁽⁴⁾ - University of Padua, Thrombotic and Hemorrhagic Diseases Unit Department of Medicine, Padova, Italia⁽⁵⁾ - Policlinic of Tor Vergata, Virology Unit, Roma, Italia⁽⁶⁾

Dynamic expression of CD169 in blood cells and circulating microvesicles as a potential diagnostic marker of immune response in COVID-19 and PASC individuals.

MARIALAURA FANELLI¹, VITA PETRONE¹, ROSSELLA CHIRICO¹, CHRISTIAN MARACCHIONI¹, MARTINA GIUDICE¹, ELISABETTA TETI², LUIGI COPPOLA², CHIARA SORACE³, MARCO IANNETTA^{2,3}, MARTA ZORDAN^{2,3}, PIETRO VITALE³, ALEXANDRE LUCAS⁴, CLAUDIA MARIA RADU⁵, LOREDANA SARMAI^{2,3}, EMANUELA BALESTRIERI¹, SANDRO GRELLI^{1,6}, CLAUDIA MATTEUCCI¹, ANTONELLA MINUTOLO¹.

¹ Department of Experimental Medicine, University of Rome Tor Vergata, Rome, 00133, Italy; ² Infectious Diseases Clinic, Tor Vergata Hospital, Rome 00133, Italy; ³ Department of Systems Medicine, University of Rome Tor Vergata, Rome 00133, Italy; ⁴ We-Met platform, Institut des Maladies Métaboliques et Cardiovasculaires (I2MC), plateau We-Met, Inserm UMR1297 and Université Paul Sabatier, Toulouse, France; ⁵Thrombotic and Hemorrhagic Diseases Unit Department of Medicine – DIMED University of Padua, Italy; ⁶ Virology Unit, Policlinic of Tor Vergata, Rome, 00133, Italy;

Introduction: Viral infections induce CD169 expression as an antiviral response through type I IFN signaling. CD169 has been found highly overexpressed in the blood of COVID-19 patients (COV), and we have demonstrated its correlation with immune dysfunction and respiratory outcomes in COV. The COVID-19 is characterized by a high inflammatory response and immune dysregulation that persist even after SARS-CoV-2 infection, generating the post-acute sequelae of SARS-CoV-2 infection (PASC). Considering the COV immune dysregulation and the repercussions in PASC individuals, the identification of new biomarkers that can characterize COVID-19 and predict its long-term effects is needed. Based on our recent evidences supporting the close implication of CD169 expression in COVID-19, the aim of the present study was to extend the evaluation of these potential biomarkers also in PASC, to identify possible associations also with important alterations following the infection and potentially underlining the post-infection sequelae. The study also aimed to investigate the CD169 in association to the immune dysfunction mirrored at circulating

level, in particularly focusing on plasma circulating microvesicles (MVs). Materials and methods: Flow cytometry was used for the evaluation of the median fluorescence intensity ratio of CD169 between monocytes and lymphocytes (CD169 RMFI) in blood samples from 60 COV, 33 PASC and 43 healthy donors (HD). Leukocytes subpopulations and MVs were characterized for HLA-DR+CD169 proteins expression. Serum inflammatory markers (IL-1beta, CXCL10, IL-8, I-CAM, V-CAM) were assessed by Ella Automated Immunoassay System. The different markers analyzed were associated with clinical and biochemical parameters in COV and PASC. Results: CD169 RMFI was found significantly higher in COV than in HD and PASC. Among the leukocyte populations, monocytes showed a significantly higher percentage of HLA-DR+CD169+ in COV and PASC than in HD and correlated with CXCL10 and number of platelets. In plasma, the percentage and number of HLA-DR+CD169+ circulating MVs were significantly elevated in COV and PASC compared to HD and correlated with coagulation factors. Discussion and conclusions: These data suggest a potential role of MVs as a contributing factor to COVID-19 disease and post-infective chronic inflammation. This scenario opens new possibilities for studying the use of CD169 at cellular and MVs level as markers and potential cofactors of COVID-19 complexity and the long-term complications observed in PASC.

133 - Fecal calprotectin values in pediatric patients with bacterial and viral diarrhoea: An observational study.

BRUNELLA BRESCIA⁽¹⁾ - CLAUDIA CICINO⁽¹⁾ - AIDA GIANCOTTI⁽¹⁾ - GIORGIO SETTIMO BARRECA⁽¹⁾ - FRANCESCA TRIMBOLI⁽¹⁾ - MARTA PANTANELLA⁽¹⁾ - SIMONA GIGLIOTTI⁽¹⁾ - EMANUELA LARATTA⁽¹⁾ - CONCETTA ZANGARI⁽¹⁾ - VALENTINA TANCRE⁽¹⁾ - GRAZIA PAVIA⁽¹⁾ - NADIA MARASCIO⁽¹⁾ - ANGELA QUIRINO⁽¹⁾ - GIOVANNI MATERA⁽¹⁾

Università degli studi "Magna Graecia" di Catanzaro, Azienda Ospedaliero-Universitario "R. Dulbecco" presidio "Mater Domini", Dipartimento Scienze della Salute, Catanzaro, Italia⁽¹⁾

ABSTRACT

Fecal calprotectin values in pediatric patients with bacterial and viral diarrhoea: An observational study.

Authors: BRUNELLA BRESCIA¹, CLAUDIA CICINO¹, AIDA GIANCOTTI¹, GIORGIO S BARRECA¹, FRANCESCA TRIMBOLI¹, MARTA PANTANELLA¹, SIMONA GIGLIOTTI¹, EMANUELA LARATTA¹, CONCETTA ZANGARI¹, VALENTINA TANCRE¹, GRAZIA PAVIA¹, NADIA MARASCIO¹, ANGELA QUIRINO¹ AND GIOVANNI MATERA¹

Affiliations: ¹Department of Health Sciences, Institute of Clinical Microbiology, "Magna Graecia" University of Catanzaro, Italy.

Introduction: Acute Infectious Diarrhoea (AID) is one of the most common disease in pediatric patients, mainly caused by viruses (40% to 60%) and bacteria (2% to 15%). Faecal calprotectin (FCP) is a very sensitive non-invasive marker of gastrointestinal tract inflammation that was used to differentiate between organic and AID. The aims of this study were to evaluate the circulation of enteric pathogens in children aged 1-18 years with suspected AID and to determine the values of fecal calprotectin (FCP) in predicting bacterial/viral diarrhoea.

Materials and methods: Our study included pediatric patients aged 1-18 years with diarrhoea symptoms attending to University Hospital of Catanzaro, from October 2022 to June 2023. A total of 117 stool samples were collected and analyzed to detect gastrointestinal pathogens, including bacteria, viruses and parasites, using the FilmArray® BioFire® Gastrointestinal Panel (FA-GP). The FCP was determined by Chemiluminescence immunoassay (CLIA). Statistical analysis was performed by T. test. P. value <0.05 was considered significant.

Results: Overall, 47/117 (52%) stool sample were FA-GP positive for at least one microorganism. Bacterial agents were detected in 28 pediatric patients (60%), while viral agents were detected in 14 pediatric patients (30%). Five patients (10%) were infected with both pathogens. Regarding viral targets circulation, Sapovirus was the most prevalent (7/15, 47%), followed by Norovirus GI/GII (4/15, 26%), Astrovirus (2/15, 13%), Adenovirus F 40/41 (1/15, 7%) and Rotavirus (1/15, 7%). About bacterial agents, Clostridium difficile and Enteropathogenic E. coli (EPEC) were predominant (11/33, 34% and 10/33, 30% respectively); followed by Enteraggregative E. coli (EAEC) (7/33, 21%), Enterotoxigenic E.coli (ETEC), Salmonella spp., Shigella, Shiga-like toxin-producing toxin E.coli (STEC), Y. enterocolitica (1/33, 3% respectively). FCP values were higher in bacterial infection (mean 174.80 ± 135.57 µg/g) compared to viral infected patients (60.06 ± 44.81 µg/g). The results

revealed that most of the FA-GP-positive children had faecal calprotectin concentration significantly high (p-value=0.004).

Discussions and conclusions: The FCP is a sensitive marker of intestinal inflammation whose detection is relatively simple and non-invasive. In our study we reported a FCP values significantly increasing in bacterial infection compared to viral infection. Nevertheless, it is important to conduct further study on a larger number of pediatric patients in order to evaluate the evidence supporting the use of FCP in AID.

151 - Differential diagnosis for fever and rash as a key strategy to achieve the goal of eliminating Measles and Rubella

CLARA FAPPANI⁽¹⁾ - MARIA GORI⁽¹⁾ - SILVIA BIANCHI⁽¹⁾ - MARA TERRANEO⁽¹⁾ - ERICA BILARDI⁽¹⁾ - DANIELA COLZANI⁽¹⁾ - ELISABETTA TANZI⁽¹⁾ - MARTA CANUTI⁽²⁾ - ANTONELLA AMENDOLA⁽¹⁾

Università degli Studi di Milano, Dipartimento di Scienze della Salute, Milano, Italia⁽¹⁾ - Università degli Studi di Milano, Department of Pathophysiology and Transplantation, Milano, Italia⁽²⁾

Differential diagnosis for fever and rash as a key strategy to achieve the goal of eliminating Measles and Rubella

CLARA FAPPANI^{1,2,3}, MARIA GORI^{1,3}, SILVIA BIANCHI^{1,3}, MARA TERRANEO¹, ERICA BILARDI¹, DANIELA COLZANI¹, ELISABETTA TANZI^{1,3,4}, MARTA CANUTI^{3,4,5}, ANTONELLA AMENDOLA^{1,3,4}

¹Department of Health Sciences, Università degli Studi di Milano, Milan, Italy; ²Department of Clinical Sciences and Community Health, Università degli Studi di Milano, Milan, Italy;

³Coordinate Research Centre EpiSoMI (Epidemiology and Molecular Surveillance of Infections), Università degli Studi di Milano, Milan, Italy; ⁴Centre for Multidisciplinary Research in Health Science (MACH), Università degli Studi di Milano, Milan, Italy; ⁵Department of Pathophysiology and Transplantation, Università degli Studi di Milano, Milan, Italy.

Introduction

Several etiological agents could be associated with an exanthem and, in the quest to eliminate measles virus (MeV) and rubella virus (RuV), every suspected case must be properly identified and diagnosed. As Subnational Reference Laboratory of the measles and rubella surveillance network MoRoNet in Milan (Northern Italy), from the beginning of the surveillance activities (March 2017) to December 2022, we investigated 978 measles and rubella suspected cases (fever and rash): 668 tested positive for MeV and none was positive for RuV; 310 cases tested negative for both MeV and RuV infection (discarded) and were further investigated for 8 viral pathogens commonly associated with morbilliform rash.

Materials and methods

Differential diagnosis was carried out on 289 measles discarded cases (median age 20 years) whose samples were still available (228 oropharyngeal swabs and 61 urine samples). The nucleic acids of Human Herpesviruses 6 and 7 (HHV-6 and HHV-7), Parvovirus B19 (B19V), Enterovirus (EV), Epstein-Barr virus (EBV), Human Adenovirus (HAdV), Human Cytomegalovirus (HCMV), and Severe Acute Respiratory Syndrome-Coronavirus-2 (SARS-CoV-2) were detected by commercial multiplex real-time PCR or in house assays.

Results

At least one pathogen associated with rash was detected in 188 discarded cases (65%) with HHV-7 being the most frequently identified pathogen (32%), followed by EBV (28%), HHV-6 (12%), HAdV (7%), B19V (6%), HCMV (4%), SARS-CoV-2 (4%), and EV (3%). No difference in the number of detected infections over time was observed and infections were identified in all age groups. As expected, most HHV-6, EV, HAdV, and HCMV positive cases were found in children aged 0-4 years (59%, 88%, 50%, and 67% of positive samples, respectively) and HHV-7 was most frequent in the 15-39 age group (56% considering all infections).

Multiple infections with 2-4 pathogens were observed in 75 cases (40%). Particularly, most co-infection involved herpesviruses (95%).

Discussion and conclusions

In the light of the World Health Organization measles elimination goal, the introduction of additional laboratory methods for differential diagnosis to screen for other pathogens is required for the final classification of clinically compatible cases.

The screening panel used in this study allowed to increase the percentage of virus-positive cases from 68% to 88%, allowing to clarify viral involvement and epidemiology, improve diagnosis, and strengthen surveillance activities. As all investigated pathogens were detected, this diagnostic panel was a suitable tool to complement MeV and RuV surveillance activities.

174 - *Fusarium Solani* fungemia in pediatric patient with acute B-lymphoblastic leukemia

GIORGIA LA COSTA⁽¹⁾ - VITTORIANA DE LAURENTIIS⁽²⁾ - ENRICA M. RANIERI⁽¹⁾ - MICHELE MASTRIA⁽¹⁾ - MARA LORUSSO⁽¹⁾ - STEFANIA GARZONE⁽¹⁾ - FRANCESCO DE LEONARDIS⁽³⁾ - VITTORIO GRECO MIANI⁽³⁾ - RAFFAELE DEL PRETE⁽¹⁾ - CARMELA DE CARLO⁽²⁾ - LUIGI RONGA⁽²⁾ - STEFANIA STOLFA⁽¹⁾ - FEDERICA ROMANELLI⁽²⁾ - ELEONORA SPARAPANO⁽²⁾ - LUCIA NISI⁽²⁾ - MARIA TERESA MONTAGNA⁽¹⁾ - ADRIANA MOSCA⁽¹⁾

Università degli studi di Bari Aldo Moro, Dipartimento interdisciplinare di Medicina sezione Microbiologia e Virologia, Università Aldo Moro, Bari, Italia⁽¹⁾ - UOC Microbiology and Virology, University Aldo Moro, Dipartimento interdisciplinare di Medicina sezione Microbiologia e Virologia, Università Aldo Moro, Bari, Italia⁽²⁾ - Pediatric Hematology and Oncology, University Hospital of Policlinico, Bari, Italia⁽³⁾

Fusarium solani fungemia in pediatric patient with acute B-lymphoblastic leukemia

Giorgia La Costa³, Vittoriana De Laurentiis¹, Enrica M. Ranieri³, Michele Mastria³, Mara Lorusso³, Stefania Garzone³, Francesco De Leonardis², Vittorio Greco Miani², Raffaele Del Prete³, Carmela De Carlo¹, Luigi Ronga¹, Stefania Stolfa³, Federica Romanelli¹, Eleonora Sparapano¹, Lucia Nisi¹, Maria Teresa Montagna³, Adriana Mosca³

1 UOC Microbiology and Virology, University "Aldo Moro", Bari, Italy

2 Pediatric Hematology and Oncology - University Hospital of Policlinico - Bari Piazza G. Cesare,11, Bari, Italy

3 Section of Microbiology, Interdisciplinary Department of Medicine (DIM), School of Medicine, University of Bari " Aldo Moro", Bari, Italy

Introduction

In recent years, invasive fungal infections have increased in immunosuppressed individuals especially with hematological malignancies and undergoing chemotherapy. *Fusarium solani* species complex (FSSC) can lead to keratitis and onychomycosis up to most severe systemic infections with high morbidity and mortality. Early diagnosis is important due to its natural resistance to most antifungal agents. We report a case of a 12-year-old male patient with previously diagnosis of acute B lymphoblastic leukemia with disseminated FSSC blood infection, admitted in April 2023 to the pediatric ward of oncohematology.

Materials and methods

On admission the young patient presented febrile neutropenia and elevated PCR values therefore, blood cultures were performed and empirical therapy (acyclovir, cefepime and fluconazole) was administered. After 5 days blood cultures were repeated for feverish rise and observation of erythematous lesions on patient's limbs.

Results

At the admission, blood cultures were negative while those performed after 5 days resulted positive for FSSC (Time To Positivity = 3 days). Identification of the fungi was performed by MALDI-TOF and phenotypic methods. Antimycotic therapy was chanced empirically to Voriconazole twice/day. After two weeks, the patient was discharged with both blood cultures and PCR. One month later, of Voriconazole therapy the patient had normal transaminase values with healing skin lesions.

Discussion and conclusions

The incidence of FSSC infections has increased in recent years, probably due to the escalation of chemotherapy and the widespread use of antibacterial agents, which are risk factors for disseminated FSSC infection in hematological patients. Our case shows how early diagnosis and systemic use of voriconazole prevented the infection from worsening and improved the patient's outcome.

177 - SARS-CoV-2 infection in healthy school-age children

MATTEO DOMENICO MARSIGLIA⁽¹⁾ - SILVIA ANCONA⁽²⁾ - EMERENZIANA OTTAVIANO⁽²⁾ - FRANCESCA TRIVA⁽²⁾ - PRISCILLA PASUTTO⁽²⁾ - MASSIMILIANO SERGIO
FRANCESCO CAMPAGNOLI⁽²⁾ - SILVIA BIANCHI⁽²⁾ - ELISA BORGHI⁽²⁾

Università degli Studi di Milano, Dipartimento di Scienze Biomediche e Cliniche, Milano, Italia⁽¹⁾ -
Università degli Studi di Milano, Dipartimento di Scienze della Salute, Milano, Italia⁽²⁾

Abstract

Title: SARS-CoV-2 infection in healthy school-age children

Authors:

MATTEO D. MARSIGLIA^{1,2}, SILVIA ANCONA¹, EMERENZIANA OTTAVIANO¹,
FRANCESCA TRIVA¹, PRISCILLA PASUTTO¹, MASSIMILIANO S. F. CAMPAGNOLI¹,
SILVIA BIANCHI¹, ELISA BORGHI¹.

1. Department of Health Sciences, Università degli Studi di Milano, Milan (Italy);

2. Department of Biomedical and Clinical Sciences, Università degli Studi di Milano, Milan (Italy).

Introduction

Children generally have asymptomatic SARS-CoV-2 infection or mild COVID-19 symptoms. However, data on asymptomatic or paucisymptomatic infections among healthy school-age children are scant, particularly in the post-quarantine period after the loosening of containment measures. Schools provide a highly controlled environment to monitor the spread of the virus. The aim of the study was to evaluate SARS-CoV-2 variant and antibody prevalence in the pediatric population. To increase kids' acceptability, non-invasive tools for sample collections were used.

Materials and Methods

The study was conducted in primary (6-10 years old) and middle (11-14 years old) schools in the surrounding area of Milan (Italy). Saliva samples, Dried Blood Spots (DBS), and online clinical questionnaires were collected in the school setting at two-time points, December 2022 (t0) and March 2023 (t1). SARS-CoV-2 RNA in saliva samples was detected by real-time RT-PCR (COVID-19 HT Screen, Clonit). DBS samples were tested for IgG anti-SARS-CoV-2 nucleocapsid (anti-N) protein (Luminex xMAP SARS-CoV-2 Multi-Antigen IgG assay). The concordance of IgG titres between serum and DBS was previously validated on paired samples from healthy controls.

Results

352 school-age children participated in the study. One-hundred ninety-one students (54.3%) were from primary schools, while 161 (45.7%) were from middle schools. At t0, 300 saliva samples and 292 DBS were collected and processed. Only one child tested positive for SARS-CoV-2. 222 out of 292 (76.0%) students were anti-N IgG positive. At t1, 288 saliva samples and 279 DBS were collected. Two children tested positive for SARS-CoV-2. 15 (5.1%) students, previously negative for

anti-N IgG, seroconverted. Up to date, we collected 154 clinical questionnaires. Clinical data from 86 out of 237 (36.3%) anti-N IgG positive students were available. Thirty-nine out of 86 (45.3%) students did not report a previous COVID-19 diagnosis, despite the seropositivity. Unfortunately, variants were not evaluated because of the high cycle threshold values detected in the positive samples.

Discussion and Conclusions

Saliva and DBS samples were in general well accepted by children and families, and the school resulted a suitable setting to monitor SARS-CoV-2 infections. The low number of positive cases is probably due to the high seroprevalence in the studied population. The combination of molecular and serological testing allowed recognizing 20 otherwise undiagnosed infections. The merge of clinical and serological data showed that almost half of school-age children infected by SARS-CoV-2 were asymptomatic.

188 - Self-sampling as alternative strategy to detect Human Papillomavirus (HPV) and other sexually transmitted pathogens in women referred to colposcopy

CHIARA GIUBBI⁽¹⁾ - MARIANNA MARTINELLI⁽¹⁾ - MARIA LETIZIA DI MEO⁽²⁾ - RUTH CHINYERE NJOKU⁽³⁾ - FEDERICA PERDONI⁽¹⁾ - ERIKA BRUNO⁽¹⁾ - ROSARIO MUSUMECI⁽¹⁾ - FABIO LANDONI⁽²⁾ - ROBERT FRUSCIO⁽²⁾ - CLEMENTINA ELVEZIA COCUZZA⁽¹⁾

University of Milano-Bicocca, Department of Medicine and Surgery, Monza, Italia⁽¹⁾ - ASST Monza, Fondazione IRCCS San Gerardo dei Tintori, Monza, Italia⁽²⁾ - University of Sassari, Department of Biomedical Science, Sassari, Italia⁽³⁾

Self-sampling as alternative strategy to detect Human Papillomavirus (HPV) and other sexually transmitted pathogens in women referred to colposcopy

CHIARA GIUBBI¹, MARIANNA MARTINELLI¹, MARIA L. DI MEO², RUTH C. NJOKU³, FEDERICA PERDONI¹, ERIKA BRUNO¹, ROSARIO MUSUMECI¹, FABIO LANDONI^{1,2}, ROBERT FRUSCIO^{1,2}, CLEMENTINA E. COCUZZA¹

¹Department of Medicine and Surgery, University of Milano-Bicocca, 20900, Monza, Italy;

²Fondazione IRCCS San Gerardo dei Tintori, ASST Monza, 20900, Monza, Italy;

³Department of Biomedical Science, University of Sassari, 07100, Sassari, Italy.

Introduction: While self-sampling as alternative strategy to detect Human Papillomavirus (HPV) is being increasingly introduced in clinical practice, a recently published flowchart for the diagnosis of cervicitis in Italy (AMCLI, Percorsi Diagnostici) suggests the use of cervical samples for the molecular identification of pathogens mainly responsible for the disease (*C. trachomatis*, *N. gonorrhoeae*, *T. vaginalis* and *M. genitalium*). This study aims to assess the possible application of self-sampling procedures as an alternative to detect sexually transmitted infections (STIs) in women referred to colposcopy.

Materials and Methods: Clinician-collected cervical samples, self-collected vaginal and urine specimens were collected from 276 women undergoing colposcopy at San Gerardo Hospital, Monza following a recent abnormal cervical cytology result. All samples were tested using Microlab Nimbus platform for either nucleic acid extraction and PCR plate preparation for the detection of 14 high-risk HPV (hrHPV) genotypes and 7 other STIs (*C. trachomatis* (CT), *N. gonorrhoeae* (NG), *T. vaginalis* (TV), *M. hominis* (MH), *M. genitalium* (MG), *U. urealyticum* (UU), *U. parvum* (UP)) using, respectively, AnyplexTMII HPV and AnyplexTMII STI-7e (Seegene). Cohen's κ was used to evaluate the analytical agreement.

Results: 210/276 (76.1%) cervical and 223/276 (80.8%) vaginal specimens were hrHPV positive, with HPV16 and HPV31 being the hrHPV genotypes more frequently detected. A concordance of 95.65% for the detection of hrHPV was demonstrated between cervical and vaginal specimens with an almost perfect agreement ($\kappa=0.899$). 134/276 (48.6%) of the cervical samples and 156/276 (56.4%) of the vaginal swabs were positive for at least one of the other STIs investigated. In both types of specimen, UP, UU and MH were the pathogens more commonly identified. In general, for most of the sexually transmitted pathogens, the detection rate was higher in vaginal swabs. Three women showed TV infection in both cervical and vaginal specimens. No women showed infection from NG. For all the STIs investigated, a substantial agreement between the different types of sample was reached.

Discussion and Conclusions: These preliminary results confirm the good performance of self-sampling in the detection of hrHPV and provide more insights into its possible application for the

analysis of other sexually transmitted pathogens. Future analysis, including testing of urine specimens, will permit to better evaluate the distribution of sexually transmitted infections in different anatomical sites.

200 - Comparison of two detection methods for the quantitative detection of circulating Human Papillomavirus (HPV) from plasma and saliva of patients with head and neck cancer.

RUTH CHINYERE NJOKU⁽¹⁾ - MARIANNA MARTINELLI⁽²⁾ - CHIARA GIUBBI⁽²⁾ - FEDERICA PERDONI⁽²⁾ - CLEMENTINA ELVEZIA COCUZZA⁽²⁾ - CONSIGLIA PADUANO⁽³⁾ - DAVIDE SMUSSI⁽³⁾ - CRISTINA GURIZZAN⁽³⁾ - CLAUDIA ASSONI⁽³⁾ - LUIGI LORINI⁽³⁾ - PAOLO BOSSI⁽³⁾

University of Sassari, Department of Biomedical Science, Sassari, Italia⁽¹⁾ - University of Milano-Bicocca, Department of Medicine and Surgery, Monza, Italia⁽²⁾ - University of Brescia, ASST Spedali Civili, Division of Medical Oncology, Department of Medical and Surgical Specialties, Radiological Sciences and Public Health, Brescia, Italia⁽³⁾

Topic: Approcci diagnostici

Comparison of two detection methods for the quantitative detection of circulating Human Papillomavirus (HPV) from plasma and saliva of patients with head and neck cancer.

RUTH C. NJOKU^{1,2}, MARIANNA MARTINELLI¹, CHIARA GIUBBI¹, FEDERICA PERDONI¹, CRISTINA GURIZZAN³, CONSIGLIA PADUANO³, LUIGI LORINI³, DAVIDE SMUSSI³, CLAUDIA ASSONI³, PAOLO BOSSI³, CLEMENTINA E. COCUZZA¹

¹ Department of Medicine and Surgery, University of Milano-Bicocca, Monza, Italy

² Department of Biomedical Science, University of Sassari, Sassari, Italy.

³ Division of Medical Oncology, Department of Medical and Surgical Specialties, Radiological Sciences and Public Health, University of Brescia, ASST Spedali Civili, Brescia, Italy

Introduction

In Italy the incidence of HPV-associated oropharyngeal squamous cell carcinoma (OPC) (as percentage of all OPC) has been reported to have increased from 16.7% in 2000-2006 to 46.1% in 2013-2018. Currently, the gold standard for diagnosis involves tissue biopsy, but the analysis of circulating HPV DNA in plasma as well as in saliva shows potential in aiding early detection. An ongoing study aims to compare two HPV DNA detection methods, namely droplet digital PCR (ddPCR) and quantitative real-time PCR (qPCR), in terms of their accuracy in detecting and quantifying HPV DNA in plasma and saliva of patients with a recent diagnosis of HPV-associated OPC.

Materials and Methods

As part of an ongoing longitudinal study (Identify Study), blood and saliva samples were obtained from patients with locally advanced and histologically confirmed HPV-associated OPC at the time of diagnosis. Blood samples collected using Cell-Free DNA BCT® tubes (Streck) and saliva samples collected using FLOQSwab (Copan) and resuspended in 1mL eNAT (Copan). Nucleic acids were extracted using NucliSENS easyMAG (bioMérieux). To detect and quantify circulating HPV16 tumor DNA, two different assays were employed: OncoPredict HPV assay (Hiantis) based on qPCR and an in house ddPCR assay for HPV 16. The starting volume of nucleic acid extract used for HPV DNA detection was 5µl for qPCR for both sample types and 1µl saliva and 3µl plasma for ddPCR.

Results

We analysed 10 patients with an HPV-positive, stage III-IV OPC before starting curative treatment. In saliva, HPV16 was detected in all 10 OPC patients (100%) using qPCR and 7/10 (70%) showed positivity with ddPCR. In 9 out of the same 10 OPC patients, also Plasma could be analyzed: HPV16 was detected 100% by qPCR and in 89% (8/9) by ddPCR. HPV16 viral loads detected in saliva ranged from 3.18 - 3.36E+05 viral copies/ml with qPCR and 100 - 1.09E+05 viral copies/ml. In plasma, circulating HPV16 viral loads ranged from 7.77 - 6.20E+03 viral copies/ml and from 26.72 - 8.02E+03 viral copies/ml using qPCR and ddPCR, respectively.

Discussion and Conclusions

The preliminary study results have demonstrated high rates HPV16 DNA positivity in both saliva and plasma samples of patients with HPV-associated OPC. Furthermore, both qPCR and ddPCR showed comparable HPV16 DNA viral load detection in both sample types, suggesting that commercially available, less labour-intensive qPCR assays could in the future be validated for their use in the diagnosis and follow-up of patients with HPV-associated OPC.

Funded by Fondazione Spedali Civili, Brescia and University of Milano-Bicocca, Monza.

205 - Analysis of antibody titre decay in the mother-infant dyad exposed to congenital *Toxoplasma gondii* infection

SIMONA TRIMARCO⁽¹⁾ - ANGELA SPAGNUOLO⁽¹⁾ - FRANCESCO FRALLICCIARDI⁽¹⁾ - ADRIANO ROMANO⁽¹⁾ - ELENA SCAGLIONE⁽²⁾ - FRANCESCA CARRATURO⁽³⁾ - ILARIA CARUCCIO⁽¹⁾ - ASSUNTA RUSSO⁽¹⁾ - MARIA STABILE⁽¹⁾ - ROSA D'AURIA⁽¹⁾ - MARIA CONCETTA CUOMO⁽¹⁾ - CHIARA PAGLIUCA⁽⁴⁾ - ROBERTA COLICCHIO⁽²⁾ - MARIATERESA VITIELLO⁽²⁾ - SERENA SALOMÈ⁽³⁾ - FRANCESCO RAIMONDI⁽³⁾ - PAOLA SALVATORE⁽⁵⁾

Department of Integrated Activity of Laboratory Medicine and Transfusion, Complex Operative Unit of Clinical Microbiology, University Hospital Federico II, Naples, Italia⁽¹⁾ - Dep. of Int. Act. of Lab. Med. and Tran. Complex Operative Unit of Clinic. Microb.; Department of Molecular Medicine and Medical Biotechnology, University of Naples Federico II, Naples, Italia⁽²⁾ - Department of Translational Medical Sciences, Division of Neonatology, University Hospital Federico II, Naples, Italia⁽³⁾ - Department of Molecular Medicine and Medical Biotechnology, University of Naples Federico II, Naples, Italia⁽⁴⁾ - Dep. of Int. Act. of Lab. Med. and Tran. UOC Clinic. Microb.; Dep. of Mol. Med. and Med. Biotec.; CEINGE Franco Salvatore; Task Force on Microb. Stud., University of Naples Federico II, Naples, Italia⁽⁵⁾

Analysis of antibody titre decay in the mother-infant dyad exposed to congenital *Toxoplasma gondii* infection

SIMONA TRIMARCO¹, ANGELA SPAGNUOLO¹, FRANCESCO FRALLICCIARDI¹, ADRIANO ROMANO¹, ELENA SCAGLIONE^{1,2}, FRANCESCA CARRATURO³, ILARIA CARUCCIO¹, ASSUNTA RUSSO¹, MARIA STABILE¹, ROSA D'AURIA¹, MARIA C. CUOMO¹, CHIARA PAGLIUCA², ROBERTA COLICCHIO^{1,2}, MARIATERESA VITIELLO^{1,2}, SERENA SALOME³, FRANCESCO RAIMONDI³, PAOLA SALVATORE^{1,2,4,5}

¹Department of Integrated Activity of Laboratory Medicine and Transfusion, Complex Operative Unit of Clinical Microbiology, University Hospital Federico II, Naples, Italy; ²Department of Molecular Medicine and Medical Biotechnology, University of Naples Federico II, Naples, Italy; ³Department of Translational Medical Sciences, Division of Neonatology, University of Naples Federico II, Naples, Italy; ⁴CEINGE, Advanced Biotechnologies Franco Salvatore s.c.ar.l., Naples, Italy; ⁵Task Force on Microbiome Studies, University of Naples Federico II, Naples, Italy.

Introduction: *Toxoplasma gondii* is an obligate intracellular protozoan parasite that infects all warm-blooded vertebrates. Human infections are primarily caused by ingesting undercooked meat containing viable tissue cysts or by ingesting food or water contaminated with oocysts shed in the faeces from infected cats. Usually, the infection is asymptomatic because the human immune system is able to avoid the disease, but in particular conditions like HIV infection, immunosuppression therapies, or pregnancy the disease appears to be symptomatic. Infection acquired during pregnancy can lead to maternal-foetal transmission. Congenital Toxoplasmosis (CT) is an important cause severe and permanent neurological deficits, although at birth most infants appear asymptomatic. For infants exposed in utero to *T. gondii*, current guidelines recommend monitoring the specific antibody titer until 12 months of age. In this study, it was investigated the antibody titer decay in the mother-infant

dyad. **Materials and Methods:** To investigate the antibody titer decay in the mother-infant dyad, a population-based cohort study of neonates referred for prenatal exposure to *T. gondii* from January 2014 to December 2020 attending our institution, was conducted. Paired serum samples from the mother and the neonate were collected for determination of anti-Toxoplasma antibodies levels. The anti-Toxoplasma antibodies IgG and IgM, and IgG-Avidity in patients' serum, were measured with an automated quantitative test using the VIDAS Instrument. For the qualitative detection of anti-Toxoplasma IgA, the in vitro diagnostic kit PLATELIA™ TOXO IgA TMB was used. **Results:** A total of 670 neonates, referred to the Perinatal Infection Unit of the University Federico II of Naples, have been analyzed. 636 (95%) completed the serological follow up until 12 months. Specific IgG antibodies negativization occurred in 628 (98.7%) within 5 months. At 9 and 12 months, all patients had negative IgG. An initial neonatal IgG antibody titer ≥ 200 IU/ml was associated with a longer time to negativization. Maternal IgG antibody titer ≥ 200 IU/ml at childbirth was also associated to delayed time to negativization in the infant. Specific antibody negativization was irreversible in all patients. **Discussion and Conclusions:** Lower anti-Toxoplasma antibody titers detected at birth in the mother-infant-dyad lead to an earlier and irreversible negativization. This information allows for customization of the infant follow up program and avoids invasive and expensive tests.

209 - Comparative Analysis of miRNA Expression Profiles in HPV-Negative and HPV-Positive Cancer Cell Lines: Implications for Tumorigenesis

MARINA POTESÀ ⁽¹⁾ - VALENTINA ROGLIA ⁽¹⁾ - FEDERICO IACOVELLI ⁽¹⁾ - MARCO CIOTTI ⁽²⁾ - ANNA CRISCUOLO ⁽³⁾ - ANTONELLA MINUTOLO ⁽⁴⁾ - CARLA MONTESANO ⁽¹⁾

Università di Roma Tor Vergata, Dipartimento di Biologia, Roma, Italia ⁽¹⁾ - Policlinico Tor Vergata, Unità di Virologia, Roma, Italia ⁽²⁾ - Università di Roma Tor Vergata, Dipartimento di Ginecologia, Roma, Italia ⁽³⁾ - Università di Roma Tor Vergata, Dipartimento di Medicina Sperimentale, Roma, Italia ⁽⁴⁾

Comparative Analysis of miRNA Expression Profiles in HPV-Negative and HPV-Positive Cancer Cell Lines: Implications for Tumorigenesis

MARINA POTESÀ ^{1,2*}, VALENTINA ROGLIA ^{1*}, FEDERICO IACOVELLI ¹, MARCO CIOTTI ³, ANNA CRISCUOLO ⁴, ANTONELLA MINUTOLO ⁵ AND CARLA MONTESANO ^{1,2}.

1. Department of Biology University of Rome Tor Vergata, Rome, Italy.
2. UNESCO Interdisciplinary Chair in Biotechnology and Bioethics.
3. Virology Unit, Polyclinic Tor Vergata, Rome, Italy.
4. Department of Gynecology, University of Rome Tor Vergata, Rome, Italy.
5. Department of Experimental Medicine, University of Rome Tor Vergata, Rome, Italy.

*These authors contributed equally to this work

Introduction: Cervical cancer (CC) is one of the most common cancers in women worldwide, and persistent infection with high-risk human papillomavirus (HR-HPV) is widely recognized as the major cause of CC. However, approximately 5% of all CC are HPV-negative, and they are usually associated with diagnosis at an advanced stage and poor prognosis. MicroRNAs (miRs) play a critical role in various cellular processes, including tumorigenesis, and their dysregulation has been implicated in numerous cancers. This study aimed to investigate the difference in the expression levels of miRs involved in tumorigenesis in HPV-negative and HPV-positive cancer cell lines, compared to healthy donors. This investigation focused on elucidating potential differences in miR expression profiles between these groups and identifying miRs that could serve as potential biomarkers or therapeutic targets. **Materials and Methods:** The expression profiles of 84 hsa-miRs known to be involved in tumorigenesis was analyzed with the Human Cancer Pathway RT₂ Profiler PCR Array, in three distinct tumor cell line C33A (HPV-), HeLa (HPV18+) and SiHa (HPV16+). Normal specimens from cervical swab residual samples of HPV and PAP test negative (CSRS) cells were obtained from 44 patients, and their miRs expression patterns were used as reference control. In addition, a comprehensive bioinformatic analysis was performed to unravel the potential gene targets of these 84 hsa-miRs analyzed, involved in three crucial pathways: oxidative stress, inflammation, and the NFκB pathway. **Results:** Our findings revealed that the majority of the miRs investigated were overexpressed in all cell lines, especially in HeLa (75) and C33A (72), whereas 47 were overexpressed in SiHa. Forty miRNAs were overexpressed in all three cell lines when compared to CSRS; however, the degree of miRs overexpression varied greatly between the different tumor cell lines. C33A HPV-negative cell lines showed a different expression of 11 miRs compared to HPV-positive cell lines; 8 of these had a common expression in both HPV+ cell lines. Interestingly, bioinformatic analysis showed that these 8 miRs had important targets, such as TNF-alpha, TLR6, C5 complement factor, and IFN-gamma, which are involved in the immune response to infections and tumor development. We identified a panel of miRNAs that exhibited differential expression patterns that could potentially contribute to the distinct pathogenesis of these cancer subtypes. **Discussion and Conclusions:** Investigating the functional roles of these dysregulated

miRNAs will provide valuable insights into the underlying mechanisms of tumorigenesis, in both HPV-negative and HPV-positive CC, shedding light on potential biomarkers and therapeutic targets for these malignancies.

216 - Contribution of T2 Magnetic Resonance (T2MR ®) technology for the early detection of ESKAPE pathogens in ICU patients: a two years experience in Palermo, Italy

DOMENICO GRACEFFA ⁽¹⁾ - SALVATORE ANTONINO DISTEFANO ⁽¹⁾ - GIOVANNI GIAMMANCO ⁽²⁾ - ANDREA CORTEGIANI ⁽³⁾ - SALVATORE CAMPANELLA ⁽⁴⁾ - ANGELICA ANCONA ⁽⁴⁾ - MARTA CAPUTO ⁽⁴⁾ - PIETRO MIRASOLA ⁽⁴⁾ - CELESTINO BONURA ⁽²⁾

Università degli Studi di Palermo, Azienda Ospedaliera Universitaria Policlinico “Paolo Giaccone” di Palermo - U.O.C Microbiologia e Virologia, Palermo, Italia ⁽¹⁾ - Università degli Studi di Palermo, Dipartimento di Promozione della Salute, Materno Infantile, Medicina Interna e Specialistica di eccellenza (PROMISE) “G. D’Alessandro”, Palermo, Italia ⁽²⁾ - Università degli Studi di Palermo, Azienda Ospedaliera Universitaria Policlinico “Paolo Giaccone” di Palermo - U.O.C Anestesia Rianimazione e Terapia Intensiva, Palermo, Italia ⁽³⁾ - Università degli Studi di Palermo, Dipartimento di Discipline Chirurgiche Oncologiche e Stomatologiche (Di.Chir.On.S.), Palermo, Italia ⁽⁴⁾

Contribution of T2 Magnetic Resonance (T2MR ®) technology for the early detection of ESKAPE pathogens in ICU patients: a two years experience in Palermo, Italy

Domenico Graceffa^{1,3}, Salvatore A. Distefano³, Giovanni Giammanco^{1,3}, Andrea Cortegiani^{2,4}, Salvatore Campanella⁴, Angelica Ancona⁴, Marta Caputo⁴, Pietro Mirasola⁴ and Celestino Bonura^{1,3}

¹Dipartimento di Promozione della Salute, Materno Infantile, Medicina Interna e Specialistica di eccellenza (PROMISE) “G. D’Alessandro” – Università degli Studi di Palermo

²Azienda Ospedaliera Universitaria Policlinico “Paolo Giaccone” di Palermo - U.O.C Anestesia Rianimazione e Terapia Intensiva

³Azienda Ospedaliera Universitaria Policlinico “Paolo Giaccone” di Palermo - U.O.C Microbiologia e Virologia

⁴Dipartimento di Discipline Chirurgiche Oncologiche e Stomatologiche (Di.Chir.On.S.) – Università degli Studi di Palermo

Introduction

Bloodstream infections (BSIs) are a major cause of global morbidity and mortality and are increasingly frequent. BSIs can progress to sepsis and septic shock. The acquisition of antimicrobial resistance genes reduces therapeutic options and is fairly frequent in the pathogens belonging to the ESKAPE group that are most frequently involved in BSIs in critically ill patients. T2 Magnetic Resonance technology, compared to Blood Cultures (BC), enables a more rapid and sensitive detection directly from whole blood samples of ESKAPE bloodstream pathogens.

Materials and Methods

T2Bacteria Panels were tested on whole blood from 85 patients with sepsis or septic shock who were admitted from 1 January 2021 to 31 December 2022 to the General Intensive Care Unit of Azienda Ospedaliera Policlinico “Paolo Giaccone”, a University Hospital in Palermo, Italy. BC were performed at the onset of sepsis/septic shock, according to SEPSIS-3 definition in parallel with the T2 test. Positive BC were subjected to Gram staining microscopy and solid-medium subcultures. The

MS MALDI-TOF system was routinely used for identifying microorganisms isolated from BC, whereas the Phoenix system was used for susceptibility testing.

Results

85 T2Bacteria tests were performed on whole blood from ICU patients with sepsis or septic shock. Overall, one or more causative agent(s) were identified by the T2Bacteria assay in 41% of episodes (35/85), whereas negative and invalid results were obtained in 57% (49/85) and 1% (1/85) of episodes, respectively. The concordance rate between T2Bacteria and BC was 81% (69/85), with 20 concordant positive and 49 concordant negative episodes. The results of T2Bacteria tests, as well as their concordance with BC results show that, excluding contaminants, 48 ESKAPE pathogens were identified by the T2Bacteria assay (two microorganisms were identified in 4/35 and four microorganisms in 3/35 episodes, respectively) whereas 25 microorganisms grew from accompanying BC. When two or more causative agent(s) were identified by the T2Bacteria assay, at least one of them grew from concomitantly collected BC and the other(s) could be isolated from different sources of infection. Overall, blood pathogens coverage by T2Bacteria panel was 88% (22/25), whereas the discordant results in 12% (3/25) of episodes, were linked to non ESKAPE pathogens.

Conclusions

Our data show the usefulness of the T2 assays in terms of accurate diagnosis, directly from whole blood, for the identification of pathogens faster (in 3-5 hours) than with standard blood culture-based method, enabling physicians to reduce time to appropriate antimicrobial therapy.

217 - Innovative phage-based sensor for the rapid detection of staphylococcus aureus

SARA BOLOGNINI⁽¹⁾ - ZAHRAALSADAT YOUSEFNIAJEJAHROMI⁽²⁾ - CLAUDIA CAMPOBASSO⁽¹⁾ - NOEMI POMA⁽¹⁾ - CATERINA FERRETTI⁽¹⁾ - DARIA BOTTAI⁽¹⁾ - ANDREA BONINI⁽²⁾ - LAURA RINDI⁽³⁾ - FABIO DI FRANCESCO⁽²⁾ - ARIANNA TAVANTI⁽¹⁾ - MARIAGRAZIA DI LUCA⁽¹⁾

Università di Pisa, Dipartimento di Biologia, Pisa, Italia⁽¹⁾ - Università di Pisa, Dipartimento di Chimica Industriale, Pisa, Italia⁽²⁾ - Università di Pisa, Dipartimento di Ricerca Traslationale e delle nuove tecnologie in medicina e chirurgia, Pisa, Italia⁽³⁾

Innovative phage-based sensor for the rapid detection of Staphylococcus aureus

SARA BOLOGNINI¹, ZAHRAALSADAT YOUSEFNIAJEJAHROMI², CLAUDIA CAMPOBASSO^{1,3}, NOEMI POMA¹, CATERINA FERRETTI¹, DARIA BOTTAI¹, ANDREA BONINI², LAURA RINDI⁴, FABIO DI FRANCESCO², ARIANNA TAVANTI¹, MARIAGRAZIA DI LUCA¹

¹Department of Biology, University of Pisa, Pisa, Italy; ²Department of Chemistry and Industrial Chemistry, University of Pisa, Pisa, Italy; ³Department of Biosystems, Laboratory of Gene Technology, KU Leuven, Leuven, Belgium; ⁴Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa, Pisa, Italy

Introduction Rapid detection of pathogenic bacteria is key to the microbiological diagnosis process and to address the antibiotic therapy avoiding the abuse of broad-spectrum molecules. Recent developments in detection methods are mainly based on electrochemical biosensors which carry a recognition element required for the selective detection of the pathogen. Due to the high selectivity for their bacterial hosts, bacteriophages might be considered powerful biorecognition elements for the rapid and specific identification of bacterial pathogens from biological samples. This work aimed to develop a phage-based impedimetric sensor for the rapid detection of *S. aureus* from clinical samples.

Materials and Methods A novel isolated *S. aureus* bacteriophage Zeno (3×10^5 PFU) was covalently immobilized onto a screen-printed gold electrode, using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and N-Hydroxysuccinimide (NHS) as crosslinkers. Electrochemical Impedance Spectroscopy (EIS) was used to study the electrochemical response of the biosensor interface and the bacterial capture process. The phage-based biosensor was incubated for 5, 10 and 20 min in the presence of different amount of *S. aureus* MRSA ATCC 43300 cells (from 10^0 to 10^8 CFU/mL), before measuring the impedance variation. A negative sample without bacteria and samples with either *P. aeruginosa* or *E. faecalis* were also included to evaluate the sensor selectivity. The ability of phage-based sensor to detect *S. aureus* at different pH (7.4 and 5.5) was also tested.

Results The EIS biosensor functionalised with 3×10^5 PFU Zeno was able to specifically detect *S. aureus* with a linear range from 10^1 to 10^4 CFU/mL within 20 minutes under physiological pH, with a limit of detection (LOD) of 1.2 CFU/mL. The lack of the impedimetric signal observed when the electrode was incubated with other bacterial species, such as *P. aeruginosa* and *E. faecalis* confirmed the specificity of the biosensor for *S. aureus*. In presence of acidic pH of 5.5, biosensor was also able to detect bacteria with a linear range from 10^1 - 10^4 CFU/mL with a LOD of 1.55 CFU/mL.

Discussion and Conclusion In this study, an innovative phage Zeno-based EIS biosensor for rapid detection of *S. aureus* was developed. Experiments for bacterial detection in different biologic matrix are ongoing. This detection tool represents a proof of concept for the development of phage-based biosensors for the detection of other bacterial pathogens.

225 - Evaluation of syndromic SeeGene Allplex GI panels for detection of pathogens associated with acute gastroenteritis.

ARIANNA SUCATO⁽¹⁾ - FLORIANA BONURA⁽¹⁾ - CHIARA FILIZZOLO⁽¹⁾ - MARIANGELA PIZZO⁽¹⁾ - FRANCESCA DI BERNARDO⁽²⁾ - ANTONINA COLLURA⁽²⁾ - DONATELLA FERRARO⁽¹⁾ - GIOVANNI M. GIAMMANCO⁽¹⁾ - SIMONA DE GRAZIA⁽¹⁾

Università degli studi di Palermo, Dipartimento di Promozione della Salute, Materno-Infantile, di Medicina Interna e Specialistica di Eccellenza “G. D’Alessandro”, Palermo, Italia⁽¹⁾ - ARNAS, Ospedale Civico e Di Cristina, Unità Operativa di Microbiologia e Virologia, Ospedale Civico e Di Cristina, ARNAS, Palermo, Italia⁽²⁾

Evaluation of syndromic SeeGene Allplex GI panels for detection of pathogens associated with acute gastroenteritis.

ARIANNA SUCATO¹, FLORIANA BONURA¹, CHIARA FILIZZOLO¹, MARIANGELA PIZZO¹, FRANCESCA DI BERNARDO², ANTONINA COLLURA², DONATELLA FERRARO¹, GIOVANNI M. GIAMMANCO¹, SIMONA DE GRAZIA¹

¹Department of Health Promotion Sciences, Maternal and Infant Care, Internal Medicine and Medical Specialties “G. D’Alessandro”, University of Palermo, Palermo, Italy;

²Unit of Microbiology and Virology, National Relevance and High Specialization Hospital Trust ARNAS Civico, Di Cristina, Benfratelli, Palermo, Italy.

Introduction

Acute gastroenteritis (AGE) is a leading cause of morbidity and mortality globally, resulting in high public health costs. Diarrheal disease in children can be the result of bacterial, viral, or parasitic infections. The fast detection of enteric pathogens allows the timely planning of appropriate therapy and control measures and is essential to prevent the spread of these infectious agents. This study aimed to evaluate the diagnostic performance of a multiplex molecular assay (SeeGene Allplex GI Virus Panel) simultaneously detecting Norovirus, Rotavirus, Adenovirus, Astrovirus, and Sapovirus, comparing the results to Real-time PCR reference methods recommended for surveillance activities for enteric viruses. In addition, the set of virus-negative samples by reference methods was further investigated using Seegene Allplex GI Panels 2-3-4 (13 bacteria and 6 protozoa) and Helminth panel (9 targets) in order to assess their potential to reduce the diagnostic gap of AGE.

Materials and Methods

A total of 161 stool samples collected from children hospitalized with AGE at the "G. Di Cristina" Children's Hospital, Palermo, Italy, in 2022 and previously tested by reference PCR methods were retrospectively investigated. Extraction was performed with Nimbus platform and Allplex GI Panels were used according to the manufacturer protocols (Seegene, Seoul, Korea). The Cohen's kappa coefficient (k) was calculated to evaluate performances agreement.

Results

A total of 129 samples (80.1%) were positive for at least one virus. Co-infections were detected in 25 patients (19.4%) involving two and three viruses in 22 and 3 cases, respectively, and even four viruses in a single sample. The multiplex panels and reference methods showed a good agreement for Norovirus GII (k= 0.67) while agreement was moderate for Astrovirus (k= 0.59), Adenovirus (k= 0.42) and Rotavirus (k= 0.47). Such low agreements were related to a higher sensibility of commercial

assay compared to reference molecular methods used for surveillance activities. The molecular investigations for bacteria and protozoa allowed to detect an etiologic agent in almost 45% (22/49) of virus-negative samples (17 bacteria and 5 protozoa).

Discussion and Conclusions

Our results suggested that Allplex GI panels can be recommended as a valid method for diagnosis of viral, bacterial, and protozoan gastroenteritis, allowing to significantly reduce the diagnostic gap. The panel-based assays have the advantages of a rapid turnaround time and of detection of a large number of microorganisms but pose challenges for the interpretation of results, particularly for high Ct values and co-infections. Close interaction between clinicians and laboratories will be needed to tailor the results to individual clinical cases.

246 - Multidrug-resistant bacteria surveillance in intensive care unit: the importance of a molecular approach

LIVIA TARTAGLIONE ⁽¹⁾ - ANDREA BUSNI ⁽¹⁾ - ELISA MATARAZZO ⁽¹⁾ - ELENA NICOLINI ⁽¹⁾ - ALESSANDRA ARMILLIS ⁽¹⁾ - ELENA PASTORI ⁽¹⁾ - DIANA FANTI ⁽¹⁾ - ALESSANDRA BIELLI ⁽¹⁾ - CHIARA S. VISMARA ⁽¹⁾

ASST Grande Ospedale Metropolitano Niguarda, ASST Grande Ospedale Metropolitano Niguarda, Milano, Italia ⁽¹⁾

Multidrug-resistant bacteria surveillance in intensive care unit: the importance of a molecular approach

LIVIA TARTAGLIONE, ANDREA BUSNI, ELISA MATARAZZO, ELENA NICOLINI, ALESSANDRA ARMILLIS, ELENA PASTORI, DIANA FANTI, ALESSANDRA BIELLI, CHIARA S. VISMARA

Clinical Microbiology, ASST GOM Niguarda, Milan, Italy

Introduction

Carbapenem-resistant Enterobacterales (CRE), carbapenem resistant *Acinetobacter baumannii* complex (CRAB) and *Pseudomonas aeruginosa* cause severe problems in healthcare facilities worldwide. MDR infections are associated with faster spread from patient to patient and higher mortality rate, thus molecular detection of carbapenemases could be a useful tool for rapid patient's management. We evaluated the performance of a commercial real time PCR (rt-PCR) versus our conventional culture-based approach in order to evaluate its introduction as screening method for diagnostic purpose.

Materials and methods

From May 2022 to March 2023, 414 rectal swabs obtained from 215 intensive care unit (ICU) patients of Niguarda Hospital (Milan, Italy) were simultaneously tested using both the rt-PCR and the conventional method, using CHROMID® Carba agar (bioMérieux, France). Positive cultures were further tested by a lateral flow immunoassay (L.F.I.) NG-Test® CARBA 5 (NG Biotech, France) to confirm carbapenemase production. The rt-PCR assay used was the Allplex™ Entero-DR Assay (Seegene, South Korea) for detection of the 3 major antibiotic resistance mechanisms (CPE, VRE, ESBL) in association with a research use only (RUO) real time PCR detecting *A. baumannii* complex and *P. aeruginosa*.

Results

Out of 414, 67 were positive only for *A. baumannii* complex, 100 only for *P. aeruginosa* and 35 were positive for both with RUO assay. Of these 74.5% and 70.3% were also culture positive for *A. baumannii* complex and for *P. aeruginosa* respectively. By using rt-PCR, we found: 35 NDM, 16 KPC, 2 OXA-48 and 4 VIM positive swabs. All the NDM were also positive for *A. baumannii* complex with RUO assay. The culture examination allowed us to confirm the presence of *A. baumannii* complex NDM producing strains in 24 of 28 NDM positive swabs by identification of colonies by MALDI TOF and confirmation of carbapenemase presence by using L.F.I. CARBA 5 on bacterial colonies. The positivity of 16 KPC and 1 OXA-48 swabs was confirmed by isolation of *Klebsiella pneumoniae* and *Escherichia coli* respectively and confirmation with L.F.I. (KPC and OXA-48 positives with CARBA 5). In contrast, 4 VIM, 4 NDM, 1 OXA-48 and 1 KPC positive swabs were culture negative.

Discussion and conclusions

Our data show higher sensitivity and faster execution of the molecular test compared to culture and might suggest its use as screening method for rapid identification of MDR carriers, while culture could be a reflex test. The implementation of molecular surveillance measures in critical care settings is required to limit the spread of these organisms and to improve infection control measures. The study results also underscore the need to extend the search for carbapenemase to include CRAB in order to avoid possible outbreaks.

251 - Advanced Bacterial Detection in a Simulated Bloodstream Infections through Digital PCR: A Proof of Concept for Enhanced Diagnostic Accuracy

ALESSIA MIRABILE⁽¹⁾ - CARMELO BONOMO⁽¹⁾ - EMANUELE NICITRA⁽¹⁾ - STEFANIA STEFANI⁽¹⁾ - DAFNE SAMANTHA IRENE BONGIORNO⁽¹⁾ - NICOLÒ MUSSO⁽¹⁾

Università degli studi di Catania, Dipartimento di Scienze Biomediche e Biotecnologiche (BIOMETEC), Catania, Italia⁽¹⁾

Advanced Bacterial Detection in a simulated Bloodstream Infections through Digital PCR: A Proof of Concept for Enhanced Diagnostic Accuracy

ALESSIA MIRABILE¹, CARMELO BONOMO¹, EMANUELE NICITRA¹, STEFANIA STEFANI¹, DAFNE S.I. BONGIORNO¹, NICOLÒ MUSSO².

¹Department of Biomedical and Biotechnological Science (BIOMETC), Section of Microbiology, University of Catania, Via Santa Sofia 97, Catania, Italy

²Department of Biomedical and Biotechnological Science (BIOMETC), Medical Biochemistry, University of Catania, Via Santa Sofia 97, Catania, Italy

Introduction: Bacterial infections pose significant challenges in healthcare, necessitating rapid and reliable diagnostic methods. Traditional PCR-based methods have limitations, particularly in cases where the bacterial target load is low, leading to delayed interventions and increased morbidity especially in cases of sepsis. Digital Polymerase Chain Reaction (dPCR) is an innovative molecular biology technique that has revolutionized the field of nucleic acid analysis detection and offers promising avenues for improving early diagnosis and patient outcomes. This work aims to show the bacterial detection in blood samples in order to highlight the high sensitivity and accuracy of dPCR.

Material and Method: DNA extraction of a clinical strain of *Staphylococcus aureus* was performed, followed by a dPCR assay to assess the limit of detection of the technique. Four specific targets (*gyrB*, *kataA*, *pdhA*, *sodA*) present in single copies in *S. aureus* genome, as well as 16s rDNA, were tested using scalar DNA amount (from 4ng to 0.004ng) employing QIAcuity One dPCR platform. Subsequently several bacteria-spiked blood samples from a healthy donor were performed, in order to simulate a bloodstream infection. Bacterial dilutions were performed inoculating from 1.74×10^8 to 1 CFU. Total DNA was extracted from samples with 10^8 CFU and from 10^3 up to 1 CFU. A negative control (whole blood with no bacteria) was included. dPCR was performed for the absolute quantification of three different targets (16s rDNA, *gyrB* and *gapdh*) for all spike-in samples.

Results: A linear correlation with a $R^2 = 0,99$ for all target was shown between target concentration (from 3284 to 3.4 copies/ μ L) and the starting amount of bacterial DNA (from 0.4 ng to 0.0004 ng) [Fig.1]. Similarly, a linear correlation with $R^2 = 0,99$ was also observed for spike-in samples while our internal control target (*gapdh*) remained stable compared to the negative control sample [Fig.2].

Discussions and conclusions: dPCR has exhibited potential in monitoring the efficacy of antibiotic treatments, tracking bacterial persistence in blood samples. These preliminary results show the advantages of dPCR in bacterial detection such as its high sensitivity, ability to quantify low microbial loads, resistance to PCR inhibitors commonly present in complex clinical samples as well as overcoming steric hindrance of human genomic. This work highlights the potential of dPCR to enhance the sensitivity and accuracy of bacterial identification in clinical samples, especially when

the microbial load is low. In conclusion, dPCR could become a valuable tool to break down conventional culture times allowing the early detection of bacteria in blood samples.

This study is partly supported by the European Union – NextGenerationEU within the National Recovery and Resilience Plan (NRRP) PE13 INF-ACT.

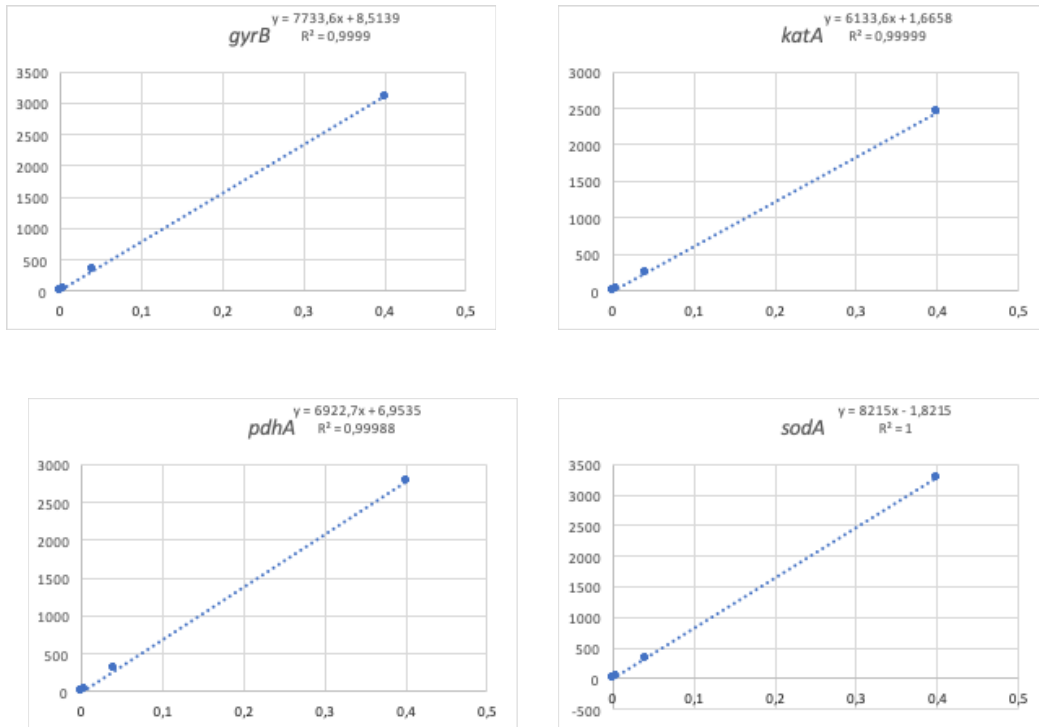


Figure 1. Linear correlation between *S. aureus* specific genic target load (copies/μl) and starting DNA amount (ng).

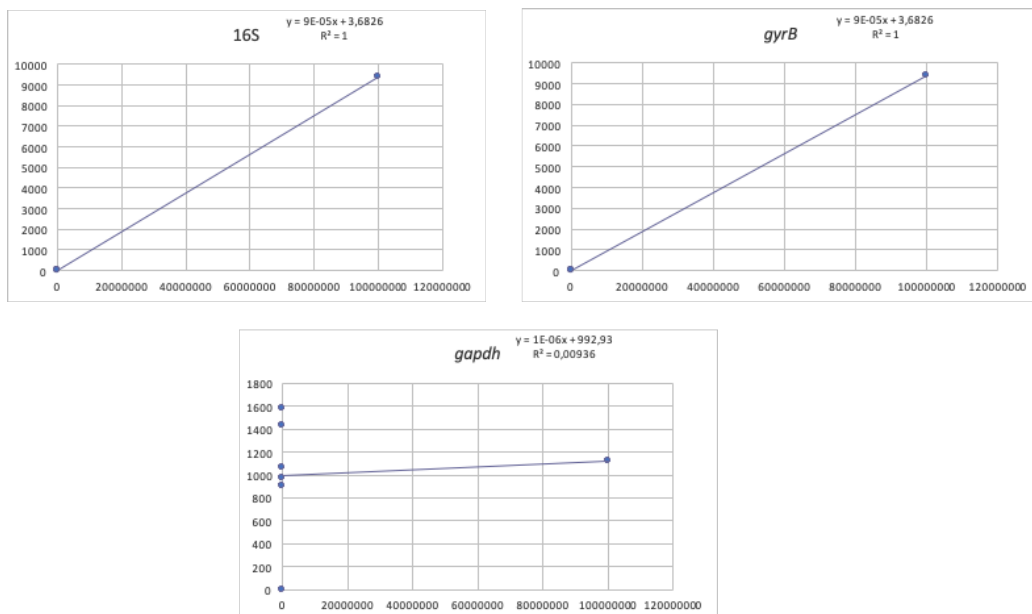


Figure 2. 16S, gyrB and gapdh load (copies/μl) in differently concentrated spike-in blood samples.

266 - Preventing early-onset group B streptococcus (GBS-EOD) invasive infection in the new-born: a national survey, Italy, 15 December 2022 to 12 February 2023

MICHELA SABBATUCCI ⁽¹⁾ - PIERANGELO CLERICI ⁽²⁾ - CRETI ROBERTA ⁽¹⁾

ISS, Department Infectious Diseases, Rome, Italia ⁽¹⁾ - Italian Clinical Microbiologists Association, AMCLI, ITALY, Italia ⁽²⁾

Preventing early-onset group B streptococcus (GBS-EOD) invasive infection in the new-born: a national survey, Italy, 15 December 2022 to 12 February 2023

MICHELA SABBATUCCI ^{1,3}, PIERANGELO CLERICI ², ROBERTA CRETI ³

Unit 5, Directorate General Health Prevention Communicable Diseases and International Prophylaxis, Ministry of Health, Rome, Italy

President of the Italian Clinical Microbiologists Association (AMCLI)

Department of Infectious Diseases, Istituto Superiore di Sanità, Roma, Italy

Introduction

Group B Streptococcus (GBS) vaginal/rectal colonisation in pregnancy is the main risk factor for the development of neonatal early-onset invasive infection (GBS-EOD). In Italy, pregnant women who test positive at antenatal GBS screening (AGBSS) are given intrapartum antibiotic prophylaxis (IAP). If performed on schedule (no more than five weeks before delivery), with the correct method (vaginal and rectal sampling), and with the appropriate culture procedure (enrichment in broth before inoculation on selective medium), the result of AGBSS is extremely reliable. In Italy, previous data indicated that AGBSS was performed following different protocols by the microbiology laboratories. This can lead to false-negative AGBSS results and higher risk of GBS-EOD.

Materials and Methods

A national survey was promoted by the Istituto Superiore di Sanità, in collaboration with the Ministry of Health and the Italian Clinical Microbiologists Association (AMCLI). We distributed the survey through an online password-protected questionnaire active from 15 December 2022 to 12 February 2023. The questionnaire included 22 questions in order to collect information on the microbiological method used for performing AGBSS, on the total incidence of GBS-EOD in the years 2018-2022 and cases from GBS-negative mothers.

Results

All the 20 Italian Regions (R) and the 2 autonomous provinces (AP) participated to the survey with a total of 222 healthcare facilities by completing the questionnaire. A total of 189 (85.5%) healthcare facilities declared the availability of a local microbiological protocol for AGBSS. It is performed starting at the 35th (35.8%) or 36th week (50.5%) of pregnancy. Almost all the facilities performed both vaginal and rectal swab (98.6%). Liquid broth enrichment before plating on solid selective medium was performed by 58.5% of the laboratories, while 39.2% cultured the sample directly on plate and 1.4% used molecular methods only. Prevalence of maternal GBS colonisation varied largely between Regions ranging from 5.6% in Apulia to 28.1% in Emilia-Romagna. Overall, incidence of GBS-EOD was 0.43 per 1,000 newborns during the study period. The number of GBS-EOD cases from mothers who had resulted negative to AGBSS was at least 30%.

Discussion and Conclusions

Over one third of the respondents cultured samples directly on plate without prior broth enrichment, increasing possible false negative results. This could also possibly explain the large variability in

the prevalence of GBS-colonised pregnant women and the notable proportion of GBS-EOD cases from negative mothers, indicating the urgent need of a national GBS-EOD prevention guideline for the harmonisation of the microbiological method to limit GBS-EOD burden in our country.

269 - Laboratory strategy for the rapid diagnosis of leishmaniasis: experience in Palermo University hospital.

MARIANGELA PIZZO ⁽¹⁾ - TERESA MARIA ASSUNTA FASCIANA ⁽¹⁾ - ROBERTA VIRRUSO ⁽²⁾ - GIUSEPPE MINÌ ⁽²⁾ - GAETANO D'ALESSANDRO ⁽²⁾ - CHIARA MASSARO ⁽¹⁾ - LUCA PIPITÒ ⁽¹⁾ - ANTONIO CASCIO ⁽¹⁾ - GIOVANNI M. GIAMMANCO ⁽¹⁾ - CINZIA CALÀ ⁽¹⁾

Università degli studi di Palermo, Promozione della Salute, Materno-Infantile, di Medicina Interna e Specialistica di Eccellenza "G. D'Alessandro" (PROMISE), Palermo, Italia ⁽¹⁾ - A.O.U.P. "Paolo Giaccone", Palermo, U.O.C di Microbiologia, Virologia e Parassitologia, Palermo, Italia ⁽²⁾

Laboratory strategy for the rapid diagnosis of leishmaniasis: experience in Palermo University hospital.

MARIANGELA PIZZO*, TERESA M. A. FASCIANA*, ROBERTA VIRRUSO**, GIUSEPPE MINÌ**, GAETANO D'ALESSANDRO**, CHIARA MASSARO*, LUCA PIPITÒ*, ANTONIO CASCIO*, GIOVANNI M. GIAMMANCO*, CINZIA CALÀ*

*Department of Health Promotion, Mother and Child Care, Internal Medicine and Medical Specialties (PROMISE), University of Palermo, Palermo, Italy

** U.O.C. of Microbiology, Virology and Parassitology, A.O.U.P. "Paolo Giaccone", Palermo, Italy

Introduction

Leishmaniasis is a vector-borne disease caused by protozoan of the genus *Leishmania* and transmitted by female sandflies. Human leishmaniasis is a public health problem especially in the Mediterranean basin and Sicily is considered endemic for *L. infantum*. The spectrum of disease, including tegumentary lesions or visceral disease, depends on the immune status of the host and immune-inflammatory responses. Immunosuppression, mostly HIV-related, is one of the strongest risk factors for the onset of clinical disease and the response to pharmacological treatment. Although several laboratory assays have been developed for diagnosis of leishmaniasis, currently no diagnostic approach is considered a gold standard. The aim of our study was to evaluate a serological commercial test for the rapid diagnosis of leishmaniasis, compared to molecular method on clinical samples collected over a period of two and a half years.

Materials and methods

From January 2021 to June 2023, 126 patients were admitted to the Policlinico Universitario "P. Giaccone" in Palermo, for suspected leishmaniasis, mostly at the Infectious Diseases Unit. Visceral leishmaniasis (VL) was suspected in 111 patients (36% HIV-related), and cutaneous leishmaniasis (CL) in 15 immunocompetent patients. All patients were screened for serum antibodies by automated indirect chemiluminescent immunoassay (Virclia® IgG+IgM monotest) and were also evaluated for *Leishmania* by Real Time PCR kit (Clonit) on either blood (111 VL) or skin biopsy (15 CL).

Results

Of the 126 patients, 34 were positive by serological test, but only 14 of them were confirmed by PCR (10 blood, 4 biopsy). A negative serological result was observed in 90 patients, confirmed by PCR in 88 cases. The 2 discordant patients with CL were positive only by amplification of DNA from biopsy. Finally, 2 symptomatic CL patients showed an indeterminate serological result and a positive PCR.

Discussion and Conclusions

Although for leishmaniasis diagnosis, coupling serological investigations and molecular methods represent the best approach in terms of sensitivity, however, from our experience, serological analysis, conducted with VirClia monotest, allowed to obtain rapid results, in just 50 minutes, that were concordant with molecular methods in 81% of cases. In the group of VL patients with positive serology, molecular investigation was not always successful possibly due to low parasite load. Instead for CL diagnosis molecular method on skin biopsies showed to be crucial and should not be excluded especially in the early stages of the disease, when antibodies are not yet detectable. Close cooperation between the clinician and the microbiologist is necessary to ensure correct interpretation of serological results based on the clinical data.

294 - Screening of carriage of Carbapenem-resistant Enterobacterales (CRE), Acinetobacter baumannii (CRAB), and Pseudomonas aeruginosa (CRPsA) in a hospital setting by culture and molecular tests.

CALOGERO RASPANTI ⁽¹⁾ - WALTER RAZETE ⁽²⁾ - NOELE SPURIA ⁽²⁾ - CINZIA CALÀ ⁽¹⁾ - NICOLETTA DE LUCA ⁽²⁾ - RITA IMMORDINO ⁽²⁾ - ROBERTA VIRRUSO ⁽²⁾ - GIOVANNI M. GIAMMANCO ⁽¹⁾ - TERESA M.A. FASCIANA ⁽¹⁾

Department of Health Promotion, Mother and Child Care, Internal Medicine and Medical Specialties., University of Palermo, Palermo, Italia ⁽¹⁾ - Microbiology and Virology Unit., AOU Policlinico “P. Giaccone”, Palermo, Italia ⁽²⁾

Introduction

Carbapenem-resistant Enterobacterales (CRE), Acinetobacter baumannii (CRAB), and Pseudomonas aeruginosa (CRPsA) represent an alarming problem. The morbidity associated with CRE, CRAB and CRPsA infections is usually high, with a relevant clinical and economic impact and therapeutic options for these microorganisms are often limited to a few drugs. Several approaches to reduce the global burden of these infections have been proposed, among which hospital screening and surveillance protocols. In this context, rectal screening for CRE, CRAB and CRPsA carriage in high-risk patients represents an useful method to limit their spread. The aim of this study was to evaluate the use of molecular tests in comparison with the culture-based method for rectal screening.

Materials and Methods

The study was conducted at the Microbiology and Virology Unit, AOU Policlinico “P. Giaccone”, between November 2022 and January 2023. A total of 97 non-duplicated rectal swab specimens were collected. For the isolation of CRE, CRAB and CRPsA strains from rectal swab specimens, an aliquot from each specimen was inoculated on BBL CHROMagar CPE and on MacConkey II Agar (Becton Dickinson). If swab tested positive, any suspected colony was identified using the MS MALDI-ToF (Bruker Daltonics Inc) and the class of carbapenemase was determined by immunochromatography method. The molecular analysis was performed with a commercial Real-Time PCR assay (AB Analitica), according to the manufacturer's instructions.

Results

A total of 97 surveillance rectal swabs underwent evaluation by culture and molecular analysis. By culture method, the prevalence detected for CRE was around 3% (3/97), the prevalence of detection for CRAB was 7% (7/97) while 10% (10/97) of the swabs were excluded from analysis for absence of growth on MacConkey II Agar. The molecular test provided 92 valid results over 97 tested specimens. The remaining five samples were excluded from analysis for lack of amplification due to probable presence of inhibitors. In detail, the prevalence detected for KPC-producing Enterobacterales was around 10% (10/97), the prevalence of detection of CRAB was 20% (20/97) while bla_{VIM} ed bla_{IMP} and bla_{OXA-48} was detected respectively in 1% (1/97) of the cases. In addition, the prevalence of the gene mcr was detected in 2% (2/97) of the cases.

Discussion and Conclusions

Molecular assays represent a valuable diagnostic tool as they allow the rapid screening of colonized patients. Our results showed that a higher percentage of colonization was detected using molecular

analysis with a dramatic decrease in turnaround time. Additionally, this method ensured an higher sample adequacy thanks to an internal control system able to monitor the influence of possible inhibitory activities.

300 - Extraction-free RT-qPCR for the detection of SARS-CoV-2: modification of a commercial assay protocol reduces turnaround time.

EDOARDO MANDALÀ⁽¹⁾ - CATERINA AMATO⁽²⁾ - WALTER RAZETE⁽¹⁾ - GAETANO D'ALESSANDRO⁽¹⁾ - NOELE SPURIA⁽¹⁾ - SHARON RUSSO⁽¹⁾ - SIMONA DE GRAZIA⁽²⁾ - GIOVANNI MARIA GIAMMANCO⁽²⁾

UOC Microbiologia e Virologia, Azienda Ospedaliera Universitaria Policlinico (AOUP) "P.Giaccone", Palermo, Italia⁽¹⁾ - Università degli studi di Palermo, Promozione della Salute, Materno-Infantile, Medicina Interna e Specialistica d'Eccellenza "G. D'Alessandro", Palermo, Italia⁽²⁾

Extraction-free RT-qPCR for the detection of SARS-CoV-2: modification of a commercial assay protocol reduces turnaround time.

EDOARDO MANDALA¹, CATERINA AMATO², WALTER RAZETE¹, GAETANO D'ALESSANDRO¹, NOELE SPURIA¹, SHARON RUSSO¹, SIMONA DE GRAZIA², GIOVANNI M. GIAMMANCO²

¹U.O.C. of Microbiology and Virology, A.O.U.P. "Paolo Giaccone", Palermo, Italy;

²Department of Health Promotion Sciences, Maternal and Infant Care, Internal Medicine and Medical Specialties "G. D'Alessandro", University of Palermo, Palermo, Italy

Introduction

The global state of emergency for COVID-19 caught most laboratories unprepared for the need to consistently increase their capacity to perform molecular tests, both in terms of throughput and turnaround time (TAT). Use of an automated extraction platform (REp) was the proposed standard protocol for high-quality performance, but turnaround time with full batch can exceed 4 hours. Here we propose an extraction-free modified protocol (EFp2.0) for the Allplex SARS-CoV-2 Assay RT-qPCR capable of consistently reducing TAT without quality loss. The aim of our study was to compare the performance of the novel shortened EFp2.0 to the conventional (REp) and extraction-free methods (EFp).

Materials and Methods

A panel of 131 nasopharyngeal swabs previously tested for SARS-CoV-2 by conventional Seegene protocol (REp) with fully positive result (118) at various cycle thresholds (Ct) or with one or two targets detected (13; presumptive positive (pp)) were selected for EFp2.0 validation (tab1). Conventional Seegene protocol (REp) included extraction and amplification of four gene targets (E, N and S/RpRd) by Allplex SARS-CoV-2 Assay (Seegene). Samples were then stored at -80°C before being tested in duplicate using the two extraction free protocols, hereby called EFp and EFp2.0. In detail, 15 µL of clinical samples diluted (1:4) in 45 µL of RNase-free water, for EFp, were treated as previously described by Blairon (2021) for virus inactivation and cell-lysis (EFp), or were directly amplified for the proposed EFp2.0. Both pre-treated (EFp) and untreated (EFp2.0) samples were amplified using Allplex Seegene kit.

Results

Among the 118 samples detected as positive for SARS-CoV-2 by the REp, EFp and EFp2.0 identified 86 (72.9%) and 90 (76.3%) SARS infections, respectively. Therefore, EFp produced 16 (13.5%) false negative and 16 (13.5%) pp, while EFp2.0 produced 12 (10.2%) false negative and 16 (13.5%) pp. However, all pp and false negative samples produced by the EF protocols

corresponded to samples originally showing a Ct >35 with the REp. Among the 13 pp by the REp, EFp and EFp2.0 confirmed the result in 8 (61.5%) and 3 (23%) samples, respectively. Overall, comparing the results obtained, we observed that EFp and EFp2.0 showed a delay of approximately 3 and 2 Cts compared to REp, respectively. Finally, skipping the inactivation and cell lysis phases, TAT of the EFp2.0 allowed a time saving of at least 30 min compared to standard EFp.

Discussions and Conclusions

These results demonstrate that EFp2.0 is more efficient than the EFp for Ct < 35 samples. Indeed, EFp2.0 detects positive samples with higher sensibility and at earlier Cts. While for the Ct >35 and pp samples, more missing gene targets were observed with EFp2.0. However, based to the knowledge on SARS-CoV-2 pathogenesis, pp patients could be considered non-infectious and EFp2.0 results could be more fitted to clinical management. The reduction of TAT obtained with the EFp2.0 we propose could be of value to better handle high throughput demand in case of pandemics.

307 - Assessment of increased bla_{KPC} gene dosage by digital PCR for the rapid prediction of meropenem/vaborbactam resistance in *Klebsiella pneumoniae* clinical strains

NOEMI AIEZZA⁽¹⁾ - ALBERTO ANTONELLI⁽¹⁾ - MARCO COPPI⁽¹⁾ - VINCENZO DI PILATO⁽²⁾ - TOMMASO GIANI⁽¹⁾ - GIAN MARIA ROSSOLINI⁽¹⁾

University of Florence, Department of Experimental and Clinical Medicine, University of Florence; Microbiology and Virology Unit, Florence Careggi University Hospital, Florence, Italia⁽¹⁾ - University of Genoa, Department of Surgical Sciences and Integrated Diagnostics, University of Genoa; Microbiology and Virology Unit, Florence Careggi University Hospital, Florence, Italia⁽²⁾

Abstract

Assessment of increased bla_{KPC} gene dosage by digital PCR for the rapid prediction of meropenem/vaborbactam resistance in *Klebsiella pneumoniae* clinical strains

NOEMI AIEZZA^{1,3}, ALBERTO ANTONELLI^{1,3}, MARCO COPPI^{1,3}, VINCENZO DI PILATO^{2,3}, TOMMASO GIANI^{1,3}, GIAN MARIA ROSSOLINI^{1,3}

¹Department of Experimental and Clinical Medicine, University of Florence, Florence, Italy;

²Department of Surgical Sciences and Integrated Diagnostics, University of Genoa, Genoa, Italy;

³Microbiology and Virology Unit, Florence Careggi University Hospital, Florence, Italy

Introduction. Infections caused by carbapenemase-producing Enterobacterales (CPE) represent a global concern. Among them, *K. pneumoniae* strains producing KPC- (KPC-KP), NDM- or OXA-48 have been increasingly isolated in Europe with KPC-KP being most prevalent worldwide. The limited armamentarium against these microorganisms has led the introduction of novel β -lactam- β -lactamase inhibitor combinations (BLICs), such as meropenem/vaborbactam (MER/VAB), which are now among the most used strategies for the treatment of KPC-KP infections. Despite the promising activity of MER/VAB, several resistance mechanisms have been already described. Among them, the increase of bla_{KPC} copy-number associated with porin mutations, seems to be the leading cause of MER/VAB resistance in clinical strains. The aim of this study was to evaluate digital droplets-based PCR (ddPCR) for the rapid assessment of bla_{KPC} increased gene dosage and prediction of MER/VAB resistance. **Materials and Methods.** A total of 6 well-characterized clinical isolates of KPC-producing *K. pneumoniae*, collected from three different Italian hospitals, with known genome sequence, MER/VAB phenotype and KPC carbapenemase specific activity were included in the study. Overall, 5 of 6 strains showed a MER/VAB resistant phenotype (with a MIC=32 mg/L or MIC=>64 mg/L), for whom the genomic analysis revealed also an increased bla_{KPC} copy-number compared to chromosome. Selected strains presented different bla_{KPC} gene dosage (one, two or three copies) and bla_{KPC} genetic context (harbored on pKpQIL plasmid or other mobile genetic elements). *K. pneumoniae* BAA-2814, presenting a susceptible phenotype to MER/VAB was used as control strain. The absolute bla_{KPC} gene dosage was determined using ddPCR (Bio-Rad® QX-100) in parallel with a target specific for pKpQIL-like plasmid and rpsL housekeeping gene. **Results.** For each isolate, it was possible to identify the bla_{KPC}/pKpQIL ratio indicative of the bla_{KPC} copy-number in pKpQIL plasmid, pKpQIL/rpsL ratio, representing the copy-number of the pKpQIL plasmid respect to the chromosome and bla_{KPC}/rpsL ratio, evaluating the number of bla_{KPC} copies compared to the housekeeping gene, respectively. The instrument

correctly revealed an increased bla_{KPC} gene dosage in the 5 MER/VAB-resistant. Specifically, two strains exhibited a duplication of bla_{KPC}-carrying transposon, one presented a higher pKpQIL-like plasmid/chromosome ratio and the last two a combination thereof compared to MER/VAB susceptible controls. Discussion and Conclusion. In conclusion, ddPCR proved to be a reliable tool for the rapid (~3 hours) estimation of MER/VAB resistance from isolated colonies. Further experiments will be necessary for the evaluation of this method from clinical samples.

314 - Concomitant syndromic diagnosis of monkeypox and other vesicular viruses in patients with skin and genital lesions

MARIA BEATRICE VALLI ⁽¹⁾ - MARTINA RUECA ⁽¹⁾ - GIULIA MATUSALI ⁽¹⁾ - ENRICO GIRARDI ⁽²⁾ - EMANUELE NICASTRI ⁽³⁾ - ANDREA ANTINORI ⁽⁴⁾ - FABRIZIO MAGGI ⁽¹⁾

Istituto Nazionale Malattie Infettive "L.Spallanzani" I.R.C.C.S., Laboratorio di Virologia e laboratori di Biosicurezza, Roma, Italia ⁽¹⁾ - Istituto Nazionale Malattie Infettive "L.Spallanzani" I.R.C.C.S., Direzione Scientifica, Roma, Italia ⁽²⁾ - Istituto Nazionale Malattie Infettive "L.Spallanzani" I.R.C.C.S., UOC MI Alta Intensità e Cura, Roma, Italia ⁽³⁾ - Istituto Nazionale Malattie Infettive "L.Spallanzani" I.R.C.C.S., UOC IMMUNODEFICIENZE VIRALI, Roma, Italia ⁽⁴⁾

Concomitant syndromic diagnosis of monkeypox and other vesicular viruses in patients with skin and genital lesions

Valli Maria Beatrice, Martina Rueca, Giulia Matusali, Enrico Girardi, Emanuele Nicastri, Andrea Antinori and Fabrizio Maggi

Introduction

Since May 2022, more than 87,000 laboratory-confirmed cases of mpox virus (MPXV) infection have been reported in more than 100 countries. Prior to the current multi-country outbreak, human-to-human transmission was considered rare and self-limiting outside the endemic regions of Central and West Africa. Therefore, the rapid increase of non-imported MPXV cases in non-endemic countries has raised concerns about the route of transmission in humans. The evidence that most cases are men that have sex with men and the identification of MPXV in semen and urethral secretions suggest a sexual transmission. This hypothesis is supported by the finding of a high percentage of patients with one or more concomitant sexually transmitted infections (STIs). Consequently, the observed clinical features strongly support the importance of differential diagnoses for STIs and other vesicular viruses in order to eventually identify their occurrence in negative and positive MPXV samples.

Materials and Methods

Specimens of vesicular lesions collected from the skin and genital tract of patients presenting at INMI Spallanzani for MPXV diagnosis were retrospectively tested by using a novel molecular syndromic Vesicular Viruses Panel (VVP) assay. The VVP test can detect in a single reaction the West African and Congo Basin MPXV and five other viral pathogens that cause similar skin manifestations: herpes simplex virus 1 (HSV1), herpes simplex virus 2 (HSV2), varicella-zoster virus (VZV), enterovirus (EV), and human herpesvirus 6 (HHV6).

Results

We tested 36 vesicular swabs (21 were collected from anal, perianal, and genital ulcers). Of the 23 samples that tested positive for MPXV by using the routine diagnostic real-time PCR test, 1 (4%) resulted negative to all pathogens, and 22 (96%) were confirmed as virus-positive by the VVP assay. Of these latter positive samples, 19 (86%) revealed a single MPXV infection, while three (14%) were positive for at least one other virus (1 for HSV2, 1 for EV, and 1 for HSV2 and HHV6). Six samples that were found negative for MPXV DNA with both the routine rtPCR and VVP tests resulted positive for other viruses included in the VVP (1 EV, 1 HSV1, 2 HSV2, and 2 VZV).

Discussion and Conclusions

The use of a syndromic vesicular assay can be useful for simultaneously testing MPXV and other viruses for a rapid differential diagnosis and for revealing cases of coinfection. Despite the limited number of samples analyzed, our data reveal 13.6% of coinfections, highlighting the importance of a

wider screening in the population with high-risk sexual behaviour in cases where MPXV is suspected (and vice versa) to ensure a comprehensive aetiology and an adequate treatment.

324 - Relationship between Cycle threshold value and N antigen amount in SARS-CoV-2 variants

MARIA ANTONELLA ZINGAROPOLI ⁽¹⁾ - ROBERTA CAMPAGNA ⁽²⁾ - BENEDETTA GENNENZI ⁽³⁾ - ELISABETTA LAZZARI ⁽⁴⁾ - CHIARA NONNE ⁽²⁾ - DANIELE EMANUELE COMPAGNINO ⁽²⁾ - MARIA GEMMA LEONE ⁽²⁾ - GERMANA SFARA ⁽²⁾ - SARA FARAONE ⁽³⁾ - GUIDO ANTONELLI ⁽²⁾ - OMBRETTA TURRIZIANI ⁽²⁾

Università La Sapienza, Dipartimento di Sanità Pubblica e Malattie Infettive, Roma, Italia ⁽¹⁾ -
Università La Sapienza, Dipartimento di Medicina Molecolare, Roma, Italia ⁽²⁾ - Policlinico
Umberto I, Unità di Microbiologia e Virologia, Roma, Italia ⁽³⁾ - INMI Lazzaro Spallanzani,
Laboratorio di Virologia, Roma, Italia ⁽⁴⁾

Relationship between Cycle threshold value and N antigen amount in SARS-CoV-2 variants
MARIA A. ZINGAROPOLI 1, ROBERTA CAMPAGNA 2, BENEDETTA GENNENZI 3,
ELISABETTA LAZZARI 4 CHIARA NONNE 2, DANIELE E. COMPAGNINO 2, MARIA G.
LEONE 2, GERMANA SFARA 2, SARA FARAONE 3, GUIDO ANTONELLI 2, OMBRETTA
TURRIZIANI 2

Affiliation

1 Department of Public Health and Infectious diseases, Sapienza University of Rome, Italy; 2
Department of Molecular Medicine, Sapienza University of Rome, Italy; 3 Microbiology and
Virology Unit, Sapienza University Hospital Policlinico Umberto I, Rome, Italy; Virology Unit,
INMI, Lazzaro Spallanzani, Rome, Italy

Introduction

The presence of mutations in the SARS-CoV-2 virus can potentially impact test performance. Next to PCR, antigen tests are used to assess SARS-CoV-2 infection. These assays recognize the nucleoprotein N and the result is expressed as INDEX value or COI. Currently, Cycle threshold (Ct) values allow an estimation of the viral load and INDEX or COI is used to estimate the ‘amount’ of N antigen (Ag) within a sample. This study aimed to evaluate whether Ct and INDEX values differed significantly between wild-type SARS-CoV-2 and viral variants.

Materials and methods

The variant of concern (VOC) was identified through commercial PCR kits, Sanger sequencing or Next Generation Sequencing. N Ag amount was tested by chemiluminescent assay (SARS-CoV-2 Antigen, Siemens) and expressed as INDEX value. SARS-CoV-2 RNA was detected by the AltoStar RT-PCR kit 1.0 (Altona diagnostics) and the FTD SARS-CoV-2 Assay (Fast Track Diagnostics). Both assays provide a qualitative result, and a Ct value inversely proportional to the amount of viral RNA present in tested samples.

Results

Comparing Ct values, Delta showed a median value significantly lower than other VOCs. To identify whether a difference in N antigen amount existed among SARS-CoV-2 variants, we evaluated the Ag INDEX value of 144 samples. Each VOC was stratified into two groups, according to Ct values: Ct values ≤ 21 and Ct values > 21 . In the group with Ct ≤ 21 Delta showed a significantly lower value of Ag INDEX when compared to Wuhan strain ($p=0.0367$). In the group with Ct values > 21 , Gamma was the variant with the highest INDEX median, while Omicron was the variant with the lowest median. Interestingly, for Omicron variant a high percentage of discordant samples (positive to molecular assay and negative to antigenic test) was observed. To further evaluate this discordance, 169 NPS, all identified as Omicron, were analyzed. Samples with Ct values between 21 and 35 were selected. Among the 169 Omicron NPS, 63% showed detectable levels of RNA and undetectable levels of N antigen.

Discussion and Conclusions

From the differences found between Ct values between VOCs we can speculate that patients infected with Delta had higher viral load than those infected with other VOCs. Furthermore, Delta showed a lower amount of antigen N when the viral load was high, suggesting that not all the transcripts produced are encapsidated. Omicron variant showed the highest percentage of discordance between PCR and antigen test results. This may be explained hypothesizing that either PCR test detects viral transcripts that may not be associated with nucleocapsid protein, or that the presence of many mutations on the N protein causes a reduced sensitivity of antigen tests.

326 - RT-QuIC detection prion seeding activity in spiked human tears

SARAH VASCELLARI ⁽¹⁾ - CHRISTINA D. ORRÙ ⁽²⁾ - GIANLUIGI ZANUSSO ⁽³⁾ - ANNA POLEGGI ⁽⁴⁾ - FRANCO CARDONE ⁽⁴⁾ - GIOVANNI COSSU ⁽⁵⁾ - ANTONELLA MANDAS ⁽⁶⁾ - FABRIZIO ANGIUS ⁽¹⁾ - ALDO MANZIN ⁽¹⁾ - BYRON CAUGHEY ⁽²⁾

University of Cagliari, Department of Biomedical Sciences, Cagliari, Italia ⁽¹⁾ - National Institute of Allergy and Infectious Diseases (NIAID), National Institute of Health (NIH), Laboratory of Neurological Infections and Immunity, Rocky Mountain Laboratories, Hamilton, Montana, Stati Uniti D'america ⁽²⁾ - University of Verona, Department of Neuroscience, Biomedicine and Movement, Verona, Italia ⁽³⁾ - Istituto Superiore di Sanità, Department of Neuroscience, Roma, Italia ⁽⁴⁾ - A.O. Brotzu, S. C. Neurology and Stroke Unit, Cagliari, Italia ⁽⁵⁾ - University of Cagliari, Department of Medical Sciences and Public Health, Cagliari, Italia ⁽⁶⁾

RT-QuIC detection prion seeding activity in spiked human tears

¹SARAH VASCELLARI, ²CHRISTINA D. ORRÙ, ³GIANLUIGI ZANUSSO, ⁴ANNA POLEGGI, ⁴FRANCO CARDONE, ⁵GIANNI COSSU, ⁶ANTONELLA MANDAS, ¹FABRIZIO ANGIUS, ¹ALDO MANZIN, ²BYRON CAUGHEY

¹Department of Biomedical Sciences, University of Cagliari, Cagliari, Italy; ²Laboratory of Neurological Infections and Immunity (LNII), Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases (NIAID), National Institute of Health (NIH), Hamilton, MT, United States; ³Department of Neuroscience, Biomedicine and Movement, University of Verona, Verona, Italy; ⁴Department of Neuroscience, Istituto Superiore di Sanità, Rome, Italy; ⁵S. C. Neurology and Stroke Unit, A.O. Brotzu, Cagliari, Italy; ⁶ Department of Medical Sciences and Public Health, University of Cagliari, Cagliari, Italy.

Introduction: Over the last years, advancement in the development of new diagnostic approaches for prion disorders have been made. Among these, the real time quaking-induced conversion (RT–QuIC) assay is part of the diagnostic criteria for sporadic Creutzfeldt-Jakob disease (CJD). Although combined RT-QuIC analyses of cerebrospinal fluid (CSF) and olfactory mucosa have been shown to be 100% sensitive, the interest in alternative easily collectable diagnostic biofluids remains. Recently, detection of prions in human tears was reported and sparked interest in this biofluid as a new potential diagnostic specimen for prion disease. In this study, we further optimized the protocol for elution and detection of sCJD and GSS prion seeds (PrP^{sCJD} and PrP^{GSS}) from Schirmer's strips, outlining strategies to overcome assay inhibition associated with tears and achieve more rapid detection.

Methods: Tear samples were collected from human healthy individuals using the Schirmer's strip test and stored at -20°C. For spiking experiments, tear-soaked strips were spiked with serial dilutions of brain homogenates from genetic or sporadic CJD patients and non-CJD controls (e.g., Alzheimer's disease). Prion seeds were eluted from the strips with different concentration of NaCl, or 0.002% SDS solution. Proteinase K (PK) digestion was implemented for 30 minutes at 37°C. Eluted spiked tears were used to seed in RT-QuIC reactions in which Hamster 90-231 was the substrate and an incubation temperature was 50-55°C.

Results: Our preliminary results suggest that NaCl treatment of PrP^{sCJD}/PrP^{GSS} spiked tear-soaked strips allows for more efficient prion seeds elution compared to 0.002% SDS buffer. sCJD prion

seeding activity was detectable in 10~20 hours. Furthermore, the addition of a PK digestion step to remove inhibitory proteins from tears improved prion seeds' detection time by at least 5 hours. Conclusions: Our preliminary experiments provide evidence that a NaCl treatment of Schirmer's strips allows for more efficient elution of prions and suggests that PK digestion can be used to overcome the inhibitory effect of some proteins. These results suggest that tears have the potential to be used as diagnostic biofluid that harbor prion seeding activity.

02 Controllo microbiologico dell'ambiente

26 - Antimicrobial activities of functionalized enamelled-coated surfaces

HAMZA UR REHMAN ⁽¹⁾ - FRANCESCA RUSSO ⁽²⁾ - MASSIMO CALOVI ⁽²⁾ - BERENICE FURLAN ⁽¹⁾ - STEFANO ROSSI ⁽²⁾ - ORIETTA MASSIDDA ⁽¹⁾

Department of Cellular, Computational, and Integrative Biology, University of Trento, Trento, Italia
⁽¹⁾ - Department of Industrial Engineering, University of Trento, Trento, Italia ⁽²⁾

Antimicrobial activities of functionalized enamelled-coated surfaces

Hamza Ur Rehman¹, Francesca Russo², Massimo Calovi², Berenice Furlan¹, Stefano Rossi², Orietta Massidda¹

¹Department of Cellular, Computational, and Integrative Biology, University of Trento, Trento, Italy; ²Department of Industrial Engineering, University of Trento, Trento, Italy

1. Introduction: Frequently touched surfaces (FTS) that are contaminated with pathogens are one of the main sources of nosocomial infections, which commonly include hospital-acquired and healthcare-associated infections (HAIs). FTS also play a leading role in the spreading of pathogens in high-traffic public places. Consequently, attention has been devoted to developing novel methods to tackle this problem. Porcelain enamel coatings (silica-based inorganic coating), that meets the modern criteria of durability and environmental sustainability because of their unique properties such as high chemical resistance and corrosion protection, could be a valid solution for application in healthcare settings and public spaces, where cleaning and disinfection are often required. In this work we report the development of functionalized enamelled-coated-surfaces supplemented with 1% wt. AgNO₃ (SA) and 2% wt. AgNO₃ (SB) and evaluate their antimicrobial efficacy. 2. Materials and methods: Surface roughness and thickness were investigated through optical and scanning electron microscopic observations. After this characterization, the antimicrobial properties of these surfaces were evaluated against relevant pathogens including the Gram-positive *Staphylococcus aureus* (ATCC 6538) and the Gram-negative *Escherichia coli* (ATCC 8739) using the ISO 22196:2011 standard. All experiments were conducted in triplicates. 3. Results: Both SA and SB showed an antibacterial activity against *S. aureus* and *E. coli* after 24 hours with respect to the untreated control. Consistently, SA reduced *E. coli* growth to 30, 98, and 100% and *S. aureus* growth to 6, 20, and 100% after 2, 8, and 24 hours of exposure, respectively. SB significantly inhibited *E. coli* growth to 0 after 8 and 24 hours and reduced *S. aureus* growth to 18, 68, and 100 % after 2, 8, and 24 hours, respectively. Moreover, both SA and SB reduced *C. albicans* growth to 80 and 98%, respectively, after 24 hours of contact. Longer exposure (48 and 72 hours) led to a larger inhibitory effect up to 99.9%. 4. Discussion and conclusions: Taken together, these results demonstrate that enamelled-coated surfaces possess a remarkable antibacterial activity against *S. aureus* and *E. coli*. SA and SB significantly reduced the growth of both bacteria to 0 at 24 hours and with SB the growth of *E. coli* was already halted to 0 after 8 hours, indicating that a higher AgNO₃ concentration would be more rapidly bactericidal over time. Finally, both SA and SB demonstrated a fungistatic effect after 24 hours and, likely, a fungicidal effect against *C. albicans* after 48 and 72 hours.

66 - Shaping the subway microbiome by a probiotic-based sanitation during the COVID-19 emergency

MARIA D'ACCOLTI ⁽¹⁾ - IRENE SOFFRITTI ⁽¹⁾ - FRANCESCA BINI ⁽¹⁾ - ELEONORA MAZZIGA ⁽¹⁾ - ANTONELLA VOLTA ⁽²⁾ - MATTEO BISI ⁽²⁾ - SANTE MAZZACANE ⁽²⁾ - ELISABETTA CASELLI ⁽¹⁾

University of Ferrara, 1Department of Chemical, Pharmaceutical and Agricultural Sciences, and LTTA; 2 CIAS Research Center, University of Ferrara, Ferrara, Italia ⁽¹⁾ - University of Ferrara, CIAS Research Center, University of Ferrara, Ferrara, Italia ⁽²⁾

Shaping the subway microbiome by a probiotic-based sanitation during the COVID-19 emergency

MARIA D'ACCOLTI ^{1,2}, IRENE SOFFRITTI ^{1,2}, FRANCESCA BINI ^{1,2}, ELEONORA MAZZIGA ^{1,2}, ANTONELLA VOLTA ², MATTEO BISI ², SANTE MAZZACANE ², ELISABETTA CASELLI ^{1,2}

¹Department of Chemical, Pharmaceutical and Agricultural Sciences, and LTTA, University of Ferrara, Ferrara, Italy; ²CIAS Research Center, University of Ferrara, Ferrara, Italy;

Introduction: The COVID-19 pandemics has highlighted how much the public transportation environment, such as subways, may be important for transmission of potential pathogens. Consistent with this, based on massive use of chemical disinfection has been mandatorily introduced during the SARS-CoV-2 emergency, and is still in place. However, chemical disinfectants have a temporary action and could worsen both pollution and antimicrobial resistance (AMR) concerns. Alternatively, a probiotic-based sanitation (PBS) was recently shown to provide effective and long-term pathogen control (including SARS-CoV-2) without worsening AMR spread. Thus, our study was aimed to assess the PBS effect on the subway microbiome, compared to chemical disinfectants, during the COVID-19 pandemics.

Materials and Methods: Two underground trains were enrolled in the study: one train (control) continued to receive conventional chemical sanitation, whereas the other train received PBS in substitution of chemical disinfection. The trains' microbiome was characterized by both culture-based (CFU count) and molecular methods, including 16S rRNA NGS for bacteriome characterization, qPCR microarray for resistome characterization, and digital droplet PCR for SARS-CoV-2 monitoring.

Results: The results showed a stable >80% reduction of bacterial/fungal pathogens ($P < 0.001$), of AMR ($P < 0.01$) and of SARS-CoV-2 presence ($P < 0.01$) in the PBS-treated train compared with the chemically disinfected control train. NGS bacteriome profiling evidenced diverse clusters in the air vs. surface microbiomes, meanwhile demonstrating the specific action of PBS against pathogens rather than the entire train bacteriome.

Discussion and Conclusions: The data presented provide the first direct assessment of the impact of different sanitation procedures on the subway microbiome, allowing a better understanding of its composition and dynamics and showing that a biological sanitation approach may be highly effective in counteracting pathogens and AMR spread in our increasingly urbanized and interconnected environment.

79 - Characterization of the pathogenic potential of the beach sand microbiome and assessment of quicklime as a remediation tool.

FRANCESCA BINI ⁽¹⁾ - IRENE SOFFRITTI ⁽¹⁾ - MARIA D'ACCOLTI ⁽¹⁾ - ELEONORA MAZZIGA ⁽¹⁾ - ANTONELLA VOLTA ⁽²⁾ - MATTEO BISI ⁽²⁾ - SILVIA ROSSI ⁽³⁾ - FRANCESCO VIROLI ⁽⁴⁾ - MARCELLO BALZANI ⁽⁴⁾ - MARCO PETITTA ⁽⁵⁾ - SANTE MAZZACANE ⁽²⁾ - ELISABETTA CASELLI ⁽¹⁾

University of Ferrara, Section of Microbiology, Department of Chemical, Pharmaceutical and Agricultural Sciences and LTTA; CIAS Research Center, Ferrara, Italia ⁽¹⁾ - University of Ferrara, CIAS Research Center, Ferrara, Italia ⁽²⁾ - Building and Construction Cluster of the Emilia Romagna Region, Building and Construction of the Emilia Romagna Region, Bologna, Italia ⁽³⁾ - University of Ferrara, TekneHub department of Architecture, Ferrara, Italia ⁽⁴⁾ - University 'La Sapienza', Department of Earth Sciences, Roma, Italia ⁽⁵⁾

Topic: Controllo microbiologico dell'ambiente

Characterization of the pathogenic potential of the beach sand microbiome and assessment of quicklime as a remediation tool

FRANCESCA BINI ^{1,2}, IRENE SOFFRITTI ^{1,2}, MARIA D'ACCOLTI ^{1,2}, ELEONORA MAZZIGA ^{1,2}, ANTONELLA VOLTA ², MATTEO BISI ², SILVIA ROSSI ³, FRANCESCO VIROLI ⁴, MARCELLO BALZANI ⁴, MARCO PETITTA ⁵, SANTE MAZZACANE ² AND ELISABETTA CASELLI ^{1,2}

¹Section of Microbiology, Department of Chemical, Pharmaceutical and Agricultural Sciences, and LTTA, University of Ferrara, Ferrara, Italy; ²Cias Research Center, University of Ferrara, Ferrara, Italy; ³Building and Construction Cluster of the Emilia Romagna Region, Bologna, Italy; ⁴TekneHub, Department of Architecture, University of Ferrara, Ferrara, Italy; ⁵Department of Earth Sciences, University "La Sapienza", Rome, Italy

Introduction: Beach sand may act as a reservoir for potential human pathogens, posing a public health risk, especially in highly populated and industrialized areas. Despite this, the microbiological monitoring is usually performed by assessing only water quality, whereas sand microbiome is rarely investigated to determine beach quality. Being the beaches of the northern Adriatic Sea in Italy in close contact with water estuaries of two large rivers (Po and Reno rivers), likely collecting pollutants from many animal farms and urban cities along their ways, the aims of this study were to assess the level of microbial contamination in the sand of a beach located between the two river estuaries. In parallel, in the search for effective and safe decontamination methods, we tested the possible use of quicklime as a possible rapid sand decontamination method.

Materials and Methods: Sand samples collected from a beach of the North Adriatic Sea (Cesenatico, Forlì-Cesena, Italy) were analyzed by both conventional culture-based microbiological methods (CFU counts) and molecular methods (Whole Genome Sequencing (WGS) and quantitative qPCR

microarrays), in order to obtain a comprehensive picture of the microbial community present in sampled sand. Antimicrobial activity of quicklime (calcium oxide, CaO) was evaluated in vitro and then on controlled field, analyzing the impact of CaO treatment on both potential human pathogens and environmental microbes.

Results: The characterization of the sand microbiome, showed the presence of $>10^3$ /g of Enterobacteriaceae, *Pseudomonas aeruginosa*, Enterococci, and Staphylococci, with significant prevalence of human pathogens including drug-resistant strains. The potential of quicklime as a restoring method, was confirmed both in vitro and on-field, showing that the addition of 1-3% quicklime (w/w) to sand provided an up to -99% of bacteria, fungi, and viruses, in a dose- and time-dependent manner, till 45 days post-treatment.

Discussion and Conclusions: Data suggest that accurate monitoring of sand microbiome may be essential, besides water, to assess beach quality and safety. Moreover, first evidences of quicklime potential for sand decontamination are provided, suggesting its usage as a possible way to restore the microbiological quality of sand in highly contaminated areas.

82 - Molecular surveillance of the pediatric hospital microbiome: strategies for infection prevention and control

CAROLINA CASON⁽¹⁾ - GIUSEPPINA CAMPISCIANO⁽¹⁾ - NUNZIA ZANOTTA⁽¹⁾ - LUANA ALDEGHERI⁽¹⁾ - SABRINA SCOLZ⁽²⁾ - ELISABETTA CASELLI⁽³⁾ - MANOLA COMAR⁽¹⁾

Institute for Maternal and Child Health – IRCCS "Burlo Garofolo", Department of Advanced Translational Microbiology, Trieste, Italia⁽¹⁾ - Institute for Maternal and Child Health – IRCCS "Burlo Garofolo", Sanitary Direction, Trieste, Italia⁽²⁾ - University of Ferrara, Section of Microbiology, Department of Chemical, Pharmaceutical and Agricultural Sciences, and LTTA, Ferrara, Italia⁽³⁾

Molecular surveillance of the pediatric hospital microbiome: strategies for infection prevention and control

CAROLINA CASON^{1,2}, GIUSEPPINA CAMPISCIANO^{1,2}, NUNZIA ZANOTTA¹, LUANA ALDEGHERI^{1,2}, SABRINA SCOLZ³, ELISABETTA CASELLI^{4,5}, MANOLA COMAR^{1,2}

¹Department of Advanced Translational Microbiology, Institute for Maternal and Child Health – IRCCS "Burlo Garofolo", Trieste, Italy; ²Department of Medical, Surgical, and Health Sciences, University of Trieste, Trieste, Italy; ³Sanitary Direction, Institute for Maternal and Child Health – IRCCS "Burlo Garofolo", Trieste, Italy; ⁴Section of Microbiology, Department of Chemical, Pharmaceutical and Agricultural Sciences, and LTTA, University of Ferrara, Ferrara, Italy; ⁵CIAS Research Centre, University of Ferrara, Ferrara, Italy;

Introduction: Antibiotic resistance is a complex phenomenon influenced by multiple factors, resulting in the emergence of Healthcare-Associated Infections (HAIs) in hospitalized patients. HAIs are characterized by the increasing prevalence of multi-drug resistant strains. The hospital environment acts as a reservoir for drug-resistant pathogens, particularly on high-contact surfaces, increasing the risk of cross-contamination. This study introduces the application of molecular methods in monitoring the microbiome of pediatric hospital settings and emphasizes the integration of these insights into targeted interventions. Materials and Methods: From December 2019 to February 2023, 325 environmental swabs were collected from 12 wards at the Institute for Maternal and Child Health – IRCCS "Burlo Garofolo", Trieste, Italy, following a new protocol designed to monitor high-risk wards, defining the methodology, the sampling points and the timing after disinfection. Using a molecular multiplex platform, *Staphylococcus* spp., *S. aureus*, *Streptococcus* spp., *S. pneumoniae*, *S. agalactiae*, *S. pyogenes*, *L. monocytogenes*, *Enterococcus* spp., *P. aeruginosa*, *A. baumannii*, *N. meningitidis*, *S. maltophilia*, *E. coli*, *K. pneumoniae*, *S. marcescens*, *Enterobacteriaceae* species, *Proteus/Morganella* spp., *Candida* spp., *C. albicans*, and resistance genes including *mecA*, *vanA*, *vanB*, *blaSHV*, *blaCTX-M*, and fifteen carbapenem resistance genes were investigated. Results: The monitoring protocol demonstrated its effectiveness by providing rapid and comprehensive results compared to Next-Generation Sequencing (NGS) methods. Regular collection of environmental swabs allowed for periodic assessment of the resident microbiome. The most frequently detected microorganisms included *Staphylococci*, *Streptococcus* spp., *P. aeruginosa*, and *Candida* spp. Additionally, carbapenemase resistance genes (*GES*, *VIM*, *NDM*) and the methicillin resistance gene (*mecA*) were frequently identified, particularly in the

surgical and pediatric clinical departments. Door handles and sinks were found to be the most contaminated surfaces. Samples obtained after sanitation interventions exhibited reduced microorganisms and resistance genes, confirming the effectiveness of the hospital's sanitation protocols and handwashing practices. Discussion and Conclusion: This molecular surveillance study has provided valuable insights into the pediatric hospital microbiome and the presence of resistance genes, even during outbreaks. These findings have significant implications for informed decision-making and the implementation of targeted interventions to prevent and control infections, mitigating the risk of healthcare-associated infections and ensuring a safer healthcare environment for pediatric patients.

90 - Potential Use of a Combined Bacteriophage-Probiotic Sanitation System to Control Microbial Contamination and AMR in Healthcare Settings: A Pre-Post Intervention Study

ELEONORA MAZZIGA ⁽¹⁾ - MARIA D'ACCOLTI ⁽¹⁾ - IRENE SOFFRITTI ⁽¹⁾ - FRANCESCA BINI ⁽¹⁾ - LUCA ARNOLDO ⁽²⁾ - ANTONELLA VOLTA ⁽³⁾ - MATTEO BISI ⁽³⁾ - PAOLA ANTONIOLI ⁽⁴⁾ - PATRIZIA LAURENTI ⁽⁵⁾ - WALTER RICCIARDI ⁽⁵⁾ - SARA VINCENTI ⁽⁶⁾ - SANTE MAZZACANE ⁽³⁾ - ELISABETTA CASELLI ⁽¹⁾

University of Ferrara, Department of Chemical, Pharmaceutical and Agricultural Sciences and LTTA; CIAS Research Center, Ferrara, Italia ⁽¹⁾ - University of Udine, Department of Medicine, Udine, Italia ⁽²⁾ - University of Ferrara, CIAS Research Center, Ferrara, Italia ⁽³⁾ - S. Anna University Hospital, Department of Infection Prevention Control and Risk Management, Ferrara, Italia ⁽⁴⁾ - Department of Health Sciences and Public Health, Section of Hygiene, Catholic University of the Sacred Heart, Department of Woman and Child Health and Public Health, Fondazione Policlinico Universitario A. Gemelli IRCCS, Roma, Italia ⁽⁵⁾ - Fondazione Policlinico Universitario A. Gemelli IRCCS, Department of Woman and Child Health and Public Health, Roma, Italia ⁽⁶⁾

Topic: Controllo microbiologico dell'ambiente

Potential Use of a Combined Bacteriophage-Probiotic Sanitation System to Control Microbial Contamination and AMR in Healthcare Settings: A Pre-Post Intervention Study

ELEONORA MAZZIGA ^{1,2}, MARIA D'ACCOLTI ^{1,2}, IRENE SOFFRITTI ^{1,2}, FRANCESCA BINI ^{1,2}, LUCA ARNOLDO ³, ANTONELLA VOLTA ², MATTEO BISI ², PAOLA ANTONIOLI ⁴, PATRIZIA LAURENTI ^{5,6}, WALTER RICCIARDI ^{5,6}, SARA VINCENTI ⁶, SANTE MAZZACANE ², ELISABETTA CASELLI ^{1,2}

¹Department of Chemical, Pharmaceutical and Agricultural Sciences, and LTTA, University of Ferrara, Ferrara, Italy; ²CIAS Research Center, University of Ferrara, Ferrara, Italy; ³Department of Medicine, University of Udine, Udine, Italy; ⁴Department of Infection Prevention Control and Risk Management, S. Anna University Hospital, Ferrara, Italy; ⁵Department of Health Sciences and Public Health, Section of Hygiene, Catholic University of the Sacred Heart, Rome, Italy;

⁶Department of Woman and Child Health and Public Health, Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy

Introduction: Microbial contamination in the hospital environment is a major concern for public health, since it significantly contributes to the onset of healthcare-associated infections (HAIs), which are further complicated by the alarming level of antimicrobial resistance (AMR) of HAI-associated pathogens. Chemical disinfection to control bioburden has a temporary effect and can favor the selection of resistant pathogens, as observed during the COVID-19 pandemic. Instead, probiotic-based sanitation (probiotic cleaning hygiene system, PCHS) was reported to stably abate pathogens, AMR, and HAIs. PCHS action is not rapid nor specific, being based on competitive exclusion, but the addition of lytic bacteriophages that quickly and specifically kill selected bacteria was shown to improve PCHS effectiveness. This study aimed to investigate the effect of such combined probiotic-phage sanitation (PCHS-ph) in two Italian hospitals, targeting staphylococcal contamination.

Material and Methods: The pre-post interventional study included three phases: first 4-week period during which hospitals maintained the conventional chemical-based sanitizing procedures (T0), a 4-week period during which PCHS replaced the chemical sanitation (T1), a final 6-week period during which phages were added to PCHS sanitation in the bathrooms of 12/24 randomly selected rooms, while the remaining rooms received only PCHS without phages (T2, PCHS-ph period). Microbial bioburden was monitored by conventional culture-based CFU count, Sb-1 bacteriophage load was evaluated by specific quantitative qPCR and microarrays were used for the assessment of the resistome of the contaminating population.

Results: The results showed that PCHS-ph could provide a significantly higher removal of staphylococci, including resistant strains, compared with disinfectants (-76% , $p < 0.05$) and PCHS alone (-50% , $p < 0.05$). Extraordinary sporadic chlorine disinfection appeared compatible with PCHS-phi, while frequent routine chlorine usage inactivated the probiotic/phage components, preventing PCHS-phi action.

Discussion and Conclusions: Since the control of microbial contamination continues to be a major issue in the management of the pandemic emergency, collected data highlight the potential of a biological sanitation for better control of the infectious risk in healthcare facilities, without worsening pollution and AMR concerns.

131 - EVALUATION OF LEGIONELLA PNEUMOPHILA IN WATER SAMPLES AND COMPARISON WITH LEGIOLERT/REAL-TIME PCR

IGNAZIO ARRIGO ⁽¹⁾ - TERESA FASCIANA ⁽²⁾ - ELENA GALIA ⁽¹⁾ - MARIA RITA TRICOLI ⁽³⁾
- MARIO PALERMO ⁽⁴⁾ - LUCIA CANNOVA ⁽²⁾ - GIUSEPPE GALLINA ⁽⁵⁾ - ANNA
GIAMMANCO ⁽³⁾

LABORATORIO DI RIFERIMENTO REGIONALE PER LA LEGIONELLOSI, A.O.U.P
"P.GIACCONE", PALERMO, Italia ⁽¹⁾ - DIPARTIMENTO PROMISE, A.O.U.P "P.GIACCONE"
/UNIVERSITA' DI PALERMO, PALERMO, Italia ⁽²⁾ - DIPARTIMENTO PROMISE, A.O.U.P
"P.GIACCONE", PALERMO, Italia ⁽³⁾ - DASOE ASSESSORATO REGIONALE SALUTE,
UNIVERSITA' DI PALERMO, PALERMO, Italia ⁽⁴⁾ - DISCIPLINE CHIRURGICHE,
ONCOLOGICHE E STOMATOLOGICHE, A.O.U.P "P.GIACCONE" /UNIVERSITA' DI
PALERMO, PALERMO, Italia ⁽⁵⁾

Evaluation of Legionella pneumophila contamination in water samples and comparison with
Legiolert/real-time PCR

IGNAZIO ARRIGO¹, TERESA FASCIANA², ELENA GALIA¹, MARIA RITA TRICOLI²,
MARIO PALERMO³, LUCIA CANNOVA², GIUSEPPE GALLINA⁴, ANNA GIAMMANCO²

¹Regional Reference Laboratory for Clinical Environmental Surveillance and Control of
Legionellosis, Palermo, Italy;

²Department of Health Promotion, Mother and Child Care, Internal Medicine and Medical
Specialties, University of Palermo, Italy;

³Sicilian Health Department, Public Health and Environmental Risks Service, University of
Palermo, Italy;

⁴Department of Surgical Oncological and Stomatological Disciplines, University of Palermo, Italy.

Introduction: Monitoring of Legionella pneumophila (Lp) populations in environmental water is very important for public health. Culture on selective media is currently the reference method, performed in accordance with the standard method, ISO 11731, the results are obtained 1-2 weeks after the initiation of culture. Furthermore, one reason for which false negative results might often occur is the overgrowth of non-Legionella bacteria. By contrast, real-time PCR methods, can provide results within only 24-48 h after sampling and it can be an alternative tool for rapid and accurate detection of Lp in water samples. An additional promising method is the Legiolert culture test based on a most probable number (MPN), a liquid culture method based on bacterial enzyme detection.

Materials and methods: A total of 25 water samples were collected, from a hospital located in Palermo (Sicily), in 2-L sterile bottles. 1L of each sample was analyzed by culture method, 1L by real-time PCR qualitative test and only 100 mL of potable water using the Legiolert method. After filtration, bacteria collected on the membranes were resuspended in 10 ml of the original water sample and 0,1 mL was spread on BCYE agar. While as for molecular method, DNA was extracted using a commercially available kit according to the manufacturer's instructions, PCR was performed with Applied Biosystems QuantStudio 96-well (Thermo Fisher Scientific). Finally, a Legiolert blister reagent was added to the 100 mL of sample and poured into a Quanti-tray/Legiolert tray and incubated at 37°C in a moist chamber with 5% CO₂.

Results: Legionella spp. was found in 16/25 of the samples analyzed by culture method. In particular, among 16 positive samples was detected often mixed Lp (serogroup 1 and serogroup 2-

14) and/or *Legionella* spp. cultures. *Legionella* presence in positive samples ranged from 150 CFU/L to 30000 CFU/L. Real-time PCR revealed the presence of Lp in 22/25 water samples with cycle threshold (Ct) value ranged from 26,6 Ct to 40,2 Ct. While Legiolert test highlights 19/25 positive samples according to culture method.

Discussion and conclusions: The data generated in this study showed congruence between culture method ISO 11731/Legiolert, in fact water samples detected as positive only by Legiolert test had low MPN count. The observed difference in detection between real-time PCR and culture methods were due to the high sensitivity of the molecular method. This result supports the data found in the literature, indicating that culture method frequently underestimates the presence of Lp in water samples.

230 - Tracking SARS-CoV-2 RNA in urban wastewater through diverse biomarkers

ERIKA BRUNO⁽¹⁾ - MARIANNA MARTINELLI⁽¹⁾ - CHIARA GIUBBI⁽¹⁾ - FEDERICA PERDONI⁽¹⁾ - ARIANNA AZZELLINO⁽²⁾ - ANDREA TUROLLA⁽²⁾ - DANILO CEREDA⁽³⁾ - EMANUELA AMMONI⁽³⁾ - ELISABETTA SUFFREDINI⁽⁴⁾ - GIUSEPPINA LA ROSA⁽⁵⁾ - ANDREA FRANZETTI⁽⁶⁾ - ROSARIO MUSUMECI⁽¹⁾ - CLEMENTINA ELVEZIA COCUZZA⁽¹⁾ - FRANCESCA MALPEI⁽²⁾

University of Milano - Bicocca, Department of Medicine and Surgery, Monza, Italia⁽¹⁾ - Politecnico di Milano, Department of Civil and Environmental Engineering, Milano, Italia⁽²⁾ - Regione Lombardia, Sicurezza alimentare, nutrizione e qualità delle acque destinate al consumo umano, Milano, Italia⁽³⁾ - Istituto Superiore di Sanità, Department of Food Safety, Nutrition and Veterinary Public Health, Roma, Italia⁽⁴⁾ - Istituto Superiore di Sanità, Department of Environment and Health, Roma, Italia⁽⁵⁾ - University of Milano - Bicocca, Department of Earth and Environmental Sciences, Milano, Italia⁽⁶⁾

Title: Tracking SARS-CoV-2 RNA in urban wastewater through diverse biomarkers

ERIKA BRUNO^{1,2}, MARIANNA MARTINELLI¹, CHIARA GIUBBI¹, FEDERICA PERDONI¹, ARIANNA AZZELLINO³, ANDREA TUROLLA³, DANILO CEREDA⁴, EMANUELA AMMONI⁴, ELISABETTA SUFFREDINI⁵, GIUSEPPINA LA ROSA⁶, ANDREA FRANZETTI², ROSARIO MUSUMECI¹, CLEMENTINA E. COCUZZA¹, FRANCESCA MALPEI³.

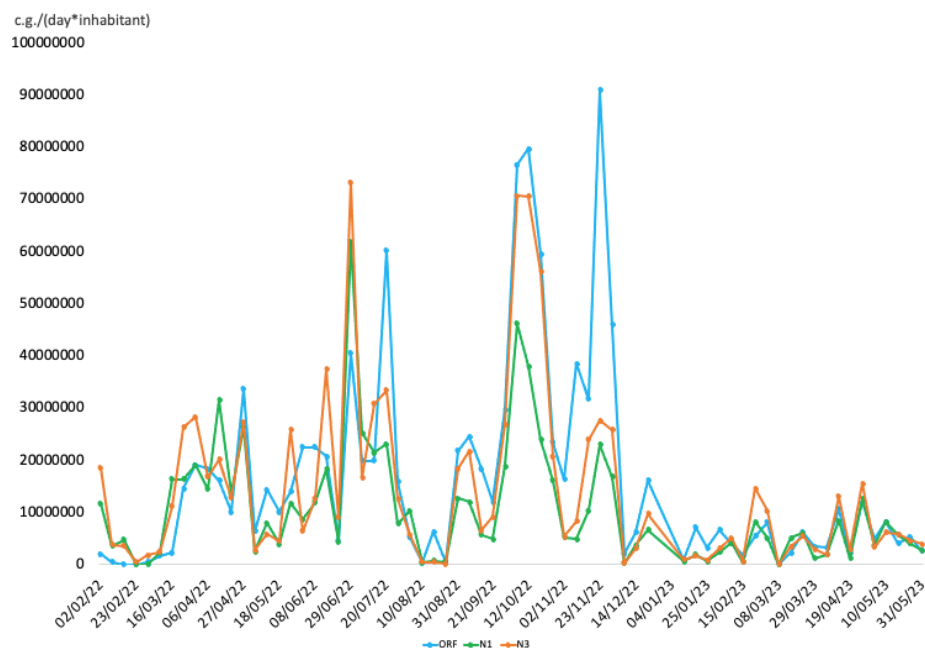
¹Department of Medicine and Surgery, University of Milano-Bicocca, Monza, Italy; ²Department of Earth and Environmental Sciences, University of Milano-Bicocca, Milano, Italy; ³Department of Civil and Environmental Engineering, Politecnico di Milano, Milano, Italy; ⁴Sicurezza alimentare, nutrizione e qualità delle acque destinate al consumo umano, UO Prevenzione, Regione Lombardia, Piazza Città di Lombardia, Milano; ⁵Department of Food Safety, Nutrition and Veterinary Public Health, Istituto Superiore di Sanità, Rome, Italy; ⁶Department of Environment and Health, Istituto Superiore di Sanità, Rome, Italy

Introduction: Wastewater-based epidemiology (WBE) has emerged as a promising approach for the detection and monitoring of viral pathogens, such as the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), the causative agent of the Corona Virus Disease-19 (COVID-19). Aim of this study was to detect the presence of SARS-CoV-2 RNA in Sondrio Wastewater Treatment Plant (WWTP), as a part of a national on-going surveillance study, coordinated by the Italian National Institute of Health (Istituto Superiore di Sanità – ISS), entitled “SARI Project - Sorveglianza Ambientale di SARS-CoV-2 attraverso i Reflui urbani in Italìa”.

Materials and Methods: Wastewater samples (24h composite, 500 mL) were collected weekly in the WWTP inlet, from February 2022 to May 2023. According to the SARI project protocol, the samples were concentrated by centrifugation for 2h at 12000×g and 4°C using PEG 8000. Nucleic acid extraction was performed using the NucliSens EasyMag system (bioMérieux). SARS-CoV-2 ORF1b (nsp14) gene, along with two other biomarkers, N1 and N3 genes, was detected by one-step real time RT-PCR for viral quantification.

Results: The results provided the SARS-CoV-2 viral load, expressed as viral genome copies/equivalent inhabitant per day, and normalized according to flow rate and number of inhabitants.

Discussion and Conclusions: The three lines, representing the three biomarkers (ORF1b in blue, N1 in green and N3 in orange), displayed similar trends in the quantitative determination of SARS-CoV-2 genomic copies per day per inhabitant. The detection of SARS-CoV-2 RNA in wastewater samples showed positivity rates 95.9% for ORF1b (66 positive samples out of 68), 95,6% for N1 gene (65/68) and 98.5% for N3 gene (67/68). The highest SARS-CoV-2 concentrations were detected in two different periods during the surveillance, more specifically in June 2022 for N1 and N3 genes ($6,2E+07$ and $7,3E+07$ c.g./day*inhabitant, respectively) and in November 2022 for ORF1b ($9,1E+07$ c.g./day*inhabitant). In conclusion, the monitoring of SARS-CoV-2 in wastewater, using multiple biomarkers, represents an important surveillance tool for tracking COVID-19 pandemic waves.



318 - Investigation of SARS-CoV-2 RNA in wastewater and bivalve mollusks from the Campania Region

ANNALISA LOMBARDI ⁽¹⁾ - ANTONIA VOLI ⁽²⁾ - ANDREA MANCUSI ⁽³⁾ - SANTA GIRARDI ⁽³⁾ - YOLANDE THÉRÈSE ROSE PROROGA ⁽³⁾ - BIANCAMARIA PIERRI ⁽⁴⁾ - RENATO OLIVARES ⁽⁵⁾ - LUIGI COSSENTINO ⁽⁵⁾ - ELISABETTA SUFFREDINI ⁽⁶⁾ - GIUSEPPINA LA ROSA ⁽⁷⁾ - GIOVANNA FUSCO ⁽⁸⁾ - ANTONIO PIZZOLANTE ⁽⁴⁾ - AMALIA PORTA ⁽²⁾ - MARIA TRIASSI ⁽¹⁾ - FRANCESCA PENNINO ⁽¹⁾ - ALESSANDRA TOSCO ⁽²⁾

Università degli Studi di Napoli "Federico II", Dipartimento di Sanità Pubblica, Napoli, Italia ⁽¹⁾ - Università di Salerno, Dipartimento di Farmacia, Fisciano, Italia ⁽²⁾ - Istituto Zooprofilattico Sperimentale del Mezzogiorno, Dipartimento Coordinamento di Sicurezza Alimentare, Portici, Italia ⁽³⁾ - Istituto Zooprofilattico del Mezzogiorno, Centro di Referenza Nazionale per l'Analisi e Studio di Correlazione tra Ambiente, Animale e Uomo, Portici, Italia ⁽⁴⁾ - Agenzia Regionale Protezione Ambientale Campania, ARPAC, Napoli, Italia ⁽⁵⁾ - Istituto Superiore di Sanità, Dipartimento di Sicurezza Alimentare, Nutrizione e Sanità Pubblica Veterinaria, Roma, Italia ⁽⁶⁾ - Istituto Superiore di Sanità, Dipartimento Ambiente e Salute, Roma, Italia ⁽⁷⁾ - Istituto Zooprofilattico Sperimentale del Mezzogiorno, Dipartimento di Sanità Animale, Portici, Italia ⁽⁸⁾

Investigation of SARS-CoV-2 RNA in wastewater and bivalve mollusks from the Campania Region
ANNALISA LOMBARDI ^{1, †}, ANTONIA VOLI ^{2, †}, ANDREA MANCUSI ³, SANTA GIRARDI ³, YOLANDE T. R. PROROGA ³, BIANCAMARIA PIERRI ⁴, RENATO OLIVARES ⁵, LUIGI COSSENTINO ⁵, ELISABETTA SUFFREDINI ⁶, GIUSEPPINA LA ROSA ⁷, GIOVANNA FUSCO ⁸, ANTONIO PIZZOLANTE ⁴, AMALIA PORTA ², MARIA TRIASSI ¹, FRANCESCA PENNINO ^{1, #}, ALESSANDRA TOSCO ^{2, #}

† These authors contributed equally to this work.

These authors contributed equally to this work.

¹ Department of Public Health, University "Federico II", Via Sergio Pansini 5, 80131 Naples, Italy;

² Department of Pharmacy, University of Salerno, Via Giovanni Paolo II 132, 84084 Fisciano, Italy;

³ Department of Food Security Coordination, Istituto Zooprofilattico Sperimentale del Mezzogiorno, Via Salute 2, 80055 Portici, Italy; ⁴ Centro di Referenza Nazionale per l'Analisi e Studio di Correlazione tra Ambiente, Animale e Uomo, Istituto Zooprofilattico Sperimentale del Mezzogiorno, Via Salute 2, 80055 Portici, Italy; ⁵ Campania Regional Environmental Protection Agency (ARPAC), Via Vicinale Santa Maria del Pianto, 80143 Naples, Italy; ⁶ Department of Food Safety, Nutrition and Veterinary Public Health, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome, Italy; ⁷ Department of Environment and Health, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome, Italy; ⁸ Department of Animal Health, Istituto Zooprofilattico Sperimentale del Mezzogiorno, Via Salute 2, 80055 Portici, Italy

Introduction

SARS-CoV-2 is a single-stranded RNA virus whose genome was found in the faeces of infected individuals and, consequently, in wastewater, via the sewage system. In addition, its presence has also been observed in bivalve mollusks, organisms that can filter large quantities of water and thus accumulate viruses within them. SARS-CoV-2 RNA can reach bivalve mollusks in different ways, including ineffective wastewater treatment. The aim of this work was to monitor the presence of

SARS-CoV-2 RNA in wastewater and bivalve mollusks from the Campania region and to assess whether there was a temporal correspondence between the results obtained in wastewater and mollusk samples to explore the possibility of using both types of samples as sentinels of the epidemiological situation.

Materials and Methods

From October 2021 to April 2022, 168 wastewater samples were collected from 6 wastewater treatment plants (WWTPs) and 57 samples of bivalve mollusks from 8 harvesting sites. Wastewater samples were collected as part of the national SARI (environmental surveillance wastewater Italy) project organized by the Italian ‘National Institute of Health’ (ISS). For sewage samples, the ORF1ab gene of SARS-CoV-2 was monitored by Real-Time RT-PCR according to the SARI protocol - revision 3. The E, RdRp and ORF1ab genes were monitored for mollusk samples by droplet digital RT-PCR (dd RT-PCR).

Results

In 92.3% of the wastewater samples, SARS-CoV-2 RNA was detected. 54.4% of the mollusk samples tested positive for at least one of the 3 monitored genes. The average daily viral load in the 6 WWTPs showed a first increase in December 2021 - January 2022, then a second increase in March - April 2022. The average monthly mollusk positivity rate showed high values from December 2021 to April 2022, with progressive bioaccumulation from December to March. On the other hand, the number of active cases in the Campania region showed an increase in December, a peak in January 2022, and a second increase in March-April 2022.

Discussion and Conclusions

A higher rate of positivity was observed in sewage than in mollusks. This suggests the ability of sewage treatment in WWTPs in reducing the presence of SARS-CoV-2 RNA. In addition, the presence of the virus in mollusks suggests that a viral parameter, as well as a bacterial parameter (as stipulated in current Italian legislation), should also be monitored in the wastewater leaving the WWTP. The temporal correspondence between viral RNA in wastewater and mollusks suggests the possibility of using both samples as sentinels of the epidemiological situation.

321 - Detection and characterization of carbapenemase producing *Enterobacterales* from wastewater samples

FABIO MORECCHIATO⁽¹⁾ - LORENZO PACI⁽¹⁾ - CLAUDIA NICCOLAI⁽²⁾ - MARCO COPPI⁽²⁾ - ALBERTO ANTONELLI⁽²⁾ - SIMONA POLLINI⁽²⁾ - MATTEO RAMAZZOTTI⁽³⁾ - TOMMASO LOTTI⁽⁴⁾ - CLAUDIO LUBELLO⁽⁴⁾ - GIAN MARIA ROSSOLINI⁽²⁾

Department of Experimental and Clinical Medicine, University of Florence, Firenze, Italia⁽¹⁾ - Department of Experimental and Clinical Medicine; Microbiology and Virology Unit, University of Florence; Careggi University Hospital, Florence, Italia⁽²⁾ - Department of Biochemical Sciences, University of Florence, Florence, Italia⁽³⁾ - Department of Civil and Environmental Engineering - (DICEA), University of Florence, Florence, Italia⁽⁴⁾

Detection and characterization of carbapenemase producing *Enterobacterales* from wastewater samples

Fabio Morecchiato¹, Lorenzo Paci¹, Claudia Niccolai^{1,2}, Marco Coppi^{1,2}, Alberto Antonelli^{1,2}, Simona Pollini^{1,2}, Matteo Ramazzotti³, Tommaso Lotti⁴, Claudio Lubello⁴, Gian Maria Rossolini^{1,2}

¹ Department of Experimental and Clinical Medicine, University of Florence, Florence, Italy

² Microbiology and Virology Unit, Careggi University Hospital, Florence, Italy

³ Department of Biochemical Sciences, University of Florence, Florence, Italy

⁴ Department of Civil and Environmental Engineering - (DICEA), University of Florence, Florence, Italy.

Introduction. The diffusion of multi-drug resistant (MDR) pathogens represents a significant threat to healthcare worldwide. Among MDR, carbapenemase-producing *Enterobacterales* (CPE) are among the most impactful and difficult to treat pathogens in both nosocomial- and community-based infections. The diffusion of MDR bacteria and mechanisms of antimicrobial resistance could be tracked using wastewaters, an approach named wastewater-based epidemiology (WBE), which gained new attention during the last years. The aim of this study was the detection and characterization of CRE from wastewater samples collected in different treatment plants in Tuscany.

Materials and Methods. Wastewater samples collected from six different wastewater treatment plants in Tuscany (Arezzo, Firenze, Grosseto, Pistoia, Prato and Siena) during the period November 2022 – February 2023 were considered. 50 mL of sample were centrifuged at 4000 RPM for 10 minutes, then 10 µL of pellet were plated on CHROMID™ CARBA SMART (bioMérieux, France) selective agar medium for carbapenem-resistant *Enterobacterales*. Bacterial identification was performed with MALDI Biotyper® (Bruker Corporation, USA) mass spectrometry. Carbapenemase genes (*bla_{NDM}*, *bla_{KPC}*, *bla_{OXA-48-like}*, *bla_{VIM}* and *bla_{IMI}*) were detected with multiplex real-time polymerase chain reaction. Antimicrobial susceptibility testing (AST) were performed by broth microdilution with MICRONAUT AST-SYSTEMS (MERLIN Diagnostika GmbH, Germany). Whole genome sequencing (WGS) analysis were performed with Illumina technology on the first isolates for each centre.

Results. A total of 109 wastewater samples were collected during the study period, for which 27 were positive for at least one of the bacterial pathogens under investigation. 27 different CPE strains

were identified and characterized, including: 10 *Escherichia coli* (5 NDM-producer, 3 OXA-48-like, 1 KPC and 1 VIM), 8 *Klebsiella pneumoniae* (6 KPC, 1 OXA-48-like, 1 KPC and VIM co-producer), 4 *Citrobacter freundii* (2 KPC and 2 VIM), 2 *Klebsiella oxytoca* (1 KPC and 1 KPC and VIM co-producer), 2 *Enterobacter cloacae* complex (VIM-producer), and 1 *Enterobacter mori* (IMI-producer). AST results were comparable with resistance mechanisms detected. WGS analysis revealed the presence of a high-risk clone ST147 of *K. pneumoniae* and a *E. mori* carrying a novel bla_{IMI-2-like} variant.

Discussion and Conclusions. This study showed the presence of CRE pathogens in wastewaters from different districts in Tuscany, highlighting their potential risk of diffusion in the environment. WBE applied for this purpose could represent a suitable option for monitoring and tracking their spread.

03 Fisiologia e metabolismo microbico

25 - Characterization of Mutants Involved in the Regulation of Cell Wall Synthesis and Remodeling in *Acinetobacter baumannii*

BERENICE FURLAN⁽¹⁾ - ALESSANDRA MARTORANA⁽²⁾ - ALESSANDRA POLISSI⁽²⁾ -
MICHAEL WHALEN⁽³⁾ - ORIETTA MASSIDDA⁽¹⁾

Department of Cellular, Computational and Integrative Biology, Università di Trento, Trento, Italia

⁽¹⁾ - Department of Pharmacological and Biomolecular Sciences, Università di Milano, Milano,

Italia ⁽²⁾ - Institute of Biophysics, National Research Council CNR, Trento, Italia ⁽³⁾

Characterization of Mutants Involved in the Regulation of Cell Wall Synthesis and Remodeling in *Acinetobacter baumannii*

Berenice Furlan¹, Alessandra Martorana², Alessandra Polissi², Michael B. Whalen³, Orietta Massidda¹

¹Department of Cellular, Computational and Integrative Biology (CIBIO), University of Trento, Trento, Italy; ²Department of Pharmacological and Biomolecular Sciences, University of Milano, Milano, Italy; ³Institute of Biophysics (IBF), National Research Council (CNR), Trento, Italy

1. Introduction: The emergence and spread of nosocomial pathogens, characterized by multi- or pan-resistance to antibiotics and by a tremendous ability of intrahospital dissemination, is of extreme critical relevance. Among the "ESKAPE" pathogens, *Acinetobacter baumannii* has been listed as a priority for which R&D strategies are urgently needed to develop novel therapies. The cell envelope is an essential structure that maintains cellular integrity and provides protection against external stresses. Thus, targeting pathways involved in the regulation and remodeling of peptidoglycan (PG), the major component of the bacterial cell wall, and its intimate, integrated function with the outer membrane (OM) can lead to the identification of new targets with lower evolutionary pressure and thereby less likely to promote development of resistance. In this work, we phenotypically characterize *A. baumannii* knock-out mutants defective in selected non-essential proteins involved in PG synthesis regulation and remodeling under physiological and stress conditions to explore proteins targets that can affect fitness, virulence and antibiotic resistance. 2. Materials and Methods: Transposon inactivated isogenic mutants of the *A. baumannii* clinical strain AB5075 were phenotypically characterized for growth, viability and morphology. In addition, their resistance profile to several classes of antibiotics was evaluated using the Epsilometric test (E-test) method with a panel of antibiotics mainly targeting the cell envelope, and further confirmed by a broth microdilution method to determine the Minimal Inhibitory Concentration (MIC) and the Minimal Bactericidal Concentration (MBC). SDS/EDTA and lysozyme sensitivity assays were performed to screen for strain-specific phenotypes upon stress treatments and checkerboard assays were used to look for synergistic effects in combinations. 3. Results: *A. baumannii* mutants inactivated for penicillin-binding proteins (PBPs), L,D-transpeptidases and putative regulators of PG synthesis displayed clear morphological defects, despite that none of the mutants showed a significant difference in growth and viability in comparison to the WT strain. When treated with SDS/EDTA, *A. baumannii* exhibited an overall high-level sensitivity to SDS with mutant-specific

differences already at low concentrations. Moreover, the analyses of the MICs confirmed a general profile of resistance to many of the antibiotics tested with the exception of polymyxins. Possible heteroresistance was also observed when the strains were tested with carbapenems. 4. Discussion and conclusion: Overall, these findings highlight the importance of understanding how enzymes involved in PG synthesis regulation and remodeling are orchestrated to allow proper growth and division under stress conditions. Besides its interest as a clinically relevant and antibiotic resistant pathogen, *A. baumannii* provides a unique Gram-negative model to study the relationships between enzymes and regulators involved in cell wall synthesis and remodeling.

195 - Culture-independent methods contributed to elucidate mechanisms for O₂ tolerance in the bacterial genus *Sulfurimonas*

MASSIMILIANO MOLARI⁽¹⁾ - GUNTER WEGENER⁽¹⁾ - ANTJE BOETIUS⁽¹⁾

HGF-MPG Joint Research Group for Deep-Sea Ecology and Technology,, Max Planck Institute for Marine Microbiology, Bremen, Germania⁽¹⁾

Topic: Physiology and microbial metabolism

Culture-independent methods contributed to elucidate mechanisms for O₂ tolerance in the bacterial genus *Sulfurimonas*

Massimiliano Molari^{1,2}, Gunter Wegener^{1,3}, and Antje Boetius^{1,2,3}

¹ HGF-MPG Joint Research Group for Deep-Sea Ecology and Technology, Max Planck Institute for Marine Microbiology, Bremen, Germany.

² HGF-MPG Joint Research Group for Deep-Sea Ecology and Technology, Alfred Wegener Institute for Polar and Marine Research, Bremerhaven, Germany

³ MARUM Center for Marine Environmental Sciences, University of Bremen, Bremen, Germany

Introduction

The genus *Sulfurimonas* belongs to the phylum Campylobacterota (former class Epsilonproteobacteria). The described members of the genus *Sulfurimonas* occupy habitats defined by moderate temperatures, elevated hydrogen sulfide concentrations (μM to mM) and low and high variable oxygen concentrations ($<20\%$; with optimal growth at 1-8%). Yet, abundant *Sulfurimonas* 16S rRNA gene sequences have also been reported in non-buoyant stage of hydrothermal plumes, which consist mostly of cold ($<4^\circ\text{C}$) and oxygen-saturated ($>20\%$) seawater with highly dilute admixtures of hydrothermal fluid ($<0.01\%$). In this study, we applied culture-independent methods to investigate the distribution and physiology of *Sulfurimonas* in the hydrothermal plumes of Gakkel Ridge (Central Arctic Ocean).

Materials and Methods

A conductivity-temperature-depth (CTD) rosette equipped with 24 12-L Niskin bottles was used to collect water samples for DNA extraction and for cell counts. Microbial biomass for metatranscriptomics was collected by filtering in situ large volume of seawater (ca. 200 L) using in situ pumps (McLane) equipped with polycarbonate filters ($0.2\ \mu\text{m}$ pore size). DNA and RNA were extracted in MPI laboratory using commercial kits, and RNA extracts were treated to remove co-extracted DNA. The hypervariable V3–V4 region of the bacterial 16S rRNA gene was amplified and sequenced on a MiSeq platform (Illumina; 2×300 paired reads). For metagenomes and metatranscriptomics the libraries were sequenced on a MiSeq platform (Illumina; 2×300 paired reads) and a HiSeq1500 platform (Illumina; 1×150 paired reads), respectively. Customized bioinformatic pipelines and several software were used to obtain metagenome assembled genomes and to perform oligotyping, phylogenetic, and comparative genomic analyses.

Results

We found that a previously uncharacterized *Sulfurimonas* taxon is abundant and active in oxygen-saturate hydrothermal plumes. According to the standards for microbial uncultivated species, we

propose to name this uncultivated (U) taxon ‘^USulfurimonas pluma’. ^US. pluma uses hydrogen as primary energy source to fix carbon via reverse tricarboxylic acid (rTCA), and oxygen as sole electron acceptor. Compared to cultivated Sulfurimonas strains, ^US. pluma do not have nitrate, nitrite, nitric oxide and sulfide:quinone reductase. Furthermore ^US. pluma encode for more oxygen tolerant enzymes involved in rTCA, and transcribed both cbb3-type and caa3-type cytochrome c oxidases genes. The presence and highly expression of hemerythrin-like genes, and superoxide reductase and peroxidases genes are further solutions to reduce intracellular O₂ concentrations.

Discussion and Conclusions

In this study we identified an uncharacterized aerobic Sulfurimonas species inhabiting non-buoyant hydrothermal plumes and fluids, and subsurface aquifers. The genome of ^US. pluma shows substantial genome reduction (17–40%), including the loss of genus-specific functional genes (that is, for denitrification) and acquisition of genes allowing growth in pelagic oxygen-saturated environments.

329 - *Pseudomonas aeruginosa* exploits zinc-binding metallophores to cope with host nutritional immunity strategies.

EMMA MICHETTI ⁽¹⁾ - FRANCESCA PACELLO ⁽¹⁾ - VALERIO SECLI ⁽²⁾ - ANDREA BATTISTONI ⁽¹⁾ - SERENA AMMENDOLA ⁽¹⁾

Università di Roma Tor Vergata, Dipartimento di Biologia, Roma, Italia ⁽¹⁾ - Ospedale Bambino Gesù, Dipartimento di Oncoematologia, Terapia Genica e Cellulare, Roma, Italia ⁽²⁾

Pseudomonas aeruginosa exploits zinc-binding metallophores to cope with host nutritional immunity strategies.

Emma Michetti (1), Francesca Pacello (1), Valerio Secli (2), Andrea Battistoni (1), Serena Ammendola (1)

(1) Department of Biology, Tor Vergata University of Rome, Rome, Italy; (2) Department of Onco-Hematology, Gene and Cell Therapy, Bambino Gesù Children's Hospital-IRCCS, Rome, Italy

1. Introduction

Innate immunity includes numerous strategies to limit bacterial proliferation based on the reduction of transition metals availability at the sites of infection. Among these, particularly effective are those based on the action of calprotectin (CP), the most abundant neutrophilic protein, which efficiently sequesters zinc and other divalent metals. Among human pathogens, *Pseudomonas aeruginosa* displays a particularly remarkable ability to withstand CP-induced metal starvation and to obtain zinc through specialized metal uptake systems that significantly contribute to its ability to infect the host and colonize the lung of CF patients. Zinc import is primarily ensured by the Zur-regulated operons *ZnuABC* and *ZrmABCD*, the latter being responsible for the synthesis, secretion and uptake of the metallophore pseudopaline. Here we show that CP starves *P. aeruginosa* of zinc and that a structural analogue of pseudopaline, the plant metallophore nicotianamine (NA), competes with CP for zinc binding.

2. Materials and Methods

P. aeruginosa strain PA14 and mutant strains *znuAzrmA* and *znuAzrmB* (unable to import zinc through the *ZnuABC* and pseudopaline) were used to analyze the response to CP. Transcription analysis was performed by RT-PCR on RNA samples from PA14 grown in a medium containing CP. Plasmid *pzrmApTZ110* was used as a reporter of intracellular zinc status by measuring the activity of beta-galactosidase expressed from the *zrmA* promoter. The activity of the purified recombinant metallo- β -lactamase VIM-1 was analyzed using the nitrocefin test.

3. Results

We observed that CP induces both zinc and iron starvation in PA14, as shown by the overexpression of Zur-controlled genes and, to a lesser extent, some iron-uptake-related genes. We also observed that the ability of wild-type PA14 to resist CP is mediated by its zinc-uptake systems, among which pseudopaline contributes significantly, as shown by the growth comparison between wild-type and mutant strains. Supplementation of the medium with NA decreases the CP-mediated induction of *zrmA*, probably through the ability of this chelating molecule to interfere with the stability of zinc association to CP. In support of this view, we have found that NA interferes in vitro with the enzymatic activity of the zinc-dependent β -lactamase VIM-1.

4. Discussion and Conclusions

This study demonstrates that CP induces zinc deficiency in *P. aeruginosa* and that NA, a molecule structurally similar to pseudopaline, can alleviate the metal requirement by promoting zinc uptake even in the presence of CP. Our data indicate that metallophores released by bacteria can extract protein-bound zinc, thereby contributing to *P. aeruginosa* resistance to host innate immunity mechanisms.

04 Microbiota

20 - Repression of a potent genotoxin via amino acid supplementation

SOFIA SANDALLI⁽¹⁾ - JENNIFER HALLAM⁽¹⁾ - GILLIAN DOUCE⁽¹⁾ - ANDREW ROE⁽¹⁾

School of Infection and Immunity, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, Regno Unito⁽¹⁾

Repression of a potent bacterial genotoxin via amino acid supplementation

SOFIA SANDALLI¹, JENNIFER C. HALLAM¹, GILLIAN R. DOUCE¹, ANDREW J. ROE¹

¹School of Infection and Immunity, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, United Kingdom

Many *Escherichia coli* (*E. coli*) strains of the B2 phylogroup produce colibactin, a potent genotoxin biosynthesised by the *pks* island. In eukaryotic cells, colibactin induces DNA damage, chromosomal instability, cell-cycle arrest, and is strongly implicated in the development of colorectal cancer.

The ability of proteinogenic L-amino acids and their corresponding D-enantiomers to repress colibactin production was measured via transcription of the *clbB* gene - necessary for the biosynthesis of the genotoxin - through a *pclbB*-GFP reporter-assay in various types of growth media. The most inhibitory amino acids were validated by RT-qPCR and were selected for further analyses in the colibactin-producing *E. coli* strain, Nissle 1917. Analyses included HeLa cell infections to test change in colibactin-driven megalocytosis, and alkaline gels to measure variation in cross-linked DNA induced by colibactin.

Our results demonstrate the inhibitory effect of several amino acids on colibactin production, with particular repression seen by D-Serine in several *pks*⁺ strains. We observed that D-Serine reduces the cytopathic responses typically observed during infection of HeLa cells with *pks*⁺ strains. Levels of γ -H2AX were reduced 2.75-fold in infected cells supplemented with D-Serine, and exposure of *pks*⁺ *E. coli* to D-Serine caused a reduction in host cell senescence observable at 72 hours post infection. The levels of cross-linked DNA were reduced by up to 0.64-fold in the presence of D-Serine.

Our results show the potential of D-Serine as a preventative in colibactin-associated disease and implicate several other amino acids as possible therapeutic candidates.

35 - Characterization of the fecal microbiome in pediatric patients affected with chronic recurrent multifocal osteomyelitis: a pilot study

GIUSEPPINA CAMPISCIANO⁽¹⁾ - SARA DELLA PAOLERA⁽²⁾ - NUNZIA ZANOTTA⁽¹⁾ - CAROLINA CASON⁽¹⁾ - ANDREA TADDIO⁽²⁾ - MANOLA COMAR⁽¹⁾

Department of Advanced Translational Microbiology, Institute for Maternal and Child Health - IRCCS Burlo Garofolo, Trieste, Italia⁽¹⁾ - Department of Pediatrics, Institute for Maternal and Child Health - IRCCS Burlo Garofolo, Trieste, Italia⁽²⁾

Topic: Microbiota

Characterization of the fecal microbiome in pediatric patients affected with chronic recurrent multifocal osteomyelitis: a pilot study

GIUSEPPINA CAMPISCIANO^{1,3}, SARA DELLA PAOLERA², NUNZIA ZANOTTA¹, CAROLINA CASON^{1,3}, ANDREA TADDIO^{2,3}, MANOLA COMAR^{1,3}

¹Department of Advanced Translational Microbiology, Institute for Maternal and Child Health - IRCCS Burlo Garofolo, Trieste, Italy; ²Department of Pediatrics, Institute for Maternal and Child Health - IRCCS Burlo Garofolo, Trieste, Italy; ³Department of Medical Surgical and Health Sciences, University of Trieste, Trieste, Italy.

Introduction: chronic nonbacterial osteomyelitis (CNO), also called chronic recurrent multifocal osteomyelitis (CRMO), primarily affects children and adolescents and encompasses a spectrum of symptoms including monofocal or multifocal autoinflammatory bone lesions. CRMO mice models have suggested an interplay between microbes and bone erosions: an increase of intestinal Prevotella has been observed which, if reduced by dietary and antibiotic intervention, leads to disease-ameliorating effects by the reduction of secreted inflammatory mediators. In our pilot study, we profiled the gut microbiome of patients with a pre-established diagnosis of CRMO and of healthy children (HC) with no evidence of inflammatory diseases. Materials and Methods: stool samples were collected at the Institute for Maternal and Child Health IRCCS Burlo Garofolo, Trieste, Italy, and at the Sick Kids Children Hospital, Toronto, Canada, for the subsequent sequencing of the V1-V3 region of the 16S rRNA gene on the Ion Torrent PGM platform. Sequencing data were analyzed with QIIME2 (version 2022.2) to perform the alpha (Chao1 and observed ASVs) and beta diversity (Bray-Curtis) calculation. Differences in alpha diversity values were tested using the Mann-Whitney test whereas changes in beta diversity using a pairwise PERMANOVA test with 999 permutations. Results: we evaluated 14 CRMO patients (5 males, 9 females) with a median age of 13 years (10–14 years) and 21 HC (12 males, 9 females) with a median age of 10 years (9–12 years). In terms of alpha diversity and bacterial composition, no significant differences were observed between CRMO and HC groups. Though, an increasing trend of Streptococcus salivarius and Escherichia coli abundances in CRMO patients was observed. This increasing trend was also retained among CRMO patients when distinguished based on the phase of the disease, with Escherichia increasing in the active phase of the disease (n=5). Discussion and Conclusions: although our observations cannot demonstrate a direct causal role between gut microbiome composition and CRMO, we can speculate that the observed intestinal dysbiotic trend could be a consequence of the imbalanced inflammatory response. As a commensal of the oral and intestinal microbiota, Streptococcus salivarius, slightly increased in CRMO, protects the host by counteracting gut inflammation. In contrast, in our cohort, a slight increase in proinflammatory Escherichia has been observed specifically in the active phase of CRMO. This increase has been previously described especially under inflammatory conditions. In mice models, this increase has

been modulated by changes in diet regimen. Future studies are warranted to better understand the possible role of gut microbiome in the context of CRMO.

36 - Exploring the interplay between oral microbiome, inflammatory factors and glycemic control in Type 1 Diabetes Mellitus.

LUANA ALDEGHERI⁽¹⁾ - EULALIA CATAMO⁽²⁾ - NUNZIA ZANOTTA⁽¹⁾ - GIUSEPPINA CAMPISCIANO⁽¹⁾ - CAROLINA CASON⁽¹⁾ - ANDREA CONTI⁽²⁾ - GIANLUCA TORNESE⁽³⁾ - ANTONIETTA ROBINO⁽²⁾ - MANOLA COMAR⁽¹⁾

SSD R Diagnostica Avanzata Microbiologica Traslazionale, Istituto materno infantile IRCCS Burlo Garofolo, Trieste, Italia⁽¹⁾ - SC Genetica Medica, Istituto materno infantile IRCCS Burlo Garofolo, Trieste, Italia⁽²⁾ - SS Endocrinologia, auxologia e diabetologia, Istituto materno infantile IRCCS Burlo Garofolo, Trieste, Italia⁽³⁾

Title: Exploring the interplay between oral microbiome, inflammatory factors and glycemic control in Type 1 Diabetes Mellitus

LUANA ALDEGHERI¹⁻², EULALIA CATAMO³, NUNZIA ZANOTTA¹, GIUSEPPINA CAMPISCIANO¹⁻², CAROLINA CASON¹⁻², ANDREA CONTI³, GIANLUCA TORNESE⁴, ANTONIETTA ROBINO³, MANOLA COMAR¹⁻²

¹SSD R Diagnostica Avanzata Microbiologica Traslazionale, Istituto materno infantile IRCCS Burlo Garofolo, Trieste, Italia; ²Dipartimento di Scienze Mediche e Chirurgiche e della Salute, Università degli Studi di Trieste, Trieste, Italia; ³SC Genetica Medica, Istituto materno infantile IRCCS Burlo Garofolo, Trieste, Italia; ⁴SS Endocrinologia, auxologia e diabetologia, Istituto materno infantile IRCCS Burlo Garofolo, Trieste, Italia.

Introduction: Type 1 diabetes mellitus (T1DM) is a metabolic disorder characterized by the autoimmune destruction of pancreatic beta cells and consequently insulin deficiency and hyperglycemia. Over time, this condition causes organ damage with micro and macrovascular complications, such as nephropathy, neuropathy and cardiovascular diseases. The management of glycemic control is a complex mechanism that depends on several genetic and environmental factors. Recent evidence also indicated that, among the environmental factors, the imbalance in the microbiota may play a role, leading to systemic inflammation and destruction of the pancreatic beta cells. Therefore, this study aims to explore the complex interaction between bacterial composition, inflammatory response, diabetes-related hormones in the oral district and glycemic control in a cohort of children and young adults with T1DM. Materials and Methods: A total of 54 young individuals (aged 8-22 years) with a minimum disease duration of 1 year were recruited. Data on demographic and clinical characteristics were collected from each participant: i.e., age, sex, puberty, age of onset, disease duration. According to glycated hemoglobin (HbA1c) values, T1DM participants were classified into two groups: HbA1c values <7.5% (58 mmol/mol) and HbA1c ≥7.5%. The concentration of 27 immune proteins and 10 diabetes-related hormones was assessed in saliva samples using the Luminex 200 platform. Furthermore, the oral microbiome was investigated using molecular metabarcoding, involving amplification and sequencing of the 16S rRNA gene through Illumina technology. Results: Statistical analysis showed a significant increase ($p<0.05$) in the concentration of pro-inflammatory factors, such as Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF), Platelet-Derived Growth Factor-bb (PDGF-bb) and Interleukin-15 (IL-15), in saliva samples from T1DM individuals with poor glycemic control (HbA1c >7.5%) compared to those with an optimal one (HbA1c ≤7.5%). Additionally, T1DM individuals with poor glycemic control showed higher levels of the diabetes-related hormone leptin in their saliva. The analysis of the oral microbiome did not uncover any significant differences in bacterial composition between the

two selected groups. Discussion and Conclusion: The preliminary results of this study shed light on the potential involvement of immune and hormonal factors in glycemic control. To establish a probable correlation between the oral microbiome and glycemic control, further analysis with a larger cohort of patients is necessary. Investigating the correlation between these factors and glycemic control is crucial to prevent the development of long-term diabetes-related complications that may lead to disability or fatal outcomes.

44 - Fecal sample collection and room-temperature storage using swabs with preservation medium enables deep characterization of the human gut metaproteome

ALESSANDRO TANCA⁽¹⁾ - MARCELLO ABBONDIO⁽¹⁾ - LAURA DE DIEGO⁽¹⁾ - MARIA ANTONIETTA DELEDDA⁽¹⁾ - SERGIO UZZAU⁽¹⁾

Dipartimento di Scienze Biomediche, Università di Sassari, Sassari, Italia⁽¹⁾

Fecal sample collection and room-temperature storage using swabs with preservation medium enables deep characterization of the human gut metaproteome

ALESSANDRO TANCA, MARCELLO ABBONDIO, LAURA DE DIEGO, MARIA ANTONIETTA DELEDDA, SERGIO UZZAU

Dipartimento di Scienze Biomediche, Università di Sassari, Sassari, Italy

Introduction

Standardization of sample collection and storage protocols is a key requirement when dealing with high-complexity clinical samples such as human feces. Different types of swabs with preservation media are used in clinical microbiology for collection, storage and transport of fecal samples. To the best of our knowledge, the suitability of medium-containing swabs for metaproteomic analysis of fecal samples has not been yet investigated so far. Here, we evaluated the ability of the eNAT® swabs (Copan) to preserve gut metaproteome information compared to a gold-standard control.

Materials and methods

Four volunteers produced a stool sample and self-collected four equal aliquots: two were collected using a swab and stored in a preservation medium, both provided with the eNAT® system (SPM); the other two were collected using the spatula of a standard stool container and stored in the container itself (SSC). One SPM and one SSC sample per subject were immediately subjected to protein extraction; the second SPM and SSC sample from each subject were stored at RT and at -80°C, respectively, for 14 days. Protein extraction and digestion were performed according to established bead beating- and FASP-based protocols, respectively. Peptide mixtures were analyzed by liquid chromatography-tandem mass spectrometry. Mass spectra were searched against gut microbial and human sequence databases using Proteome Discoverer (Thermo). Statistical analysis was carried out using a two-tailed paired Student's t-test.

Results

The number of peptides identified in SPM samples was significantly higher compared to that reached analyzing SSC samples. This difference was mainly due to an increase in the number of microbial peptides, while host peptide amounts were similar. No significant differences could be observed neither between SPM samples stored for few minutes or two weeks, nor between fresh and snap-frozen SSC samples. The Firmicutes:Bacteroidetes ratio increased slightly in SPM compared to SSC samples.

Discussion and Conclusions

This pilot study provides original data about the performance of the eNAT® system in collecting, stabilizing and storing fecal samples for downstream metaproteomics. In spite of a lower protein yield, SPM samples outperformed SSC samples in terms of peptide identifications. Even at the cost of a slight information loss in the Bacteroidetes sub-metaproteome, the eNAT® collection and storage system, requiring neither temperature-controlled containers nor freezing, might be a promising solution to be applied in large-cohort metaproteomic investigations.

54 - Effects of *Clostridioides difficile* infection on the structure and functions of the human gut microbiota

LAURA DE DIEGO⁽¹⁾ - ALESSANDRO TANCA⁽¹⁾ - MARCELLO ABBONDIO⁽¹⁾ - SERGIO UZZAU⁽¹⁾

Università degli Studi di Sassari, Dipartimento di Scienze Biomediche, Sassari, Italia⁽¹⁾

Effects of *Clostridioides difficile* infection on the structure and functions of the human gut microbiota

LAURA DE DIEGO¹, ALESSANDRO TANCA¹, MARCELLO ABBONDIO¹, SERGIO UZZAU¹

¹Department of Biomedical Sciences, University of Sassari, Sassari, Italy

Preferred presentation method: Poster

Topic: Metaproteomics in Health and Disease

1. Introduction

Clostridioides difficile is a spore forming pathobiont that colonizes the human intestine. Germination and toxins production, most commonly occurring after use of antibiotic medications, lead to diarrhea, pseudomembranous colitis and toxic megacolon, with an imbalance of the gut microbiota taxonomic structure. No studies have yet described the functional and the taxonomic variations occurring in the gut microbiota in the context of *C. difficile* disease.

Our aim was to profile the intestinal metaproteome functional features specifically associated to *C. difficile* colonization and toxigenic activity.

2. Materials and Methods

The study involved 80 subjects under antibiotic treatment with clinical symptoms attributable to *C. difficile* infection. The subjects were divided into three groups based on the results of the diagnostic test conducted on fecal samples for *C. difficile*: negative (N, 30 patients), testing negative for antigens and positive for toxins genes (Tg, 20 patients), and positive to both antigen and genes (Tp, 30 patients).

Proteins were extracted from fecal samples and processed according to a modified filter-assisted sample preparation (FASP) protocol. Peptide mixtures were analyzed by liquid chromatography and tandem mass spectrometry followed by bioinformatic analyses with the Proteome Discover bioinformatic platform (Thermo). Subsequently, Unipept and eggNOG-mapper were used for taxonomic and functional annotation, respectively, while data aggregation at various taxonomic and functional levels, was performed by an in-house software.

Richness, alpha diversity and beta diversity were calculated using peptide, taxon and function abundance data. Comparison of taxon and function abundances between groups was performed using the Student's t-test.

3. Results

No differences were observed between the three sample groups regard to richness. On the other hand, a higher functional alpha diversity was measured in the N group compared to the other groups (Tg and Tp).

Total abundance of Anaerostipes peptides was significantly higher in the microbiota of Tp patients (compared to N), whereas that of Ruminococcus peptides was significantly higher in the microbiota of N patients (compared to Tp).

Moving to functional results, total abundance of peptides associated with multidrug resistance (specifically related to the MexAB-OprM efflux pump) and histidine metabolism were found

significantly higher in the microbiota of patients belonging to the Tp group when compared to those from the N and Tg group, respectively.

4. Discussion and Conclusions

Our data highlight the impact of *C. difficile* presence on both the taxonomic and functional features of the gut microbiota.

Specifically, metaproteomic analyses enabled to identify an enrichment of specific metabolic pathways and antibiotic resistance mechanisms as part of the impaired gut microbiota physiology in CDI. Noteworthy, these functional changes appear dependent by substantial *C. difficile* toxin production and not by the sole mucosa colonization.

56 - Preliminary evaluation of fecal microbiota transplantation by oral capsules in patients with liver cirrhosis and hepatic encephalopathy refractory to conventional treatment

GIANLUCA QUARANTA⁽¹⁾ - FRANCESCA ROMANA PONZIANI⁽²⁾ - ALESSANDRA GUARNACCIA⁽¹⁾ - CHIARA AGRILLO⁽¹⁾ - FEDERICA IANNARELLI⁽¹⁾ - CARLO AIROLA⁽²⁾ - FRANCESCO SANTOPAULO⁽²⁾ - GIOVANNI FANCELLO⁽¹⁾ - FLAVIO DE MAIO⁽¹⁾ - MAURIZIO SANGUINETTI⁽¹⁾ - LUCA MASUCCI⁽¹⁾

FONDAZIONE POLICLINICO A. GEMELLI, DIPARTIMENTO DI MICROBIOLOGIA E VIROLOGIA, ROMA, Italia⁽¹⁾ - FONDAZIONE POLICLINICO A. GEMELLI, Digestive Disease Center-CEMAD, ROMA, Italia⁽²⁾

Preliminary evaluation of fecal microbiota transplantation by oral capsules in patients with liver cirrhosis and hepatic encephalopathy refractory to conventional treatment

GIANLUCA QUARANTA¹, FRANCESCA R. PONZIANI^{3,4}, ALESSANDRA GUARNACCIA¹, CHIARA AGRILLO¹, FEDERICA IANNARELLI¹, CARLO AIROLA³, FRANCESCO SANTOPAULO³, GIOVANNI FANCELLO¹, FLAVIO DE MAIO¹, MAURIZIO SANGUINETTI^{1,2}, LUCA MASUCCI^{1,2}

1 Department of Laboratory and Infectious Sciences, A. Gemelli University Hospital IRCCS, 00168 Rome, Italy.

2 Department of Basic Biotechnological Sciences, Intensivological and Perioperative Clinics, Catholic University of Sacred Heart, 00168 Rome, Italy.

3 Digestive Disease Center-CEMAD, Fondazione Policlinico Universitario Agostino Gemelli IRCCS, 00168 Rome, Italy.

4 Translational Medicine and Surgery Department, Università Cattolica del Sacro Cuore, 00168 Rome, Italy.

Introduction

Gut bacteria exert multiple functions at systemic level. A fascinating example is the gut-brain-liver axis. This crosstalking is disrupted in hepatic encephalopathy (HE). HE is a complication of cirrhosis featured by brain dysfunctions. In HE, gut barrier alteration lead to a bacterial overgrowth and a catabolites overproduction. In this scenario, proinflammatory species increase while beneficial decrease. HE standard therapy includes lactulose and antibiotics. We evaluated the effectiveness of fecal microbiota transplantation (FMT) via colonoscopy and oral capsules administration.

Materials and Methods

The study includes three patient groups: 1) colonoscopy plus capsules; 2) capsules; 3) lactulose. Healthy donors were selected by screening tests on stool and blood samples. 250 mL of donor fecal solution has been infused in the cecum of patients by colonoscopy. FMT capsules have been prepared filling gastro-resistant capsules with 400 µl of fecal slurry. Capsules were stored at -80°C and monthly delivered to the patients. The dosage was 1 capsule twice/day for 12 months. Metagenomics, fecal calprotectin and serum ammonium dosages were evaluated. Neurological improvements were tested with Psychometric hepatic encephalopathy score tests (PHES).

Results

We report preliminary findings for three patients (PZ1, PZ2, PZ3) belonging to the group 1 and one patient (PZ4) from group 3. Patients were evaluated at three timepoints: baseline (T0), after one month (T1) and after three months (T3) from FMT. PZ1 showed high abundance of Fusobacteria which decreased after treatment. PZ2 showed high abundance of Proteobacteria at T0, which decreased at T3. Bacteroidetes percentage increased after treatment. PZ3 has shown after three months, an increase of Bacteroidetes and a decrease of Proteobacteria compared to baseline. PZ4 did not shown phyla modification except for Fusobacteria which increased at T3. We also evaluated the relative abundance of beneficial bacteria *Faecalibacterium prausnitzii* and *Bacteroides thetaiotaomicron* which increased in group 1. We focused on *Streptococcus salivarius*, an ammonium producer: it appeared more abundant in control patient. Fecal calprotectin and serum ammonium dosages did not shown modulation. PHES tests have shown a general improvements for both groups.

Discussion and Conclusion

The aim of our project is to fill the significant lack of HE treatment options and candidate FMT as therapeutic tool. Oral capsules may be safe, less troublesome for the patient. We need to expand analysis to a larger number of patients and focusing on the microbiota typing in order to identify species related to a state of remission. This may represent the first step toward a personalized therapy.

57 - Dynamics of Gut Microbiota and Clinical Variables after Medium (3 months) and Long-Term (6-12 months) Ketogenic and Mediterranean Diets in Drug-Naïve Patients with Type 2 Diabetes Mellitus and Obesity

VANESSA PALMAS⁽¹⁾ - ANDREA DELEDDA⁽²⁾ - VITOR HEIDRICH⁽³⁾ - GIUSEPPINA SANNA⁽¹⁾ - LORENZO PUGLIA⁽²⁾ - MICHELE FOSCI⁽²⁾ - GIULIA CAMBARAU⁽²⁾ - ALESSIO LAI⁽⁴⁾ - MARIETTA MELIS⁽²⁾ - ELISABETTA LOI⁽²⁾ - ANDREA LOVISELLI⁽²⁾ - FERNANDA VELLUZZI⁽²⁾ - ALDO MANZIN⁽¹⁾

Università degli Studi di Cagliari, Dipartimento di Scienze Biomediche, MONSERRATO (CA), Italia⁽¹⁾ - Università degli Studi di Cagliari, Department of Medical Sciences and Public Health, MONSERRATO (CA), Italia⁽²⁾ - Instituto de Química, Universidade de São Paulo, Departamento de Bioquímica, São Paulo, Brasile⁽³⁾ - P.O. Binaghi, DIABETOLOGIA, CAGLIARI, Italia⁽⁴⁾

TOPIC: MICROBIOTA

51th National Congress of the Italian Society of Microbiology (SIM), 24-27 September 2023, Cagliari

Dynamics of Gut Microbiota and Clinical Variables after Medium (3 months) and Long-Term (6-12 months) Ketogenic and Mediterranean Diets in Drug-Naïve Patients with Type 2 Diabetes Mellitus and Obesity

VANESSA PALMAS¹, ANDREA DELEDDA², VITOR HEIDRICH³, GIUSEPPINA SANNA¹, LORENZO PUGLIA², MICHELE FOSCI², GIULIA CAMBARAU², ALESSIO LAI⁴, MARIETTA MELIS², ELISABETTA LOI², ANDREA LOVISELLI², FERNANDA VELLUZZI², ALDO MANZIN¹

¹ Department of Biomedical Sciences, Microbiology and Virology Unit, University of Cagliari, I-09042, Monserrato (CA), Italy;

² Department of Medical Sciences and Public Health, Obesity and Endocrinology Units, University of Cagliari, 09124 Cagliari, Italy;

³ Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo, São Paulo, Brazil;

⁴ P.O. Binaghi, Diabetologia, ASSL Cagliari, Italy.

Introduction: Type 2 diabetes mellitus (T2DM) is a progressive chronic metabolic disease that has increasingly spread worldwide, increasing mortality mainly due to cardiovascular complications. Lifestyle improvement through diet and physical activity is, together with drug treatment, the cornerstone of T2DM management. The Mediterranean diet (MD) is usually recommended, but recently scientific societies have promoted a very low-calorie ketogenic diet (VLCKD) multiphase protocol, for its benefit on body weight and metabolic parameters. Diet is known to affect gut microbiota (GM), but only a few studies have investigated the effects of VLCKD on GM, especially in T2DM.

Materials and Methods: We have extended the results obtained from a previous study, which had evaluated the effect of nutritional intervention (NI) up to three months, to a 12-month follow-up in the same patients. We have compared the anthropometric and metabolic parameters, quality of life, and the GM of 11 patients with recently diagnosed T2DM and overweight or obesity, randomly assigned to a VLCKD (KETO group: 6 patients) or hypocaloric MD (MEDI group: 5 patients); data were recorded at baseline (T0), after two (T2), three (T3), six (T6) and twelve months (T12) of NI. The GM analysis was performed through Next Generation Sequencing of the V3 and V4 hypervariable region of the 16S rRNA gene on the MiSeq Illumina platform.

Results: The comparison of the anthropometric parameters between T0 and T6, showed a significant reduction of the BMI and waist circumference values in both groups, but to greater extent in the KETO group (BMI: -5.8 vs -1.7 kg/m²; p=0.006; WC: -15.9 vs -5.2 cm; p=0.011), while at T12 a slight increase of the same variables was observed. As for the metabolic variables, a significant improvement of HbA1c (6.7% vs 5.5% p=0.02) and triglycerides (158 vs 95 mg/dl p=0.04) values was found only in the KETO group at T6. Finally, only in the KETO group the physical component of quality of life, assessed by means of the SF-36, showed a significant improvement. The results showed that VLCKD had significant effects on GM composition over time, while MD showed no significant results at T6 nor T12. In the KETO group, a significant increase in beneficial microbial taxa such as Verrucomicrobiota phylum and its members Verrucomicrobiae, Verrucomicrobiales, Akkermansiaceae and Akkermansia species was observed until T6, while their significant reduction was observed from T6 to T12. Moreover, alterations linked to some metabolic pathways were found exclusively in the KETO group.

Discussion and Conclusions: Both dietary approaches allowed patients to improve their health state, but VLCKD has shown better results on anthropometric and metabolic parameters as well as on GM profile, especially up to T6 of NI.

67 - Effect on sub-gingival microbiome of diverse subgingival air-polishing in the treatment of advanced periodontitis: a randomized controlled clinical trial.

IRENE SOFFRITTI ⁽¹⁾ - MARIA D'ACCOLTI ⁽¹⁾ - MAGDA MENSI ⁽²⁾ - ROBERTO FARINA ⁽³⁾ - MARIA ELENA GUARNELLI ⁽³⁾ - CHIARA FABBRI ⁽³⁾ - FRANCESCA BINI ⁽¹⁾ - ELEONORA MAZZIGA ⁽¹⁾ - LEONARDO TROMBELLI ⁽³⁾ - ELISABETTA CASELLI ⁽¹⁾

University of Ferrara, Department of Chemical, Pharmaceutical and Agricultural Sciences, LTTA and CIAS Research Center, Ferrara, Italia ⁽¹⁾ - University of Brescia & ASST Degli Spedali Civili Di Brescia, Section of Periodontics, School of Dentistry, Department of Surgical Specialties, Radiological Science and Public Health & U.O.C. Odontostomatologia, Brescia, Italia ⁽²⁾ - University of Ferrara & Azienda Unità Sanitaria Locale (AUSL) di Ferrara, Research Centre for the Study of Periodontal and Peri-Implant Diseases & Operative Unit of Dentistry, Ferrara, Italia ⁽³⁾

Topic: Microbiota

Effect on sub-gingival microbiome of diverse subgingival air-polishing in the treatment of advanced periodontitis: a randomized controlled clinical trial.

IRENE SOFFRITTI ¹, MARIA D'ACCOLTI ¹, MAGDA MENSI ^{2,3}, ROBERTO FARINA ^{4,5}, MARIA ELENA GUARNELLI ^{4,5}, CHIARA FABBRI ^{4,5}, FRANCESCA BINI ¹, ELEONORA MAZZIGA ¹, LEONARDO TROMBELLI ^{4,5}, ELISABETTA CASELLI ¹

¹Department of Chemical, Pharmaceutical and Agricultural Sciences, LTTA and CIAS Research Center, University of Ferrara, Ferrara, Italy; ²Section of Periodontics, School of Dentistry, Department of Surgical Specialties, Radiological Science and Public Health, University of Brescia, Brescia, Italy; ³U.O.C. Odontostomatologia - ASST Degli Spedali Civili Di Brescia, Brescia, Italy; ⁴Research Centre for the Study of Periodontal and Peri-Implant Diseases, University of Ferrara, Ferrara, Italy; ⁵Operative Unit of Dentistry, Azienda Unità Sanitaria Locale (AUSL), Ferrara, Italy

Introduction: Periodontal disease is associated with dysbiosis of the oral microbiome and prevalence of specific bacteria in the subgingival biofilm. Scarce evidence exists on the effect of subgingival air-polishing applied during treatment of severe periodontitis on the periodontal microbial community. Thus, our study aimed to evaluate the effect on the subgingival microbiome of air-polishing during non-surgical treatment of deep bleeding pockets in advanced periodontitis patients.

Materials and methods: Forty patients with III/IV grade periodontitis were enrolled; 5–9 mm gingival pockets showing bleeding on probing were selected as experimental sites. All patients underwent a full-mouth session of erythritol powder supragingival air-polishing and ultrasonic instrumentation. Test group received additional subgingival air-polishing. Samples were collected from experimental sites at baseline and 3 months following treatment. The periodontal microbial community was analyzed by a real-time quantitative PCR microarray.

Results: In the test group, compared to the control group, significant increase of health-related species was observed (*Abiotropha defectiva*, *Capnocytophaga sputigena*, and *Lautropia mirabilis*), together with the decrease of periodonto-pathogens (*Catonella morbi*, *Filifactor alocis*, *Porphyromonas endodontalis*, *Tannerella forsythia* and *Treponema Denticola*). By contrast, in the control group, only decrease of *F. alocis*, *T. forsythia*, and *T. socranskii*. were statistically significant.

Discussion and Conclusions: The results suggested that the addition of subgingival erythritol powder treatment could influence more deeply the recovery of a healthy microbiome, by causing a shift of the periodontal microbiome toward eubiosis, compared to conventional treatment.

69 - Urinary microbiota and type II diabetes: a randomized clinical trial

MARCO CALVIGIONI⁽¹⁾ - EDOARDO BIANCALANA⁽²⁾ - DILETTA MAZZANTINI⁽¹⁾ -
FRANCESCO CELANDRONI⁽¹⁾ - ANNA SOLINI⁽²⁾ - EMILIA GHELARDI⁽¹⁾

Universita' di Pisa, Dipartimento di Ricerca Traslazionale e Nuove Tecnologie in Medicina e Chirurgia, Pisa, Italia⁽¹⁾ - Universita' di Pisa, Dipartimento di Patologia Chirurgica, Medica, Molecolare e dell'Area Critica, Pisa, Italia⁽²⁾

Urinary microbiota and type II diabetes: a randomized clinical trial

MARCO CALVIGIONI¹, EDOARDO BIANCALANA², DILETTA MAZZANTINI¹,
FRANCESCO CELANDRONI¹, ANNA SOLINI², EMILIA GHELARDI¹

¹ Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa, Pisa, Italy; ² Department of Surgical, Medical, and Molecular Pathology and Critical Care Medicine, University of Pisa, Pisa, Italy.

Introduction. As other microbial communities residing in the human body, urinary microbiota may be influenced in terms of composition and functions by drug therapy. In this study, the urinary microbiota from healthy individuals was compared with that of patients suffering from type II diabetes. The oral administration of two different hypoglycaemic therapies commonly prescribed as adjuvant treatment to Metformin in type II diabetes was also investigated to clarify if urinary microbial communities were perturbed in correspondence with the treatments.

Materials and Methods. 25 healthy controls (age 51-73 yo, 10 males, 15 females) and 50 patients with type II diabetes (age 39-82 yo, 34 males, 16 females) were enrolled in the trial. Subjects from the type II diabetes group were randomly divided and subjected to treatment A or treatment B for 12 weeks. Middle-late stream of early morning urines were collected before (T₀) and after (T₁) treatment. The composition of the urinary microbiota at T₀ and T₁ was quantitatively and qualitatively evaluated by 16S rDNA-targeting phylum- and genus-specific Real-Time qPCRs and V3-V4 16S rDNA Illumina sequencing, respectively.

Results. As a result of the compositional analysis, differences in the urinary microbiota between healthy and type II diabetic individuals were evidenced, with diabetic patients displaying higher total bacterial loads and levels of Firmicutes and Actinobacteria than healthy controls. Further differences within the two groups were also highlighted in dependence of gender. Specific treatment-dependent shifts in microbial composition at the phylum, genus, and species levels were highlighted at T₁ in comparison with T₀, leading to significant differences among treatment A and treatment B.

Discussion and Conclusions. Our results indicate that the urinary microbiota actually differs between healthy and type II diabetic individuals, probably due to highest levels of glucose in urines of diabetic subjects. Moreover, the two hypoglycaemic treatments resulted being not equal in shaping the microbiota composition of diabetic subjects, thus suggesting that opting for the therapeutic approach with the lowest impact on the local microbial communities could be recommendable for better clinical outcomes.

70 - Metaproteomic changes in the human gut microbiota following an antibiotic treatment for the eradication of *Helicobacter pylori*

MARCELLO ABBONDIO⁽¹⁾ - ALESSANDRO TANCA⁽¹⁾ - LAURA DE DIEGO⁽¹⁾ - ROSANGELA SAU⁽¹⁾ - GIOVANNI MARIO PES⁽²⁾ - MARIA PINA DORE⁽²⁾ - SERGIO UZZAU⁽¹⁾

Università di Sassari, Dipartimento di Scienze Biomediche, Sassari, Italia⁽¹⁾ - Università di Sassari, Dipartimento di Medicina, Chirurgia e Farmacia, Sassari, Italia⁽²⁾

Metaproteomic changes in the human gut microbiota following an antibiotic treatment for the eradication of *Helicobacter pylori*

MARCELLO ABBONDIO¹, ALESSANDRO TANCA¹, LAURA DE DIEGO¹, ROSANGELA SAU¹, GIOVANNI M. PES², MARIA PINA DORE², SERGIO UZZAU¹

¹Dipartimento di Scienze Biomediche, Università di Sassari, Sassari, Italy

²Dipartimento di Medicina, Chirurgia e Farmacia, Università di Sassari, Sassari, Italy

Introduction

Knowledge of gut microbiota (GM) impact on host metabolism, immunity and neuroendocrine functions has recently assumed a paramount importance in clinical settings. GM homeostasis is strongly endangered by antimicrobial treatment. However, to date, there is a lack of information on how antimicrobial therapies affect the GM functions and their complex relationships with the host physiology. Here, we evaluate the impact of a standardized therapy for the eradication of *Helicobacter pylori* on the GM functions with a metaproteomic approach.

Materials and Methods

Ten patients diagnosed with *H. pylori* infection and undergoing a successful antibiotic therapy based on tetracycline and metronidazole (supplemented with rabeprazole and bismuth) were recruited in the study. Feces were collected immediately before starting the therapy (T0), at 10 days of therapy (T1) and 30 days after the end of the therapy (T2). Proteins were extracted from feces and subjected to tryptic digestion with the filter-aided sample preparation (FASP) protocol. Peptides were identified and quantified by liquid chromatography-tandem mass spectrometry (LC-MS/MS) followed by bioinformatic analysis with Proteome Discoverer. Differential analysis between time points (paired t-test) was performed using Perseus at taxonomic, functional and taxonomic-functional levels.

Results

Eradication of *H. pylori* by antimicrobial treatment was accompanied by a significant decrease of the microbial/host peptide relative abundance ratio, consistently with a reduction of the microbial biomass within the fecal sample, as well as by a microbial richness and alpha-diversity reduction. We found that specific genera (i.e., *Enterococcus*, *Klebsiella*, and *Proteus*) were less impaired by antibiotics treatment and that major taxa like *Bacteroides* showed a reassortment of their proteome. At T1, GM showed a reduction of several functions with a relative increase of those involved in cell wall/membrane biogenesis and nucleotide transport/metabolism. Finally, an increase of key microbial functions (i.e., outer membrane porin) occurred at T1. All patients showed recovery of the GM functions at T2.

Discussion and Conclusions

In this study we highlighted the functional reassortment of the GM in patients undergoing antimicrobial therapy. Our results demonstrate that, when an investigation of GM antimicrobial treatment effects is required, metaproteomics can reveal the reduction of microbial peptide abundance, the residual functions of different microbial taxa, and the specific variations of key GM

functions, explaining therapy side effects on host metabolism and their interindividual difference in clinical outcome.

73 - Profiling gut microbiota in patients undergoing laparoscopic R-en-Y vs One Anastomosis Gastric Bypass

FLAVIO DE MAIO⁽¹⁾ - CRISTIAN E. BORU⁽²⁾ - GIULIA SANTARELLI⁽³⁾ - DANILA CAPOCCIA⁽²⁾ - NUNZIO VELOTTI⁽⁴⁾ - ORNELLA VERRASTRO⁽⁵⁾ - BRUNELLA CAPALDO⁽⁶⁾ - MARIO MUSELLA⁽⁴⁾ - MAURIZIO SANGUINETTI⁽¹⁾ - MARCO RAFFAELLI⁽⁵⁾ - FRIDA LEONETTI⁽²⁾ - GIANFRANCO SILECCHIA⁽²⁾ - GIOVANNI DELOGU⁽⁷⁾

Fondazione Policlinico Universitario Agostino Gemelli, Department of Laboratory and Infective Sciences, Faculty of Medicine, Roma, Italia⁽¹⁾ - Università di Napoli "Federico II", Department of Medico-Surgical Sciences and Biotechnologies, UOC Diabetologia Universitaria, SM Goretti Hospital, Latina, University "La Sapienza" of, Napoli, Italia⁽²⁾ - Università Cattolica del Sacro Cuore, Department of Sciences of Basic Biotechnologies, Intensive and Perioperative Clinics, Section of Microbiology, Roma, Italia⁽³⁾ - Università di Napoli "Federico II", Department of Advanced Biomedical Sciences, Napoli, Italia⁽⁴⁾ - Fondazione Policlinico Universitario Agostino Gemelli, Division of Endocrine and Metabolic Surgery, Roma, Italia⁽⁵⁾ - Università di Napoli "Federico II", Department of Clinical Medicine and Surgery, Napoli, Italia⁽⁶⁾ - Mater Olbia Hospital, Mater Olbia Hospital, Olbia, Italia⁽⁷⁾

Profiling gut microbiota in patients undergoing laparoscopic R-en-Y vs One Anastomosis Gastric Bypass

Authors

Flavio De Maio¹, Cristian E. Boru³, Giulia Santarelli², Danila Capoccia³, Nunzio Velotti⁴, Ornella Verrastro⁵, Brunella Capaldo⁶, Mario Musella⁴, Maurizio Sanguinetti^{1,2}, Marco Raffaelli⁵, Frida Leonetti³, Gianfranco Silecchia³, Giovanni Delogu^{2,7}

Affiliations

¹Department of Laboratory and Infective Sciences, Faculty of Medicine, Fondazione Policlinico A. Gemelli IRCCS, Università Cattolica del Sacro Cuore, Rome, Italy; ²Department of Sciences of Basic Biotechnologies, Intensive and Perioperative Clinics, Section of Microbiology, Università Cattolica del Sacro Cuore, Rome, Italy; ³Department of Medico-Surgical Sciences and Biotechnologies, UOC Diabetologia Universitaria, SM Goretti Hospital, Latina, University "La Sapienza" of Rome, Italy; ⁴Department of Advanced Biomedical Sciences, University of Naples "Federico II", Naples, Italy; ⁵Division of Endocrine and Metabolic Surgery, Fondazione Policlinico A. Gemelli IRCCS, Università Cattolica del Sacro Cuore, Rome, Italy; ⁶Department of Clinical Medicine and Surgery, University of Naples "Federico II", Naples, Italy; ⁷Mater Olbia Hospital, Olbia, Italy

Introduction

R-en-Y gastric bypass (RYGB) and one anastomosis gastric bypass (OAGB/MGB) represent two of the most used laparoscopic bariatric/metabolic procedures. The metabolic and physiological consequences shape the short- and long-term outcomes of bypass surgeries, and the variations in the gut microbiota composition may be a possible key driver. No studies properly describe gut microbiota changes following RYGB and OAGB/MGB in a longitudinal cohort.

Materials and methods

We enrolled patients with severe obesity, randomized between OAGB/MGB or RYGB. Fecal and blood samples were collected, pre- (T0) and 12 months postoperatively (T1). Gut microbiota was determined by V3-V4 16S rRNA regions sequencing and homemade bioinformatic pipeline based on Qiime2 and R packages. We compared OAGB/MGB vs RYGB microbiota profiles shift postoperatively and its impact on metabolic and nutritional status.

Results

An overall significant variation was detected in anthropometric and serum nutritional parameters at 1 year, although no differences were found in total weight loss and body mass index among the two studies groups. Samples collected at T1 showed significant decrease in microbial diversity. While Firmicutes and Verrucomicrobia phyla did not significantly change, Actinobacteria and Proteobacteria relative abundances increased compared to Bacteroidetes. Interestingly, we observed a significant increase of the Firmicutes/Bacteroidetes (F/B) ratio one year post surgery, mostly due to a decrease in Bacteroidetes abundance. We found out that at T1 serum levels of glucose and Vitamin D were positively and inversely correlated with Bacteroidetes and Firmicutes, respectively. We classified samples based on T1 microbial signature identifying three clusters. Whereas cluster A and B appeared similar in terms of alpha diversity, cluster C showed a decrease. Interestingly, an opposite trend can be depicted by *Bacteroides*, *Bifidobacterium*, *Prevotella* and *Roseburia* that were increased; and *Streptococcus* that conversely is reduced in cluster A and B compared to cluster C.

Discussion and conclusions

Bariatric surgery promotes a dramatic change in gut microbiota composition one year after surgery, even though the by-pass technique per se did not impact on microbial signature. Firmicutes may be not properly affected, while each nutritional modification may impact on Bacteroidetes phylum. The increase in Proteobacteria may be associated with an improvement of metabolic functions and lowering of pro-inflammatory status. Unfortunately, we reported conflicting about possible biomarkers. However, we identified three clusters of patients showing different microbial signatures that it will be evaluated in the long-term to disclose eventual impact on clinical outcomes.

75 - Comparison of methods for processing fecal protein extracts on a multiwell plate for metaproteomic analysis

MARIA ANTONIETTA DELEDDA⁽¹⁾ - MARCELLO ABBONDIO⁽¹⁾ - LAURA DE DIEGO⁽¹⁾ - ALESSANDRO TANCA⁽¹⁾ - SERGIO UZZAU⁽¹⁾

Università degli studi di Sassari, Dipartimento di Scienze Biomediche, Sassari, Italia⁽¹⁾

Comparison of methods for processing fecal protein extracts on a multiwell plate for metaproteomic analysis

MARIA ANTONIETTA DELEDDA, MARCELLO ABBONDIO, LAURA DE DIEGO, ALESSANDRO TANCA, SERGIO UZZAU

Dipartimento di Scienze di Scienze Biomediche, Università di Sassari, Sassari, Italy

Introduction

Metaproteomics provides a detailed picture of the biological functions active in complex microbial communities. Increasing levels of standardization and automation in sample preparation methods for metaproteomics is needed, as it would enhance their applicability to large-scale studies and the comparability of different datasets. The aim of this study was to compare different high-throughput, automatable sample preparation methods for the metaproteomic analysis of multiple fecal samples. Study design included different multiwell plate versions of FASP (Filter-aided sample preparation), SP3+iST (Solid-phase-enhanced sample preparation+in-StageTips), and S-Trap (Suspension trapping) methods.

Materials and methods

Fecal protein extracts from 8 healthy subjects were used to test 5 multiwell plates, namely 3 for the FASP method (a MultiScreen® plate with a 10-kDa cutoff, named MS-10, and two generic plates with 10- and 30-kDa cutoffs, named P10 and P30, respectively), 1 for the SP3+iST method and 1 for the S-Trap method. Protein extracts were loaded in triplicate in each plate and processed for clean-up, digestion and peptide elution. Peptide mixtures were separated by liquid chromatography and analyzed with the Thermo Explorer 480 mass spectrometer. Bioinformatic analysis was carried out using the Proteome Discoverer platform. Statistical analysis was carried out using a paired Student's t-test.

Results

The efficiency of the five methods was primarily evaluated by measuring the average number of peptides identified with each method (for each subject). Accordingly, MS10 and SP3+iST methods significantly outperformed the other methods. Furthermore, the number of microbial genera detected was largely comparable in four plates, with the only S-Trap showing a lower taxonomic richness. A similar trend was observed for microbial functions. Principal Component Analysis showed a strong inter-individual variability; however, samples from the same subjects processed with MS10 and SP3+iST plates clustered together. No clear differences were seen between methods in terms of distribution of the identified proteins according to their molecular weight. Finally, the highest level of reproducibility between replicates was measured for SP3+iST, followed by MS10 and S-Trap.

Discussion and conclusions

Among the five methods compared in this study, SP3+IST and MS-10 multiwell plates reach the best results in terms of efficiency, sensitivity and reproducibility. Associating our data with technical and economic considerations (e.g., protocol duration and costs of plates and reagents) will help researchers choose the best method for large-scale application of human gut metaproteomics.

147 - Altered oropharyngeal microbiota in common variable immunodeficiency (CVID) patients

MARIA GIUFRE'⁽¹⁾ - FEDERICA PULVIRENTI⁽²⁾ - TANCREDI M. PENTIMALLI⁽³⁾ - ROMINA CAMILLI⁽¹⁾ - CINZIA MILITO⁽⁴⁾ - MARINA CERQUETTI⁽¹⁾ - ANNALISA PANTOSTI⁽¹⁾ - ISABELLA QUINTI⁽⁴⁾

Istituto Superiore di Sanita', Malattie Infettive, Roma, Italia⁽¹⁾ - Reference Centre for Primary Immune Deficiencies, Azienda Ospedaliera Universitaria Policlinico Umberto I, Roma, Italia⁽²⁾ - Berlin Institute for Medical Systems Biology (BIMSB), Max Delbrück Center for Molecular Medicine (MDC), Berlin, Germania⁽³⁾ - "Sapienza" University of Rome, Italy, Department of Molecular Medicine, Roma, Italia⁽⁴⁾

Altered oropharyngeal microbiota in common variable immunodeficiency (CVID) patients

MARIA GIUFRE'¹, FEDERICA PULVIRENTI², TANCREDI M. PENTIMALLI³, ROMINA CAMILLI¹, CINZIA MILITO⁴, MARINA CERQUETTI¹, ANNALISA PANTOSTI¹, ISABELLA QUINTI^{2,4}

1Department of Infectious diseases, Istituto Superiore di Sanità, Rome, Italy; 2Reference Centre for Primary Immune Deficiencies, Azienda Ospedaliera Universitaria Policlinico Umberto I, Rome, Italy; 3Berlin Institute for Medical Systems Biology (BIMSB), Max Delbrück Center for Molecular Medicine (MDC), Berlin, Germany; 4Department of Molecular Medicine, "Sapienza" University of Rome, Italy

1. Introduction The respiratory tract microbiota is essential for human health and well-being and is driven by genetic, lifestyle and environmental factors. Common Variable Immunodeficiency (CVID) is a primary antibody deficiency characterized by low levels of immunoglobulins and increased susceptibility to infections. Mucosal IgA is crucial to a robust host-microbial symbiosis, allowing the colonisation of defined mucosal niches by commensal species through the exclusion of exogenous competitors. Therefore, mucosal immunity alterations in CVID might impact the respiratory tract ecosystem. Here, we investigated the bacterial composition of the upper respiratory tract by molecular methods in adults with CVID. 2. Materials and Methods Oropharyngeal samples from 72 CVID adult patients and 26 age-matched controls were analysed by sequencing the V3-V4 regions of the bacterial 16S ribosomal RNA gene. Microbiota profiles were investigated in terms of taxonomic assignment, most abundant taxa and relative abundance, using the Quantitative Insights Into Microbial Ecology (QIIME) pipeline. Differentially abundant taxa have been identified and used to build a dysbiosis index. Microbiome alterations between CVID and healthy status were assessed using a machine learning model trained on microbial abundance data. 3. Results The oropharyngeal microbiota of CVID patients showed lower alpha- and beta-diversity compared to healthy controls. The alpha diversity analysis by Chao1 index indicated that the microbial diversity was lower in CVID patients, especially in the subgroup with undetectable IgA levels, age > 40 years and severe/moderate chronic obstructive pulmonary disease (COPD). At taxonomic levels, in CVID we observed a decrease in the families Prevotellaceae, Fusobacteriaceae, Veillonellaceae, Campylobacteraceae and Flavobacteriaceae, generally correlated with a healthy status. Moreover, in CVID patients we observed a relatively increased abundance of the order Lactobacillales including the family Streptococcaceae, respect to controls. The dysbiosis index was higher in CVID patients than in HD and was inversely associated with the alpha diversity. Patients with undetectable IgA serum levels or with moderate/severe COPD showed a higher relative abundance of the genera Haemophilus and Streptococcus compared to those with serum IgA levels > 0.01 g/L or with no

COPD, respectively. 4. Discussion and Conclusions Our data showed that adult CVID patients had an altered respiratory microbial ecosystem enriched with potentially pathogenic bacteria and decreased commensal bacteria. Moreover, our findings highlight IgA as a potential driver of upper respiratory tract microbiota homeostasis. Treatment aimed to replace mucosal IgA and possibly reduce upper respiratory infections by immunobiotics should gain attention.

159 - Preliminary insights in the gut microbiome profile of immigrant patients from African countries

MARIANNA MARANGI ⁽¹⁾ - GIORGIA PALLADINO ⁽²⁾ - ROSELLA DE NITTIS ⁽³⁾ - SILVIA TURRONI ⁽²⁾ - SIMONE RAMPELLI ⁽²⁾ - MAURIZIO MARGAGLIONE ⁽¹⁾ - MARCO CANDELA ⁽²⁾

Università di Foggia, Dipartimento di Medicina Clinica e Sperimentale, Foggia, Italia ⁽¹⁾ -
Università di Bologna, Dipartimento di Farmacia e Biotecnologie, Bologna, Italia ⁽²⁾ - Unità di
Microbiologia e Virologia, Ospedale Riuniti, Foggia, Italia ⁽³⁾

Preliminary insights in the gut microbiome profile of immigrant patients from African countries

MARIANNA MARANGI¹, GIORGIA PALLADINO², ROSELLA DE NITTIS³, SILVIA TURRONI², SIMONE RAMPELLI², MAURIZIO MARGAGLIONE¹, MARCO CANDELA²

¹Department of Clinical and Experimental Medicine, University of Foggia, Viale Luigi Pinto, 71122, Foggia, Italy; ²Department of Pharmacy and Biotechnology, University of Bologna, Italy; ³Microbiology and Virology Unit, Ospedali Riuniti, Viale Luigi Pinto, 71122, Foggia, Italy.

Introduction: It is well known that the gut microbiome composition (GMC) has a key role for the human health and that it is influenced from geographic origin and lifestyle. While the GMC has been well characterized for some human populations, especially from USA and European countries, little is known about the GMC of most populations from African continent. Within the international contest, Italy, and in particular the Southern Italy, is well known to be the area with the highest density of immigrant populations, mainly from African countries. For these populations, who's the geographical origin is uncertain, the GMC is still so far to be characterized.

Material and methods: In an attempt to advance our knowledge of the GMC of some African populations and infer a clearer origin, we preliminary characterized, by 16S rRNA amplicon sequencing, the GMC of n. 79 immigrant patients coming from different region of Sub-Saharan Africa and temporarily hospitalized in an asylum seekers center (Borgo Mezzanone, Foggia, Apulia Region, Italy). Then the GMC obtained was compared with those already characterized of some population from USA, Europe and some African countries as Nigeria, Cameroun, and Tanzania. In the comparison analysis two variables as origin (i.e., western states vs non-western states) and lifestyle (i.e., rural vs urban population) were taken into consideration.

Results: The comparison between the GMC of several populations, taking both origin and lifestyle into account, showed that geographical origin has a greater effect on the groups separation than lifestyle, as rural and urban populations from the same state (e.g., USA or Canada) group together on the basis of their origin. However, an intermediate situation occurs for the Nigerian samples, for which three rural individuals cluster together with the other rural African samples, while the individuals living in the city overlap more with the rest of the urban samples of western origin. With respect to these results, the GMC of immigrants overlaps in part with populations considered western and in part with non-western populations.

Discussion and Conclusion: Geographical origin appears to be the main factor influencing the GMC of different worldwide populations. However, populations lifestyle should also be taken into account as a factor in shaping GMC, as demonstrated in the case of Nigerian population and by the distribution of the immigrants' dataset when compared to western and non-western populations. These results thus pose the preliminary basis to exploit the characterization and profiling of the human GM to infer

the geographical origin and lifestyle of immigrant populations of unclear origin, despite further investigations are still needed to implement such a prediction model.

179 - Oral and blood microbiota relate to interferon and endothelial genes expression during COVID-19

LETIZIA SANTINELLI⁽¹⁾ - GIULIA RADOCCIA⁽¹⁾ - MATTEO FRACELLA⁽²⁾ - LEONARDO SORRENTINO⁽²⁾ - MANOLA COMAR⁽³⁾ - FEDERICA FRASCA⁽²⁾ - LUCA MADDALONI⁽¹⁾ - GINEVRA BUGANI⁽¹⁾ - ALESSANDRA PIERANGELI⁽²⁾ - ANNA TERESA PALAMARA⁽⁴⁾ - GUIDO ANTONELLI⁽²⁾ - CLAUDIO MARIA MASTROIANNI⁽¹⁾ - GABRIELLA D'ETTORRE⁽¹⁾ - CAROLINA SCAGNOLARI⁽²⁾ - VALERIO IEBBA⁽³⁾ - SERENA SCHIPPA⁽¹⁾

Sapienza University of Rome, Department of Public Health and Infectious Diseases, Rome, Italia⁽¹⁾ - Sapienza University of Rome, Department of Molecular Medicine, Laboratory of Virology, Rome, Italia⁽²⁾ - University of Trieste, Department of Medical Sciences, Trieste, Italia⁽³⁾ - National Institute of Health, Department of Infectious Diseases, Rome, Italia⁽⁴⁾

Oral and blood microbiota relate to interferon and endothelial genes expression during COVID-19

LETIZIA SANTINELLI¹, GIULIA RADOCCIA¹, MATTEO FRACELLA², LEONARDO SORRENTINO², MANOLA COMAR³, FEDERICA FRASCA², LUCA MADDALONI¹, GINEVRA BUGANI¹, ALESSANDRA PIERANGELI², ANNA T. PALAMARA⁴, GUIDO ANTONELLI², CLAUDIO M. MASTROIANNI¹, GABRIELLA d'ETTORRE², CAROLINA SCAGNOLARI², VALERIO IEBBA³ AND SERENA SCHIPPA¹

¹ Department of Public Health and Infectious Diseases, Sapienza University of Rome, Rome, Italy;

² Department of Molecular Medicine, Laboratory of Virology, Istituto Pasteur Italia, Sapienza University of Rome, Rome, Italy;

³ Department of Medical Sciences, University of Trieste, Trieste, Italy;

⁴ Department of Infectious Diseases, National Institute of Health, Rome, Italy.

Introduction: SARS-CoV-2 infection has been associated with a relevant alteration of the resident bacterial community within the gut of COVID-19 patients. In this context, the role of the oral and blood microbiota and their relationship with interferon (IFN) pathways and endothelial cell genes remains unclear. We characterized the oral and blood microbiota and their interaction with the transcriptional expression of multiple IFN and endothelial genes in hospitalized COVID-19 patients. **Material and methods:** 16S rRNA gene sequencing was applied to the oral and blood samples from COVID-19 patients and compared with that of healthy volunteers (HV). Alpha-diversity was calculated at the species level, and beta-diversity was calculated using the Bray-Curtis dissimilarity measure. Partial least square discriminant analysis (PLS-DA) and the importance of variables (VIP) plot were used to identify the most discriminating species. The transcript levels of IFN and endothelial genes were assessed by Real-Time PCR assays. **Results:** Evaluation of alpha-diversity showed an increase in the biodiversity, measured by Shannon index, and species richness, in both oral swabs and blood samples of COVID-19 patients when compared to HV. A separation of COVID-19 microbial community from those of HV was assessed through beta-diversity both in blood and oral samples. Highly discriminant bacterial species of oral swabs of COVID-19 patients were *Streptococcus periodontium*, *Veillonella dispar*, *Streptococcus sinensis*, *Staphylococcus epidermidis*, and *Flavobacterium succinicans*, while two keystone bacterial species, *Sphingomonas paucimobilis* and *Cutibacterium acnes*, were distinctive for blood. Three clusters of bacterial species in the oral district were negatively correlated with mRNAs levels of almost all genes analyzed,

mainly with IFN-lambda1 and positively correlated with IFN-alpha, IFN-beta, IFN-lambda-2, IFN-lambda-3, MxA, ISG15, ISG56, and IFI27 transcript levels. By contrast, five different clusters of bacterial species at blood level were negatively correlated with IFN-alpha, IFN-beta, ISG56, IFN-lambda-1 and IFN-lambda-3 transcripts levels and positively correlated with IFI27, ISG15, MxA, IFN-gamma, CDH5, ITGA2B, ITGAL, ITGB3, TMBS3, IFNAR1, IFNAR2, VCAN and ICA.

Discussion and conclusions: This study evidenced a distinctive oral and blood microbiota in hospitalized COVID-19 patients, with a definite structural network in relation to IFNs and endothelial cells pathways. These results might help to identify specific bacterial consortia as disease biomarkers and/or new therapeutic targets to improve management and treatment of COVID-19 to diminish virus-induced inflammation.

186 - Phenotypic characterization and whole genome analysis of *Staphylococcus aureus* isolates colonizing the skin microbiota of patients with severe atopic dermatitis.

FRANCESCA SIVORI⁽¹⁾ - ILARIA CAVALLO⁽¹⁾ - MAURO TRUGLIO⁽¹⁾ - GIORGIA FABRIZIO⁽¹⁾ - FLAVIO DE MAIO⁽²⁾ - MAURIZIO SANGUINETTI⁽²⁾ - ALESSIA CAVALLO⁽³⁾ - EMANUELA CAMERA⁽³⁾ - ANTONIO CRISTAUDO⁽⁴⁾ - FULVIA PIMPINELLI⁽¹⁾ - ENEA GINO DI DOMENICO⁽⁵⁾

Microbiology and Virology, San Gallicano Dermatological Institute, IRCCS, ROMA, Italia⁽¹⁾ - Dipartimento di Scienze di Laboratorio e Infettivologiche, Fondazione Policlinico Universitario "A. Gemelli", IRCCS, ROMA, Italia⁽²⁾ - Laboratory of Cutaneous Physiopathology, San Gallicano Dermatological Institute, IRCCS, ROMA, Italia⁽³⁾ - Clinical Dermatology, San Gallicano Dermatological Institute, IRCCS, ROMA, Italia⁽⁴⁾ - Department of Biology and Biotechnology "C. Darwin", Sapienza University of Rome, ROMA, Italia⁽⁵⁾

Phenotypic characterization and whole genome analysis of *Staphylococcus aureus* isolates colonizing the skin microbiota of patients with severe atopic dermatitis.

Francesca SIVORI¹, Iliaria CAVALLO¹, Mauro TRUGLIO¹, Giorgia FABRIZIO¹, Flavio DE MAIO², Maurizio SANGUINETTI², Alessia CAVALLO³, Emanuela CAMERA³, Antonio CRISTAUDO⁴, Fulvia PIMPINELLI¹, Enea G. DI DOMENICO⁵

¹ Microbiology and Virology, San Gallicano Dermatological Institute, IRCCS, Rome, Italy; ² Dipartimento di Scienze di Laboratorio e Infettivologiche, Fondazione Policlinico Universitario "A. Gemelli" IRCCS, Rome, Italy; ³ Laboratory of Cutaneous Physiopathology, San Gallicano Dermatological Institute, IRCCS, Rome, Italy; ⁴ Clinical Dermatology, San Gallicano Dermatological Institute, IRCCS, Rome, Italy; ⁵ Department of Biology and Biotechnology "C. Darwin" Sapienza University of Rome, 00185 Rome, Italy.

Introduction. *Staphylococcus aureus* is a major skin colonizer during atopic dermatitis (AD) flares. Although host genetic risk factors are crucial to AD initiation, *S. aureus* significantly worsens disease severity. This study systematically investigates the skin physiology parameters, the composition of the stratum corneum lipidome, changes in the skin microbiome, and the factors contributing to *S. aureus* persistence in AD.

Materials and methods. We analyzed the transepidermal water loss (TEWL), the stratum corneum (SC) hydration, the composition of the stratum corneum lipidome, and microbiome variations in the lesional (LE) and non-lesional (NL) skin sites of 16 patients with severe AD and healthy control subjects (HS). In addition, a genotypic and phenotypic characterization was also performed to determine the adhesion, biofilm, and related regulatory genes in *S. aureus* isolates.

Results. We found significant differences in TEWL, SC, and lipid composition between AD patients and HS. Moreover, impaired skin barrier function was associated with significant variations in both microbiome diversity and composition. The skin of AD patients showed higher relative abundances of *S. aureus* and *S. lugdunensis* compared to HS. Besides, the increase in *S. aureus* and *S. lugdunensis* was negatively correlated with *Cutibacterium acnes* prevalence. Methicillin-resistant *S. aureus* (MRSA), sequence type 22 (ST22), accounted for 14.3% of the isolates and were resistant to 43.7% of all tested antibiotics. In addition, genes related to adhesion and biofilm formation were highly enriched in *S. aureus* isolates. Accordingly, *S. aureus* isolates were more efficient in early adhesion, biomass, and extracellular DNA production when compared with the *S. aureus* ATCC 6538 reference strain.

Discussion and Conclusions. Our findings indicate that an impaired skin barrier function is linked with a site-specific dysbiosis in AD compared to HS. Furthermore, derangement of small molecule and lipid metabolome together with dysbiosis selects for *S. aureus* isolates enriched in adhesins and biofilm-related genes. This investigation may contribute to designing reliable strategies for preventing and removing *S. aureus* colonization and reducing disease severity in AD.

196 - Effect of food supplements on gut microbiota in obese patients: preliminary results of zimba trial

ALICE CARAMASCHI⁽¹⁾ - SIMONETTA BELLONE⁽²⁾ - VALENTINA ANTONIOTTI⁽³⁾ - GIORGIA NOVELLO⁽⁴⁾ - ROBERTA RICOTTI⁽²⁾ - VALENTINA MANCIOPPI⁽²⁾ - FLAVIA PRODAM⁽³⁾ - ELISA BONA⁽¹⁾

Università del Piemonte Orientale, Dipartimento per lo Sviluppo Sostenibile e la Transizione Ecologica, Vercelli, Italia⁽¹⁾ - Università del Piemonte Orientale, Dipartimento di Scienze della Salute, SCU Pediatrica, Novara, Italia⁽²⁾ - Università del Piemonte Orientale, Dipartimento di Scienze della Salute, Novara, Italia⁽³⁾ - Università del Piemonte Orientale, Dipartimento di Scienze e Innovazione tecnologica, Alessandria, Italia⁽⁴⁾

Effect of food supplements on gut microbiota in obese patients: preliminary results of zimba trial

ALICE CARAMASCHI¹, SIMONETTA BELLONE⁴, VALENTINA ANTONIOTTI³, GIORGIA NOVELLO², ROBERTA RICOTTI⁴, VALENTINA MANCIOPPI⁴, FLAVIA PRODAM³, ELISA BONA^{1,5}

¹ Dipartimento per lo Sviluppo Sostenibile e la Transizione Ecologica, Università del Piemonte Orientale, Vercelli, Italy

² Dipartimento di Scienze e Innovazione Tecnologica, Università del Piemonte Orientale, Vercelli, Italy

³ Dipartimento di Scienze della Salute, Università del Piemonte Orientale, Novara, Italy

⁴ Dipartimento di Scienze della Salute, SCU Pediatrica, Novara, Italy

⁵ Centro Universitario per la Ricerca Traslazionale sulle Malattie Autoimmuni ed Allergiche, UPO-CAAD, Novara, Italy

INTRODUCTION. Obesity is a multifactorial problem now widespread throughout the world. Recent studies suggest that human microbiota can modulate the pathophysiology of obesity. Several microbes can ferment the non-digestible carbohydrates, such as prebiotics, influencing human health. This relationship of microbiota in causing obesity needs exploration. **MATERIALS AND METHODS.** Zimba project is a placebo-controlled clinical trial that study the efficacy of the association of Zinc, Myoinositol and GOS vs administration only GOS (placebo) in pediatric obesity. NGS analysis of 16S rDNA was performed according to Torre et al., 2022. Raw sequences were processed by MicroBAT software. Statistical analysis was performed using MicrobiomeAnalyst software. **RESULTS.** We analyzed 165 bacterial metagenomes in which alpha diversity showed not statistically significant differences but changes in beta diversity was observed. The prebiotic intake supplemented with Zn induced a significant increase in Firmicutes, Bifidobacterium sp., Faecalibacterium prausnitzii, unclassified Lactosporaceae, Ruminococcus and Dorea longicatena, and decrease in Bacteroides vulgatus and Parabacteroides distasonis in respect to baseline. **DISCUSSION AND CONCLUSION.** Some of these bacterial species are essential for a healthy microbiota homeostasis but they could being modulate by Zn presence. Further analyses linked to the clinical and metabolic responses will be necessary to validate the proposed mechanisms at the base of the microbiota modulation.

207 - *Mycobacterium avium* subspecies *paratuberculosis* (MAP) infection, and its impact on gut microbiome of individuals with Multiple Sclerosis

HAJRA ASHRAF⁽¹⁾ - UMER ZEESHAN IJAZ⁽²⁾ - LEONARDO ANTONIO SECHI⁽¹⁾

University of Sassari, Department of Biomedicine, Sassari, Italy⁽¹⁾ - University of Glasgow, University of Glasgow, Glasgow, United Kingdom⁽²⁾

Mycobacterium avium subspecies *paratuberculosis* (MAP) infection, and its impact on gut microbiome of individuals with Multiple Sclerosis

Hajra Ashraf¹, Umer Z. Ijaz^{2,3,4}, Leonardo A. Sechi^{1,5}

¹*Department of Biomedical Sciences; University of Sassari, Sassari, Italy

²Water & Environment Research Group, University of Glasgow, Mazumdar-Shaw Advanced Research Centre, Glasgow, United Kingdom

³National University of Ireland, Galway, University Road, Galway, Ireland

⁴Department of Molecular and Clinical Cancer Medicine, University of Liverpool, Liverpool, United Kingdom

⁵Complex Structure of Microbiology and Virology; AOU Sassari, Sassari, Italy

Introduction

There is a growing literature suggesting the role of gut microbiome in the etiology of Multiple sclerosis (MS) with development of disease being a multifactorial interaction of gut, brain, and the immune system. Whilst gut microbiology is a well-explored topic in MS in the context of healthy controls, there are hardly any studies that link microbiome of MS to *Mycobacterium avium* subspecies *paratuberculosis* (MAP) infection. Therefore, in this study, we further characterize the MS patients as MAP positive and negative, and try to understand their microbial profiles by considering their exposome (i.e., psycho-social, dietary and lifestyle).

Methods

Bacterial (16S rRNA) microbiome faecal samples (n=69) from Sardinia, Italy, divided into three categories, Healthy Controls (HC) (n=24), MS MAP+ (n=27), and MS MAP- (n=18), were collected, for two time points, T1, and T2, a month apart. These were processed to generate Operational Taxonomic Units (OTUs) (VSEARCH pipeline), along with their phylogeny, and taxonomic information (SILVA v138), as well as their predictive metabolic potential (PICRUST2). On this dataset, we have applied several multivariate statistical algorithms in R to explore their diversities (alpha and beta), core microbiome, and taxonomic/functional differential analyses.

Results

Using ANOVA, and accounting for subjects, alpha diversity differences were observed in taxonomic diversity (HC and MAP- at T1, $p < 0.001$ Chao Richness; HC T1 and MAP- T2, $p < 0.05$ Shannon Entropy), and not in functional diversities (MetaCyc pathways). In terms of beta diversity, using numerous distances in PERMANOVA, microbial profiles differ between HC, MAP-, and MAP+: Bray-Curtis (accounting for compositional differences, 4% variability, $p < 0.001$), Unweighted UniFrac (accounting for phylogenetic differences, 4% variability, $p < 0.001$), and Hierarchical Meta-Storms (accounting for functional differences, 5% variability, $p = 0.006$). Using Species Abundance Occupancy Diagrams, and fitting Sloan Prokaryotic Neutral Model, we have been able to identify core microbiome: those that are neutral, those that are selected by the hosts, and those that were based on dispersal limitation. Core phyla shared between MAP+ and MAP- belonged to Actinobacteria, Bacteroidata, Verrucomicrobiota, Firmicutes, with additional Desulfobacterota, and Proteobacteria observed in MAP+ only. Using a Quasi Conditional Association Test for Clusters, Archaea were over represented in MS samples, particularly in MAP+. These belonged to the genus *Methanobrevibacter* (also confirmed by additional DESeq2 analyses). Using contingency analyses, we are able to identify discriminatory patterns between MAP statuses and sources of variability, e.g., antibiotics usage, fish consumption, coffee, weight change, medications (Gilenya), and living locations. Using CODA-LASSO algorithm, we were able to find Ezakiella and PWY-6284: Superpathway of unsaturated fatty acid biosynthesis (*E. Coli*) as significantly positive association with EDSS score in MAP+, and Coprococcus and PWY-3941: beta-alanine biosynthesis II as significantly positive association with EDSS score in MAP- individuals.

Discussion and Conclusions

Overall, the results suggest altered microbiota between MAP+ and MAP- individuals suggesting dietary intervention to modulate gut microbiome, and that differences were more pronounced in terms of compositional changes than the functional potential of microbial communities.

243 - Gut microbiota profile in CDKL5 deficiency disorder patients

EMERENZIANA OTTAVIANO ⁽¹⁾ - ORNELLA XYNOMILAKIS ⁽²⁾ - CAMILLA CECCARANI ⁽³⁾ - SILVIA ANCONA ⁽¹⁾ - PAOLA TOGNINI ⁽⁴⁾ - AGLAIA VIGNOLI ⁽⁵⁾ - ELISA BORGHINI ⁽¹⁾

Universita' Degli Studi Di Milano, Dip. Scienze Della Salute, Milano, Italia ⁽¹⁾ - Universita' Degli Studi Di Milano, Dip. Scienze Biochimiche E Cliniche, Milano, Italia ⁽²⁾ - Consiglio Nazionale Delle Ricerche (Cnr), Istituto Tecnologie Biomediche, Milano, Italia ⁽³⁾ - Università Di Pisa, Department Of Translational Research And New Technologies In Medicine And Surgery, Pisa, Italia ⁽⁴⁾ - Asst Gom Niguarda, Ospedale Niguarda Ca' Granda, Milano, Italia ⁽⁵⁾

Gut Microbiota Profile In Cdkl5 Deficiency Disorder Patients

Emerenziana Ottaviano¹, Ornella Xynomilakis², Camilla Ceccarani⁵, Silvia Ancona¹, Paola Tognini³, Aglaia Vignoli⁴, Elisa Borghi¹

1- Department Of Health Sciences, Università Degli Studi Di Milano, Milan, Italy;

2- Department Of Biochemical And Clinical Sciences, Università Degli Studi Di Milano, Milan, Italy;

3- Department Of Translational Research And New Technologies In Medicine And Surgery, University Of Pisa, Pisa, Italy.

4- Neuropsychiatry Of Childhood And Adolescence, Asst Gom Niguarda, Ospedale Niguarda Ca' Granda, Milan, Italy;

5-Institute Of Biomedical Technologies, National Research Council, (Cnr), Segrate, Milan, Italy.

Introduction

CDKL5 deficiency disorder (CDD) is a rare neurodevelopmental disease caused by mutations in the X-linked cyclin-dependent kinase-like 5 CDKL5 gene, classified as a developmental epileptic encephalopathy. Epilepsy is a prominent feature and is often severe. Gastrointestinal problems are also common in CDD, together with subclinical immune dysfunction, and participate in clinical worsening. Studies on other neurodevelopmental disorders suggest that gut microbiota (GM) alterations could participate in associated comorbidities by acting through the gut-microbiota-brain axis. Up to date, no studies describe the GM composition in CDD patients. Hence, we aimed at investigating whether a distinct GM characterises CDD patients.

Materials and methods

We enrolled 17 CDD patients (mean age 10.9 ± 10.6 years; 15 females, 2 males) and 17 healthy controls (HC), siblings or parents /mean age 28.5 ± 17.2 years). For each participant we collected stool samples, clinical data through dedicated scales, and dietary nutritional parameters. GM was characterised by sequencing of the V3-V4 hypervariable region of the 16S rRNA bacterial gene on Illumina platform. Dietary food records were analysed using the commercial software Metadieta (Meteda, Italy). Seizures, sleep disorders, and other clinical data were assessed by online calls carried out by the research team.

Results

Dietary analysis showed a lower daily energy intake in CDD subjects. Nevertheless, we did not find differences in the percentage of micro- and macronutrients compared with HC. Although the number of patients was low, since the disorder is particularly rare, a trend toward a decrease in the gut microbial biodiversity was reported for CDD. The correlation analysis with clinical symptoms showed a decrease in alpha-diversity related to the lack of deambulation and the presence of sleep disorders. The unweighted Unifrac analysis (beta-diversity) showed a significant clustering of CDD patients' from HC. CDD microbial communities were enriched in the genera *Erysipelatoclostridium*, *Eggerthella*, *Enterococcus* and depleted in *Eubacterium*, *Roseburia*, *Odoribacter* and *Dorea*.

Discussion and conclusion

Our study demonstrates the existence of differences in the composition of the gut microbiota in CDD patients. In particular, some of the enriched genera in CDD have been previously reported in drug-resistant epilepsy. As dietary regimens, such as the ketogenic diet, have been demonstrated to reduce seizure occurrence in refractory epilepsy, interventions aimed at restoring gut microbiota composition could represent a therapeutic option for ameliorating CDD patients' quality of life by reducing comorbidities associated with the disease.

303 - Dynamics of nasopharyngeal tract mycobiota in COVID-19 patients

VERONICA FOLLIERO ⁽¹⁾ - CARLO FERRAVANTE ⁽¹⁾ - FEDERICA DELL'ANNUNZIATA ⁽¹⁾ - ROSARIO BRANCACCIO ⁽¹⁾ - ELENA ALEXANDROVA ⁽¹⁾ - DOMENICO DI ROSA ⁽¹⁾ - JESSICA LAMBERTI ⁽¹⁾ - DOMENICO PALUMBO ⁽¹⁾ - VALERIA CRUDELE ⁽²⁾ - ROBERTA MANENTE ⁽¹⁾ - RITA GRECO ⁽³⁾ - GIOVANNI BOCCIA ⁽¹⁾ - PASQUALE PAGLIANO ⁽¹⁾ - ALESSANDRO WEISZ ⁽¹⁾ - FRANCESCA RIZZO ⁽¹⁾ - GIANLUIGI FRANCI ⁽¹⁾

University of Salerno, Department of Medicine, Surgery and Dentistry, Scuola Medica Salernitana, Salerno, Italia ⁽¹⁾ - University Hospital of Campania "Luigi Vanvitelli, UOC of Virology and Microbiology, Napoli, Italia ⁽²⁾ - AORN Sant 'Anna and San Sebastiano, UOSD Microbiology, Caserta, Italia ⁽³⁾

Dynamics of nasopharyngeal tract mycobiota in COVID-19 patients

Veronica Folliero¹, Carlo Ferravante¹, Federica Dell'Annunziata¹, Rosario Brancaccio¹, Elena Alexandrova¹, Domenico Di Rosa¹, Jessica Lamberti¹, Domenico Palumbo¹, Valeria Crudele², Roberta Manente¹, Rita Greco³, Giovanni Boccia¹, Pasquale Pagliano¹, Alessandro Weisz¹, Francesca Rizzo¹, Gianluigi Franci¹

1. Department of Medicine, Surgery and Dentistry "Scuola Medica Salernitana", University of Salerno, Salerno, Italy;

2. UOC of Virology and Microbiology, University Hospital of Campania "Luigi Vanvitelli", Naples, Italy

3. UOSD Microbiology - AORN Sant 'Anna and San Sebastiano, Caserta, Italy.

Introduction: The nasopharyngeal tract harbours a complex microbial community that plays a crucial role in maintaining mucosal host homeostasis. Emerging evidence suggests that SARS-CoV-2 infection disrupts the human nasopharyngeal microbiome, with significant implications for disease progression and severity. While the impact of SARS-CoV-2 on bacterial communities has been extensively studied, our understanding of its effects on the mycobiota (fungal community) remains limited. Investigating the dynamics of fungal diversity in COVID-19 patients can enhance our understanding of disease pathogenesis, facilitate the development of innovative treatment strategies, and improve disease management. **Materials and Methods:** Nasopharyngeal swabs were collected from 55 COVID-19 positive patients exhibiting different symptom profiles during three distinct waves of COVID-19 in the Campania Region. Among the patients, 39 had mild symptoms, 6 had moderate symptoms, and 10 had severe symptoms. Sequencing was performed using the NextSeq 500 platform. Fungal taxonomy profiling was conducted by utilising the RefSeq comprehensive fungal genomes/protein database. **Results:** Our analysis revealed significant variations in fungal diversity based on the wave of sample collection and the severity of the disease. The nasopharyngeal mycobiota of patients from the second wave of infection exhibited a greater richness of species. In contrast, nasopharyngeal fungal diversity remained stable during the first and third waves of sample collection. The reduced mycobiota diversity observed in the first and third waves may be attributed to the stringent restrictions imposed during the initial year of the pandemic and in the post-summer periods when the positivity rate was high. Additionally, the phylum Ascomycota and its subphylum Saccharomycotina, as well as the phylum Basidiomycota, were more abundant in patients with severe symptoms compared to those with mild symptoms. Several species within these phyla are known to cause co-infections that exacerbate the symptoms of infectious diseases. **Discussion and Conclusions:** Our findings indicate that SARS-CoV-2 infection significantly alters the mycobiota of the nasopharyngeal tract, influencing disease progression and severity. Understanding the mycobiota composition in COVID-19 patients and the factors that influence it can contribute to improved disease

management. Our results highlight the variability of mycobiota diversity based on disease severity and the wave of sample collection. The phyla Basidiomycota and Ascomycota were found to be more abundant in patients with severe symptoms compared to those with mild symptoms. Furthermore, fungal population diversity was greater during the second wave, while it remained relatively stable during the first and third waves of sample collection, suggesting a dynamic impact of the disease on the fungal microbiome. Based on our research, COVID-19 induces significant dysbiosis of the fungal microbiome, which may contribute to disease pathogenesis. Understanding the underlying mechanisms of this dysbiosis can aid in the development of effective treatments.

305 - Dynamics of nasopharyngeal tract mycobiota in COVID-19 patients

VERONICA FOLLIERO ⁽¹⁾ - CARLO FERRAVANTE ⁽¹⁾ - FEDERICA DELL'ANNUNZIATA ⁽¹⁾ - ROSARIO BRANCACCIO ⁽¹⁾ - ELENA ALEXANDROVA ⁽¹⁾ - DOMENICO DI ROSA ⁽¹⁾ - JESSICA LAMBERTI ⁽¹⁾ - DOMENICO PALUMBO ⁽¹⁾ - VELERIA CRUDELE ⁽²⁾ - ROBERTA MANENTE ⁽¹⁾ - RITA GRECO ⁽³⁾ - GIOVANNI BOCCIA ⁽¹⁾ - PASQUALE PAGLIANO ⁽¹⁾ - ALESSANDRO WEISZ ⁽¹⁾ - FRANCESCA RIZZO ⁽¹⁾ - GIANLUIGI FRANCI ⁽¹⁾

University of Salerno, Department of Medicine, Surgery and Dentistry, Scuola Medica Salernitana, Salerno, Italia ⁽¹⁾ - University Hospital of Campania "Luigi Vanvitelli, UOC of Virology and Microbiology, Napoli, Italia ⁽²⁾ - AORN Sant 'Anna and San Sebastiano, UOSD Microbiology, Caserta, Italia ⁽³⁾

Dynamics of nasopharyngeal tract mycobiota in COVID-19 patients

Veronica Folliero¹, Carlo Ferravante¹, Federica Dell'Annunziata¹, Rosario Brancaccio¹, Elena Alexandrova¹, Domenico Di Rosa¹, Jessica Lamberti¹, Domenico Palumbo¹, Valeria Crudele², Roberta Manente¹, Rita Greco³, Giovanni Boccia¹, Pasquale Pagliano¹, Alessandro Weisz¹, Francesca Rizzo¹, Gianluigi Franci¹

1. Department of Medicine, Surgery and Dentistry "Scuola Medica Salernitana", University of Salerno, Salerno, Italy;

2. UOC of Virology and Microbiology, University Hospital of Campania "Luigi Vanvitelli", Naples, Italy

3. UOSD Microbiology - AORN Sant 'Anna and San Sebastiano, Caserta, Italy.

Introduction: The nasopharyngeal tract harbours a complex microbial community that plays a crucial role in maintaining mucosal host homeostasis. Emerging evidence suggests that SARS-CoV-2 infection disrupts the human nasopharyngeal microbiome, with significant implications for disease progression and severity. While the impact of SARS-CoV-2 on bacterial communities has been extensively studied, our understanding of its effects on the mycobiota (fungal community) remains limited. Investigating the dynamics of fungal diversity in COVID-19 patients can enhance our understanding of disease pathogenesis, facilitate the development of innovative treatment strategies, and improve disease management. **Materials and Methods:** Nasopharyngeal swabs were collected from 55 COVID-19 positive patients exhibiting different symptom profiles during three distinct waves of COVID-19 in the Campania Region. Among the patients, 39 had mild symptoms, 6 had moderate symptoms, and 10 had severe symptoms. Sequencing was performed using the NextSeq 500 platform. Fungal taxonomy profiling was conducted by utilising the RefSeq comprehensive fungal genomes/protein database. **Results:** Our analysis revealed significant variations in fungal diversity based on the wave of sample collection and the severity of the disease. The nasopharyngeal mycobiota of patients from the second wave of infection exhibited a greater richness of species. In contrast, nasopharyngeal fungal diversity remained stable during the first and third waves of sample collection. The reduced mycobiota diversity observed in the first and third waves may be attributed to the stringent restrictions imposed during the initial year of the pandemic and in the post-summer periods when the positivity rate was high. Additionally, the phylum Ascomycota and its subphylum Saccharomycotina, as well as the phylum Basidiomycota, were more abundant in patients with severe symptoms compared to those with mild symptoms. Several species within these phyla are known to cause co-infections that exacerbate the symptoms of infectious diseases. **Discussion and Conclusions:** Our findings indicate that SARS-CoV-2 infection significantly alters the mycobiota of the nasopharyngeal tract, influencing disease progression and severity. Understanding the mycobiota

composition in COVID-19 patients and the factors that influence it can contribute to improved disease management. Our results highlight the variability of mycobiota diversity based on disease severity and the wave of sample collection. The phyla Basidiomycota and Ascomycota were found to be more abundant in patients with severe symptoms compared to those with mild symptoms. Furthermore, fungal population diversity was greater during the second wave, while it remained relatively stable during the first and third waves of sample collection, suggesting a dynamic impact of the disease on the fungal microbiome. Based on our research, COVID-19 induces significant dysbiosis of the fungal microbiome, which may contribute to disease pathogenesis. Understanding the underlying mechanisms of this dysbiosis can aid in the development of effective treatments.

316 - In vitro study of various bacterial strains from human microbiota cultured in different types of advanced 3D substrates.

EMANUELA PELUSO⁽¹⁾ - DANIELA PACHECO⁽²⁾ - SEBASTIAO VAN UDEN⁽²⁾ - SONJA VISENTIN⁽³⁾ - PAOLA PETRINI⁽⁴⁾ - LIVIA VISAI⁽⁵⁾

Università degli studi di Pavia, Department of molecular medicine, Pavia, Italia⁽¹⁾ - Bac3Gel Lda, TagusPark—Edificio Inovacao II, Porto Salvo, Portogallo⁽²⁾ - Università degli studi di Torino, Department of Molecular Biotechnology and Health Science, Torino, Italia⁽³⁾ - Politecnico di Milano, Department of Chemistry, Materials and Chemical Engineering, Milano, Italia⁽⁴⁾ - Università degli studi di Pavia, Department of Molecular Medicine, Pavia, Italia⁽⁵⁾

In vitro study of various bacterial strains from human microbiota cultured in different types of advanced 3D substrates.

EMANUELA PELUSO¹, DANIELA PACHECO², SEBASTIÃO VAN UDEN², SONJA VISENTIN³, PAOLA PETRINI⁴, LIVIA VISAI^{1,5}

¹Department of Molecular Medicine, Center for Health Technologies, UdR INSTM, University of Pavia, Pavia, Italy; ²Bac³Gel Lda, TagusPark—Edificio Inovacao II, Porto Salvo, Portugal;

³Department of Molecular Biotechnology and Health Science, University of Torino, Torino, Italy;

⁴Department of Chemistry, Materials and Chemical Engineering "Giulio Natta", Politecnico di Milano, Milano, Italy; ⁵Medicina Clinica-Specialistica, UOR5 Laboratorio di Nanotecnologie, ICS Maugeri, IRCCS, Pavia, Italy.

1.Introduction.Trillions of microbes have evolved with and continue to live on and within human beings. A population of these “good” germs gradually colonizes the human body from birth and forms the classic so-called “normal microbial flora”, currently reported as microbiota. Host–microbe interactions occur primarily along mucosal surfaces; mucus is a human-designed barrier that covers all organs interfacing with the outside world, specific to each environment of the human body with a unique set of microorganisms. However, most of the microbial diversity in any environment has not yet been cultured. In nature, bacteria grow and develop by forming biofilms. Most of the experiments carried out in vitro are realized by using the planktonic culturing method, failing to recreate how bacteria grow and develop in real life. By copying key properties of the human mucus, Bac³Gel® (bac3gel.com) is a newly generation of advanced 3D substrates for culturing microorganisms, exhibiting micro-gradients that recreate several types of human habitat.

2.Materials and Methods. For this purpose, a set of seven different bacterial strains has been used: E. coli, E. faecalis, L. monocytogenes, A. baumannii, K. pneumoniae, S. aureus and P. aeruginosa. Each of these has a specific human environment in which it is preferentially expressed, or like the so-called ESKAPEs (Enterococcus spp, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, Enterobacter spp), these can colonize anywhere in the human organism. After carrying out the infection for 24 and 48 hours, gels and planktonic cultures have been analyzed with a newly technique, called spot plating. Qualitative analyses were conducted in order to highlight populations of live and dead bacteria on the gels.

3.Results. Gel has no negative impact on bacterial growth, proving that Bac³Gel in vitro technology allows the growth of all the bacterial strains as an environment very similar to the human body.

4.Discussion and conclusions. What truly changes is the association of bacteria organized as

filamentous-like structures that appeared in a brown coloration easily distinguished by naked eye from the transparent structure of Bac³Gel. This may be associated with the different gradients present simultaneously in gel's structure, while this is not present in planktonic cultures in which all bacteria grow under the same conditions of oxygen and nutrients. Examination of the seven bacteria strains under the confocal microscope showed remarkable viability, persisting even at 48 hours. Bac³Gel® can offer a 3D substrate for laboratory cultivation of difficult-to-cultivate bacteria by providing different nutrient conditions, oxygen tension and viscoelastic properties in a single structure.

323 - Bacterial, fungal and viral gut microbiota signatures predict immunotherapy response in cutaneous and mucosal melanoma patients

VALERIO IEBBA⁽¹⁾ - SATORU YONEKURA⁽²⁾

Università di Trieste, Dipartimento Universitario Clinico di Scienze Mediche Chirurgiche e della Salute, Trieste, Italia⁽¹⁾ - Kyoto University Graduate School of Medicine, Kyoto, Japan., Department of Dermatology, Kyoto, Giappone⁽²⁾

Bacterial, fungal and viral gut microbiota signatures predict immunotherapy response in cutaneous and mucosal melanoma patients

Valerio Iebba¹, Satoru Yonekura²

AFFILIATIONS

1 Department of Medical, Surgical and Health Sciences, University of Trieste, Trieste, Italy.

2 Department of Dermatology, Kyoto University Graduate School of Medicine, Kyoto, Japan.

Introduction: Over the past decade, accumulating evidence points to the clinical impact of the intestinal microbiota on therapeutic outcome of immune checkpoint inhibitors (ICI). Although specific fecal bacterial species have been associated with beneficial responses in meta-analyses, no clear consensus exists on a bacterial, fungal or viral fingerprint identifying responsiveness to immunotherapy in melanoma patients. **Materials and Methods:** We analyzed seven different cohorts of fecal shotgun metagenomes from cutaneous melanoma patients (n=407) retrieved from the literature and added a new cohort of mucosal melanoma patients (n=33) aiming at disentangling, through dedicated bioinformatic pipelines and machine-learning analyses, the microbial signatures of non-responders (NR) or responders (R) to ICI. In addition to taxonomical recognition, bacterial genes and pathways were taken into account to build a predictive model. We also reconstructed ecosystem networks in responders and non-responders to ICI and detected patterns of microbial species leading to a diagnostic tool predicting the individual response to immunotherapy. **Results:** Employing dedicated bioinformatic pipelines, we found 458 bacterial, 180 fungal and 75 viral species, along with 384 bacterial pathways. Using a leave-one-dataset-out (LODO) and a random forest machine learning algorithm, we were able to find robust fecal microbial signatures able to predict the NR status, with averaged area-under-curve (AUC) values ranging from 0.65 to 0.78. Notably, using the bacterial genetic signatures, and not the pathway-based, raised the AUC values of roughly 8%, leading to an averaged AUC of 0.85 for a specific subset of microbes and genes (patent pending). The sensitivity, specificity, positive and negative predictive value of this signature were 81%, 75%, 67% and 68%, respectively. **Discussion and Conclusions:** We here disclose a combination of microbial and genetic signatures able to predict the non-responsiveness to ICI in both cutaneous, the most prevalent, and mucosal melanoma patients. The individual basis of the prediction raises the possibility to use this combined signature in precision-medicine within the melanoma setting, which has an incidence of more than 300k cases per year.

325 - A metagenomic cross-cohort analysis of bacterial virulence factors in colorectal cancer

VALERIO IEBBA ⁽¹⁾ - SATORU YONEKURA ⁽²⁾

Università di Trieste, Dipartimento Universitario Clinico di Scienze Mediche Chirurgiche e della Salute, Trieste, Italia ⁽¹⁾ - Kyoto University Graduate School of Medicine, Kyoto, Japan., Department of Dermatology, Kyoto, Giappone ⁽²⁾

A metagenomic cross-cohort analysis of bacterial virulence factors in colorectal cancer

Valerio Iebba¹, Satoru Yonekura²

AFFILIATIONS

1 Department of Medical, Surgical and Health Sciences, University of Trieste, Trieste, Italy.

2 Department of Dermatology, Kyoto University Graduate School of Medicine, Kyoto, Japan.

Introduction: Colorectal cancer (CRC) is a disease, caused by multi-etiological factors, affecting more than 2 million people worldwide. The last decade witnessed the discovery within CRC of fecal microbial (FM) members able to trigger and drive a prolonged cancer-related cellular inflammation. CRC-related microbial members, intermingled within a network of interactions able to self-segregate into communities (“guilds”), produce virulence factors (VFs) as adhesins, invasins, antimicrobial resistances and genotoxins, able to exacerbate intestinal inflammation and the overall microbial oncogenic potential. Materials and Methods: We analyzed fecal shotgun metagenomics samples from eleven different cohorts of colorectal cancer patients (n=1729) and healthy volunteers (n=2533) retrieved from the literature. We employed dedicated pipelines to: i) depict taxonomical differences; ii) build metagenome-assembled genomes (MAGs) and related functional annotation; iii) using specific databases for known VFs in order to build networks of co-occurring pathogenicity profiles. Results: Using a leave-one-dataset-out (LODO) and a random forest model, we found higher biodiversity and richness in CRC fecal samples, with higher relative abundances of the known bacterial species statistically related to CRC progression, such as *Fusobacterium nucleatum* and the recently patented (by the author) species discovered as pan-cancer biomarkers (*Clostridium hathewayi*, *Enterocloster asparagiforme*, *Enterocloster bolteae*). Through a network analysis, we depicted the existence of six communities, named species-interacting groups (SIGs), and after applying a cohort-coded color we found that CRC species clustered in two communities, at a significant distance from controls ($p < 0.001$). We then analyzed the VFs with dedicated databases and algorithms, highlighting the higher prevalence of bacterial siderophores and related genotoxins (such as colibactin) in CRC samples ($p = 0.002$). Through MAGs phylogeny reconstruction, we made a genomic comparison of the pathogenicity island pks, which encodes for colibactin, depicting its distribution and modular complexity within the CRC-related pathogens. Discussion and Conclusions: This study, applied to CRC setting but extendible to other cancers, would pave the way for a VFs-oriented examination of the pathogenic potential of microbial members related to cancer.

05 Modelli matematici ed epidemiologia/Epidemiologia molecolare

15 - Genomic epidemiology of *K. pneumoniae* carbapenemase-producing *Klebsiella pneumoniae* (KPC-Kp) strains from three Northern-Italy hospitals and investigation of intra- and inter- hospitals genetic diver

VALERIA FOX⁽¹⁾ - DAVIDE MANGIONI⁽²⁾ - AGNESE COMELLI⁽³⁾ - SILVIA RENICA⁽¹⁾ - MARIANNA ROSSI⁽⁴⁾ - MADDALENA CASANA⁽⁵⁾ - LUDOVICA MELA⁽³⁾ - ALESSANDRA BIELLI⁽⁶⁾ - CHIARA S. VISMARA⁽⁶⁾ - PAOLO BONFANTI⁽⁴⁾ - BARBARA BOTTAZZI⁽⁷⁾ - BARBARA CASSANI⁽⁷⁾ - PAOLA MORELLI⁽⁵⁾ - CARLO F. PERNO⁽⁸⁾ - ANDREA GORI⁽⁹⁾ - ANTONIO MUSCATELLO⁽³⁾ - ALESSANDRA BANDERA⁽²⁾ - CLAUDIA ALTERI⁽¹⁾

Department of Oncology and Hemato-Oncology, University of Milan, Milan, Italia⁽¹⁾ - Infectious Diseases Unit, Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, & Department of Pathophysiology and Transplantation, University of Milan, Milan, Italia⁽²⁾ - Infectious Diseases Unit, Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italia⁽³⁾ - Infectious Diseases Unit, San Gerardo Hospital, Monza, Italia⁽⁴⁾ - Infectious Diseases Unit, Humanitas Clinical and Research Center IRCCS, Rozzano, Italia⁽⁵⁾ - Complex Unit of Clinical Microbiology, Niguarda Hospital, Milan, Italia⁽⁶⁾ - Humanitas Clinical and Research Center, IRCCS, Rozzano, Italia⁽⁷⁾ - Multimodal Research Area, Bambino Gesù Children Hospital IRCCS, Rome, Italia⁽⁸⁾ - Department of Pathophysiology and Transplantation, University of Milan, & Division of Infectious Diseases, L. Sacco University Hospital, Milan, Italia⁽⁹⁾

Category: Modelli matematici ed epidemiologia/Epidemiologia molecolare

Title: Genomic epidemiology of *K. pneumoniae* carbapenemase-producing *Klebsiella pneumoniae* (KPC-Kp) strains from three Northern-Italy hospitals and investigation of intra- and inter- hospitals genetic diversity

Authors: Valeria Fox¹, Davide Mangioni^{2,3}, Agnese Comelli², Silvia Renica¹, Marianna Rossi⁴, Maddalena Casana⁵, Ludovica Mela², Alessandra Bielli⁶, Chiara S. Vismara⁶, Paolo Bonfanti⁴, Barbara Bottazzi⁷, Barbara Cassani⁷, Paola Morelli⁵, Carlo F. Perno⁸, Andrea Gori^{3,9}, Antonio Muscatello², Alessandra Bandera^{2,3}, Claudia Alteri¹

¹Department of Oncology and Hemato-Oncology, University of Milan, Milan, Italy

²Infectious Diseases Unit, Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy

³Department of Pathophysiology and Transplantation, University of Milan, Milan, Italy

⁴Infectious Diseases Unit, San Gerardo Hospital – Monza, Italy

⁵Infectious Diseases Unit, Humanitas Clinical and Research Center IRCCS, Rozzano - Milan, Italy

⁶Complex Unit of Clinical Microbiology, Niguarda Hospital, Milan, Italy

⁷Humanitas Clinical and Research Center IRCCS, Rozzano – Milan, Italy

⁸Multimodal Research Area, Bambino Gesù Children Hospital IRCCS, Rome, Italy

⁹Division of Infectious Diseases, L. Sacco University Hospital, Milan, Italy

Introduction

K. pneumoniae carbapenemase-producing *Klebsiella pneumoniae* (KPC-Kp) is a leading cause of hospital-acquired infections globally, thus its genomic surveillance is crucial for virulence and drug-resistance monitoring and outbreak containment.

Materials and methods

A genomic analysis was performed on 87 KPC-Kp strains isolated from 3 hospitals in Northern-Italy in 2019-2021. KPC-Kp strains whole genome was sequenced to assess sequence types (STs), capsular and lipopolysaccharide loci, and characterize resistome, virulome and mobilome. Maximum Likelihood method and Minimum Spanning Tree (MST) based on CoreSNP alignment were used to determine strains correlations and identify putative transmission chains.

Results

Predominant STs included ST307 (35, 40.2%), ST512/1519 (sharing 1 allele difference, 15, 17.2%), ST20 (12, 13.8%), and ST101 (7, 8.1%), while remaining isolates (13, 14.9%) belonged to STs represented by less than 5 isolates. Carbapenemase genes were present in all strains, always on a Tn4401a transposon, and included KPC-3 (48, 55.2%), KPC-2 (38, 43.7%) and KPC-33 (1, 1.2%), the latter conferring resistance to ceftazidime/avibactam (CZA). Seven (8.1%) KPC-Kp strains carried multiple Tn4401a copies and displayed higher meropenem (MEM) and CZA resistance compared to those carrying 1 copy (median[IQR] MIC of 32[32-32] vs 10[8-32] mg/ml for MEM, $p=0.035$, and 7[6.5-7.5] vs 1.5[1-2] mg/ml for CZA, $p=0.002$). Virulence factors included siderophores enterobactin (87, 100%), yersiniabactin (53, 60.9%) and aerobactin (11, 12.6%), and a truncated *rmpA2*, a gene normally associated to hypermucoidy (5, 5.8%). Of note, aerobactin and *rmpA2* were almost exclusively present in ST101 ($p<0.001$ both) and ST512/1519 ($p=0.020$ and $p=0.165$, respectively), on the same plasmid. The median(IQR) number of plasmids per strain was 4(3-6), with ST20 and ST101 carrying the highest number (7[7-7] and 6[4-7], respectively, $p<0.001$). By ML and MST, strains belonging to ST307, ST512/1519 and ST258 did not show evidence of transmission chains or clustering based on hospital. Strains belonging to ST20 all came from the same hospital and 10/12 strains were found to belong to a single cluster, characterized by a lower median[IQR] n° of SNPs (7[5-11] vs 414[413-417], $p<0.001$). Five out of 7 ST101 strains came from the same hospital, and 4 of these formed a cluster with lower median[IQR] n° of SNPs (7.5[5-10] vs 176[123-177], $p=0.028$).

Discussion and Conclusions

Our findings provide in-depth insights into circulating KPC-Kp in Northern-Italy, allowing to discriminate STs against resistome and virulome, and to address new perspectives on intra- and inter-hospital circulation dynamics. These results lay the ground for the definition of STs at higher risk of clonal dissemination.

93 - Microorganisms from human clinical samples as a tool for technological innovation: creation of a shared platform of the UNITO collections.

LORENZA CAVALLO ⁽¹⁾ - JANIRA ROANA ⁽¹⁾ - ANTONIO CURTONI ⁽¹⁾ - ALESSANDRO BONDI ⁽¹⁾ - NARCISA MANDRAS ⁽¹⁾ - MATTIA GENCO ⁽¹⁾ - IOLANDA PERUGINI ⁽²⁾ - DANIELA SCALAS ⁽³⁾ - LUCA COCOLIN ⁽⁴⁾ - FRANCESCA MENOTTI ⁽¹⁾ - VALERIA PRIGIONE ⁽²⁾ - VIVIAN TULLIO ⁽¹⁾ - CRISTINA VARESE ⁽²⁾ - CRISTINA COSTA ⁽¹⁾

UNIVERSITA' DI TORINO, DIP SCIENZE DELLA SANITA' PUBBLICA E PEDITRICHE, TORINO, Italia ⁽¹⁾ - UNIVERSITA' DI TORINO, SCIENZE DELLA VITA E DEI SISTEMI, TORINO, Italia ⁽²⁾ - UNIVERSITA' DI TORINO, Dipartimento di Scienze Veterinarie, TORINO, Italia ⁽³⁾ - UNIVERSITA' DI TORINO, Dipartimento di Scienze Agrarie, Forestali e Alimentari, TORINO, Italia ⁽⁴⁾

Microorganisms from human clinical samples as a tool for technological innovation: creation of a shared platform of the UNITO collections.

LORENZA CAVALLO¹, JANIRA ROANA¹, ANTONIO CURTONI^{1,2}, ALESSANDRO BONDI^{1,2}, NARCISA MANDRAS¹, MATTIA GENCO¹, IOLANDA PERUGINI³, DANIELA SCALAS⁴, LUCA COCOLIN⁵, FRANCESCA MENOTTI¹, VALERIA PRIGIONE³, VIVIAN TULLIO¹, CRISTINA VARESE³, CRISTINA COSTA^{1,2}

¹Department of Public Health and Pediatrics, University of Turin, Italy; ²Microbiology and Virology Unit, University Hospital Città della Salute e della Scienza di Torino, Italy; ³Department of Life Sciences and Systems Biology, University of Turin, Italy; ⁴ Microbiology and Infectious Diseases Unit, Department of Veterinary Sciences, University of Turin, Italy; ⁵ Department of Agricultural, Forest and Food Sciences, University of Turin, Italy.

Introduction. The Turin University Culture Collection - TUCC – is the microbial collection edited by the University of Turin (Italy), result of the collaboration of four departments: MUT collection at the Life Sciences and Systems Biology department (DBIOS), MiAGR collection at the Agricultural, Forest and Food Sciences department (DISAFA), MiBAT collection at the Public Health and Pediatrics department (DSSPP) and MiVET at the Veterinary Science department (DSV). In particular, MiBAT collection represents an important resource for clinical research and for sharing knowledge with other universities, in Italy and abroad. Microorganisms, such as yeasts, filamentous fungi, and bacteria, are mainly isolated from human clinical sample (skin, urine, blood, bronchoalveolar lavage, etc) derived from community or hospitalized patients. The aim of this project is to guarantee the conservation and valorization of a huge number of microbial strains that play relevant roles in different sectors of our country.

Materials and Methods. Bacterial and fungal strains identification was performed on overnight subcultures both using matrix-assisted laser desorption ionization-time of flight mass spectrometry and Next Generation Sequencing using the 16S rRNA gene. Strains were sequenced on Illumina sequencing platform. Furthermore, microorganisms were stored and preserved by using different techniques including cryopreservation in glycerol (-152°C), cryopreservation through cryovials (-80°C) and lyophilization.

Results. To date, the MiBAT collection includes more than 60 bacteria clinical strains, as well as 40 different strains of mold and 30 strains of yeast. In particular, multidrug-resistant bacteria such as

extended-spectrum-beta-lactamase-producing Enterobacteriaceae, *Klebsiella pneumoniae* serin-carbapenemase, New-Delhi metallo-beta-lactamase, Verona integron-encoded metallo-beta-lactamase producing Enterobacteriaceae, methicillin-resistant *Staphylococcus aureus* and vancomycin and teicoplanin resistant *Enterococcus faecium*, are included. Additionally, the collection includes also multidrug-resistant fungal pathogens such as *Candida auris*. All microorganisms are available for research use via the TUCC portal (www.tucc.unito.it), a publicly accessible web portal.

Discussion and Conclusions. TUCC provides important services for the academic world, industry, and institutions; it brings together the University's microbial resources, equipment, and staff, optimizing management and ensuring access to both resources and services that individual structures can provide. Finally, TUCC ensures the proper administration of complex legal procedures in order to comply with national and international norms governing genetic resource access, distribution, and exploitation.

106 - Human enterovirus surveillance in the Marche Region (Italy)

KATIA MARINELLI ⁽¹⁾ - STEFANO MENZO ⁽²⁾ - SARA CAUCCI ⁽²⁾

SOSD VIROLOGIA, Azienda Ospedaliera Universitaria delle Marche, ANCONA, Italia ⁽¹⁾ -
dipartimento di scienze biomediche e salute pubblica, università politecnica delle Marche,
ANCONA, Italia ⁽²⁾

Human enterovirus surveillance in the Marche Region (Italy)

KATIA MARINELLI ², SARA CAUCCI ¹ AND STEFANO MENZO^{1,2}

¹Department of Biomedical Sciences and Public Health, Università Politecnica delle Marche,
Ancona, Italy;

²Virology Laboratory, Azienda Ospedaliera Universitaria delle Marche, Ancona, Italy;

Abstract

1.Introduction

Enteroviruses are ubiquitous non-enveloped viruses, usually responsible of mild and transient systemic infections, with a vast syndromic spectrum as they can affect the respiratory tract, the skin, the gastrointestinal tract, the nervous system, the serous membranes and muscle. The most severe forms of the disease can be observed in newborns and in immunosuppressed patients. The aim of our Laboratory was to study the molecular epidemiology of enterovirus types and the different clinical syndromes associated with enterovirus infections.

2.Materials and methods

Clinical samples: feces, CSF, nasopharyngeal swabs, blood were analyzed by an in-house nested RT-PCR assay targeting a sequence in the VP1 region and subsequently genotyped by Sanger sequencing technique. Sequences were aligned with CustalV and phylogenetic reconstruction was obtained by Maximum Likelihood on the MEGA11 software package.

3.Results

736 consecutive samples relating to 523 enterovirus-infected patients, either outpatients or hospitalized in various wards of Region Marche during the period 1 June 2010-31 May 2023 were examined. Pediatric population was involved in 70% of cases of infection. Phylogenetic analysis showed that enterovirus infections followed a seasonal pattern with micro-epidemics recurring especially in summer in clear monophyletic clusters. Interestingly, major epidemic clusters were observed in the summer in seaside resorts. As for other viral infections, after the lower circulation during the COVID pandemic years, a surge in enterovirus infections observed in the first months of 2023, compared to 2019 (+100%). In the global cohort, the most frequently identified types were: Echovirus 30, Coxsackievirus A6, Coxsackievirus B5, Echovirus 6, albeit each year different types could prevail. Clinical syndromes most frequently associated with the infections were: 219 meningitis, 95 exanthematous diseases, 11 neonatal sepsis, 4 myocarditis, 82 respiratory infections. In our cohort we recorded 2 fatal outcomes for neonatal sepsis (Echovirus 11 and Coxsackie B4) and 2 in children for myocarditis (enterovirus 90).

4.Discussion and conclusions

Enterovirus infections are usually associated to mild disease, and in the absence of serologic data, their circulation in the population is difficult to assess. However, incidence is high in the pediatric population, and some strains are associated to potentially life threatening disease and have epidemic potential, especially in crowded seaside resorts, where sewage systems may become insufficient.

Molecular monitoring of enteroviral infections is therefore granted, in order to promptly identify epidemic clusters, especially this year, when a significant epidemic peak is to be expected.

114 - Frequency and Variation of Viral Respiratory Infections in Pre- and During - COVID-19 Pandemic: A 7-year experience in a teaching hospital of Southern Italy

GRAZIA PAVIA⁽¹⁾ - GIUSEPPE GUIDO MARIA SCARLATA⁽¹⁾ - CLAUDIA CICINO⁽¹⁾ - MICHELE MANNO⁽¹⁾ - MARIA GIUSEPPINA STILLITANO⁽¹⁾ - GIORGIO SETTIMO BARRECA⁽¹⁾ - ANGELO GIUSEPPE LAMBERTI⁽¹⁾ - AIDA GIANCOTTI⁽¹⁾ - LUIGIA GALLO⁽¹⁾ - RAFFAELLA SINOPOLI⁽¹⁾ - ELENA COLOSIMO⁽¹⁾ - NADIA MARASCIO⁽¹⁾ - ANGELA QUIRINO⁽¹⁾ - GIOVANNI MATERA⁽¹⁾

Università degli Studi "Magna Graecia" di Catanzaro, Azienda Ospedaliero-Universitaria "R. Dulbecco" Presidio "Mater Domini", Catanzaro, Dipartimento Scienze della Salute, Catanzaro, Italia⁽¹⁾

Frequency and Variation of Viral Respiratory Infections in Pre- and During -COVID-19 Pandemic: A 7-year experience in a teaching hospital of Southern Italy

GRAZIA PAVIA¹, GIUSEPPE G.M. SCARLATA¹, CLAUDIA CICINO¹, MICHELE MANNO¹, MARIA G. STILLITANO¹, GIORGIO S. BARRECA¹, ANGELO G. LAMBERTI¹, AIDA GIANCOTTI¹, LUIGIA GALLO¹, RAFFAELLA SINOPOLI¹, ELENA COLOSIMO¹, NADIA MARASCIO¹, ANGELA QUIRINO¹ AND GIOVANNI MATERA¹

¹ Department of Health Sciences, Unit of Clinical Microbiology, "Magna Græcia" University Hospital of Catanzaro; Catanzaro, Italy

Introduction: COVID-19 pandemic had an indirect effect on typical seasonal patterns of Viral Respiratory Infections (VRIs) worldwide. Since VRIs prevalence may affect clinical prediction and management resources, the aim of this study was to evaluate the impact of COVID-19 pandemic on frequency and circulation of respiratory viruses in a Teaching Hospital of Southern Italy in pre- and during-COVID-19 pandemic periods. **Materials and Methods:** A total of 3484 nasopharyngeal swabs from patients with suspected VRIs attending to University Hospital, between January 2017 and May 2023, were analyzed by BioFire® RP2.1 Plus and QIAstat-Dx® Respiratory SARS-CoV-2 panels. The samples processed before March 15, 2020, were considered "pre-COVID-19" and those from March 15, 2020 to May 5, 2023, were considered "during-COVID-19". Statistical analysis was performed by ChiSquare test ($p \leq 0.05$). **Results:** Overall, 725/3484 (21%) patients were positive for at least one respiratory virus, among them 439 (61%) were male, with mean age of 61 years (range 18-98). The "pre-COVID-19" positive samples were 115/369 (32%), while "during-COVID-19" positive samples were 610/3115 (19.5%). Male patients were the most affected (65% vs. 60%) and mean age was overlapped (63 vs. 61 age) among both groups, respectively. Age stratification showed a positivity rate significantly higher in >65 age group (64% vs. 55%, $p \leq 0.05$) for both periods considered, respectively. Regarding viral targets circulation in "pre-COVID-19" group, Influenza A Virus was the most prevalent (59%) followed by Human Rhinovirus (HRV) (14%). Interestingly, the spread of SARS-CoV-2 (56%, 350/610) from March 2020 onwards has led to marked and significant decrease of Influenza A Virus (59% vs. 4%; $p = 0.0001$) circulation, followed by a mild increase of HRV (14% vs. 25%, $p = 0.046$). Viral co-infections were unchanged in both groups (about 5%). In "pre-COVID-19" samples, the major percentage (60%, 3/5) of viral co-infection had combinations of HRV plus Adenovirus and HRV plus Respiratory Syncytial Virus

(RSV). In "during-COVID-19" group, HRV plus SARS-CoV-2 (35%, 13/37) was the main viral co-infection. Discussion and Conclusions: COVID-19 pandemic and public health implementation of non-pharmaceutical interventions, had mitigated VRIs transmission. In agreement with scientific literature, we observed a notable decreasing of seasonal circulation of Influenza A Virus. Interestingly, we observed that RSV epidemiology remained unaltered in pre- and during-COVID-19 pandemic period and, in addition, we reported a predominant co-circulation of HRV with SARS-CoV-2. Our study highlights the importance of characterizing epidemiological patterns of VRIs to control infection, especially in patients more susceptible to severe complications.

137 - A novel epidemiological tool: Fourier Transform infrared spectroscopy (FT-IR) for real time tracing of *Klebsiella pneumoniae* producing NDM-5 and NDM-1/OXA-48-like enzymes

ELENA ADDIS⁽¹⁾ - MATTIA SCARAZZAI⁽¹⁾ - ANNA BERTONCELLI⁽¹⁾ - DAVIDE GIBELLINI⁽¹⁾ - ANNARITA MAZZARIOL⁽¹⁾

Università di Verona, Dipartimento di Diagnostica e Sanità Pubblica, Verona, Italia⁽¹⁾

A novel epidemiological tool: Fourier Transform infrared spectroscopy (FT-IR) for real time tracing of *Klebsiella pneumoniae* producing NDM-5 and NDM-1/OXA-48-like enzymes

ELENA ADDIS¹, MATTIA SCARAZZAI¹, ANNA BERTONCELLI¹, DAVIDE GIBELLINI¹, ANNARITA MAZZARIOL¹

¹Department of Diagnostics and Public Health, University of Verona, Verona, Italy

Introduction. Extensively drug-resistant (XDR) *Klebsiella pneumoniae* (KPN) is globally recognised as a major cause of difficult-to-treat healthcare-acquired infections; the rapid identification of XDR strains plays a key role for the rapid isolation of patients carrying these bacteria. However, patient isolation fails to completely avoid the spread of bacteria, thus it is important to rapidly analyze the clonality of isolates belonging to the same species. The aim of this study is to analyze the clonal relationships among XDR KPN clinical strains, with a new analytical method, named Fourier Transform-Infrared Spectroscopy (FT-IR). In order to evaluate the reliability of data obtained from FT-IR analysis, the strains were also analyzed with a DNA-based typing method: Pulse Field Gel Electrophoresis (PFGE). Additionally, virulence factors and bla_{NDM} variants were evaluated to increase data reliability.

Materials and methods. This study focused on 18 XDR KPN clinical isolates collected in 2022/2023 in Verona hospitals. Carbapenemases production was investigated by NG-test® CARBA5 (NG-biotech, France). bla_{NDM} variants were discriminated by sequencing (Eurofins Genomics, Germany). The presence of different virulence factors was investigated with PCR analysis, using primers that target RmpA, RmpA2, IucA, IroB and Peg-344 genes. KPN isolates were analyzed with two different typing methods in order to investigate the clonal relationship: PFGE and FT-IR. FT-IR analysis was performed with IR Biotyper® system (Bruker Daltonics, Germany) from strains grown on Columbia Blood Agar (37°C, 24h±1h). Data analysis of acquired spectra was performed considering only carbohydrates region and applying hierarchical cluster analysis (HCA), Principal Component Analysis (PCA) and Linear Discriminant Analysis (LDA).

Results. All isolates harbour NDM enzyme, and seven co-harbour OXA-48-like enzyme. bla_{NDM} sequencing revealed variants NDM-1 and NDM-5. Virulence factors investigation revealed the presence of RmpA/A2 and Peg-344 genes in two out of three FT-IR sub-clusters. FT-IR analysis showed the presence of two main clusters, differently from PFGE analysis that identifies only one cluster. Moreover, FT-IR analysis discriminated the presence of three sub-clusters, according to the presence of isolates that harbour respectively NDM-1/OXA-48-like, and NDM-5 carbapenemases.

Discussion and conclusions. To reduce the spreading of XDR KPN strains, real time epidemiological analysis may play a key role. FT-IR analysis overcomes the time-consuming issue of genetic based typing methods, supplementing genotyping. Although PFGE identified only one cluster, FT-IR discriminated three sub-clusters, according to epidemiological facts and patient transfers in different hospital wards.

150 - The mosaic of Adenovirus variants circulating in 2022-2023 in hospitalized patients.

FEDERICA ANNA MARIA GIARDINA ⁽¹⁾ - GUGLIELMO FERRARI ⁽²⁾ - ANTONINO MARIA GUGLIELMO PITROLO ⁽²⁾ - ANTONIO PIRALLA ⁽²⁾ - FAUSTO BALDANTI ⁽¹⁾

Department of Clinical, Surgical, Diagnostic and Pediatric Sciences, University of Pavia, Pavia, Italia ⁽¹⁾ - Microbiology and Virology Department, Fondazione IRCCS Policlinico San Matteo, Pavia, Italia ⁽²⁾

The mosaic of Adenovirus variants circulating in 2022-2023 in hospitalized patients.

Federica A.M. Giardina¹, Guglielmo Ferrari², Antonino M.G. Pitrolo², Antonio Piralla², Fausto Baldanti^{1,2}.

¹ Department of Clinical, Surgical, Diagnostic and Pediatric Sciences, University of Pavia, Pavia, Italy.

² Microbiology and Virology Department, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy.

Introduction. Human Adenovirus (hAdVs) is usually associated with mild respiratory infections. However, it could cause severe syndromes, especially among children and elderly people or those with underlying comorbidities, thus requiring hospitalization and respiratory support. This study aimed to: I) investigate hAdV infections among adults and children with respiratory syndromes and II) evaluate the circulation of different hAdV genotypes in one year surveillance period.

Materials and Methods. All respiratory samples collected from June 2022 to June 2023 at Microbiology and Virology Department, Fondazione IRCCS Policlinico San Matteo, Pavia were tested for a panel of respiratory viruses including human Adenovirus. hAdV-positive samples with a viral load higher than 10³ copies/ml were then amplified with a specific nested PCR targeting the hexon gene to assess the correct genotype.

Results. A total of 4,097 respiratory samples, collected from as many patients, were analyzed for diagnostic routine during the study period and 282 of them (6.9%), including 14 (5%) bronchoalveolar lavages and 268 (95%) nasal swabs, were positive for hAdV. Among positive patients, 151 (53.5%) were males; the median age was 3 years (range 0-81). In particular, 244/282 (86.5%) were less than 18 years old and 176/244 (72.1%) of them were admitted to the pediatric Emergency Room of our hospital; 32 of them (13.1%) were admitted to the pediatric ward, 8 (3.3%) to the pediatric oncohematology department and 5 (2%) were admitted to the Neonatal Intensive Care Unit (NICU). All the remaining pediatric subjects (23/244, 9.4%) were outpatients referring to several clinics in the hospital. Among adults subject (n=38), 9/38 (23.7%) were admitted to the Pneumology department, 7 (18.4%) to the Infectious Diseases department e 7 to the Hematology department. In 97/282 (34.5%) patients, hAdV viral load was lower than 10³ copies/ml; in 91 (32.3%) was between 10³ and 10⁵ copies/ml; in 88 (31.2%) was higher than 10⁵ copies/ml. For 6 (2%) patients, quantitative viral load was not available. To date, genotyping was performed for 102/179 (57%) of all samples with viral load higher than 10³ and it was successful for 83 cases (81.4%). In particular, hAdV B3 was detected in 50/83 cases (60.2%), C2 in 14 cases (16.9%), C1 in 13 cases (15.7%) and C5 in 3 cases (3.6%). Notably, almost all C1, C2 and C5 cases were detected in the period June 2022-March 2023, while all B3, with few exceptions, cases were detected in the period March-June 2023.

Discussion and conclusion. Children are at high risk for hAdV infection and very often present high viral loads and severe clinical symptoms. Moreover, several hAdV strains have been detected circulating in different months throughout the study period.

172 - Genomic analysis of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates from bloodstream infections in Italy: a pilot study

MICHELA PAGNOTTA ⁽¹⁾ - GIULIA ERRICO ⁽¹⁾ - CHRISTINA MERAKOU ⁽¹⁾ - FABIO D'AMBROSIO ⁽¹⁾ - SARA GIANCRISTOFARO ⁽¹⁾ - ROMINA CAMILLI ⁽¹⁾ - MARIA DEL GROSSO ⁽¹⁾ - MONICA MONACO ⁽¹⁾

Istituto Superiore di Sanità, Department of Infectious Diseases, Rome, Italia ⁽¹⁾

Genomic analysis of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates from bloodstream infections in Italy: a pilot study

MICHELA PAGNOTTA¹, GIULIA ERRICO¹, CHRISTINA MERAKOU^{1,2}, FABIO D'AMBROSIO¹, SARA GIANCRISTOFARO¹, ROMINA CAMILLI¹, MARIA DEL GROSSO¹, MONICA MONACO¹, AND THE MRSA GENOMIC SURVEILLANCE PILOT STUDY GROUP

¹Department of Infectious Diseases, Antibiotic-Resistance and Special Pathogens Unit, Istituto Superiore di Sanità, Rome, Italy; ²ECDC fellowship Programme, Public Health Microbiology path (EUPHEM), European Centre for Disease Prevention and Control (ECDC), Stockholm, Sweden.

Introduction. Methicillin-resistant *Staphylococcus aureus* (MRSA) represents a global threat in both healthcare and community settings. Italy is one of the European countries with the highest percentage of MRSA isolates from invasive infections (30% in 2021). In the framework of a cross sectional observational pilot study of genomic surveillance of MRSA conducted in Italy in 2022, isolates from bloodstream infections (BSIs) were analyzed in order to evaluate microbiological and molecular features. **Materials and Methods.** From July-October 2022, up to ten consecutive non-duplicated clinical isolates of MRSA from BSIs were collected and sent to ISS from 32 hospital laboratories (16 Regions) participating in the national antibiotic-resistance surveillance system AR-ISS. Species identification and susceptibility to antibiotics was performed by hospital laboratories according to their routine. WGS using an Illumina platform was performed at ISS. **Results.** A total of 202 MRSA isolates were collected. The isolates were found to be resistant to levofloxacin (78.3%, 123/157), erythromycin (45.9%, 90/196), and less frequently to tetracycline (15.0%, 24/160), gentamicin (12.8%, 22/172), clindamycin (12.4%, 23/185), teicoplanin (1.1%, 2/175), and daptomycin (0.5%, 1/189). All isolates were susceptible to ceftaroline, linezolid, tigecycline and vancomycin. Preliminary WGS analysis was conducted on 58 isolates from 16 centers from 11 Regions. ResFinder analysis showed that all MRSA harbored the *mecA* gene, 55.2% carried macrolide resistance genes [mainly *erm*(C)], 22.4% aminoglycoside resistance genes [mainly *aac*(6')-*aph*(2'') and *aph*(3')-III], and 10.3% tetracycline resistance genes [mainly *tet*(K)]. Point mutations conferring resistance to quinolones (*gyrA* and *grlA* genes) were detected in 82.8% of the isolates. MLST analysis showed that the 58 MRSA belonged to 9 different Sequence Type (ST)s, the most detected clones being ST22 (72.4%), ST5 (6.9%) and ST1 (5.2%). Further WGS analysis is ongoing. **Discussion and Conclusions.** This study showed that MRSA isolates were confirmed to be resistant to different classes of antibiotics. By preliminary WGS analysis, ST22 confirmed to be the most common clone circulating in Italy. Genomic surveillance is needed to highlight changes in MRSA molecular epidemiology and to contribute to the prevention and control of clonal transmission in healthcare setting in Italy.

190 - Machine learning model based on MALDI-TOF mass spectra for prediction of ceftazidime resistance in Escherichia coli strains isolated from bloodstream infections

SIMONE AMBRETTI⁽¹⁾ - DONATELLA LOMBARDI⁽¹⁾ - ALESSIA BERTOLDI⁽¹⁾ - CRISTINA MARIA CROVARA PESCE⁽¹⁾ - ETTORE ROCCHI⁽²⁾ - MAURO FACCIN⁽²⁾ - CLAUDIA SALA⁽²⁾ - GASTONE CASTELLANI⁽²⁾

U.O. Microbiologia, IRCCS AOU di Bologna Policlinico S.Orsola, Bologna, Italia⁽¹⁾ - Department of Medical and Surgical Sciences, Università di Bologna, Bologna, Italy, Bologna, Italia⁽²⁾

Machine learning model based on MALDI-TOF mass spectra for prediction of ceftazidime resistance in Escherichia coli strains isolated from bloodstream infections

SIMONE AMBRETTI^{1,2}, DONATELLA LOMBARDO¹, ALESSIA BERTOLDI¹, CRISTINA MARIA CROVARA PESCE¹, ETTORE ROCCHI², MAURO FACCIN², CLAUDIA SALA², GASTONE CASTELLANI²

¹Microbiology Unit, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy

²Department of Medical and Surgical Sciences, Università di Bologna, Bologna, Italy

Introduction Escherichia coli is the most prevalent gram-negative pathogen causing bloodstream infections. Antimicrobial resistance (AMR) is clinically relevant in this species because of the spread of extended spectrum beta-lactamases (ESBL), determining resistance to third generation cephalosporins. The rapid detection of this phenotype is fundamental to allow a timely and appropriate antimicrobial therapy for septic patients. Matrix-assisted laser desorption ionization–time-of-flight mass spectrometry (MALDI-TOF MS) is currently widely used in clinical microbiology laboratories for rapid, reliable and cost-effective species identification of bacterial isolates and different protocols have been evaluated to be implemented into the diagnostic workflow of positive blood cultures. Aim of this study is the development of a machine learning (ML) model based on evaluation of whole MALDI-TOF MS profile for prediction of ceftazidime resistance in E.coli strains, as phenotypic marker of ESBL production in positive blood cultures. **Materials and Methods** Our study included 80 clinical isolates of E.coli collected from routinary diagnostic workflow of blood cultures performed in bacteriology lab of S.Orsola University Hospital. Briefly, positive bottles (Becton Dickinson) were sub-cultured onto chocolate agar for few hours and MALDI-TOF MS species identification was performed using Maldi Biotyper Sirius system (Bruker Daltonics), after a preparation method including the extraction step with formic acid. Antimicrobial susceptibility testing was performed using Microscan Walkaway (Beckman Coulter) panels for gram-negative pathogens. The raw whole mass spectra (m/z range from 2k to 20k Dalton) were collected in order to develop a ML model for prediction of ceftazidime resistance, as one of the most relevant drugs for phenotypic detection of ESBL-producing E.coli. The spectra were pre-processed and divided into a training set (60 spectra) and a testing set (20 spectra). Different ML models were used and finally their predictions have been subjected to the soft voting algorithm to achieve a single prediction. The final voting classifier was evaluated using the area under the receiver operating characteristic curve (AUROC). Moreover very major error (VME) rates was evaluated. **Results** The rate of ceftazidime resistance of E.coli tested strains was 40%. The voting classifier performed on the testing set produces an AUROC value equal to 0.94. The rate of VME was 5% (one of resistant spectra have been misclassified as susceptible). **Discussion and Conclusions** Our preliminary results show that ML models applied to MALDI-TOF MS could be considered as promising tools in order to

achieve a fast and reliable prediction of highly relevant antimicrobial resistance phenotypes. Further studies including higher number of strains, possibly from different regions, are needed to confirm these data. Moreover the analysis of discriminating informative peaks could be useful to better understand the rationale of ML models.

214 - Emergence of high-level gentamicin resistance in *Streptococcus agalactiae* hypervirulent serotype IV strains

ROBERTA CRETI⁽¹⁾ - MONICA IMPERI⁽¹⁾ - SIMONA RECCHIA⁽¹⁾ - GIOVANNA ALFARONE⁽¹⁾ - GIOVANNI GHERARDI⁽²⁾

Istituto Superiore di Sanità, Dipartimento di Malattie infettive, roma, Italia⁽¹⁾ - Università Campus Bio-Medico, Facoltà Dipartimentale di Medicina e Chirurgia, roma, Italia⁽²⁾

Emergence of high-level gentamicin resistance in *Streptococcus agalactiae* hypervirulent serotype IV strains

ROBERTA CRETI¹, MONICA IMPERI¹, SIMONA RECCHIA¹, GIOVANNA ALFARONE¹, GIOVANNI GHERARDI²

¹ Dipartimento di Malattie Infettive, Reparto di Antibiotico-Resistenza e Patogeni Speciali, Istituto Superiore di Sanità, Roma, Italy; ² Unità di Scienze Batteriologiche Applicate, Facoltà Dipartimentale di Medicina e Chirurgia, Università Campus Bio-Medico, Roma, Italy.

Introduction

Streptococcus agalactiae (Group B Streptococci, GBS) is intrinsically resistant to gentamicin and no recommendation for testing high-level gentamicin resistance (HLGR) is present in the guidelines issued by the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST). Only the French EUCAST reports breakpoints for gentamicin for beta-haemolytic streptococci. HLGR-GBS are, however, emerging and need to be closely monitored.

Methods

Four-hundred and thirty-three GBS strains isolated from different sources were tested for HLGR both by disk diffusion (disk content for gentamicin 120 µg, according to the interpretative criteria for zone diameter for enterococci of CLSI) and the presence of *aac(6')-Ie-aph(2'')-Ia* gene by Polymerase Chain reaction (PCR). Clonality was assessed by MultiLocus sequence Typing (MLST). Whole genome sequencing of HLGR-GBS strains was performed by the Nanopore MinIon technology and high-quality reads were "de novo" assembled.

The bacterial collection included strains from invasive GBS disease (112 isolates from adults and 104 isolates from neonates) and from pregnant carrier women (217 isolates).

Results

Twenty GBS strains (4.6%) presented HLGR. The aminoglycoside resistance was asymmetrically diffused between strains from adults or neonates (adult invasive strains: 6 HLGR isolates (5.35%); pregnant carrier women: 11 HGLR isolates (5.06%); neonates: 3 HLGR isolates (2.9%).

Notably, fourteen out of 20 HLGR GBS strains (70%) represented an emergent clonal lineage (serotype IV, Sequence type 1010, clonal complex 452), present both in adults and neonates.

These HLGR GBS strains possessed also the *hvgA* gene, encoding an important invasin with meningeal tropism that, generally, is not present in serotype IV GBS strains.

This clonal lineage presented an IS256 element upstream of the *aac(6')-Ie-aph(2'')-Ia* gene and a truncated IS256 element downstream of HLGR gene. This structure has not been detected in HLGR enterococci.

Conclusions

The clinical significance of the emergence of HLGR in GBS is still unknown. High-level aminoglycosides resistance abrogates the enhanced bactericidal activity of the combined use of ampicillin with gentamicin that is the empirical therapy for neonatal sepsis and GBS meningitis. The high prevalence of serotype IV, mainly in adult GBS, is an emerging data. Moreover, the finding the

about half of serotype IV GBS strains of our collection belonged to the clonal expansion of a HLAR virulent lineage, is a novel report.

248 - Retrospective epidemiological study of candidemia at San Martino Hospital (HSM), Genoa, Italy, 2019-2022

ELISA COSTA⁽¹⁾ - GIULIA CODDA⁽¹⁾ - EDWARD WILLISON⁽¹⁾ - LUCA CALABRESE⁽¹⁾ - VINCENZO DI PILATO⁽¹⁾ - ANNA MARCHESE⁽¹⁾

DISC, Università di Gneova, Genova, Italia⁽¹⁾

Retrospective epidemiological study of candidemia at San Martino Hospital (HSM), Genoa, Italy, 2019-2022

Elisa Costa^a, Giulia Codda^a, Edward Willison^a, Luca Calabrese^a, Vincenzo Di Pilato^a, Anna Marchese^a

^aDISC, Microbiology Unit, University of Genoa, Italy

INTRODUCTION

Candida spp. is an important cause of bloodstream infections (BSI), accounting for high mortality (from 30% to 70%). The emergence of different resistance profiles have made *Candida* spp. infections problematic for patient management and outcome. The aim of the study was to describe the epidemiology of *Candida* spp. causing candidemia at HSM from January 2019 to December 2022, analysing the trend of species distribution in vitro susceptibility to antifungal drugs.

MATERIALS AND METHODS

All data were provided from patients with positive blood culture for *Candida* spp.. For each patient, only the first episode of *Candida* BSI was recorded.

RESULTS

In the study period, a total of 1287 candidemia episodes were diagnosed. The yearly cases were: 222 in 2019, 306 in 2020, 379 in 2021, 318 in 2022 and the prevalence over blood culture-positive episodes occurred in a single year are: 4.5 % in 2019, 6.2% in 2020, 13.5% in 2021 and 6.5% in 2022. In 2019 and 2020, *C. albicans* and *C. parapsilosis* were the main species accounting for candidemia (84% and 77%, annually). In 2021 and 2022 the prevalence of *C. auris* raised to 15% and 12.6%, respectively, whilst for *C. albicans* /*C. parapsilosis*, was 65.7 % and 67.6%.

In addition, data analysis revealed that *C. parapsilosis* resistance to azoles and in particular to fluconazole decreased between 2019 and 2022 as annually shown: 64.7%, 56%, 53% and 50%. In *C. auris*, resistance to fluconazole was appreciable in 100% of cases and, in 2022, 2 echinocandin-resistant isolates (5%) were detected.

DISCUSSION

This report shows a fluctuating trend in candidemia across the COVID-19 pandemic. Whilst *C. albicans* /*C. parapsilosis* accounted for most (80%) of candidemia in 2019 and 2020, a significant epidemiological shift was observed since 2021, owing to a decreased prevalence of *C. albicans* /*C. parapsilosis* (65.7%), and to a concurrent increase in candidemia (13.5%) and in the *C. auris* prevalence (15%) following the outbreak recognized in our institution. To likely explain these trends, several hypotheses have been postulated: - During the COVID-19 pandemic, we experienced an increased use of disinfectants commonly used in the nosocomial environment with limited functionality against *C. auris* but effective with the others; - Competition for the same ecological niche may subsist between different *Candida* spp. with an advantageous fitness for *C. auris*; - Antifungal prophylaxis in at-risk and immunocompromised patients for *C. auris* may have affected the transmission of *C. parapsilosis* and *C. albicans*; - The existing ICU capacity had been overwhelmed by COVID-19 patients, likely favoring the spread colonisation of *C. auris*. Further

investigations are needed to fully corroborate such hypotheses and to understand the factors underlying this trend.

287 - IMPACT OF THE SARS-COV-2 PANDEMIC ON THE CIRCULATION OF ENTERIC VIRUSES IN CHILDREN IN PALERMO

CHIARA FILIZZOLO ⁽¹⁾ - MARIANGELA PIZZO ⁽¹⁾ - GIUSEPPA LUISA SANFILIPPO ⁽¹⁾ - FLORIANA BONURA ⁽¹⁾ - CHIARA MASCARELLA ⁽¹⁾ - ARIANNA SUCATO ⁽¹⁾ - FRANCESCA DI BERNARDO ⁽²⁾ - ANTONINA COLLURA ⁽²⁾ - GIOVANNI MAURIZIO GIAMMANCO ⁽¹⁾ - SIMONA DE GRAZIA ⁽¹⁾

Dipartimento di Promozione della Salute, Materno-Infantile, di Medicina Interna e Specialistica di Eccellenza “G. D’Alessandro” (PROSAMI), Università degli Studi di Palermo, Palermo, Italia ⁽¹⁾ - Unità Operativa di Microbiologia e Virologia, Ospedale Civico e Di Cristina, ARNAS, Palermo, Italy, Palermo, Italia ⁽²⁾

IMPACT OF THE SARS-COV-2 PANDEMIC ON THE CIRCULATION OF ENTERIC VIRUSES IN CHILDREN IN PALERMO

CHIARA FILIZZOLO^a, MARIANGELA PIZZO^a, GIUSEPPA LUISA SANFILIPPO^a, FLORIANA BONURA^a, CHIARA MASCARELLA^a, ARIANNA SUCATO^a, FRANCESCA DI BERNARDO^b, ANTONINA COLLURA^b, GIOVANNI M. GIAMMANCO^a, SIMONA DE GRAZIA^a

^aDipartimento di Promozione della Salute, Materno-Infantile, di Medicina Interna e Specialistica di Eccellenza “G. D’Alessandro” (PROSAMI), Università di Palermo, Palermo, Italy

^bUnità Operativa di Microbiologia e Virologia, Ospedale Civico e Di Cristina, ARNAS, Palermo, Italy

Introduction:

The COVID-19 pandemic emergence posed the need to implement restriction measures in order to reduce SARS-CoV-2 circulation. Use of masks, social distancing and implementation of sanitary hygiene measures caused an impact on the circulation of several pathogens including enteric viruses, usually transmitted by direct or indirect faecal transmission, and related to high rates of hospitalization for acute gastroenteritis (AGE), especially in young children. The aim of this study was to evaluate the epidemiological impact of lockdown measures on the circulation of the main agents of viral AGE comparing their prevalence during COVID-19 pandemic (P2: 2020-2021) with data from the two previous years (P1: 2018-2019).

Materials and Methods

During four consecutive years of enteric viruses surveillance, a total of 1758 stool samples were collected, 1102 in P1 and 656 in P2, from children admitted to the Children's Hospital "G. Di Cristina" in Palermo for AGE and were screened for rotavirus (RVA), norovirus (NoV), adenovirus (AdV F40-41) and astrovirus (AstV) by RT-RealTime-PCR. Positive samples were genotyped to differentiate viral variants.

Results

Among the 1758 samples tested, an enteric virus was detected in 40.35% of patients during P1 and in 23.81% in P2. From spring 2020 to autumn 2021, a 40.47% reduction in virological tests for AGE was observed. Among the viral agents tested, NoV prevalence dropped from 21.01% in P1 to 6.61% in P2 although GII.P16/GII.4 remained the prevalent genotype over the whole study period. The

prevalence of RVA remained stable in the two periods (10.57% and 9.46%, respectively) but the G3P[8] equine-like genotype was replaced by G1P[8]. AdV F40-41 cases remained steadily around 7% prevalence (6.95% in P1 and 7.11% in P2), while AstV-1a circulated at low prevalence (1.89% vs 0.64%).

Discussion and Conclusions

During the pandemic period a marked reduction in requests for diagnostic tests for enteric viruses was observed, probably reflecting the limited number of hospitalizations for AGE. Apparently, the lockdown restrictions limited the spread of enteric infections but were particularly effective on the circulation of NoV, the main responsible for AGE. A shift in RVA genotypes was also observed with the re-emergence of G1P[8] strains that had disappeared since vaccine introduction. Surveillance activity will be of particular value in the next future to monitor epidemiological changes, including the emergence/re-emergence of different genotypes, following the loss of immunity due to the limited circulation of enteric viruses during COVID-19 pandemic.

301 - Epidemiological surveillance of SARS-CoV-2 variants using NGS during pandemic waves in Verona

RICCARDO CECCHETTO ⁽¹⁾ - **EMIL TONON** ⁽¹⁾ - **ERICA DIANI** ⁽¹⁾ - **ANNA LAGNI** ⁽¹⁾ - **VIRGINIA LOTTI** ⁽¹⁾ - **NIOLETTA MEDAINA** ⁽²⁾ - **GIONA TURRI** ⁽²⁾ - **MOSÈ FAVARATO** ⁽³⁾ - **DAVIDE GIBELLINI** ⁽¹⁾

Università Di Verona, Dip. Diagnostica E Sanità Pubblica, Verona, Italia ⁽¹⁾ - Ospedale G.B. Rossi, Uoc Microbiologia, Verona, Italia ⁽²⁾ - Aulss 3, Uosd Diagnostica Molecolare E Genetica, Venezia, Italia ⁽³⁾

Epidemiological surveillance of SARS-CoV-2 variants using NGS during pandemic waves in Verona

RICCARDO CECCHETTO^{1,2}, **EMIL TONON**^{1,2}, **ERICA DIANI**¹, **ANNA LAGNI**¹, **VIRGINIA LOTTI**¹, **NIOLETTA MEDAINA**², **GIONA TURRI**², **MOSE' FAVARATO**³, **DAVIDE GIBELLINI**^{1,2}

¹ Department of Diagnostic and Public Health, Verona University, 37134 Verona, Italy; ² UOC Microbiology, AOUI Verona, 37134 Verona, Italy; UOSD of Molecular Diagnostics and Genetics, AULSS 3 Serenissima, Venice, Italy.

Introduction.

The availability of new technologies for deep sequencing including Next Generation Sequencing (NGS) provides a consistent analysis of viral genome variations. The epidemiological determination of SARS-CoV-2 viral genome changes during the different pandemic waves, displayed the genome evolution and the subsequent onset of variants over time, often associated with a different impact on viral transmission and disease severity.

Materials and Methods

Viral RNAs were extracted with a semiautomated instrument from 913 positive nasopharyngeal swabs collected at AOUI Verona from February 2022 to June 2023. The sequencing was performed in samples with Ct<32 determined with Allplex SARS-CoV-2 Assay (Seegene). The NGS procedure was carried out with Illumina COVIDSeq Assay (Illumina, San Diego, CA, USA) with ARTIC v4 and v4.1 primer pool. The bioinformatic analysis was performed using Integrative Genomics Viewer (IGV) tool to control sample reads distribution, Pangolin COVID-19 Lineage Assigner, Nextclade tool by Nextstrain to identify mutations and lineages and Usher for phylogenetic studies.

Results

We investigated the SARS-CoV-2 positive samples between the beginning of the Italian pandemic to June 2023. 913 samples were successfully analyzed by NGS technique between February 2022 and June 2023. The 6 pandemic waves detected in AOUI Verona confirmed the impact of variants displayed in Italy. In our samples we detected principally BA.2, BQ.1, BA.5.1, BA.5.2 and, more recently, XBB.1 and its subvariants. Interestingly, we have detected some clusters or specific and particular variants such as XE variant.

Discussion and conclusion

Our data indicate the flow of SARS-CoV-2 genome evolution during the SARS-CoV-2 pandemic. It is noteworthy that the detection of particular sequences and mutations of ORF8 region observed even before of the last wave may suggest some genomic evolution avenues to obtain a useful balance between the host biology and SARS-CoV-2 impact. In fact, the last dominant variants are able to improve its transmission and sometimes the viral replication without an increase of clinical impact and mortality rate. NGS methodologies could be implemented as supporting systems in epidemiological analysis in nearly real time, to predict the progress of the pandemic over time. With this approach, further development of worldwide pandemic could be studied and monitored.

322 - SARS-CoV-2 variants in Marche region: evolution, mutations, and variability.

CARLA ACCIARRI⁽¹⁾ - ROBERTA LONGO⁽¹⁾ - BENEDETTA CORVARO⁽¹⁾ - SARA CAUCCI⁽¹⁾ - STEFANO MENZO⁽¹⁾

Università Politecnica delle Marche, Dipartimento di Scienze Biomediche e Sanità Pubblica, Ancona, Italia⁽¹⁾

SARS-CoV-2 variants in Marche region: evolution, mutations, and variability.

CARLA ACCIARRI¹, ROBERTA LONGO¹, SARA CAUCCI¹, BENEDETTA CORVARO¹, STEFANO MENZO^{1,2}

1. Dipartimento di Scienze Biomediche e Sanità Pubblica Università, Politecnica delle Marche, Ancona, Italia

2. SOSD Virologia, Azienda Ospedaliero Universitaria delle Marche, Ancona; Italia

1. Introduction: In December 2019, Severe Acute Respiratory Syndrome-2 (SARS-CoV-2) caused an outbreak in Wuhan, China. The virus spread rapidly across the world causing billions of infections. This led to the accumulation of mutations, despite the proofreading activity of the virus, potentially affecting various biological properties, some of which were fixed in new variants. Most of these occurred in the S gene, allowing the spike protein to gain infectious potential and evade humoral response. Mutations in other genes are less characterized, and their effect is not completely known. Here we describe the flow of SARS-CoV-2 mutations occurring over time during the pandemic in the Marche region, estimating the selective pressure on all genes, including non-structural ones.

2. Materials and methods: Viral RNA was automatically extracted from nasopharyngeal swabs from patients found positive for SARS-CoV-2 with high viral load. The library for the Next Generation Sequencing (NGS) was prepared by a microfluidic handler, then sequenced and the sequences were analyzed with bioinformatic tools.

3. Results: SARS-CoV-2 variants in our region followed the global pattern with ancestral lineages in 2020, first variants of concern/interest (VOC/VOI) in 2021 and the rise of Omicron from late 2021. As opposed to Delta lineages, there was a sharp switch between Omicron variants, different clades only coexisting for a limited amount of time. To better understand the phylogenetic link between variants, we built a phylogenetic tree of the whole genome sequences. Most of mutations were synonymous, not determining a change into aminoacidic sequence. SARS-CoV-2 tends to increase the number of its non-synonymous mutations over time, and besides canonical mutations we found frequent sporadic mutations, particularly in gene S, some of which common across variants, meaning that the virus has some hotspot for mutations. Unlike the S gene, strict conservative evolution was demonstrated for most non-structural genes. However, a slight positive pressure was found in a few small non-structural genes (particularly NSP6), whose biological impact is being investigated.

4. Discussion and conclusions: SARS-CoV-2 is evolving over time to adapt to humans as well to other mammalian species, accumulating mutations, mostly in gene S gene. Other genes, like those implied in replication or other non-structural genes, are remarkably conserved across variants. In these genes, only a small number of changes are tolerated, probably as they confer an important evolutionary advantage. Understanding the biological significance of these mutations, some of which may constitute the basis for new variants, could shed light in the biology of the virus and provide insight for novel antiviral strategies.

330 - Characterization of a genome database of 837 isolates assigned to 72 distinct species in the *Acinetobacter* genus using the Pasteur Multi-locus sequencing type (MLST) scheme.

ANTONELLA MIGLIACCIO⁽¹⁾ - JAMES BRAY⁽²⁾ - MICHELE INTOCCIA⁽³⁾ - MARIA STABILE⁽¹⁾ - GIOVANNI SCALA⁽³⁾ - KEITH A. JOLLEY⁽²⁾ - SYLVAIN BRISSE⁽⁴⁾ - RAFFAELE ZARRILLI⁽¹⁾

University of Naples “Federico II”, Department of Public Health,, Naples, Italia⁽¹⁾ - University of Oxford, United Kingdom, Department of Biology,, Oxford, Stati Uniti D'america⁽²⁾ - University of Naples Federico II, Italy, Department of Biology,, Naples, Italia⁽³⁾ - Biodiversity and Epidemiology of Bacterial Pathogens,France, Institut Pasteur, Paris, Francia⁽⁴⁾

Characterization of a genome database of 837 isolates assigned to 72 distinct species in the *Acinetobacter* genus using the Pasteur Multi-locus sequencing type (MLST) scheme.

Antonella Migliaccio¹, James Bray², Michele Intoccia³, Maria Stabile^{4,5}, Giovanni Scala³, Keith A. Jolley², Sylvain Brisse⁶ and Raffaele Zarrilli¹

¹ Department of Public Health, University of Naples “Federico II”, Naples, Italy, ² Department of Biology, University of Oxford, Oxford, United Kingdom, ³ Department of Biology, University of Naples Federico II, Naples, Italy, ⁴ Department of Chemical Sciences, University of Naples Federico II, Naples, Italy, ⁵ Complex Operational Unit of Clinical Microbiology, University Hospital “Federico II”, Naples, Italy, ⁶ Biodiversity and Epidemiology of Bacterial Pathogens, Institut Pasteur, Paris, France

Background

Non-baumannii *Acinetobacter* species are increasingly isolated in the clinical setting and the environment. The aim of the present study was to analyze a genome database of 837 *Acinetobacter* spp. isolates, which includes 798 non-baumannii *Acinetobacter* genomes, in order to define concordance of classification and discriminatory power of 7-gene MLST, 53-gene MLST and single-nucleotide polymorphisms SNPs phylogenies.

Methods

Bacterial genomes were manually selected from the PubMLST database. Pasteur MLST and ribosomal MLST were performed using BIGSdb software. Phylogenies were performed on Pasteur MLST or ribosomal MLST concatenated alleles, or SNPs extracted from core genome alignment using RAxML under the GTR-GAMMA model. The phylogenetic trees were visualized using iTol v6 software. The resistance genes were detected using ABRicate software.

Results

The Pasteur MLST scheme was able to identify and genotype 72 species in the *Acinetobacter* genus, with classification results concordant with the ribosomal MLST scheme. The discriminatory power and the genotyping reliability of Pasteur MLST scheme was assessed in comparison to genome-wide SNP phylogeny on 535 non-baumannii *Acinetobacter* genomes assigned to *Acinetobacter pittii*, *Acinetobacter nosocomialis*, *Acinetobacter seifertii* and *Acinetobacter lactucae* (heterotypic synonym of *Acinetobacter dijkshoorniae*), which are the most clinically relevant non-baumannii species of the *A. baumannii* group. The Pasteur MLST and SNP phylogenies were congruent (Robinson-Fould test) and grouped genomes into four monophyletic and six non-monophyletic clades in *A. pittii*, and one each in *A. seifertii*, respectively. Also, *A. lactucae* genomes were grouped into one non-monophyletic

clade within *A. pittii* genomes. The SNP phylogeny of *A. nosocomialis* genomes showed a heterogeneous population and did not correspond to Pasteur MLST phylogeny, which identified two monophyletic and three non-monophyletic clades.

Acquired antimicrobial resistance genes belonging to at least three different antimicrobial classes were identified in 122 isolates assigned to 21 distinct species in the *Acinetobacter* genus. Also, the presence of a class D oxacillinase, which is a naturally occurring enzyme in several *Acinetobacter* species, was found in 482 isolates assigned to 35 *Acinetobacter* species.

Conclusions

The Pasteur MLST scheme is a useful genotyping tool to identify *Acinetobacter* species and to analyze the population structure of the *A. baumannii* group.

06 Molecole naturali ad azione antimicrobica/Nuovi approcci antimicrobici

1 - Importin alpha/beta-dependent nuclear transport of human parvovirus B19 nonstructural protein 1 is essential for viral replication

GUALTIERO ALVISI⁽¹⁾ - ELISABETTA MANARESI⁽²⁾ - EMILY, M. CROSS⁽³⁾ - MIKAYLA HOAD⁽³⁾ - NASIM AKBARI⁽¹⁾ - SILVIA PAVAN⁽¹⁾ - DARYL ARIAWAN⁽⁴⁾ - GLORIA BUA⁽²⁾ - GAYLE, F. PETERSEN⁽³⁾ - JADE FORWOOD⁽³⁾ - GIORGIO GALLINELLA⁽²⁾

Università di Padova, Dipartimento di Medicina Molecolare, Padova, Italia⁽¹⁾ - Università degli Studi di Bologna, Dipartimento di Farmacia e Biotecnologie, Bologna, Italia⁽²⁾ - Charles Sturt University, School of Dentistry and Medical Sciences, Wagga Wagga, Australia⁽³⁾ - Macquarie University, Dementia Research Centre, Sydney, Australia⁽⁴⁾

Importin alpha/beta-dependent nuclear transport of human parvovirus B19 nonstructural protein 1 is essential for viral replication

GUALTIERO ALVISI^{1*}, ELISABETTA MANARESI², EMILY M. CROSS³, MIKAYLA HOAD³, NASIM AKBARI¹, SILVIA PAVAN¹, DARYL ARIAWAN⁴, GLORIA BUA², GAYLE F. PETERSEN³, JADE FORWOOD³, GIORGIO GALLINELLA²

¹Department of Molecular Medicine, University of Padova, 35121 Padova, Italy; ² Department of Pharmacy and Biotechnology, University of Bologna, 40138 Bologna, Italy; ³ School of Dentistry and Medical Sciences, Charles Sturt University, Wagga Wagga, NSW, 2650, Australia; ⁴Dementia Research Centre, Macquarie University, Sydney, NSW, 2109, Australia

* Correspondence to: gualtiero.alvisi@unipd.it

Introduction: Human parvovirus B19 (B19V) is a major human pathogen causing a variety of diseases, characterized by a selective tropism to human progenitor cells in bone marrow. In similar fashion to all Parvoviridae members, the B19V ssDNA genome is replicated within the nucleus of infected cells through a process which involves both cellular and viral proteins. Among the latter, a crucial role is played by non-structural protein (NS)1, a multifunctional protein involved in genome replication and transcription, as well as modulation of host gene expression and function. Despite the localization of NS1 within the host cell nucleus during infection, little is known regarding the mechanism of its nuclear transport pathway. **Methods:**

In this study we undertake structural, biophysical, and cellular approaches to characterize this process. **Results:** Quantitative confocal laser scanning microscopy (CLSM), gel mobility shift, fluorescence polarization and crystallographic analysis identified a short sequence of amino acids (GACHAKKPRIT-182) as the classical nuclear localization signal (cNLS) responsible for nuclear import, mediated in an energy and importin (IMP) alpha/beta-dependent fashion. Structure-guided mutagenesis of key residue K177 strongly impaired IMP alpha binding, nuclear import, and viral gene expression in a minigenome system. Further, treatment with ivermectin, an antiparasitic drug interfering with the IMP alpha/beta dependent nuclear import pathway, inhibited NS1 nuclear accumulation and viral replication in infected UT7/Epo-S1 cells. **Discussion:** Thus, NS1 nuclear transport is a potential target of therapeutic intervention against B19V induced disease.

11 - A heparan sulfate proteoglycan binding tetrapeptide successfully inhibits SARS-CoV-2 Omicron replication

CAMILLA BIBA⁽¹⁾ - LORENZO DEPAU⁽¹⁾ - LIA FIASCHI⁽¹⁾ - JLENIA BRUNETTI⁽¹⁾ - ILENIA VARASI⁽¹⁾ - CHIARA FALCIANI⁽¹⁾ - MAURIZIO ZAZZI⁽¹⁾ - ILARIA VICENTI⁽¹⁾ - LUISA BRACCI⁽¹⁾

Univesity of Siena, Department of Medical Biotechnologies, Siena, Italia⁽¹⁾

A heparan sulfate proteoglycan binding tetrapeptide successfully inhibits SARS-CoV-2 Omicron replication

CAMILLA BIBA, LORENZO DEPAU, LIA FIASCHI, JLENIA BRUNETTI, ILENIA VARASI, CHIARA FALCIANI, MAURIZIO ZAZZI, ILARIA VICENTI AND LUISA BRACCI

Department of Medical Biotechnologies, University of Siena, Siena, Italy

Introduction The emergence of the SARS-CoV-2 Omicron variant has led to increased transmissibility and immune escape, due to the high number of mutations in the Spike (S) region, particularly in the receptor binding domain (RBD). Further, the mechanism of Omicron entry has evolved into endocytic dependent internalization, as opposed to membrane fusion dependent internalization predominant with previous variants. In addition, a possible interaction of membrane Heparan Sulfate Proteoglycans (HSPGs) with SARS-CoV-2 during virus internalization has been recently suggested. The aim of this work was to determine the role of HSPGs in the infectivity of the Omicron variant, and the efficacy of an HSPG binding tetrapeptide (LB-1) in inhibiting Omicron entry in a live virus cell-based model. **Materials and Methods** LB-1 was synthesized in a tetra-branched form on a multiple automated synthesizer by standard Fmoc chemistry. Caco-2 cells were infected for 24h with Omicron BA.1 and Delta SARS-CoV-2 strains. After incubation, the virus supernatant was transferred on the highly permissive reporter cell line VERO E6. The half-maximal Tissue Culture Infectious Dose was determined after 24h in VERO E6 measuring the viral nucleocapsid expression by ELISA assay. The experiments were performed with and without treatment with heparinase to remove the HSPGs chains. The half-maximal cytotoxic concentration of LB-1 was determined by luminescence. To determine the antiviral activity, serial dilutions of LB-1 starting from the not-toxic dose, were incubated with a fixed amount of BA.5, BA.1 and Delta strains (MOI=0.01) for 1h at 37°C on pre-seeded Caco-2 cells. After incubation, the virus-peptide mixture was removed, and fresh LB-1 was added. Viral supernatants were transferred in VERO E6, and the half-maximal inhibitory concentration of LB-1 (IC₅₀) was measured by ELISA as previously described. Each experiment included a mock infection control, a virus control and two reference inhibitors with known IC₅₀ (remdesivir and a previously tested human immune serum). **Results** LB-1 inhibited cell infection by BA.1 and BA.5 with 21.5 ± 2.8 and 19.5 ± 1.7 μ M IC₅₀, respectively. By contrast, LB-1 had no effect against Delta variant indicating a crucial role for HSPGs in cell infection by Omicron but not by Delta. Heparinase treated cells were not permissive to Omicron infection, confirming the role of HSPGs, while a modest impact was measured on Delta (4.6-fold reduction). **Discussion and Conclusions** The prototype HSPG binding tetrapeptide LB-1 selectively inhibited the replication of SARS-CoV-2 Omicron. The role of HSPGs in Omicron infection and this proof-of-concept results support further development of HSPGs targeting agents as a novel strategy to block SARS-CoV-2 infection.

27 - All-Trans Retinoic Acid Effect on *Candida albicans* growth and biofilm formation

TERENZIO COSIO (1) - ENRICO SALVATORE PISTOIA (1) - GAETANA COSTANZA (1) - ELENA CAMPIONE (2) - ROBERTA GAZIANO (1)

Department of Experimental Medicine, Tor Vergata University of Rome, 00133 Rome, Italy;; Department of Experimental Medicine, Tor Vergata University of Rome, 00133 Rome, Italy;; Roma, Italia (1) - Department of Systems Medicine, Dermatology Unit, Tor Vergata University Hospital, 00133 Rome, Italy;; Department of Systems Medicine, Dermatology Unit, Tor Vergata University Hospital, 00133 Rome, Italy;; Roma, Italia (2)

All-Trans Retinoic Acid Effect on *Candida albicans* growth and biofilm formation

Terenzio COSIO^{1,2}, Enrico S. PISTOIA³, Gaetana COSTANZA³, Elena CAMPIONE², Roberta GAZIANO³

¹Department of Experimental Medicine, PhD Course in Microbiology, Immunology, Infectious Diseases, and Transplants (MIMIT), University of Rome Tor Vergata, 00133 Rome, Italy;

²Department of Systems Medicine, Dermatology Unit, Tor Vergata University Hospital, 00133 Rome, Italy;

³Department of Experimental Medicine, University of Rome Tor Vergata, 00133 Rome, Italy;

Introduction: *Candida albicans* (*C. albicans*) is the most common fungal pathogen causing recurrent mucosal and life-threatening systemic infections. The ability to switch from yeast to hyphae and produce biofilm are the key virulence determinants of this fungus. In fact, *Candida* biofilms on medical devices represent the major risk factor for nosocomial bloodstream infections. Novel antifungal strategies are required given the severity of systemic candidiasis, especially in immunocompromised patients, and the lack of effective anti-biofilm treatments. Retinoids have gained attention recently due to their antifungal properties.

Material and methods: The present study aimed at evaluating the *in vitro* effects of different concentrations (300 to 18.75 µg/mL) of All-trans Retinoic Acid (ATRA), a vitamin A metabolite, on *Candida* growth and biofilm formation.

Results: ATRA completely inhibited the fungal growth, by acting as both fungicidal (at 300 µg/mL) and fungistatic (at 150 µg/mL) agent. Furthermore, ATRA was found to negatively affect *Candida* biofilm formation in terms of biomass, metabolic activity and morphology, in a dose-dependent manner, and intriguingly, its efficacy was as that of amphotericin B (AmB) (2-0.12 µg/mL). Additionally, transmission electron microscopy (TEM) analysis showed that at 300 µg/mL ATRA induced plasma membrane damage in *Candida* cells, confirming its direct toxic effect against the fungus.

Conclusion: Altogether, the results suggest that ATRA has a potential for novel antifungal strategies aimed at preventing and controlling biofilm-associated *Candida* infections.

28 - Isolation and characterization of novel mycobacteriophages and evaluation of their lytic activity against *Mycobacterium abscessus*

MARIAGRAZIA DI LUCA ⁽¹⁾ - ENRICO CASU ⁽²⁾ - CLAUDIA CAMPOBASSO ⁽¹⁾ - CATERINA FERRETTI ⁽¹⁾ - SARA BOLOGNINI ⁽¹⁾ - FABIO FILIPPINI ⁽²⁾ - GIULIA FREER ⁽²⁾ - ARIANNA TAVANTI ⁽¹⁾ - LAURA RINDI ⁽²⁾

Università di Pisa, Dipartimento di Biologia, Pisa, Italia ⁽¹⁾ - Università di Pisa, Dipartimento di Ricerca Traslazionale e delle Nuove Tecnologie in Medicina e Chirurgia, Pisa, Italia ⁽²⁾

Isolation and characterization of novel mycobacteriophages and evaluation of their lytic activity against *Mycobacterium abscessus*

MARIAGRAZIA DI LUCA¹, ENRICO CASU², CLAUDIA CAMPOBASSO^{1,3}, CATERINA FERRETTI¹, SARA BOLOGNINI¹, FABIO FILIPPINI², GIULIA FREER², ARIANNA TAVANTI¹, LAURA RINDI²

¹Department of Biology, University of Pisa, Pisa, Italy; ²Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa, Pisa, Italy; ³ Department of Biosystems, KU Leuven, Leuven, Belgium

Introduction

Mycobacterium abscessus is a rapidly growing mycobacterium associated with several diseases in humans, of which lung disease is the most common, particularly in patients with bronchiectasis, chronic obstructive pulmonary disease and cystic fibrosis. During the last years, *M. abscessus* has appeared as an emerging pathogen, with an increasing number of reported cases worldwide. *M. abscessus* is one of the most drug-resistant mycobacteria, thus stressing the need for new and more effective treatment options. Phage therapy is based on the use of bacteriophages for the treatment of bacterial infection and offers a promising alternative approach against *M. abscessus* infections. This study aims to isolate novel mycobacteriophages, describe their genetic and biological characteristics and evaluate their capacity to infect and kill *M. abscessus* strains.

Materials and Methods

Forty-two environmental samples were collected for the isolation of phages using *M. smegmatis* mc² 155 as host. Plaques with different phenotypes were isolated and propagated in the host. Phage genome was extracted, sequenced by Illumina technology and annotated. The phages were characterized by lisogeny screening and one-step growth experiments. Then, their lytic activity against *M. abscessus* clinical strains was also tested by spot assay.

Results

Fourteen phage clones with an apparent different plaque morphology were selected from two soil samples and propagated in *M. smegmatis* mc² 155. Whole genome sequencing led to the identification of three genetically distinct mycobacteriophages, belonging to two novel species. From their genome analysis, they were found to carry genes involved in lysogeny such as integrase and repressor. The ability of phages to carry out a lysogenic cycle was also confirmed by an in vitro assay. The three novel phages identified do not carry any gene for drug resistance or virulence traits.

Their one-step growth curves showed that one of the phages has a remarkably fast replication cycle of about 30 minutes. Finally, the host range of all phage clones was evaluated against 16 *M. abscessus* clinical strains. The phage clones were able to infect all tested bacterial strains, including isolates associated with disseminated infection and multidrug resistance.

Discussion and conclusions

Three new mycobacteriophages were identified and characterized. Overall, the properties observed for such phages, including the absence of resistance or virulence genes, the fast replication cycle and the lytic activity against *M. abscessus* clinical strains, suggest their potential application in managing *M. abscessus* infections.

31 - A promising antimicrobial peptide against some virulence features of *Acinetobacter baumannii* clinical strains

ROSANNA PAPA (1) - IRENE PARIS (1) - MARIKA TRECCA (1) - ESTHER IMPERLINI (2) -
FRANCESCO BUONOCORE (2) - MARCO ARTINI (1) - LAURA SELAN (1)

Sapienza Università, Dipartimento di Sanità Pubblica e Malattie Infettive, Roma, Italia (1) -
University of Tuscia, Department for Innovation in Biological, Agro-Food and Forest Systems,
Viterbo, Italia (2)

Acinetobacter baumannii is a Gram-negative multidrug-resistant nosocomial pathogen and it is recognized as a major cause of hospital acquired infections (HAI). According to the World Health Organization, *A. baumannii* is one of the most resistant pathogens belonging to ESKAPE group (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *A. baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* spp.). The main infections caused by this pathogen are ventilator-associated pneumonia (VAP), meningitis, osteomyelitis, bloodstream, skin and soft tissue and burn infections. The eradication of *A. baumannii* is very complicated for its ability to: i) form biofilm, ii) survive desiccation, iii) make motility on medical implantable devices. Among the different virulence factors, outer membrane protein (OmpA) biofilm-associated protein (Bap), type IV pili and Csu pili are mainly involved in biofilm formation. As already mentioned, another virulence factor is the bacterial motility; despite to *A. baumannii* has been classified as a non-mobile pathogen, it is able to perform two types of motilities: the twitching, led by type IV pili, and the surface motility, appendages-independent. These are only some of the features that contribute to the multi-drug resistance of this pathogen. The increasing resistance to conventional antibiotics has become a global health crisis. Antimicrobial peptides (AMPs) have been identified as new potential drugs to replace or integrate classical antibiotics, as they show a broad spectrum of activity against human bacterial pathogens. Antarctica marine life is an excellent source for novel drugs of biotechnological interest, including AMPs. The icefish *Chionodraco hamatus*, a particular Antarctic marine organism, produces an AMP, named chionodracine. Different mutants from the original peptide were successfully designed based on its scaffold to improve the antibacterial activity. In particular, the peptide named KHS-Cnd, showed a high capacity to kill ESKAPE pathogens. In our previous work, KHS-Cnd has been efficiently tested against *P. aeruginosa* clinical isolates from cystic fibrosis patients. Therefore, in this study, we analyzed the minimal concentration able to inhibit bacterial growth (MIC), the inhibition of motility and the effect on biofilm formation of KHS-Cnd against *A. baumannii* clinical strains.

32 - Eskape pathogens: looking for alternative strategies from an exotic source

ROSANNA PAPA ⁽¹⁾ - MARIKA TRECCA ⁽¹⁾ - IRENE PARIS ⁽¹⁾ - CATERINA D'ANGELO ⁽²⁾ -
ERMENEGILDA PARRILLI ⁽²⁾ - MARCO ARTINI ⁽¹⁾ - LAURA SELAN ⁽¹⁾

Sapienza Università, Dipartimento di Sanità Pubblica e Malattie Infettive, Roma, Italia ⁽¹⁾ - Federico II University, Department of Chemical Sciences, Napoli, Italia ⁽²⁾

ESKAPE pathogens are the major cause of nosocomial infections. These pathogens have the ability of escaping biocidal action of antimicrobials and the immune system response. As a result, ESKAPE pathogens are responsible for more than 40% of infections in intensive care units and pose an economic burden, especially in low- and middle-income countries. ESKAPE pathogens exhibit resistance to drug using different mechanisms, including drug inactivation by irreversible enzyme cleavage, drug-binding site alteration, diminution in permeability of drug or drug efflux increment to reduce accumulation of drug as well as biofilm production.

The scientific community has shown significant interest in using novel strategies to counteract the virulence of these pathogens. Anti-virulence drugs do not necessarily kill bacterial cells but prevent bacterial pathogenesis by targeting their virulence traits, and they can be used to combat the emergence of antibiotic-resistant pathogens. The use of anti-virulence strategies results in less evolutionary pressure, thus reducing the development of resistant strains. In this approach, the anti-ESKAPE drug administered would interfere with virulence factors instead of bacterial vitality, thus leading to the development of new strategies for the prevention and control of infections.

Recently, several agents of natural origin isolated from fungi, bacteria and plants have shown anti-virulence features against some bacterial species, including ESKAPE. In this context, we chose to explore an exotic and unusual ecological niche represented by Antarctic marine habitat. Marine microorganisms possess a vast diversity of metabolic capabilities, since their ecological niche forces them to abruptly adapt to the diverse range of physical and chemical conditions of rapidly changing marine ecosystems. Cold-adapted marine bacteria represent an unexploited reservoir of biodiversity endowed with an interesting chemical repertoire, also applicable in the pharmaceutical field.

In this work we analyzed the effect of supernatant derived from four different Antarctic bacteria, against a collection of 70 clinical ESKAPE pathogens. In particular, antibiofilm effects of Antarctic bacterial culture supernatants were examined on ESKAPE pathogens either during biofilm development by adding it to the medium at the beginning of the growth (pre-adhesion period), or after biofilm formation (mature biofilm). Firstly, to exclude an effect of supernatants on bacterial viability, their antimicrobial activity was tested. The obtained results have shown the great potential of Antarctic bacteria as producers of molecules capable of counteracting the phenomenon of biofilm formation of bacterial species of significant clinical interest.

41 - Virucide activity of nanocomposites based on resin included clays for sanitization of highly touched surfaces

FRANCESCO RICCHI ⁽¹⁾ - FRANCESCO LIPANI ⁽¹⁾ - ISABELLA MARCHESI ⁽¹⁾ - ANNALISA BARGELLINI ⁽¹⁾ - FABIO TATEO ⁽²⁾ - ANTONELLA MANSI ⁽³⁾ - CLAUDIO CERPELLI ⁽¹⁾

Università degli Studi di Modena e Reggio Emilia, Facoltà di Medicina e Chirurgia, Modena, Italia

⁽¹⁾ - Consiglio Nazionale delle Ricerche, Istituto di Geoscienze e Georisorse, Padova, Italia ⁽²⁾ -

INAIL, Dipartimento di Medicina, Epidemiologia, Igiene del Lavoro e Ambientale, Roma, Italia ⁽³⁾

Virucide activity of nanocomposites based on resin included clays for sanitization of highly touched surfaces

Francesco Ricchi¹, Francesco Lipani¹, Isabella Marchesi², Annalisa Bargellini², Fabio Tateo³, Antonella Mansi⁴, Roberta Bertani⁵, Claudio Cermelli¹

¹ Dipartimento Chirurgico, Medico, Odontoiatrico e di Scienze Morfologiche con Interesse Trapiantologico, Oncologico e di Medicina Rigenerativa, Università di Modena e Reggio Emilia, Modena, Italy; ² Dipartimento di Scienze Biomediche, Metaboliche e Neuroscienze, Sezione di Sanità Pubblica, Università di Modena e Reggio Emilia, Modena, Italy; ³ Istituto di Geoscienze e Georisorse, Consiglio Nazionale delle Ricerche, Padova, Italy; ⁴ Dipartimento di Medicina Epidemiologia, Igiene del Lavoro e Ambientale, INAIL Centro Ricerche Monte Porzio Catone, Roma, Italy; ⁵ Dipartimento di Ingegneria Industriale, Università di Padova, Padova, Italy

Introduction

The evergrowing problem antibiotic and disinfectant resistance disclosed by microorganisms imposes the search of new approaches for infection containment. Nanocomposites represent a promising alternative. These are solid materials made of a raw matrix including nanoparticles of the active compound with a dimension in the order of nanometers. Clays have been demonstrated to have antibacterial properties and are used in the traditional medicine. Little is known about its virucide activity. Therefore, the aim of this work is to investigate the virucide activity of nanostructured clays included in epoxide resins.

Materials and Methods

The antiviral activity was tested on Herpes Simplex Virus-1 (Hsv-1), Coronavirus OC-43 (CoV-OC43) and Adenovirus-5 (AdV) with different types of clays, both natural and modified, included or not in blocks of epoxide resins. A biological assay (virus yield reduction) was used in order to rescue infectious particles and not only uninfected genetic material. Briefly, the epoxide blocks were soaked in a suspension of the different viruses for 1h and 24h. At the end, the viral load of rescued virus was assessed by end point titration.

Results

Initially, the potential virucide activity of powdered clays was tested but no results were obtained because of technical problems related to clay resuspension. Interestingly, epoxide resin without nanostructured clays showed a significant antiviral efficacy at 24h exposition (> 1Log reduction) against HSV-1 and CoV-OC43 but not AdV-5. The inclusion of different types of nanostructured clays caused an increase in viral reduction only in one case, whereas with the other clays a decrease of the antiviral efficacy showed by the epoxide resin per se was observed.

Discussion

These preliminary results show that nanocomposites containing nanostructured clays included in epoxide resin have a virucide activity. An interesting finding is the intrinsic antiviral activity of epoxide resin, suggesting that this incorporating matrix is an ideal support for clay nanocomposites. The decrease in virucide activity of some clays included in epoxide resins may be due to the interactions between resin and clay hindering the release of the active compounds.

43 - Isolation of Human Monoclonal Antibodies Against Pan-Drug Resistant *Klebsiella pneumoniae*

SORAYA SOLEDAD BOSCH⁽¹⁾ - VITTORIA ZUCCONI GALLI FONSECA⁽¹⁾ - EMANUELE ROSCIOLI⁽¹⁾ - GIULIA CARDINALI⁽¹⁾ - GIUSEPPE MACCARI⁽²⁾ - GIAMPIERO BATANI⁽¹⁾ - IDA PACIELLO⁽¹⁾ - CLAUDIA SALA⁽¹⁾ - ANNA KABANOVA⁽¹⁾ - GIUSY TISEO⁽³⁾ - FRANCESCO MENICHETTI⁽³⁾ - MARCO FALCONE⁽³⁾ - RINO RAPPUOLI⁽⁴⁾

Fondazione Toscana Life Sciences, Monoclonal Antibody Discovery Lab, Siena, Italia⁽¹⁾ -
Fondazione Toscana Life Sciences, Data Science for Health (DASCH) Lab, Siena, Italia⁽²⁾ -
Department of Clinical and Experimental Medicine, Infectious Diseases Unit, University of Pisa, Pisa, Italia⁽³⁾ - Fondazione Biotechnopolo di Siena, Monoclonal Antibody Discovery Lab, Siena, Italia⁽⁴⁾

Isolation of Human Monoclonal Antibodies Against Pan-Drug Resistant *Klebsiella pneumoniae*

SORAYA S. BOSCH¹, VITTORIA ZUCCONI GALLI FONSECA^{1,2}, EMANUELE ROSCIOLI¹, GIULIA CARDINALI^{1,2}, GIUSEPPE MACCARI³, GIAMPIERO BATANI¹, IDA PACIELLO¹, CLAUDIA SALA¹, ANNA KABANOVA¹, GIUSY TISEO⁴, FRANCESCO MENICHETTI⁴, MARCO FALCONE⁴, RINO RAPPUOLI^{1,5}

¹Monoclonal Antibody Discovery Lab, Fondazione Toscana Life Sciences, Siena, Italy; ²University of Siena, Siena, Italy; ³Data Science for Health (DASCH) Lab, Fondazione Toscana Life Sciences, Siena, Italy; ⁴Department of Clinical and Experimental Medicine, Infectious Diseases Unit, University of Pisa, Pisa, Italy; ⁵Fondazione Biotechnopolo di Siena, Siena, Italy

1. Introduction

Klebsiella pneumoniae (Kp) is a major cause of healthcare-associated infections responsible for increased morbidity and mortality worldwide. For a long time, multidrug resistance (MDR) and hypervirulence were considered two non-overlapping phenotypes, as carbapenem-resistant *K. pneumoniae* (CRKp) strains are much less virulent and hypervirulent *K. pneumoniae* (hvKp) strains are usually susceptible to antibiotics. However, an increasing number of strains have been identified that integrate both phenotypes in recent years, creating MDR strains that result in devastating clinical outcomes.

2. Materials and Methods

In this study, we are working with Kp clinical isolates from the outbreak in Tuscany, which started in 2018 and is ongoing, with bacteria belonging to ST147, the most representative clone in the outbreak. Human monoclonal antibodies (mAbs) were isolated from patients with bloodstream infection caused by bla_{NDM-1}-carrying Kp. Several in vitro experiments were carried out to characterize these mAbs, including serum bactericidal assays (SBA), opsonophagocytic assays (OPA), and confocal fluorescence microscopy.

3. Results

More than 200 Kp-binding mAbs were isolated from human samples, and their initial characterization resulted in a shortlist of 24 highly bactericidal candidates. The IC₅₀ values of these mAbs ranged from 1-32,000 ng/mL in SBA. Moreover, some were cross-reactive against several strains of Kp, such as those carrying bla_{NDM-1}, bla_{NDM-5}, and bla_{NDM-9}. Furthermore, OPA showed different levels of bacterial phagocytosis promoted by the various mAbs. Additional studies are ongoing to identify the cognate antigens.

4. Discussion and Conclusions

Currently, there are no vaccines or alternative therapies against Kp. Immunotherapy with mAbs for the treatment of infectious diseases is receiving increasing attention, with several mAbs in clinical phases; however, experimental strategies for effective development have not yet been optimized for bacterial pathogens. We established an antigen-agnostic approach to efficiently screen human mAbs, starting from the blood of patients who recovered from Kp infection. We successfully isolated extremely potent candidates against pan-drug-resistant ST147 bla_{NDM-1}-carrying Kp clinical isolates. Overall, this study exemplifies a rationally designed preclinical approach for developing and characterising innovative immunotherapies to target antimicrobial-resistant pathogens and address the silent pandemic caused by MDR species.

49 - Azole-loaded mucosomes as multi drug delivery platform to counteract infections caused by *Candida albicans*.

NARCISA MANDRAS (1) - LORENZA CAVALLO (1) - OLGA VALENTINA GARBERO (2) - FABRIZIO CAREDDU (2) - FRANCESCA MENOTTI (1) - FABIO LONGO (1) - ELEONORA MANISCALCO (1) - VALERIA ALLIZOND (1) - CLAUDIA PAGANO (1) - JANIRA ROANA (1) - ANNA MARIA CUFFINI (1) - SONJA VISENTIN (2) - GIULIANA BANCHE (1)

Università di Torino, Dipartimento di Scienze della Sanità Pubblica e Pediatriche, Torino, Italia ⁽¹⁾ -
Università di Torino, Dipartimento di Biotecnologie Molecolari e Scienze della Salute, Torino, Italia ⁽²⁾

Azole-loaded mucosomes as multi drug delivery platform to counteract infections caused by *Candida albicans*.

NARCISA MANDRAS¹, LORENZA CAVALLO¹, OLGA V. GARBERO², FABRIZIO CAREDDU², FRANCESCA MENOTTI¹, FABIO LONGO¹, ELEONORA MANISCALCO¹, VALERIA ALLIZOND¹, JANIRA ROANA¹, CLAUDIA PAGANO¹, ANNA M. CUFFINI¹, SONJA VISENTIN², GIULIANA BANCHE¹

¹Department of Public Health and Pediatrics, University of Torino, Turin, Italy; ²Department of Molecular Biotechnology and Health Science, University of Torino, Turin, Italy

Introduction. Today there is a clear need to reinvent the way in which current drugs are delivered, focusing on different mechanisms of action and improving the efficiency of delivery systems to make them more selective for the specific body district and the type of targeted environment or microorganism. By mimicking our primary defensive mechanism, the mucus, we used mucin glycoproteins to develop a new system with multiple potential applications, including drug delivery. The aim of this project is to assess the potential antifungal effect of a novel category of nanosystems, named mucosomes, which consist of glycosylated ad mucoadhesive nanoparticles composed of mucins, alone or coupled with azoles as multi drug delivery platform to counteract infections caused by *Candida albicans*.

Materials and methods. The antimicrobial activity of mucosomes (NPs) prepared with mucine from bovine submaxillary gland (BSM) and porcine gastric mucin (PGM), and drugs (fluconazole-FLU, itraconazole-ITZ and voriconazole-VOR) were determined by a broth microdilution assay in 96-well plates, according to EUCAST document E.DEF 7.3.1, against two strains of *C. albicans*, one resistant and one sensitive to posaconazole. Furthermore, an in vitro time-kill assay on *C. albicans*, monitored for 24, 48, 72 and 120 hours, was performed by incubating yeasts in sterile sampling tubes alone (control growth) or with different formulations: FLU or ITZ or VOR loaded NPs (1xMIC concentration); NPs without azoles; PGM or BSM.

Results. BSM/PGM-NPs alone did not show significant activity against *C. albicans* strains. On the contrary, mucosome formulations loaded with drugs showed efficacy against the two tested strains with MIC ranges of 2.5-4.7 µg/ml for NPs-ITZ, 2.49->39.77 µg/ml for NPs-VOR and 12.49->49.93 µg/ml for NPs-FLU. Furthermore, the time kill kinetics confirmed that NPs were particularly effective

to reduce yeast growth. According to cell counts, azole loaded NPs displayed long term antimicrobial efficacy against *C. albicans* significantly inhibiting yeast growth up to 120 hours. Intriguingly, NPs-ITZ were generally more effective, than NPs-VOR and NPs-FLU.

Discussion and Conclusions. The possibility to deliver drugs using mucosomes may offer several advantages over conventional systems in terms of mucoadhesive properties and targeted delivery. Drugs would be released more closely to pathogens, limiting their adverse effects and maximizing their effectiveness. Taken together, these results support the hypothesis that mucosomes are capable antifungal devices and proper drug loaded mucosomes might be a promising strategy for the treatment of *C. albicans* infections.

50 - New inhibitors of Carbonic Anhydrases from *Pseudomonas aeruginosa*: antimicrobial and antibiofilm activities with respect to ciprofloxacin

BEATRICE MARINACCI⁽¹⁾ - ANDREA ANGELI⁽²⁾ - SIMONE CARRADORI⁽¹⁾ - NOEMI MENCARELLI⁽¹⁾ - VALENTINA PUCA⁽¹⁾ - BENEDETTA PELLEGRINI⁽¹⁾ - SANTOLO FRANCATI⁽³⁾ - CLAUDIU T. SUPURAN⁽²⁾ - ROSSELLA GRANDE⁽¹⁾

Università degli Studi "G. d'Annunzio" Chieti-Pescara, Dipartimento di Farmacia, Chieti, Italia⁽¹⁾ -
Università degli Studi di Firenze, Dipartimento NEUROFARBA, Firenze, Italia⁽²⁾ - Alma Mater
Studiorum Università di Bologna, Dipartimento di Scienze e Tecnologie Agro-Alimentari, Bologna,
Italia⁽³⁾

New inhibitors of Carbonic Anhydrases from *Pseudomonas aeruginosa*: antimicrobial and
antibiofilm activities with respect to ciprofloxacin

BEATRICE MARINACCI^{1,2}, ANDREA ANGELI³, SIMONE CARRADORI¹, NOEMI
MENCARELLI¹, VALENTINA PUCA¹, BENEDETTA PELLEGRINI¹, SANTOLO FRANCATI⁴,
CLAUDIU T. SUPURAN³, ROSSELLA GRANDE¹

¹ Department of Pharmacy, "G. d'Annunzio" University of Chieti-Pescara, Chieti, Italy;

² Department of Innovative Technologies in Medicine & Dentistry, "G. d'Annunzio" University of
Chieti-Pescara, Chieti, Italy;

³ Neurofarba Department, University of Florence, Sesto Fiorentino (FI), Italy;

⁴ Department of Agricultural and Food Sciences, University of Bologna, Viale Fanin, 42, Bologna,
40127, Italy

Introduction. *Pseudomonas aeruginosa* is an opportunistic pathogen commonly associated with nosocomial infections. The most affected patients are those with respiratory disorders such as pneumonia and cystic fibrosis. These conditions are of particular concern given that *P. aeruginosa* is known to display a high rate of antibiotic resistance. Indeed, in 2017 the World Health Organization included carbapenem-resistant *P. aeruginosa* in the critical-priority category of pathogens for which new antimicrobials are urgently needed. One of the most promising strategies is to find novel bacterial targets, therefore, 42 new molecules were synthesized and characterized to evaluate the possible inhibition of *P. aeruginosa* growth by interacting with innovative targets such as bacterial Carbonic Anhydrases (CAs).

Materials and methods. The Minimum Inhibitory Concentration (MIC) was determined via the broth microdilution method and alamarBlue assay. The Minimal Bactericidal Concentration (MBC) was determined by the Colony Forming Unit (CFU) count. Among the compounds, MC11 and MC176 showed satisfactory MIC values compared to ciprofloxacin, therefore, they were selected to evaluate their potential antibiofilm activity. *P. aeruginosa* mature biofilm was treated with different concentrations of the selected compounds, corresponding to 1xMIC, 2xMIC and 3xMIC. The Minimum Biofilm Eradication Concentration (MBEC) was determined via alamarBlue, CFU count and Crystal Violet (CV) assay. Finally, the toxicity of both compounds was evaluated using *Galleria mellonella* in vivo model. Different concentrations of the tested compounds were administered to the larvae which were monitored for 4 days to score mortality.

Results. The tested compounds showed a heterogeneous activity profile with MIC values ranging from 0.5 to 128 µg/ml. Encouraging results were obtained for the compounds MC11 and MC176 that showed a MIC of 0.5 µg/ml which is closer to the one of ciprofloxacin (0.25 µg/ml). The two molecules also displayed the same MBC value corresponding to 1 µg/ml. The MBEC confirmed their efficacy in reducing *P. aeruginosa* biofilm: MC11 displayed a MBEC of 2xMIC (1 µg/ml) while MC176 showed a ciprofloxacin-like behavior with a MBEC of 3xMIC (1.5 µg/ml). The in vivo toxicity assay proved the safety of the two compounds: no death was recorded even in the *G. mellonella* larvae treated with the highest compounds concentration corresponding to 16.6 mg/kg.

Discussion and conclusion. The data obtained in this study proved that MC11 and MC176 can be considered a new promising approach to counteract *P. aeruginosa* infections. Many studies promote the use of selective CAs inhibitors as an alternative strategy to eradicate *P. aeruginosa* infections and limit the spread of antibiotic resistance.

52 - Silver-doped composite modified scaffolds to impart both anti-microbial features and enhanced biological properties in bone regenerative medicine.

FRANCESCA MENOTTI ⁽¹⁾ - FABIO LONGO ⁽¹⁾ - SARA COMINI ⁽¹⁾ - ROSARIA SPARTI ⁽¹⁾ - SARA SCUTERA ⁽¹⁾ - LORENZA CAVALLO ⁽¹⁾ - BARTOLOMEO COPPOLA ⁽²⁾ - ELISA FIUME ⁽²⁾ - ELEONORA MANISCALCO ⁽¹⁾ - CLAUDIA PAGANO ⁽¹⁾ - ANNA MARIA CUFFINI ⁽¹⁾ - NARCISA MANDRAS ⁽¹⁾ - PAOLA PALMERO ⁽²⁾ - GIULIANA BANCHE ⁽¹⁾ - VALERIA ALLIZOND ⁽¹⁾

University of Torino, Department of Public Health and Pediatrics, Torino, Italia ⁽¹⁾ - Politecnico of Torino, Department of Applied Science and Technology, Torino, Italia ⁽²⁾

Silver-doped composite modified scaffolds to impart both anti-microbial features and enhanced biological properties in bone regenerative medicine.

FRANCESCA MENOTTI¹, FABIO LONGO¹, SARA COMINI¹, ROSARIA SPARTI¹, SARA SCUTERA¹, LORENZA CAVALLO¹, BARTOLOMEO COPPOLA², ELISA FIUME², ELEONORA MANISCALCO¹, CLAUDIA PAGANO¹, ANNA MARIA CUFFINI¹, PAOLA PALMERO², GIULIANA BANCHE¹, VALERIA ALLIZOND¹

¹Department of Public Health and Pediatrics, University of Torino, Turin, Italy; ²Department of Applied Science and Technology, Politecnico of Torino, Turin, Italy

Introduction. The regeneration of damaged or lost osseous structures without the need for permanent implants can be achieved using biodegradable and bioresorbable scaffolds that can provide structural and biomechanical stability allowing the natural tissue restoration. Biomaterials based on biodegradable polymers, such as poly ϵ -caprolactone (PCL) complexed with bioactive inorganic materials (e.g., hydroxyapatite, HA, and β -tricalcium phosphate, β -TCP), owing these properties, are emerging as a promising innovation tool for bone regeneration applications. Notably, prosthetic joint infection (PJI) following orthopaedic implant surgery are worsened by the increasing microbial resistance to the antibiotics commonly used in such cases, and represents an ever-growing concern. Thus, the biomaterial functionalization with metal ions - such as silver - can constitute an important resource to overcome the microbial-resistance issue thanks to their broad spectrum of antimicrobial properties. This work aimed to combine the antibacterial properties of silver, with those of PCL, added with BCP, for medical applications in which good tissue regeneration and antimicrobial effects are required.

Materials and methods. PCL and BCP/PCL porous constructs functionalized with low percentages ($\sim 1.0\%$) of silver (Ag) were developed by salt-leaching method (by using sodium chloride, NaCl, or sodium nitrate, NaNO₃ as pore formers) and characterized from the morphological/microstructural point of view by means of Field Emission Scanning Electron Microscopy (FESEM), while the phase composition was assessed by X-Ray Diffraction (XRD). The anti-bacterial experiments were executed against *Staphylococcus aureus*, *S. epidermidis* and *Escherichia coli* by means of inhibition halo and adhesion assays. Cytotoxicity tests on human fetal osteoblasts (Saos-2 cells) were also performed.

Results. FESEM analysis showed that the samples were characterized by square-shaped macropores, whose average dimension was in agreement with that of the starting salt. The presence of PCL and BCP phases were confirmed by XRD. The appearance of an inhibition halo around the silver-

functionalized PCL scaffolds for all the three microorganisms, and a significant ($p<0.05$) decrease on both adherent and planktonic bacteria demonstrated the Ag^+ release from the 3D constructs. Furthermore, the viability and proliferation of Saos-2 cells was not hampered by the PCL scaffolds enriched with the lowest silver percentages.

Discussion and Conclusions. Due to the combined antimicrobial, osteoproliferative and biodegradable properties, the PCL-based scaffolds enriched with silver showed good potential for bone tissue engineering and offer a promising strategy, as an ideal microbial anti-adhesive tool for reduction in PJIs and antimicrobial molecules-targeted delivery.

POSTER presentation

Presenting Author

Professor Valeria Allizond

Department of Public Health and Pediatrics

Via Santena 9 - 10126 Torino

Tel. 011.670.5644

Fax 011.236.5644

E-mail: valeria.allizond@unito.it

55 - Small molecules and PROTACs to inhibit and degrade HPV oncoproteins

CHIARA BERTAGNIN⁽¹⁾ - LORENZO MESSA⁽¹⁾ - MATTEO PAVAN⁽²⁾ - JENNY DESANTIS⁽³⁾ - BEATRICE MERCORELLI⁽¹⁾ - MATTIA STURLESE⁽²⁾ - MARTA CELEGATO⁽¹⁾ - TOMMASO D'ANGELI⁽³⁾ - GABRIELE CRUCIANI⁽³⁾ - LAWRENCE BANKS⁽⁴⁾ - LAURA GORACCI⁽³⁾ - STEFANO MORO⁽²⁾ - ARIANNA LOREGIAN⁽¹⁾

Department Of Molecular Medicine, University Of Padua, Padua, Italia⁽¹⁾ - Department Of Pharmaceutical And Pharmacological Sciences, University Of Padua, Padua, Italia⁽²⁾ - Department Of Chemistry, Biology And Biotechnology, University Of Perugia, Perugia, Italia⁽³⁾ - International Centre For Genetic Engineering And Biotechnology, Area Science Park, Trieste, Italia⁽⁴⁾

Small molecules and PROTACs to inhibit and degrade HPV oncoproteins

CHIARA BERTAGNIN¹, LORENZO MESSA¹, MATTEO PAVAN², JENNY DESANTIS³, BEATRICE MERCORELLI¹, MATTIA STURLESE², MARTA CELEGATO¹, TOMMASO D'ANGELI³, GABRIELE CRUCIANI³, LAWRENCE BANKS⁴, LAURA GORACCI³, STEFANO MORO², ARIANNA LOREGIAN¹.

¹ Department of Molecular Medicine, University of Padua, Padua, Italy; ² Department of Pharmaceutical and Pharmacological Sciences, University of Padua, Padua, Italy; ³ Department of Chemistry, Biology, and Biotechnology, University of Perugia, Perugia, Italy; ⁴ International Centre for Genetic Engineering and Biotechnology (ICGEB), Trieste, Italy.

Introduction

High-Risk human papillomaviruses (HR-HPVs) are responsible worldwide for virtually all cases of cervical carcinoma and several other types of cancers. Despite substantial advancement in anti-HPV vaccination, no specific anticancer drug to treat HPV-associated tumors exists yet in the clinics. The carcinogenic potential of HR-HPVs relies on the activity of the two major viral oncoproteins, E6 and E7, which are therefore considered as attractive targets for drug development. In this context, the development of specific anti-E6/anti-E7 inhibitors could be coupled to the emerging PROteolytic TARgeting Chimeras (PROTACs) technology, in order to enhance their potency by inducing E6 and E7 degradation besides their inhibition.

Materials and Methods

We performed two in silico screenings of small-molecule libraries to search for compounds directed against the E6/p53 or the E7/PTPN14 interaction. Hit compounds were tested for their ability to inhibit the E6/p53 or the E7/PTPN14 binding by in vitro and cell-based assays, and for specific cytotoxic activity against HPV-positive cancer cells by MTT assays. The p53 and PTPN14 rescue in HPV-positive cells was assessed by Western Blot and immunofluorescence. Expression of p53 and YAP target genes was measured by RealTime qPCR. Compounds' antitumoral activity was investigated through cell proliferation assays, clonogenic assays, transwell migration and invasion assays, wound healing assays, and 3D spheroid formation assays. Synergism between Cpd12 and standard chemotherapeutic drugs was investigated by combination studies. PROTAC-induced E6 degradation was assessed through Western Blot.

Results

Among the hits emerged from the two in silico screenings, we identified two compounds - Cpd12

and Cpd20 - that specifically block the E6/p53 and E7/PTPN14 interaction, respectively, and inhibit the growth of HPV-positive cells without affecting HPV-negative cancer cells and non-tumoral cells. Treatment of HPV-positive cells with Cpd12 led to the rescue of p53 levels and upregulation of p53 target genes. Cpd20 was able to restore PTPN14 protein levels, leading to the relocalization of YAP in the cytoplasm and its consequent inactivation. Both compounds were able to inhibit the migration, invasion, and stem potential of HPV-positive cells, thus showing promising antitumoral activity. Cpd12 was also shown to synergize with standard chemotherapeutic drugs. Finally, Cpd12 was exploited for the design of PROTACs targeting E6 to degradation. Preliminary results showed that PRO-HPV-04 can lead to a dose-dependent decrease in E6 levels besides p53 upregulation.

Discussion and Conclusions

We identified first-in-class inhibitors of the E6/p53 and E7/PTPN14 interactions, providing the proof-of-principle that these interactions can be targeted by means of small molecules. We plan to develop PROTAC-based anti-E6 and anti-E7 drugs that could be the basis for specific anti-HPV therapies.

60 - A small-molecule inhibitor of flaviviral NS3-NS5 interaction with broad-spectrum activity and efficacy in vivo

BEATRICE MERCORELLI ⁽¹⁾ - MARTA CELEGATO ⁽¹⁾ - MATTIA STURLESE ⁽²⁾ - VIVIAN VASCONCELOS COSTA ⁽³⁾ - MARTA TREVISAN ⁽¹⁾ - ANGELICA SAMER LALLO DIAS ⁽⁴⁾ - INGREDY BEATRIZ SOUZA PASSOS ⁽³⁾ - CELSO MARTINS QUEIROZ-JUNIOR ⁽³⁾ - LORENZO MESSA ⁽¹⁾ - ANNAGIULIA FAVARO ⁽²⁾ - CHIARA BERTAGNIN ⁽¹⁾ - STEFANO MORO ⁽²⁾ - MAURO MARTINS TEIXEIRA ⁽⁴⁾ - ARIANNA LOREGIAN ⁽¹⁾

Università di Padova, Dipartimento di Medicina Molecolare, Padova, Italia ⁽¹⁾ - Università di Padova, Dipartimento di Scienze del farmaco, Padova, Italia ⁽²⁾ - Universidade Federal de Minas Gerais, Belo Horizonte, Brazil, Departamento de Morfologia, ICB, Belo Horizonte, Brasile ⁽³⁾ - Universidade Federal de Minas Gerais, Belo Horizonte, Brazil, Departamento de Bioquímica e Imunologia, ICB, Belo Horizonte, Brasile ⁽⁴⁾

Categoria: Molecole naturali ad azione antimicrobica/Nuovi approcci antimicrobici

A small-molecule inhibitor of flaviviral NS3-NS5 interaction with broad-spectrum activity and efficacy in vivo

Beatrice Mercorelli^a, Marta Celegato,^a Mattia Sturlese,^b Vivian Vasconcelos Costa,^c Marta Trevisan,^a Angélica Samer Lallo Dias,^d Ingredy Beatriz Souza Passos,^c Celso Martins Queiroz-Junior,^c Lorenzo Messa,^a Annagiulia Favaro,^b chiara bertagnin,^a Stefano Moro,^b Mauro Martins Teixeira,^d Arianna Loregian,^a

^ADepartment of Molecular Medicine, University of Padua, Padua, Italy;

^BMolecular Modeling Section (MMS), Department of Pharmaceutical and Pharmacological Sciences, University of Padua, Padua, Italy;

^CDepartamento de Morfologia, ICB, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil;

^DDepartamento de Bioquímica e Imunologia, ICB, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil.

Introduction. Every year, Dengue virus (DENV) causes hundred million infections worldwide that can result in dengue disease and severe dengue. Two other mosquito-borne flaviviruses, i.e., Zika virus (ZIKV) and West Nile virus (WNV) are responsible of prolonged outbreaks and are associated with severe neurological diseases, congenital defects, and eventually death. All these three viruses, despite their importance for global public health still lack a specific drug treatment. Here, we describe the structure-guided discovery of small molecules with pan-flavivirus antiviral potential.

Materials and Methods. A virtual screening of ~1 million structures targeting the NS3-NS5 interaction surface of different flaviviruses was performed taking into account the crystal structures of NS5 of DENV, ZIKV, and WNV and predicting by molecular dynamic simulation the binding of a small molecule to a conserved site in flaviviral NS5. Thirty virtual hit compounds were then tested by an ELISA assay to investigate their ability to interfere with the NS3-NS5 interaction in vitro and in DENV-2 infected cells by plaque reduction assays to inhibit virus replication. Two nontoxic hit molecules were then subjected to further investigation by analyzing their pan-dengue antiviral activity, activity against other mosquito-borne flaviviruses and unrelated ssRNA viruses, the reduction of viral protein expression, and the activity in disease-relevant ZIKV-infected neural stem cells. Moreover, NMR studies were performed to validate NS5 as the target and in vivo studies in an animal model of DENV infection were also conducted.

Results. Two molecules inhibited the interaction between DENV NS3 and NS5 in vitro, the replication of all DENV serotypes as well as ZIKV and WNV in infected cells, while being inactive

against other unrelated ssRNA viruses. Both hit compounds exhibited low propensity to select resistant viruses. By NMR studies, we confirmed the interaction of the two hit compounds to DENV-2 NS5. Remarkably, one molecule demonstrated efficacy in a mouse model of dengue by reducing peak viremia, viral load in target organs, and associated tissue pathology.

Discussion and conclusions. This study provides the proof-of-concept that targeting flaviviral NS3-NS5 interaction is an effective therapeutic strategy able to reduce virus replication in vivo and discloses new chemical scaffolds that could be further developed, thus providing a significant milestone in the development of much awaited broad-spectrum anti-flaviviral drugs.

71 - Graphene Quantum Dots as promising nanomaterials against Mycobacterium tuberculosis infection

GIULIA SANTARELLI⁽¹⁾ - FLAVIO DE MAIO⁽²⁾ - GIORDANO PERINI⁽³⁾ - VALENTINA PALMIERI⁽³⁾ - ALESSANDRO SALUSTRI⁽¹⁾ - ROBERTO ROSATO⁽¹⁾ - IVANA PALUCCI⁽²⁾ - MICHELA SALI⁽²⁾ - MASSIMILIANO PAPI⁽³⁾ - GIOVANNI DELOGU⁽⁴⁾

Università Cattolica del Sacro Cuore, Department of Sciences of Basic Biotechnologies, Intensive and Perioperative Clinics, Section of Microbiology, Roma, Italia⁽¹⁾ - Fondazione Policlinico Universitario Agostino Gemelli, Department of Laboratory and Infective Sciences, Faculty of Medicine, Roma, Italia⁽²⁾ - Università Cattolica del Sacro Cuore, Dipartimento di Neuroscienze, Roma, Italia⁽³⁾ - Mater Olbia Hospital, Mater Olbia Hospital, Olbia, Italia⁽⁴⁾

Graphene Quantum Dots as promising nanomaterials against Mycobacterium tuberculosis infection

Authors

Giulia Santarelli¹, Flavio De Maio², Giordano Perini^{3,4}, Valentina Palmieri^{4,5}, Alessandro Salustri¹, Roberto Rosato¹, Ivana Palucci^{1,2}, Michela Sali^{1,2}, Massimiliano Papi^{3,4}, Giovanni Delogu^{2,6}

Affiliations

¹Department of Sciences of Basic Biotechnologies, Intensive and Perioperative Clinics, Section of Microbiology, Università Cattolica del Sacro Cuore, Rome, Italy; ²Department of Laboratory and Infective Sciences, Faculty of Medicine, Fondazione Policlinico A. Gemelli IRCCS, Università Cattolica del Sacro Cuore, Rome, Italy; ³Fondazione Policlinico Universitario “A. Gemelli”, IRCCS, Rome, Italy; ⁴Dipartimento di Neuroscienze, Università Cattolica del Sacro Cuore, 00168 Rome, Italy; ⁵Istituto dei Sistemi Complessi, CNR, Rome, Italy; ⁶Mater Olbia Hospital, Olbia, Italy

Introduction

Tuberculosis (TB) remains one of the most alarming infectious diseases worldwide. The emergence and spread of drug-resistant Mycobacterium tuberculosis (Mtb) strains lead to the development of new drugs and therapeutic strategies. Carbon-based nanomaterials (CNMs) have showed a promising activity against mycobacteria. We have demonstrated that graphene oxide (GO) entraps mycobacteria in the extracellular milieu and stimulates ROS production. Unfortunately, GO revealed a significant toxicity in human peripheral blood mononuclear cells (PBMCs). Among CNMs, Graphene Quantum Dots (GQDs) are of particular interest in nanotechnology because of photo-physical and chemical properties and their interaction with biological membranes.

Materials and methods

GQDs anti-mycobacterial properties were evaluated against Mtb in both axenic cultures (at low concentration and at exponential growth) and in in vitro infection models. We assayed the direct activity, using a non-functionalized (L-GQDs) and two functionalized GQDs (NH₂-GQDs and COOH-GQDs), at the final concentration of 50 µg/ml, alone or in combination with isoniazid (INH), amikacin (AMK) and linezolid (LZD), at minimal inhibitory concentration. To assess bacterial survival, colony forming units (CFUs) were determined. Given the proven efficacy of GQDs to

perturb the cell membrane and to modify its permeability, we tested the GQDs-drugs activity in in vitro models of Mtb infection. Firstly, GQDs cytotoxicity was assessed in murine macrophages (J774) and on PBMCs. In our models, Mtb infected cells (MOI 1), were previously treated GQDs before drug administration.

Results

Our results showed that GQDs did not exert neither trapping activity nor a mycobactericidal effect when used to treat axenic cultures. CFUs measured at 6-24 hours post treatment or until 21 days (for Mtb at exponential growth) did not show significant differences when GQDs were used alone or in combination with the selected drugs.

J774 were infected and treated with GQDs before administration of the selected drugs. CFUs measured 24 hours later highlighted that AMK and INH increased they efficacy on infected macrophages. In the second experimental setting, when infected PBMCs were GQDs treated, before drug administration, no significant variation in CFUs was detected. In both models, GQDs alone did not show any toxic activity.

Discussion and conclusions

GQDs-drugs appeared to be a potential innovative anti-TB treatment at the forefront in fighting emerging antibiotic-resistant Mtb strains. Although GQDs did not have any trapping or direct antimycobacterial effect, as demonstrated for GO, their ability to change cell membrane permeability, ameliorating drug cellular intake may be an important source to contain Mtb replication.

78 - Citrus fruit polyphenols for fighting antibiotic resistance

DILETTA MAZZANTINI⁽¹⁾ - MARIACRISTINA MASSIMINO⁽¹⁾ - MARCO CALVIGIONI⁽¹⁾ - FRANCESCO CELANDRONI⁽¹⁾ - EMILIA GHELARDI⁽¹⁾

Università di Pisa, Dipartimento di Ricerca Traslazionale e delle Nuove Tecnologie in Medicina e Chirurgia, Pisa, Italia⁽¹⁾

Citrus fruit polyphenols for fighting antibiotic resistance

DILETTA MAZZANTINI¹, MARIACRISTINA MASSIMINO¹, MARCO CALVIGIONI¹, FRANCESCO CELANDRONI¹, EMILIA GHELARDI¹

¹Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa, Pisa, Italy.

1. Introduction. Nowadays, antibiotic resistance represents a global health emergency and research is progressively focusing on the development of new therapeutic options to face up bacterial infections. Polyphenols, natural molecules extracted from plants with a wide-spectrum antimicrobial activity, represent promising candidates in the management of bacterial infections, being suitable to be used alone or in combination with existing antibiotics. In this study, the antimicrobial activity of a mixture of polyphenols extracted from citrus fruits (named B) was investigated. In addition, the ability of B to enhance the activity of antibiotics toward multidrug-resistant bacteria and the potential development of resistance to B were evaluated.

2. Materials and Methods. Minimal inhibitory concentrations (MICs) of B against reference and multidrug-resistant strains (*Staphylococcus aureus*, *Staphylococcus epidermidis*, *Klebsiella pneumoniae*, and *Escherichia coli*) were determined by broth microdilution. Minimal Bactericidal concentrations (MBCs) were also identified. Time kill tests were performed by inoculating bacteria in 2-fold the MIC of B and by plating aliquots on solid media at selected time points. The checkerboard assay was used to test the interaction of B with antibiotics. To evaluate the in vitro development of resistance to B, *S. aureus* and *K. pneumoniae* reference strains were sub-cultured 10 times on plates containing 0.5-fold the MIC of B. After the 5th and the 10th transfer, bacteria were seeded on plates containing 2-fold the MIC of B to isolate resistant colonies.

3. Results. B was active at low concentrations and displayed bactericidal effects against all the tested microbes. Most of the reduction in *S. aureus* and *K. pneumoniae* viability occurred within the first minute of exposure and no residual living cells were obtained starting from 10 minutes of incubation. Experiments are still ongoing to evaluate the interaction of B with antibiotics and to investigate the development of resistance to B in Gram-positive and Gram-negative bacteria.

4. Discussion and Conclusions. B showed a rapid bactericidal effect and a wide-spectrum antimicrobial activity, also extended to multidrug resistant bacteria. Therefore, B appears to be a promising candidate in managing infections sustained by multidrug-resistant bacteria.

This research was supported by PNRR THE - Tuscany Health Ecosystem - Spoke 7 – Innovating Translational Medicine - Sub-project 5- Innovative models for management of infections caused by antibiotic-resistant bacteria (Project code: ECS00000017; CUP: I53C22000780001).

94 - Improving Gene-Editing delivery to fight viral infections

ALESSANDRO DE CARLI⁽¹⁾ - DOMENICO FAVARO⁽²⁾ - PIOTR BARSKI⁽³⁾ - VITTORIA RAFFA⁽⁴⁾ - MAURO PISTELLO⁽⁵⁾ - MICHELE LAI⁽²⁾

Università di Siena, Dipartimento di Biotecnologie Mediche, Siena, Italia⁽¹⁾ - Università di Pisa, Dipartimento di Medicina Traslazionale, Pisa, Italia⁽²⁾ - ProChimia Surfaces Sp. z o.o., Research and Development ProChimia Surfaces, Gdynia, Polonia⁽³⁾ - Università di Pisa, Dipartimento di Biologia, Pisa, Italia⁽⁴⁾ - U.O. Virologia Universitaria, Azienda Universitaria Ospedaliera Pisana, Pisa, Italia⁽⁵⁾

Improving Gene-Editing delivery to fight viral infections

ALESSANDRO DE CARLI^{1,5}, DOMENICO FAVARO¹, PIOTR BARSKI⁴, VITTORIA RAFFA³, MAURO PISTELLO^{1,2}, MICHELE LAI¹

¹Department of Translational Research and New Technologies in Medicine and Surgery, Retrovirus Center and Virology Section, University of Pisa, Pisa, Italy;

²Laboratory of Virology Unit, Pisa University Hospital, Pisa, Italy;

³ Department of Biology, Nanomedicine and Molecular Biology Section, University of Pisa, Pisa, Italy;

⁴ProChimia Surfaces, Gdynia, Poland;

⁵Department of Medical Biotechnologies, University of Siena, Siena, Italy.

Introduction: CRISPR-Cas13 is a recently discovered endonuclease belonging to the Cas protein family that targets single-stranded RNA. This enzyme already proved to powerfully eradicate RNA virus infections, however, its delivery into cells poses challenges. To address this limitation, we functionalized gold nanoparticles (Au_NPs) with Cas13 and evaluate delivery, specificity, and antiviral potency against SARS-CoV-2 and ZIKV. The study was supported by the I-GENE project, which has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement N° 862714.

Materials and Methods: Viral loads were evaluated intracellularly and extracellularly using qRT-PCR and confocal microscopy. Nano-formulation Localization: Au_NPs functionalized with fluorescent Cas9 were followed by confocal live imaging. Enzymatic Kinetics and System Delivery: Huh-7 cells were prior infected and then treated with Au_NPs functionalized against SARS-CoV-2 and ZIKV genomes. Intracellular viral load was assessed by qRT-PCR. Antiviral Efficacy Assessment: The efficacy of lipofection-delivered or Au_NPs-associated system was evaluated at various time points after SARS-CoV-2 and ZIKV infection.

Results: gRNA Selection: (>1 log) reduction observed for SARS-CoV-2 replication when Nucleocapsid protein, RNA-dependent RNA polymerase, and Non-structural protein 3 were targeted. Similar results were observed for ZIKV when Capsid protein and Non-structural proteins 1, 4A, and 4B were targeted. Nano-formulation Localization: Au_NPs-Cas9 spontaneously enters cells and localizes in the cytoplasm within 1h, localizing into cytoplasmic vesicles and nucleus. Enzymatic Kinetics and Delivery System Evaluation: Initial reduction (<1 log) observed after 8-10 hours of treatment, followed by viral load recovery after 24 hours with lipofection or Au_NPs delivery. Antiviral Efficacy Evaluation: Reduction (<1 log) observed after a single treatment with Au_NPs administered prior to, during, or after infection. Lipofection shows approximately 1 log reduction after a single treatment post-infection.

Discussion and Conclusions: Our results demonstrate the advantageous nature of the Au₂₅ NPs delivery system as it spontaneously transports the CRISPR-Cas13 system into the cell. The enzymatic kinetics indicate a temporally restricted action, suggesting a potential need for periodic cell treatment. The reduction in viral load, although not highly efficient, provides a basis for further studies to optimize this novel antiviral strategy.

97 - Effect of the Human Lactoferricin-Derived Peptide hLF 1-11 on *Candida albicans* and *Staphylococcus* spp. mixed biofilms.

IACOPO FRANCONI⁽¹⁾ - COSMERI RIZZATO⁽¹⁾ - ARIANNA TAVANTI⁽²⁾ - ANTONELLA LUPETTI⁽¹⁾

Università di Pisa, Dipartimento di Ricerca Traslationale e delle Nuove Tecnologie in Medicina e Chirurgia, Pisa, Italia⁽¹⁾ - Università di Pisa, Dipartimento di Biologia, Pisa, Italia⁽²⁾

Effect of the Human Lactoferricin-Derived Peptide hLF 1-11 on *Candida albicans* and *Staphylococcus* spp. mixed biofilms.

Iacopo Franconi¹, Cosmeri Rizzato², Arianna Tavanti², Antonella Lupetti¹

¹Dipartimento di Ricerca Traslationale e delle Nuove Tecnologie in Medicina e Chirurgia, Università di Pisa, Pisa, Italy;

²Dipartimento di Biologia, Università di Pisa, Pisa, Italy.

Introduction: Biofilm-producing microorganisms represent a major healthcare problem. *Candida albicans* and *Staphylococcus* spp. have shown the highest tendency of biofilm formation, both alone and in association with each other. Among all novel therapeutic approaches for the treatment of microbial biofilms, antimicrobial peptides seem to be a promising option. The present study aimed at analyzing the inhibitory activity of the N-terminal lactoferrin-derived peptide (hLF 1-11) against both *C. albicans* and *Staphylococcus epidermidis*/*Staphylococcus aureus* monomicrobial and polymicrobial biofilms.

Methods: hLF 1-11 anti-biofilm activity effects on biofilm formation inhibition and eradication via evaluation of biomass reduction, metabolic activity, cell viability and observation of cell morphology on confocal fluorescence microscopy.

Results: hLF 1-11 at concentrations ≥ 88 $\mu\text{g/mL}$ effectively inhibited biofilm formation in all the tested strains and their combinations. The morphologic analysis showed the disappearance of the hyphal cellular form of *C. albicans* after treatment both in monomicrobial and polymicrobial biofilms. The effect of hLF 1-11 treatment on the biofilm eradication was a significant decrease on metabolic activity and a 20% reduction of the biomass of *C. albicans* - *S. aureus* mixed biofilms, the morphological analysis showed a biofilm still structured as the untreated one but with abundant presence of dead cells.

Discussion and Conclusions: hLF 1-11 treatment is of potential clinical relevance, representing a promising novel approach to target the difficult to treat polymicrobial *C. albicans* – *S. aureus*, *C. albicans* – *S. epidermidis* infections. These mixed models represent an in-vitro microbiological association that mimic in-vivo biofilm conditions, which are frequently encountered in clinical practice. Eradication strategies are an unmet need in current clinical practice; therefore, prevention of surface colonization and biofilm formation in general is the recommended approach. To this point, based on the evidence presented in this study, it appears reasonable to further investigate hLF 1-11 as a possible therapeutic option in biofilm prevention.

99 - Humanized monoclonal antibody targeting a pre-fusion domain of the viral fusion protein stabilizes the extended state of the measles viral fusion process

ROBERTA DELLA MARCA ⁽¹⁾ - OLIVIA HARDER ⁽²⁾ - MASSIMILIANO GALDIERO ⁽¹⁾ - STEFAN NIEWIESK ⁽²⁾ - MATTEO POROTTO ⁽¹⁾

Università degli studi della Campania Luigi Vanvitelli, Dipartimento di Medicina Sperimentale, Napoli, Italia ⁽¹⁾ - Department of Veterinary Biosciences, College of Veterinary Medicine, The Ohio State University, Columbus, Ohio, Stati Uniti D'america ⁽²⁾

Humanized monoclonal antibody targeting a pre-fusion domain of the viral fusion protein stabilizes the extended state of the measles viral fusion process.

Roberta Della Marca^{1,2,3}, Olivia Harder⁴, Massimiliano Galdiero³, Stefan Niewiesk⁴, Matteo Porotto^{1,2,3} *

¹Center for Host-Pathogen Interaction, Columbia University Vagelos College of Physicians and Surgeons, New York, New York, USA

²Department of Pediatrics, Columbia University Vagelos College of Physicians and Surgeons, New York, New York, USA

³Department of Experimental Medicine, University of Campania 'Luigi Vanvitelli', 81100 Caserta, Italy

⁴ Department of Veterinary Biosciences, College of Veterinary Medicine, The Ohio State University, Columbus, Ohio, USA

Introduction: Measles virus (MeV) infection is significant public health threat despite the availability of a vaccine due to hesitation in developed countries, therefore, effective antivirals are in high demand. MeV infection requires the presence of two glycoproteins: the hemagglutinin (H) and the fusion (F) proteins. The H protein interacts with the receptor and activates the F protein to catalyze membrane merging. First, the F pre-fusion state extends into a transient intermediate that interacts with the cell membrane. This intermediate then collapses to the post-fusion state. We identified a monoclonal antibody (mAb,77.1) that targets the MeV F protein and efficiently prevents infection in vivo. Here we assessed its mechanism of action compared it with two other fusion inhibitors: a small molecule (3g) that prevents F activation and an inhibitor peptide (HRC) that stabilizes the transient intermediate of the fusion.

Materials and Methods: To determine the mechanism of action of 77.1 mAb, we investigated at what stage in the fusion process it acts through a triggering assay. In addition to 77.1, we used HRC and 3g. For these experiments, we used a chimeric receptor binding protein (H-HN), that binds sialic acid-bearing receptors on red blood cells (RBC) and the HN-sialic acid binding is regulated by a small molecule (zanamivir). Cells co-expressing H-HN and MeV F were mixed with sialic acid receptor-bearing RBCs in the presence or absence of the 77.1 mAb, HRC peptide, or 3g compound. After incubation at 37°C for 60' (to permit F activation), zanamivir was added to reverse the receptor interaction. We determined the amount of RBCs in three groups: (i) Released into the medium by zanamivir, indicating that they had been attached only through H-HN, not by F insertion into the target cell membrane. (ii) Not released by zanamivir, indicating that F had been activated and inserted into the target RBC membrane (but not fused). Finally, (iii) RBC undergoing fusion and the F activation process proceeded past the transitional intermediate to achieve fusion.

Results: The RBC triggering assay showed that the 3g compound prevented the F activation. For both 77.1 and HRC peptide exerted their antiviral activity by stabilizing the F protein after its activation. The HRC peptide binds to F only upon activation and cannot interact with the pre-fusion F. In distinction, the 77.1 mAb tightly binds to a pre-fusion epitope in F, allows for the F membrane insertion but prevents the further F refolding required for membrane fusion.

Discussion and Conclusion: mAb 77.1 shows promise as potential MeV immune prophylaxis via specific targeting of the pre-fusion MeV F structure and stabilizing an extended intermediate of the fusion process.

110 - *Pseudomonas* spp.: a new ally to fight drug resistance?

MARCELLO MURGIA⁽¹⁾ - LUISA MARRAS⁽²⁾ - VALENTINA CORONEO⁽¹⁾

Dipartimento di Scienze Mediche e Sanità Pubblica, Università di Cagliari, Cagliari, Italia⁽¹⁾ -
Servizio laboratorio analisi chimico cliniche-microbiologiche, ASL Cagliari, Cagliari, Italia⁽²⁾

Pseudomonas spp.: a new ally to fight drug resistance?

MARCELLO MURGIA¹, LUISA MARRAS², VALENTINA CORONEO¹

¹Dipartimento di Scienze Mediche e Sanità Pubblica, Università di Cagliari, Italia;

²Servizio laboratorio analisi chimico cliniche-microbiologiche ASL Cagliari, Cagliari, Italia

Introduction.

The improper and massive use of biocides among the population has been found to enhance human resistance to drugs, whilst promoting the development of new and emerging microorganisms.

The health emergency caused by this resistances induce the international scientific community to researching new inhibitory molecules.

The aim of this work was to evaluate the fungicidal activity of fenazin-1-carboxylic acid synthesized by *Pseudomonas* spp. isolated from samples of *Pleurotus eryngii*.

Materials and Methods.

For this study, the control samples were taken from mushrooms without contamination, which subsequently have been contaminated through a simulation and placed to incubate at 25°C for 72 hours. It was then observed the inhibitory effect around the inoculation point of other fungal species previously presented. Subsequently, the aqueous extract obtained from a suspension of 2 ml of *P. fluorescens* with OD600 (1 McF) and the filtered solution (45-micrometre polycarbonate) were tested for antifungal activity. The applied method was that of Zygadlo and Guzmán with a modification in *Rhizopus* spp. and *Aspergillus brasiliensis*. 12 mL of Potato Dextrose Agar (PDA) was poured into the Petri dish and 6 mL of PDA was poured on the cover. Subsequently, six paper disks were placed up and down on the PDA medium in each Petri dish. Two different experiments were carried out: six filtered (F) and six no filtered (NF). Samples with the mycelia in PDA and paper disk impregnated with distilled sterile water were used as the negative control. The effectiveness of the treatments was evaluated by measuring the average diametric growth of the mycelium at 4, 8, and 12 days after the inoculation. The percentage of inhibition was calculated according to the following equation of Zygadlo et al. ($I = 100 (C - T) C^{-1}$ where I is inhibition, C is average diameter of fungi grown in PDA + water, and T is the average diameter of fungi cultivated in PDA + F and NF).

Results.

The resistance of *Mucor* spp. was observed, whilst it was highlighted on a smaller percentage on *Fusarium* spp., (38%), *Paecilomyces* spp., (44%), and *Penicillium* spp. (19%). With the modified method it was possible to highlight the complete inhibition of *R. spp.* and *A. brasiliensis* with 16%.

Discussion and Conclusions.

The inhibitory action of phenazine-1-carboxylic acid, as reported by other authors, is effective against the growth of moulds in the food chain. This is important to prevent problems associated with the production process and Public health.

111 - Antimicrobial activity of novel arginine- and proline-rich peptides obtained through a bioinformatic approach

TECLA CIOCIOLA⁽¹⁾ - LAURA GIOVATI⁽¹⁾ - LORENZA ARTESANI⁽¹⁾ - STELLA GARCIA COLOMBAROLLI⁽²⁾ - GIADA BIANCHETTI⁽³⁾ - GIUSEPPE MAULUCCI⁽³⁾ - STEFANIA CONTI⁽¹⁾ - ALBERTO VITALI⁽²⁾

University of Parma, Department of Medicine and Surgery, Parma, Italia⁽¹⁾ - National Research Council of Italy, Istituto di Scienze e Tecnologie Chimiche (SCITEC-CNR), Rome, Italia⁽²⁾ - Fondazione Policlinico Universitario A. Gemelli IRCCS; Università Cattolica del Sacro Cuore, Department of Neuroscience, Rome, Italia⁽³⁾

Antimicrobial activity of novel arginine- and proline-rich peptides obtained through a bioinformatic approach

TECLA CIOCIOLA¹, LAURA GIOVATI¹, LORENZA ARTESANI¹, STELLA GARCIA COLOMBAROLLI², GIADA BIANCHETTI^{3,4}, GIUSEPPE MAULUCCI^{3,4}, STEFANIA CONTI¹, ALBERTO VITALI²

¹Department of Medicine and Surgery, Unit of Biomedical, Biotechnological and Translational Sciences, University of Parma, Parma, Italy; ²National Research Council of Italy, Istituto di Scienze e Tecnologie Chimiche (SCITEC-CNR), Rome, Italy; ³Department of Neuroscience, Section of Biophysics, Università Cattolica del Sacro Cuore, Rome, Italy; ⁴Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy.

Introduction

Microbial infections still constitute a major problem for public health, mainly in relation to the spread of antimicrobial resistance. Furthermore, the ability of bacterial and fungal cells to form biofilms has been associated with high rates of morbidity and mortality, as well as with severe resistance to most available antimicrobials. Research and development of new anti-infective drugs, stagnating for many years, have thus become a global priority. In this scenario, antimicrobial peptides (AMPs) are gaining increasing interest as an alternative or complement to currently available antimicrobial agents. Proline-rich AMPs, almost exclusively of animal origin, are of particular interest due to their peculiar mode of action.

Materials and Methods

A search for new arginine- and proline-rich peptides from plants has been carried out with a bioinformatic approach by sequence alignment and the use of antimicrobial prediction tools. The search resulted in two peptide candidates that were synthesized by solid-phase chemistry and evaluated for their cytotoxic, hemolytic and structural properties by consolidated experimental methods. The *in vitro* antimicrobial activity was investigated against planktonic and biofilm-structured yeasts (*Candida* spp.) and bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*), including resistant isolates. The effect of peptides' treatment on microbial cells was studied by confocal microscopy.

Results

The selected peptides, named Ma1 and Ma2, showed a similar potent antimicrobial activity with half-maximal effective concentration values in the micromolar range, without toxic effects on mammalian cells. Their mode of action appeared to be linked to a membrane perturbation mechanism, as suggested by killing kinetics and the results of *in vitro* vesicles perturbation assay, and confocal

microscopy observations. Circular dichroism measurements revealed a predominant random coil arrangement for both peptides.

Discussion and Conclusions

Interestingly, Ma1 and Ma2 seemed to exert a membrane-perturbation effect, while most proline- and arginine-rich AMPs of animal origin act on intracellular targets. Further studies will be needed to evaluate the in vivo therapeutic activity of these peptides, or their derivatives, in suitable experimental infection models. The Ma2 peptide, that showed more potent activity, represents a promising candidate for developing new antimicrobial drugs to be used as an alternative treatment to microbial infections, mainly caused by biofilms.

112 - Characterization of four novel bacteriophages targeting multi-drug resistant *Klebsiella pneumoniae* strains of sequence type 147 and 307

GRETA PONSECCHI⁽¹⁾ - LAURA SCARFONE⁽¹⁾ - NOEMI POERIO⁽¹⁾ - TOMMASO OLIMPIERI⁽¹⁾ - ALBERTO ANTONELLI⁽²⁾ - MARCO COPPI⁽²⁾ - EUGENIO PACCAGNINI⁽³⁾ - PIETRO LUPETTI⁽³⁾ - GIAN MARIA ROSSOLINI⁽²⁾ - MAURIZIO FRAZIANO⁽¹⁾ - MARCO MARIA D'ANDREA⁽¹⁾

University of Rome "Tor Vergata", Department of Biology, Rome, Italia⁽¹⁾ - University of Florence, Department of Experimental and Clinical Medicine, Florence, Italia⁽²⁾ - University of Sienna, Department of Life Sciences, Sienna, Italia⁽³⁾

Characterization of four novel bacteriophages targeting multi-drug resistant *Klebsiella pneumoniae* strains of sequence type 147 and 307

GRETA PONSECCHI^{1,2}, LAURA SCARFONE¹, NOEMI POERIO¹, TOMMASO OLIMPIERI¹, ALBERTO ANTONELLI^{3,4}, MARCO COPPI^{3,4}, EUGENIO PACCAGNINI⁵, PIETRO LUPETTI⁵, GIAN MARIA ROSSOLINI^{3,4}, MAURIZIO FRAZIANO¹, MARCO M. D'ANDREA¹

¹ Dept. of Biology, University of Rome "Tor Vergata", Rome, Italy; ² PhD Program in Evolutionary Biology and Ecology, Dept. of Biology, University of Rome "Tor Vergata", Rome, Italy; ³ Dept. of Experimental and Clinical Medicine, University of Florence, Florence, Italy; ⁴ Microbiology and Virology Unit, Florence Careggi University Hospital, Florence, Italy; ⁵ Dept. of Life Sciences, University of Sienna, Sienna, Italy.

Introduction: the development of alternative antimicrobial strategies is deemed to be a high priority to deal with the challenge caused by the spread of multidrug-resistant (MDR) bacteria in clinical settings. According to several international organizations, phages or components thereof are one of these possible options that could be useful to prevent or treat bacterial infections. Among the drug-resistant bacteria, carbapenem resistant *Klebsiella pneumoniae* (CR-Kp) are particularly worrisome, given the extensive MDR profiles, their pandemic dissemination and primary role in healthcare associated and life-threatening infections. In this study we isolated and characterised four lytic bacteriophages targeting two major high-risk clones of CR-Kp circulating in hospital environments, i.e., those belonging to Sequence Type (ST) 307 and ST147. Materials and Methods: wastewater samples collected from hospitals located in Central Italy were screened for the presence of phages by using a previously characterized collection of *K. pneumoniae* clinical isolates as hosts and the top-agar overlay technique. Host specificity and infection efficiency was assessed by spot test and efficiency of plating, respectively. Dynamic of bacterial infections was determined by the one-step growth curve method. Phages were visualized through transmission electron microscopy (TEM) and their genomes were obtained and analysed by a Next Generation Sequencing approach followed by bioinformatics analysis. Results: four bacteriophages, named GP-1, GP-2, GP-4 and GP-5, have been isolated, purified and produced at high titres. Collectively, two phages were able to selectively lyse 12/14 *K. pneumoniae* strains of ST307, while the other two were active only against all the tested *K. pneumoniae* strains of ST147 (n=12). Phages maintain an overall good stability to temperature and pH changes and were characterized by infection cycles having latency periods ranging from 10 to 50 minutes and burst sizes of 10-100 PFU. Results from TEM analysis and genome sequencing demonstrated that the four phages were of different families and allowed to rule out the presence of antibiotic resistance genes, virulence factors or toxins. Discussion and Conclusions: considering their strictly lytic nature and their high selectivity towards two of the

major high-risk clones of *K. pneumoniae*, the isolated phages can be considered as good candidates for their evaluation in animal models as members of cocktails for applications to treat or prevent severe infections caused by CR-Kp strains.

113 - Evaluation of the antibiotic activity of grapefruit seed extract in edible mushrooms contaminations

MARCELLO MURGIA ⁽¹⁾ - ALESSANDRO BANCHIERO ⁽¹⁾ - LUISA MARRAS ⁽²⁾ -
VALENTINA CORONEO ⁽¹⁾

Dipartimento di Scienze Mediche e Sanità Pubblica, Università di Cagliari, Cagliari, Italia ⁽¹⁾ -
Servizio laboratorio analisi chimico cliniche-microbiologiche, ASL Cagliari, Cagliari, Italia ⁽²⁾

Evaluation of the antibiotic activity of grapefruit seed extract in edible mushrooms contaminations
MARCELLO MURGIA¹, ALESSANDRO BANCHIERO¹, LUISA MARRAS², VALENTINA
CORONEO¹

¹Dipartimento di Scienze Mediche e Sanità Pubblica, Università di Cagliari, Italia;

²Servizio laboratorio analisi chimico cliniche-microbiologiche ASL Cagliari, Cagliari, Italia
Introduction.

Due to its intrinsic characteristics, *Pleurotus eryngii* is a mushroom highly susceptible to fungal contamination and bacteriosis, causing large economic losses to producers of edible mushrooms. Many of these microorganisms produce biofilm, a complex extracellular polymeric matrix structure in which are contained resistant to antibiotics and disinfectant bacteria. The focus of this work was to find different substances to prevent the growth of bacteriosis by *Pseudomonas* spp. and *P. fluorescens* isolated in samples of *P. eryngii*.

Materials and Methods.

The Kirby-Bauer test was applied to evaluate antibiotic activity, using a grapefruit seed extract in ATCC strains that produce biofilm: *Staphylococcus aureus* 6538, *S. aureus* MRS (wild type), *P. aeruginosa* 9027, *P. fluorescens* (wild type), *Escherichia coli* 25922 and *Candida albicans* 2091. It was then added the micro method of dilutions in broth and the antibiofilm activity by applying the crystal violet assay, reported by S. Stepanovic, using sterile 96-well microplates. Subsequently, the grapefruit seed extract was atomized on the mushroom growth substrate surface after and before sowing.

Results.

From the results obtained it emerged that grapefruit seed extract is highly effective. No action was exerted in *C. albicans*. The inhibition of *S. aureus* was highlighted with 17.60%, 1.40% to *S. aureus* MRS, 0.15% to *P. aeruginosa*. In addition, *E. coli* was inhibited with 28% and *P. fluorescens* with 1.15%.

Discussion and Conclusions.

Through this study, the antibiofilm activity of grapefruit seed extract was observed. The inhibition percentages obtained are satisfactory to *P. spp.* causing bacteriosis in *P. eryngii*; moreover, it has emerged that this substance is more effective in the preliminary phase of the sowing of the fungal spores in the substrate, thus inhibiting the development of the bacteriosis.

121 - Effect of fish skin mucous derived from bathypelagic fish *Centrolophus niger* (Gmelin, 1789) on *Escherichia coli* strains

VIRGINIA FUOCHI⁽¹⁾ - SALVATORE FURNARI⁽¹⁾ - LUCIA LONGHITANO⁽¹⁾ - FRANCESCO TIRALONGO⁽²⁾ - DANIELE TIBULLO⁽¹⁾ - PIO MARIA FURNERI⁽¹⁾

Università di Catania, BIOMETEC, Catania, Italia⁽¹⁾ - Università di Catania, Dip. di Scienze Biologiche, Geologiche e Ambientali, Catania, Italia⁽²⁾

Effect of fish skin mucous derived from bathypelagic fish *Centrolophus niger* (Gmelin, 1789) on *Escherichia coli*

VIRGINIA FUOCHI¹, SALVATORE FURNARI¹, LUCIA LONGHITANO², FRANCESCO TIRALONGO³, DANIELE TIBULLO² E PIO MARIA FURNERI¹

1 Dipartimento di Scienze Biomediche e Biotecnologiche, Sez. di Microbiologia, Università di Catania, Catania, Italia; 2 Dipartimento di Scienze Biomediche e Biotecnologiche, Sez. di Biochimica medica, Università di Catania, Catania, Italia; 3 Dipartimento di Scienze Biologiche, Geologiche e Ambientali, Sez. Biologia Animale, Università di Catania, Catania, Italia.

Introduction: *Centrolophus niger*, commonly known as the Black Oreo, is a species of fish found mainly in the deep waters of the Atlantic and Indian Oceans but also in the Mediterranean Sea. This fish possesses a distinct anatomy and physiology that contribute to its survival in harsh marine environments, and one remarkable adaptation is the production of a mucous layer that covers its skin. The mucous is a complex mixture of proteins, glycoproteins, and polysaccharides, which form a protective barrier against various environmental threats, including bacteria. The mucous contains bioactive compounds with antimicrobial properties, making it an interesting area of study for biomedical applications. For this purpose, the evaluation of the antibacterial activity of the mucous extract of this peculiar fish was carried out. Material and Methods: Mucous samples were collected from *C. niger* individuals and processed with acetonitrile to obtain an extract. Then, the antibacterial activity was evaluated against a panel of bacterial strains, including both Gram-positive and Gram-negative bacteria. Standard microbiological technique such as minimum inhibitory concentration (MIC) determination was employed to assess the efficacy. Results: Preliminary results showed significant inhibitory effects on both Gram-positive and Gram-negative among the tested strains, but *Escherichia coli* was found to be highly susceptible to this antibacterial action. These findings suggested that the extract possesses effective antibacterial properties specifically targeting this clinically relevant pathogen associated with various infections, including urinary tract infections, gastrointestinal disorders, and systemic infections. Discussion and Conclusions: The mucous of *C. niger* has exhibited a broad-spectrum antibacterial effect, targeting *E. coli* strains. This characteristic is important in the current era of antibiotic resistance, where the development of new antibacterial agents is urgently needed. The exploration of fish mucous as a potential source for novel antimicrobial compounds holds promise for addressing this global health challenge. In conclusion, the mucous of *C. niger* showcases remarkable antibacterial properties. Further research is warranted to elucidate the underlying mechanisms of its antibacterial effects and to isolate and characterize the bioactive compounds responsible for this activity. Harnessing the potential of *C. niger* mucous may lead to the development of effective antibacterial therapies and contribute to combating the rising threat of antibiotic resistance in the medical field.

124 - Antimicrobial activity of in silico designed synthetic peptides against biofilms of antibiotic-resistant *Escherichia coli* isolates

LORENZA ARTESANI⁽¹⁾ - TECLA CIOCIOLA⁽¹⁾ - ALICE VISMARRA⁽²⁾ - CRISTINA BACCI⁽²⁾ - STEFANIA CONTI⁽¹⁾ - LAURA GIOVATI⁽¹⁾

University of Parma, Department of Medicine and Surgery, Parma, Italia⁽¹⁾ - University of Parma, Department of Veterinary Science, Parma, Italia⁽²⁾

Antimicrobial activity of in silico designed synthetic peptides against biofilms of antibiotic-resistant *Escherichia coli* isolates

LORENZA ARTESANI¹, TECLA CIOCIOLA¹, ALICE VISMARRA², CRISTINA BACCI², STEFANIA CONTI¹, LAURA GIOVATI¹

¹Department of Medicine and Surgery, Biomedical, Biotechnological and Translational Sciences Unit, University of Parma, Parma, Italy; ²Department of Veterinary Science, Parasitology Unit, University of Parma, Parma, Italy

Introduction

Bacterial resistance to beta-lactam antibiotics, with special reference to new generation cephalosporins, is a public health problem of primary concern. In *Escherichia coli*, resistance against these antibiotics is mainly mediated by extended-spectrum-beta-lactamases (ESBL). ESBL-producing *E. coli* are associated with an array of health-threatening hospital and community acquired, as well as foodborne, infections. These infections may also be biofilm-related, thus more difficult to treat because of the higher tolerance to conventional antibiotics and host immune response. Therefore, there is an urgent need of new drugs active against microbial biofilms of ESBL-producing *E. coli*. In this regard, antimicrobial peptides have attracted increasing attention as new drug candidates.

Materials and Methods

Based on structure-function characteristics of previously published peptides, four novel peptide sequences with potentially enhanced antimicrobial activity were identified through a bioinformatic approach. The peptides were synthesized by solid-phase technique and their antibacterial properties were assessed in vitro against planktonic cells and biofilms of different *E. coli* strains using colony forming unit and crystal violet assays, confocal laser scanning microscopy (CLSM), and scanning electron microscopy (SEM). Structural features and interactions with bacterial cells components were determined by circular dichroism and fluorescence spectroscopy. Cytotoxicity against mammalian cells was also assessed.

Results

Micromolar concentrations of the four peptides showed antibacterial activity against both sensitive and ESBL-producing *E. coli* planktonic cells, without showing toxicity on mammalian cells. A differential inhibitory effect against *E. coli* biofilm was detected by crystal violet assay. Quantitative fluorescence intensity analysis of 3D reconstructed CLSM images of treated biofilm showed a significant eradicating effect and reduction of viable bacteria. CLSM studies with fluorescent peptides demonstrated their ability to penetrate within *E. coli* cells, while SEM revealed gross morphological alterations in treated bacteria. As assessed by spectroscopic studies, peptides may firstly interact with *E. coli* superficial oligosaccharides, then with cell membranes.

Discussion and Conclusions

Four antimicrobial peptides active against planktonic ESBL-producing *E. coli*, also showed a potent anti-biofilm activity. Killing kinetics seemed to exclude a disruption of cell membrane as the

primary mechanism of action, suggesting instead an interaction with intracellular targets whose identification could lead to the development of novel antimicrobials active against biofilm-forming *E. coli*, including drug-resistant strains.

128 - Novel fungicidal thiazole derivatives inhibit the growth of *Candida albicans* by ergosterol depletion.

GIANLUIGI CABIDDU⁽¹⁾ - RITA MELEDDU⁽²⁾ - LAURA DEMURU⁽²⁾ - ELIAS MACCIONI⁽²⁾
- ALESSANDRO DE LOGU⁽¹⁾

Università degli Studi di Cagliari, Dipartimento di Scienze della Vita e dell'Ambiente - Sezione di Scienze Farmaceutiche, Farmacologiche e Nutraceutiche, Cagliari, Italia⁽¹⁾ - Università degli Studi di Cagliari, Dipartimento di Scienze della Vita e dell'Ambiente - Sezione di Scienze del Farmaco, Cagliari, Italia⁽²⁾

Novel fungicidal thiazole derivatives inhibit the growth of *Candida albicans* by ergosterol depletion.

GIANLUIGI CABIDDU^a, RITA MELEDDU^b, LAURA DEMURU^b, ELIAS MACCIONI^b,
ALESSANDRO DE LOGU^a

^aDepartment of Environmental and Life Sciences, Section of Pharmaceutical, Pharmacological and Nutraceutical Sciences, University of Cagliari, Cagliari, Italy; ^bDepartment of Environmental and Life Sciences, Section of Drug Sciences, University of Cagliari, Cagliari, Italy.

Introduction.

Over the past decades, fungal infections have substantially increased. *C. albicans* accounts for most of these infections, especially in hospitalized and AIDS patients. The current therapeutic armamentarium against systemic infections consists of four drug classes: polyenes, 5-fluorocytosine, azoles, and echinocandins. Azoles and in particular Fluconazole (FLC) are used due to their efficacy and low toxicity, but the wide exposure to these agents has led to an increase of drug resistance. Azoles act by depleting ergosterol production, leading to plasma membrane disruption. However, azoles are mainly fungistatic. Thus, to discover novel antifungal agents, we designed, synthesized, and studied a small library of potential ergosterol synthesis inhibitors. All the new derivatives were evaluated against *C. albicans* to assess their antimicrobial effect and mode of action.

Materials and Methods.

Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) were determined for each compound by the broth microdilution method and colony forming units count respectively. Cytotoxicity assays were also performed on Vero cell line by the tetrazolium-based colorimetric MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay. Ergosterol quantification methods were performed according to literature reported methods, and results were determined spectrophotometrically.

Results.

Tested compounds were active against *C. albicans* at low MICs and MFCs (concentrations ranging between 0.39 and 1.5 µg/mL for both parameters). The compound with the lowest MIC and MFC was selected to study the mode of action. Spectrophotometric analysis results show a decrease of ergosterol content in cultures of *C. albicans* treated with the compound with respect to untreated control cultures. These compounds also show low toxicity on Vero cells (> 500 µg/mL).

Discussion and Conclusions.

Results show that the studied compounds are active against *C. albicans* with MIC values comparable to those of FLC and other azoles. However, our compounds are fungicidal with MFC values identical or close to their MIC values. Absorbance spectra obtained after ergosterol extraction of treated and untreated samples, confirmed our hypothesis that these compounds inhibit ergosterol production. Since

the inhibition of ergosterol biosynthesis determined for the compounds occurred at concentrations like those determined for FLC, a further mechanism of action could be hypothesized which explains the fungicidal effect. These results encouraged us to further investigate such compounds to elucidate their biological activity. Moreover, we will extend our experiments on structurally related derivatives to further enhance antifungal efficacy and better clarify the mode of action.

138 - The inhibitory effects of *Lactobacillus gasseri* 1A-TV cell-free supernatant against ESBL and carbapenemase producing *Klebsiella pneumoniae*

GAIA VERTILLO ALUISIO ⁽¹⁾ - MARIA LINA MEZZATESTA ⁽¹⁾ - STEFANIA STEFANI ⁽¹⁾ - MARIA SANTAGATI ⁽¹⁾

Università di Catania, Dipartimento di Scienze Biomediche e Biotecnologiche, Catania, Italia ⁽¹⁾

The inhibitory effects of *Lactobacillus gasseri* 1A-TV cell-free supernatant against ESBL and carbapenemase producing *Klebsiella pneumoniae*

GAIA VERTILLO ALUISIO¹, MARIA LINA MEZZATESTA¹, STEFANIA STEFANI, MARIA SANTAGATI¹

¹Department of Biomedical and Biotechnological Sciences (BIOMETEC), Section of Microbiology, University of Catania, Catania, Italy

Introduction: *Klebsiella pneumoniae* is one of the most concerning MDR pathogens with an incidence of up to 50% of ESBL-producing and carbapenem-resistant clinical strains. Colonization and carriage of carbapenem-resistant *K. pneumoniae* (CRKP) are also considered to be the main reason for recurrent infections in critically ill patients. Several studies have reported beneficial effects exerted by novel strategies based on probiotics and their products as preventive therapies against MDR pathogens, in order to guarantee a better outcome for hospitalized and immunocompromised patients. In our previous study, we firstly isolated and characterized *Lactobacillus gasseri* 1A-TV for its antimicrobial activity against a wide range of Gram -positive and -negative pathogens. In this study, we evaluated the anti-ESBL and carbapenemase producing *K. pneumoniae* effects exerted by the *L. gasseri* 1A-TV cell-free supernatant (CFS).

Materials and Methods: 1A-TV genomic sequencing was performed with Illumina Miseq and assembled with SPAdes v3.14.0. RAST was applied for genome annotation and BAGEL4 was used for prediction of potential bacteriocins. The inhibitory activity of *L. gasseri* 1A-TV was first tested against seven *K. pneumoniae* (n.2 ESBL-producing strains; n.2 KPC-3 KP; n.2 co-producing NDM-OXA-48 KP and a non-resistant strain as control) by the antagonism test and quantified by the agar spot test. The antibacterial effect of 1A-TV CFS was evaluated by time-killing curve assays tested at different time intervals: 0, 4, 6, 8 and 24 h against six *K. pneumoniae* included in this study.

Results: The genome of *L. gasseri* 1A-TV amounted to 2,018,898 bp with 34.9% GC. A total of 1,937 putative protein coding sequences, 55 tRNA and 4 rRNA were detected by RAST. Genome mining by BAGEL predicted two bacteriocin biosynthetic gene clusters (BBGCs): BBGC1 contained two class IIc bacteriocins, namely blp-like and gassericin A, while BBGC2 carried the bacteriocin helveticin J belonging to class III. 1A-TV showed strong inhibitory activity against ESBL and carbapenemase producing *K. pneumoniae* by both the agar spot assay and the deferred antagonism test. Furthermore, time-killing tests showed a bacteriostatic effect of 1A-TV CFS at 4, 6, 8 hours, while at 24 hours it exhibited a strong bactericidal effect against all MDR *K. pneumoniae* strains.

Discussion and Conclusions: Our findings demonstrate the inhibitory effect of *L. gasseri* 1A-TV CFS against the ESBL- and carbapenemase producing *K. pneumoniae*, strengthening the concept of using probiotic bioactive natural products to protect the host against the MDR *K. pneumoniae* strains. These results also support novel therapeutic strategies as new postbiotics for the prevention and treatment of multidrug-resistant organism colonization or infection.

141 - Activity of a newly designed gallium-siderophore complex (GaS1) against lung isolates of *A. fumigatus* in an in vitro air-liquid interface lung infection model

SEMIH ESIN⁽¹⁾ - BRUNELLA GRASSIRI⁽²⁾ - MAGDALENA PIATEK⁽³⁾ - LEWIS M. O'FERRALL⁽⁴⁾ - JOHANNES A. SAKE⁽⁵⁾ - CARSTEN EHRHARDT⁽⁵⁾ - DARREN M. GRIFFITH⁽⁴⁾ - KEVIN KAVANAGH⁽³⁾ - ANNE MARIE HEALY⁽⁵⁾ - ANNA M. PIRAS⁽²⁾ - GIOVANNA BATONI⁽¹⁾

Università di Pisa, Dipartimento di Ricerca Traslationale e delle nuove tecnologie in Medicina e Chirurgia, Pisa, Italia⁽¹⁾ - Università di Pisa, Dipartimento di Farmacia, Pisa, Italia⁽²⁾ - Maynooth University, Department of Biology, Maynooth, Irlanda⁽³⁾ - University of Medicine and Health Sciences, Department of Chemistry, RCSI, Dublin, Irlanda⁽⁴⁾ - Trinity Collage Dublin, School of Pharmacy and Pharmaceutical Sciences, Dublin, Irlanda⁽⁵⁾

Activity of a newly designed gallium-siderophore complex (GaS1) against lung isolates of *A. fumigatus* in an in vitro air-liquid interface lung infection model

SEMIH ESIN¹, BRUNELLA GRASSIRI², MAGDALENA PIATEK^{3,6}, LEWIS M. O'FERRALL^{4,6}, JOHANNES A. SAKE⁵, CARSTEN EHRHARDT⁵, DARREN M. GRIFFITH^{4,6}, KEVIN KAVANAGH^{3,6}, ANNE MARIE HEALY^{5,6}, ANNA M. PIRAS² AND GIOVANNA BATONI¹

¹Department of Translational Research and New Technologies in Medicine and Surgery and

²Department of Pharmacy, University of Pisa, Pisa, Italy.

³Department of Biology, Maynooth University, Maynooth, Co Kildare, Ireland.

⁴Department of Chemistry, RCSI, University of Medicine and Health Sciences, Dublin 2, Ireland.

⁵School of Pharmacy and Pharmaceutical Sciences, Trinity College Dublin, Dublin, Ireland.

⁶SSPC The Science Foundation Ireland Research Centre for Pharmaceuticals, Ireland.

Introduction: In late 2022, *Aspergillus fumigatus* was included by the World Health Organization in the critical priority group of fungal pathogens that are associated with serious risk of mortality and morbidity in humans. Pulmonary aspergillosis is particularly severe in patients with cystic fibrosis (CF) in which it is clearly associated with airway damage and lung function decline. Azole-resistant *A. fumigatus* is an emerging threat that hampers our ability to successfully treat patients with aspergillosis, necessitating the identification of new treatment options against azole-resistant isolates. In this study, we aimed to test the activity of a newly designed gallium-siderophore complex (GaS1) against lung isolates of *A. fumigatus* in an in vitro lung infection model mimicking the conditions found in the CF airways.

Materials and Methods: Three *A. fumigatus* strains (AF1, AF2 and AF3) were isolated from respiratory specimens. To reproduce the CF lung conditions, the MIC values of GaS1 were preliminarily tested in a host-cell compatible medium (RPMI 1640, 2mM glutamine) in the presence of an artificial sputum medium (ASM). Monolayers of NCI-H441 cells (human distal lung epithelium) were grown in 96-well plates as undifferentiated epithelium or on Transwell® inserts at the air liquid interface (ALI). Monolayers were infected with 5×10^3 conidia/well of AF3 and treated with different concentrations of GaS1. After 48 h, macroscopic and microscopic observation of the monolayers was performed, while fungal load was evaluated by plating serial dilutions of treated and untreated samples on Sabouraud agar.

Results: GaS1 tested in a concentration range of 0.016-0.5 mg/mL inhibited the growth of all three *A. fumigatus* isolates in a complex host-environment mimicking medium at the non-toxic concentration of 0.063 mg/mL. Monolayers of NCI-H441 cells in the presence of 20% ASM supported the growth of the clinical isolate AF3 at levels even higher than those observed in the same medium without cells. GaS1 exhibited a marked and dose-dependent anti-fungal activity against AF3 in the presence of both un-differentiated and differentiated lung epithelial cells at the ALI. In both experimental systems evident hyphal growth was visible following 48 h of culture in untreated samples, while in GaS1 treated samples nearly no growth of AF3 was detected at the macroscopic and microscopic level.

Discussion and Conclusions: The novel gallium-siderophore complex, GaS1, has shown remarkable activity against clinical isolates of *A. fumigatus* at concentrations that are non-toxic for NCI-H441 cells. Therefore, GaS1 could represent a promising adjuvant candidate for the treatment of *A. fumigatus* infected CF patients.

158 - Synergistic antibacterial effect of colistin in combination with human albumin nanoparticles on multidrug resistant *Acinetobacter baumannii* isolates

SARA SCUTERA⁽¹⁾ - FRANCESCA MENOTTI⁽¹⁾ - ELEONORA MANISCALCO⁽¹⁾ - MONICA ARGENZIANO⁽²⁾ - ILARIO FERROCINO⁽³⁾ - GABRIELE BIANCO⁽⁴⁾ - ROBERTA CAVALLI⁽²⁾ - VIVIANA CAFISO⁽⁵⁾ - TIZIANA MUSSO⁽¹⁾

University of Torino, Department of Public Health and Pediatric Sciences, TORINO, Italia⁽¹⁾ - University of Torino, Department of Drug Science and Technology, TORINO, Italia⁽²⁾ - University of Torino, Department of Agricultural, Forest and Food Sciences, TORINO, Italia⁽³⁾ - University Hospital Città della Salute e della Scienza di Torino, Microbiology and Virology Unit, TORINO, Italia⁽⁴⁾ - University of Catania, Department of Biomedical and Biotechnological Sciences, CATANIA, Italia⁽⁵⁾

Synergistic antibacterial effect of colistin in combination with human albumin nanoparticles on multidrug resistant *Acinetobacter baumannii* isolates

SARA SCUTERA¹, FRANCESCA MENOTTI¹, ELEONORA MANISCALCO¹, MONICA ARGENZIANO², ILARIO FERROCINO³, GABRIELE BIANCO⁴, ROBERTA CAVALLI², VIVIANA CAFISO⁵ AND TIZIANA MUSSO¹

¹Department of Public Health and Pediatric Sciences, University of Torino, Torino, Italy; ²Department of Drug Science and Technology, University of Torino, Torino, Italy; ³Department of Agricultural, Forest and Food Sciences, University of Torino, Torino, Italy; ⁴Microbiology and Virology Unit, University Hospital Città della Salute e della Scienza di Torino, Torino, Italy; ⁵Department of Biomedical and Biotechnological Sciences, University of Catania, Catania, Italy

Introduction Evolution of resistance to last-resort antibiotics, such as colistin (Col), in multidrug-resistant (MDR) Gram-negative bacteria (GNB), is a relevant public health problem.

Nanotechnology is a promising strategy to counteract resistant bacterial infections. We developed a new formulation of human albumin nanoparticles (haNPs) loaded with Col able to counteract Col-resistant (Col R) GNB, including *Acinetobacter baumannii* (Ab). Investigating the mechanisms involved in this effect, we found that haNPs might behave as efflux pump inhibitor (EPI). The aim of this study was to evaluate the synergistic effect of haNPs in combination with Col or other antibiotics and investigate the inhibitory effect of haNPs on efflux pump activity. **Materials and methods:** Six clinical MDR-Col R Ab strains were phenotypically characterized and subjected to WGS analysis to identify Col resistance mechanisms. Ab ATCC 19606 was used as a Col-sensitive (Col S) control. To evaluate the antimicrobial efficacy of haNPs and their ability to enhance Col activity, checkerboard assays were performed. Moreover, to verify the effect as EPI, the MIC of Col in association with sub MIC concentration of haNPs was determined in parallel with the action of the inhibitor carbonyl cyanide 3 chlorophenylhydrazone (CCCP). The MIC value of ethidium bromide (EtBr), a typical substrate for efflux pumps, was also determined with and without haNPs. A preliminary study with antibiotics other than Col was performed. **Results:** The genomic characterization of the strains indicate the presence of SNPs in the Col-R associated genes *pmrA/B* and *galU*. All strains displayed high frequency of ARGs. We found that haNPs potentiate Col activity against both susceptible and MDR Ab strains with fractional inhibitory concentration (FIC) indexes (FICIs) of 0.117 and 0.078 for ATCC 19606 and the Col-R strain A, respectively. For Col-R strain A the Col MIC was reduced from 125 µg/mL to 15,6 µg/mL when used in combination with 16,8 µg/mL (albumin concentration) of haNPs. No antimicrobial activity was found for haNPs alone (≤ 4,3 mg/ml). In the Col-full R Ab, phenotypically characterized for the presence of efflux

pumps, haNPs function as EPI as demonstrated by a reduction of at least 8 folds for Col MIC, and of 2-4 folds for EtBr MIC. Preliminary results on the action of haNPs on the fluoroquinolone Ciprofloxacin were performed demonstrating a MIC reduction of 2-4 folds. Discussion and Conclusions: The contribution of different categories of efflux pumps to antimicrobial resistance in Ab has been described. According to our results, haNPs exhibit efflux pumps inhibitory activity, which may be one of the possible mechanisms of their antibacterial activity against MDR Col R Ab strains. The biocompatible formulation of haNPs holds considerable promise for implementing novel strategy based on combination with other antibiotics to counteract the action of MDR bacteria.

160 - Bithiazole compounds in the treatment of virus/bacteria co-infections

GIULIA RADOCCIA (1) - MARTA DE ANGELIS (1) - SOFIA VENDITTI (1) - REBECCA DE GREGORIO (1) - FRANCESCA BARBIERI (2) - MARIA GRAZIA MARTINA (2) - MARCO RAD (2) - SERENA SCHIPPA (1) - LUCIA NENCIONI (1)

La Sapienza University of Rome, Department of Public Health and Infectious Diseases, Roma, Italia (1) - University of Parma, Department of Food and Drug Sciences, Parma, Italia (2)

Bithiazole compounds in the treatment of virus/bacteria co-infections.

GIULIA RADOCCIA^{1,§}, MARTA DE ANGELIS^{1,2,§}, SOFIA VENDITTI¹, REBECCA DE GREGORIO¹, FRANCESCA BARBIERI³, MARIA GRAZIA MARTINA³, MARCO RAD³, SERENA SCHIPPA^{1,*}, LUCIA NENCIONI^{1,*}

1 Department of Public Health and Infectious Diseases, Sapienza University of Rome, Rome, Italy;

2 Department of Molecular Medicine, Laboratory of Virology, Sapienza University of Rome, Rome, Italy;

3 Department of Food and Drug Sciences, University of Parma, Viale delle Scienze, 27/A, 43124, Parma, Italy

Introduction: The presence, in the respiratory tract, of concomitant bacterial and viral infections, including that by influenza virus, is found in 20-30% of patients and is often associated with increased risk of shock, mechanical ventilation, and mortality. To date, due to increasing antibiotic resistance, the search for new strategies to effectively combat co-infections is of particular interest. The aim of the study was to evaluate antibacterial and antiviral activity of synthesized thiazole compounds and to test their efficacy in the context of Influenza virus A and *Staphylococcus aureus* co-infection. **Methods:** A selection of bithiazole antivirals (active against enteroviruses) was analyzed for (i) antibacterial activity versus *S. aureus* ATCC 6538 in sessile and planktonic form; (ii) cytotoxicity against the lung epithelial cell line A549; (iii) antiviral activity against influenza virus A/Puerto Rico/8/1934/H1N1 (PR8); (iv) the ability to impair viral /bacterial co-infection on A549 cell line. **Results:** Among the compounds tested, only MR335 showed significant antibacterial activity, and the rest of the assays were performed with this compound. The results showed that MR335 had: antibacterial activity when used at concentrations >100 µM in liquid culture; antibacterial activity on forming and preformed biofilms at concentrations of 500 and 300 µM; no cytotoxic activity against the A549 cell line at concentrations <100 µM. The antiviral activity of compound MR335 was assayed at noncytotoxic concentrations on the A549 cell line, showing efficacy at concentrations ≤40 µM. In the model of co-infection, the incubation of influenza A virus-infected cells with *S. aureus* significantly increased bacterial invasive ability. Treatment with MR335 at the concentration of 40 µM (effective concentration in knocking down viral titer, noncytotoxic, but not active toward bacteria), was able to counteract co-infections (*S. aureus*/influenza A virus), significantly decreasing the invasive capacity of *S. aureus*. **Discussion and conclusions:** Our results showed that compound MR335 had both antibacterial and antiviral activity, but at different concentrations. In addition, it showed the ability to counteract *S. aureus*/influenza A virus PR8 coinfection on the lung epithelial cells, by negatively interfering with *S. aureus* invasive capacity and viral titer. It is important to note that this ability was found at sub-inhibitory antibacterial concentrations and is probably due to the compound's antiviral activity which, by lowering the viral titer, blocks the synergistic cooperation between virus and bacteria. Our results show for the first time the virus/bacteria coinfection-fighting activity of a bithiazole derivative (MR335) in the context of the lung cell line A549.

165 - Antiviral activity of the non-steroidal anti-inflammatory drug indomethacin against Respiratory Syncytial Virus

CATERINA TRAMONTOZZI⁽¹⁾ - SIMONE LA FRAZIA⁽¹⁾ - ANNA RICCIO⁽¹⁾ - SILVIA PAUCIULLO⁽¹⁾ - MARIA GABRIELLA SANTORO⁽¹⁾

Università di Roma "Tor Vergata", Dipartimento di biologia, Roma, Italia ⁽¹⁾

Antiviral activity of the non-steroidal anti-inflammatory drug indomethacin against Respiratory Syncytial Virus

CATERINA TRAMONTOZZI¹, SIMONE LA FRAZIA¹, ANNA RICCIO¹, SILVIA PAUCIULLO¹ and M. GABRIELLA SANTORO¹.

¹Department of Biology, University of Rome Tor Vergata, Rome, Italy.

Introduction: Respiratory syncytial virus (RSV) is a leading cause of lower respiratory tract disease in young children and elderly people. Two protein-based RSV vaccines recently received FDA approval for adults aged 60 years and older. While Palivizumab is administered as an immune prophylaxis, currently there is no definitive treatment for RSV infection. Indomethacin (INDO), a traditional cyclooxygenase-1 and -2 inhibitor with potent anti-inflammatory, analgesic and antipyretic properties, is known to possess antiviral activity against several viral pathogens, and has been recently used successfully in the treatment of COVID-19 patients. Herein we investigated the effect of INDO on human RSV (hRSV) replication, using the RSV A2 strain as a model. **Materials and methods:** Human HEp-2 carcinoma, alveolar type II-like epithelial lung A549 cells, bronchial epithelial 16HBE cells and MRC-5 lung fibroblasts were infected with hRSV (0.1 or 1 TCID₅₀/cell) for 2h at 37°C. INDO, dissolved in ethanol, was diluted in culture medium at the time of use. At different times after infection, virus yield was determined by TCID₅₀ infectivity assay, and cell viability was determined by MTT assay. Viral and cellular proteins were evaluated by Western-blot and immunofluorescence analysis. RSV genomic RNA (gRNA) levels were determined by qRT-PCR. **Results:** INDO was found to possess a potent antiviral activity against hRSV, causing a dose-dependent decrease of infectious virus yield at submicromolar concentrations (IC₅₀: 0.47µM; SI>1000). The antiviral activity was independent of the cell type. Treatment with INDO at different stages of the virus infection cycle resulted in significant RSV yield reduction when treatment was started after virus adsorption, whereas treatment of cells before infection or during the adsorption period did not affect virus replication. Analysis of viral proteins and RNA shows that INDO reduces RSV fusion F protein and RNA levels. This effect was associated with drug-induced phosphorylation of the translation initiation factor eIF2α at Ser51, as previously described in different RNA virus models. **Discussion and Conclusions:** Globally, RSV is responsible for approximately 60.000 in-hospital deaths annually in children younger than 5 years of age, and is an increasing cause of morbidity and mortality in immunocompromised patients and the elderly. In addition to vaccines, drugs for the treatment of acute infections are urgently needed. The results described herein indicate that INDO is effective against RSV infection in vitro, acting at post-entry level and causing a block of viral RNA and protein expression in infected cells, and suggesting a possible beneficial effect of INDO in the treatment of RSV infections.

167 - Screening of a panel of compounds as anti-Sars-CoV-2 antiviral drugs

SILVIA NOTTOLI⁽¹⁾ - CARLO DI MARZO⁽²⁾ - PAOLA QUARANTA⁽²⁾ - MICHELE LAI⁽²⁾ - ROBERTA IBBA⁽³⁾ - SIMONA SESTITO⁽⁴⁾ - SANDRA PIRAS⁽³⁾ - ANTONIO CARTA⁽³⁾ - GIULIA FREER⁽²⁾

unità operativa di virologia, Azienda Ospedaliera Universitaria Pisana, Pisa, Italia⁽¹⁾ - Centro Retrovirus, Dipartimento di ricerca traslazionale e nuove tecnologie in medicina e chirurgia, Università di Pisa, Pisa, Italia⁽²⁾ - Dipartimento di medicina, chirurgia e farmacia, Università di Sassari, Sassari, Italia⁽³⁾ - Dipartimento di chimica, fisica, matematica e scienze naturali, università di sassari, Sassari, Italia⁽⁴⁾

Screening of a panel of compounds as anti-Sars-CoV-2 antiviral drugs

Topic: Nuovi approcci antimicrobici

SILVIA NOTTOLI¹⁻², CARLO DI MARZO², PAOLA QUARANTA², MICHELE LAI², ROBERTA IBBA³, SIMONA SESTITO⁴, SANDRA PIRAS³, ANTONIO CARTA³, GIULIA FREER².

¹ Virology Operative Unit, Pisa University Hospital, Pisa, Italy; ² Retrovirus Center, Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa, Pisa, Italy; ³ Department of Medicine, Surgery and Pharmacy, University of Sassari, Sassari, Italy;

⁴ Department of Chemical, Physical, Mathematical and Natural Sciences, University of Sassari, Sassari, Italy.

Introduction

A virus later known as SARS-CoV-2 spread across China late in 2019 causing a very contagious respiratory infection that shortly thereafter caused a pandemic, infecting more than 640 million people all over the globe (WHO - December 2022). Since the very beginning of the pandemic, both academia and industry directed most of their efforts through the identification of vaccines which effectively stemmed the infections. Although vaccination has changed the outcome of the disease for many people, the identification of drugs against SARS-CoV-2 are still a priority for researchers, because the virus has shown to be highly variable. In this context, we screened an in-house library of small molecules to evaluate whether they could inhibit SARS-CoV-2 replication. These molecules were chosen because they had shown antiviral activity against the replication of various viruses.

Materials and Methods

We evaluated compound cytotoxicity of the panel of compounds on CALU-3 and VERO-TMPRSS cells using WST-8 cell viability assay. We therefore analysed the effect of our compounds on the replication stage of SARS-CoV-2, and for this, we used a simple cytopathic effect (CPE) method, which involves the observation of CPE as an indicator of virus growth, was used for roughly evaluating the antiviral activity of individual compounds against SARS-CoV-2 infection. The IC₅₀, as well as the corresponding selectivity index (SI) were calculated after cells were infected with multiplicity of infection (MOI) of 1 with SARS-CoV-2 in the presence of scalar concentrations of drugs. Time-of-addition experiments were performed by adding the selected drugs before, during and 2 or 6 h after infection and evaluating the change in viral yield. We performed western blots and Real-Time qPCR to quantify viral yield reduction.

Results

From this extensive library, we were able to select 9 compounds that inhibit Sars-CoV-2 replication. These most promising compounds were also tested to calculate their IC₅₀, resulting in the range from 10 to 50 micromolar.

Discussion and Conclusions

Nine compounds showed anti-SARS-CoV-2 activity and were further characterized for their potency against; they may be considered as lead compounds for future improvement aimed at COVID-19 treatment. We plan to characterize them for their cytotoxicity on different cell lines and their antiviral efficacy against different SARS-CoV-2 variants.

189 - Activity evaluation of phage SAU1593 with levofloxacin or erythromycin against Staphylococcus aureus strains isolated from diabetic foot infections

ALESSIO CORRAINI ⁽¹⁾ - ROSA AURICCHIO ⁽¹⁾ - ANNA BERTONCELLI ⁽¹⁾ - ANNARITA MAZZARIOL ⁽¹⁾

Università di Verona, Dipartimento di Diagnostica e Sanità Pubblica, Verona, Italia ⁽¹⁾

Activity evaluation of phage SAU1593 with levofloxacin or erythromycin against Staphylococcus aureus strains isolated from diabetic foot infections

ALESSIO CORRAINI, ROSA AURICCHIO ANNA BERTONCELLI, ANNARITA MAZZARIOL

Department of Diagnostics and, Public Health, University of Verona Italy

Introduction. Diabetes mellitus (DM) is a chronic disease where hyperglycaemia favours the development of chronic vascular complications, including Diabetic Foot Infection (DFI). Staphylococcus aureus represent one of the main pathogen of DFI. The aim of the study was to extract active phage from wastewater samples coming from the Verona University Hospitals, and to evaluate its activity against 100 S. aureus previously characterized strains isolated from patients with DFI in combination with antibiotics.

Material and methods. After three extractions without any results, a pool of active phages was correctly isolated with protocols modification. The activity of newly extracted phages was assessed by performing plaque assays on all the strains involved in the study, evaluating lytic plaques produced on the bacterial strains inoculated in a solid medium. Plaque Forming Unit (PFU) was used to phage titration. Phage kinetics was performed by 24 hour growth curves of strain alone, with levofloxacin or erythromycin at MIC value, with phage at 10^{-7} PFU, or with combination of antibiotic and phage.

Results. Out of 100 tested S. aureus strains, it was observed that the phages had a lytic action, determined by the production of a clear and sharp lytic plaque, on 16 strains. 46 out of 100 strains showed a turbid plaque, 38 out of 100 strains showed no plaque. Subsequently, 16 confirmed strains with clear lytic plaque were selected for the PFU assay. 12 bacterial strains were found to be phage-sensitive showing a phage titre higher than 10^{-7} PFU and were selected for phage kinetics. growth-curve. Out of 12 tested strains, the combination of phage and antibiotic produced a synergistic effect, resulting in the inhibition of bacterial growth and, in some cases, in the death of the strain, in 8 samples. For the other 4, however, the combination of the two agents produced an effect less than or equal compared to the phage or the antibiotic tested individually.

Discussion and conclusions. We were able to develop a protocol that allows the correct extraction of active phages against S. aureus from wastewater samples. The results of the growth curves evaluation showed that in the majority of samples (8 out of 12) the combination of the two therapeutic agents produced a significant synergistic effect that resulted in the inhibition of bacterial growth and, in some cases, even in the death of the bacterial strain; as opposed to the two agents administered separately. These results lay the groundwork for further investigations into phage therapy and the use of phages in support of antibiotic therapy for the treatment of chronic infections such as DFI.

192 - Antibiotic resistance, a global health problem: search for alternative antimicrobial molecules and Superbug Awareness For Education (SAFE) activities.

EMANUELA ROSCETTO⁽¹⁾ - UMBERTO GALDIERO⁽¹⁾ - MARTINA AVERSA⁽¹⁾ - MARIATERESA MANZONI⁽¹⁾ - ANNA FIORETTI⁽¹⁾ - CIRO RAUCH⁽¹⁾ - MARIA ROSARIA CATANIA⁽¹⁾

Università Federico II di Napoli, Dipartimento di Medicina Molecolare e Biotecnologie Mediche, Napoli, Italia⁽¹⁾

Antibiotic resistance, a global health problem: search for alternative antimicrobial molecules and Superbug Awareness For Education (SAFE) activities.

EMANUELA ROSCETTO, UMBERTO GALDIERO, MARTINA AVERSA, MARIATERESA MANZONI, ANNA FIORETTI, CIRO RAUCH, MARIA R. CATANIA.

Department of Molecular Medicine and Medical Biotechnology, University Federico II of Naples, via Pansini 5, 80131 Napoli, Italy

INTRODUCTION - The current difficulty in treating infections caused by multi-drug resistant bacteria has generated a global health emergency that requires complex interventions aimed at a limited and correct use of antibiotics already in use, also through the awareness of citizens, but at the same time it needs new bioactive molecules. In this scenario, the exploration of the environmental microbiota is a promising approach in the discovery of new natural antimicrobials. Our study aims at the isolation and identification of strains with antimicrobial activity from soil samples taken from high school students participating in the European project SAFE (Superbug Awareness For Education).

MATERIALS AND METHODS - The collection of samples took place in various Neapolitan areas and in the WWF Cratere degli Astroni Oasis. Eighty soil samples were diluted and seeded onto R2A plates. The screening of the colonies obtained to evaluate their antimicrobial activity was carried out by diffusion test in agar on a TSA plate previously seeded with pathogenic bacteria of clinical interest. The bioactive strains were identified by MALDI-TOF mass spectrometry and/or 16S rRNA sequencing. The supernatant of massive cultures of selected strains was tested on Gram + and/or Gram - pathogens by micro-dilution in broth.

RESULTS - The 716 strains isolated in this study were screened for their antibacterial properties against *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Klebsiella pneumoniae*. The 44 strains resulting bioactive belonged to the genera: *Pseudomonas*, *Achromobacter*, *Stenotrophomonas*, *Bacillus*, *Aeromonas*, *Streptomyces*, *Paenibacillus*, *Chryseobacterium*. Seven strains for which no antibiotic activity appeared to be documented in the literature were selected for further testing in different growth media. The supernatant of a 96-hour massive culture in MH broth of a *Streptomyces avidini* strain showed a 90% inhibiting effect on the growth of the test strains.

DISCUSSION AND CONCLUSIONS - Although the acquisition of antibiotic resistance is a natural process, today the phenomenon has reached critical dimensions. SAFE activities provided the opportunity to collect a large number of soil samples from very different sites and study the activity of the bacteria present in them. Our work has to make school and university students aware of the problems due to antibiotic resistance. Starting from soil samples we have been able to select strains

of different species showing a promising antibiotic activity against multi-resistant pathogens. Further studies are underway to extract and purify the bioactive secondary metabolites.

203 - Zeno_omega: a novel therapeutic phage-based formulation evolved for the treatment of multi-drug resistant *Staphylococcus aureus*

CLAUDIA CAMPOBASSO⁽¹⁾ - SARA BOLOGNINI⁽¹⁾ - JEROEN WAGEMANS⁽²⁾ - LAURA RINDI⁽³⁾ - ARIANNA TAVANTI⁽¹⁾ - ROB LAVIGNE⁽²⁾ - MARIAGRAZIA DI LUCA⁽¹⁾

Università di Pisa, Dipartimento di Biologia, Pisa, Italia⁽¹⁾ - KU Leuven, Department of Biosystems, Leuven, Belgio⁽²⁾ - Università di Pisa, Dipartimento di Ricerca Traslationale e delle Nuove Tecnologie in Medicina e Chirurgia, Pisa, Italia⁽³⁾

Zeno_omega: a novel therapeutic phage-based formulation evolved for the treatment of multi-drug resistant *Staphylococcus aureus*

CLAUDIA CAMPOBASSO^{1,2}, SARA BOLOGNINI¹, JEROEN WAGEMANS², LAURA RINDI³, ARIANNA TAVANTI¹, ROB LAVIGNE², MARIAGRAZIA DI LUCA¹

¹Department of Biology, University of Pisa, Pisa, Italy; ²Department of Biosystems, Laboratory of Gene Technology, KU Leuven, Leuven, Belgium; ³Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa, Pisa, Italy

Introduction The spreading of multi-drug resistant bacteria led to the need of new strategies to treat microbial infections. In this scenario, phage therapy, which is based on the use of bacteriophages, is gaining more and more interest. Aim of the work was to select a therapeutic phage with a broad host range versus *Staphylococcus aureus* strains and to develop a protocol to improve phage lytic activity to prevent the selection of bacterial resistant clones.

Materials and Methods Phage Zeno was isolated from wastewater samples using *S. aureus* ATCC 43300 as host. The isolated phage Zeno was characterized genotypically (Illumina sequencing, genome annotation, functional predictions) and phenotypically (assessing its replication cycle, stability at different temperature and pH, host range). A protocol of phage evolution to adapt Zeno to its host was developed through eight rounds of 5-day co-incubation each. After the last round, the mutations occurred in the phage population, renamed Zeno_omega, were assessed by sequencing the genome of eight single plaques. The lytic activity of the mutated phages was compared to the wild type one by treating *S. aureus* cells with phages (multiplicity of infection, MOI=1) for 24h and assessing the cell growth by CFU/ml counting.

Results Phage Zeno is a new myovirus species, belonging to Kayvirus genus. Its replication cycle is strictly lytic and no genes coding for antibiotic resistant traits, virulence factors and toxins were identified. Moreover, three endolysins (two amidases and one transglycosylase) were annotated and a depolymerase was predicted. The phage showed a fast and efficient replication cycle, with a short latent period of 20' (to lyse the first infected cells) and a burst size achieved after 3h. Zeno showed high stability to both extreme temperatures (up to 50°C) and pH (ranging from 4 to 11). Finally, it showed a wide host range at strain level, being active against 20 out of 21 *S. aureus* clinical isolates with a different geographic origin. After 24h incubation, the evolved Zeno_omega determined a 4log₁₀ reduction of *S. aureus* CFU/ml with respect to the untreated control, while the wild type phage caused no CFU/ml reduction. The genome analysis of the evolved phages revealed the occurrence of point mutations/insertions, mainly in structural proteins, such as a protein involved in cell recognition, the

predicted depolymerase, determining the partial degradation of the cell wall, and the major tail sheath protein, mediating phage DNA injection.

Discussion and Conclusions Due to its wide host range, its high stability, and its bactericidal efficacy, the evolved Zeno_omega is considered a potential therapeutic phage candidate for phage therapy in compassionate use.

204 - In vitro antibacterial effects of Thyme essential oil against Salmonella enterica group B

MARTINA DI ROSARIO ⁽¹⁾ - GIUSEPPE MANTOVA ⁽¹⁾ - DANIELA SATERIALE ⁽²⁾ - BEATRICE BOTTI ⁽¹⁾ - ELENA SCAGLIONE ⁽³⁾ - CHIARA PAGLIUCA ⁽¹⁾ - CATERINA PAGLIARULO ⁽²⁾ - MARIATERESA VITIELLO ⁽³⁾ - PAOLA SALVATORE ⁽⁴⁾ - ROBERTA COLICCHIO ⁽³⁾

University of Naples Federico II, Department of Molecular Medicine and Medical Biotechnology, University of Naples Federico II, Naples, Italia ⁽¹⁾ - University of Sannio, Benevento, Italy, Department of Science and Technology, University of Sannio, Via F. De Sanctis snc, 82100 Benevento, Italy, Naples, Italia ⁽²⁾ - University of Naples Federico II, Dpt. of Mol. Med. and Med. Biotec., Uni. of Naples Federico II; Dpt. of Int. Act. of Lab. Med. and Tra., C. O.U of Cli. Micro., Uni. Hosp. Fed. II, Naples, Italia ⁽³⁾ - University of Naples Federico II, Dpt. of Mol. Med. and Med. Biotec; Dpt. of Int. Act. of Lab. Med. and Tra., C.O.U of Cli. Micro; Task Force on Micro. Stud. ; Ceinge Franco Salvatore, Naples, Italia ⁽⁴⁾

In vitro antibacterial effects of Thyme essential oil against Salmonella enterica group B

MARTINA DI ROSARIO¹, GIUSEPPE MANTOVA¹, DANIELA SATERIALE², BEATRICE BOTTI¹, ELENA SCAGLIONE^{1,3}, CHIARA PAGLIUCA¹, CATERINA PAGLIARULO², MARIATERESA VITIELLO^{1,3}, PAOLA SALVATORE^{1,3,4,5}, ROBERTA COLICCHIO^{1,3}

¹Department of Molecular Medicine and Medical Biotechnology, University of Naples Federico II, Naples, Italy; ²Department of Science and Technology, University of Sannio, Via F. De Sanctis snc, 82100 Benevento, Italy; ³Department of Integrated Activity of Laboratory Medicine and Transfusion, Complex Operative Unit of Clinical Microbiology, University Hospital Federico II, Naples, Italy; ⁴Task Force on Microbiome Studies, University of Naples Federico II, Naples, Italy; ⁵CEINGE, Advanced Biotechnologies Franco Salvatore s.c.ar.l., Naples, Italy.

Introduction: Foodborne diseases have been defined as a growing public health problem worldwide. The main etiological agents of these diseases have been identified in bacterial pathogens, such as Salmonella spp., whose control in the food processing environment is considered a major challenge. The use of safe conservation methods that allow to prevent the deterioration of meat products and the spread of foodborne illness outbreaks is crucial. Aromatic plants and their derivatives, such as essential oils (EOs), possess in vitro antimicrobial activity. The aim of present study was to evaluate the antimicrobial properties of thyme essential oil (Thy-EO), that has shown a potential in the infectious disease treatment or as alternative food additive, in order to improve the quality and the shelf-life of perishable foods. Materials and methods: The agar-well diffusion assay was performed to evaluate in vitro antibacterial activity, measuring the diameter of inhibition zone of Thy-EO against Salmonella enterica group B. The sensibility of S. enterica to increasing concentrations of Thy-EO (2.5-100 µL/mL) was determined by microdilution method. In addition, to assess the bactericidal or bacteriostatic activity of selected essential oil, time-killing assays were performed at values corresponding to the minimum inhibition concentration (MIC), and 2, 4-fold the MIC. Results: The Thy-EO showed a significant inhibitory activity, as demonstrated by the diameters of inhibition zones exerted by low amount of EO (>10mm). Accordingly, quantitative evaluation of the antimicrobial activity of Thy-EO against clinical isolate was confirmed by value of MIC and minimum bactericidal

concentration (MBC) corresponding to 2.5 $\mu\text{L/mL}$. Furthermore, Thy-EO determined a total decrease in bacterial growth throughout the observation period, starting with concentration of EO corresponding to MIC value until 4xMIC. Discussion and Conclusion: The findings obtained by in vitro microbiological assay, demonstrate that the Thy-EO compounds were able to inhibit the growth of foodborne pathogens with a dose and time dependent manner, and also suggest the possible use of the selected oil in a cellular infection system, to evaluate the ability of Thy-EO to reduce the number of viable intracellular bacteria. These encouraging results suggest that Thy-EO can be considered a natural antimicrobial agent to control the growth of foodborne pathogens.

206 - Antimicrobial activity of WMR-K and WMR-K+gH625 against *Salmonella enterica* group B

ELENA SCAGLIONE⁽¹⁾ - CHIARA PAGLIUCA⁽²⁾ - GIUSEPPE MANTOVA⁽²⁾ - MARTINA DI ROSARIO⁽²⁾ - CHIARA OLANDESE⁽²⁾ - ANNARITA FALANGA⁽³⁾ - ROBERTA COLICCHIO⁽¹⁾ - STEFANIA GALDIERO⁽⁴⁾ - PAOLA SALVATORE⁽⁵⁾ - MARIATERESA VITIELLO⁽¹⁾

University of Naples Federico II, Department of Molecular Medicine and Medical Biotechnology; Dep. of Int. Act. of Lab. Med. and Trans. UOC Clinical Microbiology, Naples, Italia⁽¹⁾ - University of Naples Federico II, Department of Molecular Medicine and Medical Biotechnology, Naples, Italia⁽²⁾ - University of Naples Federico II, Department of Agriculture, Naples, Italia⁽³⁾ - University of Naples Federico II, Department of Pharmacy, Naples, Italia⁽⁴⁾ - University of Naples Federico II, Dep. of Mol. Med. and Med. Biotec.; Dep. of Int. Act. of Lab. Med. and Trans. UOC Clin. Micr.; CEINGE Franco Salvatore; Task Force Microb. Studies, Naples, Italia⁽⁵⁾

Antimicrobial activity of WMR-K and WMR-K+gH625 against *Salmonella enterica* group B

ELENA SCAGLIONE^{1,2}, CHIARA PAGLIUCA¹, GIUSEPPE MANTOVA¹, MARTINA DI ROSARIO¹, CHIARA OLANDESE¹, ANNARITA FALANGA³, ROBERTA COLICCHIO^{1,2}, STEFANIA GALDIERO⁴, PAOLA SALVATORE^{1,2,5,6}, MARIATERESA VITIELLO^{1,2}

¹Department of Molecular Medicine and Medical Biotechnology, University of Naples Federico II, Naples, Italy; ²Department of Integrated Activity of Laboratory Medicine and Transfusion, Complex Operative Unit of Clinical Microbiology, University Hospital Federico II, Naples, Italy; ³Department of Agriculture, University of Naples Federico II, Naples, Italy; ⁴Department of Pharmacy, University of Naples Federico II, Naples, Italy; ⁵CEINGE, Advanced Biotechnologies s.c.ar.l., Naples, Italy; ⁶Task Force on Microbiome Studies, University of Naples Federico II, Naples, Italy.

Introduction. Antibiotic resistance is a growing worldwide problem and it is currently essential to identify new antimicrobial molecules to improve or replace traditional antibiotics. It is known that antimicrobial peptides show a broad-spectrum activity against both gram-positive and gram-negative bacteria. Recent studies have demonstrated that an analogue of a marine antimicrobial peptide, WMR-K, has potent antimicrobial activity against *Candida albicans* and *Klebsiella pneumoniae*. Furthermore, literature data have demonstrated that cell penetrating peptides, such as short cationic and amphipathic peptides, have been used to facilitate cell membrane passage and intracellular drug delivery. In particular, the gH625 peptide was identified as a membrane perturbing domain in glycoprotein H of Herpes simplex virus 1, that can traverse and deliver several molecules across cell membranes in in vitro system. The aim of the present study has been to evaluate the antibacterial activity of WMR-K, able to allow self-assembly for intracellular delivery, and WMR-K conjugated to the gH625 peptide (WMR-K+gH625) against *Salmonella enterica* group B, a facultative intracellular enteropathogen. The cytotoxicity activity of WMR-K and WMR-K+gH625 was investigated in order to employ these peptides in an in vitro infection system in eukaryotic cell lines infected with *S. enterica*. **Materials and Methods.** The minimum inhibition concentration (MIC) and the minimum bactericidal concentration (MBC) of WMR-K and WMR-K+gH625 against selected bacterial strain were determined testing increasing concentration of both peptides (5µM-200µM) by broth microdilution test. Time kill kinetics for the selected peptides were evaluated against *S. enterica*

both in bacterial culture medium (Tryptone Soya Broth, TSB) and in a cellular culture medium (Dulbecco's Modified Eagle Medium, DMEM). The cytotoxicity of peptides was evaluated through MTT assay in human cells line. Results. The obtained experimental data highlighted the antibacterial efficacy of WMR-K and WMR-K+gH625 against *S. enterica*, with MIC values of 5 μ M for WMR-K+gH625 and of 50 μ M for WMR-K, respectively. Time kill-kinetics assays have highlighted that both peptides exert their activity within the first hour of contact. Discussion and Conclusion. Although preliminary data, the obtained results suggest a promising antibacterial activity of WMR-K+gH625, compared to WMR-K alone, against *S. enterica* group B strain. These data encourage to investigate the ability of gH625 to improve the intracellular delivery of WMR-K into an in vitro infection system in eukaryotic cell lines infected with *S. enterica* group B.

208 - Isolation and characterization of novel bacteriophages active against clinical strains of *Klebsiella pneumoniae*

ELISA FAUSTI⁽¹⁾ - CLAUDIA CAMPOBASSO⁽²⁾ - MARIANNA LUONGO⁽²⁾ - SELENIA BANCHERI⁽²⁾ - DARIA BOTTAI⁽²⁾ - CESIRA GIORDANO⁽³⁾ - SIMONA BARNINI⁽³⁾ - ARIANNA TAVANTI⁽²⁾ - MARIAGRAZIA DI LUCA⁽²⁾

Università Roma Tor Vergata, Dipartimento di Medicina Sperimentale e Chirurgia, Roma, Italia⁽¹⁾ - Università di Pisa, Dipartimento di Biologia, Pisa, Italia⁽²⁾ - Azienda Ospedaliera Universitaria Pisana, Ospedale di Cisanello, Unità di Microbiologia, Pisa, Italia⁽³⁾

Isolation and characterization of novel bacteriophages active against clinical strains of *Klebsiella pneumoniae*

ELISA FAUSTI¹, CLAUDIA CAMPOBASSO^{2,3}, MARIANNA LUONGO², SELENIA C. BANCHERI², DARIA BOTTAI², CESIRA GIORDANO⁴, SIMONA BARNINI⁴, ARIANNA TAVANTI^{2,3}, MARIAGRAZIA DI LUCA²

¹Department of Experimental Medicine and Surgery, University of Rome Tor Vergata, Rome, Italy; Department of Biology, ²Unit of Genetics and Microbiology, University di Pisa, Italy; ³Department of Biosystems, Laboratory of Gene Technology, KU Leuven, Leuven, Belgium; ⁴Microbiology Unit, Azienda Ospedaliera Universitaria Pisana, Pisa, Italy.

Introduction

Multi-drug resistant bacteria, including carbapenem-resistant *Klebsiella pneumoniae*, are difficult to be treat, causing great concern because of the limited therapeutic options available. Phage therapy is based on the use of bacteriophages to target pathogenic bacteria and represents a promising innovative therapeutic strategy. The aim of this study was to genotypically characterise novel isolated *K. pneumoniae* phages and to assess their lytic activity.

Materials and Methods

Phages were isolated from environmental samples (i.e. soil and wastewater), using *K. pneumoniae* clinical strains as hosts. The isolated phages were genotypically characterized through genome sequencing and annotation. The phage host range was assessed by spot assay against a panel of 49 *K. pneumoniae* strains, including both carbapenemase-producing (KPC) and New-Delhi beta-metallolactamase (NDM-1) strains. The killing kinetics of two selected phages (Multiplicity of infection - MOI =1 and 10), alone or in combination, was tested versus its host at 3, 6 or 24h. The viability of bacteria after phage treatment was determined by CFUs (Colony Forming Units)/mL counting.

Results

Three novel phages, phi15, phi24 and phi25, were identified. The genome analysis revealed that all of them were myovirus belonging to the Drulisvirus genus. They all shared a virulent nature, and no antibiotic-resistance genes were detected in their genomes. The broad host range profile analysis highlighted that the three phages had a different host spectrum only versus New-Delhi beta-

metallolactamase (NDM-1) *Klebsiella pneumoniae* strains. When phi15 and phi24 were tested against its host, after 3 hours incubation, a $3\log_{10}$ reduction in comparison to the untreated control was achieved with both phages tested alone (at both MOIs) and a $4\log_{10}$ reduction was observed when phages were combined. Nevertheless, bacteria growth increased over time, reaching a level comparable to the untreated control after 24 hours, with all treatment conditions.

Discussion and conclusions

The new isolated phages showed suitable features to be used for human phage therapy to target multi-drug resistant *K. pneumoniae* infections, thanks to their virulent nature, lytic activity against multiple strains and lack of antibiotic-resistance genes. In addition, the in vitro assays showed interesting results with respect to the application of phage cocktails and/or combinations of phages and antibiotics to increase the treatment efficacy in terms of magnitude and timing of CFU/mL reduction. Further experiments testing phages in combination with different classis of antibiotics are ongoing.

212 - Synergistic effects of carvacrol in combination with antibiotics against *Acinetobacter baumannii*, *Escherichia coli* and *Pseudomonas aeruginosa*

GIOVANNA GINESTRA ⁽¹⁾ - ANDREANA MARINO ⁽¹⁾ - LOREDANA FERRERI ⁽²⁾ -
GIUSEPPE GRANATA ⁽²⁾ - GRAZIA M.L. CONSOLI ⁽²⁾ - ANTONIA NOSTRO ⁽¹⁾

Università degli Studi di Messina, Dipartimento di Scienze Chimiche, Biologiche, Farmaceutiche ed Ambientali, Messina, Italia ⁽¹⁾ - C.N.R., Istituto di Chimica Biomolecolare, Catania, Italia ⁽²⁾

Synergistic effects of carvacrol in combination with antibiotics against *Acinetobacter baumannii*, *Escherichia coli* and *Pseudomonas aeruginosa*

GIOVANNA GINESTRA¹, ANDREANA MARINO¹, LOREDANA FERRERI², GIUSEPPE GRANATA², GRAZIA M.L. CONSOLI², ANTONIA NOSTRO¹

¹Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Italy; ²Institute of Biomolecular Chemistry (ICB-CNR) Catania, Italy

Introduction. The emergence of antimicrobial-resistance is a major global health threat requiring novel therapeutic strategies. Among components of the essential oils, carvacrol has significant antimicrobial properties that make it promising agent in the fight against drug-resistant human pathogens. The present study aimed to determine the activity of carvacrol in combination with antibiotics against Gram-negative bacteria and the advantage of their co-delivery by loading into nanostructured systems. **Materials and Methods.** The bacteria used in this study were as follows: multidrug-resistant *Acinetobacter baumannii* DSM 106838, *Escherichia coli* ATCC 10536, ESBLs producing *E. coli* DSM 105388, *Pseudomonas aeruginosa* ATCC 9027 and VIM-2 producing *P. aeruginosa* DSM 102273. The MICs of carvacrol, ofloxacin, gentamicin and meropenem were determined by the broth microdilution method according to CLSI. The interaction of carvacrol with antibiotics was investigated using the checkerboard test. The combination was evaluated by determining the fractional inhibitory concentration index (FICI) as follows: synergistic effect $FICI \leq 0.5$; additive effect $FICI > 0.5 - \leq 1$; indifference $FICI > 1 - < 2$; antagonism $FICI \geq 2$. The antibiofilm activity of carvacrol and ofloxacin alone and in association was also determined. **Results.** The MIC values were found in the following ranges: 0.015-0.5% for carvacrol; 0.03-128 micrograms/mL for ofloxacin; 0.5-128 micrograms/mL for gentamicin and 0.25-256 micrograms/mL for meropenem. *A. baumannii*, *E. coli* DSM 105388 and *P. aeruginosa* DSM 102273 were found to be resistant to ofloxacin and gentamicin and *A. baumannii* and *P. aeruginosa* DSM 102273 to meropenem. The synergistic combinations ($FICI = 0.37 - 0.50$) were: carvacrol-ofloxacin against *A. baumannii* and *P. aeruginosa* DSM 102273; carvacrol-gentamicin against *E. coli* ATCC 10536 and both *P. aeruginosa* strains; carvacrol-meropenem against *A. baumannii*. Carvacrol-ofloxacin combination showed additive effect towards *P. aeruginosa* ATCC 9027 and both *E. coli* strains and also inhibitory activity against biofilm formation of *A. baumannii* and *P. aeruginosa* DSM 102273. **Discussion and conclusions.** The results extend the knowledge on carvacrol and its potential to fight the emergence of antibiotic-resistance. The synergistic effects of carvacrol in combination with antibiotics allow to reduce the minimum effective dose of antimicrobials and moderate their negative effects. The most

effective combinations will be trapped into nanosized micelles with a polycationic surface to obtain greater stability and controlled release of the active components.

221 - Complex Electromagnetic Fields: eco-sustainable strategy to counteract the resistant *Candida albicans* infections

SILVIA DI LODOVICO⁽¹⁾ - PAOLA DI FERMO⁽²⁾ - MORENA PETRINI⁽²⁾ - EMIRA D'AMICO⁽²⁾ - SARA D'ARCANGELO⁽¹⁾ - FIRAS DIBAN⁽¹⁾ - LUIGINA CELLINI⁽¹⁾ - MARA DI GIULIO⁽¹⁾ - SIMONETTA D'ERCOLE⁽²⁾

University "G. d'Annunzio" Chieti-Pescara, Department of Pharmacy, Chieti, Italia⁽¹⁾ - University "G. d'Annunzio" Chieti-Pescara, Department of Medical, Oral and Biotechnological Sciences, Chieti, Italia⁽²⁾

Complex Electromagnetic Fields: eco-sustainable strategy to counteract the resistant *Candida albicans* infections

SILVIA DI LODOVICO¹, PAOLA DI FERMO², MORENA PETRINI², EMIRA D'AMICO², SARA D'ARCANGELO¹, FIRAS DIBAN¹, LUIGINA CELLINI¹, MARA DI GIULIO¹, SIMONETTA D'ERCOLE²

¹Department of Pharmacy, University "G. d'Annunzio" Chieti-Pescara, 66100, Chieti, Italy.

²Department of Medical, Oral and Biotechnological Sciences, University of "G. d'Annunzio" Chieti-Pescara, 66100, Chieti, Italy.

2773/2800Introduction: The worrying antimicrobial resistance is a global challenge including a wide number of human pathogenic yeasts such as *Candida* spp. The use of novel technologies such as Complex Electromagnetic Fields-CMFs represents a new eco-sustainable strategy to counteract the infection associated to anti-fungal resistant *C. albicans*. The aim of this study was twofold: to evaluate the anti-virulence (anti-biofilm, fluidity and permeability assays) effect of CMFs against clinical anti-fungal resistant *C. albicans*; to study the CMFs effect (toxicity, ROS production, wound healing assay, morphology, adhesion and collagen production) on Human Gingival Fibroblasts-HGF.

Materials and Methods: For the study, two different CMFs programs were used: stress and antibacterial programs. The CMFs anti-virulence effect against anti-fungal resistant *C. albicans* S5 was performed in terms of: i) anti-biofilm by biomass quantification with Crystal violet staining, CFU/ml determination, Live/Dead assay; ii) membrane fluidity change by Laurdan Generalized Polarization-GPexc; iii) membrane permeability by using Propidium Iodide-PI. The CMFs effect on HGF was carried out by: i) viability assay by MTT; ii) ROS production; iii) wound healing assay; iv) morphology by toluidine-blue staining and SEM; v) production of collagen by Picrosirius red staining. Results: The CMFs affected the *C. albicans* biofilm with significant biomass (64.37%±10.80 stress and 15.63%±7.40 antibacterial) and CFU/ml (99.19%±0.06 stress, 5.19%±2.03 antibacterial) reductions obtained with stress program in respect to the control. The stress CMFs was used for the fluidity and permeability with a remarkable effect on the fluidity *C. albicans* membrane change (GPexc: -0.14 CTR and -0.06 CMFs) without an effect on the permeability (the CMFs fluorescence intensity was lower than the CTR). The MTT assay showed no CMFs negative effects on the viability of HGF with a major ROS production with the antibacterial program at 3 and 24h in respect to stress program and control. For the wound healing assay, stress program showed the best effect in terms of the rate migration at 24h. The toluidine-blue staining observations showed the typical morphology of cells and the presence of elongated and spindle-shaped with cytoplasmic extensions and lamellipodia

was observed at SEM. The CMFs antibacterial program increased the production of collagen with respect to control and stress program. Discussion and Conclusion: CMFs showed a relevant anti-virulence action against *C. albicans*, no cytotoxicity effects on HGF and a high HGF migration rate, representing a novel eco-sustainable strategy to counteract the yeast biofilm infections.

 Funded by the European Union – Next Generation EU, MUR, UdA, SCIAMI.

232 - A preliminary study on bacterial control strains to assess the synergistic effect of new heteroaryl-ethylene molecules in combination with antibiotics.

PAOLO GIUSEPPE BONACCI ⁽¹⁾ - CARMELO BONOMO ⁽¹⁾ - DALIDA ANGELA BIVONA ⁽¹⁾ - EMANUELE NICITRA ⁽¹⁾ - CARMELA BONACCORSO ⁽²⁾ - DAFNE SAMANTHA IRENE BONGIORNO ⁽¹⁾ - STEFANIA STEFANI ⁽¹⁾ - NICOLÒ MUSSO ⁽¹⁾

Università degli studi di Catania, Dipartimento di scienze biomediche e biotecnologiche (BIOMETEC), Catania, Italia ⁽¹⁾ - Università degli studi di Catania, Dipartimento di Scienze Chimiche, Catania, Italia ⁽²⁾

A preliminary study on bacterial control strains to assess the synergistic effect of new heteroaryl-ethylene molecules in combination with antibiotics.

PAOLO G. BONACCI¹, CARMELO BONOMO¹, DALIDA A. BIVONA¹, EMANUELE NICITRA¹, CARMELA BONACCORSO², DAFNE BONGIORNO¹, STEFANIA STEFANI¹ E NICOLÒ MUSSO¹.

¹Department of Biomedical and Biotechnological Sciences (BIOMETEC), Università degli Studi di Catania, Via S. Sofia, 97, 95123 Catania, Italy

²Department of Chemical Sciences, Università degli Studi di Catania, Viale Andrea Doria 6, 95125 Catania, Italy

Introduction Antimicrobial resistance represents a major burden for public health worldwide, increasing the need for the development of novel antimicrobials and alternative treatment strategies. In this work, antibacterial activity of six newly synthesized compounds was evaluated using nine ATCC strains, including 3 *Staphylococcus aureus*, 1 *Pseudomonas aeruginosa*, 1 *Enterococcus faecalis*, 1 *Salmonella enterica*, 1 *Escherichia coli*, 1 *Acinetobacter baumannii*, and 1 *Klebsiella pneumoniae*. Moreover, PB4, an already published compound, was employed to examine its possible synergism with GC-VI-70, that showed a good cytotoxicity/MIC profile on Gram-positive, as well as with six well known antibiotics (linezolid, gentamicin, ampicillin, erythromycin, rifampin, and imipenem). The cytotoxicity of these compounds was explored to discover any side effect on Caco2 human cells (ATCC HTB-37).

Materials and Methods The antibacterial activity of the compounds, alone and in combination, was evaluated determining the Minimum Inhibitory Concentration (MIC) through Broth Microdilution. In the combination with antibiotics, PB4 was used at a fixed concentration of 0,125 µg/mL (0.2 µM), whereas in the combination with GC-VI-70 was used at a concentration corresponding to the MIC of each specific strain. MTT assay at 24 and 48 hours post treatment, was performed on Caco2 cells, and IC₅₀ values were determined both for single and combined treatments.

Results PB4 induced a significant rifampin MIC reduction for USA 300 *S. aureus* (4-fold log reduction) and ATCC 29212 *E. faecalis* (2-fold log reduction). The combination PB4-gentamycin resulted in a 2-fold log MIC reduction for ATCC 700603 *K. pneumoniae*, ATCC 27853 *P. aeruginosa*, and ATCC 29212 *E. faecalis*. Furthermore, GC-VI-70 showed a synergic effect with PB4 against Gram-positive bacteria, decreasing PB4 MIC values for Gram-positive bacteria (3 logs for ATCC 29212 *E. faecalis* and 2 logs for ATCC 12598 *S. aureus*). All values are listed in table 1 and 2. Cell metabolism was not affected by antibiotic treatment after 48 hours (Figure 1). IC₅₀ values showed no significance differences in PB4-antibiotics treatments compared to PB4 alone (Figure 2).

Discussions and Conclusions The results show the promising antimicrobial activity of the GC-VI-70 and PB4 heteroaryl-ethylenes, emphasizing the potential of new compounds in fighting antimicrobial resistance. Our study highlights the relevance of combination therapy that represents a strategy to reduce the overall dosage of antibiotics, minimizing the risk of side effects and the selective pressure exerted on bacteria responsible for the development of new resistance mechanisms. Further investigations are ongoing to gain deeper insights on their specific mechanisms of action.

			PB4		BCM4		BCM6		GC-VII-39		GC-VI-70		GC-VII-50		GC-VI-10	
	Species	Strain	mg/L	μM	mg/L	μM	mg/L	μM	mg/L	μM	mg/L	μM	mg/L	μM	mg/L	μM
GRAM +	<i>Staphylococcus aureus</i>	ATCC 29213	0.125	0.24	128	349.5	4*	8.2	128	243.6	16	26.6	0.5*	1.1	128	168.9
	<i>Staphylococcus aureus</i>	ATCC 12598	0.25	0.49	64	174.7	4*	8.23	32	60.9	16	26.6	0.25*	0.5	64	84.4
	<i>Staphylococcus aureus</i>	USA 300	≤0.125	≤0.24	64	174.7	8*	16.4	>128	>243.6	4*	6.65	2*	4.6	128	168.9
	<i>Enterococcus faecalis</i>	ATCC 29212	0.5	0.98	≥128	≥349.5	32	65.93	16	30.45	2*	3.32	8*	18.5	4*	5.2
	<i>Klebsiella pneumoniae</i>	ATCC 700603	64	125.1	>128	≥349.5	>128	>263.7	≥128	≥243.6	>128	>212.7	>128	>297.4	>128	>168.9
GRAM -	<i>Escherichia coli</i>	ATCC 25922	2	3.91	≥128	≥349.5	128	263.7	≥128	128	212.7	64	148.7	>128	>168.9	>168.9
	<i>Salmonella enterica</i>	ATCC 14028	64	125.1	>128	≥349.5	>128	>263.7	≥128	≥243.6	>128	>212.7	>128	>297.4	>128	>168.9
	<i>Pseudomonas aeruginosa</i>	ATCC 27853	>128	>250.2	>128	≥349.5	>128	>263.7	≥128	≥243.6	>128	>212.7	>128	>297.4	>128	>168.9
	<i>Acinetobacter baumannii</i>	ATCC 17978	≤0.125	≤0.24	≥128	≥349.5	64	131.8	≥128	≥243.6	32	53.19	16	37.18	>128	>168.9

Table 1: MIC values of heteroaryl-ethylene compounds on ATCC strains. All values are expressed in mg/L and μM

	Species	Strain	LNZ	LNZ + PB4	RD	RD + PB4	E	E + PB4	CN	CN + PB4	AMP	AMP + PB4	IMI	IMI + PB4
GRAM +	<i>Staphylococcus aureus</i>	ATCC 29213	4	4	0.125	0.125	0.5	0.5	0.5	0.5	2	8	-	-
	<i>Staphylococcus aureus</i>	ATCC 12598	4	4	0.125	0.125	0.5	0.5	0.5	0.5	0.125	0.125	-	-
	<i>Staphylococcus aureus</i>	USA 300	2	1	0.125*	0.006*	32	32	0.5	0.5	8	8	-	-
	<i>Enterococcus faecalis</i>	ATCC 29212	4	2	0.25*	0.06*	2	1	4*	1*	0.25	0.125	-	-
	<i>Klebsiella pneumoniae</i>	ATCC 700603	-	-	-	-	-	-	8*	2*	> 128	> 128	0.03	0.03
GRAM -	<i>Escherichia coli</i>	ATCC 25922	-	-	-	-	-	-	0.5	0.5	8	8	0.06	0.06
	<i>Salmonella enterica</i>	ATCC 14028	-	-	-	-	-	-	1	1	2	2	0.06	0.06
	<i>Pseudomonas aeruginosa</i>	ATCC 27853	-	-	-	-	-	-	2*	0.5*	> 128	> 128	0.25	0.25
	<i>Acinetobacter baumannii</i>	ATCC 17978	-	-	-	-	-	-	2	2	32	32	0.06	0.06

Table 1: MIC values of antibiotics alone and combined to PB4. All values are expressed in mg/L

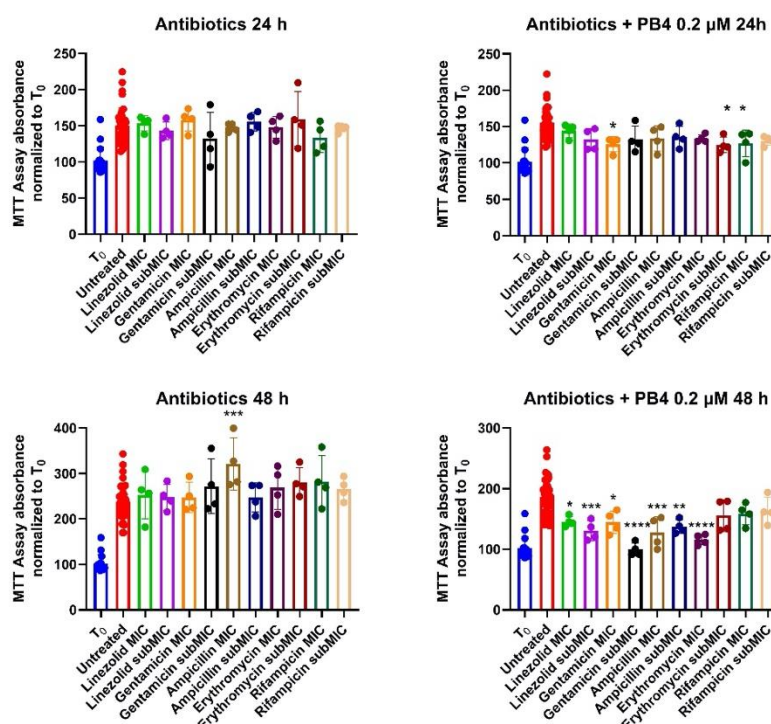


Figure 1: Caco2 cell growth, 24 and 48 hours after treatments with antibiotics alone and in combination with PB4 (0.2 μM). All values are normalized to T0.

Gentamicin	MIC	150.4	241.0	140.0	141.1
	subMIC	132.3	271.7	132.4	99.2
Ampicillin	MIC	147.7	321.1	133.8	127.7
	subMIC	155.6	247.5	135.6	137
Erythromycin	MIC	148.2	269	133.6	117.5
	subMIC	150.5	280.0	134.0	115.0

Table 3: Caco2 IC₅₀ values at 2 and 48 hours after treatments with antibiotics alone and in combination with PB4

236 - Ligand-free AgNPs: an innovative antimicrobial strategy

MARIA VITTORIA MORONE⁽¹⁾ - FEDERICA DELL' ANNUNZIATA⁽¹⁾ - ANNALISA CHIANESE⁽¹⁾ - VERONICA FOLLIERO⁽²⁾ - CARLA ZANNELLA⁽¹⁾ - ANNA DE FILIPPIS⁽¹⁾ - GIANLUIGI FRANCI⁽²⁾ - ANTONIO MORONE⁽³⁾ - MASSIMILIANO GALDIERO⁽¹⁾

Dipartimento di Medicina Sperimentale, Università degli studi della Campania "Luigi Vanvitelli", Napoli, Italia⁽¹⁾ - Department of Medicine, Surgery and Dentistry "Scuola Medica Salernitana", Università di Salerno, Baronissi, Italia⁽²⁾ - Istituto struttura della materia U.O. di Tito Scalo, Consiglio Nazionale Delle Ricerche, Tito Scalo, Italia⁽³⁾

Ligand-free AgNPs: an innovative antimicrobial strategy

Maria Vittoria Morone¹, Federica Dell'Annunziata^{1,2}, Annalisa Chianese¹, Veronica Folliero², Carla Zannella¹, Anna De Filippis¹, Gianluigi Franci², Antonio Morone³, Massimiliano Galdiero¹.

¹ Department of Experimental Medicine, University of Campania "Luigi Vanvitelli", 80138 Naples, Italy.

² Department of Medicine, Surgery and Dentistry "Scuola Medica Salernitana" University of Salerno, 84081 Baronissi, Italy.

³ National Research Council, Institute of Structure of Matter U.O. di Tito Scalo, 85050, Italy

Introduction: In recent times, infectious diseases, caused by pathogenic microbes (bacteria, viruses, fungi, protozoa, parasites), have become a great burden on the world economy as well as on public health. Novel preventive antiviral strategies and new antibacterial drugs need to be developed, trying to control antimicrobial resistance spread. Due to their unique properties, silver nanoparticles (AgNPs) could be considered a valid alternative to counteract the phenomenon of drug-resistance. In this study we present an innovative method for preparing ligand-free Ag nanoparticles (LF-AgNPs) and evaluate their antimicrobial properties.

Materials and Methods: LF-AgNPs were produced by Pulsed Laser Ablation in Liquid (PLAL) and were characterized by Total X Ray Fluorescence, Zeta Potential, Transmission Electron Microscopy (TEM), and Nanoparticle Tracking Analysis (NTA). Antiviral activity was evaluated against Herpes Simplex Virus type 1, severe acute respiratory syndrome coronavirus 2 and poliovirus type 1, by plaque reduction assays and confirmed by Real-time PCR and fluorescence microscopy. The antibacterial activity was tested by minimal inhibitory concentration (MIC) method against *Staphylococcus aureus* (S.aureus 6538) and *S. aureus* multidrug-resistant (S.aureus MDR), as Gram positive bacteria, and against *Escherichia coli* (E.coli 11229) and *E.coli* Extended Spectrum Beta-Lactamase (E.coli ESBL) as Gram negative ones. In order to investigate LF-AgNPs antibacterial mechanism, a time killing assay was conducted. Finally, the ability of AgNPs to inhibit biofilm formation and destroy the preformed matrix, was evaluated by crystal violet assay against *E. coli* and *S. aureus*, ATCC biofilm producer strains.

Results: AgNPs appeared spherical in shape and 20-100 nm sized in diameter. Regarding the antiviral activity, AgNPs interfered directly with the viral particles at an early stage of infection against all tested viruses. Furthermore, their antibacterial effect was remarkable against both *S.aureus* MDR and *E. coli* ESBL while AgNPs were bacteriostatic against *S.aureus* ATCC and bactericidal against *E. coli*

ATCC strains. Their antimicrobial activity was also directed against biofilm formation of *S.aureus* and *E.coli* ATCC biofilm producers, since AgNPs were able to inhibit it ranging from 1.2×10^7 to 2.9×10^6 by about 50%. Finally, AgNPs degraded the mature biofilm of *S.aureus* and *E.coli* ATCC biofilm producers by about 50% in the range from 1.2×10^7 to 2.9×10^6 .

Conclusions: Antiviral and antibacterial action of LF-AgNPs are a promising and innovative strategy against emerging antimicrobial resistance phenomena, anyway it needs of further investigation to better understand their mechanism.

237 - New imidazolium alkaloids from the marine bacterium *Shewanella aquimarina* as innovative antimicrobial strategies.

ROSA GIUGLIANO⁽¹⁾ - ANNALISA AMBROSINO⁽¹⁾ - CARMINE BUONOCORE⁽¹⁾ - GERARDO DELLA SALA⁽²⁾ - PIETRO TEDESCO⁽²⁾ - CARLA ZANNELLA⁽¹⁾ - GIANLUIGI FRANCI⁽³⁾ - ANNA DE FILIPPIS⁽¹⁾ - DONATELLA DE PASCALE⁽²⁾ - MASSIMILIANO GALDIERO⁽¹⁾

Università degli Studi della Campania "Luigi Vanvitelli", dipartimento medicina sperimentale, Napoli, Italia⁽¹⁾ - stazione zoologica Anton Dohrn, 2. Department of Marine Biotechnology, Napoli, Italia⁽²⁾ - University of Salerno, Department of Medicine, Surgery and Dentistry, Salerno, Italia⁽³⁾

New imidazolium alkaloids from the marine bacterium *Shewanella aquimarina* as innovative antimicrobial strategies.

Rosa Giugliano¹, Annalisa Ambrosino¹, Carmine Buonocore², Gerardo Della Sala², Pietro Tedesco², Carla Zannella¹, Gianluigi Franci³, Anna De Filippis¹, Donatella de Pascale², Massimiliano Galdiero¹.

¹Department of Experimental Medicine, University of Campania "Luigi Vanvitelli", 80138 Naples, Italy

²Department of Ecosustainable Marine Biotechnology, Stazione Zoologica Anton Dohrn, Via Ammiraglio Acton, 55, 80133 Naples, Italy

³Department of Medicine, Surgery and Dentistry "Scuola Medica Salernitana", University of Salerno, 84081 Baronissi, Italy

INTRODUCTION: The continuous outbreak of drug-resistant bacterial and viral infections imposes the need to search for new drug candidates. Marine natural products from bacteria still inspire the design of pharmaceuticals. Indeed, marine bacteria have unique metabolic flexibility to inhabit each ecological niche, thus expanding their biosynthetic ability to assemble unprecedented molecules. Combining the One-Strain-Many-Compounds approach and tandem mass spectrometry molecular networking unveiled a *Shewanella aquimarina* strain as a source of novel imidazolium alkaloids.

MATERIALS AND METHODS: Antibacterial activity was evaluated against several strains of *Staphylococcus aureus* through the liquid microdilution assay. All the steps of biofilm formation were investigated with the crystal violet method. On the other hand, to assess the antiviral potential, the plaque reduction assay was performed. Finally, liquid survival assay was utilized to evaluate the activity against *Caenorhabditis elegans*.

RESULTS: The alkaloid mixture was shown to a) exert antibacterial activity against antibiotic-resistant *S. aureus* clinical isolates with a MIC value of 100 µg/mL, b) have synergistic effects with tigecycline and linezolid, c) interfere with the biofilm formation of *S. aureus* 6538 and MRSA. Moreover, the mixture showed antiviral activity against enveloped and non-enveloped viruses. Indeed, it inhibited entry of human coronavirus HCoV-229E and herpes simplex viruses into human cells and inactivated poliovirus PV-1 in post-infection assay at 200 µg/mL. Finally, at the same

concentration, the fraction showed anthelmintic activity against the *C. elegans*, causing 99% mortality after 48h.

DISCUSSION AND CONCLUSION: The enriched fraction of imidazolium alkaloids showed antibacterial, antibiofilm, antiviral, and anthelmintic activities and enhanced antimicrobial effects of antibiotics currently included in clinical settings, thus representing a valid ally in the fight against the microbial drug resistance. Notably, we proved here for the first time that this alkaloids mixture inactivates members of the Herpesviridae and Coronaviridae families, likely disrupting the viral envelope as a cationic surfactant. The broad-spectrum activities of these compounds is partially due to their biosurfactant behavior and make them promising candidates for breaking down drug-resistant infectious diseases.

238 - Anti-staphylococcal and anti-biofilm properties of the cationic peptide Orechromicin-1 from *Oreochromis niloticus*

FRANCESCA PALMA⁽¹⁾ - MARINA ACUNZO⁽¹⁾ - ANNALISA CHIANESE⁽¹⁾ - ERICA PANICO⁽²⁾ - NUNZIANNA DOTI⁽³⁾ - ALESSANDRA MONTI⁽³⁾ - ALESSANDRA FUSCO⁽¹⁾ - CARLA ZANNELLA⁽¹⁾ - ANNA DE FILIPPIS⁽¹⁾ - GIOVANNA DONNARUMMA⁽¹⁾ - MASSIMILIANO GALDIERO⁽⁴⁾

Università degli Studi della Campania "Luigi Vanvitelli", Dipartimento di Medicina Sperimentale, Napoli, Italia⁽¹⁾ - Università degli Studi della Campania "Luigi Vanvitelli", Azienda Ospedaliera Universitaria, Sezione di Microbiologia e Virologia, Napoli, Italia⁽²⁾ - Istituto di Biostrutture e Bioimaging, Consiglio Nazionale delle Ricerche, Napoli, Italia⁽³⁾ - Università degli Studi della Campania "Luigi Vanvitelli", Dipartimento di Medicina Sperimentale, Napoli, Italia⁽⁴⁾

Anti-staphylococcal and anti-biofilm properties of the cationic peptide Orechromicin-1 from *Oreochromis niloticus*

FRANCESCA PALMA¹, MARINA ACUNZO¹, ANNALISA CHIANESE^{1,2}, ERICA PANICO², NUNZIANNA DOTI³, ALESSANDRA MONTI³, ALESSANDRA FUSCO¹, CARLA ZANNELLA^{1,2}, ANNA DE FILIPPIS¹, GIOVANNA DONNARUMMA^{1,2}, MASSIMILIANO GALDIERO^{1,2}.

¹Department of Experimental Medicine; University of Campania "Luigi Vanvitelli"; 80138 Naples; Italy.

²Section of Microbiology and Virology; University Hospital "Luigi Vanvitelli" of Naples, Naples, Italy.

³Institute of Biostructures and Bioimaging (IBB), National Research Council (CNR), 80134 Naples, Italy.

Introduction: Drug resistance and biofilm formation frequently account for the treatment failure of bacterial infections. One of the most important genera of pathogens, especially in the nosocomial setting, is *Staphylococcus*. *Staphylococcus aureus* (*S. aureus*) and *Staphylococcus epidermidis* (*S. epidermidis*), the most common isolated species, are frequently involved in tissue and medical devices infections, causing acute and chronic diseases. Hence, new antimicrobial molecules development is a pressing mission for scientific community. Natural cationic antimicrobial peptides (CAMP) are short molecules modulating immune response in virtually every life form. They have been widely described as broad-spectrum antimicrobial agents thanks to their positive-charged and amphipathic structure able to interact with outer surface of bacteria. Fish like tilapia (*Oreochromis niloticus*) represent a fruitful resource of CAMP, oreochromicins, that have been poorly studied so far. In this context, we evaluated the antibacterial and anti-biofilm properties of oreochromicin-1 (Oreoch-1) against *S. aureus* and *S. epidermidis*.

Materials and Methods: The cytotoxicity profile of Oreoch-1 was analyzed by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay on Caco-2 cell line in the range of concentrations from 100 to 1.56 µg/mL. The broth microdilution method and the crystal violet (CV) staining were exploited to assess the anti-staphylococcal activity. LIVE/DEAD evaluation and the analysis of some biofilm-related genes expression via RT-PCR was performed to support the peptide anti-biofilm effect.

Results: The peptide showed a low cytotoxicity, below 24% at 25 µg/mL. Oreoch-1 registered a minimum inhibitory concentration (MIC) of 25 and 12.5 µg/mL for *S. aureus* and *S. epidermidis*, respectively, exerting a bacteriostatic activity against both strains. CV staining highlighted a significant biofilm inhibitory activity only against *S. aureus*, impaired significantly at MIC and sub-MIC values. Microscopy and molecular analysis of *S. aureus* biofilm confirmed the anti-staphylococcal activity of the peptide.

Discussion and Conclusions: To our knowledge, this is the first report on the antimicrobial and anti-biofilm properties of Oreoch-1. In conclusion, our interesting data highlight Oreoch-1 as a new potential anti-staphylococcal agent. As biofilms persist as the main concern in clinical settings, this peptide may serve as a potential candidate drug molecule against biofilm-forming *S. aureus*.

242 - Effect of resveratrol on antibiofilm action against *Pseudomonas aeruginosa* clinical strains

MARIA RITA TRICOLI ⁽¹⁾ - ANNA GIAMMANCO ⁽²⁾ - VIVIANA DE CARO ⁽³⁾ - IGNAZIO ARRIGO ⁽¹⁾ - GIUSEPPE ANGELLOTTI ⁽³⁾ - TERESA FASCIANA ⁽⁴⁾

Laboratorio Microbiologia, A.O.U.P "P.Giaccone", Palermo, Italia ⁽¹⁾ - Università Degli Studi Di Palermo, Promise, Palermo, Italia ⁽²⁾ - Università Degli Studi Di Palermo, Scienze E Tecnologie Biologiche Chimiche E Farmaceutiche, Palermo, Italia ⁽³⁾ - Dipartimento Promise, A.O.U.P "P.Giaccone" /Università Di Palermo, Palermo, Italia ⁽⁴⁾

Effect of resveratrol on antibiofilm action against *Pseudomonas aeruginosa* clinical strains

Maria Rita Tricoli, Anna Giammanco, Viviana De Caro, Ignazio Arrigo, Giuseppe Angelotti, Teresa Fasciana

INTRODUCTION Antibiotic resistance is a serious public health problem due to excessive and unnecessary use of antibiotics. An mechanism that can make the action of antibiotic drugs ineffective in treating of infectious diseases is the formation of bacterial biofilms, described also in MDR-*P.aeruginosa* strains. The discovery and development of alternative therapeutic strategies that present novel pathways against *P. aeruginosa* infections are required. The purpose of this work was to study the action of resveratrol (3, 4', 5 trihydroxystilbene), a naturally occurring polyphenol characterized by several properties including preventing biofilm formation. However, the low bioavailability of molecule limits its use at a therapeutic level. In order to increase its efficiency we conjugated resveratrol (RSV) with nanostructured lipid vectors (NLC) and evaluated the antibiofilm action of RSV and RSV-NLC on clinical *P.aeruginosa* strains.

MATERIALS AND METHODS Fifty clinical isolates of *P.aeruginosa* from different hospitals were examined. The strains were cultured on tryptose agar and then the antibiofilm action of RSV and RSV-NLC (16µg/ml) was evaluated using polystyrene microplates. Specifically, the strains were incubated at 37 °C for 24 h with the molecules to be assayed, washed with PBS, stained with Cristal Violet (0.5%), and finally the optical density (OD) was read at 570 nm with Multiscan. Treated and untreated strains were repeated in triplicate.

RESULTS The clinical isolates of *P. aeruginosa* examined (n.50) were 42% (n.21) high biofilm producers (strongly adherent), 26% (n.13) medium biofilm producer (moderately adherent) and 32% (n.16) low biofilm producers (weakly adherent). Of the strains found to be strongly adherent following treatment with RSV, they showed the following results: 4.76% remained strongly adherent (n.1), while 71.43% showed a reduction in biofilm production and became moderately adherent, and only 23.81% were classified as weakly adherent. The strains when treated with RSV- NLC only in one case strain did not reduce biofilm production, in the 47.62% (10/21) were moderately adherent, the remaining of the isolates showed a significant reduction in biofilm by classifying themselves as weakly adherent.

DISCUSSION From the analysis of the data, the resveratrol seems to show a good action on gram negatives bacteria. However, when RSV is linked to NLC the antibiofilm action is high, probably due to better penetration of polyphenol. We believe that RSV-NLC could be used in therapy associated to antimicrobial to avoid the onset of MDR strains and the possible side effects of drugs when used at high doses.

244 - Pilocarpine hydrochloride promotes *Candida albicans* cell membrane damage leading to reactive species production and apoptosis

EMERENZIANA OTTAVIANO⁽¹⁾ - SILVIA ANCONA⁽¹⁾ - MICHELE DEI CAS⁽²⁾ - ELISA BORGHI⁽¹⁾

Universita' Degli Studi Di Milano, Dip. Scienze Della Salute, Milano, Italia⁽¹⁾ - Università Degli Studi Di Milano, Dip. Scienze Della Salute, Milano, Italia⁽²⁾

Title: Pilocarpine hydrochloride promotes *Candida albicans* cell membrane damage leading to reactive species production and apoptosis

Authors:

Emerenziana Ottaviano¹, Silvia Ancona¹, Michele V. Dei Cas¹, Elisa Borghi¹.

1. Department of Health Sciences, Università degli Studi di Milano, Milan (Italy);

Introduction

Biofilm formation is a relevant feature in *Candida albicans* (Ca) virulence, allowing the fungus to persist on indwelling devices, to colonize tissues, and to develop antimicrobial tolerance. Active treatments to overcome biofilm recalcitrance are thus necessary to develop new antifungal strategies. Pilocarpine hydrochloride (PHCl), a general muscarinic agonist, inhibits Ca biofilm formation in vitro and in vivo, in the *Galleria mellonella* invertebrate model. Morphological analysis showed a PHCl-dependent reduction in adhesion, yeast filamentation, and biomass production, suggesting alterations of several pathways involved in the biofilm formation process. We postulated that PHCl could affect the yeast cell wall integrity signaling. To test our hypothesis, we evaluated, on Ca treated with PHCl, i) the lipid content, ii) the ROS formation, and iii) apoptosis.

Materials and Methods

Lipids from pellets of planktonic and biofilm-organized Ca SC5314 cells, treated or not for 24 hours with PHCl 25 μ M, were collected and extracted by methanol/chloroform mixture and measured in a Shimadzu UPLC coupled with a Triple TOF 6600 Sciex equipped with Turbo Spray IonDrive. ROS formation and apoptosis were evaluated by flow cytometry (FACSVerse, Beckman Coulter, Brea, CA, USA). For apoptosis, yeast cells were labeled with APC Annexin V and propidium iodide (PI). For ROS production, dihydroethidium (DHE) labelling was used.

Results

The transition between the planktonic and biofilm status showed per se a strong increase in lipids involved in cell wall remodeling. PHCl treatment caused a decrease in lipids content to a level even lower than those found in planktonic cells. In particular, ergosterol and its derivatives were strongly depleted. PHCl led to phosphatidylserine externalization, an apoptosis hallmark, and increased ROS formation.

Discussion and conclusions

Our study showed that PHCl interferes with ergosterol biosynthesis, which ultimately affects plasma membrane integrity and increase yeast apoptosis. Despite a variety of mechanisms can trigger Ca apoptosis, we found that PHCl treatment induces an increase in ROS production. Because in

mammals PHCl has a predominance for the muscarinic receptor M3 and M1, both associated with calcium influx, studies investigating the role of this ion will further elucidate PHCl mechanisms leading to Ca apoptosis.

249 - Cryogel based on b-cyclodextrin and a maltol derivative for antimicrobial applications

VIRGINIA FUOCHI ⁽¹⁾ - CHIARA ZAGNI ⁽²⁾ - SALVATORE FURNARI ⁽¹⁾ - VINCENZO PATAMIA ⁽²⁾ - GIUSEPPE FLORESTA ⁽²⁾ - ANTONIO RESCIFINA ⁽²⁾ - PIO MARIA FURNERI ⁽¹⁾

Università di Catania, BIOMETEC, Catania, Italia ⁽¹⁾ - Università di Catania, Dip. Scienze del Farmaco e della Salute, Catania, Italia ⁽²⁾

Cryogel based on b-cyclodextrin and a maltol derivative for antimicrobial applications

VIRGINIA FUOCHI¹, CHIARA ZAGNI², SALVATORE FURNARI¹, VINCENZO PATAMIA², GIUSEPPE FLORESTA², ANTONIO RESCIFINA² E PIO MARIA FURNERI¹

1 Dipartimento di Scienze Biomediche e Biotecnologiche, Sez. di Microbiologia, Università di Catania, Catania, Italia; 2 Dipartimento di Scienze del Farmaco e della Salute, Università di Catania, Catania, Italia

Introduction: It is acknowledged that microbial infections and antibiotic resistance represent a severe economic and health risk to society. These facts have led to the development of several new techniques for impeding crucial biological processes in microbial cells. One of these techniques' centers on the use of metal-chelating agents, which can specifically disrupt the microorganism's vital metal metabolism by obstructing metal uptake and bioavailability for critical reactions. Additionally, a wide range of nanomaterials have been made available by nanotechnology for possible uses in the antibacterial industry. This complex field is being shaped by antimicrobial nanoparticles, which are also being investigated as therapeutic and drug delivery tools. **Materials and Methods:** The synthesized cryogels were characterized by various techniques to determine their physicochemical properties. Fourier-transformed infrared spectroscopy (FTIR) was used to determine the functional groups of the cryogels. The morphology of the samples was investigated by scanning electron microscopy (SEM). ICP-MS was used to investigate the chelation capabilities of the material. To assess the antimicrobial activity of the cryogels, bacterial pathogens such as *Staphylococcus aureus* ATCC 29213, *Pseudomonas aeruginosa* ATCC 27853 and *Klebsiella pneumoniae* ATCC 600703 were tested. For this purpose, an evaluation of the growth rate was performed compared to the control. **Results:** A new drug delivery system based on b-cyclodextrin-maltol derivative was designed and produced. HEMA, N,N'-methylene-bisacrylamide/maltol derivative monomer has been synthesized and co-polymerized with a b-cyclodextrin acrylic monomer. The results of the antimicrobial assay suggested that cryogels had a great inhibitory power against the tested bacteria. **Discussion and Conclusions:** The 3Dporous materials containing hydrophobic cavities capable of drug delivery properties and still retaining the capability of iron chelation was proved as affective antimicrobial. This non-conventional approach was successfully designed as a proof of concept of a dual acting antibacterial nanomaterial with drug delivery properties and iron depletion capabilities obtained thanks to the maltol derivative.

258 - Antiviral activity of lipophilic extracts derived from *Lavandula austroapennina* organs

ANNALISA AMBROSINO ⁽¹⁾ - CLAUDIA GRAVINA ⁽²⁾ - MARIAVITTORIA MORONE ⁽¹⁾ - PRAGATI RAJENA MORE ⁽¹⁾ - FRANCESCO FOGLIA ⁽¹⁾ - SIMONA PICCOLELLA ⁽²⁾ - CARLA ZANNELLA ⁽¹⁾ - ASSUNTA ESPOSITO ⁽¹⁾ - SEVERINA PACIFICO ⁽²⁾ - ANNA DE FILIPPIS ⁽¹⁾ - MASSIMILIANO GALDIERO ⁽¹⁾

University of Campania "Luigi Vanvitelli", Department of Experimental Medicine, Napoli, Italia ⁽¹⁾
- University of Campania "Luigi Vanvitelli", Department of Environmental, Biological and Pharmaceutical Sciences and Technologies, Caserta, Italia ⁽²⁾

Antiviral activity of lipophilic extracts derived from *Lavandula austroapennina* organs.

Introduction Medicinal and aromatic plants (MAPs) are historically of interest for their therapeutic properties. Among them, species from *Lavandula* genus (Lamiaceae), are broadly investigated for their antioxidant, anti-inflammatory, antimicrobial efficacy. In a framework aimed at the recovery and enhancement of medicinal plants endemic to the territory of the Cilento and Vallo di Diano National Park, *Lavandula austroapennina* N.G. Passal., Tundis and Upon has aroused interest.

Materials and Methods. Plants of *Lavandula austroapennina* were dissected in its organs (corolla, calyx, leaves, stem, and roots), subjected to extraction in n-hexane by ultrasound-assisted maceration and chemically analyzed by ultra-high-performance chromatography coupled to high resolution mass spectrometry (UHPLC-ESI-QqTOF-MS/MS tools). Subsequently, 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) assay was performed to verify the possible cytotoxicity of the organ extracts of *L. austroapennina* towards Vero cell line. The antiviral activity was evaluated by plaque reduction assay (PRA) with different schema of treatment (co-treatment, virus pre-treatment, post-treatment, and cell pre-treatment), against members of Herpesviridae, Coronaviridae and Picornaviridae family.

Results. The extracts varied in their relative content of fatty acids and ursane and oleanane-type triterpenes. In particular, the oleanolic acid content appeared to increase in the order corolla < leaf < stem. Although all the extracts did not show cytotoxic effects, the antiviral efficacy was particularly important for the stem extract, capable to completely inhibit the tested viruses at low doses, especially in virus pre-treatment. The analysis of the antiviral activity in a non-enveloped virus PV-1 allows to assert that the extracts from the organs of *L. austroapennina*, and especially the stem extract, affect the viral envelope.

Discussion and Conclusions. Our extracts appeared to be more effective when virus pre-treatment experiment was carried out, suggesting they are able to interfere with the viral entry. Data herein collected suggested that *L. austroapennina* organs differently induce viral suppression acting at virus replication first levels, through disruption of the pathogen envelope. In fact, fatty acids, which represent the most abundant component for almost all the extracts investigated, are capable of inducing the inactivation of some enveloped viruses. This evidence suggests a positive outcome for the use of apolar mixtures from *L. austroapennina*. This study underlines how much knowledge of the medicinal plant heritage of a territory is a harbinger of promising discoveries in the health field.

264 - Exploring the Inhibitory Potential of oreochromicin peptide against viruses with different Classes of Fusion Proteins

BIANCA MARIA NASTRI ⁽¹⁾ - ANNALISA CHIANESE ⁽¹⁾ - CARLA CAPASSO ⁽¹⁾ -
NUNZIANNA DOTI ⁽²⁾ - ALESSANDRA MONTI ⁽²⁾ - VALENTINA IOVANE ⁽³⁾ - SERENA
MONTAGNARO ⁽⁴⁾ - UGO PAGNINI ⁽⁴⁾ - CARLA ZANNELLA ⁽¹⁾ - ANNA DE FILIPPIS ⁽³⁾ -
MASSIMILIANO GALDIERO ⁽¹⁾

University of Campania "Luigi Vanvitelli", Department of Experimental Medicine, Naples, Italia ⁽¹⁾
- National Research Council (CNR), Institute of Biostructures and Bioimaging (IBB), Naples, Italia
⁽²⁾ - University of Naples "Federico II", Department of Agricultural Sciences, Naples, Italia ⁽³⁾ -
University of Naples "Federico II", Department of Veterinary Medicine and Animal Productions,
Naples, Italia ⁽⁴⁾

Exploring the Inhibitory Potential of oreochromicin peptide against viruses with different Classes of Fusion Proteins

Introduction. Antimicrobial peptides (AMPs) are innate defense mechanism, providing protection against a wide range of pathogens including bacteria, fungi, and viruses. Oreochromicin, derived from *Tilapia* (*Oreochromis* spp.), is a natural AMPs not yet explored as antiviral agent. Viral fusion proteins can mediate both virus-cell fusion leading to infection and pathological cell-cell fusion, they are increasingly viewed as targets for antiviral intervention. The aim of this study is focused on evaluation of potential Oreochromicin as drugs with antiviral properties, interacting with viruses belonged to different classes of viral membrane-fusion proteins. The three viral classes describe distinct surface fusion proteins found on enveloped viruses, each with unique structures and mechanisms of action.

Materials and Methods. Cell viability was assessed using the MTT assay and viral plaque reductions assays have been conducted to evaluate the peptide antiviral activity against viruses from three classes. First, Vero SLAM cells were employed to investigate the potential against canine distemper virus (CDV; class I). BHK-21 cells were utilized to test oreochromicin effectiveness against Schmallenberg virus (SBV; class II). Lastly, MDBK cells were exposed to oreochromicin to assess its efficacy against caprine herpes virus 1 and bovine herpes virus 1 (CpHV-1; BoHV-1; class III). The assay involved direct exposure of the viruses to oreochromicin followed by infection of the respective cell lines. After 1 hour of incubation, cells were washed to remove all the unattached virus, and then were overlaid with a viscous medium for 48 hours. Plaque formation was analyzed after staining with crystal-violet solution.

Results. Oreochromicin showed a remarkable inhibitory activity against viruses from different classes. In detail, it reached the 50% inhibitory concentration (IC₅₀) at 1.5 μ M, 3 μ M and 6 μ M for CDV, SBV, and animal herpesviruses, respectively, by inhibiting the early phases of the infection acting directly on the viral particles.

Discussion and Conclusions. These findings highlighted the promising antiviral efficacy of oreochromicin, suggesting its potential as antiviral agent. The peptide demonstrated a broad-spectrum antiviral activity, effectively targeting viruses with diverse membrane fusion protein classes and so suggesting that it targets a common feature or mechanism in the viral fusion and entry process. By

evaluating the specific inhibitory capabilities of oreochromicin, we need to improve our results evaluating pathway involved in interaction between tested AMP and viral fusion proteins. Our future studies will be focused with Oreochromicin interaction with other viruses belonged to three fusion protein classes

277 - Antimicrobial and antioxidant properties of vesicles loaded with an extract obtained from olive leaves

ILENIA DELOGU ⁽¹⁾ - INES CASTANGIA ⁽²⁾ - SILVIA PUXEDDU ⁽¹⁾ - ELISA RAGA ⁽¹⁾ - MARIA LETIZIA MANCA ⁽²⁾ - ALDO MANZIN ⁽¹⁾ - FABRIZIO ANGIUS ⁽¹⁾

Università Degli Studi di Cagliari, Dipartimento Scienze Biomediche, Cagliari, Italia ⁽¹⁾ - Università Degli Studi di Cagliari, Dipartimento di Scienze della Vita e dell'Ambiente, Cagliari, Italia ⁽²⁾

Antimicrobial and antioxidant properties of vesicles loaded with an extract obtained from olive leaves

ILENIA DELOGU ¹, INES CASTANGIA ², SILVIA PUXEDDU ¹, ELISA RAGA ¹, MARIA LETIZIA MANCA ², ALDO MANZIN ^{1,3}, FABRIZIO ANGIUS ¹

¹ Department of Biomedical Sciences, Section of Microbiology and Virology, University of Cagliari, Cagliari, Italy

² Department of Life and Environmental Sciences, Section of Pharmaceutical Sciences, University of Cagliari, Cagliari, Italy

AOU Policlinico "D. CASULA", APPLIED MICROBIOLOGY LAB, MONSERRATO (CA), Italy

Introduction

Olive leaves (*Olea europaea* L.) are considered a by-product of olive cultivation and processing. The olive pruning and harvesting for oil production and edibility generate a considerable volume of leaves and their transformation in beneficial and health-promoting foods, cosmeceuticals, additives, or nutraceuticals is an innovative challenge. It is known that major phenolic constituents of olive leaves (e.g., oleuropein) are responsible for the pharmacological properties and several studies reported their antiviral, antioxidant, antifungal, anti-inflammatory and antibacterial activity. Here we evaluated the antimicrobial properties of aqueous dispersion of olive leaves extract (OLE) and OLE-loaded vesicles against common pathogens with the aim of finding valid formulations to complement the common medications for skin infections.

Materials and methods

The OLE phytochemicals were incorporated into specifically designed phospholipid vesicles enriched with co-solvents to ease topical application (i.e., liposomes, ethosomes and glycerophospholiposomes) and their chemical-physical properties (i.e., mean diameter, surface charge, stability) and technological (incorporation efficiency) were characterized. Furthermore, the biocompatibility of the dispersion or OLE-loaded vesicles and their ability to protect cells from toxic effects (i.e., hydrogen peroxide [HP]) were evaluated on keratinocytes. We evaluate the OLE formulations' ability to inhibit viral infections using Herpes simplex virus type 1 (HSV-1); furthermore, microbes responsible for common skin infections such as Gram-positive (*S. aureus* and *S. epidermidis*), Gram-negative (*E. coli*) and fungi (*C. albicans*) were challenged to evaluate their susceptibility.

Results

All formulations were 100 nm sized and homogeneously dispersed with a 90% efficiency of OLE incorporation, highly biocompatible and effective in protecting cells from HP-induced oxidative stress. The OLE formulations produced have been specifically designed for topical use and their

efficacy were also supported by the cytotoxicity on epithelial cells. Although only high concentrations of OLE dispersion proved to be effective on Gram positive bacteria, the antiviral activity against HSV-1 has proved to be outperforming protecting the cell monolayer by the HSV-1 cytopathic effect during infection at all the concentrations of the formulations tested.

Discussion and conclusion

Overall, these preliminary data pointed out the promising suitability of phytocomplex-loaded vesicles obtained from olive by-product as a new and easy alternative strategy for topical application suggesting their possible combination with common drug to treat skin lesions caused by the association of oxidative stress and viral infections.

280 - Ionic liquids as a new effective antimicrobial approach

Silvia Puxeddu (1) - Ilenia Delogu (1) - Alessandra Scano (2) - Sarah Vaschellati (1) - Giuseppe Pala (1) - Guido Ennas (2) - Aldo Manzin (1) - Fabrizio Angius (1)

Università degli studi di Cagliari, Dipartimento di Scienze Biomediche, Cagliari, Italia (1) -

Università degli studi di Cagliari, Dipartimento di chimica e scienze geologiche, Cagliari, Italia (2)

Ionic liquids as a new effective antimicrobial approach

SILVIA PUXEDDU 1, ILENIA DELOGU 1, ALESSANDRA SCANO 3,4, SARAH VASCELLARI 1, GIUSEPPE PALA 1, GUIDO ENNAS 3,4, ALDO MANZIN 1,2, FABRIZIO ANGIUS 1

1 Department of Biomedical Sciences, Unit of Microbiology and Virology, University of Cagliari, Cagliari, Italy

2 AOU Policlinico "D. Casula", Applied Microbiology Lab, Monserrato (CA), Italy

3 Department of Chemical and Geological Sciences, University of Cagliari, Cagliari, Italy

4 Cagliari Research Unit of the National Consortium of Materials Science and Technology (INSTM), Cagliari, Italy

Introduction

The undisputed emergence and spread of drug-resistance is progressively limiting the treatment of common infections accounting for the urge in the development of new antibiotics or further alternatives. To this effort, promising antimicrobials should act by non-specific mechanisms to overcome the emergence of resistance strategies (e.g., mutations, gene expression) such as disrupting the cell wall resulting in cell leakage and death. Ionic liquids (ILs), also known as room-temperature molten salt, are compounds consisting of organic cations and inorganic or organic anions. Features such as non-volatility, non-flammability, low melting points, high thermal stability, adjustable physico-chemical properties and antimicrobial activity, make the use of ILs very interesting in several applications. Although ILs are endowed with these promising properties, scientists are divided concerning their use as antimicrobials due to stereotypes and misconceptions on bioavailability and toxicity of these compounds. In this study, we analyzed the antimicrobial activity of commercial ILs against microorganisms that usually cause common infections.

Materials and methods

Two commercial ILs, namely IL-1 ([bmim][PF₆]) and IL-2 ([C₁₀-mim][NTf₂]) were tested against a panel of Gram-positive (i.e., *Staphylococcus aureus* and *Enterococcus faecalis*) and Gram-negative bacteria (i.e., *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Escherichia coli*), and fungi (i.e., *Candida albicans*). Microbes were challenged with different IL concentrations to assess antimicrobial resistance/susceptibility by agar diffusion, inclusion and microdilution assays. Cytotoxicity was also evaluated in VERO cells.

Results

In our experimental conditions, both IL-1 and IL-2 showed a potent antimicrobial activity against all microorganisms, with an extraordinary effect at very low concentrations obtained against *C. albicans* and *K. pneumoniae*, though IL-1 and IL-2 showed a modest cytotoxic effect on VERO cells.

Discussion and conclusion

Overall, our preliminary data indicate that the ILs tested here revealed to be highly effective against all investigated microorganisms albeit with a grade of variability. Therefore, we speculate they may act by a nonspecific mechanism on the cell wall altering its structure and leading to death, which could avoid the development of drug-resistance mechanisms. Moreover, ILs showed a moderate cytotoxic effect on mammalian cells that must be considered for future in vivo use based on the disease etiology.

282 - Screening of novel anti-HIV drugs as inhibitors against Sars-CoV-2 and HSV-2 viruses

SILVIA NOTTOLI⁽¹⁾ - CARLO DI MARZO⁽²⁾ - MARIA SIDOTI⁽¹⁾ - PAOLA QUARANTA⁽³⁾ - PAOLA MAZZETTI⁽¹⁾ - GIULIA FREER⁽²⁾ - ELENA DREASSI⁽⁴⁾ - MAURO PISTELLO⁽¹⁾

unità operativa di virologia, Azienda Ospedaliera Universitaria Pisana, Pisa, Italia⁽¹⁾ - Centro Retrovirus, Dipartimento di ricerca traslazionale e nuove tecnologie in medicina e chirurgia, Università di Pisa, Pisa, Italia⁽²⁾ - Istituto di Neuroscienze, Cnr, Pisa, Italia⁽³⁾ - Dipartimento di biotecnologie, Università di Siena, Siena, Italia⁽⁴⁾

Screening of novel anti-HIV drugs as inhibitors against Sars-CoV-2 and HSV-2 viruses

Topic: Nuovi approcci antimicrobici

SILVIA NOTTOLI¹, CARLO DI MARZO², MARIA SIDOTI¹, PAOLA QUARANTA⁴, PAOLA MAZZETTI¹, GIULIA FREER², ELENA DREASSI³, MAURO PISTELLO^{1,2}

¹Virology Operative Unit, Pisa University Hospital, Pisa, Italy; ²Retrovirus Center, Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa, Pisa, Italy; ³Department of Biotechnologies, University of Siena, Siena, Italy; ⁴Institute of Neuroscience, CNR, Pisa, Italy.

Introduction

This work was conducted as part of Tuscavir, an interdisciplinary consortium with the goal of conducting research and providing qualified services for the development of novel antiviral therapies. The project led to the development of several small molecules targeting different steps of HIV viral replication. The present study aims to evaluate the breadth of antiviral activity of these compounds against SARS-CoV-2 and HSV-2.

Materials and Methods

We analysed the effect of the preparations on SARS-CoV-2 replication using a method that involves the observation of cytopathic effect (CPE) as an indicator of virus growth. This assay allows us to evaluate the antiviral activity and cytotoxicity of individual compounds at increasing concentrations against SARS-CoV-2 and HSV-2 infections. We then confirmed the cytotoxicity by performing a WST-8 cell viability assay on different cell lines. The antiviral activity of selected molecules against SARS-CoV-2 and HSV-2 was confirmed using a dilution limitation assay or a plaque assay. To confirm drug efficacy, we performed Western blots and real-time qPCR.

Results

The IC₅₀ and the corresponding selectivity index (SI) were determined, and the efficacy was compared. To investigate the mechanism of action of the compounds that showed activity, experiments were performed with selected compounds at the time of addition by adding the compounds before, during, and after infection and evaluating the change in viral yield.

Discussion and Conclusions

From this library, we were able to select several compounds that inhibit the replication of Sars-CoV-2 or HSV-2. These promising compounds were also tested to calculate their IC₅₀. They can be considered as lead compounds for future improvement of COVID -19 treatment. We plan to characterize their antiviral activity against different SARS-CoV-2 variants.

286 - Resveratrol derivatives, promising strategy for innovative therapeutic schemes to tackle the *Helicobacter pylori* persistent infections

MARA DI GIULIO ⁽¹⁾ - PAOLA DI FERMO ⁽¹⁾ - FIRAS DIBAN ⁽¹⁾ - SARA D'ARCANGELO ⁽¹⁾ - BARBARA DE FILIPPIS ⁽¹⁾ - SILVIA DI LODOVICO ⁽¹⁾ - SIMONETTA D'ERCOLE ⁽²⁾ - EMANUELA DI CAMPLI ⁽¹⁾ - LUIGINA CELLINI ⁽¹⁾

Università "G.d'Annunzio" Chieti-Pescara, Dipartimento di Farmacia, Chieti, Italia ⁽¹⁾ - Università "G.d'Annunzio" Chieti-Pescara, Dipartimento di Scienze mediche, orali e biotecnologiche, Chieti, Italia ⁽²⁾

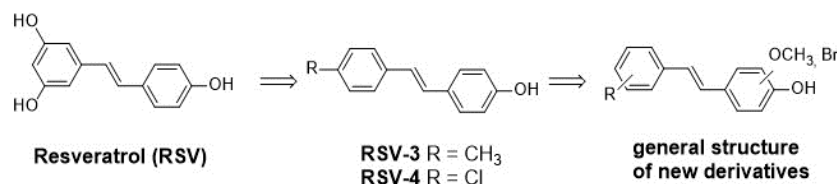
Resveratrol derivatives, promising strategy for innovative therapeutic schemes to tackle the *Helicobacter pylori* persistent infections

MARA DI GIULIO ¹, PAOLA DI FERMO ¹, FIRAS DIBAN ¹, SARA D'ARCANGELO ¹, BARBARA DE FILIPPIS¹, SILVIA DI LODOVICO ¹, SIMONETTA D'ERCOLE ², EMANUELA DI CAMPLI ¹, LUIGINA CELLINI ¹

¹ Department of Pharmacy, University of "G. d'Annunzio" Chieti-Pescara, Italy; ² Department of Medical, Oral and Biotechnological Sciences, University "G. d'Annunzio" Chieti-Pescara, Italy

Introduction. Over recent years, resveratrol (RSV, figure) attracted great attention for its biological multi-activities like anti-inflammatory, anti-carcinogenesis, including antimicrobial activity, although the in vivo application is limited for its low bioavailability. In our recent work, we demonstrated the antibacterial and anti-virulence effects (biofilm reduction and swarming motility inhibition) of RSV and new synthesized RSV-phenol derivatives (RSV-3 and 4, figure), with a higher bioavailability, alone and combined with levofloxacin-LVX against resistant *H. pylori* clinical strains.

Materials and Methods. In this work, we explored the effect of different substituents on the RSV two aromatic rings. The general structure of the molecules is shown in the figure. They were evaluated in terms of the antibacterial (MIC/MBC) and anti-virulence effects (biofilm reduction and swarming motility inhibition) of new synthesized RSV-phenol derivatives, against resistant *H. pylori* clinical strains. Moreover, the toxicity on eucariotic cells of the more active RSV derivatives was also evaluated as well as the antioxidant properties.



Figure

Results. The results showed an increased antibacterial action of the new RSV derivatives with lower MIC (3.25 – 12,5 µg/ml) in respect to the lead compounds against clinical resistant strains. The best RSV derivatives, significantly reduced the biofilm formation, affected the bacterial membrane permeability and the gene expression of the main *H. pylori* virulence factors. Preliminary studies show low toxicity on eucariotic fibroblast cells.

Conclusion. The alarming phenomenon of the multidrug resistance in *H. pylori* underlines the need to search novel strategies to improve the eradication rate. Overall, our results underline the anti-*H. pylori* effect of RSV-derivatives, representing interesting candidates for innovative therapeutic schemes to tackle the *H. pylori* antibiotic resistance.

This work was funded by a Research Grant PRIN 2022 Project code: 2022LTYW84 from MUR, Italy.

306 - Outer membrane vesicles derived from Gram-Negative bacteria: biological vectors for innovative antiviral therapies

SANDRA RUFOLO ⁽¹⁾ - FEDERICA DELL'ANNUNZIATA ⁽¹⁾ - FRANCESCA MENSITIERI ⁽¹⁾ - VERONICA FOLLIERO ⁽¹⁾ - GIULIA GAUDINO ⁽¹⁾ - MASSIMILIANO GALDIERO ⁽²⁾ - FABRIZIO DAL PIAZ ⁽¹⁾ - VIVIANA IZZO ⁽¹⁾ - GIANLUIGI FRANCI ⁽¹⁾

Department of Medicine, Surgery and Dentistry "Scuola Medica Salernitana", University Salerno, Salerno, Italia ⁽¹⁾ - Department of Experimental Medicine, University of Campania Luigi Vanvitelli, Napoli, Italia ⁽²⁾

Outer membrane vesicles derived from Gram-Negative bacteria: biological vectors for innovative antiviral therapies

Federica Dell'Annunziata^{1,2}, Francesca Mensitieri¹, Veronica Folliero¹, Giulia Gaudino¹, Sandra Rufolo¹, Massimiliano Galdiero^{2,3}, Fabrizio Dal Piaz¹, Viviana Izzo¹, Gianluigi Franci^{1,4}.

¹ Department of Medicine, Surgery and Dentistry "Scuola Medica Salernitana", University of Salerno, 84081 Baronissi Salerno, Italy

² Department of Experimental Medicine, University of Campania Luigi Vanvitelli, 80138 Naples, Italy

³ Complex Operative Unity of Virology and Microbiology, University Hospital of Campania "Luigi Vanvitelli", 80138 Naples, Italy

⁴ Clinical Pathology and Microbiology Unit, San Giovanni di Dio e Ruggi D'Aragona University Hospital, 84126 Salerno, Italy

Introduction: Introduction: The COVID-19 pandemic caused by SARS-CoV2 has underscored the urgent demand for innovative antiviral therapies. Peptides, with their broad-spectrum antimicrobial efficacy, have emerged as potential candidates, although their in vivo stability and bioavailability remain challenging. To address this, outer membrane vesicles (OMVs) derived from Gram-negative bacteria have garnered attention as promising drug delivery vectors, leveraging their efficient drug loading and targeted delivery capabilities. In this study, we aimed to enhance the stability of the AR23 peptide by expressing it on the surface of Escherichia coli OMVs, while preserving its potent antiviral activity.

Materials and Methods: The E. coli BL21(DE3) strain was transformed with a plasmid encoding the AR23 peptide attached to the cytolysin A (ClyA) protein as a membrane scaffold. OMVs were purified from both wild-type E. coli and E. coli-AR23 using centrifugation, filtration, and ultracentrifugation. OMVs were quantified and the presence of the ClyA-AR23 chimera was confirmed through mass spectrometry. The in vitro antiviral activity of AR23 and AR23-OMVs was evaluated against Herpes Simplex Type 1 using viral pretreatment assays and confirmed by plaque reduction assay and real-time PCR.

Results: Proteomic analysis confirmed the presence of the ClyA-AR23 chimera in the recombinant OMVs. AR23-OMVs exhibited dose-dependent antiviral activity, with 60% inhibition at 40 µg/mL. This was accompanied by a 0.5-fold reduction in the expression of the UL54 gene, which encodes the ICP27 protein involved in viral mRNA splicing and nuclear export. Non-functionalized vesicles showed no significant antiviral activity.

Discussion and Conclusions: The limited in vivo stability of peptides poses a significant challenge for their therapeutic application, emphasizing the need for innovative strategies to maintain their antiviral activity while improving stability. Our study demonstrates the potential of outer membrane vesicles (OMVs) as ideal vectors to address the bioavailability limitations associated with peptide-based therapies. By utilizing OMVs, we were able to enhance the stability of the AR23 peptide while preserving its potent antiviral activity. These findings highlight the promising role of OMVs in delivering peptides for enhanced antiviral activity, providing a valuable approach for future antiviral therapy development.

317 - PYED-1: new molecule able to re-sensitize colistin-resistant *Acinetobacter baumannii* strains

MARIA STABILE⁽¹⁾ - ANTONELLA MIGLIACCIO⁽²⁾ - ANNA ESPOSITO⁽³⁾ - ANNALISA GUARAGNA⁽³⁾ - RAFFAELE ZARRILLI⁽²⁾ - ELIANA DE GREGORIO⁽⁴⁾

Università, Complex Operational Unit of Clinical Microbiology, University Hospital “Federico II”, Napoli, Italia⁽¹⁾ - Università, Department of Public Health, University of Naples “Federico II”, Napoli, Italia⁽²⁾ - Università, Department of Chemical, Materials and Production Engineering, University of Naples “Federico II”, Napoli, Italia⁽³⁾ - Università, Department of Molecular Medicine and Medical Biotechnology, University of Naples “Federico II”, Napoli, Italia⁽⁴⁾

PYED-1: new molecule able to re-sensitize colistin-resistant *Acinetobacter baumannii* strains

MARIA STABILE^{1,2}, ANTONELLA MIGLIACCIO³, ANNA ESPOSITO⁴, ANNALISA GUARAGNA⁴, RAFFAELE ZARRILLI³ AND ELIANA DE GREGORIO^{2,5}

¹Department of Chemical Sciences, University of Naples “Federico II”, Naples, Italy; ²Complex Operational Unit of Clinical Microbiology, University Hospital “Federico II”, Naples, Italy;

³Department of Public Health, University of Naples “Federico II”, Naples, Italy; ⁴Department of Chemical, Materials and Production Engineering, University of Naples “Federico II”, Naples, Italy;

⁵Department of Molecular Medicine and Medical Biotechnology, University of Naples “Federico II”, Naples, Italy

Introduction: *Acinetobacter baumannii* is a Gram-negative pathogen associated with multidrug (MDR) resistance and hospital outbreaks of infection. Due to increasing resistance to multiple classes of antibiotics and the ability of this bacterium to form highly structured biofilms, *A. baumannii* infections are difficult to treat successfully. This makes the search for new antimicrobial strategies mandatory. Many natural steroids have been demonstrated to exhibit broad biological functions. Recently, the corticosteroid PYED-1 (pregnadiene-11-hydroxy-16 α ,17 α -epoxy-3,20-dione-1) showed significant antimicrobial activity against bacterial and fungal pathogens. The aim of this study was to investigate the effects of synthetic precursor of deflazacort (DFZ) on the growth of *A. baumannii* cells as well as on the formation and persistence of biofilms.

Materials and Methods: The MIC values were measured by broth microdilution method, while the activity against bacterial biofilms was tested by crystal violet and tetrazolium salt reduction assay. The combination effects between PYED-1 and antibiotics were assessed by checkerboard assay. The transcription of selected virulence-related genes was verified by qRT-PCR. In-vivo toxicity of PYED-1 was determined using *Galleria mellonella* larvae.

Results: The compound was screened against *A. baumannii* reference strains and colistin-resistant clinical isolates belonging to different sequence types. Among all compounds, PYED-1 proved to be the most active molecule against *A. baumannii* strains, showing rapid time-dependent kinetics of bacterial killing. Also, PYED-1 displayed a synergistic effect with colistin against MDR *A. baumannii* isolates. Interestingly, the combination of colistin with PYED-1 caused a reversal of antibiotic resistance. Then, we investigated the effect of PYED-1 on *A. baumannii* biofilms. Sub-MIC values of PYED-1 disrupted preformed biofilms, decreasing both biofilm biomass and viability of *A.*

baumannii biofilm cells. Transcriptional analysis was used to elucidate the mechanisms responsible for the inhibition of growth and biofilm formation. At sub-inhibitory concentration, the expression of biofilm- and virulence- associated genes was significantly suppressed after PYED-1 treatment. The in-vivo toxicity experiments of PYED-1 in *Galleria mellonella* larvae show the non-toxicity of the compound.

Discussion and Conclusions: The results revealed that PYED-1, in combination with colistin, exerts a strong antimicrobial activity against *A. baumannii* growth and has also the potential to restore the effectiveness of colistin against MDR strains. PYED-1 acts as a good antibacterial as well as antibiofilm agent and may represent a novel potential molecule for treatment of biofilm-related *A. baumannii* infections.

327 - Potential of Mesenchymal Stromal Cells secretome as host-directed therapy for mycobacterial infection

FRANCESCO PAGLIONE⁽¹⁾ - IVANA PALUCCI⁽²⁾ - CATALDO MARIA MANNAVOLA⁽¹⁾ - ALESSANDRO SALUSTRI⁽²⁾ - FLAVIO DE MAIO⁽³⁾ - ANTONIETTA ROSA SILINI⁽⁴⁾ - ORNELLA PAROLINI⁽⁵⁾ - GIOVANNI DELOGU⁽⁶⁾

Istituto di Microbiologia, Fondazione Policlinico Universitario “A. Gemelli”, IRCSS, Roma, Italia
(¹) - Department of Sciences of Basic Biotechnologies, Intensive and Perioperative Clinics, Section of Microbiology, Università Cattolica del Sacro Cuore, Roma, Italia (²) - Department of Laboratory and Infective Sciences, Faculty of Medicine, Fondazione Policlinico A. Gemelli IRCCS, Università Cattolica del Sacro Cuore, Roma, Italia (³) - Centro di Ricerca "E. Menni", Fondazione Poliambulanza - Istituto Ospedaliero, Brescia, Italia (⁴) - Department of Life Science and Public Health, Università Cattolica del Sacro Cuore, Roma, Italia (⁵) - Medicina di laboratorio, Mater Olbia Hospital, Olbia, Italia (⁶)

Potential of Mesenchymal Stromal Cells secretome as host-directed therapy for mycobacterial infection

Francesco Paglione¹, Ivana Palucci^{2,3}, Cataldo M. Mannavola¹, Alessandro Salustri², Flavio De Maio³, Antonietta R. Silini⁵, Ornella Parolini^{1,4}, Giovanni Delogu^{3,6}

¹ Fondazione Policlinico Universitario “A. Gemelli”, IRCSS, Rome, Italy;

² Department of Sciences of Basic Biotechnologies, Intensive and Perioperative Clinics, Section of Microbiology, Università Cattolica del Sacro Cuore, Rome, Italy;

³ Department of Laboratory and Infective Sciences, Faculty of Medicine, Fondazione Policlinico A. Gemelli IRCCS, Università Cattolica del Sacro Cuore, Rome, Italy;

⁴ Department of Life Science and Public Health, Università Cattolica del Sacro Cuore, 00168 Rome, Italy

⁵ Centro di Ricerca "E. Menni", Fondazione Poliambulanza - Istituto Ospedaliero, Brescia, Italy

⁶ Mater Olbia Hospital, Olbia, Italy;

Introduction. Mycobacteria are characterized by their ability to infect macrophages and other host cells, evade intracellular degradation and form an intracellular replicative niche. Due to these characteristics, the therapies against these bacteria are often long and ineffective. As part of new therapies against mycobacteria, the use of Mesenchymal Stromal Cells (MSCs) is gaining interest. Indeed, given their phagocyte-enhancing properties, MSCs could be used as adjuvant therapy against infections by mycobacteria, particularly those caused by multi drug resistant (MDR)/extensively drug-resistant (XDR) Mycobacterium tuberculosis (Mtb) and nontuberculous mycobacteria (NTM). Materials and methods. Four mycobacteria species were examined: Mycobacterium smegmatis (M. smegmatis), Mycobacterium avium, Mtb (H37RV) and Mycobacterium bovis (BCG). To assess direct action on mycobacteria, MSCs supernatants have been added to increasing concentrations in the growth medium. The immunomodulatory action was evaluated with a T cell stimulation assay on whole blood. Blood samples from patients with latent Mtb infection (LTBI) were stimulated with RD1-antigens, mitogen, or recombinant Heparin-Binding Hemagglutinin Adhesin (rHBHA) for 16 hours at 37°C in the presence or not of increasing concentration of MSCs supernatants or medium

control only. Lastly, to assess the effects on macrophages, THP-1 cells were infected with the four different strains examined and the colony forming units (CFUs) were evaluated at 4 hours post infection. At this time point, the cells were treated with increasing concentrations (vol/vol) of the MSCs supernatant or medium control only. CFUs were evaluated at 4 days post infection. Results. The MSCs supernatants did not show a direct effect on mycobacteria at any of the concentrations tested. In addition, the results obtained from the T cell stimulation assay in whole blood have confirmed the immunomodulatory activity of MSC showing an inhibition of the Interferon-gamma-~~(INF-gamma)~~ secretion starting at low concentrations of MSC supernatant. THP-1 cells infected with mycobacteria and conditioned with MSC supernatants showed an increased antimycobacterial activity, as measured by the reduction in intracellular CFUs of *M. smegmatis*, BCG and *Mtb* H37Rv. At certain concentrations the reduction in mycobacterial CFU is like that achieved by antibiotic treatment. Conversely, activity against *M. avium* is observed only when the MSCs supernatant is used at the highest concentration. ~~Instead, such an important CFU lowering is observed only at lower concentrations regarding MAC.~~

Discussion. Our results s-suggest that MSCs can exert antimicrobial properties by enhancing the antimycobacterial-killing power of macrophages. Identification of the key components responsible for this activity may pave the way for the development of tailored host-directed therapies.

331 - The Secondary Resistome to Oxacillin and Tetracycline in MRSA ST398

NADER ABDELMALEK ⁽¹⁾ - MOSAED SALEH ALOBAIDALLAH ⁽²⁾ - SALLY WAHEED YOUSIEF ⁽¹⁾ - ALESSANDRO TANCA ⁽¹⁾ - JOHN EMERDAHL OLSEN ⁽²⁾ - SALVATORE RUBINO ⁽¹⁾ - BIANCA PAGLIETTI ⁽¹⁾

Università, Università di Sassari/Dipartimento di Scienze Biomediche, Sassari, Italia ⁽¹⁾ - University of Copenhagen, Department of Veterinary and Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Danimarca ⁽²⁾

The Secondary Resistome to Oxacillin and Tetracycline in MRSA ST398

Nader Abdelmalek ¹, Mosaed S. Alobaidallah ², Sally W. Yousief ¹, Alessandro Tanca ¹, John E. Olsen ², Salvatore Rubino ¹, Bianca Paglietti ¹

¹ Department of Biomedical Sciences, University of Sassari, Sassari, Italy

² Department of Veterinary and Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Frederiksberg, Denmark

Introduction: Antimicrobial resistance (AMR) is a critical global health issue impacting both human and livestock populations. Methicillin-resistant *Staphylococcus aureus* (MRSA) is a common multidrug-resistant bacterium and has been classified as a high-priority pathogen for new antibiotic development. Restoring susceptibility to existing antimicrobials holds promise in combating AMR, as complete resistance expression relies on helper enzymes. Our study aims to identify these helper genes/proteins involved in MRSA's oxacillin and tetracycline resistance, which could serve as potential targets for restoring susceptibility. **Materials and Methods:** We screened a large-scale transposon mutant library of MRSA ST398 using transposon-directed insertion site sequencing technology (TraDis) after separate exposures to ½ minimum inhibitory concentration of oxacillin and tetracycline. Label-free quantitative proteomics was performed on wild-type MRSA ST398 after antibiotic exposure, and mass spectrophotometer output was processed using Proteome Discoverer, and statistical analysis was conducted using Perseus v1.6. **Results:** The high-throughput transposon mutagenesis assay identified 50 and 141 non-essential genes potentially contributing to oxacillin and tetracycline resistance, respectively. Statistical analysis and their involvement in biological processes guided the selection of genes for validation through knockout experiments. Proteomics analysis revealed significant differential protein expression, particularly following oxacillin exposure and a limited overlap between genomic and proteomic findings underscores the importance of integrating these technologies to fully comprehend the resistance mechanism. **Conclusion:** Our study provides insights into identifying helper genes/proteins associated with oxacillin and tetracycline resistance in MRSA. Through high-scale transposon mutagenesis and proteomics, we offer valuable information about the secondary resistome and potential targets for combating antimicrobial resistance. These findings pave the way for developing novel strategies to restore MRSA's susceptibility to existing antimicrobials, addressing the urgent global health concern of AMR.

07 One Health

2 - Zoonotic pathogenic microorganisms from the marine environment: A One Health perspective

MARIANNA MARANGI⁽¹⁾ - CATERINA LANFREDI⁽²⁾ - SABINA AIROLDI⁽²⁾

Università di Foggia, Dipartimento di Medicina Clinica e Sperimentale, Foggia, Italia⁽¹⁾ - Tethys Research Institute, Tethys Research Institute, Milano, Italia⁽²⁾

Zoonotic pathogenic microorganisms from the marine environment: A One Health perspective

MARIANNA MARANGI¹, CATERINA LANFREDI², SABINA AIROLDI²

¹Department of Clinical and Experimental Medicine, University of Foggia, Viale Luigi Pinto, 71122, Foggia, Italy; ²Tethys Research Institute, Viale G.B. Gadio 2, 20121 Milano, Italy

Introduction: Over the past three decades, it has become increasingly clear that the emerging and/or re-emerging zoonotic infectious diseases originate in animals, especially wild ones. Moreover, the main drivers of their occurrence are associated with human activities, including changes in ecosystems and land use, agricultural intensification, urbanisation, travel, and international trade. Understanding the ecology of each emerging/re-emerging zoonotic disease requires a collaborative, multidisciplinary approach, crossing the boundaries of animal, human and environmental health, in order to undertake a risk assessment and develop response and control plans.

Material and Methods: As part of a long term research project on cetacean ecology, conducted in the Pelagos Sanctuary, Ligurian Sea (north-western Mediterranean Sea), a total of n. 35 faecal samples from individuals of three different species of baleen whales (fin whale, n. 11) and toothed whales (sperm whale, n. 18; long-finned pilot whale, n. 5) were collected between 2000 and 2022. Faecal samples were then subjected to coprological examination, in order to identify eggs/cysts/oocysts of potential pathogenic microorganisms, and to DNA extraction and molecular analysis to genetically characterise the detected microorganisms.

Results: By microscopic examination, cysts and oocysts of zoonotic intestinal protozoan parasites have been identified in the faecal samples of fin, sperm, and long-finned pilot whales. In particular, *Giardia duodenalis* has been identified in five and three samples of fin and sperm whales, respectively. *Cryptosporidium* was identified in four and two samples of sperm and long-finned pilot whale, respectively and *Blastocystis* was identified in five and two samples of sperm and pilot whale, respectively. By molecular analysis and sequencing *C. parvum*, *G. duodenalis* Assemblage A and *Blastocystis* ST3 were genetically characterized.

Discussion and conclusions: This is the first study in which zoonotic protozoan parasites were detected in three different species of free-ranging baleen and toothed whales and the first record of *C. parvum* and *Blastocystis* in long-finned pilot whales. This survey also provides a baseline investigation of the possible implications of anthropogenic activities, possibly due to the spread of these microorganisms in coastal waters contaminated by sewage, agricultural and urban discharges, shared between humans and wildlife. These results give an overview of potential zoonotic microorganisms circulating in Mediterranean marine megafauna, indicating the need for more integrated research to understand the associated risk and to prevent human and marine ecosystem exposure to these invasive anthropogenic pathogenic microorganisms.

162 - Echinococcus granulosus sensu lato in hunters and hunting dogs from southern Italy: a One Health approach

ANNA MOREA ⁽¹⁾ - GIOVANNI SGROI ⁽²⁾ - FRENCESCO BUONO ⁽³⁾ - STEFANO SCARCELLI ⁽³⁾ - ELISA CASTALDO ⁽³⁾ - FRANCESCO LOCANTORE ⁽¹⁾ - FRANCESCA INDRACCOLO ⁽¹⁾ - ANGELA STUFANO ⁽¹⁾ - VALENTINA SCHINO ⁽¹⁾ - PIERO LOVREGGIO ⁽¹⁾ - NICOLA D'ALESSIO ⁽²⁾ - VINCENZO VENEZIANO ⁽³⁾ - DOMENICO OTRANTO ⁽⁴⁾ - ROBERTA IATTA ⁽¹⁾

University of Bari Aldo Moro, Interdisciplinary Department of Medicine, Bari, Italia ⁽¹⁾ - Istituto Zooprofilattico Sperimentale del Mezzogiorno, Istituto Zooprofilattico Sperimentale del Mezzogiorno, Napoli, Italia ⁽²⁾ - University of Naples Federico II, Naples, Department of Veterinary Medicine, Napoli, Italia ⁽³⁾ - University of Bari Aldo Moro, Department of Veterinary Medicine, Bari, Italia ⁽⁴⁾

Echinococcus granulosus sensu lato in hunters and hunting dogs from southern Italy: a One Health approach

ANNA MOREA¹, GIOVANNI SGROI², FRANCESCO BUONO³, STEFANO SCARCELLI³, ELISA CASTALDO³, FRANCESCO LOCANTORE¹, FRANCESCA INDRACCOLO¹, ANGELA STUFANO¹, VALENTINA SCHINO¹, PIERO LOVREGGIO¹, NICOLA D'ALESSIO², VINCENZO VENEZIANO³, DOMENICO OTRANTO⁴, ROBERTA IATTA¹

¹Interdisciplinary Department of Medicine, University of Bari Aldo Moro, Bari, Italy; ²Department of Animal Health, Istituto Zooprofilattico Sperimentale del Mezzogiorno, Portici (Naples), Italy; ³Department of Veterinary Medicine, University of Naples Federico II, Naples, Italy; ⁴Department of Veterinary Medicine, University of Bari “Aldo Moro

Introduction

Cystic echinococcosis (CE) by *Echinococcus granulosus sensu lato* (s.l.) is a zoonotic neglected tropical disease with a worldwide distribution. CE is endemic in Italy, involving mainly rural and pastoral communities, where small ruminants represent the main intermediate hosts. In recent decades, wild boars dramatically increased in urban and peri-urban areas, are involved in the dissemination of zoonotic pathogens including *E. granulosus* s.l. In this scenario, hunters may be at risk of exposure to the cestode's eggs shed by their hunting dogs (definitive hosts). Therefore, the study aimed to assess the seroprevalence of *Echinococcus* spp. in hunters, the role of hunting dogs as reservoirs of the parasite along with hunter's knowledge on the infestation's risk.

Materials and Methods

Hunters from Basilicata and Campania regions were recruited on volunteer basis during educational meetings during December 2022 and May 2023. All the participants filled out a questionnaire, enquiring about socio-demographic and anamnestic characteristics and their knowledge on EC. A blood sample was collected from each hunter and tested for *Echinococcus* spp. IgG by an ELISA

immunoenzymatic assay (Euroimmun, Germany). In addition, fecal samples from hunting dogs of the above hunters were screened for parasitological examination with mini-FLOTAC method.

Results

Out of 122 hunters, 6 (4.8%) from Basilicata region scored either positive (n=3) or borderline (n=3) for IgG anti-Echinococcus spp. Out of 170 hunting dogs, 77 (45.3%) were positive to zoonotic parasites at the coprological examination being ancylostomatids (26.4%) the most prevalent, followed by *Toxocara canis* (10,6%) and taeniids (5.8%). A hunter seropositive to *Echinococcus* spp. owned a hunting dog positive to taeniid parasites. Through the questionnaire analysis, only 31.1% and 35.2% know the transmission route of *E. granulosus* s.l. to humans and dogs, respectively, and 30.3% and 43.4% the correct practice of the disposal of hunting dog's feces and wild boar's viscera, respectively.

Discussion and Conclusions

The exposure of hunters to *E. granulosus* s.l. along with the role of their hunting dogs as reservoirs of zoonotic parasites highlight the need to promote health surveillance and educational programs for avoiding the cestode's circulation. Further molecular analysis on faecal samples positive to taeniids will be performed for parasite species identification. Hence, a One Health collaborative approach among parasitologists, veterinarians and physicians is advocated to control and prevent the spread of *E. granulosus* s.l. and the risk of infestation in the exposed population.

178 - Occurrence of ESKAPE bacteria in healthy cattle population and the related zoonotic risk

ANNA-RITA ATTILI⁽¹⁾ - ALESSANDRO GUERRINI⁽¹⁾ - FRANCESCA GIGLI⁽¹⁾ -
FRANCESCA PAOLA NOCERA⁽²⁾ - CORRADO MARIA CORRADINI⁽¹⁾ - FILOMENA
FIORITO⁽²⁾ - CLAUDIA CERRACCHIO⁽²⁾ - VINCENZO CUTERI⁽¹⁾ - LUISA DE MARTINO⁽²⁾

Università di Camerino, Scuola di Bioscienze e Medicina Veterinaria, Camerino, Italia⁽¹⁾ -
Università di Napoli Federico II, Dipartimento di Medicina Veterinaria e Produzioni Animali,
Napoli, Italia⁽²⁾

Occurrence of ESKAPE bacteria in healthy cattle population and the related zoonotic risk

ANNA-RITA ATTILI¹, ALESSANDRO GUERRINI¹, FRANCESCA GIGLI¹, FRANCESCA P.
NOCERA², CORRADO M. CORRADINI¹, FILOMENA FIORITO², CLAUDIA CERRACCHIO²,
VINCENZO CUTERI¹, LUISA DE MARTINO²

¹ School of Biosciences and Veterinary Medicine, Microbiology and Infectious Diseases,
University of Camerino, Matelica, Italy; ² Department of Veterinary Medicine and Animal
Production, Microbiology and Infectious Diseases, University of Naples “Federico II”, Naples, Italy

1. Introduction Antimicrobial-resistant ESKAPE bacteria (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species) represent a global threat to human health. Since the role of healthy and/or asymptomatic animals as carrier of ESKAPE opportunistic bacteria is less investigated, we aimed at collecting data regarding the occurrence of the ESKAPE isolates in healthy cattle population, evaluating their antimicrobial resistant profiles.

2. Materials and Methods A prospective epidemiological study was carried out in dairy farms on 180 bovine ocular, nasal, auricular, oral swabs, milk and faecal samples. Standard cultures on nutritive and selective agar media were performed and bacterial colonies were identified by MALDI-TOF MS. Susceptibility of ESKAPE strains to a panel of 30 different antibiotics belonging to 17 classes was evaluated by Kirby-Bauer and E-test methods (EUCAST, 2023). As suggested by Magiorakos et al. (2012), multidrug-resistant (MDR), extensively drug-resistant (XDR) and pandrug-resistant (PDR) isolates were defined.

3. Results *Enterococcus faecium* (n=17), *Staphylococcus aureus* (n=5), *Klebsiella pneumoniae* ssp. *pneumoniae* and *Klebsiella pneumoniae* ssp. *ozaenae* (n=15), *Acinetobacter baumannii* (n=7), *Pseudomonas aeruginosa* (n=7), and *Enterobacter cloacae* (n=12) were identified. The ESKAPE bacteria resulted susceptible to carbapenems, except *P. aeruginosa* strains, to polymyxins, sulphonamides, aminoglycosides, glycopeptides (vancomycin and teicoplanin), and *S. aureus* to β -lactams. No PDR strains were recorded, while XDR (28.6%, n=63) and MDR (68.2%) were observed with resistance towards cephalosporins (92.1%), monobactams (57.1%), fluoroquinolones (55.5%) and tetracyclines (38.1%). Nose and mouth of healthy cattle represent the anatomical sites at major zoonotic risk (RR=28.55; AR=0.93) for *A. baumannii*, *K. pneumoniae*, *E. cloacae*.

4. Discussion and Conclusions The obtained preliminary data contribute to acquire epidemiological informations about ESKAPE pathogens spread from healthy cattle population and to coordinate antimicrobial resistance surveillance in a One Health approach.

241 - Rising threat: the prevalence of *K. pneumoniae* Ceftazidime/Avibactam resistant strains in healthcare facilities

ALESSIA MIRABILE ⁽¹⁾ - DALIDA ANGELA BIVONA ⁽¹⁾ - KRISTINA NICOLE MARTIN ⁽²⁾ - DAVIDE LO PORTO ⁽³⁾ - ANDREA CONA ⁽³⁾ - FRANCESCO MONACO ⁽³⁾ - ALESSANDRA MULARONI ⁽³⁾ - STEFANIA STEFANI ⁽¹⁾ - DAFNE BONGIORNO ⁽¹⁾

Università di Catania, Department of Biomedical and Biotechnological Science, Catania, Italia ⁽¹⁾ - Università di Catania, U.O.C. Laboratory Analysis, University Hospital Policlinico-San Marco,, Catania, Italia ⁽²⁾ - ISMETT-IRCCS Istituto Mediterraneo per i Trapianti e Terapie ad Alta Specializzazione, Unit of Infectious Diseases, Palermo, Italia ⁽³⁾

Rising threat: the prevalence of *K. pneumoniae* Ceftazidime/Avibactam resistant strains in healthcare facilities

Alessia Mirabile¹, Dalida Angela Bivona¹, Kristina Nicole Martin², Davide Lo Porto², Andrea Cona², Francesco Monaco ², Alessandra Mularoni², Stefania Stefani¹ and Dafne Bongiorno¹
Department of Biomedical and Biotechnological Science, Microbiology section University of Catania

U.O.C. Laboratory Analysis, University Hospital Policlinico-San Marco, University of Catania

2. Unit of Infectious Diseases, ISMETT-IRCCS Istituto Mediterraneo per i Trapianti e Terapie ad Alta Specializzazione, Via E. Tricomi, 5, 90127 Palermo, Italy.

Introduction: The rapid emergence and dissemination of antibiotic resistance pathogens pose a significant threat to global public health. Of particular concern is the recent occurrence and diffusion in hospital settings of *Klebsiella pneumoniae* strains resistant to ceftazidime/avibactam (CZA). Although, CZA is currently considered the most successful last-resort drug for the treatment of these infections, cases of *K. pneumoniae* resistant to CZA are increasing which limits treatment options and exacerbates the challenges associated with hospital-acquired infections. We characterized the strains of *K. pneumoniae* CZA-R and investigated their spread at the Mediterranean institute for transplants and highly specialized therapies (ISMETT) located in Palermo.

Material and methods: Fifteen strains of *K. pneumoniae* were isolated between April 2021 and November 2022 from different body sites (blood, central venous catheter, urine and lower respiratory tract). Antibiotic susceptibility testing was carried out through gradient tests and whole genome sequencing (WGS) was conducted through the Illumina MiSeq platform. Resistant and virulence gene content, mutation in omp proteins, plasmid analysis and studies on phylogenetic relationships were conducted.

Results: 14 strains showed resistance to CZA, 4 were Meropenem resistant and 2 were Meropenem/Vaborbactam resistant. Data obtained from WGS showed the circulation of sequence type ST101 (12), ST307 (1), ST39 (1), ST512 (1). The strains display a plethora of resistance genes to most antibiotic families, including bla_{KPC3-31-34}, bla_{SHV1-28}, bla_{TEM181}, bla_{CTX-M-15} and bla_{OXA1-9}. Most carbapenemase genes are located on plasmids such as IncFIB (2), IncFII (14) and IncFIC (1). Numerous mutations on the Omp36-37 porins are also present. The phylogenetic analysis shows a persistence of ST101 over time, which appears to be present in the hospital throughout the period.

Discussions and Conclusion: The diffusion of CZA-R *K. pneumoniae* strains is a multifaceted process influenced by various factors. To date, cases of *K. pneumoniae* CZA-R are increasing and is important to deeper understand the mechanisms related to the reduced susceptibility to these antimicrobials. The presence of plasmids and mobile genetic elements carrying resistance genes facilitates horizontal transfer, enabling the dissemination of resistant strains among patients and the hospital setting. In conclusion, this work highlights the emergence of resistant strains, and how important the complete characterization (both phenotypic and molecular) is to deeply understand the events that underlie the acquisition of new resistance mechanisms.

This study is partly supported by PNRR Extended Partnership initiative on Emerging Infectious Diseases (PE00000007, INF-ACT).

253 - Uncovering Antibiotic Resistance in Italian Wastewater: Exploring ARB, ARGs, and Microbial Diversity in WWTPs

GIUSY BONANNO FERRARO⁽¹⁾ - CARMELO BONOMO⁽²⁾ - PAMELA MANCINI⁽¹⁾ - CAROLINA VENERI⁽¹⁾ - ROSSELLA BRIANCESCO⁽¹⁾ - ANNA MARIA COCCIA⁽¹⁾ - ELISABETTA SUFFREDINI- DAFNE BONGIORNO⁽²⁾ - NICOLÒ MUSSO⁽²⁾ - STEFANIA STEFANI⁽²⁾ - GIUSEPPINA LA ROSA⁽¹⁾

Istituto Superiore di Sanità, Dipartimento Ambiente e Salute, Roma, Italia⁽¹⁾ - Università di Catania, Dipartimento di Scienze Biomediche e Biotecnologiche, Catania, Italia⁽²⁾

Uncovering Antibiotic Resistance in Italian Wastewater: Exploring ARB, ARGs, and Microbial Diversity in WWTPs

Giusy Bonanno Ferraro¹, Carmelo Bonomo², Pamela Mancini¹, Carolina Veneri¹, Rossella Briancesco¹, Anna Maria Coccia¹, Dafne Bongiorno², Nicolò Musso², Stefania Stefani², Giuseppina La Rosa¹

¹ Department of Environment and Health, Istituto Superiore di Sanità, Rome, Italy

² Department of Biomedical and Biotechnological Science, University of Catania, Italy

Introduction Antibiotic-resistant bacteria (ARB) and antibiotic-resistant genes (ARGs) can spread across human, animal, and natural environments. Water acts as a critical medium for antibiotic resistance dissemination. Wastewater Treatment Plants (WWTPs) play a crucial role in removing contaminants, but ARB and ARGs can persist despite treatment. Monitoring WWTPs can provide insights into the community's antibiotic resistance status.

Materials and Methods Eight wastewater samples were collected from four WWTPs in the Lazio region. The samples included untreated influents and treated effluents. Four resistance genes blaKPC, blaCTX-M-1 group, tetA and sul1, and two specie specific genes rpoB and 16S rRNA, were quantified using Digital PCR. Bacterial communities were characterized using Illumina MiSeq 16S rRNA gene amplicon sequencing. Bioinformatics analysis was conducted using QIIME2. Alpha and Beta diversity indexes were calculated to evaluate changes in microbial diversity among the collected samples.

Results The absolute abundance of ARGs genome copies per liter (gc/L) could be ranked along the following order: sul1 > tetA > CTX-M > KPC. Specifically, the median values of ARGs in influents (gc/L) were as follows: sul1=1.34E+09; tetA=1.98E+08; CTX-M=9.66E+06; KPC=1.03E+06 and rpoB=2.60E+06; 16S=1.84E+10. In the effluents, they were as follows: sul1=3.26E+07; tetA=6.00E+08; CTX-M=2.32E+06; KPC=7.94E+04 and rpoB=1.73E+05; 16S=8.46E+08. In general, the average concentration values observed indicate reductions in gene abundance of less than one log. However, exceptions were found for the tetA gene, where in some cases higher values were observed in the effluent compared to the influent. In both influent and effluent samples, the three predominant phyla among the bacterial community were Proteobacteria, Bacteroidetes, and Firmicutes. The most abundant genera, in both influent and effluent samples, were Acinetobacter (10%; 4%), Flavobacterium (7%; 4%), Bacteroides (6%; 2%), Arcobacter (5%; 2%), Pseudomonas (3%; 0.8%),

and *Aeromonas* (2%; 1%). Effluent samples showed higher bacterial community diversity compared to influent samples. Statistical analysis confirmed significant differences in both alpha and beta diversity indexes between the two sample types, with p-values of 0.004.

Discussion and Conclusion In conclusion, this study sheds light on the presence and dynamics of AMR in WWTPs, highlighting the persistence of ARB and ARGs despite treatment efforts. The results emphasize the importance of ongoing surveillance and targeted interventions to mitigate the spread of AMR in both the community and the environment. This study is partly supported by the European Union NextGenerationEU within the National Recovery and Resilience Plan (NRRP) PE13 INF-ACT.

275 - Following mcr genes: from one-health surveillance to functional genomics of bacterial mobile elements

ERIKA SCALTRITI ⁽¹⁾ - CHIARA BRACCHI ⁽¹⁾ - MARTINA TAMBASSI ⁽¹⁾ - ILARIA MENOZZI ⁽¹⁾ - ALESSANDRA DODI ⁽¹⁾ - MARINA MORGANTI ⁽¹⁾ - MELISSA BERNI ⁽¹⁾ - MICRO-RER CONSORTIUM ⁽²⁾ - STEFANO PONGOLINI ⁽¹⁾ - LUCA BOLZONI ⁽¹⁾

IZSLER, Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna, Risk Analysis and Genomic Epidemiology Unit, Parma, Italia ⁽¹⁾ - Emilia Romagna, Region, Bologna, Italia ⁽²⁾

Introduction

Colistin is a polymyxinic antibiotic used to treat human Gram-negative bacteria infections. Nevertheless, intensive animal farming was the main area of use of colistin up to 2017, year of restriction of its use in veterinary medicine. Colistin resistance is mainly mediated by plasmids harboring mcr genes, potentially transferable by horizontal transmission among bacteria. Here, we provided an assessment of: (i) the circulation in Emilia Romagna Region (RER) of mcr genes before the restrictions of colistin use in animals, by using data from one-health surveillance of enteropathogens (2012-2015); (ii) the effect of the restrictions on the circulation of mcr genes in nosocomial enterobacteriaceae (Micro-RER, 2017-2020); (iii) the presence of relevant virulence determinants mcr-associated genes in mobile elements of Enterobacterales strains.

Materials and Methods

4,473 isolates of *Salmonella enterica* (SE) (from human, veterinary sources and environment; 2012-2015) were screened for colistin resistance. After the establishment of the Micro-RER surveillance by RER (2017), nosocomial enterobacteriaceae isolates with confirmed MIC ≥ 2 mg/L were tested by PCR to detect mcr genes. 49 out of 51 mcr-PCR-positive isolates were subjected to Whole-Genome Sequencing (WGS). The clonal dissemination of mcr genes and the contribution of mcr-carrying elements in the mobilization of AMR and virulence genes were evaluated. mcr-associated virulence genes were confirmed by phenotypic assays.

Results

Colistin resistance was detected in 269 out of 4,473 SE isolates (6%). mcr-1-positive human isolates were concentrated in 2015 (70%), while veterinary isolates increased from 2013 (20%) to 2014 (53.3%) and decreased in 2015 (26.7%). An high prevalence of mcr-1 in the swine-pork industry than in humans and poultry was observed. 51 out of 1164 nosocomial isolates (4.4%) were mcr-positive (50/51 for mcr-1), dropping from 24 (first half of 2018) to 3 (whole 2020). WGS on mcr-positive isolates showed the central role of the horizontal transfer of mcr genes through plasmids (mainly IncX4) or transposable elements (15/49) compared to clonal dissemination of mcr-positive strains. Notably, flu1, a gene encoding the E.coli Antigen 43 associated to biofilm formation, was found close to the mobilized colistin resistance gene mcr-1.1.

Discussion and Conclusions

This study demonstrated: (i) the presence of mcr-1 in SE isolates at least since 2012 and the gene flow from pigs to humans along the food chain; (ii) a substantial decrease in the circulation of mcr-1 genes in RER after the restrictions of colistin use in veterinary medicine; (iii) the dissemination of

mcr genes mainly driven by mobile elements rather than mcr carrying clones; (iv) the co-transfer of virulence genes, like *fla1*, and *mcr-1* genes through mobile elements of Enterobacterales strains.

296 - Ticks and tick-borne pathogens at human-domestic-wildlife interfaces in Malawi through a One Health approach

ALESSANDRA MISTRAL DE PASCALI⁽¹⁾ - CARLOTTA PASETTO⁽²⁾ - VALERIA BLANDA⁽³⁾ - DAVID KALENZI ATUHAIRE⁽⁴⁾ - AMANDA L SALB⁽⁵⁾ - CHARLOTTE BENTELEY⁽⁵⁾ - LASTON CHIMALIRO⁽⁵⁾ - ALBERTO ALBERTI⁽⁶⁾ - CARLA CACCIOTTO⁽⁶⁾ - MARTINA BRANDOLINI⁽¹⁾ - GIULIA GATTI⁽¹⁾ - MONICA CRICCA⁽¹⁾ - VITTORIO SAMBRI⁽¹⁾ - ALESSANDRA TORINA⁽³⁾ - ALESSANDRA SCAGLIARINI⁽¹⁾

Università di Bologna, Dipartimento di Scienze Mediche e Chirurgiche, Bologna, Italia⁽¹⁾ - Università di Bologna, Dipartimento di Scienze Mediche Veterinarie, Bologna, Italia⁽²⁾ - Istituto Zooprofilattico Sperimentale della Sicilia, Istituto Zooprofilattico Sperimentale della Sicilia, Palermo, Italia⁽³⁾ - African Union Centre for Tick and Tick Borne Diseases (CTTBD), Lilongwe, Malawi, African Union Centre for Tick and Tick Borne Diseases (CTTBD), Lilongwe, Malawi, Malawi, Malawi⁽⁴⁾ - Lilongwe Wildlife Trust, Lilongwe, Malawi, Lilongwe Wildlife Trust, Lilongwe, Malawi, Malawi, Malawi⁽⁵⁾ - Università di Sassari, Dipartimento di Medicina Veterinaria, Sassari, Italia⁽⁶⁾

Ticks and tick-borne pathogens at human-domestic-wildlife interfaces in Malawi through a One Health approach

ALESSANDRA M DE PASCALI^{1,2}, CARLOTTA PASETTO³, VALERIA BLANDA⁴, DAVID KALENZI ATUHAIRE⁵, AMANDA L SALB⁶, CHARLOTTE BENTLEY⁶, LASTON CHIMALIRO⁶, ALBERTO ALBERTI⁷, CARLA CACCIOTTO⁷, MARTINA BRANDOLINI^{1,2}, GIULIA GATTI^{1,2}, MONICA CRICCA^{1,2}, VITTORIO SAMBRI^{1,2}, ALESSANDRA TORINA⁴, ALESSANDRA SCAGLIARINI²

¹Unit of Microbiology, The Greater Romagna Area Hub Laboratory, Cesena, Italy;

²Department Medical and Surgical Sciences (DIMEC)—Alma Mater Studiorum, University of Bologna, Italy;

³Department of Veterinary Medical Sciences (DIMEVET)—Alma Mater Studiorum, University of Bologna, Italy;

⁴ Istituto Zooprofilattico della Sicilia, Palermo, Italy;

⁵ African Union – Centre for Tick and Tick Borne Diseases (CTTBD), Lilongwe, Malawi;

⁶ Lilongwe Wildlife Trust, Lilongwe, Malawi;

⁷ Department of Veterinary Medicine, University of Sassari, Italy

Introduction: Ticks are known vectors of pathogens of importance in human and veterinary medicine. The incidence of tick borne diseases (TBD) is increasing worldwide and is becoming a significant health issue due to its impact on public health and socio-economic field. Despite this, the role of wild animals as reservoirs of TBD is still poorly known. In Africa, wild animal populations provide a reservoir for the maintenance of numerous tick-borne pathogens such as babesiosis, theileriosis and anaplasmosis. The main objective of this study was to investigate TBDs at human-domestic-wildlife interfaces in Malawi. **Materials and methods:** A large-scale tick collection was carried out in different locations in Malawi. The ticks were stored in separate and properly labelled vials and preserved in 95% ethanol and blood was collected in tubes with EDTA and then spotted on QUIcard® FTA® Classic (Qiagen). Morphological identification was completed for all the ticks

collected, which were classified into 4 different genera. Molecular investigations were performed on ticks from African elephants (*Loxodonta africana*), cattle and humans. The samples have been tested for *Babesia* spp., *Theileria* spp., *Rickettsia* spp., *Anaplasma* spp. and Poxviridae. The detection of *Babesia* spp. and *Theileria* spp. was performed by Reverse Line Blot (RLB) and by conventional PCR drawn on 18S ribosomal RNA. DNA identification of *Rickettsia* spp. was performed by two PCRs drawn on the *ompA* gene and the *ompB* gene. The detection of *Anaplasma phagocytophilum* and *Anaplasma platys* was performed by means of a heminested PCR drawn on the *groE* gene. Finally, a PanPoxViridae Real-Time PCR was developed for the identification of Poxvirus DNA, and positives were confirmed by using conventional PCR. Results: A total of 1197 Ixodidae ticks were collected. Overall, the higher number of tick specimens were collected from elephants (N=361/1197, 30.1%). Ticks of the genus *Hyalomma* were largely predominant. A higher prevalence for piroplasms was found in cattle (14/19, 73%); with *T. mutans* (8/19, 42%), and *T. velifera* (4/19, 21%) being the most prevalent microorganisms. One elephant tick was infected with *Theileria* sp. PCR disclosed positivity for *Rickettsia* spp. in both elephant (3/30, 10%) and human (2/5, 40%) ticks. All samples tested negative for Poxvirus. Discussion and Conclusions: Questionnaires and field observations evidenced the possible circulation of other zoonoses in the study area. Given the multiplicity of areas of Africa where humans, domestic and wild animals live in close proximity, it is essential to encourage the use of a One Health approach to understand the ecology of diseases affecting human and animal health, the economy and on the conservation of endangered species.

310 - Evaluation of Antiviral Properties of Grape Pomace Extracts: A Sustainable Approach for Broad-Spectrum Viral Inhibition

ELENA IACONO (1) - CARLO DI MARZO (2) - PAOLA QUARANTA (3) - ALESSANDRA BRACA (4) - MARINELLA DE LEO (4) - GIULIA FREER (5) - MAURO PISTELLO (5) - MICHELE LAI (5)

Dipartimento di biotecnologie mediche, università di Siena, Dipartimento di biotecnologie mediche, università di Siena, Siena, Italia (1) - Università di Pisa, Università di Pisa, Centro Retrovirus, Pisa, Italia (2) - Università Sant'Anna, Università Sant'Anna, Pisa, Italia (3) - Dipartimento di Farmacia, Dipartimento di Farmacia, Pisa, Italia (4) - Università di Pisa, Centro Retrovirus, Università di Pisa, Centro Retrovirus, Pisa, Italia (5)

Title: Evaluation of Antiviral Properties of Grape Pomace Extracts: A Sustainable Approach for Broad-Spectrum Viral Inhibition

ELENA IACONO^{1,2}, CARLO DI MARZO¹, PAOLA QUARANTA¹, MAURO DI STASI³, MARINELLA DE LEO^{3,5}, EMILY CIONI³, FRANCESCA GAMBINERI⁴, GIULIANA LUMINARE⁴, ALESSANDRA BRACA³, GIULIA FREER¹, MAURO PISTELLO¹, MICHELE LAI^{1,5}.

¹Retrovirus Center, Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa, Pisa, Italy; ²Department of Medical Biotechnologies, University of Siena, Siena, Italy; ³Department of Pharmacy, University of Pisa, Pisa, Italy; ⁴Archa srl, Pisa, Italy; ⁵CISUP, University of Pisa, Pisa, Italy.

Introduction:

A circular economy promotes the concept of closed production cycles, where waste from operations is seen as a valuable resource for economically viable purposes. This approach emphasizes sustainable production practices and reducing waste disposal, which ultimately helps mitigate the environmental impact of pollution. Currently, significant research efforts are focused on finding ways to align with these principles. Natural plant extracts contain multiple compounds that exhibit antimicrobial and anti-inflammatory effects, making them promising sources for the development of novel antiviral candidates.

Aim:

The aim is to assess the antiviral and virucidal activities of grape pomace extract (PMx). Utilizing agricultural waste to produce antiviral molecules aligns with circular economy principles, offering economic and ecological benefits.

Material and Methods:

Hydroalcoholic extract obtained from grape pomace, derived from the agri-food industry of the Tuscan region, were examined for its antiviral and virucidal activities against Herpes simplex type 2 (HSV-2), Influenzavirus (IAV), Coxsackievirus B5 (Cox-B5), and Feline calicivirus (FeCV). Cytotoxicity assays were performed to determine the maximum non-toxic concentration for cells.

Viral inhibition assays were conducted on specific cell substrates to assess the antiviral activity of the extracts. Pure compounds present in the extracts were also tested to identify the specific compounds responsible for the observed inhibitory effects.

Results:

PMx demonstrated significant inhibitory effects against HSV-2, with 100% inhibition observed at certain concentrations. The extract also showed promising results against IAV, Cox-B5, and FeCV exhibiting dose-dependent inhibition. Time-of-addition studies suggested that the extract's activity targeted the virus itself through a virucidal mechanism, damaging its structure and blocking the viral cycle before entry. Further characterization of the extract's antiviral properties revealed the involvement of specific compounds, such as quercetin, gallic acid, and ethyl gallate.

Discussion and Conclusions:

The findings of this study suggest that grape pomace extracts have broad-spectrum virucidal action, inhibiting the replication and infectivity of HSV-2, IAV, Cox-B5, and FeCV. The presence of multiple bioactive compounds in the extracts may contribute to their antiviral effects, and further research is needed to identify the specific mechanisms of action. These results highlight the potential of utilizing waste materials from the wine industry to produce sustainable and non-toxic antiviral agents. The development of such agents can contribute to the expansion of effective and sustainable therapies, addressing the limited range of antivirals currently available.

312 - Distribution of Mycobacterium tuberculosis lineages and drug resistance in Campania Region

VERONICA FOLLIERO ⁽¹⁾ - CARLO FERRAVANTE ⁽¹⁾ - ANNAMARIA SALVATI ⁽¹⁾ - LAURA CRESCENZO ⁽¹⁾ - GIUSY CORVINO ⁽²⁾ - MARIA GRAZIA COPPOLA ⁽³⁾ - LUIGI ATRIPALDI ⁽³⁾ - MARIA TERESA DELLA ROCCA ⁽⁴⁾ - RITA GRECO ⁽⁴⁾ - VITTORIO PANETTA ⁽⁴⁾ - ALESSANDRO TRANFA ⁽⁵⁾ - ANNAMARIA D'ARGENIO ⁽⁵⁾ - ROSAMARIA ABATE ⁽²⁾ - ROSSELLA PERNA ⁽³⁾ - ANNALISA DEL GIUDICE ⁽³⁾ - GIUSEPPE GRECO ⁽⁶⁾ - TERESA BALDONI ⁽³⁾ - BRUNO SARNELLI ⁽⁷⁾ - VALENTINA IOVANE ⁽⁸⁾ - UGO PAGNINI ⁽⁹⁾ - GIUSEPPE IOVANE ⁽⁹⁾ - EMILIANA FINAMORE ⁽¹⁰⁾ - GIORGIO GIURATO ⁽¹⁾ - GIOVANNI NASSA ⁽¹⁾ - ALESSANDRO WEISZ ⁽¹⁾ - GIANLUIGI FRANCI ⁽¹⁾ - MASSIMILIANO GALDIERO ⁽¹⁰⁾

University of Salerno, Department of Medicine, Surgery and Dentistry "Scuola Medica Salernitana, Salerno, Italia ⁽¹⁾ - Ospedale Cardinale Ascalesi, UOC Microbiology, Napoli, Italia ⁽²⁾ - Ospedali dei Colli, Laboratory of Microbiology and Virology, Napoli, Italia ⁽³⁾ - AORN Sant 'Anna and San Sebastiano, UOSD Microbiology, Caserta, Italia ⁽⁴⁾ - San Giuseppe Moscati Hospital, Avellino, UOC Microbiology, Avellino, Italia ⁽⁵⁾ - San Giovanni di Dio e Ruggi D'Aragona University Hospital, Clinical Pathology and Microbiology Unit, Salerno, Italia ⁽⁶⁾ - Directorate General for Health Protection and Coordination of the Campania Regional Health System, ,, Napoli, Italia ⁽⁷⁾ - University of Naples Federico II, Department of Agricultural Sciences, Napoli, Italia ⁽⁸⁾ - University of Naples Federico II, Department of Veterinary Medicine and Animal Productions, Napoli, Italia ⁽⁹⁾ - University of Campania "Luigi Vanvitelli", Department of Experimental Medicine, Napoli, Italia ⁽¹⁰⁾

Distribution of Mycobacterium tuberculosis lineages and drug resistance in Campania Region
Veronica Folliero^{1*}, Carlo Ferravante^{1*}, Annamaria Salvati¹, Laura Crescenzo¹, Giusy Corvino², Mariagrazia Coppola³, Luigi Atripaldi³, Maria T. Della Rocca⁴, Rita Greco⁴, Vittorio Panetta⁴, Alessandro Tranfa⁵, Annamaria D'Argenio⁵, Rosamaria Abate², Annalisa Del Giudice³, Giuseppe Greco⁶, Teresa Baldoni³, Bruno Sarnelli⁷, Valentina Iovane⁸, Ugo Pagnini⁹, Giuseppe Iovane⁹, Emiliana Finamore¹⁰, Giorgio Giurato¹, Giovanni Nassa¹, Alessandro Weisz¹, Gianluigi Franci¹, Massimiliano Galdiero¹⁰

1. Department of Medicine, Surgery and Dentistry "Scuola Medica Salernitana", University of Salerno, Salerno, Italy;
2. UOC Microbiology - Ospedale Cardinale Ascalesi, ASL NA1, Naples, Italy;
3. Laboratory of Microbiology and Virology, Ospedali dei Colli, Naples, Italy;
4. UOSD Microbiology - AORN Sant 'Anna and San Sebastiano, Caserta, Italy;
5. UOC Microbiology - San Giuseppe Moscati Hospital, Avellino, Italy;
6. Clinical Pathology and Microbiology Unit, San Giovanni di Dio e Ruggi D'Aragona University Hospital, Salerno, Italy
7. Directorate General for Health Protection and Coordination of the Campania Regional Health System, Naples, Italy;

8. Department of Agricultural Sciences, University of Naples Federico II, Portici, Naples, Italy.
9. Department of Veterinary Medicine and Animal Productions, University of Naples Federico II, Naples, Italy;
10. Department of Experimental Medicine, University of Campania "Luigi Vanvitelli", 80138 Naples, Italy.

Introduction: Tuberculosis (TB) is a highly lethal infectious disease worldwide. Understanding the lineage distribution and drug resistance profiles of *Mycobacterium tuberculosis* (Mt) is crucial for effective TB management. Despite the Campania region being identified as a hotspot for TB infection, the evolution and transmission patterns of tuberculosis in this region are still poorly understood. Therefore, this study aimed to investigate the spread of major Mt lineages and drug resistance mechanisms in the Campania Region. **Materials and Methods:** This study involved 98 lung samples and 11 extrapulmonary samples collected from TB patients in 2019. The samples were obtained from four hospitals in the Campania region: Ascalesi Hospital (Naples) contributed 18 samples, Cotugno Hospital (Naples) contributed 66 samples, AORN S.G. Moscati (Avellino) contributed 8 samples, and AO Sant'Anna and San Sebastiano (Caserta) contributed 17 samples. *Mycobacterial* species identification was performed using IS6110-based PCR, and drug susceptibility testing for streptomycin, isoniazid, rifampicin, pyrazinamide, and ethambutol was conducted using the *Mycobacterium* Growth Indicator Tube 960 system. Genomic DNA extraction was carried out from the bacterial culture pellet using two column-based DNA extraction methods (QIAamp DNA mini kit, Qiagen, Germany). The quantity and quality of the extracted DNA were assessed using the Quant-IT DNA Assay Kit and NanoDrop ND-2000. Libraries were prepared using Illumina DNA Prep sample preparation kits. Library concentrations and sizes were determined using the Quant-IT DNA Assay Kit and Agilent 4200 TapeStation System, respectively. The resulting 109 libraries were sequenced in paired-end mode (300 bp) on the Illumina NexSeq500 platform. The quality of the sequenced reads was assessed using FastQC v0.11.3, and high-quality reads were imported into TB-profiler for lineage assignment and antimicrobial susceptibility prediction, using the *M. tuberculosis* H37Rv reference genome. **Results:** The most prevalent lineage of Mt detected was lineage 4 (n=91, 83.49%). Regarding drug resistance, 94 strains (86.3%) were found to be susceptible to all tested drugs, while 15 strains (13.7%) showed drug resistance. Among the drug-resistant *M. tuberculosis* strains, 7 were isoniazid-resistant (HR-Mt), 2 were multidrug-resistant (MDR-Mt), and 1 was extensively drug-resistant (Pre-XDR-Mt). Phenotypic drug susceptibility testing showed concordant results with the sequencing analysis for approximately 93.8% of the drugs tested. **Discussion and Conclusions:** These findings provide valuable insights into the transmission patterns and drug resistance profiles of *M. tuberculosis* in the Campania Region. The results contribute to the existing knowledge on TB and will facilitate further studies to improve the management of tuberculosis, particularly in relation to drug resistance.

313 - Methicillin-Susceptible Staphylococcus aureus wild-type and small colony variant (SCVs) isolated from osteomyelitis: genomic and expression analysis of selected genes

DALIDA ANGELA BIVONA ⁽¹⁾ - CARMELO BONOMO ⁽¹⁾ - EMANUELE NICITRA ⁽¹⁾ - EMMANUELE VENANZI RULLO ⁽¹⁾ - GIUSEPPE NUNNARI ⁽²⁾ - NICOLÒ MUSSO ⁽¹⁾ - STEFANIA STEFANI ⁽¹⁾ - DAFNE BONGIORNO ⁽¹⁾

Università di Catania, Dipartimento di Scienze Biomediche e Biotecnologiche, Catania, Italia ⁽¹⁾ -
Università di Catania, Medicina clinica e sperimentale, Catania, Italia ⁽²⁾

Methicillin-Susceptible Staphylococcus aureus wildtype and small colony variant (SCVs) isolated from osteomyelitis: genomic and expression analysis of selected genes

Dalida A. Bivona¹, Carmelo Bonomo¹, Emanuele Nicitra¹, Emmanuele Venanzi Rullo², Giuseppe Nunnari³, Nicolò Musso¹, Stefania Stefani¹, Dafne Bongiorno¹.

Department of Biomedical and Biotechnological Science, Microbiology section University of Catania

Dipartimento di Medicina Clinica Sperimentale, UOC Malattie Infettive, University of Messina

3. Dipartimento di Medicina Clinica Sperimentale, Unità Malattie Infettive, University of Catania

Background

Osteomyelitis is an infective and inflammatory process of the bone that can progress to osteonecrosis and bone destruction. Among microbiological agents, bacteria are mostly associated with human osteomyelitis. Also, surgical procedures, intravenous drug abuse and accidents can perturbate bone physiology giving rise to osteomyelitis. Staphylococcus aureus is the most isolated etiologic agent of osteomyelitis. In the present study, a S. aureus strain and its Small Colony Variants (SCVs) were isolated from a patient and phenotypically and genotypically characterize, also we selected some genes to investigate their expression profile.

Materials and Methods

S. aureus wild type (WT) and SCVs were isolated from a patient with chronic osteomyelitis. Antibiotic susceptibility test was performed in vitro via broth microdilution. Whole genome Sequencing (WGS) was performed by using Illumina DNA Prep and Illumina MiSeq. Data were analyzed using QIAGEN CLC Genomics Workbench software. Quantitative reverse transcription PCR (RT-qPCR) was performed. GraphPad Prism 9 software was used for statistical analysis, p-values set up at ≤ 0.05

Results

The *S. aureus* WT and SCVs are MSSA belonging to the Sequence Type 1. WT and SCVs were resistant to: erythromycin, penicillin G, gentamicin, levofloxacin, linezolid, tetracycline and rifampicin; and susceptible fosfomycin, oritavancin, daptomycin, vancomycin and teicoplanin. WT were also resistant to fusidic acid, instead SCVs to ceftobiprole. WGS identified identical resistome and virulome profiles for both clinical strains. Upon expression profile, the regulator genes *sigB* and *sdrE* are statistically significant up-regulated on SCVs Vs *S. aureus* WT, instead *agrA*, *psmA*, *hld*, *hla* genes are statistically significant down-regulated on SCVs Vs *S. aureus* WT.

Discussion and Conclusion

Prolonged antibiotic treatment causes MSSA to give rise to the most difficult-to-treat SCV phenotype, which is often associated with persistent and relapsing infection. Selective pressure by antibiotic treatment induces the activation of an alternative lifestyle. Instead, genomics analysis did not reveal any substantial mutation between WT and SCV, SCVs showed, when compared with WT strain, an increased expression of *sigB* regulator which promotes *sdrE* expression so favoring adhesion mechanism and, at the same time, as expected in the SCVs a decrease of *agrA*, *psmA*, *hld*, *hla* virulence gene expression so avoiding strong immune response. Inadequate identification or delayed management of *S. aureus*/SCVs infections in the context of acute osteomyelitis can severely impact disease progression. Hence, a holistic approach including appropriate characterization of isolated bacteria is necessary for the treatment of chronic osteomyelitis.

08 Patogenesi e meccanismi di virulenza microbici

4 - EXPLOITING LIPID METABOLISM BY HSV-1: A CHALLENGE TO RETHINK NEW THERAPIES FOR ALZHEIMER'S DISEASE.

Linda Trifirò ⁽¹⁾ - Camilla Albano ⁽¹⁾ - Selina Pasquero ⁽¹⁾ - Francesca Gugliesi ⁽¹⁾ - Greta Bajetto ⁽²⁾ - Weronika HEWELT-BELKA ⁽³⁾ - Erika Mina ⁽⁴⁾ - Paolo Porporato ⁽⁴⁾ - Dana Cairns ⁽⁵⁾ - David Kaplan ⁽⁵⁾ - Adam Mullis ⁽⁵⁾ - Santo Landolfo ⁽¹⁾ - Marco De Andrea ⁽¹⁾ - Valentina Dell'Oste ⁽¹⁾ - Matteo Biolatti ⁽¹⁾

Università degli Studi di Torino, Dipartimento di Scienze della Sanità Pubblica e Pediatriche, Torino, Italia ⁽¹⁾ - Università del Piemonte Orientale, CAAD Center for Translational Research on Autoimmune and Allergic Disease, Novara, Italia ⁽²⁾ - Gdańsk University of Technology, Department of Analytical Chemistry, Gdańsk, Polonia ⁽³⁾ - Università degli Studi di Torino, Dipartimento di Biotecnologie molecolari, Torino, Italia ⁽⁴⁾ - University of Arizona, Department of Immunobiology, Tucson, Arizona, Stati Uniti D'america ⁽⁵⁾

Exploiting Lipid Metabolism By Hsv-1: A Challenge To Rethink New Therapies For Alzheimer's Disease.

LINDA TRIFIRÒ^a, CAMILLA ALBANO^a, SELINA PASQUERO^a, FRANCESCA GUGLIESI^a, GRETA BAJETTO^b, WERONIKA HEWELT-BELKA^c, ERICA MINA^d, PAOLO PORPORATO^d, DANA CAIRNS^e, DAVID KAPLAN^e, ADAM MULLIS^e, SANTO LANDOLFO^a, MARCO DE ANDREA^{a,b}, VALENTINA DELL'OSTE^a, MATTEO BIOLATTI^a

^aDepartment of Public Health and Pediatric Sciences, University of Turin, Turin, Italy; ^bCAAD Center for Translational Research on Autoimmune and Allergic Disease, Novara, Italy; ^cDepartment of Analytical Chemistry, Gdańsk University of Technology, Gdańsk, Poland; ^dDepartment of Molecular Biotechnology and Health Sciences, University of Turin, Turin, Italy; ^eDepartment of Immunobiology, University of Arizona, Tucson, Arizona, USA.

Introduction. Alzheimer's disease (AD) is a neurodegenerative disorder characterized by a progressive decline in cognitive functions leading to memory loss and dementia. Only a minority of AD cases have a genetic inheritance, referred to as familial AD, and most (90%) of them are sporadic. Among the environmental risk factors that may promote the development of AD, persistent brain infections, particularly those induced by herpes simplex virus-1 (HSV-1), seem to play a key role in AD pathogenesis although the underlying mechanisms have not been fully elucidated yet. Tightly connected with AD etiopathogenesis is an alteration of lipid metabolism. Recently, CMS121 was shown to protect transgenic AD mice by reducing cognitive loss and inflammation. Interestingly, fatty acid synthase (FAS), a key enzyme in the synthesis of lipids that is increased in AD patients, was identified as a target of CMS121. Considering that cellular lipid metabolism plays a pivotal role in viral infections and that the mechanisms for the metabolic reprogramming by HSV-1 are still poorly understood, we aim at dissecting the host metabolic pathways modulated by HSV-1 in a neuronal-like cell line to identify new targets to prevent AD.

Materials and Methods. The experiments were performed in SH-SY5Y neuronal-like cells. The cells were successfully infected with HSV-1, thus representing a suitable in vitro model of HSV-1-associated neuronal pathologies. Next, cells were treated with different compounds (i.e., CMS121, C75, SSO) targeting lipid metabolism to test the antiviral activity. Moreover, to understand the link

between FAS and HSV-1 infectivity and rule out any off-target effects of the inhibitors, FAS gene expression was silenced by specific short hairpin RNA (shRNA).

Results. We demonstrated the capability of HSV-1 to upregulate the expression of the main lipogenesis enzymes. Accordingly, lipidomic analyses revealed an increase in both de novo synthesis and lipid storage following HSV-1 infection, confirming the role of HSV-1 in the deregulation of lipogenesis. In addition, we showed that the virus was still able to replicate upon treatment with compounds, but with reduced infectivity.

Discussion and Conclusions. Based on our results, we may speculate an antiviral activity of the drugs during virion assembly, leading to a block of virus production. Overall, our data unveil new aspects of HSV-1-AD interplay and uncover new potential targets to rethink new possible therapies for AD.

19 - A novel role of TMEM16A and TMEM16F in SARS-CoV-2 pathogenesis

MARTINA VIDMAR⁽¹⁾ - ILARIA SECCO⁽²⁾ - RITU GARG⁽²⁾ - MANOLA COMAR⁽¹⁾ - MAURO GIACCA⁽²⁾

Università degli Studi di Trieste, IRCCS Burlo Garofolo, SSD Diagnostica Avanzata e Microbiologia Traslazionale, Dipartimento universitario clinico di Scienze Mediche Chirurgiche e della Salute, Trieste, Italia⁽¹⁾ - King's College London, James Black Centre, Cardiovascular Department, London, Regno Unito⁽²⁾

A novel role of TMEM16A and TMEM16F in SARS-CoV-2 pathogenesis

Martina Vidmar^{1,2,3}, Iliaria Secco³, Ritu Garg³, Mauro Giacca^{1,3,4}, Manola Comar^{1,2}

1) Dipartimento Universitario Clinico di Scienze Mediche Chirurgiche e della Salute, Università degli Studi di Trieste, Trieste, Italy

2) SSD Diagnostica Avanzata e Microbiologia Traslazionale, IRCCS Burlo Garofolo, Trieste, Italy

3) Cardiovascular Science Department, James Black Centre, King's College London, London, UK

4) Molecular Medicine department, International Centre for Genetic Engineering and Biotechnology (ICGEB), Trieste, Italy

Introduction COVID-19 is a disease caused by the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). Symptoms can range from mild to severe, including lung thrombosis, acute respiratory distress syndrome, diarrhea, cytokine storm and alveolar oedema. As other viruses (including Respiratory Syncytial Virus - RSV), SARS-CoV-2 leads to syncytia formation: infected cells express SARS-CoV-2 SPIKE glycoprotein on their cell membranes and, binding their receptor, the Angiotensin Converting Enzyme 2 (ACE2), on neighboring cells, they fuse into multinucleate cells. This process seems to give advantages to viruses like a faster spread and the possibility of capturing immunity cells, as lymphocytes. Prof. Giacca's group found that the anthelmintic drug niclosamide is blocking this process, blocking TMEM16 family of proteins functions, mainly focusing on TMEM16F, a chloride channel calcium dependent and scramblase. Basing on those results, we want to investigate whether ACE2 and TMEM16A (similar in function and structure with TMEM16F) / TMEM16F directly interact and the role of TMEM16A/F in SARS-CoV-2 pathogenesis and ACE2 degradation.

Materials and Methods U2OS, HEK and Vero cells are transfected with ACE2, TMEM16A/F and SPIKE plasmids or siRNAs for TMEM16A/F; treated with Niclosamide, Bafilomycin, Cycloheximide, MG132; treated with LysoTracker or CellROX dye. Samples are then fixed in 4%PFA for immunofluorescence staining (IF) or proximity ligation assay (PLA), collected with RIPA buffer for Western Blot or co-immunoprecipitation (co-IP) or Qiazol for RNA extraction and qPCR.

Results 1) ACE2 and TMEM16A/F are interacting: according to the PLA and the co-IP results, there is interaction between those proteins and that interaction is affected by niclosamide.

2) TMEM16A and TMEM16F are degrading ACE2: when ACE2 is co-transfected in cells with TMEM16A and, in a lesser extent, TMEM16F, levels of ACE2 fall comparing to the control, while in Vero cells (which express ACE2 endogenously) the transfection of siRNAs for TMEM16A/F

increase ACE2. 3) TMEM16A/F seem to interact with SPIKE: according to co-IP, those proteins are interacting, and the interaction is affected by niclosamide too. 4) ACE2 degradation probably occurs through lysosomes. 6) Interestingly, both niclosamide and siRNAs for TMEM16A/F seem to affect the ACE2-SPIKE interaction as well.

Discussion and Conclusions We are hypothesizing a model in which ACE2 and TMEM16s are interacting, the interaction probably leads to ACE2 internalization through clathrin mediated endocytosis and then to lysosomes degradation. We want to investigate if TMEM16A/F are needed for ACE2 SPIKE-caused degradation as well, assuming that ACE2, TMEM16s and SPIKE are forming a complex important for SARS-CoV-2 pathogenesis.

22 - The protein HslJ boosts *Acinetobacter baumannii* survival against oxidative stress

CECILIA AMBROSI ⁽¹⁾ - MEYSAM SARSHAR ⁽²⁾ - MARTINA PASQUA ⁽³⁾ - CARLO ZAGAGLIA ⁽⁴⁾ - ANNA TERESA PALAMARA ⁽⁵⁾ - DANIELA SCRIBANO ⁽⁴⁾

San Raffaele Open University, IRCCS, Department of Human Sciences and Promotion of the Quality of Life, Roma, Italia ⁽¹⁾ - Bambino Gesù Children's Hospital, IRCCS, Research Laboratories, Roma, Italia ⁽²⁾ - Sapienza Università di Roma, Department of Biology and Biotechnology "C. Darwin", Roma, Italia ⁽³⁾ - Sapienza Università di Roma, Dipartimento di Sanità Pubblica e Malattie Infettive, Roma, Italia ⁽⁴⁾ - Sapienza Università di Roma, Department of Public Health and Infectious Diseases, Sapienza University of Rome, Laboratory Affiliated to Institute Pasteur Italia-Cenci Bolognetti, Roma, Italia ⁽⁵⁾

The protein HslJ boosts *Acinetobacter baumannii* survival against oxidative stress

Cecilia Ambrosi¹, Meysam Sarshar², Martina Pasqua³, Carlo Zagaglia⁴, Anna Teresa Palamara^{5,6}, and Daniela Scribano⁴

¹Department of Human Sciences and Promotion of the Quality of Life, San Raffaele Open University, IRCCS, Rome, Italy; ²Research Laboratories, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy; ³Department of Biology and Biotechnology "C. Darwin", Sapienza University, Rome, Italy; ⁴Department of Public Health and Infectious Diseases, Sapienza University, Rome, Italy; ⁵Department of Public Health and Infectious Diseases, Sapienza University of Rome, Laboratory Affiliated to Institute Pasteur Italia-Cenci Bolognetti Foundation, Rome, Italy; ⁶Department of Infectious Diseases, Istituto Superiore di Sanità, Rome, Italy.

Background: *Acinetobacter baumannii* is a life-threatening opportunistic pathogen that causes different infections, including ventilator-associated pneumonia. Due to its ability to sense and respond to the environmental stresses, *A. baumannii* can survive in hospital settings and persist during host infections. In a previous study, we identified the periplasmic protein encoded by the ABUW_2868 locus (*hslJ* gene) as being upregulated during imipenem exposure and hypothesized that it participates in the protective response against the oxidative stress. Methods: A *hslJ* mutant was attained from strain AB5075. Hydrogen peroxide assays and macrophage infections were used to determine the survival rates by colony forming units (CFU/ml) of the *hslJ* mutant in comparison with the wild type AB5075 strain. Surface hydrophobicity, biofilm-forming activity and motility in the *hslJ* mutant were also analyzed. *hslJ* transcriptional regulation is under evaluation using trans-activation experiments and protein-DNA binding assay. Results: In comparison with the wild type strain, the absence of *hslJ* gene caused a significant drop in cell survival rates of the mutant strain both upon exposure to exogenous H₂O₂ and macrophage infection. Moreover, compared to the wild type strain, the *hslJ* mutant displayed a decrease in biofilm formation and motility abilities although had a higher surface hydrophobicity. Furthermore, preliminary results suggest that expression of *hslJ* is under the control of the master regulator in defense against oxidative stress, OxyR. Conclusions: The *hslJ* gene could be part of the regulon of OxyR. Being the HslJ protein located into the periplasm, it represents the first line of defense that confers resistance to oxidative stress that *A. baumannii* faces both in disinfectants used in hospital environments and innate immune cells during infection of the lungs. These findings provide a basis to develop novel therapeutics against this new target.

40 - SARS-CoV-2 ORF3c impairs mitochondrial respiratory metabolism, oxidative stress, and autophagic flux

RACHELE CAGLIANI⁽¹⁾ - ALESSANDRA MOZZI⁽¹⁾ - MONICA OLDANI⁽²⁾ - MATILDE E. FORCELLA⁽²⁾ - CHIARA VANTAGGIATO⁽¹⁾ - DIEGO FORNI⁽¹⁾ - MANUELA SIRONI⁽¹⁾ - PAOLA FUSI⁽²⁾

Scientific Institute IRCCS E. MEDEA, Bioinformatics, Bosisio Parini, Italia⁽¹⁾ - University of Milano-Bicocca, Department of Biotechnology and Biosciences, Milano, Italia⁽²⁾

SARS-CoV-2 ORF3c impairs mitochondrial respiratory metabolism, oxidative stress, and autophagic flux

Rachele Cagliani¹, Alessandra Mozzi¹, Monica Oldani², Matilde E. Forcella², Chiara Vantaggiato², Diego Forni¹, Manuela Sironi¹ and Paola Fusi²

¹ Scientific Institute IRCCS E. MEDEA, Bioinformatics, Bosisio Parini, 23842, Italy; ² Department of Biotechnology and Biosciences, University of Milano-Bicocca, Milano, 20126, Italy.

Introduction: Coronaviruses encode a variable number of accessory proteins, which differ in sequence and number even among closely related viruses. Although these proteins are considered non-essential for coronavirus replication, they are critical to virus-host interaction, pathogenesis and virulence. SARS-CoV-2 encodes at least twelve accessory proteins, whose roles during infection have been studied. Nevertheless, ORF3c, an alternative open reading frame of ORF3a, has remained under-investigated. The ORF3c protein has been predicted to be encoded by sarbecoviruses only, including SARS-CoV-2 and batCoV RaTG13. Herein, we report the first investigation of the effect of SARS-CoV-2 ORF3c (hORF3c) and batCoV RaTG13 ORF3c (bORF3c) proteins on autophagy and lung cell mitochondrial metabolism. **Materials and Methods:** All experiments were performed on HeLa and HSAEC1 cell lines, transfected with hORF3c or bORF3c or empty vectors. Immunofluorescence and Western Blot analysis were applied to investigate cellular localization and effects on autophagic flux. The mitochondrial functionality and oxidative stress response were investigated through Agilent Seahorse XF Mito Stress, Seahorse Mito Fuel Flex Test, and XF Seahorse Glycolysis Rate Assay. **Results:** We showed that the hORF3c protein has a mitochondrial localization and alters mitochondrial metabolism, inducing a shift from glucose to fatty acids oxidation and enhanced oxidative phosphorylation. These effects result in increased ROS production and block of the autophagic flux. In particular, hORF3c affects lysosomal acidification, blocking the normal autophagic degradation process and leading to autolysosome accumulation. We also evaluated the effect on autophagy of bORF3c. In most analyses, a similar trend was observed, but the effect was definitely weaker. The two viral proteins differ only in two amino acids (R36K and K40R). Our data indicate that the 36R and 40K sites are necessary and sufficient to determine the accumulation of autophagosomes and to justify the different effect on autophagy for hORF3c and bORF3c proteins. **Discussion and Conclusions:** This is the first study in which the effect of a single SARS-CoV-2 protein on mitochondrial metabolism has been evaluated together with its direct effect on the autophagic process. We observed a different effect on autophagy for hORF3c and bORF3c proteins and we have shown that this difference depends on two amino acids in position 36 and 40. Interestingly, the amino acid R36 has also

changed in some SARS-CoV-2 variants. ORF3c is highly conserved among sarbecoviruses, suggesting that its physiological role is important for the virus. This accessory protein could be a key actor for infection and virus maintenance in the natural reservoir (i.e., bats).

61 - Identification of druggable host dependency factors shared by multiple SARS-CoV-2 variants of concern

ILARIA FRASSON⁽¹⁾ - LINDA DIAMANTE⁽¹⁾ - MANUELA ZANGROSSI⁽¹⁾ - ELENA CARBOGNIN⁽²⁾ - ANNA DALLA PIETÀ⁽³⁾ - ALESSANDRO PENNA⁽³⁾ - ANTONIO ROSATO⁽³⁾ - RANIERI VERIN⁽⁴⁾ - FILIPPO TORRIGIANI⁽⁴⁾ - CRISTIANO SALATA⁽¹⁾ - MARIA PAULA DIZANZO⁽¹⁾ - LORENZO VACCARO⁽⁵⁾ - DAVIDE CACCHIARELLI⁽⁵⁾ - MARCO MONTAGNER⁽¹⁾ - GRAZIANO MARTELLO⁽²⁾ - SARA N. RICHTER⁽¹⁾

Università di Padova, Dip. Medicina Molecolare, Padova, Italia⁽¹⁾ - Università di Padova, Dip. Biologia, Padova, Italia⁽²⁾ - Università di Padova, Dip. Scienze Chirurgiche Oncologiche e Gastroenterologiche, Padova, Italia⁽³⁾ - Università di Padova, Dipartimento di Biomedicina Comparata e Alimentazione, Padova, Italia⁽⁴⁾ - Università di Napoli, Dip Medicina Traslationale, Napoli, Italia⁽⁵⁾

Identification of druggable host dependency factors shared by multiple SARS-CoV-2 variants of concern

Ilaria Frasson,¹ Linda Diamante,^{1,2} , Manuela Zangrossi,^{1,10} Elena Carbognin,² Anna Dalla Pietà,³ Alessandro Penna,³ Antonio Rosato,^{3,4} Ranieri Verin,⁵ Filippo Torrigiani,⁵ Cristiano Salata,¹ Maria Paula Dizanzo,¹ Lorenzo Vaccaro,^{6,7} Davide Cacchiarelli,^{6,7,8} Marco Montagner,¹ Graziano Martello,² and Sara N. Richter^{1,9}

1: Department of Molecular Medicine, University of Padua, Padua 35121, Italy

2: Department of Biology, University of Padua, Padua 35131, Italy

3: Dept. of Surgery, Oncology and Gastroenterology, University of Padua, Padua 35128, Italy

4: Veneto Institute of Oncology IOV-IRCCS, Padua 35128, Italy

5: Department of Comparative Biomedicine and Food Science, University of Padua, Padua 35020, Italy

6: Telethon Institute of Genetics and Medicine (TIGEM), Pozzuoli 80078, Italy

7: Department of Translational Medicine, Federico II University, Naples 80138, Italy

8: School for Advanced Studies, University of Naples “Federico II”, Naples 80138, Italy

9: Microbiology and Virology Unit, Padua University Hospital, Padua 35128, Italy

Introduction: The high mutation rate of SARS-CoV-2 leads to the emergence of multiple variants, some of which are resistant to vaccines and drugs that target viral elements. This poses significant hurdles to the development of prophylactic approaches, as new variants partially overcome the efficacy of COVID-19 vaccines and administered antivirals. Targeting host-dependency factors, cellular proteins required for viral replication, would help avoid resistance. However, it is not clear whether different SARS-CoV-2 variants induce conserved cellular responses and exploit the same core host factors.

Materials and Methods: A high stringency CRISPR-based loss-of-function screen was used to identify host factors required for infection of human lung and colon cells by five different SARS-CoV-2 variants. The identified host factors were targeted in cells and in a mouse model of SARS-CoV-2 infection with drugs available for potential COVID-19 repurposing.

Results: We compared five variants of concern that emerged sequentially during the pandemic and found that the host transcriptional response was conserved, differing only in kinetics and magnitude. Using a genome-wide CRISPR knockout approach, we identified the host genes required for infection by each variant. Most of the genes identified were shared by multiple variants in both lung and colon cells. Hits were validated by siRNA knockdown and challenged with small molecules and repurposed FDA-approved drugs. All drugs were highly potent against all variants tested. Mechanistically, we identified ROS production as a key step in early viral replication. Antioxidants such as N-acetyl cysteine (NAC) were effective against all variants both in human lung cells and in a humanised mouse model of SARS-CoV-2 infection.

Discussion and conclusions: The aim of this study was to understand whether subsequent viral variants share the same infection kinetics and host-dependent factors, as targeting host proteins may be an effective and safe strategy against COVID-19. Our work demonstrated that the major SARS-CoV-2 variants exhibit differences in replication kinetics and magnitude, suggesting that they induce similar cellular responses upon infection. Subsequently, a set of host-dependency factors common to the variants was obtained, showing that SARS-CoV-2 relies on the redox state of the cell, but also exploits different protein kinases. A number of FDA-approved drugs and drug-like small molecules targeting the retrieved hits showed promising antiviral activity. In particular, drugs with antioxidant properties were able to inhibit SARS-CoV-2 in a mouse model of infection. Thus, combination therapy of compounds targeting host factors with direct-acting antivirals may be an effective antiviral approach.

72 - A study on cross-link formation between fibrinogen and region A of FnBPA from *Staphylococcus aureus* promoted by von Willebrand Factor-binding protein (vWbp) and tissue Transglutaminase 2 (TG2)

CHIARA MOTTA⁽¹⁾ - GIULIA BARBIERI⁽²⁾ - ANGELICA PELLEGRINI⁽¹⁾ - JOAN GEOGHEGAN⁽³⁾ - GIAMPIERO PIETROCOLA⁽¹⁾

Università di Pavia, Dipartimento di Medicina Molecolare, unità di Biochimica, Pavia, Italia⁽¹⁾ -
Università di Pavia, Dipartimento di Biologia e Biotecnologie "Lazzaro Spallanzani", Pavia, Italia
⁽²⁾ - University of Birmingham, Institute of Microbiology and Infection, Birmingham, Regno Unito
⁽³⁾

A study on cross-link formation between fibrinogen and region A of FnBPA from *Staphylococcus aureus* promoted by von Willebrand Factor-binding protein (vWbp) and tissue Transglutaminase 2 (TG2)

Chiara MOTTA¹, Giulia BARBIERI², Angelica PELLEGRINI¹, Joan GEOGHEGAN³,
Giampiero PIETROCOLA¹

¹Department of Molecular Medicine, unit of Biochemistry, University of Pavia - Pavia (Italy);

²Department of Biology and Biotechnology "Lazzaro Spallanzani", University of Pavia - Pavia (Italy); ³Institute of Microbiology and Infection, University of Birmingham – Edgbaston, Birmingham (United Kingdom)

Introduction

In the last few decades, multi-drug resistant (MDR) *Staphylococcus aureus* quickly became a major and emerging cause of healthcare-associated infections. Therefore, a research effort into discovering novel drug targets and virulence mechanisms is the first step to develop efficient therapeutic strategies. Von Willebrand factor binding protein (vWbp) was initially isolated as a bacterial receptor for the human von Willebrand factor (vWF): despite being a secreted protein, its ability to rebind to the bacterial surface through peptidoglycan and lipoteichoic acid has been proven. Furthermore, vWbp strongly contributes to *S. aureus* pathogenicity through its ability to bind and non-proteolytically activate prothrombin, resulting in Factor XIII (FXIII) activation and, ultimately, fibrin cross-linking. In this study, we investigated the incorporation of different *S. aureus* cell wall-anchored (CWA) proteins (FnBPA, FnBPB, ClfA and ClfB) into the fibrin reticule by the action of bacterially-activated FXIII, generating high-molecular weight heteropolymers. Additionally, the same analysis was also performed using tissue Transglutaminase 2 (TG2) instead of FXIII.

Material and Methods

Binding of vWbp to the bacterial surface was assessed by ELISA assay; binding to cell wall components was assessed by Dot-blot assay. The presence of reactive glutamine and lysine residues in FnBPA was assessed through the incorporation of dansyl-cadaverine and glutamine-containing peptide probes. Cross-linking of recombinant CWA proteins to fibrin(ogen) in presence of vWbp-activated FXIII or TG2 was evaluated with Western immunoblotting assays.

Results

vWbp re-associates to the bacterial surface through lipoteichoic acid and peptidoglycan, although with a preference for the latter. Only the N-terminal segment of FnBPA (N1 subdomain), which displays active glutamine residues, is directly involved in the cross-linking with fibrin(ogen). Both TG2 and vWbp-activated FXIII are able to cross-link FnBPA to fibrin(ogen), but TG2 works without vWbp involvement. No cross-links were observed with other proteins.

Discussion and Conclusions

The cross-linking site for Fbg in FnBPA was localized in N1 subdomain, which contains the functional reactive Gln103 (Q) residue, previously identified as the main lysine acceptor residue within fibrinogen. On the contrary, no cross-links were observed with ClfA, ClfB and FnBPB: in fact, multiple alignments of their N-terminal sequence with FnBPA showed that none of them possess a reactive Gln103 residue. These findings point to a unique pathogenic mechanism, in which the transglutaminase activity of vWbp-activated FXIII and/or TG2 contributes to the persistence of MDR *S. aureus* strains both in blood and host tissues.

This research was supported by EU funding within the NextGenerationEU-MUR PNRR Extended Partnership initiative on Emerging Infectious Diseases (Project no. PE000000007, INF-ACT).

85 - The SaeRS two-component system regulates the expression of group B streptococcal genes that are highly expressed in vivo

FRANCESCO COPPOLINO ⁽¹⁾ - GIUSEPPE V. DE GAETANO ⁽²⁾ - GERMANA LENTINI ⁽²⁾ - AGATA FAMÀ ⁽²⁾ - PASQUALINA LAGANÀ ⁽¹⁾ - CONCETTA BENINATI ⁽²⁾ - GIUSEPPE TETI ⁽³⁾

Università degli Studi di Messina, Dipartimento di Scienze Biomediche, Odontoiatriche e delle Immagini Morfologiche e Funzionali, Messina, Italia ⁽¹⁾ - Università degli Studi di Messina, Dipartimento di Patologia Umana, Messina, Italia ⁽²⁾ - Scylla Biotech Srl, Scylla Biotech Srl, Messina, Italia ⁽³⁾

The SaeRS two-component system regulates the expression of group B streptococcal genes that are highly expressed in vivo

FRANCESCO COPPOLINO ¹, GIUSEPPE V. DE GAETANO ², GERMANA LENTINI ², AGATA FAMÀ ², PASQUALINA LAGANÀ ¹, ARNAUD FIRON ³, GIUSEPPE TETI ⁴, CONCETTA BENINATI ^{2,4}

Departments of Biomedical, Dental and Imaging Sciences¹ and of Human Pathology and Medicine², University of Messina, Messina, Italy; ³ Microbiology Department, Biology of Gram-positive pathogens, Institut Pasteur, Paris, France; ⁴Scylla Biotech Srl, Messina, Italy.

Introduction: *Streptococcus agalactiae*, also named group B *Streptococcus* (GBS), is a commensal Gram-positive bacterium that colonizes the human gastro-intestinal tract and causes invasive infections in neonates, in the elderly and in patients with underlying chronic disease. Two-component signal transduction systems (TCSs), consisting of a sensor (S) histidine kinase and a response regulator (R), enable bacteria to adapt to a wide range of microenvironments. This work was undertaken to study the functional role of the SaeRS TCS in the activation of GBS virulence factors.

Materials and Methods: We introduced an amino acid substitution (T133A) in the *saeS* gene and a full deletion of the *saeR* gene (Δ *saeR*) to obtain, respectively, constitutively active (HKA) and constitutively inactive systems in the background of the representative clone complex 23, serotype III, NEM316 strain. The transcriptome of in vitro grown bacteria was analyzed in the mutants using RNA-seq, while in vivo gene expression was assessed by RT-qPCR using *gyrA* as the control housekeeping gene.

Results: In in vitro grown bacteria, transcriptome analysis revealed no differences in gene expression in the absence of *saeR*, while a distinctive set of upregulated genes, comprising those encoding for the SaeRS system components, PbsP and BvaP was identified in the HKA mutant. These genes, particularly *pbsP* and *bvaP* were also found to be highly expressed in vivo, after inoculation of bacteria the murine peritoneum, to levels that were considerably higher than those of genes encoding for two well-characterized virulence factors, such as *bibA* and *cylE*, used as controls. Expression of *pbsP* and *bvaP*, but not *bibA* and *cylE*, was partially reduced in the Δ *saeR* strain, which also showed a significantly reduced virulence in a model of GBS meningitis. The HKA strain was defective in its ability to persist in vivo, which prevented further analysis. However, this strain showed a markedly increased ability to adhere to and invade brain endothelial cells in vitro in a manner that was entirely *pbsP*-dependent.

Discussion and Conclusions: Collectively these data indicate that the SaeR TCS plays an important role in the pathogenesis of GBS infection, as evidenced by reduced virulence in the absence of the SaeR regulator. The ability of the SaeRS system to promote virulence likely resides in its ability to induce the upregulation of a distinctive set of genes comprising the components of the SaeRS system itself, as well as two known adhesins/invasins, particularly PbsP, which was previously shown to promote the ability of GBS to cross the blood brain barrier.

91 - Investigation of Merkel Cell Polyomavirus (MCPyV) status in Merkel Cell Carcinoma (MCC)

SARA PASSERINI⁽¹⁾ - CARLA PREZIOSO⁽²⁾ - GIULIA BABINI⁽¹⁾ - AMEDEO FERLOSIO⁽³⁾ - TERENCE COSIO⁽⁴⁾ - ELENA CAMPIONE⁽⁴⁾ - UGO MOENS⁽⁵⁾ - MARCO CIOTTI⁽⁶⁾ - VALERIA PIETROPAOLO⁽¹⁾

“Sapienza” University of Rome, Department of Public Health and Infectious Diseases, Rome, Italia⁽¹⁾ - IRCCS San Raffaele Roma, Laboratory of Microbiology of Chronic-Neurodegenerative Diseases, Rome, Italia⁽²⁾ - Tor Vergata University of Rome, Anatomic Pathology, Department of Biomedicine and Prevention, Rome, Italia⁽³⁾ - Tor Vergata University of Rome, Dermatologic Unit, Department of Systems Medicine, Rome, Italia⁽⁴⁾ - University of Tromsø—The Arctic University of Norway, Department of Medical Biology, Faculty of Health Sciences, Tromsø, Norvegia⁽⁵⁾ - Polyclinic Tor Vergata Foundation, Virology Unit, Rome, Italia⁽⁶⁾

Investigation of Merkel Cell Polyomavirus (MCPyV) status in Merkel Cell Carcinoma (MCC)

SARA PASSERINI¹, CARLA PREZIOSO², GIULIA BABINI¹, AMEDEO FERLOSIO³, TERENCE COSIO^{4,5}, ELENA CAMPIONE⁴, UGO MOENS⁶, MARCO CIOTTI⁷, VALERIA PIETROPAOLO¹

¹Department of Public Health and Infectious Diseases, “Sapienza” University of Rome, 00185 Rome, Italy; ²Laboratory of Microbiology of Chronic-Neurodegenerative Diseases, IRCCS San Raffaele Roma, 00166 Rome, Italy; ³Anatomic Pathology, Department of Biomedicine and Prevention, Tor Vergata University of Rome, 00133 Rome, Italy; ⁴Dermatologic Unit, Department of Systems Medicine, Tor Vergata University of Rome, 00133 Rome, Italy; ⁵Department of Experimental Medicine, Tor Vergata University of Rome, 00133 Rome, Italy; ⁶Department of Medical Biology, Faculty of Health Sciences, University of Tromsø—The Arctic University of Norway, 9037 Tromsø, Norway; ⁷Virology Unit, Polyclinic Tor Vergata Foundation, 00133 Rome, Italy.

Introduction: Merkel Cell Polyomavirus (MCPyV) is a double-stranded DNA virus associated with Merkel cell carcinoma (MCC), a rare but aggressive skin tumor. To improve our understanding of the role of MCPyV in MCCs, investigation of MCPyV DNA, transcripts and viral DNA integration and mutations was performed on primary tumors and regional lymph nodes from two MCC patients, one metastatic and one non-metastatic.

Material and Methods: After DNA extraction, MCPyV-DNA was searched by quantitative polymerase chain reaction (qPCR), followed by amplification of Large T Antigen (LTag), Viral Protein 1 (VP1) and Non-Coding Control Region (NCCR) by qualitative PCR. In parallel, following RNA extraction, LTag and VP1 transcripts were investigated by reverse-transcription PCR (RT-PCR). Viral integration was also studied, and full length LTag sequencing performed.

Results: qPCR revealed that the primary tumor of both patients and the lymph node of the metastatic patient were positive for MCPyV small t-antigen (sT), with an average value of 7.0×10^2 copies/ μ g. The same samples harbored LTag, NCCR and VP1 DNA. Sequencing results of NCCR and VP1 revealed a complete identity with the reference strain MCC350. As reported in most MCPyV-positive MCCs, LTag but not VP1 gene was detectable at RNA level. The two MCC primary tumors reported a truncated LTag with a conserved Retinoblastoma (Rb) protein binding site, and integration of the

viral genome occurred at 5q23.1 and 5q11.2, respectively. The metastatic lymph node, instead, did not show integration of viral DNA and a full-length LTag was observed.

Discussion and Conclusions: Results contribute to strongly support MCPyV's role in MCC pathogenesis, assuming viral integration and the expression of a truncated LT gene with a preserved LXCXE motif, able to bind and sequester Rb protein, as crucial factors in MCPyV mediated oncogenesis.

98 - SARS-CoV-2 infection induces Tau pathological signature

PAOLA QUARANTA⁽¹⁾ - MARIANNA MIGNANELLI⁽²⁾ - GIACOMO SIANO⁽²⁾ - MATTEO BIMBATI⁽²⁾ - PAOLA PERRERA⁽¹⁾ - CARMEN RITA PIAZZA⁽¹⁾ - PIETRO GIORGIO SPEZIA⁽¹⁾ - MARIO COSTA⁽³⁾ - ANTONINO CATTANEO⁽²⁾ - MAURO PISTELLO⁽¹⁾ - CRISTINA DIPRIMIO⁽³⁾

Università di Pisa, Dipartimento di Ricerca Traslazionale e Nuove Tecnologie in Medicina, Pisa, Italia⁽¹⁾ - Scuola Normale superiore, Laboratorio di Biologia Bio@SNS, Pisa, Italia⁽²⁾ - Centro Nazionale di Ricerca CNR, istituto di Neuroscienze, Pisa, Italia⁽³⁾

SARS-CoV-2 infection induces Tau pathological signature

Paola Quaranta^{1;2}, Marianna Mignanelli³, Giacomo Siano³, Matteo Bimbati³, Paola Perrera¹, Carmen Rita Piazza^{1;4}, Pietro Giorgio Spezia¹, Mario Costa², Antonino Cattaneo³, Mauro Pistello^{1;5}, Cristina Di Primio².

1. Department of Translational Research and New Technologies in Medicine and Surgery, Retrovirus Center, University of Pisa, Pisa, Italy.
2. Institute of Neuroscience, Italian National Research Council (CNR), Pisa, Italy
3. Laboratorio di Biologia Bio@SNS, Scuola Normale Superiore di Pisa, Pisa, Italy
4. Department of Medical Biotechnologies, University of Siena, Siena, Italy
5. Azienda Ospedaliera Universitaria Pisana, Virology Unit, Pisa, Italy

Introduction Covid-19 disease is much more than a simple respiratory disease since several organs can be affected with unexpected and silent deterioration including the Central Nervous System (CNS). SARS-CoV-2 infection results in a wide range of long-term neurological symptoms likely due to hidden damages in the brain and it is worryingly associated with the aggravation of Alzheimer's disease. Since few data are available about the molecular basis of these manifestations, here we investigate the mechanism of infection of CNS and the interplay between viral proteins and Tau protein.

Materials and Methods: Both SH-SY5Y neuroblastoma cells and K18-hACE c57BL/6J mice were infected with several strain variants and examined at different time points from infection for the presence of viral proteins and biomarkers of neurodegeneration by confocal microscopy and western blot.

Results We find that SARS-CoV-2 infection in both models triggers the aberrant phosphorylation of microtubule-associated protein Tau at several pathological epitopes associated with Alzheimer's disease and other tauopathies. This event increases Tau's propensity to form insoluble aggregates and alters its subcellular localization.

Discussion and Conclusions

These findings indicate that SARS-CoV-2 plays a direct role in neurodegeneration; our data support the evidence that SARS-CoV-2 infection in the CNS triggers downstream effects altering Tau function, eventually leading to the impairment of neuronal function. Our data open up a new molecular mechanism underlying post-COVID neurological manifestations and acknowledge the potential scale of the disease long-term course.

107 - Transcriptome analysis of Parvovirus B19 infected erythroid progenitor cells

ERIKA FASANO (1) - FEDERICA BICHICCHI (1) - GLORIA BUA (1) - ELISABETTA MANARESI (1) - FRANCESCA BONVICINI (1) - GIORGIO GALLINELLA (2)

Università Di Bologna, Fabit, Bologna, Italia (1) - IRCCS Azienda Ospedaliero-Universitaria Di Bologna, Unità Di Microbiologia, Bologna, Italia (2)

Transcriptome analysis of Parvovirus B19 infected erythroid progenitor cells

ERIKA FASANO¹, FEDERICA BICHICCHI¹, GLORIA BUA¹, ELISABETTA MANARESI¹, FRANCESCA BONVICINI¹, GIORGIO GALLINELLA²

¹Dept of Pharmacy and Biotechnology, University of Bologna, Bologna, Italy; ²Microbiology Unit, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy

1. INTRODUCTION

Parvovirus B19 (B19V) is a human pathogen with a worldwide prevalence, and it is characterized by a selective and narrow tropism for the erythroid lineage in the bone marrow. A VP1u (Viral Protein 1 unique region) binding coreceptor (coR) has been identified as a molecule involved in B19V entry step. Hence, in our work we aimed to characterize the outcome of viral infection and virus-induced modification on host cell transcriptome in the cell fraction of Erythroid Progenitor cells (EPCs) expressing the VP1u-coR, isolated by using fluorescence-activated cell sorting, by qPCR and RT-qPCR to assess the increment of viral DNA and viral RNAs, respectively, and by dual (virus/cell) NGS RNAseq analysis.

2. MATERIALS AND METHODS

An expanding population of EPCs was generated in vitro from peripheral blood mononuclear cells (PBMCs) by culturing in medium enriched with appropriate growth and differentiating factors. At day 8, EPCs were labeled for the expression of VP1u-coR and were sorted based on morphological parameters and fluorescence. Then, the cells were infected with a virus derived from the genomic clone CK10 and equal amounts of cell culture were collected at 2; 16 and 48 hours post infection (hpi). Thereafter, nucleic acids were extracted from cells for the subsequent analysis: qPCR; RT-qPCR and NGS RNAseq.

3. RESULTS

Starting from heterogenous unsorted EPCs (HUE) we attained nearly homogenous populations of positive sorted EPCs (PSE) and negative sorted EPCs (NSE). The cell fraction expressing the VP1u-coR was enriched up to +81.6%. For what concerns the productivity of B19V infection in the different populations, the data shows that only HUE and PSE are permissive to B19V while in NSE no viral replication neither transcription have occurred. Furthermore, we investigated cell-virus interactions via dual RNAseq analysis. The B19V transcriptome was reconstructed highlighting the use of polyA and splice processing sites. The B19 virus induced variation in the EPCs transcriptome was analyzed, revealing pathways up and down regulated during the infectious process.

4. DISCUSSION AND CONCLUSIONS

Our data suggest that the cell targeting by VP1u-coR interaction is a determinant for a productive infection process but not in a binding-dependent way, rather in the later step of B19V infection. The effective productivity of viral replication is critically linked to the intracellular environment and in turn has been shown to induce deviations from the normal cell differentiation and replicative state. These data will be valuable for a deeper understanding of the pathogenic potential of the virus and for developing new antiviral strategies.

117 - Longitudinal study of Human Polyomaviruses viruria in kidney transplant recipients

MARIA DOLCI (1) - CATERINA COLICO (2) - FEDERICO AMBROGI (3) - EVALDO FAVI (4) - LUCIA SIGNORINI (1) - MARTA PEREGO (3) - EDOARDO CAMPIOLI (3) - KEVIN KAMAU MAINA (1) - PASQUALE FERRANTE (1) - MARIANO FERRARESSO (4) - SERENA DELBUE (1)

University of Milano, Department of Biomedical, Surgical and Dental Sciences, Milano, Italia (1) - Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Department of Emergency Surgery, Milano, Italia (2) - University of Milano, Department of Clinical Sciences and Community Health, Milano, Italia (3) - Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, General Surgery and Kidney Transplantation, Milano, Italia (4)

Longitudinal study of Human Polyomaviruses viruria in kidney transplant recipients

Maria Dolci¹, Caterina Colico², Federico Ambrogi³, Evaldo Favi⁴, Lucia Signorini¹, Marta Perego³, Edoardo Campioli³, Kevin Kamau Maina¹, Pasquale Ferrante¹, Mariano Ferraresso⁴, Serena Delbue¹

¹ Laboratory of Molecular Virology, Department of Biomedical, Surgical and Dental Sciences, University of Milano, Via Carlo Pascal 36, 20133 Milano, Italy

² Department of Emergency Surgery, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy

³ Department of Clinical Sciences and Community Health, University of Milan, Milan, Italy

⁴ General Surgery and Kidney Transplantation, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy

Introduction: Immunosuppression after kidney transplantation (KTx) exposes recipients to Human Polyomaviruses (HPyVs) infections, whose natural history is still misunderstood.

Materials and Methods: Allograft biopsies, and urine from 58 donor-recipient pairs were collected before KTx and from 1 (T0) up to 540 (T9) days post KTx. Specimens were tested for JC (JCPyV), BK (BKPyV), and Merkel Cell polyomavirus (MCPyV), by quantitative Real-Time PCR. Evolution of post-KTx HPyVs viruria, and association between HPyVs recipients viruria/donor viruria, were evaluated.

Results: HPyVs were detected in 4/58 (6.89%) allograft biopsies. HPyVs viruria was present in 29/58 (50%) donors and 45/58 (77.59%) recipients. JCPyV DNA was detected in 26/58 (44.83%) donors and 25/58 recipients (43.10%), 19 of whom received kidney from JCPyV positive donor. BKPyV genome was detected in 3 (5.17%) donors and 22 (37.93%) recipients, whereas MCPyV DNA was detected in 2 (3.45%) donors, and 17 (29.31%) recipients. The median time of JCPyV, BKPyV and MCPyV first episode of replication was 1, 68, and 198 days post KTx, respectively. At T0, donor JCPyV viruria was associated with increased risk of JCPyV replication post-KTx; recipients with JCPyV positive donor showed lower risk of BKPyV replication post-KTx.

Discussion and Conclusions: the results suggested that JCPyV may be transmitted by allograft, and that its replication post KTx might prevent BKPyV reactivation.

118 - The BM 110, a hypervirulent strain of group B streptococci, exploits $\alpha_2\beta_1$ and $\alpha_3\beta_1$ integrins to invade human enterocytes

GIUSEPPE VALERIO DE GAETANO ⁽¹⁾ - GERMANA LENTINI ⁽¹⁾ - FRANCESCO COPPOLINO ⁽²⁾ - AGATA FAMÀ ⁽¹⁾ - GIAMPIERO PIETROCOLA ⁽³⁾ - GIUSEPPE TETI ⁽⁴⁾ - CONCETTA BENINATI ⁽¹⁾

Università, Dipartimento di Patologia Umana, Messina, Italia ⁽¹⁾ - Università, Dipartimento di Scienze biomediche, odontoiatriche e delle immagini morfologiche e funzionali, Messina, Italia ⁽²⁾ - Università, Dipartimento di Medicina Molecolare, Pavia, Italia ⁽³⁾ - Scylla Biotech Srl, Scylla Biotech Srl, Messina, Italia ⁽⁴⁾

The BM 110, a hypervirulent strain of group B streptococci, exploits $\alpha_2\beta_1$ and $\alpha_3\beta_1$ integrins to invade human enterocytes

Giuseppe Valerio De Gaetano ¹, Germana Lentini ¹, Francesco Coppolino ², Agata Famà ¹, Giampiero Pietrocola ³, Giuseppe Teti ⁴, Concetta Beninati ^{1,4}

¹Department of Human Pathology, University of Messina, Messina, Italy; ²Department of Biomedical, Dental and Imaging Sciences, University of Messina, Messina, Italy; ³ Department of Molecular Medicine, Biochemistry Section, University of Pavia, Pavia, Italy; ⁴ Scylla Biotech Srl, Messina, Italy.

Introduction: Streptococcus agalactiae (group B streptococcus or GBS) is one of the main causes of neonatal infections. This pathogen is capable of adhering to polarized enterocytes and invading them through their basolateral surfaces. GBS penetration into enterocytes involves a caveolae- and lipid raft-dependent endocytic pathway associated with actin re-organization. Here, we describe the basolaterally located receptors whereby GBS promotes adherence to and invasion of host intestinal cells.

Materials and Methods: We examined the “hypervirulent” CC17 BM110 strain for its ability to interact with cultured intestinal epithelial cells in adhesion and invasion assays by using anti-integrin subunits antibodies. We also performed a structured-illumination fluorescence microscopy analysis for co-localization studies on interactions between bacteria and cell surface-expressed integrins. The Caco-2 cell line was used as in vitro model for resembling the intestinal epithelium. **Results:** Fluorescence microscopy analysis showed the presence of alpha 2 (α_2) and alpha 3 (α_3) integrin subunits predominantly along the basolateral surfaces of human enterocytes. The fluorescent streptococci, colocalized with alpha subunits, are significantly inhibited in their adherence capacity by antibodies directed against α_2 and α_3 integrin subunits. Antibodies against the common beta 1 (β_1) chain, were used to understand the involvement of GBS in the internalization process. GBS binding to integrins triggers the engulfment of bacteria in actin-coated vacuoles facilitating streptococcal trafficking inside enterocytes.

Discussion and Conclusions: Our data suggest that “hypervirulent” streptococci can colonize and invade the gut by targeting $\alpha_2\beta_1$ and $\alpha_3\beta_1$ integrins in the basolateral side of enterocytes. In clinical cases of GBS-mediated Late Onset Disease (LOD), the high virulence of CC17 BM110 strain has been associated with its capacity of colonizing the infant gut before reaching the blood-brain

barrier. In light of this, our results may be useful to develop new strategies to control neonatal GBS infections by preventing colonization and invasion of the intestinal mucosa.

120 - Pseudotyped lentiviral particles may help shed light on HIV maturation

GIULIA FREER⁽¹⁾ - GIULIA LOTTINI⁽²⁾ - ERIKA PLICANTI⁽²⁾ - ALESSIA CALÒ⁽¹⁾ -
MATTEO MATTEUCCI⁽¹⁾ - MICHELE LAI⁽¹⁾

University of Pisa, Centro Retrovirus, Pisa, Italia⁽¹⁾ - University of Siena, Dept. Medical
Biotechnologies, Siena, Italia⁽²⁾

Pseudotyped lentiviral particles may help shed light on HIV maturation

GIULIA FREER¹, GIULIA LOTTINI^{1, 2}, ERIKA PLICANTI^{1, 2}, ALESSIA CALO'¹, MATTEO
MATTEUCCI¹, MICHELE LAI¹

¹ Dept. Translational Research, Retrovirus Center, University of Pisa, Pisa, Italy

² Dept. Medical Biotechnologies, University of Siena, Siena, Italy

1. Introduction

Pseudotyped lentiviral particles (PLV) are modified lentiviruses where the viral envelope proteins are replaced with those from other viruses of interest. The envelope proteins determine viral tropism, allowing them to target specific cell types for gene delivery or other experimental purposes. PLVs have become valuable tools in gene therapy, as they can deliver genetic material into specific cell types at a high efficiency.

Because widely used PLVs are based on a Human Immunodeficiency Virus (HIV) backbone, their efficiency of transduction may be dependent on a process by which newly synthesized viral particles undergo structural changes to become infectious, i.e. maturation, that in HIV involves proteolysis of Gag/Gag-pol. Indeed, HIV assembles as an immature, non-infectious particle that contains the polyprotein precursors called Gag and Gag-Pol at the cell membrane. Once virions are released by budding, Gag is subsequently cleaved by the viral protease enzyme, resulting in the production of p24 capsid protein and consequent maturation of the viral capsid. In addition matrix and nucleocapsid proteins are also produced, together with the cleavage of Pol into reverse transcriptase, integrase and protease in the mature virions.

In this work, we developed HIV-based PLVs and attempted to shed light on the events that influence maturation of the viral capsid.

1. Materials and Methods

PLV were produced by transfecting HEK 293T with different packaging plasmids and PEI. PLV were collected after 72h and precipitation was performed by adding PEG80000 and NaCl to the final concentration of 10% and 0,75 M, respectively, to cell supernatants. These were incubated at 4°C overnight and centrifuged at 10.000×g. Western blotting was performed on precipitated PLVs using antibodies against HIV p24, VSVG and SARS-CoV-2 Spike.

2. Results

An optimized protocol was set up to improve the transduction efficiency of PLVs pseudotyped with several envelope glycoproteins (Vesicular stomatitis virus, SARS-CoV-2 and others). The PLVs obtained by the best and the worst protocol were precipitated by PEG8000 and compared in terms of HIV p24 content by western blot, as a measure of their degree of maturation.

3. Discussion and Conclusions

PLVs produced by different protocols differed in yield, whereas the percentage of mature PLV particles was found to depend on envelope. Understanding the molecular mechanisms involved in HIV maturation may be crucial for the development of novel antiretroviral agents that target the maturation process.

132 - Fimbriae and Adhesins in clinical, Extremely Drug-Resistant *Providencia stuartii* Isolates producing NDM-1

VALERIO CAPITANI⁽¹⁾ - MIRIANA QUARANTA⁽²⁾ - GABRIELE ARCARI⁽³⁾ - CAROLINA MARZUILLO⁽¹⁾ - ALESSANDRA CARATTOLI⁽³⁾

Università La Sapienza, Dipartimento di Sanità Pubblica e Malattie Infettive, Roma, Italia⁽¹⁾ -

Università La Sapienza, Dipartimento di Scienze Biochimiche A. Rossi Fanelli, Roma, Italia⁽²⁾ -

Università La Sapienza, Dipartimento di Medicina Molecolare, Roma, Italia⁽³⁾

Fimbriae and Adhesins in clinical, Extremely Drug-Resistant *Providencia stuartii* Isolates producing NDM-1

Valerio Capitani¹, Miriana Quaranta², Gabriele Arcari³, Carolina Marzuillo¹, Alessandra Crattoli³

1 Dipartimento di Sanità Pubblica e Malattie Infettive; Laboratorio di Epidemiologia Molecolare; Università La Sapienza; Roma; Italia

2 Dipartimento di Scienze Biochimiche A. Rossi Fanelli; Università La Sapienza; Roma; Italia

3 Dipartimento di Medicina Molecolare; Università La Sapienza; Roma; Italia

Introduction

Providencia stuartii is an opportunistic pathogen notorious for its ability to cause respiratory and urinary tract infections. Owing to its capacity to form biofilm in catheterized patients and its intrinsic resistance to last-resort antibiotics such as imipenem, colistin and tigecyclin, it has been described as cause of outbreaks in hospital settings.

Fimbriae are the most common structure dedicated to adhesion in members of the Enterobacterales order and are considered important virulence factors. Adhesins are component of fimbriae that allow adhesion of microorganisms to human epithelia, enabling the initiation of the infection process.

Materials and Methods

Seven *P. stuartii* strains were collected at the Policlinico Umberto I Hospital in Rome during routine microbiologic processes. Isolated colonies were identified by MALDI-TOF and three were subjected to Illumina and Oxford Nanopore Technologies sequencing.

Genomes were annotated using RAST. Manual curation was performed to identify fimbrial clusters. A comparative analysis of ushers and adhesins were performed with those obtained from the NCBI Protein Database and Protein Data Bank (PDB). Phylogenetic trees were generated with IQ-TREE. UniProt was used to run sequence similarity searches, and PDB for structural homology searches. Structural domains were pinpointed with SCOP and InterPro databases. Structural models were built through the computational modeling software Rosetta.

Results

A total of 20 recognizable fimbrial clusters, belonging to the Chaperon-Uscher type and typical of the *Providencia* genus, were identified. The genes were generically annotated by programs as encoding fimbrial, uscher, and chaperone proteins, but there is a high variability on the exact type of fimbriae to which they belong, and adhesins were often misidentified.

In depth computational modeling analyses were able to identify numerous genes coding for proteins with a plausible role in the adhesion process, characteristic of the *Providencia* genus yet previously undetected in *P. stuartii* species.

Discussion and Conclusions

Structural model analyses of the identified adhesins highlighted their high similarity with proteins important for the processes of pathogenesis and biofilm formation (MrkD, FimH, LpfD, PapG, MrpH). However, *P. stuartii* fimbrial clusters and adhesins are frequently wrongly annotated, plausibly owing to their high sequence divergence with those of other Enterobacterales.

The high variety of adhesins types that can be associated with those identified in *P. stuartii*, may help explain the high ability of this microorganism to adhere and form biofilms on various surfaces, as well as to interact and infect not only the urinary system of catheterized patients, but various tissues of the human body.

144 - The serine-rich repeat glycoprotein Srr2 from *Streptococcus agalactiae* acts as an adhesin for the human fibronectin

ANGELICA PELLEGRINI⁽¹⁾ - GIULIA BARBIERI⁽²⁾ - CHIARA MOTTA⁽¹⁾ - GIAMPIERO PIETROCOLA⁽¹⁾

Università di Pavia, Dipartimento di Medicina Molecolare, Pavia, Italia⁽¹⁾ - Università di Pavia, Dipartimento di Biologia e Biotecnologie "Lazzaro-Spallanzani", Pavia, Italia⁽²⁾

The serine-rich repeat glycoprotein Srr2 from *Streptococcus agalactiae* acts as an adhesin for the human fibronectin

Angelica PELLEGRINI¹, Giulia BARBIERI², Chiara MOTTA¹, Giampiero PIETROCOLA¹

1, Department of Molecular Medicine, Unit of Biochemistry, University of Pavia, Pavia, (Italy); 2, Department of Biology and Biotechnology "Lazzaro-Spallanzani", University of Pavia, Pavia, (Italy)

Introduction: Group B *Streptococcus* (GBS) is an opportunistic pathogen that can cause severe diseases in pregnant women, newborns and elderly adults. GBS displays several virulence factors, such as adhesins that promote colonization and invasion of the host. Among these, the cell-wall anchored protein Srr2 binds fibrinogen (Fbg) through a dock, lock and latch (DLL) mechanism and plasminogen (Plg). The DLL mechanism is based on conformational changes of the N2 and N3 subdomains of Srr2 upon ligand binding. In this study, we demonstrate that GBS exploits the adhesin Srr2 to bind fibronectin (Fn), a multidomain glycoprotein ubiquitously present in the human tissues, where it promotes cell adhesion and migration. Moreover, we show that Fn promotes GBS adhesion to cervical epithelial cells' monolayer.

Materials and methods: GBS ability to bind Fn was investigated through ELISA assay using the BM110 wild-type (wt) strain and its derivative delta-srr2 mutant strain. Investigation of Srr2 mechanism of Fn binding was performed by ELISA assay using recombinant wt or mutated binding regions (BR) of Srr2, purified through affinity chromatography. The Fn domain bound by Srr2-BR was detected through dot-blot assay and western-blot of recombinant Srr2-BR-conjugated microspheres incubated with Fn or Fn fragments. Adhesion assays were conducted by incubating human cervical cell (HeLa) monolayers with mid-log phase GBS or GBS delta-srr2 cells and by plating lysed cells to enumerate adhered bacteria.

Results: We show that Srr2 promotes GBS binding to Fn. Deletion of srr2 strongly decreased GBS ability to bind Fn in ELISA assay. Moreover, the recombinant Srr2-BR binds Fn at the level of the 110 kDa cell-binding Fn fragment. Mutation of recombinant Srr2-BR did not abolish Srr2 ability to bind Fn, suggesting a mechanism of Srr2 binding of Fn that differs from the Srr2 DLL binding of Fbg. Finally, addition of exogenous Fn to HeLa cells' monolayers increased GBS adhesion levels to the host cells.

Discussion and Conclusions: Our data suggest that Srr2 plays an important role in GBS adhesion to Fn and demonstrate the importance of fibronectin in mediating GBS adhesion to the host. In light of this, our results may be useful to develop new alternative strategies to control GBS infection preventing colonization of epithelium.

This research was supported by EU funding within the NextGenerationEU-MUR PNRR Extended Partnership initiative on Emerging Infectious Diseases.

149 - *Acinetobacter baumannii* OmpA-like porins: functional characterization in bacterial physiology, antibiotic-resistance, and virulence

DANIELA SCRIBANO ⁽¹⁾ - ARIANNA POMPILIO ⁽²⁾ - ELENA CHERI ⁽³⁾ - MANUEL BELLI ⁽⁴⁾ - LUIGI SANSONE ⁽⁴⁾ - MARIO CRISTINA ⁽⁴⁾ - MEYSAM SARSHAR ⁽³⁾ - CARLO ZAGAGLIA ⁽¹⁾ - GIOVANNI DI BONAVENTURA ⁽²⁾ - ANNA TERESA PALAMARA ⁽⁵⁾ - CECILIA AMBROSI ⁽⁶⁾

Sapienza University of Rome, Department of Public Health and Infectious Diseases,, Roma, Italia ⁽¹⁾ - “G. d’Annunzio” University of Chieti-Pescara, Center for Advanced Studies and Technology (CAST), Department of Medical, Oral and Biotechnological Sciences, Chieti, Italia ⁽²⁾ - Bambino Gesù Children’s Hospital, IRCCS, Research Laboratories, Roma, Italia ⁽³⁾ - IRCCS San Raffaele Roma, MEBIC Consortium, Roma, Italia ⁽⁴⁾ - Sapienza University of Rome, Department of Public Health and Infectious Diseases, Sapienza University of Rome, Laboratory Affiliated to Institute Pasteur Italia-Cenci Bolognetti, Roma, Italia ⁽⁵⁾ - San Raffaele Open University, IRCCS, Department of Human Sciences and Promotion of the Quality of Life, Roma, Italia ⁽⁶⁾

Acinetobacter baumannii OmpA-like porins: functional characterization in bacterial physiology, antibiotic-resistance, and virulence

Daniela Scribano¹, Arianna Pompilio², Elena Cheri³, Manuel Belli⁴, Luigi Sansone⁴, Mario Cristina⁴, Meysam Sarshar³, Carlo Zagaglia¹, Giovanni Di Bonaventura², Anna Teresa Palamara^{5,6}, Cecilia Ambrosi⁷

¹Department of Public Health and Infectious Diseases, Sapienza University of Rome, Rome, Italy;

²Center for Advanced Studies and Technology (CAST), Department of Medical, Oral and Biotechnological Sciences, “G. d’Annunzio” University of Chieti-Pescara, Chieti, Italy; ³Research Laboratories, Bambino Gesù Children’s Hospital, IRCCS, Rome, Italy; ⁴MEBIC Consortium, IRCCS San Raffaele Roma, Rome, Italy; ⁵Department of Public Health and Infectious Diseases, Sapienza University of Rome, Laboratory Affiliated to Institute Pasteur Italia-Cenci Bolognetti Foundation, Rome, Italy; ⁶Department of Infectious Diseases, Istituto Superiore di Sanità, Rome, Italy; ⁷Department of Human Sciences and Promotion of the Quality of Life, San Raffaele Open University, IRCCS, Rome, Italy.

The outer membrane protein A (OmpA) plays a prominent role in biofilm formation, host cell infection, antibiotic resistance and immunomodulation in *Acinetobacter baumannii*. Combinations of OmpA and other outer membrane proteins (OMPs) enhance animal protection against *A. baumannii* infections. However, there are substantial gaps in our knowledge on *A. baumannii* OMPs. This study aimed to elucidate the role of OmpA-like proteins in *A. baumannii* strain AB5075 by means of isogenic mutants [ABUW_0505 (Δ psaB), ABUW_1015 (Δ carO), ABUW_2730 (Δ arfA), and ABUW_3045 (Δ yiaD)]. The wild type and ompA mutant were included as positive and negative controls, respectively. No differences in the antibiogram profile were detected. All mutants displayed a growth defect and lost twitching motility. Intriguingly, Δ psaB showed an increased cell stiffness and permeability. Mutations in psaB, arfA, and yiaD loci led to a reduced cell envelope thickness. All mutants but Δ psaB were less adherent to lung epithelial cells. Δ arfA demonstrated a reduced biofilm-forming activity. A decreasing gradient of in vivo virulence was observed for all the mutants. The lack of PsaB, ArfA and YiaD significantly increased stress tolerance to human sera. However, the lack of PsaB results in increased tolerance to a number of different stresses. Our data indicate that

PsaB has a crucial role in membrane fluidity that is critical in overcoming environmental stresses. ArfA contributes to the physiological cell envelope structure, aiding host cell adhesion. YiaD is involved in host-pathogen interactions. Hence, this study provides detailed clues about the role of under and not characterized yet OMPs in *A. baumannii*.

168 - Invasive meningococcal disease caused by rare capsular serogroups in Italy: genomic investigation of virulence genes associated with pathogenesis

PAOLA VACCA ⁽¹⁾ - CECILIA FAZIO ⁽¹⁾ - ARIANNA NERI ⁽¹⁾ - LUIGINA AMBROSIO ⁽¹⁾ - ANNA CARANNANTE ⁽¹⁾ - FLORIGIO ROMANO LISTA ⁽²⁾ - SILVIA FILLO ⁽²⁾ - ANDREA CIAMMARUCONI ⁽²⁾ - ANTONELLA FORTUNATO ⁽²⁾ - PAOLA STEFANELLI ⁽¹⁾

ISTITUTO SUPERIORE DI SANITA', DIPARTIMENTO MALATTIE INFETTIVE, ROME, Italia
⁽¹⁾ - ARMY MEDICAL RESEARCH CENTRE, SCIENTIFIC DEPARTMENT, ROME, Italia ⁽²⁾

Invasive meningococcal disease caused by rare capsular serogroups in Italy: genomic investigation of virulence genes associated with pathogenesis

Paola Vacca ^a, Cecilia Fazio ^a, Neri Arianna ^a, Luigina Ambrosio ^a, Anna Carannante ^a, Florigio R. Lista ^b, Silvia Fillo ^b, Andrea Ciammaruconi ^b, Antonella Fortunato ^b, and Paola Stefanelli ^a.

^a Department Infectious Diseases, Vaccine-Preventable Diseases (VPD) - Reference Lab Unit, Istituto Superiore di Sanità, Rome Italy; ^b Scientific Department-Army Medical Research Centre, Rome, Italy

Introduction

The polysaccharide capsule is one of the major virulence factor of *Neisseria meningitidis*. The majority of invasive *N. meningitidis* isolates are encapsulated, with serogroups A, B, C, Y, W and X being the most responsible of invasive diseases (IMD). In contrast, meningococci classified as nongroupable (NG), including the capsul null locus (cnl), and those belonging to other serogroups E, H, L, Z are rarely reported. Globally, IMD due to rare serogroups are often associated with immunocompromised patients, although cases have been reported in patients with no known immunocompromising conditions. The aim was to characterize the rare invasive capsular group meningococci causing IMD in Italy and to investigate a set of genes associated with pathogenesis. A set of virulence factors such as adhesins, lipooligosaccharide endotoxins (LOS), and iron acquisition systems are also reported and evaluated.

Materials and Methods

Bacterial isolates and biological samples from IMD are collected and characterized at the Department of Infectious Diseases of Istituto Superiore di Sanità. Serogroup was molecularly determined. Genotypic profiles and genomic comparison were obtained using PubMLST platform (<http://pubmlst.org/neisseria/>).

Results

From 2005 to 2022, 13 invasive meningococcal diseases (IMD) caused by rare serogroups have been identified: 7 NG, 4 cnl, and 2 serogroup E. Two patients were vaccinated against meningococcal serogroups A, C, W, Y. Sepsis and meningitis were the main clinical presentations. All patients survived, except for one. However, no data regarding the immune status of patients affected were available. Meningococci showed different genotypic profiles belonging to clonal complexes cc23, cc60, cc175, cc1136, and cc1157. Genomic comparison with an international collection of NG, cnl and capsule group E genomes (both invasive and carriage meningococcal genomes available on PubMLST database) confirmed the clusterization by clonal complexes, with intermixed invasive and carriage genomes. Furthermore, genomic analysis of a set of genes mostly associated with virulence patterns such as those encoding for pilus, LOS, and iron acquisition highlighted the presence of genes already known among the most frequent invasive meningococci serogroups.

Discussion and Conclusions

The reported IMD caused by rare meningococcal isolates presented the main virulence genes associated with the invasiveness of meningococci. Detailed molecular surveillance is key in monitoring changes in the epidemiology and pathogenicity of invasive meningococcal disease. Further work is needed to determine if the subunit protein antimeningococcal B vaccines might provide broader protection against these rare meningococci.

170 - The study of the Unfolded Protein Response and Herpes Simplex Virus infection in neuronal cell line

PAOLA PERRERA ⁽¹⁾

Università di Pisa, Dipartimento di Ricerca Traslationale, Pisa, Italia ⁽¹⁾

The study of the Unfolded Protein Response and Herpes Simplex Virus infection in neuronal cell line

PAOLA PERRERA ^{1,2}, CARMEN RITA PIAZZA ^{2,3}, PAOLA QUARANTA ², MICHELE LAI ², GIULIA FREER ², MAURO PISTELLO ^{2,4}

Affiliations: ¹Department of Translational Research, Retrovirus Center and Virology Section, University of Pisa, Pisa, Italy; ²Centro Retrovirus, University of Pisa, Pisa, Italy; ³Department of Medical Biotechnologies, University of Siena, Siena, Italy; ⁴Virology Operative Unit, University Hospital of Pisa, Pisa, Italy.

Introduction: Several stress stimuli such as calcium depletion, hypoxia, DNA damage, chemical agent, accumulated proteins, and viral infection activate the Unfolded Protein Response (UPR). This mechanism, mediated by ATF6, IRE1 and p_{er}2 α , preserves cellular homeostasis due to the attenuation of protein synthesis, the increase chaperone synthesis, the expand of endoplasmic reticulum surface area, and the degradation of misfolded proteins; if the UPR is unable to restore the homeostasis, the cell activates apoptosis. The rapid synthesis of viral proteins, during cell infection, causes an activation of the UPR but this mechanism could limit viral replication. For this reason, many viruses have evolved strategies to manipulate different aspects of the host response.

Herpes Simplex Virus 1 (HSV1) is a widely spread neurotropic virus and its lytic cycle occurs at the level of epithelial cells; after primary infection, the virus becomes latent in neurons of the peripheral nervous system and can be periodically reactivated. This work aims to analyze the UPR in neuron cell line after HSV1 infection. We will examine if the cellular factors involved in the response follow the infection and how the virus modulate the host response to promote its replication.

Materials and Methods: As a model to evaluate the interact between this mechanism and the virus, we used neuroblastoma cells and we differentiated the cells with Retinoic Acid; after one week of differentiation, we infected the cells with HSV1 and collected the cells at different time point after infection. We used western blot and RT-PCR experiments to investigate the proteins involved in the UPR and the viral proteins of Herpes Simplex Virus to understand how the virus can use this cellular machinery for its replicative cycle.

Results: HSV1 infection modulates the proteins involved in the UPR already in the first hours of infection. In particular, the ATF6 and IRE1 pathways are reduced; on the contrary, the phosphorylation status of the eif2 α factor was found to be increased. We are trying to understand which Herpes Simplex Virus protein is involved in this modulation.

Discussion and Conclusion: The UPR is a mechanism that maintains cellular homeostasis, but it is dysregulated in many pathologies, such as neurodegenerative diseases. HSV1 has an important impact on the nervous system for several reasons; it is a neurotropic virus; it establishes a lifelong latent infection in neurons, and it may be associated with neurodegeneration. Several studies show that HSV1, upon reactivation, could reach the central nervous system and bring neuronal damage like that found in neurodegeneration. The study of the UPR could become an important link between viral infection and neurodegenerative diseases.

175 - Unveiling the role of NAAA enzyme and peroxisome regulation in HSV-1 infection

CAROLINA FILIPPONI⁽¹⁾ - ELENA IACONO⁽²⁾ - VERONICA LA ROCCA⁽³⁾ - GIULIA SCIANDRONE⁽¹⁾ - ALESSANDRO DE CARLI⁽²⁾ - GIULIA FREER⁽¹⁾ - MAURO PISTELLO⁽¹⁾ - MICHELE LAI⁽¹⁾

Università di Pisa, Dipartimento di Ricerca Traslazionale e delle Nuove Tecnologie in Medicina e Chirurgia, Pisa, Italia⁽¹⁾ - Università di Siena, Dipartimento di Biotecnologie Mediche, Siena, Italia⁽²⁾ - Institute of Life Sciences, Scuola Superiore Sant'Anna, Pisa, Italia⁽³⁾

Unveiling the role of NAAA enzyme and peroxisome regulation in HSV-1 infection

CAROLINA FILIPPONI¹, ELENA IACONO^{1,2}, VERONICA LA ROCCA^{1,3}, GIULIA SCIANDRONE¹, ALESSANDRO DE CARLI^{1,2}, GIULIA FREER¹, MAURO PISTELLO^{1,4}, MICHELE LAI¹

¹Retrovirus Center, Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa, Pisa, Italy; ²Department of Medical Biotechnologies, University of Siena, Siena, Italy; ³Institute of Life Sciences, Sant'Anna School of Advanced Studies, Pisa, Italy; ⁴Department of Clinical and Experimental Medicine, University of Pisa, Pisa, Italy.

Introduction: Peroxisomes are highly dynamic and multifunctional organelles that play crucial roles in controlling fatty acid oxidation, reactive oxygen species detoxification, phospholipid synthesis, and innate immune responses. Despite their significant importance in cellular metabolism, peroxisome biology is understudied in the context of viral infections. Herpes Simplex Virus 1 (HSV-1) replication has been found to depend on peroxisome proliferation, with Peroxisome Proliferator-Activated Receptor alpha (PPAR-alpha) being the most implicated member of the PPAR family. The endogenous ligand most commonly associated with PPAR-alpha is Palmitoylethanolamide (PEA), and its regulation is controlled by the N-Acylethanolamine Acid Amidase (NAAA) enzyme, which catalyzes the hydrolysis of PEA into palmitic acid and ethanolamine. Based on these findings, our research focuses on investigating the role of NAAA enzyme and the mechanisms underlying peroxisome regulation during HSV-1 productive infection and during latency.

Materials and Methods: We infected SH-SY5Y, and A549 cells using HSV-1. We performed qRT-PCR to evaluate transcript abundance of NAAA, PPAR-alpha and peroxins (PEXs), and we confirmed the results using western blots on cell lysates. We performed confocal microscopy using Operetta CLS to analyze the number HSV-1 infected cells and the number and shape of peroxisomes.

Results: We observed a 3-fold increase in PPAR-alpha expression 24 or 48h after HSV-1 infection, accompanied by an upregulation in the expression of peroxins involved in peroxisome biogenesis. Surprisingly, contrary to our initial expectations, we discovered a remarkable over ~300 times upregulation of NAAA expression that increases at the late stage of infection (48h). Despite this, NAAA ablation coincides with a 3-fold increased HSV-1 viral load, suggesting a possible role of NAAA enzyme in the cell defense mechanism against HSV-1 infection.

Discussion: In conclusion, our study provides novel insights into the complex interplay between peroxisome dynamics, PPAR-alpha, NAAA and HSV-1 infection. The upregulation of PPAR-alpha and NAAA expression, as well as the modulation of peroxins, highlight the importance of peroxisomes in the cellular response to viral infections. It is possible to speculate that NAAA might

be a host target for HSV-1 and its activity might regulate persistence and latency of Herpesviridae family.

181 - In vivo virulence study of *Listeria monocytogenes* with truncated Internalin A in *Galleria mellonella* larvae

MARIA GORI⁽¹⁾ - EMERENZIANA OTTAVIANO⁽¹⁾ - MATTEO MARSIGLIA⁽²⁾ - SILVIA ANCONA⁽¹⁾ - SILVIA BIANCHI⁽¹⁾ - ELISABETTA TANZI⁽¹⁾ - ELISA BORGHI⁽¹⁾

Università degli Studi di Milano, Dipartimento di Scienze della Salute, Milano, Italia⁽¹⁾ - Università degli Studi di Milano, Department of Biomedical and Clinical Sciences, Milano, Italia⁽²⁾

In vivo virulence study of *Listeria monocytogenes* with truncated Internalin A in *Galleria mellonella* larvae

MARIA GORI¹, EMERENZIANA OTTAVIANO¹, MATTEO D. MARSIGLIA², SILVIA ANCONA¹, SILVIA BIANCHI¹, ELISABETTA TANZI¹, ELISA BORGHI¹

1. Department of Health Sciences, Università degli Studi di Milano, Milan (Italy);

2. Department of Biomedical and Clinical Sciences, Università degli Studi di Milano, Milan (Italy)
Introduction

Listeria monocytogenes (Lm) is one of the most virulent foodborne pathogens causing septicemia, meningitis, abortion, and death in susceptible populations. A key role in determining the virulence of Lm is played by Internalin A (InlA), a protein necessary to cross the intestinal barrier and establish a systemic infection. The truncated InlA form resulting from inlA premature stop codons (PMSCs) mutations is typically associated with attenuated virulence and is common among hypovirulent sequence types (STs). Yet, truncated InlA is reported to enhance biofilm formation. We aimed at assessing differences in the virulence and biofilm formation of Lm strains with truncated and full-length InlA.

Materials and Methods

We selected 4 clinical Lm strains isolated from severe cases of listeriosis identified as ST2 (hypervirulent) or ST121 (hypovirulent) carrying truncated or full-length InlA. For the biofilm assay, a standard inoculum of 10⁶ cells/mL in BHI has been used and biofilm biomass quantified by CV staining. Lm virulence was assessed in vivo using the *Galleria mellonella* (Gm) invertebrate model. Gm larvae/group were inoculated into the hemocoel with Lm suspension (approximately 5×10⁵ cells/larva), incubated at 37 °C up to 3 days and scored daily for survival. Larvae injected with phosphate buffered saline (PBS) were used as a control.

Results

The ability to form biofilm in vitro resulted stronger in ST121 strains, regardless the InlA form. On the contrary, the in vivo mortality was higher in larvae injected with strains harbouring full-length InlA than in those injected with truncated form of InlA (Figure 1). ST2 strain with full-length InlA exhibited the highest virulence, killing most of the larvae within 24 hours and 100% within 72 hours.

Discussion and Conclusions

Despite the direct injection of Lm suspension into the larval hemocoel may bypass the need for InlA function, our results in Gm demonstrated differences in virulence between Lm strains, with a higher killing potential in those carrying full-length InlA. ST2 strains showed a higher and faster killing capacity as compared to ST121, thus confirming its higher virulence potential in human infections. Truncated InlA is expected to attenuate Lm virulence, but infections with Lm carrying InlA protein

with a PMSC is not sufficient to prevent severe form of listeriosis. Biofilm formation was shown to be dependent on ST more than the presence of the truncated InlA. Overall, these results underline the need to better understand Lm virulence mechanisms and will serve as a basis for future works investigating the role of other virulence factors in Lm pathogenicity.

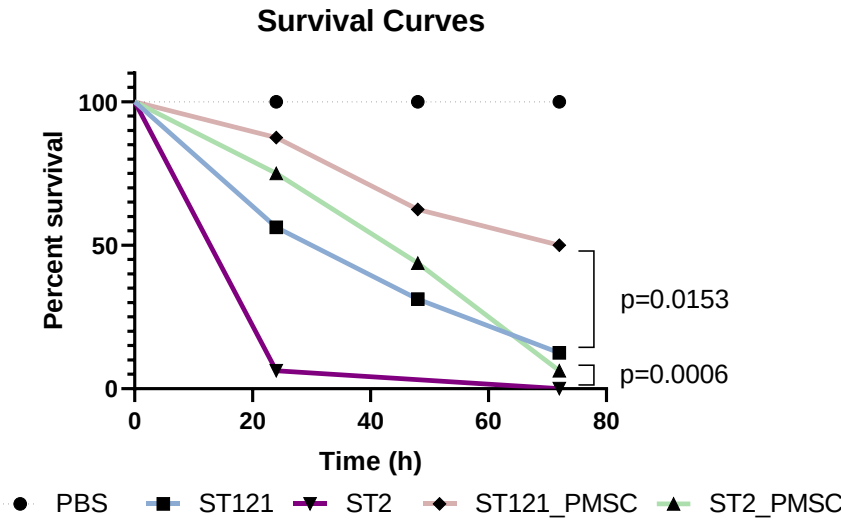


Figure 1. *G. mellonella* larvae infected with *Lm* with full-length Internalin A or the truncated form (PMSC).

194 - Analysis of viral variants associated with high-risk Human Papillomavirus (HPV) persisting infection

MARIANNA MARTINELLI⁽¹⁾ - CHIARA GIUBBI⁽¹⁾ - CHIARA VILLA⁽¹⁾ - RUTH NJOKU⁽²⁾ - FEDERICA PERDONI⁽¹⁾ - MARIA LETIZIA DI MEO⁽³⁾ - ROSARIO MUSUMECI⁽¹⁾ - ILLARI SECHI⁽²⁾ - NARCISA MURESU⁽²⁾ - ANDREA PIANA⁽²⁾ - ROBERT FRUSCIO⁽¹⁾ - FABIO LANDONI⁽¹⁾ - CLEMENTINA COCUZZA⁽¹⁾

University of Milano-Bicocca, Department of Medicine and Surgery, Monza, Italia⁽¹⁾ - University of Sassari, Department of Biomedical Sciences, Sassari, Italia⁽²⁾ - ASST Monza, San Gerardo Hospital, Monza, Italia⁽³⁾

Analysis of viral variants associated with high-risk Human Papillomavirus (HPV) persisting infection

MARIANNA MARTINELLI¹, CHIARA GIUBBI¹, CHIARA VILLA¹, RUTH C. NJOKU^{1,2}, FEDERICA PERDONI¹, MARIA LETIZIA DI MEO³, ROSARIO MUSUMECI¹, ILLARI SECHI², NARCISA MURESU², ANDREA F. PIANA², ROBERT FRUSCIO^{1,3}, FABIO LANDONI^{1,3}, CLEMENTINA E. COCUZZA¹

¹ Department of Medicine and Surgery, University of Milano-Bicocca, Monza, Italy;

² Department of Biomedical Sciences, University of Sassari, Sassari, Italy;

³ San Gerardo Hospital, ASST Monza, Monza, Italy;

Introduction

Cervical cancer is well known to be related to persisting infection of high-risk Human Papillomavirus (hrHPV). hrHPV infections are very common among women population but just a very few cases continue to persist and to lead to the development of pre-malignant lesions and cancer. Different potential biomarkers related to hrHPV infection persistence are under investigation and one of them is the possible role of intragenotype viral variants in hrHPV infections. This ongoing study aims to analyse the different hrHPV viral variants in women with persistent infection.

Materials and Methods

Presently, 335 women referred for colposcopy at the Colposcopy Clinic, San Gerardo Hospital (Monza, Italy) have been enrolled in the study and 41 returned for the first and second follow-up (FU) visit at different time points depending on the clinical outcome. Cervical samples were collected from each woman using L-shaped Endo/Esocervical FLOQSwab (Copan) and tested using Anyplex™II HR HPV (Seegene) assay to detect hrHPV genotype-specific infection. Viral variants of HPV16, HPV18, HPV31 and HPV52 have presently being investigated. Previously described PCR protocols were used to obtain amplicons of Upper Regulatory Region (URR), E6 and/or E7. Phylogenetic tree analysis was performed using MEGA 7 bioinformatics software.

Results

Overall, hrHPV positivity was found in 65.1% (218/335) of women referred to colposcopy. Persisting infection was found in 41.5% (17/41) of women and HPV16 and HPV31 the two most common persisting genotypes (7/17, 41.1% and 4/17, 23.5%; respectively). Preliminary data

obtained from sequences analysis of samples collected from women with persisting infections showed a predominance of A2 lineage for HPV16, A4 for HPV18, C for HPV31 and A1 for HPV52, respectively.

Discussion and Conclusions

Preliminary results have shown a high percentage of persisting infections among women referred to colposcopy and followed up, even after treatment. This study contributes to the implementation of data regarding the molecular epidemiology of HPV lineage variants. Future analysis on a greater number of samples and functional studies to understand how specific mutations can be linked to persisting infections will permit to better understand the role of HPV intra-type variants in cervical lesions progression.

202 - Sudden and unexplained death of an 18-month-old healthy boy due to interstitial pneumonia with multiple superinfections

ANNA-LISA CAPRIA ⁽¹⁾ - MARIA ELENA FILICE ⁽²⁾ - PIETRO GIORGIO SPEZIA ⁽³⁾ - MICHELE LAI ⁽³⁾ - VINCENZO NARDINI ⁽²⁾ - PAOLA MAZZETTI ⁽¹⁾ - ARIANNA PRECISI O PROCISSI ⁽⁴⁾ - MAURO PISTELLO ⁽¹⁾

Pisa University Hospital, Virology Division, Pisa, Italia ⁽¹⁾ - Pisa University Hospital, Pathological Anatomy Division 2, Pisa, Italia ⁽²⁾ - University of Pisa, Department of Translation, Retrovirus Center and Virology Section, Pisa, Italia ⁽³⁾ - Pisa University Hospital, Transplant Laboratory, Pisa, Italia ⁽⁴⁾

Sudden and unexplained death of an 18-month-old healthy boy due to interstitial pneumonia with multiple superinfections

Anna-Lisa Capria¹, Maria Elena Filice³, Pietro Giorgio Spezia², Michele Lai², Vincenzo Nardini³, Paola Mazzetti¹, Arianna Precisi o Procissi ⁴, Mauro Pistello^{1,2}

¹ Virology Division, Pisa University Hospital, Pisa, Italy;

² Department of Translation, Retrovirus Center and Virology Section, University of Pisa, Pisa, Italy;

³ Pathological Anatomy Division 2, Pisa University Hospital, Pisa, Italy;

⁴ Transplant Laboratory, Pisa University Hospital, Pisa, Italy.

Introduction:

In early autumn 2019 an emergency call was performed for a child that failed to wake up; he experienced a febrile episode and loss of appetite. Despite advanced cardiac resuscitation, was pronounced dead. An autopsy was requested to determine the cause of death.

Materials and methods:

On October 11th 2019, a comprehensive autopsy was conducted on the child's body. X-ray imaging assessed lung condition. Macroscopic examination on all body cavities revealed pleural effusion and enlarged lungs and brain; samples of tissues for histological examination was carried out. Various samples, such as swabs, blood, CSF, pleural effusions and lung biopsies, underwent microbiological and virological analyses. One year later, paraffin-embedded lung sections were tested for the presence of SARS-CoV-2 RNA and N and S proteins using real-time PCR (qRT-PCR)(Allplex Seegene) and immunofluorescence with High-content confocal microscopy (Operetta CLS). Analysis of whole blood for IgG and IgM antibodies against SARS-CoV-2 was also conducted to assess immunological response against SARS-CoV-2 infection.

Results:

X-rays showed honeycombing of the lungs. Histological analysis showed interstitial pneumonia and DAD with acute inflammation and fungal hyphae, spores and bacterial colonies in the alveolar spaces. Microbiological and virological investigations identified *S. aureus* in bronchoaspiration and lung biopsy, *E. coli* K1 in CSF and lung biopsy, and *C. albicans* in lung biopsy and blood. qRT-PCR detected low levels of positivity for the E and N genes of SARS-CoV-2. Confocal microscopy

confirmed the presence of the SARS-CoV-2 N protein in lung tissue but no positivity for the S protein. Moreover, no anti-SARS-CoV-2 IgG or IgM antibodies were found.

Discussion and Conclusions:

Despite the positive molecular test for SARS-CoV-2, the viral load was low to reach NGS sequencing. Besides, the quality of RNA extract from paraffin-embedded tissues limits further analysis. While fluorescence microscopy provided valuable insights, the presence of the N protein and the absence of S protein staining do not definitively exclude the possibility of cross-reactivity with fluorescent antibodies. The lack of an antibody response may be attributed to the limited time available for antibody development, as the child succumbed to the disease before an adequate immune response could occur.

In conclusion, the final diagnosis indicates an unexpected infant death due to hyperacute pneumonia, characterised by the presence of granulocytic infiltrate in the alveoli, interstitial lymphocytic infiltrate in the surrounding areas and sporadic cases of fungal pneumonia. It is also highly likely that co-existing SARS-CoV-2 infection played a significant role in the child's rapid death.

213 - *Neisseria meningitidis* activates pyroptotic pathways in a mouse model of meningitis: role of a two-partner secretion system.

CHIARA PAGLIUCA⁽¹⁾ - ELENA SCAGLIONE⁽²⁾ - GIUSEPPE MANTOVA⁽¹⁾ - SILVIA CATERINA RESTA⁽³⁾ - CATERINA PAGLIARULO⁽⁴⁾ - CECILIA BUCCI⁽³⁾ - PIETRO ALIFANO⁽³⁾ - PAOLA SALVATORE⁽⁵⁾ - ROBERTA COLICCHIO⁽²⁾

University of Naples Federico II, Department of Molecular Medicine and Medical Biotechnology, Naples, Italia⁽¹⁾ - University of Naples Federico II, Department of Molecular Medicine and Medical Biotechnology; Dep. of Int. Activity of Laboratory Medicine and Trans. UOC Clinical Microbiology, Naples, Italia⁽²⁾ - University of Salento, Department of Biological and Environmental Sciences and Technologies, Lecce, Italia⁽³⁾ - University of Sannio, Department of Science and Technology, Benevento, Italia⁽⁴⁾ - University of Naples Federico II, Department of Molecular Medicine and Medical Biotechnology; Dep. of Int. Act. of Lab. Med. and Trans. UOC Clin. Micr.; CEINGE Franco Salvatore, Naples, Italia⁽⁵⁾

Neisseria meningitidis activates pyroptotic pathways in a mouse model of meningitis: role of a two-partner secretion system.

CHIARA PAGLIUCA¹, ELENA SCAGLIONE^{1,2}, GIUSEPPE MANTOVA¹, SILVIA C. RESTA³, CATERINA PAGLIARULO⁴, CECILIA BUCCI³, PIETRO ALIFANO³, PAOLA SALVATORE^{1,2,5}, ROBERTA COLICCHIO^{1,2}.

¹Department of Molecular Medicine and Medical Biotechnology, University of Naples Federico II, Naples, Italy; ²Department of Integrated Activity of Laboratory Medicine and Transfusion, Complex Operative Unit of Clinical Microbiology, University Hospital Federico II, Naples, Italy; ³Department of Biological and Environmental Sciences and Technologies, University of Salento, Lecce, Italy; ⁴Department of Science and Technology, University of Sannio, Benevento, Italy; ⁵CEINGE, Advanced Biotechnologies s.c.ar.l., Naples, Italy.

Introduction. There is evidence that in infected cells in vitro the meningococcal HrpA/HrpB two-partner secretion system (TPS) mediates the exit of bacteria from the internalization vacuole and the docking of bacteria to the dynein motor resulting in the induction of pyroptosis, a form of pro-inflammatory cell death which may be involved in the inflammation characteristic of invasive meningococcal disease. In this study we set out to study the role of the HrpA/HrpB TPS in establishing meningitis and activating pyroptotic pathways in a suitable animal model. **Materials and Methods.** We have used an animal model of meningitis based on intracisternal inoculation of BALB/c mice that were infected with a reference serogroup C meningococcal strain and an isogenic hrpB knockout mutant. Survival experiments and bacterial load over time in brain, spleen and liver, were recorded in mice infected with the wild-type and the hrpB mutant strains. To characterize the brain damage in mice infected with the reference serogroup C meningococcal strain and with hrpB mutant, histological analysis and Western blot analysis for pyroptosis markers were performed. **Results.** Survival experiments confirmed the important role of HrpA/HrpB TPS in meningococcal pathogenesis. Compared to the wild-type strain, the ability of this mutant to replicate in brain and spread systemically was impaired. Evaluation of brain damage showed a marked reduction in cerebral hemorrhages and inflammation in mice infected with the mutant in comparison with the levels in those challenged with the wild-type strain. Consistent with these data, Western blot analysis demonstrated that *N. meningitidis* activates both canonical and non-canonical inflammasome

pathways of the pyroptosis in mouse brain, and that the activation of these pathways was attenuated in the *hrpB* mutant. In particular, activation of caspase-11, caspase-1, and gasdermin-D was markedly reduced in the mutant compared to the wild-type strain. Furthermore, consistent with previous findings suggesting an inhibition of apoptosis in cells infected in vitro with *N. meningitidis*, caspase-3, an “executioner” caspase on which both intrinsic and extrinsic apoptotic pathways converge, was not activated in infected animals. In contrast, caspase-7, another “executioner” caspase, which at variance with caspase-3 is processed by caspase-1, was activated in the mouse brain infected with the wild-type strain and, to a much lesser extent, with the *hrpB* mutant suggesting an involvement in pathways other than apoptosis. Discussion and Conclusion. These findings help to clarify the role of pyroptosis in invasive meningococcal disease and open the possibility to evaluate the use of pyroptosis inhibitors in adjunctive therapy.

222 - Parvovirus B19 infection in a co-culture model of erythroid progenitor cells and mesenchymal stromal cells

GLORIA BUA⁽¹⁾ - CHIARA GAMBERINI⁽²⁾ - PASQUALE MARRAZZO⁽²⁾ - LAURA BONSI⁽²⁾
- FRANCESCO ALVIANO⁽³⁾ - GIORGIO GALLINELLA⁽¹⁾

Università di Bologna, Dipartimento di Farmacia e Biotecnologie, Bologna, Italia⁽¹⁾ - Università di Bologna, Dipartimento di Scienze Mediche e Chirurgiche, Bologna, Italia⁽²⁾ - Università di Bologna, Dipartimento di Scienze Biomediche e Neuromotorie, Bologna, Italia⁽³⁾

Parvovirus B19 infection in a co-culture model of erythroid progenitor cells and mesenchymal stromal cells

GLORIA BUA¹, CHIARA GAMBERINI², PASQUALE MARRAZZO², LAURA BONSI²,
FRANCESCO ALVIANO³, GIORGIO GALLINELLA¹

¹Department of Pharmacy and Biotechnology, University of Bologna, Bologna, Italy; ²Department of Medical and Surgical Sciences, University of Bologna, Bologna, Italy; ³Department of Biomedical and Neuromotor Sciences, University of Bologna, 40126 Bologna, Italy

Introduction

Parvovirus B19 (B19V) is a human pathogenic virus with a specific tropism for bone marrow erythroid progenitors cells (EPCs). EPCs located in the bone marrow interact with a specific microenvironment referred to as the stem cell niche, where mesenchymal stromal cells (MSCs) are important components and show a significant influence on EPCs differentiation. Thus, the aim of this study is to characterize B19V infection in a co-culture system of in vitro differentiating EPCs and MSCs.

Materials and Methods

EPCs, generated from PBMCs, were added to a layer of bone marrow-derived MSCs (BM-MSCs). Cells were infected with B19V at a moi of 1000 and viral replication and expression analysed by qPCR, qRT-PCR and immunofluorescence assays at different time post infection. The dynamics of B19V infection were investigated in the different cellular populations: i) EPCs, ii) MSCs, iii) non-adherent EPCs collected from the co-culture supernatant, iv) adherent EPCs in contact with MSCs.

Results

Results indicated that the sub-fractions of co-cultured EPCs sustain B19V infection at a different extent, suggesting a distinct pattern of permissiveness. In particular, in non-adherent EPCs we observed an extended increase of viral DNA between 2 and 72 hpi (+2.75 Log) comparable to what observed in infected EPCs in standard conditions, while viral replication reached a plateau between 24 and 48 hpi in non-adherent EPCs, with an overall increase of B19V DNA of 1.58 Log at 72 hpi. Analysis of viral transcription and protein expression confirm the diverse permissiveness observed among co-cultured cells, with a lower transcriptional activity and protein expression detected in non-adherent EPCs. MSCs are confirmed to be a non-productive environment with no significant viral replication observed; however, they can allow a persistence presence of B19V DNA.

Discussion and Conclusions

The different permissiveness to B19V infection among the co-cultured EPCs could be related to a diverse dynamics of differentiation of adherent EPCs in respect of non-adherent EPCs, influenced by the direct contact with the MSC monolayer. In conclusion, co-culture system of EPCs and MSCs are a good model to mimic the hematopoietic niche and investigate the relationship between cellular characteristics and B19V permissiveness.

229 - Cervical lesions and HPV oral infection: is there a relation?

ARIANNA SUCATO ⁽¹⁾ - MICHELA BUTTÀ ⁽¹⁾ - NICOLA SERRA ⁽²⁾ - RITA IMMORDINO ⁽³⁾ -
GIUSEPPINA CAPRA ⁽¹⁾

Università degli studi di Palermo, Dipartimento di Promozione della Salute, Materno-Infantile, di Medicina Interna e Specialistica di Eccellenza “G. D’Alessandro”, Palermo, Italia ⁽¹⁾ - Università Federico II di Napoli, Dipartimento della salute pubblica, Napoli, Italia ⁽²⁾ - Policlinico, Paolo Giaccone, Palermo, Unità di Microbiologia e Virologia, Ospedale Policlinico P. Giaccone, Palermo, Italia ⁽³⁾

Cervical lesions and HPV oral infection: is there a relation?

ARIANNA SUCATO¹, MICHELA BUTTÀ¹, NICOLA SERRA², RITA IMMORDINO³ AND
GIUSEPPINA CAPRA^{1,3}

¹ Department of Health Promotion, Mother and Child Care, Internal Medicine and Medical Specialties (ProMISE) “G. D’Alessandro”, University of Palermo, Palermo, Italy;

²Department of Public Health, University Federico II of Naples, Naples, Italy;

³Microbiology and Virology Unit. Polyclinic Hospital “P. Giaccone”, Palermo, Italy.

Introduction

Human papillomavirus (HPV) infection is the most common sexually transmitted disease (STD) in both sexes worldwide. Persistent HPV infection, usually with high-risk genotypes (hrHPV), is a potential risk factor for the development of cervical high-grade squamous intraepithelial lesions (HSIL) and cervical cancer and head and neck squamous cell carcinoma (HNSCC). There is no confirmed evidence of cervical-oral transmission, and the link between oral and cervical HPV infection is still debated. This study aims to evaluate the potential association between cervical alterations and possible viral transmission to the oral cavity in women with suspected HPV infection.

Materials and Methods

A total of 281 female patients were tested for cervical and oral HPV infection at the Microbiology and Virology Unit (Polyclinic Hospital “P. Giaccone”, Palermo, Italy). Oral rinses and cervical scrapings were provided for each patient, and HPV detection and genotyping were performed using the INNO-LiPA HPV Genotyping Extra II kit (Fujirebio). Pap test results were available for all the patients.

Results

The prevalence of cervical and oral HPV was 73.7% (207/281) and 7.1% (20/281), respectively. Among the cervical-positive women, 32.9% (68/207) had multiple infections and 67.1% (139/207) had a single infection, while hrHPV genotype infection was found in 87.0% (180/207) of cases. In contrast, only single infections occurred in the oral-positive women, and 65.0% (13/20) hrHPV genotype infections were found. In addition, 95% (19/20) of the oral-positive women had a concurrent cervical infection, i.e., simultaneous HPV infection in the cervix and oral cavity. In particular, concordance between detected hrHPV genotypes was found in 57.9% (11/19), and type-specific concordance of both low-risk genotypes (lrHPV) and hrHPV genotypes was found in 54.5% (6/11). According to the Pap test result, 57.9% (11/19) of women with concurrent infection had cervical cytological abnormalities, of which 10.5% (2/19) were HSIL, while the remaining 42.1% (8/19) were negative for cervical lesions.

Discussion and Conclusions

There are few studies examining the concurrency and concordance of cervical and oral HPV infection. In this study, nearly 60% of infections affecting both the cervical and oral sites

simultaneously show a cervical abnormality on the Pap smear. The data is absolutely preliminary given the small number of concomitant infections. It will be necessary to expand the cohort of patients to establish a possible association between infection of both sites and greater severity of lesions, and to clarify the possible role on cervical-oral transmission.

231 - Eating the enemy: Mycoplasma strategies to evade Neutrophil Extracellular Traps (NETs) promoting bacterial nucleotides uptake and inflammatory damage.

CARLA CACCIOTTO⁽¹⁾ - ROSANNA ZOBBA⁽¹⁾ - ALBERTO ALBERTI⁽¹⁾

Università degli Studi di Sassari, Dipartimento di Medicina Veterinaria, Sassari, Italia ⁽¹⁾

Eating the enemy: Mycoplasma strategies to evade Neutrophil Extracellular Traps (NETs) promoting bacterial nucleotides uptake and inflammatory damage.

CARLA CACCIOTTO^{1;2}, ROSANNA ZOBBA¹, AND ALBERTO ALBERTI^{1;2}

¹Dipartimento di Medicina Veterinaria, Università degli Studi di Sassari, Sassari, Italy

²Mediterranean Center for Disease Control, Università degli Studi di Sassari, Sassari, Italy

Introduction

Neutrophil Extracellular Traps (NETs) release is a recently described antimicrobial mechanism for bacterial extracellular killing. NETs offer new perspective of host-microbe interplay and on the role of innate immune response in the pathogenesis of long-term syndromes. The role of NETs in mycoplasma infections gained interest since very few virulence factors are known in Mycoplasmas and also suggest a possible contribution of mycoplasmas in autoimmune diseases.

Materials and Methods

Literature on Mycoplasmas and NETs is reviewed by focusing on mechanisms underlying the induction of NETs release, their dismantling, and the utilization of nucleosides in nucleotides salvage pathway.

Results

The release of NETs is an innate immune mechanism aimed to trap microbes and prevent their dissemination. Upon stimulus, neutrophils initiate the disintegration of their nucleus and granules membranes and the decondensed chromatin, coupled with cytoplasmic and granules proteins, are released in the extracellular compartment as web-like structures. Several studies demonstrated Mycoplasmas ability to induce NETosis both in vivo and ex vivo in animal and human models. Mycoplasmas surface lipoproteins are PAMPs which interact with the innate immune cells and trigger NETs release with their lipopeptide portions through the interaction with the TLR2. As a defence, Mycoplasmas dismantle NETs DNA backbone with their surface-located nucleases and uptake resulting host-derived nucleosides through their membrane transporters. Since mycoplasmas often cause persistent infections, a constant cycle of NETs release-disassembling-mycoplasma growth can take place, causing the persistence of an inflammatory status in the host. This condition was associated to post-infection inflammatory diseases, such as chronic respiratory diseases, mastitis, pelvic and urogenital inflammatory pathologies, and autoimmune and immunosuppressive diseases.

Discussion and Conclusions

NETs induction and disassembling allow mycoplasmas counteracting early clearance by reducing the efficacy of phagocytosis. This mechanism complements mycoplasma absence of nucleotide de novo synthesis by nucleotides essential to mycoplasma replication. By using their surface-located nucleases, mycoplasmas turn neutrophils into a source of essential food (nucleotides), basically by “eating the enemy”, promoting the persistence in the host and possibly contributing to the development of many inflammatory chronic disease.

247 - Development and characterisation of a mouse model of invasive Non-Typhoidal Salmonella (iNTS) disease

ELENA PETTINI⁽¹⁾ - FABIO FIORINO⁽²⁾ - VALENTINA MOCCI⁽¹⁾ - STEFANO LAZZI⁽³⁾ - SUSANNA RICCI⁽¹⁾ - GIANNI POZZI⁽¹⁾ - DONATA MEDAGLINI⁽¹⁾

University of Siena, Department of Medical Biotechnologies, Laboratory of Molecular Microbiology and Biotechnology (L.A.M.M.B.), Siena, Italia⁽¹⁾ - LUM University "Giuseppe Degennaro", Department of Medicine and Surgery, Bari, Italia⁽²⁾ - University of Siena, Department of Medical Biotechnologies, Siena, Italia⁽³⁾

Development and characterisation of a mouse model of invasive Non-Typhoidal Salmonella (iNTS) disease

ELENA PETTINI¹, FABIO FIORINO^{1,2}, VALENTINA MOCCI¹, STEFANO LAZZI³, SUSANNA RICCI¹, GIANNI POZZI¹, DONATA MEDAGLINI¹

¹ Laboratory of Molecular Microbiology and Biotechnology (L.A.M.M.B.), Department of Medical Biotechnologies, University of Siena, Siena, Italy; ² Department of Medicine and Surgery, LUM University "Giuseppe Degennaro", Bari, Italy; ³ Department of Medical Biotechnologies, University of Siena, Siena, Italy

Introduction. Invasive Non-Typhoidal Salmonella (iNTS) infection is a major cause of death in sub-Saharan Africa and currently no licensed vaccine is available. Salmonella enterica serovar Typhimurium (S. Typhimurium) generally causes gastroenteritis/enterocolitis in immunocompetent individuals. However, in immunocompromised subjects, it is often associated with invasive infection that presents as septicemia and/or meningitis with no or limited enteric symptoms. In this study we have developed an iNTS disease mouse model using the well-known iNTS clinical isolate D23580 (ST313).

Materials and Methods. C57BL/6 susceptible mice were infected by the intragastric route (i.g.) without pretreatment with streptomycin in order not to perturb the intestinal mouse microbiota. The D23580 strain was compared to the S. Typhimurium gastroenteritis-causing reference strain ATCC 14028. Mice were infected i.g. with 10⁷ colony forming units (cfu)/mouse of either strain and monitored for a week after challenge. Survival and clinical scores were recorded daily. Bacterial loads in blood, spleen, liver, mesenteric lymph nodes, gall bladder, gut (terminal ileum, cecum and distal colon) and faeces were analysed 3, 5 and 7 days post-infection (p.i.) in SS agar plates with antibiotic selection. The inflammatory responses in the different intestinal portions were investigated by histopathological analysis, while cytokine/chemokine levels in serum were quantified using Luminex immunoassay.

Results. Survival of mice infected with the D23580 iNTS isolate dropped to 67% at day 7 p.i., while no mice died following infection with the 14028 strain. Surviving mice infected with D23580 also showed weight loss and elevated clinical scores. A week after i.g. challenge, all mice infected with D23580 presented with a severe systemic infection with high bacterial loads in the spleen, liver and gall bladder (10⁶-10⁹ cfu/organ), while bacterial dissemination of the 14028 strain occurred in half of the mice and was characterised by 100-fold lower cfu counts. Interestingly, at day 5 p.i., no D23580

bacteria were found in mouse faeces in comparison to animals infected with the ATCC strain, emphasising the invasive potential of the iNTS isolate D23580.

Discussion and Conclusions. In the present study, we have developed a mouse model of iNTS disease that closely mimics the invasive infection in humans using the clinical isolate D23580 and compared to the enterocolitis ATCC strain 14028. Histopathology results may further elucidate the diverse pathogenic behaviors (enterocolitis vs. invasive non-typhoid) of the two *S. Typhimurium* strains. The present model may represent a valuable tool to study host-pathogen interactions and to evaluate protection conferred by vaccine candidates against iNTS

268 - HSV-1 exploits extracellular vesicles to disseminate neurotoxic proteins within the brain

Virginia Protto⁽¹⁾ - Mariya T. Miteva⁽²⁾ - Filomena Iannuzzi⁽³⁾ - Domenica D. Li Puma⁽⁴⁾ - Maria E. Marcocci⁽²⁾ - Anna T. Palamara⁽¹⁾ - Giovanna De Chiara⁽³⁾

Istituto Superiore di Sanità, Dipartimento di Malattie Infettive, Roma, Italia⁽¹⁾ - Sapienza Università di Roma, Dipartimento di Sanità Pubblica e Malattie Infettive Lab Affiliato a Istituto Pasteur Italia-Fondazione Cenci Bolognetti, Roma, Italia⁽²⁾ - Centro Nazionale delle Ricerche, Istituto di Farmacologia Traslazionale, Roma, Italia⁽³⁾ - Università Cattolica del Sacro Cuore, Dipartimento di Neuroscienze, Roma, Italia⁽⁴⁾

HSV-1 exploits extracellular vesicles to disseminate neurotoxic proteins within the brain

VIRGINIA PROTTO¹, MARIYA T. MITEVA², FILOMENA IANNUZZI³, DOMENICA D. LI PUMA⁴, MARIA E. MARCOCCI², ANNA T. PALAMARA¹, GIOVANNA DE CHIARA³

1 Department of Infectious Diseases, Istituto Superiore di Sanità, Rome, Italy; 2 Department of Public Health and Infectious Diseases, Sapienza University of Rome, Laboratory affiliated to Istituto Pasteur Italia-Fondazione Cenci Bolognetti, Rome, Italy; 3 Institute of Translational Pharmacology, CNR, Rome, Italy; 4 Department of Neuroscience, Università Cattolica del Sacro Cuore, Rome, Italy, Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy;

Introduction Growing evidence supports the role of Herpes Simplex Virus-1 (HSV-1) infection in the pathogenesis of Alzheimer's disease (AD). Indeed, viral replication is known to induce the accumulation of AD-related neurotoxic proteins in neuronal cells, such as phosphorylated tau protein (ptau) and amyloid beta peptides (Abeta), and we recently demonstrated the effect of multiple viral reactivations on the accumulation of tau-related brain damages in an in vivo mouse model of recurrent viral infection. Interestingly, in AD extracellular vesicles (EVs) are known to promote the spreading of Abeta and ptau among neuronal cells, likely contributing to the dissemination of neurotoxic proteins within the brain. Indeed, recent studies reported that aggregated forms of tau can be transmitted among neurons and that exogenous aggregates of tau could enter inside cells acting as seeds for the aggregation of the endogenous protein. Here, we investigated whether HSV-1 infection could promote the spread of ptau among neuronal cells via EVs, thus propagating HSV-1-induced tau-dependent damage within the brain.

Methods Primary neurons were isolated from embryo brains of wild type (wt) and tau KO mice. EVs were purified following mock- or HSV-1 infection (ctr-EV and HSV-EV, respectively) from supernatants of SH-SY5Y neuroblastoma cells, wt primary neurons or N2A neuroblastoma cells that were previously transfected with human tau tagged with GFP (htau^{GFP}). For recipient assay, EVs isolated from htau^{GFP}-transfected cells, were treated with UV rays to deactivate the viral particles that could be loaded in EVs, and then layered on tau KO neurons. Brain EVs were isolated by ultracentrifugation from cerebral tissues of HSV-1- and mock-infected mice. Cell lysates and EVs were analyzed in WB for protein content and the efficacy of HSV-1 infection was evaluated by standard plaque (SPA).

Results First, we characterized the tau species secreted in association with EVs after HSV-1 infection of neuroblastoma cells or primary neurons, assessing that HSV-1 infection in vitro promotes the release of high molecular weight tau. Moreover, by analyzing EVs isolated from HSV-1 and mock-infected htau^{GFP}-transfected cells, we found that they can transmit HSV-1-induced ptau to recipient tau KO neurons. Specifically, tau KO neurons layered with HSV-EVs uptake higher levels of p-htau^{GFP} compared with ctr-EVs-treated cells. Finally, by exploiting an in vivo model of recurrent HSV-1

infection, we determined that multiple virus reactivations upregulate the release of exosomal tau in the brain of infected mice.

Conclusions Our data highlight that HSV-1 could enhance ptau propagation among neuronal cells, thus identifying a new mechanism by which HSV-1 may likely contribute to neurodegeneration.

271 - Phenotypic and genotypic characterization of *Acinetobacter baumannii* clinical isolates: yesterday and today

ASTRI DWYANTI TAGUEHA⁽¹⁾ - DANIELA SCRIBANO⁽¹⁾ - CARTESIO D'AGOSTINI⁽²⁾ - CARLOTTA FIORILLA⁽³⁾ - MEYSAM SARSHAR⁽⁴⁾ - DOLORES LIMONGI⁽⁵⁾ - SILVIA IANNARELLI⁽¹⁾ - ZUZANA MICIAKOVA⁽⁶⁾ - ANNA TERESA PALAMARA⁽⁷⁾ - CECILIA AMBROSI⁽⁵⁾

Sapienza University of Rome, Department of Public Health and Infectious Diseases, Rome, Italia⁽¹⁾ - University of Rome Tor Vergata, Department of Experimental Medicine, Rome, Italia⁽²⁾ - Policlinico Tor Vergata, Laboratory of Clinical Microbiology, Rome, Italia⁽³⁾ - Bambino Gesù Children's Hospital, IRCCS, Research Laboratories, Rome, Italia⁽⁴⁾ - San Raffaele Open University, IRCCS, Department of Human Sciences and Promotion of the Quality of Life, Rome, Italia⁽⁵⁾ - Sapienza University of Rome, Department of Molecular Medicine, Rome, Italia⁽⁶⁾ - Sapienza University of Rome, Department of Public Health and Infectious Diseases, Laboratory Affiliated to Institute Pasteur Italia-Cenci Bolognetti, Rome, Italia⁽⁷⁾

Phenotypic and genotypic characterization of *Acinetobacter baumannii* clinical isolates: yesterday and today

Astri D. Tagueha¹, Daniela Scribano¹, Cartesio D'Agostini^{2,3}, Carlotta Fiorilla³, Meysam Sarshar⁴, Dolores Limongi⁵, Silvia Iannarelli¹, Zuzana Miciakova⁶, Anna Teresa Palamara^{7,8}, Cecilia Ambrosi⁵

¹Department of Public Health and Infectious Diseases, Sapienza University of Rome, Rome, Italy; ²Department of Experimental Medicine, University of Rome Tor Vergata, via Montpellier 1, Rome, Italy; ³Laboratory of Clinical Microbiology, Policlinico Tor Vergata, viale Oxford 81, Rome, Italy; ⁴Research Laboratories, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy; ⁵Department of Human Sciences and Promotion of the Quality of Life, San Raffaele Open University, IRCCS, Rome, Italy; ⁶Department of Molecular Medicine, Sapienza University of Rome; ⁷Department of Public Health and Infectious Diseases, Sapienza University of Rome, Laboratory Affiliated to Institute Pasteur Italia-Cenci Bolognetti Foundation, Rome, Italy; ⁸Department of Infectious Diseases, Istituto Superiore di Sanità, Rome, Italy.

Background: *Acinetobacter baumannii* are highly adaptable bacteria that are able to change their lifestyle deeply to conquer and persist within nosocomial environments. As a consequence, *A. baumannii* clinical isolates undergo local clonal expansion showing a wide range of different phenotypes to quickly adapt to environmental/host changes. Therefore, as a step forward in understanding its changing under antibiotic selective pressure, this study aims to compare the resistome, virulome, and phenotypic properties of strains collected over the past decade to more recent ones. Methods: A total of 30 clinical *A. baumannii* isolates were collected from Policlinico Tor Vergata, during 2010-2023. Isolates were collected mainly from respiratory and urine specimens. Antibiotic susceptibility was determined by minimum inhibitory concentration according to EUCAST. Biofilm-forming ability was evaluated by crystal violet staining; motility, protease activity, and invasion to human alveolar cells were also performed. The reference strains ATCC 17978 and ATCC 19606 were included as controls. Results: All isolates were non-susceptible to various agents of carbapenem, aminoglycoside, fluoroquinolones, β -lactamase, and folate pathway inhibitors, while none was colistin-resistant. Preliminary results showed that 60% of

strains isolated from respiratory infections displayed moderate to strong biofilm-forming activity compared to those from urinary sites. This ability allows bacteria to promote their lifespan on abiotic surfaces, i.e., ventilators, catheters, or surgical tools. Motility is involved in persisting in a stressed environment; only 20% of the isolates exhibited marked twitching motility on soft agar plates, 53% had some degree of motility, whereas the remaining 27% were not motile. All strains showed no protease activity on skim milk agar plates. The ability to invade human A549 epithelial cells varied among all strains, whereas 17% of respiratory isolates have greater colony forming units/ml after 48 h of incubation compared to control cells. Conclusions: Current work with whole-genome sequencing is ongoing; these analyses will provide the genomic profile associated with antimicrobial resistance, virulence factors, multi-locus sequence types, and the relationship among isolates. Characterizing their chromosomal and plasmid-encoded resistance and virulence traits could help us understand the mechanisms behind the genetic mobilization and spread of these genes among these opportunistic pathogens and clues about their evolution as a response to environmental stress conditions. This comparative study could spotlight valuable information on preventing transmission in a healthcare environment.

292 - *Klebsiella pneumoniae*-OMVs activate death-signaling pathways in human bronchial epithelial host cells (BEAS-2B)

FEDERICA DELL'ANNUNZIATA ⁽¹⁾ - ELENA CIAGLIA ⁽¹⁾ - VERONICA FOLLIERO ⁽¹⁾ - VALENTINA LOPARDO ⁽¹⁾ - ANNA MACIAG ⁽²⁾ - ILARIA COSIMATO ⁽³⁾ - MASSIMILIANO GALDIERO ⁽⁴⁾ - ANNIBALE ALESSANDRO PUCA ⁽¹⁾ - GIANLUIGI FRANCI ⁽¹⁾

Department of Medicine, Surgery and Dentistry, University Salerno, Salerno, Italia ⁽¹⁾ - Cardiovascular Research Unit, MultiMedica, Milan, Italia ⁽²⁾ - Clinical Pathology and Microbiology Unit, San Giovanni di Dio e Ruggi D'Aragona University Hospital, Salerno, Italia ⁽³⁾ - University of Campania Luigi Vanvitelli, Department of Experimental Medicine, Napoli, Italia ⁽⁴⁾

Klebsiella pneumoniae-OMVs activate death-signaling pathways in human bronchial epithelial host cells (BEAS-2B)

Federica Dell'Annunziata^{1,2}, Elena Ciaglia¹, Veronica Folliero¹, Valentina Lopardo¹, Anna Maciag³, Iliara Cosimato^{4,5}, Massimiliano Galdiero^{2,5}, Annibale Alessandro Puca^{1,3}, Gianluigi Franci^{1,5}

¹ Department of Medicine, Surgery and Dentistry "Scuola Medica Salernitana", University of Salerno, 84081 Baronissi Salerno, Italy

² Department of Experimental Medicine, University of Campania Luigi Vanvitelli, 80138 Naples, Italy

³ Cardiovascular Research Unit, IRCCS MultiMedica, 20138 Milan, Italy

⁴ Clinical Pathology and Microbiology Unit, San Giovanni di Dio e Ruggi D'Aragona University Hospital, 84126 Salerno, Italy

⁵ Complex Operative Unity of Virology and Microbiology, University Hospital of Campania "Luigi Vanvitelli", 80138 Naples, Italy

Introduction: Apoptotic programmed cell death serves a crucial role in protecting mammalian cells from infection by activating pathways through membrane receptors that bind bacterial components. Outer membrane vesicles (OMVs) released by Gram-negative bacteria can also trigger these mechanisms by delivering various bacterial molecules to host cells. This study focuses on investigating the role of *Klebsiella pneumoniae*-OMVs in activating death mechanisms in human bronchial epithelial cells (BEAS-2B).

Materials and Methods: OMVs from *K. pneumoniae* ATCC 10038 were purified using centrifugation, filtration, and ultracentrifugation. The purified OMVs were characterized through scanning electron microscopy (TEM), nanoparticle tracking analysis (NTA), and Sodium Dodecyl Sulphate-PolyAcrylamide Gel Electrophoresis (SDS-PAGE). Cell viability was assessed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) assay. Nuclear changes in apoptotic cells were monitored by 4',6-diamidino-2-phenylindole staining, while AnnexinV/Propidium Iodide staining and flow cytometry were used to evaluate the percentage of early and late apoptotic cells. Western blot analysis confirmed the expression of key apoptotic markers.

Results: Purified *K. pneumoniae*-OMVs exhibited a spherical morphology with a diameter of 137.2 ± 41 nm and a vesicular density of 7.8×10^9 particles/mL. Exposure of cell monolayers to 50 μ g of *K. pneumoniae*-OMV for 14 hours resulted in approximately 25% cytotoxicity and 41.15-41.14% of cells undergoing early and late apoptosis. Fluorescence microscopy revealed reduced cellular density, the presence of apoptotic bodies, chromatin condensation, and nuclear membrane blebbing in residual cells. Activation of caspases-3 and -9 and dysregulation of BAX, BIM, and Bcl-xL indicated the

involvement of mitochondria-dependent apoptosis. Furthermore, a decrease in antioxidant enzymes (superoxide dismutase, catalase, and glutathione peroxidase) suggested endoplasmic reticulum stress and the potential formation of reactive oxygen species.

Discussion and Conclusions: The findings demonstrate that OMVs induce cellular stress conditions and activate cell death mechanisms. The induction of *K. pneumoniae*-OMV mediated apoptosis could contribute to generalized tissue damage and inflammation. This study provides significant insights into the comprehensive understanding of *K. pneumoniae*-OMV induced cell apoptosis.

309 - The role of human DDX3 helicase in HSV-2 infection

CARMEN RITA PIAZZA⁽¹⁾ - GIULIA LOTTINI⁽¹⁾ - PAOLA PERRERA⁽²⁾ - CRISTINA DI PRIMIO⁽³⁾ - MARIANNA MIGNANELLI⁽⁴⁾ - PIETRO GIORGIO SPEZIA⁽²⁾ - MICHELE LAI⁽⁵⁾ - GIULIA FREER⁽⁵⁾ - PAOLA QUARANTA⁽⁵⁾ - MAURO PISTELLO⁽⁶⁾

Università di Siena, Dipartimento di Biotecnologie Mediche, Siena, Italia⁽¹⁾ - Università di Pisa, Dipartimento di Ricerca Traslazionale, Pisa, Italia⁽²⁾ - CNR di Pisa, Istituto di Neuroscienze, Pisa, Italia⁽³⁾ - Scuola Normale Superiore, Laboratorio di Biologia Bio@SNS, Pisa, Italia⁽⁴⁾ - Università di Pisa, Centro Retrovirus, Pisa, Italia⁽⁵⁾ - Università Ospedale di Pisa, Unità Operativa di Virologia, Pisa, Italia⁽⁶⁾

The role of human DDX3 helicase in HSV-2 infection.

Authors: CARMEN R. PIAZZA^{1,2}, GIULIA LOTTINI^{1,2}, PAOLA PERRERA^{2,3}, CRISTINA DI PRIMIO⁴, MARIANNA MIGNANELLI⁵, PIETRO G. SPEZIA^{2,3}, MICHELE LAI², GIULIA FREER², PAOLA QUARANTA², MAURO PISTELLO^{2,6}

Affiliations: ¹Department of Medical Biotechnologies, University of Siena, Siena, Italy; ²Centro Retrovirus, University of Pisa, Pisa, Italy; ³Department of Translational Research, Retrovirus Center and Virology Section, University of Pisa, Pisa, Italy; ⁴Institute of Neuroscience, CNR of Pisa, Pisa, Italy; ⁵Laboratorio di Biologia Bio@SNS, Scuola Normale Superiore, Pisa, Italy; ⁶Virology Operative Unit, University Hospital of Pisa, Pisa, Italy.

Introduction: The human protein DDX3 is a DEAD box ATP-dependent RNA helicase that regulates transcription, mRNA maturation and translation. DDX3 is also involved in replication of several RNA viruses such as Coxsackie B5 (CV-B), while it is not effective against viruses with negative sense single-stranded RNA (ssRNA-) genome. In addition, it seems that DDX3 inhibition impacts on DNA virus replication. To further clarify the role of DDX3 protein in viral infection, we investigate the role of DDX3 during Herpes Simplex virus 2 (HSV-2) infection. Materials and Methods: To assess whether the DDX3 protein was involved in viral replication, viral inhibition assays were developed using compounds that inhibit DDX3. A549 cells were infected with the HSV-2 strain MS and, after viral adsorption, the DDX3 inhibiting compounds were added and monitored for 48 hours post-infection (PI). The antiviral activity was calculated as IC₅₀. Production and localization of capsid and envelope proteins, as well as of DDX3 were analyzed by Western Blot and immunofluorescence, respectively. Additionally, were conducted gene expression experiments and silencing of DDX3 using siRNA, followed by viral yield analysis. Results: During infection with HSV-2 MS, a decrease in the intensity of the DDX3 band was observed at 48 hours PI in Western Blot analysis, even though expression of the relative gene did not change as compared to uninfected cells. The initially diffuse localization of DDX3 became more patchy after HSV-2 infection and appeared to colocalize with gD envelope protein. Furthermore, trying to understand in which phase of viral replication the interaction between HSV-2 and DDX3 occurs, we also tested the major capsid protein, ICP5. No obvious interaction between DDX3 and ICP5 proteins was observed. Discussion and Conclusions: DDX3 is likely involved in HSV-2 infection. From these results, interaction between DDX3 and HSV-2 does not occur during the encapsidation process but is anyhow involved as judged by diminution of DDX3 level during viral infection. It could be hypothesized that the virus sequesters and enrolls DDX3 in the import-export process of viral transcripts.

328 - Inhibiting immunoregulatory amidase NAAA blocks ZIKV maturation in Human Neural Stem Cells

MICHELE LAI⁽¹⁾ - VERONICA LA ROCCA⁽¹⁾ - ELENA IACONO⁽¹⁾ - ALESSANDRO DE CARLI⁽¹⁾ - DOMENICO FAVARO⁽¹⁾ - FABIO FILIPPINI⁽¹⁾ - PIETRO GIORGIO SPEZIA⁽¹⁾ - NICOLA CLEMENTI⁽²⁾ - GIULIA FREER⁽¹⁾ - MAURO PISTELLO⁽¹⁾

Università di Pisa, Nuove tecnologie in Medicina e Chirurgia, Pisa, Italia⁽¹⁾ - San Raffaele, Laboratory of medical microbiology and virology, milano, Italia⁽²⁾

Inhibiting immunoregulatory amidase NAAA blocks ZIKV maturation in Human Neural Stem Cells
Michele Lai, Veronica La Rocca, Elena Iacono, Carolina Filipponi, Alessandro De Carli, Domenico Favaro, Giulia Lottini, Nicola Clementi, Giulia Freer, Mauro Pistello.

Introduction. Recent evidence suggests that lipids play a crucial role in viral infections beyond their traditional functions of supplying envelope and energy, and creating protected niches for viral replication. In the case of Zika virus (ZIKV), it alters host lipids by enhancing lipogenesis and suppressing β -oxidation to generate viral factories at the endoplasmic reticulum (ER) interface. This discovery prompted us to hypothesize that interference with lipogenesis could serve as a dual antiviral and anti-inflammatory strategy to combat the replication of positive sense single-stranded RNA (ssRNA+) viruses. To test this hypothesis, we examined the impact of inhibiting N-Acylethanolamine acid amidase (NAAA) on ZIKV-infected human Neural Stem Cells. NAAA is responsible for the hydrolysis of palmitoylethanolamide (PEA) in lysosomes and endolysosomes. Inhibition of NAAA results in PEA accumulation, which activates peroxisome proliferator-activated receptor- α (PPAR- α), directing β -oxidation and preventing inflammation.

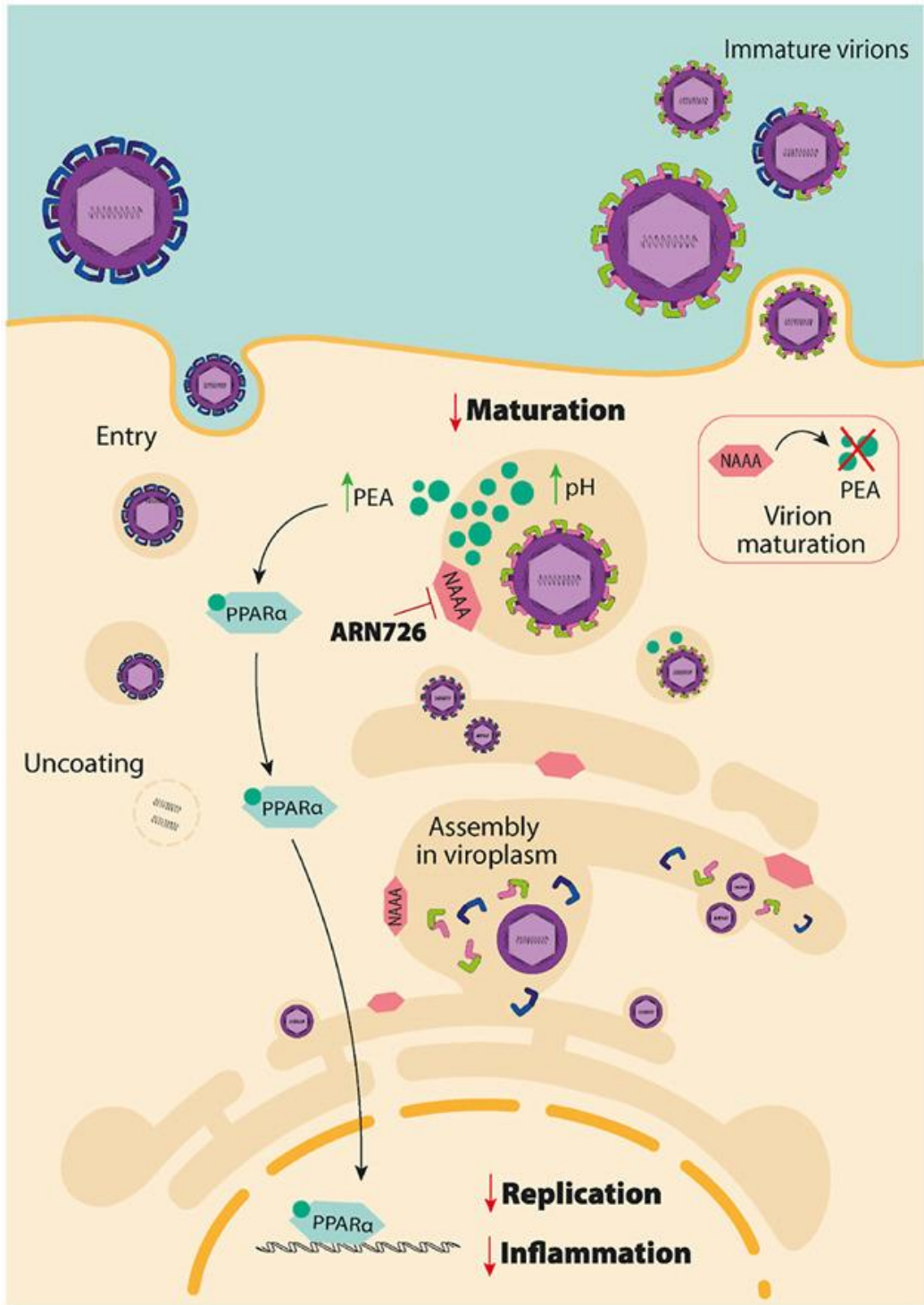
Results. Firstly, we found that ZIKV infection increases NAAA expression and the enzyme colocalizes with ZIKV viroplasms. Our findings indicate that inhibiting NAAA through gene-editing or drugs moderately reduces ZIKV replication by approximately one log10 in Human Neural Stem Cells. Surprisingly, NAAA inhibition leads to releasing immature virions that have lost their infectivity. This inhibition impairs furin-mediated prM cleavage, ultimately abolishing ZIKV maturation. Finally, we confirmed these findings in vivo against West-Nile Virus (WNV) by evaluating brain lesions and viral loads. Also in this case, NAAA reduced inflammation and brain infection. In summary, our study highlights NAAA as a host target for flaviviral infection.

Materials and Methods. This study was conducted using High-Content Confocal microscopy screenings (Operetta CLS, Perkin Elmer), Transcriptomic analyses, Virus yield assays and qRT-PCR quantification of viral genomes in cellular lysates or supernatants. ZIKV (Brasil strain) were used to infect neocortical Neuronal Stem Cells at 1 M.O.I. Golden Hamsters were infected with WNV, treated or not with ARN726 and followed for 10 days post-infection.

Discussion. In the present work, we set out to test the antiviral activity of ARN726, an anti-inflammatory compound that acts by inhibiting NAAA, and ended up uncovering that, although inhibiting inflammation decreases ZIKV replication, the most significant effect of NAAA inhibition

of ZIKV virion release is obtained by inhibiting maturation. Data were also confirmed in vivo on another flavivirus, WNV. In summary, this work unveils NAAA as a critical host factor not only for ZIKV replication, but for Flaviviruses in general and for all those viruses that rely only on Furin for their maturation.

Graphical Abstract:



09 Patogeni emergenti e riemergenti

3 - Prevalence of gastrointestinal pathogens and COVID-19 in patients with diarrhoea referring to a University Hospital

MARIANNA MARANGI⁽¹⁾ - ROSELLA DE NITTIS⁽²⁾ - MAURIZIO MARGAGLIONE⁽¹⁾ - FABIO ARENA⁽¹⁾

Università di Foggia, Dipartimento di Medicina Clinica e Sperimentale, Foggia, Italia⁽¹⁾ - Azienda Ospedaliero-Universitaria "Policlinico Riuniti", SSVD Microbiologia e Virologia, Foggia, Italia⁽²⁾

Prevalence of gastrointestinal pathogens and COVID-19 in patients with diarrhoea referring to a University Hospital

MARIANNA MARANGI¹, ROSELLA DE NITTIS², MAURIZIO MARGAGLIONE¹, FABIO ARENA^{1,2}

¹Department of Clinical and Experimental Medicine, University of Foggia, Viale Luigi Pinto, 71122, Foggia, Italy; ²Microbiology and Virology Unit, Ospedali Riuniti, Viale Luigi Pinto, 71122, Foggia, Italy.

Introduction: Gastrointestinal virus, bacteria and protozoan parasites contribute significantly to the burden of illness worldwide with diarrhoea being the most common among gastrointestinal symptoms (GS). The 2019 coronavirus disease (COVID-19), caused by SARS-CoV-2 virus, has become a global pandemic with cases widespread rapidly throughout the world. While fever and cough were symptoms more frequently associated with COVID-19, diarrhoea has been reported for 32 to 61% of individuals with SARS-CoV-2 infection. A likely mechanism underlying the presence of gastrointestinal symptoms in COVID-19 positive patients is the presence of ACE2 receptors on gut epithelial cells. Therefore, it is possible that intestinal inflammation due to COVID-19 could contribute or exacerbate symptoms related to infection by “classic” gastrointestinal pathogens. Therefore, this study aimed to evaluate the prevalence of gastrointestinal pathogens and SARS-CoV-2 infection among patients with diarrhoea referring to a University Hospital in Italy.

Material and methods: From February 2022 to January 2023, patients with diarrhoea, referring to the Policlinico Universitario Ospedaliero, Foggia, Italy, were subjected to research of COVID-19 infection by AllplexTM SARS-CoV-2 (nasopharyngeal swab sample) and to research of gastrointestinal pathogens by AllplexTM GI Virus, Bacteria and Parasite (stool sample) Assay.

Results: Out of 622 patients with diarrhoea, 79 (12.7%) resulted positive and 543 (87.3%) were negative for SARS-CoV-2. Among COVID-19 positive patients, the 38% (30/79) were co-infected with one or more gastrointestinal pathogens and in particular protozoan parasites (*Blastocystis* and *Dientamoeba fragilis*) was found in 66.6% of patients, followed by bacteria (30%) (*Campylobacter*, *Salmonella* and *Clostridium*) and viral pathogens (16.6%) (*Adenovirus* and *Norovirus*). Among COVID-19 negative patients, the prevalence of gastrointestinal pathogens was of 27.1% (147/543) and in particular viral pathogens was found in 17.5% of patients, followed by bacteria (7%) and protozoan parasites (3.3%). The association between gastrointestinal pathogens (all types) and SARS-CoV-2 positivity was statistically significant ($p=0.0448$).

Discussion and Conclusion: In this retrospective study, a statistically significant association between COVID-19 and the presence of “classic” gastrointestinal pathogens was found. This difference was driven by protozoan parasites since more than half of COVID-19 presented an infection with *Blastocystis* and *D. fragilis*, following by bacterial and viral infections. Based on these results a likely interaction between the intestinal pathogens and COVID-19 could be speculated. Future study with a larger number of samples are needed to confirm this results.

14 - *Dirofilaria* in humans: a new insight into an old knowledge

ALICE NICOLosi⁽¹⁾ - STEFANO REALE⁽²⁾ - ANDREA RUSSO⁽³⁾ - ILDEBRANDO PATAMIA⁽¹⁾

Unità Operativa Complessa Laboratorio Analisi, Azienda Ospedaliera Universitaria Policlinico-San Marco, Catania, Italia⁽¹⁾ - Istituto Zooprofilattico Sperimentale della Sicilia, Istituto Zooprofilattico Sperimentale della Sicilia, Palermo, Italia⁽²⁾ - Dipartimento di Oftalmologia, Azienda Ospedaliera Universitaria Policlinico-San Marco, Catania, Italia⁽³⁾

Dirofilaria in humans: a new insight into an old knowledge

ALICE NICOLosi¹, STEFANO REALE², ANDREA RUSSO³, ILDEBRANDO PATAMIA¹

¹U.O.C. Laboratory Analysis, University Hospital Policlinico-San Marco, Catania, Italy;

²Experimental Zooprophyllactic Institute of Sicily, Palermo, Italy; ³Department of Ophthalmology, University of Catania, Catania, Italy

Introduction

Dirofilariasis is a vector-borne parasitosis caused by filarial nematode of the genus *Dirofilaria* that typically affects canids, and sometimes felids, and can be transmitted to humans by Culicidae mosquitos. *D. repens* is the main agent of subcutaneous dirofilariasis, with the predominant localization of the nematode in the periorbital area, subconjunctival or under the skin of head or neck. Italy is traditionally endemic, representing one of the countries with the greatest number of human cases so far described. The present report describes a case of ocular dirofilariasis by *D. repens* in a 75-year-old woman resident in the Eastern cost of Sicily.

Materials and Methods

On February 2022, the patient came to the Emergency Room of University Hospital Policlinico in Catania, presenting painful conjunctival hyperemia of the right eye with visual disturbances. Physical examination of the eye revealed the presence of a nematode in the superior temporal area at the subconjunctival level. The nematode was removed by conjunctival incision and sent to the Parasitology Unit for morphological identification and, then, to the Experimental Zooprophyllactic Institute of Sicily for molecular characterization.

Results

Morphological examination showed a rounded-worm with 11.5 cm in length and width of 600 µm. Hematoxylin-eosin-stained-histopathological cross-sections showed the typical features of a non gravid female of *D. repens*, with a visible genital tube, intestine, well-developed musculature and the typical longitudinal and transversal striation of the cuticle. Multiplex-PCR *cox1* amplicons from the DNA extracted from the worm tissue resulted in a band consistent with *D. repens* (479 bp), confirming the diagnosis.

Discussion and conclusions

Accidental infection with animal filarial worms in humans is a dilemma for clinicians and parasitologists throughout the world. Dirofilariasis incidence has increased worldwide, in areas where it has already been reported and in new areas of Europe, such as some Central and Eastern European countries, especially from 2015 onwards, likely related to global warming and increased

travel and movement of infected animals. Thus, *Dirofilaria* spp. can be considered a paradigmatic example of an emergent pathogen which deserves more attention, especially in the light of the rare new findings of microfilaremia in human subjects (to date, 21 cases reported in the world), whose causes or predisposing factors are currently unknown. In this context, molecular biology, associated with traditional techniques (Knott test), could acquire even greater significance, allowing a more timely and accurate diagnosis, especially in those cases which, presenting a proven microfilaremia, may require systemic therapy in combination with surgery.

18 - Screening for tick-borne and tick-associated viruses in ticks collected in Sardinia, Italy

SILVIA FABI ⁽¹⁾ - SARAH DI SOPRA ⁽²⁾ - MICHELE PACCAGNELLA ⁽²⁾ - VALENTINA CHISU ⁽³⁾ - CIPRIANO FOXI ⁽³⁾ - CLAUDIA DEL VECCHIO ⁽²⁾ - GIOVANNA MASALA ⁽³⁾ - CRISTIANO SALATA ⁽²⁾

Università di Padova (coordinata da IUSS Pavia), Dipartimento di Medicina Molecolare, Padova, Italia ⁽¹⁾ - Università di Padova, Dipartimento di Medicina Molecolare, Padova, Italia ⁽²⁾ - Istituto Zooprofilattico Sperimentale della Sardegna, Istituto Zooprofilattico Sperimentale della Sardegna, Sassari, Italia ⁽³⁾

Screening for tick-borne and tick-associated viruses in ticks collected in Sardinia, Italy

SILVIA FABI ^{1,2}, SARAH DI SOPRA ¹, MICHELE PACCAGNELLA ¹, VALENTINA CHISU ³, CIPRIANO FOXI ³, CLAUDIA DEL VECCHIO ³, GIOVANNA MASALA ³, CRISTIANO SALATA ¹

1. Dipartimento di Medicina Molecolare, Università degli studi di Padova, Via A. Gabelli, 63 – 35121 Padova, Italy; 2. Scuola Universitaria Superiore (IUSS), Palazzo del Broletto, Piazza della Vittoria, 15 – 27100, Pavia, Italy; 3. Istituto Zooprofilattico Sperimentale della Sardegna, Via Vienna, 2 - 07100 Sassari, Italy

Ticks are important vectors of many pathogens, including arboviruses also known as tick-borne viruses (TBVs). Climate changes are promoting ticks and TBVs spread with the diffusion of new pathogens, as the Crimean-Congo hemorrhagic fever virus (CCHFV) that in the last decade colonized the Southwest Europe, as indicated by human cases reported in Spain and the evidence of virus circulation in animals of Corsica (France). Sardinia is an Italian island close to Corsica, it is a site of intense passage of birds migrating along major routes between Africa and Europe, thus representing a site potentially exposed to the risk for introduction of CCHFV or other TBVs.

This study was designed to determine the presence of TBVs and/or tick-associated viruses in ticks collected in Sardinia.

A total of 217 ticks were collected from different districts belonging to the north western, south-west and central Sardinia, all areas are of great naturalistic importance and show a wide diversity in geology, vegetation, landscape features and agro-ecology. Ticks were collected from domestic animals, wild mammals, and vegetation. Three ticks were also collected from human. Morphological identification of ticks was performed by taxonomic keys.

Each sample was longitudinally incised, and only one half of tick was homogenated by mechanical tissue lyser. Nucleic acids were extracted with Maxwell® RSC48 Instrument (Promega). One aliquote was subject to one-step real-time RT-PCR for the detection of CCHFV, using AgPath-ID™ One-Step RT-PCR Kit (Thermo Scientific). Each sample was also tested for nairoviruses, phleboviruses and flaviviruses using a Pan-PCR approach. Preliminary results did not show the presence of CCHFV while the Pan-PCR-based screening is still ongoing and results will be presented.

23 - Amino acid variability of small-molecule inhibitors and mAbs target sites in globally circulating RSV strains

VALERIA FOX ⁽¹⁾ - ROSSANA SCUTARI ⁽¹⁾ - ROMINA SALPINI ⁽²⁾ - SIMONE LA FRAZIA ⁽³⁾ - ALESSANDRA BANDERA ⁽⁴⁾ - ANTONIO PIRALLA ⁽⁵⁾ - ELENA PARIANI ⁽⁶⁾ - ANDREA GORI ⁽⁷⁾ - VALENTINA SVICHER ⁽³⁾ - CLAUDIA ALTERI ⁽¹⁾

Department Of Oncology And Hemato-oncology, University Of Milan, Milan, Italia ⁽¹⁾ - Department of Experimental Medicine, University of Rome Tor Vergata, Rome, Italia ⁽²⁾ - Department of Biology, University of Rome Tor Vergata, Rome, Italia ⁽³⁾ - Infectious Diseases Unit, Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, & Department of Pathophysiology and Transplantation, University of Milan, Milan, Italia ⁽⁴⁾ - Microbiology and Virology Department, Fondazione IRCCS Policlinico San Matteo, Pavia, Italia ⁽⁵⁾ - Department of Biomedical Sciences for Health, University of Milan, Milan, Italia ⁽⁶⁾ - Department of Pathophysiology and Transplantation, University of Milan, & Division of Infectious Diseases, L. Sacco University Hospital, Milan, Italia ⁽⁷⁾

Category: Patogeni emergenti e riemergenti

Title: Amino acid variability of small-molecule inhibitors and mAbs target sites in globally circulating RSV strains

Valeria Fox¹, Rossana Scutari¹, Romina Salpini², Simone La Frazia³, Alessandra Bandera^{4,5}, Antonio Piralla⁶, Elena Pariani⁷, Andrea Gori^{5,8}, Valentina Svicher³, Claudia Alteri¹

1 Department Of Oncology And Hemato-oncology, University Of Milan, Milan, Italy

2 Department of Experimental Medicine, University of Rome Tor Vergata, Rome, Italy

3 Department of Biology, University of Rome Tor Vergata, Rome, Italy

4 Infectious Diseases Unit, Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy

5 Department of Pathophysiology and Transplantation, University of Milan, Milan, Italy

6 Microbiology and Virology Department, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy

7 Department of Biomedical Sciences for Health, University of Milan, Milan, Italy

8 Division of Infectious Diseases, L. Sacco University Hospital, Milan, Italy

Background

Respiratory Syncytial Virus (RSV) causes high morbidity and mortality in high-risk population. To date, the only EMA-approved drugs are Pavilizumab and Nirsevimab, 2 monoclonal antibodies (mAbs) against fusion (F) protein, and ribavirin, a nucleoside analogue. Small molecule inhibitors (SMIs), targeting Large polymerase (L), Nucleocapsid (N), F and Glycoprotein (G), are under evaluation.

Here, we characterized the amino acid (aa) variability in SMIs and mAbs target sites by analyzing globally circulating RSV deposited sequences.

Material and Methods

1367 full-length RSV sequences, from drug-naïve patients, considering 1 sequence/patient, were downloaded from NCBI database. RSV type was inferred by Maximum likelihood phylogenetic analysis. Genetic distance (Maximum Composite models) and aa variability (Entropy) were evaluated in SMIs and mAbs targets against RSV type, while selective pressure (dN/dS) by Fubar and MEME tools, retaining only positions under positive selection with both methods. Statistical analyses were performed by T-test and Kruskal-Wallis test.

Results

Sequences were mostly type A (845, 61.8%), isolated before 2020 (1214, 88.8%), and mainly coming from Europe (47.5%) and North- and South-America (33.9%), followed by Asia (6.7%) and other Countries (11.9%). Genetic diversity was lowest in N protein, followed by F, G and L in both RSV types ($p < 0.001$). Of note, the within-type genetic diversity of G, F, and N was lower in sequences collected after 2020 ($p < 0.001$, $p = 0.013$ and $p = 0.056$, respectively), likely due to the limited circulation during COVID-19 pandemic. No N and L positions were under positive selection, while 2 F and 6 G positions in type A, and 2 F and 1 G positions in type B were under positive selection. Different entropy between A and B types ($\Delta H > 0.1$, $p < 0.05$) was observed in 6 aa N positions (in the N-ntd and N-ctd domains), 26 F positions (8 in antigenic sites Ø, I, II and V), 76 L positions (32 in RdRp), and 105 G positions, confirming divergent evolutionary pathways of these proteins between RSV types. Notably, none of these aa corresponded to drug-resistant sites. Only 32 (3.3%) sequences carried aa mutations conferring resistance to at least one mAb or SMI. One B strain carried a combination of F mutations (K272N+F488L) conferring full-resistance to Pavilizumab and SMIs against F.

Conclusions

The genetic characterization of RSV strains globally circulating showed a lower degree of genetic variability of N protein, compared to other drug targets, and little residue variability at positions associated to drug-resistance.

Continuous efforts in monitoring RSV genomic evolution, especially regarding mAbs and SMIs targets, are needed to guide clinical decisions and future therapeutic and preventive strategies development.

29 - First case of *Chryseobacterium gallinarum* bloodstream infection: a diagnostic challenge for an emerging pathogen

Mattia Genco ⁽¹⁾ - Antonio Curtoni ⁽¹⁾ - Lorenza Cavallo ⁽²⁾ - Silvia Scabini ⁽³⁾ - Luisa Guarrasi ⁽¹⁾ - Alessandro Bondi ⁽¹⁾ - Silvia Corcione ⁽³⁾ - Paolo Bottino ⁽¹⁾ - Narcisa Mandras ⁽²⁾ - Rossana Cavallo ⁽¹⁾ - Cristina Costa ⁽¹⁾

SC Microbiologia Virologia U, Università di Torino, Torino, Italia ⁽¹⁾ - Dipartimento di Scienze della Sanità Pubblica e Pediatriche, Università di Torino, Torino, Italia ⁽²⁾ - SC Malattie Infettive U, Università di Torino, Torino, Italia ⁽³⁾

First case of *Chryseobacterium gallinarum* bloodstream infection: a diagnostic challenge for an emerging pathogen

MATTIA GENCO¹, ANTONIO CURTONI^{1,2}, LORENZA CAVALLO¹, SILVIA SCABINI³, LUISA GUARRASI¹, ALESSANDRO BONDI^{1,2}, SILVIA CORCIONE³, PAOLO BOTTINO¹, NARCISA MANDRAS¹, ROSSANA CAVALLO^{1,2}, CRISTINA COSTA^{1,2}

¹Department of Sciences of Public Health and Pediatrics, University of Turin, Turin, Italy;

²Department of Laboratory Medicine, Microbiology and Virology Unit, University Hospital Città della Salute e della Scienza di Torino, Turin, Italy;

³Department of Medical Sciences, Infectious Disease Unit, University of Turin, Turin, Italy

Introduction

Chryseobacterium spp., belongs to Flavobacteriaceae family, is rod-shaped gram-negative bacteria, glucose non-fermenting, non-motile and ubiquitously in environment. It causes human infections such as urinary tract infections (UTI) and ventriculitis and its burden is increasing over the last years. *Chryseobacterium gallinarum* was isolated for the first time in 2014 in a pharyngeal scrape sample of chicken and, until now, only one case of human infection was described in UTI of an Indian 20-year-old pregnant patient. We report the first case of bloodstream infection (BSI) caused by *C. gallinarum* in a 67-year-old female burned patient.

Materials and Methods

Positive blood cultures were subcultured on Columbia-blood-agar. Isolated colonies were identified by Bruker microflex MALDI-TOF and subsequently by 16s rRNA sequencing. Antimicrobial susceptibility testing (AST) for cefepime, ciprofloxacin, trimethoprim-sulfamethoxazole, tigecycline, was performed with Biomerieux E-test and interpreted according to PK/PD EUCAST criteria v.13.0.

Results

Firstly MALDI-TOF identification reported *C. arthrosphaera* with score >2.0. Sequencing result, in contrast, showed *C. gallinarum*. Among tested antibiotics, cefepime was the only susceptible at standard dosing regimen, while ciprofloxacin was susceptible at increased exposure. Tigecycline resulted resistant. Trimethoprim-sulfamethoxazole MIC was 0.064.

Discussion and Conclusions

In the last few years, *Chryseobacterium* spp. was isolated in different human samples. Due to their elevated number of intrinsic resistances, in particular to beta-lactams, these emerging pathogens could represent a growing public health problem, especially in immunocompromised patients.

This is the first case of *C. gallinarum* isolated from blood cultures in a septic patient. *C. gallinarum* was successfully identified with gold-standard 16s rRNA sequencing. Routinary used identification methods (phenotypic and MALDI-TOF MS), in fact, could be unable to correctly differentiate these bacteria at species level. 16s rRNA sequencing could overcome identification problems, however, for these emerging pathogens there is a limited number of reference sequences in NCBI public libraries.

As regards AST, few antimicrobials could be reported due to the limited number of potentially effective antibiotics, the EUCAST warning statement for E-test method and the insufficient evidence in trimethoprim-sulfamethoxazole interpretation.

In conclusion, *Chryseobacterium* spp, and in particular, *C. gallinarum* infections, represent an important diagnostic issue for both identification and AST determination in clinical microbiological laboratories. Due to these obstacles, *C. gallinarum* evidence and impact in human infections could be underestimated.

47 - New putative Pannonibacter species isolated from nosocomial bacteraemia in two oncological pediatric patients

FRANCESCA INDRACCOLO ⁽¹⁾ - VITTORIANA DE LAURENTIIS ⁽¹⁾ - LOREDANA CAPOZZI ⁽²⁾ - STEFANO CASTELLANA ⁽²⁾ - ANTONIO PARISI ⁽²⁾ - MICHELA PARISI ⁽³⁾ - FRANCESCO DE LEONARDIS ⁽⁴⁾ - MASSIMO GRASSI ⁽⁴⁾ - ENRICA M. RANIERI ⁽¹⁾ - RAFFAELE DEL PRETE ⁽⁵⁾ - CARMELA DE CARLO ⁽¹⁾ - LUIGI RONGA ⁽¹⁾ - STEFANIA STOLFA ⁽¹⁾ - FEDERICA ROMANELLI ⁽¹⁾ - ELEONORA SPARAPANO ⁽¹⁾ - LUIGI SANTACROCE ⁽⁵⁾ - MARIA TERESA MONTAGNA ⁽⁵⁾ - ADRIANA MOSCA ⁽⁵⁾

UOC Microbiology and Virology, Università di Bari "Aldo Moro", Bari, Italia ⁽¹⁾ - Experimental Zooprophyllactic Institute of Apulia and Basilicata, Laboratorio di Biologia Molecolare, Foggia, Italia ⁽²⁾ - Pediatria, Università degli Studi di Tor Vergata, Roma, Italia ⁽³⁾ - Pediatric Hematology and Oncology, University Hospital of Policlinico - Bari, Bari, Italia ⁽⁴⁾ - Section of Microbiology, Interdisciplinary Department of Medicine (DIM), School of Medicine, University of Bari "Aldo Moro", Bari, Italia ⁽⁵⁾

New putative Pannonibacter species isolated from nosocomial bacteraemia in two oncological pediatric patients

Francesca Indraccolo ¹, Vittoriana De Laurentiis ¹, Loredana Capozzi ², Stefano Castellana ², Antonio Parisi ², Michela Parisi ⁵, Francesco De Leonardis ³, Massimo Grassi ³, Enrica M. Ranieri ¹, Raffaele Del Prete ⁴, Carmela De Carlo ¹, Luigi Ronga ¹, Stefania Stolfa ¹, Federica Romanelli ¹, Eleonora Sparapano ¹, Luigi Santacroce ⁴, Maria Teresa Montagna ⁴, Adriana Mosca ⁴.

1 UOC Microbiology and Virology, University "Aldo Moro", Bari, Italy

2 Experimental Zooprophyllactic Institute of Apulia and Basilicata - Via Manfredonia, 20, Foggia, Italy

3 Pediatric Hematology and Oncology - University Hospital of Policlinico - Bari Piazza G. Cesare, 11, Bari, Italy

4 Section of Microbiology, Interdisciplinary Department of Medicine (DIM), School of Medicine, University of Bari "Aldo Moro", Bari, Italy

5 Pediatria-Università degli Studi di Roma Tor Vergata, Roma, Italy

Introduction

Several cases of nosocomial infections sustained by not commonly ICA-associated bacteria are reported in the literature. Pannonibacter spp. is a Gram-negative, facultative anaerobic, alkaline tolerant and environmental bacteria, which can occasionally cause infections in humans. We present a case of isolation of a putative Pannonibacter spp. isolated from two patients with nosocomial bacteremia admitted to Pediatric Haematology and Oncology of University Hospital, Policlinico, Bari. In particular, the two pediatric patients were admitted to two different floors of the same ward. Patient 1 (Pt1) was 2-years-old male child affected by T-cell acute lymphoblastic leukaemia (LLA-T) admitted on 22 July 2022 for febrile neutropenia. On 9th August, following the onset of fever (38.5°C), a blood culture was carried out resulting positive after 36 hours for a Gram-negative bacterium. Patient 2 (Pt2) was a 3-year-old male child with congenital pyelectasis of the left side with a diagnosis of neuroblastoma made in September 2020. On 23 July 2022, 7 days after admission for

COJEC induction, Pz2 had a febrile peak and a blood culture was performed, resulting positive for a gram-negative bacterium after 32 hours.

Materials and Methods

Clinical strains isolated from Pt1 and Pt2 blood cultures were tested for identification by MALDI-TOF. Sanger sequencing of the 16S rRNA gene and whole genome sequencing (WGS) were performed by the Microbiology Laboratory. Molecular Biology Laboratory of the Experimental Zooprophyllactic Institute of Apulia and Basilicata. In addition, phylogenomic analyses were performed by considering the JSpecies and TYGS genome-based taxonomy classification web-services. Susceptibility tests were performed by Vitek 2 system.

Results

MALDI-TOF analysis failed to identify the two clinical isolates. 16S ribosomal DNA sequence analysis classified the two draft genomes as belonging to *Pannonibacter phragmitetus* strain C6-19 (99.79% similarity). However, multiple genome-wide taxonomy assignment approaches, assigned to them the status of “potential new species”. Clinical isolates showed the same susceptibility patterns resulting as resistant to aminoglycosides, third and fourth-generation cephalosporins and protected penicillins and susceptible only to carbapenems.

Discussion and Conclusions

The spread of environmental bacteria in hospital wards represents a health hazard for immunocompromised or paediatric patients, as strains can gain significant antibiotic resistance. WGS proves to be of great support in the surveillance and monitoring of the spread of emerging and resistant bacterial species. In this study, it enables the identification of a new suspected circulating bacterial species.

68 - Cutibacterium acnes in shoulder arthroplasty: preliminary results on the efficacy of an intraoperative iodopovidone irrigation in reducing its contamination

FABIO LONGO ⁽¹⁾ - ELEONORA MANISCALCO ⁽¹⁾ - FRANCESCA MENOTTI ⁽¹⁾ - ALESSANDRO BONDI ⁽²⁾ - CRISTINA COSTA ⁽²⁾ - ANTONIO CURTONI ⁽²⁾ - LORENZA CAVALLO ⁽¹⁾ - CLAUDIA PAGANO ⁽¹⁾ - ANNA MARIA CUFFINI ⁽¹⁾ - ENRICO BELLATO ⁽³⁾ - FILIPPO CASTOLDI ⁽³⁾ - DAVIDE BLONNA ⁽⁴⁾ - GIULIANA BANCHE ⁽¹⁾ - VALERIA ALLIZOND ⁽¹⁾

Università degli Studi di Torino, Dipartimento di scienze della sanità pubbliche e pediatriche, Torino, Italia ⁽¹⁾ - Città della Salute e della Scienze di Torino, Unità di Microbiologia e Virologia, Torino, Italia ⁽²⁾ - A.O.U San Luigi Gonzaga, Orbassano, Unità di Ortopedia e Traumatologia, Torino, Italia ⁽³⁾ - A.O. Ordine Mauriziano di Torino, Unità di Ortopedia e Traumatologia, Torino, Italia ⁽⁴⁾

Cutibacterium acnes in shoulder arthroplasty: preliminary results on the efficacy of an intraoperative iodopovidone irrigation in reducing its contamination

FABIO LONGO^{1,2}, ELEONORA MANISCALCO¹, FRANCESCA MENOTTI¹, ALESSANDRO BONDI², CRISTINA COSTA², ANTONIO CURTONI², LORENZA CAVALLO¹, PAGANO CLAUDIA¹, ANNA MARIA CUFFINI¹, ENRICO BELLATO³, FILIPPO CASTOLDI³, DAVIDE BLONNA⁴, GIULIANA BANCHE¹, VALERIA ALLIZOND¹

¹Department of Public Health and Pediatrics, University of Torino, Turin, Italy; ²Microbiology and Virology Unit, Città della Salute e della Scienza di Torino, Turin, Italy; ³Orthopedics and Traumatology Unit, AOU San Luigi Gonzaga, Orbassano, Turin, Italy; ⁴Orthopedics and Traumatology Unit, A.O. Ordine Mauriziano di Torino, Turin, Italy.

Introduction

Cutibacterium acnes, a slow-growing anaerobic Gram-positive bacterium is increasingly recognized as one of the main microorganism causing shoulder prosthetic joint infections (PJIs) which can affect the patient's quality of life, by causing persistent pain and stiffness, dysfunctional joints, or even prosthetic loosening. Even though this commensal skin bacterium displays virulence factors triggering inflammatory response and promoting bacterial adhesion by forming biofilm, its diagnosis remains challenging. It normally lives on the surface of the skin and in sweat glands, and, during surgical access, it may be inoculated into the deep layers. Additionally, other microorganisms can be isolated from this type of surgery as well, such as Gram-positive and Gram-negative bacteria. The use of intraoperative iodopovidone irrigation at the end of surgery has been shown to be effective in reducing the rate of PJIs following both primary and revision of hip and knee arthroplasties without findings of irrigation-related complication, however it is not sufficient to totally prevent the infectious risk. However, data pertaining shoulder arthroplasty are still lacking. On these grounds, the aim of the present study is to assess if an intraoperative iodopovidone irrigation could be able to reduce microbial, in particular C. acnes, contamination in shoulder arthroplasty.

Materials and methods

Ten patients undergoing reverse total shoulder arthroplasty (RTSA) were included in the study. At the end of RTSA surgery, three different specimens were collected from the surgical field – including two

swabs from the glenosphere and humeral component and one periprosthetic tissue – thereafter, an irrigation with iodopovidone was performed and other three samples, from the same sites, were collected. After sonication, the specimens were cultured on various agar media suitable for the growth of aerobic and anaerobic bacteria, and incubated properly. After incubation, bacteriological analysis (e.g. microbial count, morphological analysis) were carried out, and the resulting predominant bacterial types were identified using MALDI Biotyper. To evidence resistant bacterial strains, antimicrobial susceptibility testing have been also performed.

Results

We preliminarily observed a reduction (~ 70%) in the anaerobic growth, as CFU/ml, for the samples collected after the post-operative irrigation respect to the pre-operative ones, and *C. acnes* was the predominant pathogen revealed. Similarly, regarding the aerobic microorganisms, we detected differences in the bacterial count from the various samples collected before and after the intra-operative irrigation in fact, only few colonies were recovered after the irrigation, and these were mainly represented by staphylococci.

Discussion and Conclusions

Given the huge number of total shoulder arthroplasty per year, a deeper understanding of the role of innovative intra-operative disinfection protocols and appropriate prophylaxis therapy could ensure a significant reduction in the infection rates contributing to a safer patient follow up and to a relevant decrease of National Health System costs.

POSTER presentation Presenting Author: Doctor Fabio Longo
Department of Public Health and Pediatrics, Via Santena 9 - 10126 Torino
Tel. 011.670.5629 E-mail: fabio.longo899@edu.unito.it

96 - Genomic dissection of SARS-CoV-2 evolution in immunocompromised patient with prolonged viral shedding

BEATRICE MOLA⁽¹⁾ - SILVIA SILENZI⁽²⁾ - LUCA RUOTOLO⁽¹⁾ - TIZIANA LAZZAROTTO⁽¹⁾ - GIADA ROSSINI⁽²⁾

Alma Mater Studiorum - Università di Bologna, Microbiologia, Dipartimento di Scienze Mediche e Chirurgiche – DIMEC Alma Mater Studiorum - Università di Bologna, Bologna, Italia⁽¹⁾ - Azienda Ospedaliero-Universitaria of Bologna, Bologna, Italy, UO Microbiologia, IRCCS Azienda Ospedaliero-Universitaria of Bologna, Bologna, Italy, Bologna, Italia⁽²⁾

Patogeni emergenti e riemergenti

Genomic dissection of SARS-CoV-2 evolution in immunocompromised patient with prolonged viral shedding

BEATRICE MOLA¹, SILVIA SILENZI², LUCA RUOTOLO¹, TIZIANA LAZZAROTTO^{1;2},
GIADA ROSSINI²

¹ Microbiologia, Dipartimento di Scienze Mediche e Chirurgiche – DIMEC Alma Mater Studiorum - Università di Bologna

² UO Microbiologia, IRCCS Azienda Ospedaliero-Universitaria of Bologna, Bologna, Italy

Introduction: The chronic infection is proposed to be one of the most accredited mechanisms for the emergence of genetically divergent SARS-CoV-2 variants. Immunocompromised patients with their low immunization coverage represent a favorable environment for long term viral persistence that could act as a reservoir for the emergence of new mutations. The purpose of this study is to provide further insight on SARS-CoV-2 evolutionary dynamics in immunocompromised patients with prolonged viral shedding paying attention to specific mutations that evolved in variants of concern (VOCs).

Materials and Methods: Between 22 May and 30 December 2022, sequential nasopharyngeal swabs (NPSs) from three severely immunosuppressed patients (case A, B and C) with a history of onco-hematological disease and prolonged SARS-CoV-2 shedding were collected. For early SARS-CoV-2 detection, all NPSs were analyzed with RT-PCR-based assay by Allplex SARS-CoV-2 Variants I and II Assay (Seegene). Eligible samples (Ct < 27) were sequenced by Next-Generation Sequencing (NGS) technology (Illumina COVIDSEQ Assay, Illumina). Data were analyzed by Illumina DRAGeNTM COVID Lineage Application and Stanford SARS-CoV-2 software.

Results: For case A, NPSs at three different time-points were collected and genome sequence analyses showed that all matched with Omicron sublineage BA.2. For case B, a SARS-CoV-2 intra-host viral evolution was observed. Comparative genome analysis of samples collected at two time-points revealed the adherence to two different Omicron sublineages, BA.2 and the BA.2.1, respectively. In case C, five samples were tested, and all belonged to BF.2 Omicron sublineage. In all cases whole-genome sequencing showed an extensive within-patient SARS-CoV-2 evolution with the appearance of some amino acid mutations (A685S in RdRp gene; R408S in S gene; T30I in E gene; Y316N in nsp2) and disappearance of some others. Interesting, T30I mutation, considered a sensitive marker mutation for persistent infection, was observed only in case B. N440K substitution, fixed in Omicron lineages, was found in two cases with an opposite trend: disappearing in case A and appearing in B. An increasing trend of R408S substitution in S gene and Q15E mutation in M gene was observed in both case A and B. Noteworthy, Case C showed the occurrence of two mutational event in the same position of RdRP gene (V792F was substituted by V792I; C799W become C799F).

Conclusions: This study supports the hypothesis whereby persistent infection in immunocompromised patients may be one of the driving forces leading to the generation of new SARS-CoV-2 variants with immune escape properties.

104 - The infection rate of respiratory viruses in pandemic SARS-CoV2 period (2019-2023): an observational study.

GAETANA COSTANZA ⁽¹⁾ - PIERPAOLO PABA ⁽¹⁾ - MARCO CIOTTI ⁽¹⁾ - DOMENICO OMBRES ⁽¹⁾ - ADA BERTOLI ⁽¹⁾ - FABBIO MARCUCCILLI ⁽¹⁾ - STEFANIA LOSCHI ⁽¹⁾ - CLAUDIO CARAPELLESE ⁽¹⁾ - NICOLA BOTTALICO ⁽¹⁾ - ROSALBA PETRUCCELLI ⁽¹⁾ - VITA PETRONE ⁽²⁾ - MARIALAURA FANELLI ⁽²⁾ - LUCIA PIREDDA ⁽³⁾ - SERGIO BERNARDINI ⁽²⁾ - EMANUELA BALESTRIERI ⁽²⁾ - CLAUDIA MATTEUCCI ⁽²⁾ - ANTONELLA MINUTOLO ⁽²⁾ - SANDRO GRELLI ⁽¹⁾

Unit of Virology, Policlinic of Tor Vergata, ROMA, Italia ⁽¹⁾ - Università degli studi di Roma "Tor Vergata", Dipartimento di Medicina Sperimentale, Roma, Italia ⁽²⁾ - Università degli studi di Roma "Tor Vergata", Dipartimento di Biologia, Roma, Italia ⁽³⁾

The infection rate of respiratory viruses in pandemic SARS-CoV2 period (2019-2023): an observational study.

GAETANA COSTANZA¹, PIERPAOLO PABA¹, MARCO CIOTTI¹, DOMENICO OMBRES¹, ADA BERTOLI^{1,2}, FABBIO MARCUCCILLI¹, STEFANIA LOSCHI¹, CLAUDIO CARAPELLESE¹, NICOLA BOTTALICO¹, ROSALBA PETRUCCELLI¹, VITA PETRONE², MARIALAURA FANELLI², LUCIA PIREDDA³, SERGIO BERNARDINI^{1,4}, EMANUELA BALESTRIERI², CLAUDIA MATTEUCCI², ANTONELLA MINUTOLO², SANDRO GRELLI^{1,2}

1. Unit of Virology, Policlinic of Tor Vergata, Rome, Italy; 2. Department of Experimental Medicine, University of Rome Tor Vergata, Rome, 00133, Italy; 3. Department of Biology, University of Rome Tor Vergata, Rome, 00133, Italy; 4. Laboratory Medicine, Policlinic of Tor Vergata, Rome, Italy.

Background: The dissemination of SARS-CoV-2 in the last years has profoundly influenced the incidence of respiratory affections, altering epidemiological curves of other pathogens, both in pediatric and adult populations. Therefore, the possibility to recognize which microorganism is the cause Acute Respiratory Infections (ARIs), is essential for monitoring and curing patients, defining therapeutic decisions, and finding adequate public health strategies. The recognition is complicated by the fact that symptoms of Coronavirus Disease 19 (COVID-19) are similar to those of ARIs.

Moreover, measures implemented by the national policy to contain the spread of SARS-CoV-2 has further influenced the changes in the positivity rates of most respiratory viruses. **Material and Methods:** this is a retrospective study investigating on infection rate of respiratory viruses in SARS-CoV-2 epidemic situation in Virology unit of “Tor Vergata” COVID-Hospital of Rome (Oct 2019-Apr 2023) using “Film Array Respiratory Panel 2”. All data collected on database and used for statistical and epidemiological analyses. **Results:** patients with suspicious of symptoms of respiratory infections were included in this study from March 2020 to April 2023. In the first year frequently detected pathogens included SARS-CoV-2 (41,4%), Inf A-B (2,4%), HCOV (2,3%) HSRV (1,5%), an inverse trend on the rate of positive cases of common respiratory viruses. In fact, at the seasonal peak of Oct 2019-Mar 2020, respiratory flu viruses are opposed to the absence of peaks in the period Oct 2020-Mar 2021. On the other hand, the epidemiological curve shows an increase and prevalence of positive cases of SARS-CoV-2 (Oct 2020-Mar 2021). The measures taken by the Italian government, such as vaccination campaigns (Jan 2021) turned out to be the key time to see the first effects on the slowing down of SARS-CoV-2 infection rate, especially in elderly. Finally, from Oct 2022 a new increase in respiratory viruses was observed that had not been

seen in 2019, especially for the Rhinoviruses. The elimination of protective regulations, such as masking, allowed these respiratory viruses to circulate again. on the other hand, the decrease in the SARS-CoV-2 circulation could also be attributed to a decrease in confirmed diagnosis. Discussion and conclusions: This study represents a picture of the changes since the outbreak of the COVID-19 pandemic both in epidemiological terms and in the policy strategies implemented. The monitoring of the positivity rates of common respiratory viruses during the 3-year observation period allowed us to capture in real time the scenario of the area surrounding one of the largest hospitals in Lazio, reflecting what has been reported in other parts of the world as well.

105 - Prevalence of *Schistosoma mansoni* monoinfection and co-infections in the Romagna region

MARTINA BRANDOLINI ⁽¹⁾ - LAURA GRUMIRO ⁽¹⁾ - ALESSANDRA MISTRAL DE PASCALI ⁽¹⁾ - IRENE ZAGHI ⁽¹⁾ - GIORGIO DIRANI ⁽¹⁾ - SILVIA ZANNOLI ⁽¹⁾ - FRANCESCA TADDEI ⁽¹⁾ - GIULIA GATTI ⁽¹⁾ - ANNA MARZUCCO ⁽¹⁾ - MARIA SOFIA MONTANARI ⁽¹⁾ - SIMONA SEMPRINI ⁽¹⁾ - MONICA CRICCA ⁽¹⁾ - VITTORIO SAMBRI ⁽¹⁾

Unit of Microbiology, AUSL Romagna, Department of Laboratory and Transfusion Medicine, Cesena, Italia ⁽¹⁾

Prevalence of *Schistosoma mansoni* monoinfection and co-infections in the Romagna region

MARTINA BRANDOLINI^{1,2}, LAURA GRUMIRO^{1,2}, ALESSANDRA MISTRAL DE PASCALI^{1,2}, IRENE ZAGHI^{1,3}, GIORGIO DIRANI¹, SILVIA ZANNOLI¹, FRANCESCA TADDEI^{1,2}, GIULIA GATTI¹, ANNA MARZUCCO¹, MARIA SOFIA MONTANARI¹, SIMONA SEMPRINI¹, MONICA CRICCA^{1,2}, VITTORIO SAMBRI^{1,2}.

Department of Laboratory and Transfusion Medicine (DiMeLabeT), Unit of Microbiology, The Greater Romagna Area Hub Laboratory, 47522 Cesena, Italy;

Department of Medical and Surgical Sciences (DIMEC), Unit of Microbiology, Alma Mater Studiorum University of Bologna, 47522 Cesena, Italy;

Department of Specialty Medicine, Unit of Infectious Diseases, Santa Maria delle Croci Hospital, 48100 Ravenna, Italy

Introduction

Schistosoma is a trematode endemic in tropical and subtropical climates. In the last years, following globalization, expanding population, growing intercontinental travel networks and refugees' migration, its prevalence has also increased in western countries. Refugees from endemic areas represent the most affected group. Tourists travelling in remote areas are also at risk.

Materials and Methods

In a monocentric retrospective observational study, we assessed the prevalence of Schistosomiasis, particularly *S. mansoni* infection, in the Romagna area (Forlì-Cesena, Rimini, and Ravenna provinces), north-eastern Italy over the period between February 2022 and May 2023. The tested population is represented by migrants and refugees from high/moderate risk areas, and travellers/expats reporting or not excluding contact with a potentially contaminated fresh water sources in an endemic area in the previous 3 to 6 months. Subjects whose clinical condition was highly suggestive of Schistosomiasis in the absence of a declared stay in an endemic area were also included. Serology testing was performed with a commercially available ELISA (Enzyme Linked Immunosorbent Assay)-based assay, NovaLisa *Schistosoma mansoni* IgG (NovaTec, NovaTec Immundiagnostica, Dietzenbach, Germany).

Results

The study involved a total of 49 subjects (38 refugees from endemic areas and 11 Italian citizens), and 11 tested positive for *S. mansoni*. Screening clinical records, chronic Hepatitis B Virus (HBV) and active *Mycobacterium tuberculosis* (TB) infections presented in an exceptionally high proportion of *Schistosoma*-positive subjects compared to *Schistosoma*-negative: 73% vs. 36% for HBV and 36% vs. 3% for TB. Statistical analysis showed a strong association between Schistosomiasis and HBV, in line with other published data linking co-infection with HBV infection

tendency to chronicisation leading to accelerated progression to advanced liver disease and failure. Analogously, active TB was positively associated with Schistosomiasis.

Discussion and Conclusions

Our study, although based on a limited cohort suggests that in light of current migratory flows, Schistosomiasis, which long remained a neglected disease, is becoming a sanitary concern in non-endemic countries. Here it represents an additional burden for local healthcare systems, which not only have to deal with (often not properly treated) Schistosomiasis, but also must face its complications, represented by concurrent chronic infections. The current situation invites to reconsider, implement and better structure screening and treatment programmes to extend surveillance to all refugees and asylum-seekers, but also to travellers, ultimately aiming to Schistosomiasis timely diagnosis, adequate treatment, and eradication.

108 - Presence of alive *Ascaris lumbricoides* in a symptomatic patient repeatedly negative for worm eggs in fecal samples

SIMONA GIGLIOTTI⁽¹⁾ - ELETTRA MANCUSO⁽¹⁾ - MARTA PANTANELLA⁽¹⁾ - MICHELE MANNO⁽¹⁾ - CRISTINA COSCO⁽²⁾ - FRANCESCA DIVENUTO⁽¹⁾ - MORENA CAMPOLO⁽¹⁾ - NORA NOEMI RASO⁽¹⁾ - ERIKA GAMBARDELLA⁽¹⁾ - CHIARA MAIDA⁽¹⁾ - NADIA MARASCIO⁽¹⁾ - PATRIZIA DOLDO⁽³⁾ - ANGELA QUIRINO⁽¹⁾ - GIOVANNI MATERA⁽¹⁾

UOC Microbiologia Clinica, Università Magna Graecia, Catanzaro, Italia⁽¹⁾ - Uo gastroenterologia ed endoscopia operativa, università magna graecia, catanzaro, Italia⁽²⁾ - UO gastroenterologia ed endoscopia digestiva, università magna graecia, catanzaro, Italia⁽³⁾

Presence of alive *Ascaris lumbricoides* in a symptomatic patient repeatedly negative for worm eggs in fecal samples.

SIMONA GIGLIOTTI¹, ELETTRA MANCUSO¹, MARTA PANTANELLA¹, MICHELE MANNO¹, CRISTINA COSCO², FRANCESCA DIVENUTO¹, MORENA CAMPOLO¹, NORA N. RASO¹, ERIKA GAMBARDELLA¹, CHIARA MAIDA¹, NADIA MARASCIO¹, PATRIZIA DOLDO², ANGELA QUIRINO¹, GIOVANNI MATERA¹.

¹Department of Health Sciences, Unit of Clinical Microbiology, "Magna Graecia" University of Catanzaro-"Mater Domini" Teaching Hospital, Catanzaro, Italy; ²Department of Experimental and Clinical Medicine, Magna Graecia University of Catanzaro, 88100 Catanzaro, Italy.

Introduction: *Ascaris lumbricoides* is a nematode that parasitizes the human gastrointestinal tract, and it is one of the most common helminthic infections worldwide. It predominates in areas of poor sanitation, particularly in rural and urban areas of tropical and subtropical limited resources countries. The infection occurs mainly in all age groups but more commonly in children, and it can be asymptomatic, causing only malnutrition and growth retardation, or symptomatic with abdominal pain, nausea, vomiting, bloating, and diarrhea. Early diagnosis of this intestinal infection is pivotal to avoid its serious and lethal complications such as gut obstruction, volvulus and perforation. Case presentation: A 61-year-old Caucasian female was admitted to the Operational Unit of Gastrointestinal Endoscopic Surgery with abdominal pain, in combination with nausea and vomiting for 2 months. The patient reported hypertension and previous UTI. The laboratory clinical investigations were done and the red blood cell count was $4 \times 10^6/\mu\text{L}$ and eosinophils were increased (12%), probably because she exhibited allergic reactions. At this point, the physician performed abdominal examination that revealed abdominal rigidity in the central and mid-abdomen. After auscultation, a silent abdomen or minimal peristalsis was noticed. The woman underwent a colonoscopy and surprisingly *A. lumbricoides* was highlighted. The worm was sent to the Operative Unit of Microbiology at the hospital "Mater Domini" of Catanzaro for assessment and was identified as *A. lumbricoides* from their macroscopic appearance and size. The female *A. lumbricoides* was cylindrical in shape, pinkish in color, 21 cm in length and 4 mm in diameter and posterior end was straight. Diagnosis of *A. lumbricoides* infections is based on the microscopic detection of eggs in stool, thus, to detect microscopically *A. lumbricoides*'s eggs, patient fecal samples collected in several times were examined using microscopy-based methods. Oddly, although *A. lumbricoides* was identified, zero eggs were found. Discussion and Conclusion: This case illustrates as in our patient was identified the parasite, although she came from a normal socioeconomic status family and despite, she has reported to have never travel in tropical and subtropical areas where unhygienic disposal of human excreta is common. This report highlights the importance of the awareness of

ascariasis and that its preventive measures should be included in all health education programs. In addition, it would be crucial the implementation of anti-helminthic diagnostic and treatment programs to prevent possible complications as well as worms spreading

129 - The cranberry (*Vaccinium macrocarpon*) extract blocks Ebola virus and Crimean-Congo hemorrhagic fever virus infection by interfering with viral entry into target cells.

DENIS PASQUAL⁽¹⁾ - MATTIA MIRANDOLA⁽¹⁾ - SOFIA APPELBERG⁽²⁾ - MASSIMO EMILIO MAFFEI⁽³⁾ - GIORGIO GRIBAUDO⁽⁴⁾ - ALI MIRAZIMI⁽⁵⁾ - CRISTIANO SALATA⁽⁶⁾ - GIULIA SIBILLE⁽³⁾ - ANNA LUGANINI⁽³⁾

University of Padua, Dip. Medicina Molecolare, Padova, Italia⁽¹⁾ - Public Health Agency of Sweden, Departement of Microbiology, Solna, Svezia⁽²⁾ - University of Turin, Dip. di Scienze della vita e biologia dei sistemi, Torino, Italia⁽³⁾ - University of Turin, Dip. Scienze della vita e biologia dei sistemi, Torino, Italia⁽⁴⁾ - Karolinska Institute, Departement of Laboratory medicine, Solna, Svezia⁽⁵⁾ - University of Padua, Dip. Medicina Molecolare, Padova, Italia⁽⁶⁾

The cranberry (*Vaccinium macrocarpon*) extract blocks Ebola virus and Crimean-Congo hemorrhagic fever virus infection by interfering with viral entry into target cells.

Autors

Denis PASQUAL¹, Mattia MIRANDOLA¹, Sofia APPELBERG², Giulia SIBILLE³, Anna LUGANINI³, Massimo E. MAFFEI³, Ali MIRAZIMI^{2,4,5}, Giorgio GRIBAUDO³, Cristiano SALATA¹.

¹Department of Molecular Medicine, University of Padova, Padova, Italy;

²Department of Microbiology, Public Health Agency of Sweden, Solna, Sweden;

³Department of Life Sciences and Systems Biology, University of Turin, Turin, Italy;

⁴Department of Laboratory Medicine, Karolinska Institutet, Stockholm, Sweden;

⁵National Veterinary Institute, 75189 Uppsala, Sweden.

1. Introduction

Emerging and reemerging viral infections represent a major concern for human and veterinary public health and there is an urgent need for the development of broad-spectrum antivirals. It has been reported that a cranberry (*Vaccinium macrocarpon*) extract, which contains high levels of A2-type proanthocyanidins (PAC-A2), inhibits influenza A and B viruses and herpes simplex virus type 1 and 2 in vitro by interfering with the adhesion/internalization of virions. In this work, we have evaluated the antiviral activity of the cranberry extract against two highly pathogenic viruses, such as Ebola Virus (EBOV) and Crimean-Congo hemorrhagic fever virus (CCHFV).

2. Materials and methods

To evaluate the antiviral activity of the cranberry extract, a recombinant Vesicular Stomatitis Virus (pVSV) vector expressing the luciferase reporter was adopted. The pVSV was pseudotyped either with the EBOV or CCHFV glycoproteins. Time-of-addition, viral attachment, and entry assays were performed on Vero CCL-81 in the presence of different concentrations of the cranberry extract. Finally, experiments with infectious EBOV and CCHFV were performed to validate the results obtained with pseudovirus.

3. Results

The cranberry extract showed an inhibitory activity against both the pVSV-EBOV and pVSV-CCHFV infection. Treating compound during the infection phase determined a significant reduction of viral infectivity. In contrast, no or only a modest inhibition was detected when cells were treated with the

extract before or after virus internalization, respectively. The antiviral activity of the cranberry extract was confirmed against the live EBOV and CCHFV.

4. Discussion and conclusion

In conclusions, the cranberry extract inhibits EBOV and CCHFV infection acting at the early stages of their replicative cycles. This broad-spectrum antiviral activity suggests this cranberry extract (or its components) as a promising antiviral candidate against emerging and re-emerging viral infections.

130 - Hepatitis E virus acute infection in an immunocompetent subject with non-alcoholic fatty liver disease.

NADIA MARASCIO⁽¹⁾ - GRAZIA PAVIA⁽¹⁾ - MARTA PANTANELLA⁽¹⁾ - CLAUDIA CICINO⁽¹⁾ - AIDA GIANCOTTI⁽¹⁾ - FRANCESCA TRIMBOLI⁽¹⁾ - GIORGIO S. BARRECA⁽¹⁾ - ANGELO G. LAMBERTI⁽¹⁾ - FABIO MONGIARDO⁽¹⁾ - MASSIMO DE SIENA⁽²⁾ - SEBASTIANO DI SALVO⁽²⁾ - TIZIANA GRAVINA⁽²⁾ - GIOVANNI MATERA⁽¹⁾ - ANGELA QUIRINO⁽¹⁾

UOC Microbiologia Clinica, Magna Graecia University, Catanzaro, Italia⁽¹⁾ - UO Epatologia, Mater Domini University Hospital, Catanzaro, Italia⁽²⁾

Hepatitis E virus acute infection in an immunocompetent subject with non-alcoholic fatty liver disease.

NADIA MARASCIO¹, GRAZIA PAVIA¹, MARTA PANTANELLA¹, CLAUDIA CICINO¹, AIDA GIANCOTTI¹, FRANCESCA TRIMBOLI¹, GIORGIO S. BARRECA¹, ANGELO G. LAMBERTI¹, FABIO MONGIARDO¹, MASSIMO DE SIENA², SEBASTIANO DI SALVO², TIZIANA GRAVINA², GIOVANNI MATERA¹ AND ANGELA QUIRINO¹.

¹Department of Health Sciences, Clinical Microbiology Unit, “Mater Domini” University Hospital, Catanzaro, Italy; ²Unit of Hepatology, “Mater Domini” University Hospital, Catanzaro, Italy.

Introduction: Hepatitis E virus (HEV) is one of the major causative agents of acute hepatitis. Four major genotypes, such as HEV1, HEV2, HEV3 and HEV4, have been identified. To date, HEV is recognized as an emerging foodborne zoonosis. Wild boars and pigs are considered the reservoirs of infection. Herein, we report a case of HEV acute hepatitis in an immunocompetent subject with non-alcoholic fatty liver disease (NAFLD). Patient and Methods: A 62-year-old man with acute hepatitis was hospitalized in April 2023. HEV IgM and IgG were detected by Enzyme Linked Fluorescent Assay (ELFA) (Biomerieux). Viral RNA from serum samples was extracted by QIAmp Viral RNA mini kit (Qiagen). HEV RNA was quantified by one-step RT-PCR Real-Time, targeting ORF3 region, detection limit of 38 copies/mL (Clonit). Hepatitis E virus ORF1 and ORF2 sequences were performed by Sanger method (Genetic Analyzer). Sequences were edited by SeqScape Software v2.5 (Applied Biosystems). Genotype/subtype was determined by phylogenetic analysis. Results: Blood tests showed alanine aminotransferase level (ALT) at 1697 IU/L, aspartate aminotransferase level (AST) at 1030 IU/L, gammaglutamyl transferase (GGT) level at 710 IU/L and bilirubinemia at 5.65 mg/dL. HEV diagnosis was based on positivity of HEV IgM (cut-off >1.0) and IgG (level: 1.360 IU/mL). HEV RNA viral load was 659 copies/mL. HEV subtype was 3c. The other causes of hepatitis, including HAV, HBV (HDV), and HCV viruses, and Herpesviridae were excluded by routinely assays. Four days post-admission, HEV RNA viral load was 71 copies/mL. Viraemia became < 38 copies/mL from day 15 to day 30, and finally 1.5 months after diagnosis HEV RNA was undetectable. Interestingly, IgM antibodies were negative one month later the diagnosis of acute infection. Patient was not treated by Ribavirin, and he only took glutathione (intravenously and then orally) and bile acids (orally). The infection resolved spontaneously. The only risk factor was eating raw meat. Discussion and Conclusions: Potential risk linked to the consumption of animal products needs to be better evaluated. In the One Health era, the systematic sampling of pork products followed by testing for HEV positivity can be potentially useful. Further studies are important to understand the circulation of viral genotypes/subtypes in specific geographical areas. The risk factors for NAFLD include components of the metabolic syndrome

and, very recently, the meat consumption associated to high seroprevalence of HEV IgG. HEV past infection and its impact on disease severity needs to be investigated in NAFLD patients. In conclusion, it is important to understand the interaction between the virus and the host to monitor the clinical outcome of patients with liver disorders.

164 - Case report of pulmonary echinococcosis in a young patient from southern Italy.

ANNA MOREA⁽¹⁾ - ANGELA DE PALMA⁽²⁾ - GIULIA DE IACO⁽²⁾ - RICCARDO P. LIA⁽³⁾ - FRANCESCO DI GENNARO⁽⁴⁾ - ANNALISA SARACINO⁽⁴⁾ - DOMENICO OTRANTO⁽³⁾ - GIUSEPPE MARULLI⁽²⁾ - ROBERTA IATTA⁽¹⁾

Sezione di Parassitologia, Università degli Studi di Bari/Dipartimento Interdisciplinare di Medicina, BARI, Italia⁽¹⁾ - Unità operativa di Chirurgia Toracica, Policlinico/Università degli Studi di Bari/Dipartimento di Medicina di Precisione e Rigenerativa e Area Ionica, BARI, Italia⁽²⁾ - Medicina Veterinaria, Università degli Studi di Bari/Dipartimento di Medicina Veterinaria, BARI, Italia⁽³⁾ - Clinica di Malattie Infettive, Policlinico/Università degli Studi di Bari/Dipartimento di Medicina di Precisione e Rigenerativa e Area Ionica, BARI, Italia⁽⁴⁾

Case report of pulmonary echinococcosis in a young patient from southern Italy

ANNA MOREA¹, ANGELA DE PALMA², GIULIA DE IACO², RICCARDO P. LIA³, FRANCESCO DI GENNARO⁴, ANNALISA SARACINO⁴, DOMENICO OTRANTO³, GIUSEPPE MARULLI², ROBERTA IATTA¹

¹Interdisciplinary Department of Medicine, University of Bari Aldo Moro, Bari, Italy;

²Department of Precision and Regenerative Medicine and Ionian Area, Thoracic Surgery Unit, University of Bari Aldo Moro, Bari, Italy;

³Department of Veterinary Medicine, University of Bari Aldo Moro, Bari, Italy;

⁴Department of Precision and Regenerative Medicine and Ionian Area, Clinic of Infectious diseases, University of Bari Aldo Moro, Bari, Italy

Introduction

Cystic echinococcosis (CE) by *Echinococcus granulosus sensu lato* (s.l.) is a widespread food-borne disease affecting mainly pastoral and rural communities where people raise livestock in close contact with dogs. Although human CE is a notifiable infectious disease in most European countries, its distribution is largely under-recorded by national health systems. Data source on incidence of CE in Europe is from national health reports and hospital records, the European Surveillance System (TESSy) and scientific international publications including observational studies and single or multiple case reports. A CE case, reported in March 2023, in a 17-year-old boy referred to the emergency room with persistent cough, fever and dyspnea is herein described.

Materials and Methods

Chest X-ray and later on a total-body CT scan showed a cystic pulmonary mass of the apical segment of the right lower lobe (12x12cm) without calcifications or vascularization. The patient underwent thoracic surgery in which cystic fluid and membrane were sampled and sent to the parasitology and histology laboratories. Microscopic analysis was performed along with molecular test by PCR on two target genes (*cox1* and *nad5*). Sequences were subsequently analyzed by CLC software and compared with those available in the GenBank database. An ELISA test (Euroimmun, Germany) was performed on serum for detection of anti-*Echinococcus* spp. IgG.

Results

Thoracic surgery by right postero-lateral thoracotomy at the fifth intercostal space was performed and the cystic mass removed by lobectomy. The patient scored negative for IgG anti-Echinococcus spp., but at the parasitological examination of the cystic membrane, prolific vesicles containing protoscolices along with protoscolices in the liquid cysts were microscopically observed. Both specimens scored positive by PCR for *cox1* and *nad5* genes, showing a 99% nucleotide identity with *E. granulosus* s.l. genotype G3 available in GenBank. Histopathological examination confirmed the 3 layers of the membrane cysts. Albendazole 400 mg PO twice daily for at least 28 days was prescribed.

Discussion and Conclusions

The patient lived in a rural area of Apulia region in close contact with animals. He did not refer any travel abroad Italy in the past. After 2-month post-surgery, he had a gradual resumption of normal activities. Although the CE is challenging, the imaging techniques are essential for a probable diagnosis, confirmed by parasitological and molecular analyses. The geographical distribution of *E. granulosus* s.l. genotypes may elucidate on the role of animals as intermediate hosts, thus establish surveillance and control program for reducing the parasite's circulation.

171 - The role of whole-genome sequencing in cluster investigation of Carbapenem-Resistant *K. pneumoniae* isolated in a northern Italy hospital

GABRIELLA TOCCI ⁽¹⁾ - VALENTINA LEPERA ⁽¹⁾ - CAMILLA REBOLI ⁽¹⁾ - ANDREA ZAPPAVIGNA ⁽¹⁾ - CHIARA GORRINI ⁽¹⁾ - CLAUDIA CORDINI ⁽¹⁾ - ROBERTA SCHIAVO ⁽¹⁾ - LORENA LA VERGATA ⁽¹⁾ - BEATRICE ZERBI ⁽²⁾ - MARIA GRAZIA SILVOTTI ⁽²⁾ - STEFANO PONGOLINI ⁽³⁾ - ERIKA SCALTRITI ⁽³⁾ - GIULIANA LO CASCIO ⁽¹⁾

Microbiology Unit, Clinical Pathology Department, AUSL Piacenza, Piacenza, Italia ⁽¹⁾ - Hospital Hygiene Unit, Medical management Department, AUSL Piacenza, Piacenza, Italia ⁽²⁾ - Risk Analysis and Genomic Epidemiology Unit, Istituto Zooprofilattico Sperimentale della Lombardia dell'Emilia Romagna (IZSLER), Parma, Italia ⁽³⁾

The role of whole-genome sequencing in cluster investigation of Carbapenem-Resistant *K. pneumoniae* isolated in a northern Italy hospital

GABRIELLA TOCCI¹, VALENTINA LEPERA¹, CAMILLA REBOLI¹, ANDREA ZAPPAVIGNA¹, CHIARA GORRINI¹, CLAUDIA CORDINI¹, ROBERTA SCHIAVO¹, LORENA LAVERGATA¹, BEATRICE ZERBI³, MARIA GRAZIA SILVOTTI³, STEFANO PONGOLINI², ERIKA SCALTRITI², GIULIANA LO CASCIO¹

¹ Microbiology Unit, Clinical Pathology Department, AUSL Piacenza, Italy; ² Risk Analysis and Genomic Epidemiology Unit, Istituto Zooprofilattico Sperimentale della Lombardia dell'Emilia Romagna (IZSLER), Parma, Italy; ³ Hospital Hygiene Unit, Medical management Department, AUSL Piacenza, Italy

Background: Carbapenemase-producing bacteria (CPE) has become a public health problem worldwide and have been placed on the list of high-priority pathogens by WHO. Prevention of transmission of these organisms has become a challenge because the resistance genes have the ability to spread and easily colonize hospitalized patients.

Aim of this work was to evaluate the usefulness of a WGS and MLST analysis of an hospital outbreak of KPC- producing *Klebsiella pneumoniae* (KPC-KP) in the Piacenza AUSL Neurorehabilitation Unit. The study aimed to evidence if the outbreak was attributable to a single clone and how this could help to suppose a possible transmission route.

Materials and methods: From January to July 2022, 5 cases of KPC-Kp colonization were detected. From August to September, 13 new colonized or infected patients were described. Rectal swabs were analyzed for CPE according to EUCAST criteria and Italian AMCLI guidelines. KPC- KP were confirmed by an immunochromatographic lateral flow assay (NG-test Carba5 -NG Biotech, Guipry, France). The KPC-KP isolates were submitted to whole genome sequencing (WGS). Molecular investigation was performed by IZSLER (Parma, Italy) on MiSeq - Illumina. All strains were also analysed in order to determine Sequence type (ST) by Multi Locus Sequence Type (MLST) investigation and clonality by SNPs analysis.

Results: KPC-KP WGS genetic analysis was performed on 16 isolates from rectal swab and 4 isolates from infected biological samples and has identified six different STs: 8 isolates were ST307, 8 were ST716 and the other STs were composed by singles isolates. The SNPs results detected the presence

of three different clusters and 4 single clones, one of which was not yet present in the comparison databases.

Discussion and conclusion: The hospital epidemiologist requires the assistance of the microbiology laboratory to subtype bacterial isolates beyond species level (epidemiological typing) for two purposes: outbreak investigation and surveillance of nosocomial infection and antibiotic resistance. Reference methods for epidemiological survey in the past were PFGE and MLST, the first today often replaced by WGS. In this study the application of WGS in a hospital outbreak of multidrug resistant *Klebsiella pneumoniae* gave the opportunity to promptly investigate and confirm possible routes of transmission in many patients, contributing to restrict the spread of the bacteria and providing the implementation of the existing Health care workers recommendation. Combining microbiology competence and new molecular technology with epidemiological hospital survey is the winning strategy to fight this troubling infections and proper managing the spread of multidrug-resistant bacteria.

218 - Novel benzimidazole derivatives as potent and selective anti Poxvirus agents

VALERIA MANCA⁽¹⁾ - GIUSEPPINA SANNA⁽¹⁾ - ROBERTA IBBA⁽²⁾ - VANESSA PALMAS⁽¹⁾ - VERONICA FOLLIERO⁽³⁾ - SANDRA PIRAS⁽²⁾ - LAURA SANNA⁽²⁾ - ANTONIO CARTA⁽²⁾ - ALDO MANZIN⁽¹⁾

Dipartimento di Scienze Biomediche, Università degli Studi di Cagliari, Monserrato, Cagliari, Italia
(¹) - Dipartimento di Medicina, Chirurgia e Farmacia, Università di Sassari, Sassari, Italia (²) -
Dipartimento di Medicina, Chirurgia e Odontoiatria, Scuola Medica Salernitana, Salerno, Italia (³)

Novel benzimidazole derivatives as potent and selective anti Poxvirus agents

VALERIA MANCA¹, GIUSEPPINA SANNA¹, ROBERTA IBBA², VANESSA PALMAS¹,
VERONICA FOLLIERO³, SANDRA PIRAS², LAURA SANNA², ANTONIO CARTA², ALDO
MANZIN¹

¹Department of Biomedical Sciences, University of Cagliari, Cittadella Universitaria, 09042, Monserrato, Cagliari, Italy;

²Department of Medicine, Surgery and Pharmacy, University of Sassari, via Muroni 23A, 07100 Sassari, Italy;

³Department of Medicine, Surgery and Dentistry "Scuola Medica Salernitana", Via Salvatore Allende, 84081, Salerno, Italy

Abstract

Introduction: Despite the impressive progress carried out in the field of biomedical sciences, in the last decades the incidence of emerging and neglected lethal viral infections mainly belonging to the Coronaviridae, Filoviridae, Arenaviridae, Bunyaviridae, and Paramyxoviridae families have considerably impaired human health.

The worldwide vaccination campaign at the end of the 1970s determined the eradication of smallpox. However, the growing number of cases of diseases linked to orthopoxvirus diseases as the recent epidemic of monkeypox zoonosis in various countries around the world, increased the need for knowledge of these viral pathogens.

There isn't a specific medication for the Monkeypox virus (MPXV) infection until now. However, several antiviral drugs used to treat Smallpox and other viral infections could be beneficial also for Monkeypox disease.

We here report the design and synthesis of new variously substituted benzimidazole derivatives and the evaluation of their cytotoxicity and antiviral activity against representatives of Orthopoxvirus genus, Vaccinia Virus (VV), closely related to variola virus and MPXV.

Materials and Methods: The cytotoxicity, the antiviral activity, and the study of mechanisms of action of the most interesting compounds were determined with a combination of cell-based and molecular experimental techniques.

Results: In our studies, new variously substituted benzimidazoles showed interesting EC₅₀ values and a cytotoxic profile in the high micromolar range.

Discussion and Conclusion: Our work shows that the tested new benzimidazoles derivatives, possess appealing activity and selectivity accompanied with low cytotoxicity. These results set a valid foundation to identify potent and selective anti-Poxvirus agents.

219 - Efficacy of dispirotripiperazine PDSTP in a golden Syrian hamster model of SARS-CoV-2 pneumonia

GIUSEPPINA SANNA⁽¹⁾ - OLGA RIABOVA⁽²⁾ - ANNA EGOROVA⁽²⁾ - ELENA KAZAKOVA⁽²⁾ - ALEXANDER LEPIOSHKIN⁽²⁾ - NATALIA MONAKHOVA⁽²⁾ - ALESSANDRA MARONGIU⁽¹⁾ - VANESSA PALMAS⁽¹⁾ - GIANLUIGI FRANCI⁽³⁾ - VADIM MAKAROV⁽²⁾ - ALDO MANZIN⁽¹⁾

Dipartimento di Scienze Biomediche, Università degli Studi di Cagliari, Monserrato, Cagliari, Italia
⁽¹⁾ - Federal Research Centre “Fundamentals of Biotechnology”, Russian Academy of Sciences, Moscow, Russia ⁽²⁾ - Dipartimento di Medicina, Chirurgia e Odontoiatria, Scuola Medica Salernitana, Università di Salerno, Salerno, Baronissi, Italia ⁽³⁾

Efficacy of dispirotripiperazine PDSTP in a golden Syrian hamster model of SARS-CoV-2 pneumonia

GIUSEPPINA SANNA¹, OLGA RIABOVA², ANNA EGOROVA², ELENA KAZAKOVA², ALEXANDER LEPIOSHKIN², NATALIA MONAKHOVA², ALESSANDRA MARONGIU¹, VANESSA PALMAS¹, GIANLUIGI FRANCI³, VADIM MAKAROV², ALDO MANZIN¹.

¹Department of Biomedical Sciences, Microbiology and Virology Unit, University of Cagliari, Cittadella Universitaria, Sp. 8, 09042 Monserrato (CA), Italy;

²Federal Research Centre “Fundamentals of Biotechnology” of the Russian Academy of Sciences (Research Centre of Biotechnology RAS), Leninsky prospect, 33, build. 2, 119071 Moscow, Russia;

³Department of Medicine, Surgery and Dentistry "Scuola Medica Salernitana", University of Salerno, 84081 Baronissi, Italy.

Abstract

Introduction: The rising incidence of viral pandemics calls for new small-molecule therapeutics that go beyond traditional approaches and targets. Dipirotripiperazine, composed of two positively charged nitrogen atoms, represents an uncommon scaffold in drug discovery campaigns, and derivative molecules are known to prevent virus infection by disrupting early host-pathogen interactions.

Materials and Methods: We evaluate the efficacy of viral adhesion-blocking dispirotripiperazine-based molecule PDSTP in vitro using a panel of coronaviruses, and in vivo using a golden Syrian hamster model of SARS-CoV-2-related pneumonia. To investigate the antiviral mechanisms of action of PDSTP, we carried out virucidal, a time-of-drug-addition, adsorption and penetration assays.

Results: PDSTP showed no in vitro activity against the alphacoronavirus HCoV-229E, but potent activity against the Betacoronaviruses tested, HCoV-OC43 and SARS-CoV-2. Virucidal activity assay results revealed that PDSTP had no direct effect on SARS-CoV-2 replication, but mechanism-of-action study supported the antiviral effect of PDSTP only at the early stages of viral infection in a heparin-like manner. This result is consistent with the previous experimental reports that heparin

inhibits SARS-CoV-2 by competitive binding to host-surface heparan sulfate proteoglycans. Taken together, our findings confirm that PDSTP might affect cell-surface heparan sulfate residues to display its antiviral activity. In an in vivo study, we observed a positive effect of PDSTP on SARS-CoV-2 infected animals. Lower SARS-CoV-2 RNA levels in the lungs and turbinates were detected in animals treated with PDSTP compared to infected animals. At the same time, a statistically significant decrease in SARS-CoV-2 infectious titer in PDSTP animal groups was observed.

Discussion and Conclusions: We demonstrated that the molecule was reasonably active against two clinical isolates affecting the early stages of the SARS-CoV-2 cycle. In a hamster model of SARS-CoV-2 pneumonia, PDSTP treatment resulted in reduced viral loads in the lungs and turbinates and milder lung tissue lesions.

Overall, here we provide proof-of-concept for the use of an adhesion-blocking molecule as a promising strategy to treat SARS-CoV-2 infection. Together with our previous findings showing that this compound inhibits various viruses that use heparan-sulfate proteoglycans to interact with host cells, we report that this approach will go beyond the traditional “one disease-one target-one drug” strategy used in current antiviral strategies. These data support PDSTP as a preclinical candidate for the treatment of COVID-19.

220 - Elevated plasma level of IP-10 /CXCL10 is correlated with severity of human West Nile virus (WNV) infection

GIADA ROSSINI⁽¹⁾ - STEFANIA VARANI⁽²⁾ - SILVIA SILENZI⁽¹⁾ - LUCA RUOTOLO⁽³⁾ - BEATRICE MOLA⁽¹⁾ - TIZIANA LAZZAROTTO⁽²⁾

UO Microbiologia, IRCCS Azienda Ospedaliero-Universitaria of Bologna, Bologna, Italia⁽¹⁾ - UO Microbiologia, IRCCS Azienda Ospedaliero-Universitaria of Bologna; Microbiologia, Dipartimento di Scienze Mediche e Chirurgiche – DIMEC Alma Mater Studiorum - Università di Bologna, Bologna, Italia⁽²⁾ - Microbiologia, Dipartimento di Scienze Mediche e Chirurgiche – DIMEC Alma Mater Studiorum - Università di Bologna, Bologna, Italia⁽³⁾

Elevated plasma level of IP-10 /CXCL10 is correlated with severity of human West Nile virus (WNV) infection

GIADA ROSSINI¹, STEFANIA VARANI^{1;2}, SILVIA SILENZI¹, LUCA RUOTOLO², BEATRICE MOLA¹, TIZIANA LAZZAROTTO^{1;2}

¹ UO Microbiologia, IRCCS Azienda Ospedaliero-Universitaria of Bologna, Bologna, Italy

² Microbiologia, Dipartimento di Scienze Mediche e Chirurgiche – DIMEC Alma Mater Studiorum - Università di Bologna, Italy

Introduction: West Nile virus (WNV) is a mosquito-borne RNA virus belonging to the genus Flavivirus. Despite the majority of WNV infections in humans are asymptomatic (80%), some infected patients develop West Nile fever (WNF, 20%) and a small subset (<1%) develops severe neuroinvasive disease (WNND), which may include meningitis, meningoencephalitis and acute flaccid paralysis.

CXCL10/Interferon-gamma-inducible protein-10 (IP-10) is a member of CXC chemokines, and is thought to play a role in the temporal development of innate and adaptive immunity in concert with Interferons. Accumulating data have indicated that IP-10/CXCL10 plays an important role in many viral infections such as Hepatitis Virus B (HBV), Hepatitis Virus C (HCV), HIV, influenza virus. IP-10/CXCL10 has a role in the control of early acute human WNV viremia but the relationship between IP-10/CXCL10 expression and neuropathological outcomes during human infection remains to be determined.

Materials and Methods: To define the kinetic of circulating IP-10/CXCL10 levels following WNV infection, the level of IP-10/CXCL10 was measured in plasma samples from patients undergoing WNV asymptomatic infection (n.9), WNF (n.6) and WNND (n.8). The plasma samples were collected longitudinally at the time of the diagnosis and at 7, 15 and 30 days after the diagnosis and the relationship between IP-10/CXCL10 levels and WNV disease severity was analysed.

Results: During the acute stage of infection, all groups of patients (asymptomatic, WNF and WNND) exhibited high plasma levels of IP-10/CXCL10. However, during the convalescent phase of infection the IP-10/CXCL10 plasma level in WNND patients was significantly higher than its corresponding level in WNF and asymptomatic individuals. For samples collected more than 26 days after symptoms onset, the median concentration of IP-10/CXCL10 in WNND patients was 4.5-fold higher than that in WNF patients ($p < 0.001$). In contrast, no significant difference was detected for IP-10/CXCL10 levels in the group of WNF patients compared to the group of asymptomatic individuals.

Discussion and Conclusions: Our findings may help to better understand the role of IP-10/CXCL10 in the development of WNND and suggest that IP10/CXCL10 could be used as indicator of severity of WNV infection. Furthermore, the observed correlation suggests that IP10/CXCL10 may be considered as potential target of immune therapy during severe WNV infection.

233 - Asymptomatic Mpox infection in subjects presenting for MVA-BN vaccine

GIULIA MATUSALI⁽¹⁾ - VALENTINA MAZZOTTA⁽²⁾ - PIERLUCA PISELLI⁽³⁾ - AURORA BETTINI⁽¹⁾ - FRANCESCA COLAVITA⁽¹⁾ - SABRINA COEN⁽¹⁾ - FRANCESCO VAIA⁽⁴⁾ - ENRICO GIRARDI⁽⁵⁾ - ANDREA ANTINORI⁽²⁾ - FABRIZIO MAGGI⁽¹⁾

Istituto Nazionale per le Malattie Infettive "Lazzaro Spallanzani", Laboratorio di Virologia, Roma, Italia⁽¹⁾ - Istituto Nazionale per le Malattie Infettive "Lazzaro Spallanzani", HIV/AIDS Unit, Roma, Italia⁽²⁾ - Istituto Nazionale per le Malattie Infettive "Lazzaro Spallanzani", Epidemiology Unit, Roma, Italia⁽³⁾ - Istituto Nazionale per le Malattie Infettive "Lazzaro Spallanzani", General Direction, Roma, Italia⁽⁴⁾ - Istituto Nazionale per le Malattie Infettive "Lazzaro Spallanzani", Scientific Direction, Roma, Italia⁽⁵⁾

Asymptomatic Mpox infection in subjects presenting for MVA-BN vaccine

GIULIA MATUSALI¹, VALENTINA MAZZOTTA², PIERLUCA PISELLI³, AURORA BETTINI¹, FRANCESCA COLAVITA¹, SABRINA COEN¹, FRANCESCO VAIA⁴, ENRICO GIRARDI⁵, ANDREA ANTINORI², FABRIZIO MAGGI¹.

1Laboratory of Virology, National Institute for Infectious Diseases Lazzaro Spallanzani IRCCS, Rome, Italy; 2 HIV/AIDS Unit, National Institute for Infectious Diseases Lazzaro Spallanzani IRCCS, Rome, Italy; 3 Epidemiology Unit, National Institute for Infectious Diseases Lazzaro Spallanzani IRCCS, Rome, Italy; 4 General Direction, National Institute for Infectious Diseases Lazzaro Spallanzani IRCCS Rome, Italy; 5 Scientific Direction, National Institute for Infectious Diseases Lazzaro Spallanzani IRCCS, Rome, Italy.

Introduction: Recent reports assessed the prevalence of undiagnosed mpox virus (MPXV) infection either by retrospective testing of clinical specimens for viral DNA and/or anti-viral antibodies presence. These studies contribute to understanding the role of asymptomatic infection in the spread of MPXV during epidemic and inter-epidemic phases. One tool for dealing with the epidemic was the launch of the vaccination campaign with the third-generation MVA-BN vaccine. In Italy, the vaccination program was targeted to high-risk categories such as laboratory personnel with possible direct exposure to orthopoxviruses (OPXV) and men who have sex with men (MSM) who met sexual habit-associated risk criteria. **Methods:** 1549 individuals received MVA-BN at our Institute between August 8th and September 9th, 1244 (80.3%) were naïve to vaccinia virus (VACV)-based vaccines. We analysed serum samples from 141 (11.3%) randomly selected VACV-vaccine naïve individuals before the vaccine administration. Samples were tested for anti-MPXV IgG and neutralizing antibodies (NAb) by immunofluorescence and plaque-reduction-neutralization tests, respectively. All were men, median age 38 years (IQR 31-43), and 70 (49.6%) were people living with HIV (PLWH). **Results:** 123 serum samples (87.2%) tested negative by both assays, and 18 (12.8%) revealed anti-MPXV IgG at different levels. Importantly, 11 of 18 (61.1%) anti-MPXV IgG positive samples had a concomitant presence of NAb. To investigate whether anti-MPXV antibodies resulted from recent exposure to the virus, we tested the samples for the presence of anti-MPXV IgM and IgA, serological markers associated with the acute and post-acute phases of infection. Anti-MPXV IgA and IgM were detected in 8 and 4 samples, respectively. Individuals #16, 17, and 18 showed the simultaneous presence of IgG/IgA/IgM/NAb, they were ART-suppressed aviremic PLWH with a CD4 count over 500 cells/mm³. The presence of serum MPXV DNA at a low level was revealed in individual #18 (36.01 Ct). **Discussions and conclusions:** Although the only detection of anti-MPXV IgG and NAb cannot exclude previous exposure to other OPXV, the simultaneous presence of IgA and/or IgM suggests a recent OPXV infection. More importantly, the presence of MPXV DNA in the

serum of subject #18 confirms MPXV infection at the time of the first MVA-BN dose. Our findings suggest the importance of monitoring people at higher risk of infection for possible cases of asymptomatic MPXV. Even if different studies report a low to relevant impact of unrecognized and asymptomatic mpox infections, there is consensus on the need for prevention and control measures that encompass enhanced surveillance, wider availability of testing, access to vaccination, and adequate risk communication strategies.

Table 1. Detection of anti-MPXV antibodies in individual #16, 17, and 18

Individual	IgG	IgM	IgA	NAbs
#16	1:80	1:20	1:20	1:160
#17	1:80	1:320	1:80	1:80
#18	1:320	1:40	1:20	1:20

und=undetected, NAbs=neutralizing antibodies

260 - Prevalence of respiratory viruses during the late pandemic and early post-pandemic months in an Italian tertiary university hospital.

FEDERICA NOVAZZI⁽¹⁾ - FRANCESCA DRAGO FERRANTE⁽²⁾ - ANGELO PAOLO GENONI⁽¹⁾ - ANDREINA BAJ⁽¹⁾ - RICCARDO CAPUANO⁽²⁾ - GIANLUCA CASSANI⁽²⁾ - ALBERTO COLOMBO⁽²⁾ - PAOLO GIGANTE⁽²⁾ - RENÈE PASCIUTA⁽²⁾ - AGOSTINO ROSSI⁽²⁾ - NICASIO MANCINI⁽¹⁾

University of Insubria, Varese, Italy, Laboratory of Medical Microbiology and Virology, Department of Medicine and Technological Innovation,, Varese, Italia⁽¹⁾ - Laboratory of Medical Microbiology and Virology,, University Hospital of Varese, Italy, Varese, Italia⁽²⁾

Prevalence of respiratory viruses during the late pandemic and early post-pandemic months in an Italian tertiary university hospital.

Federica Novazzi^{a,b}, Francesca Drago Ferrante^b, Angelo Paolo Genoni^{a,b}, Andreina Baj^{a,b}, Riccardo Capuano^b, Gianluca Cassani^b, Alberto Colombo^b, Paolo Gigante^b, Reneè Pasciuta^b, Agostino Rossi^b, Antonio Tamborini^b and Nicasio Mancini^{a,b}

^aLaboratory of Medical Microbiology and Virology, Department of Medicine and Technological Innovation, University of Insubria, Varese, Italy; ^bLaboratory of Medical Microbiology and Virology, University Hospital of Varese, Italy

Introduction: Respiratory viruses (RVs) are an important burden on human health and it is debated whether COVID-19 pandemic somehow impacted on their diffusion. In this study we report the epidemiology of non-SARS-CoV-2 RVs in upper (nasal swab: NS) and lower (bronchoalveolar lavage: BAL) respiratory samples (RSs) of patients admitted to the University Hospital of Varese, during the late pandemic and early post-pandemic months.

Material and Methods: From January 2022 to May 2023 a total of 913 RSs [629 (68.9%) NS and 284 (31.1%) BAL] were collected from patients attending primary and secondary healthcare services for acute respiratory illness (ARI). During the study period two syndromic molecular panels were used: the BioFire® Respiratory 2.1 (RP2.1) Panel for NS and the BioFire® Pneumonia plus Panel for BAL (BioMérieux, Marcy-l'Étoile, France) detecting a broad panel of pathogens commonly associated to respiratory infections.

Results: Overall, 213/913 samples (23.3%) tested positive to one or more RV, with a positivity rate of 33.8% and of 28.5% for NS and BAL, respectively. Among positive NS, in 96.2% a single RV was detected whereas two or more RVs were detected in 3.8% of cases. Influenza A (FLUA) and SARS-CoV-2 accounted for more than one third of positive cases (35.9% in total) with Respiratory syncytial virus (RSV) lagging well behind (5.6%). Only in a single NS (0.5%) two of the most common viruses (RSV and SARS-CoV-2) were detected together. Among positive NS, 94/213 (44.1%) were collected from patients admitted to the adult emergency unit (median age 55 y.o.), with 22/94 (23.4%) positive to FLUA-H3N2. Among positive BAL, in 63/81 (77.8%) a single RV was detected, whereas in the remaining 18/81 (22.2%) cases two or more RVs were detected. RSV was detected in 24/81 (29.6%) and FluA-H3N2 in 6/81 (2.1%). RSV and FluA were never detected together in a single BAL sample. Positive BAL samples were mostly collected from pediatric patients or young adults (median age 19 y.o.) , mostly (48.1 %) admitted to the pediatric intensive care unit.

Discussion and conclusion: In our study, a higher co-detection of two or more RVs was observed in lower respiratory tract samples compared to upper respiratory tract samples. This co-detection was

frequent especially in BAL samples collected from pediatric patients admitted to the ICU. The presence of “minor” RVs in co-infections possibly precipitating the clinical picture of young patients deserves attention and their pathophysiological direct and indirect role should be further investigated.

267 - Frog-derived antiviral peptides (AVPs): pan-inhibitors against respiratory viruses

CARLA ZANNELLA⁽¹⁾ - ANNALISA CHIANESE⁽¹⁾ - ROSA GIUGLIANO⁽¹⁾ - ANNALISA AMBROSINO⁽¹⁾ - ALESSANDRA MONTI⁽²⁾ - NUNZIANNA DOTI⁽²⁾ - ANNA DE FILIPPIS⁽¹⁾ - MASSIMILIANO GALDIERO⁽¹⁾

Università degli Studi della Campania "Luigi Vanvitelli", Dipartimento di Medicina Sperimentale-Sezione Microbiologia e Virologia, Napoli, Italia⁽¹⁾ - Institute of Biostructures and Bioimaging (IBB), National Research Council (CNR), Napoli, Italia⁽²⁾

Frog-derived antiviral peptides (AVPs): pan-inhibitors against respiratory viruses

Carla Zannella^{1,2}, Annalisa Chianese^{1,2}, Rosa Giugliano¹, Annalisa Ambrosino^{1,2}, Alessandra Monti³, Nunzianna Doti³, Anna De Filippis¹, Massimiliano Galdiero^{1,2}.

1 Department of Experimental Medicine, University of Campania "Luigi Vanvitelli", 80138 Naples, Italy.

2 Section of Microbiology and Virology; University Hospital "Luigi Vanvitelli" of Naples, 80138 Naples, Italy.

3 Institute of Biostructures and Bioimaging (IBB), National Research Council (CNR), 80131 Naples, Italy.

Introduction. COVID-19 outbreak has evidence the emergence of the discovery for new antiviral agents, mostly able to act against a broad spectrum of viruses. Most current antivirals are directed to inhibit specific viruses since these therapeutic molecules are designed to act on a specific viral target with the objective of interfering with a precise step in the replication cycle. Therefore, antimicrobial peptides (AMPs) have been identified as promising antiviral agents that could help to overcome this limitation and provide compounds able to act on more than a single viral family. In this scenario, AMPs with antiviral effect, also known as AVPs, have been already reported as potent inhibitors of the viral infection by affecting different stages of virus lifecycle. In the present study, we analyzed the activity of AVPs derived from skin secretions of different amphibians against a wide range of respiratory viruses, including the coronaviruses HCoV-229E and SARS-CoV-2, measles virus, human parainfluenza virus type 3 and influenza virus H1N1.

Material and methods. AVPs were synthesized using the solid-phase Fmoc chemistry method, followed by purification by reversed-phase HPLC. The toxicity was determined via the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, identifying a 50% cytotoxic concentration (CC₅₀). Different time of addition assays were performed to evaluate in which part of the viral lifecycle AVPs could be involved.

Results. The antiviral activity was investigated via plaque assays, indicating that the main target of Hylin-a1, AR-23, RV-23 and HS-1 peptides was the viral envelope. These data have been also confirmed by the lack of activity of the peptides against the naked coxsackievirus B3.

Discussion and conclusions. Considering AVPs huge therapeutic potential, further experiments are required to confirm their mechanism of action and to increase their stability by modifying the native sequence.

272 - Design of Mopeia virus fusion inhibitors on GP2 glycoprotein

ANNALISA CHIANESE⁽¹⁾ - CARLA ZANNELLA⁽¹⁾ - FRANCESCA PALMA⁽¹⁾ - ROSA GIUGLIANO⁽¹⁾ - ALESSANDRA MONTI⁽²⁾ - NUNZIANNA DOTI⁽²⁾ - ANNA DE FILIPPIS⁽¹⁾ - MASSIMILIANO GALDIERO⁽¹⁾

Università, Dipartimento di Medicina Sperimentale, Università degli studi della Campania "Luigi Vanvitelli", Napoli, Italia⁽¹⁾ - Istituto di Biostructures e Bioimaging, Consiglio Nazionale di Ricerca, Napoli, Italia⁽²⁾

Design of Mopeia virus fusion inhibitors on GP2 glycoprotein

Annalisa Chianese^{1,2}, Carla Zannella^{1,2}, Francesca Palma¹, Rosa Giugliano¹, Alessandra Monti³, Nunzianna Doti³, Anna De Filippis¹, Massimiliano Galdiero^{1,2}

1 Department of Experimental Medicine, University of Campania "Luigi Vanvitelli", 80138 Naples, Italy.

2 Section of Microbiology and Virology; University Hospital "Luigi Vanvitelli" of Naples, 80138 Naples, Italy.

3 Institute of Biostructures and Bioimaging (IBB), National Research Council (CNR), 80131 Naples, Italy.

Introduction. Lassa fever is an acute viral hemorrhagic illness occurring in West Africa, having posed a serious public health threat in many countries. The etiologic agent of Lassa fever is Lassa virus (LASV), an enveloped, single-stranded RNA virus belonging to the Arenaviridae family. So far, no approved vaccines and specific treatment options against LASV exist. To develop specific fusion inhibitors against LASV, we used a surrogate Mopeia virus (MOPV), sharing a common rodent host (*Mastomys natalensis*), and more than 75% amino acid identity with LASV. However, differently by LASV, MOPV is non-pathogenic in non-human primates, and no human case of infection has ever been reported. **Materials and methods.** Based on the structure, we designed several peptides spanning the heptad repeat 1 (HR1) and 2 (HR2) domains within the GP2 fusion protein. Then we tested their antiviral activities via cell-based assays. **Results.** The GP2 protein is a class I fusion protein containing a N-terminal fusion peptide, a N-terminal HR1 domain (residues 295-363), a linker T-loop domain, a C-terminal HR2 domain (residues 386-431), a transmembrane domain, and an intracellular domain. We found that the HR2 peptides were effective in inhibiting MOPV infection because of their higher affinity to bind with the HR1 groove. **Discussion and Conclusions.** Comparing LASV and MOPV, two very similar viruses with different pathogenic potential, may be a fruitful approach to identify immune and viral features involved in LASV pathogenesis and to identify antiviral therapeutics. These results not only suggest that MOPV provides an important basis for rational design of LASV fusion inhibitors, but also help to develop inhibitors for LASV infection by working with lower biosafety level (BSL) compared to the BSL4 required for the pathogenic virus.

295 - SARS-CoV-2 large ORF8 deletion cluster in routine NGS screening for epidemiological surveillance

EMIL TONON ⁽¹⁾ - RICCARDO CECCHETTO ⁽¹⁾ - ERICA DIANI ⁽¹⁾ - ANNA LAGNI ⁽¹⁾ - VIRGINIA LOTTI ⁽¹⁾ - NICOLETTA MEDAINA ⁽²⁾ - GIONA TURRI ⁽³⁾ - MOSÈ FAVARATO ⁽⁴⁾ - DAVIDE GIBELLINI ⁽¹⁾

UNIVERSITÀ DI VERONA, DIP.DIAGNOSTICA E SANITÀ PUBBLICA, VERONA, Italia ⁽¹⁾ - OSPEDALE G. B. RIOSSI, UOC MICROBIOLOGIA, VERONA, Italia ⁽²⁾ - OSPEDALE, UOC MICROBIOLOGIA, ERONA, Italia ⁽³⁾ - AULSS 3, UOSD of Molecular Diagnostics and Genetics, VENEZIA, Italia ⁽⁴⁾

Detection of SARS-CoV-2 large ORF8 deletion cluster in routine NGS screening for epidemiological surveillance

EMIL TONON^{1,2}, RICCARDO CECCHETTO^{1,2}, ERICA DIANI¹, ANNA LAGNI¹, VIRGINIA LOTTI¹, NICOLETTA MEDAINA², GIONA TURRI², MOSE' FAVARATO³, DAVIDE GIBELLINI^{1,2}

¹ Department of Diagnostic and Public Health, Verona University, 37134 Verona, Italy; ² UOC Microbiology, AOUI Verona, 37134 Verona, Italy; UOSD OF Molecular Diagnostics and Genetics, AULSS 3 Serenissima, Venice, Italy.

Introduction.

Next Generation Sequencing (NGS) is a technology widely used for viral sequencing. This method was employed for the epidemiological analysis of new emerging viruses and in the detection of new viral variants. NGS described the different mutations and/or recombination in SARS-CoV-2 revealed during the Covid-19 pandemic. In particular, specific mutations in S protein determined the classification of new variants and supported the comprehension of subtle correlation between variants and pathological outcomes and the strategies of viral escape. In addition to S mutations and deletions, sequencing analyses individuated important genome variations located in other regions suggesting a peculiar SARS-CoV-2 evolution. In this study, we investigated on a specific 426 bp deletion, occurring between ORF7b and ORF8, detected in 6 patients infected with BA.5.1 Omicron variant during epidemiological analysis.

Materials and Methods

All positive nasopharyngeal samples were amplified by RT-PCR by Allplex SARS-CoV-2 Assay (Seegene) and only samples with Ct<30 were analyzed by NGS platform. After the bioinformatic analysis of obtained reads that revealed the presence of 426 bp deletion, these samples were also analyzed by Sanger sequencing to confirm the previous results. We compared our sequences with all the similar sequences uploaded in GISAID. We analyzed the possible secondary structure of region involved in this deletion using RNA folding tool of the Mfold web server.

Results

We detected in 6 cases the presence of a large deletion spanning ORF7b and ORF8. This in frame deletion maintains the first 22 aa of ORF7b and the last 3 aa of ORF8 encoded proteins. All patients share the viral variant BA.5.1. Analysis of secondary structure of the region involved in deletion shows that it contains specific tandem repeats located on hairpin structures, which can facilitate rearrangement events. Interestingly all patients carrying this deletion show longer positivity period compared to patients without this deletion.

Discussion and conclusion

The 426 bp deletion between ORF7b and ORF8 causes the loss of second half of ORF7b and the absence of ORF8 encoded protein. The presence of this deletion could be related to a longer positivity period and milder symptomatology. Previous studies indicate that this deletion could be responsible of lower impact on inflammatory response and suggest that it could be an evolution strategy. Recently XBB subvariants confirm our hypothesis, in fact it contains an ORF8 with a stop codon at position 8, determining the absence of encoded protein. This aspect suggests that ORF8 does not play a crucial role in viral transmission and that probably, the absence of this protein could be advantageous for the virus adaptation to host.

10 Probiotics

116 - Targeting persister cells of *Pseudomonas aeruginosa* under conditions relevant to cystic fibrosis using culture filtrate supernatants of lactobacilli

MARTA BIANCHI ⁽¹⁾ - ESINGUL KAYA ⁽¹⁾ - SEMIH ESIN ⁽¹⁾ - GIOVANNA BATONI ⁽¹⁾ -
GIUSEPPANTONIO MAISETTA ⁽¹⁾

Università di Pisa, Dip. di Ricerca Traslazionale e delle nuove Tecnologie in Medicina e Chirurgia,
Pisa, Italia ⁽¹⁾

Targeting persister cells of *Pseudomonas aeruginosa* under conditions relevant to cystic fibrosis
using culture filtrate supernatants of lactobacilli

MARTA BIANCHI¹, ESINGÜL KAYA¹, SEMIH ESIN¹, GIOVANNA BATONI¹ AND
GIUSEPPANTONIO MAISETTA¹

¹Department of Translational Research and New Technologies in Medicine and Surgery, University
of Pisa, 56123 Pisa, Italy.

Introduction: The therapy of lung infections sustained by *Pseudomonas aeruginosa* in cystic fibrosis (CF) patients is problematic due to the presence of a sticky mucus in the airways and the formation of biofilm, which exhibits increased antibiotic tolerance. Importantly, highly tolerant subpopulations of bacteria, also known as persister cells, have been implicated in antibiotic treatment failure and in promoting the evolution of antibiotic resistance. It is widely accepted that persister bacteria play a crucial role in the recalcitrance and relapse of many bacterial infections, including *P. aeruginosa* pulmonary infections in CF patients. Among novel antimicrobial strategies, the use of probiotics and their products is emerging as a particularly attractive approach. The aim of this study was to evaluate the anti-persister activity of culture filtrate supernatants (CFS) of *Lactocaseibacillus rhamnosus* against *P. aeruginosa* in artificial sputum medium (ASM), which resembles the CF lung environment. **Materials and methods:** MICs of CFS and tobramycin towards *P. aeruginosa* were evaluated by a standard liquid susceptibility assay. Persisters of *P. aeruginosa* were obtained following two different procedures: exposing stationary-phase cultures to 200 µg/ml cyanide m-chlorophenylhydrazine (CCCP, an uncoupler able to decrease ATP synthesis and induce the persistent state) in LB medium or incubating stationary-phase cultures with high doses of tobramycin (128 µg/ml: 64-fold MIC) in ASM. Killing effect of CFS against persisters of *P. aeruginosa* (strain PaCF1, isolated from the sputum of a CF patient) was evaluated in ASM by CFU count. **Results:** MIC values of CFS and tobramycin towards PaCF1 were 1:16 and 1 µg/ml, respectively. When pretreated with CCCP, PaCF1 displayed very low susceptibility to high dose of ciprofloxacin in ASM, while it resulted susceptible to CFS diluted 1:8 (2MIC), exhibiting a 200-fold decrease in CFU number compared to the control. To obtain tobramycin-tolerant bacteria in ASM, PaCF1 was exposed to high doses of antibiotic for 24h. A further 24h-incubation of PaCF1 to high dose of tobramycin confirmed the acquisition of the tolerant state of the bacteria towards the same antibiotic, whereas the exposure for 24h of PaCF1 to CFS at 1:8 and 1:4 dilution caused a decrease of CFU number of 1 log and 5 logs, respectively. **Discussion and conclusion:** Metabolites derived from probiotics seem to represent a promising therapeutic strategy against *P. aeruginosa* in CF patients. Although the mechanisms of the antimicrobial activity of CFS have yet to be fully elucidated, we can hypothesize a role of acidic bacteriocins, peptides able to damage bacterial cytoplasmic membranes, as mediators of the killing effect of CFS against *P. aeruginosa* persisters.

135 - Exploring the antibacterial effect of *Lacticaseibacillus rhamnosus* cell-free culture supernatants against *Pseudomonas aeruginosa* in an air-liquid interface lung infection model

MARTA BIANCHI⁽¹⁾ - GIUSEPPANTONIO MAISETTA⁽¹⁾ - BRUNELLA GRASSIRI⁽²⁾ - ESINGUL KAYA⁽¹⁾ - MICHAL MALINOWSKI⁽³⁾ - ANNA MARIA PIRAS⁽²⁾ - GIOVANNA BATONI⁽¹⁾ - SEMIH ESIN⁽¹⁾

Università di Pisa, Dip. di Ricerca Traslationale e delle nuove Tecnologie in Medicina e Chirurgia, Pisa, Italia⁽¹⁾ - Università di Pisa, Dip. di Farmacia, Università di Pisa, Pisa, Italia⁽²⁾ - University of Wroclaw, Medical University of Wroclaw, Wroclaw, Polonia⁽³⁾

Exploring the antibacterial effect of *Lacticaseibacillus rhamnosus* cell-free culture supernatants against *Pseudomonas aeruginosa* in an air-liquid interface lung infection model

MARTA BIANCHI¹, GIUSEPPANTONIO MAISETTA¹, BRUNELLA GRASSIRI², ESINGÜL KAYA¹, MICHAL MALINOWSKI^{2,3}, ANNA M. PIRAS², GIOVANNA BATONI¹ AND SEMIH ESIN¹

¹Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa, Italy; ²Department of Pharmacy, University of Pisa, Italy; ³Medical University of Wroclaw, Poland.

Introduction: *Pseudomonas aeruginosa* (Pa) infections pose a significant challenge in healthcare settings, especially in cystic fibrosis (CF) patients, causing chronic lung inflammation and declining respiratory function, which complicates CF management. At the aim of developing alternative antimicrobial strategies for Pa infections in CF patients, in this study, we investigated the antibacterial effect of *Lacticaseibacillus rhamnosus* cell-free culture supernatants (CFS-LR) against Pa using an in-vitro air-liquid interface (ALI) lung infection model. In addition, we developed a liquid CFS-LR formulation to evaluate its potential for future aerosol administration.

Materials and Methods: Two Pa clinical strains, isolated from the sputum of CF patients, PaCF1 (non-mucoid phenotype) and PaCF4 (mucoid phenotype) were used. To obtain CFS-LR, culture of *L. rhamnosus* were established in De Man-Rogosa-Sharpe broth (MRSB) for 48 hours, centrifuged, and filtered through 0.22 µm filters. The ALI lung infection model was established using NCI-H441 human distal lung epithelial cells, seeded into Transwell® inserts, and differentiated with dexamethasone and insulin-transferrin-selenium. Monolayers were infected with PaCF1 and PaCF4 and treated for 7 h with CFS-LR before assessing the bacterial load as compared to un-treated samples. CFS-LR cytotoxicity against NCI-H441 cells was evaluated via the WST1 assay. To assess the inhalation profile of CFS-LR, the aerodynamic distribution of nebulized CFS-LR was evaluated using a twin-stage impinger (TSI) with an Aerogen® mesh nebulizer.

Results: In the ALI model, Pa treatment with CFS-LR resulted in significant CFU reduction of both PaCF1 (2 logs) and PaCF4 (3 logs) at the dilution of 1:8, while at the dilution of 1:4 they completely eradicated the bacteria. Cytotoxicity studies revealed a toxic effect of CFS-LR at 1:4 dilution, while the 1:8 dilution was well tolerated. Nebulization of CFS-LR resulted in 69% deposition in the second stage of the TSI, suggesting their ability to reach the deepest airways. Lyophilization of CFS-LR led to a reduction in their bactericidal activity against Pa, but cryoprotectants such as trehalose and mannitol partially restored their antimicrobial activity.

Discussion and Conclusions: The findings of this study demonstrate a marked antibacterial effect of CFS-LR against Pa in an ALI lung infection model. The ALI model closely mimics the lung environment and offers valuable insights into the effectiveness of potential therapeutic agents. Furthermore, the TSI results indicate the feasibility of using CFS-LR through aerosol delivery, allowing their targeted administration to the lungs with the potential of maximizing their effect directly at the site of infection.

146 - Different activity of *Lactobacillus* spp. against two *Proteus mirabilis* isolated clinical strains in different anatomical sites: an explorative study to improve the therapeutic approach

ALESSANDRA FUSCO⁽¹⁾ - VITTORIA SAVIO⁽¹⁾ - BRUNELLA PERFETTO⁽¹⁾ - ADRIANA CHIAROMONTE⁽¹⁾ - GIOVANNA TORELLI⁽¹⁾ - GIUSY MAVILIO⁽¹⁾ - ALBERTO ALFANO⁽²⁾ - GIOVANNA DONNARUMMA⁽¹⁾

Università della Campania Luigi Vanvitelli, Dipartimento di Medicina Sperimentale sezione Microbiologia e Microbiologia clinica, Napoli, Italia⁽¹⁾ - Università della Campania Luigi Vanvitelli, Dipartimento di Medicina Sperimentale sezione Biotecnologie e Biologia Molecolare, Napoli, Italia⁽²⁾

Different activity of *Lactobacillus* spp. against two *Proteus mirabilis* isolated clinical strains in different anatomical sites: an explorative study to improve the therapeutic approach

ALESSANDRA FUSCO¹, VITTORIA SAVIO¹, BRUNELLA PERFETTO¹, ADRIANA CHIAROMONTE¹, GIOVANNA TORELLI¹, GIUSY MAVILIO¹, ALBERTO ALFANO², GIOVANNA DONNARUMMA¹

¹Department of Experimental Medicine, Section of Microbiology and Clinical Microbiology, University of Campania "Luigi Vanvitelli", Naples, Italy

²Department of Experimental Medicine, Section of Biotechnology and Molecular Biology, University of Campania "Luigi Vanvitelli", Naples, Italy

Introduction: Urinary tract infections (UTIs) and catheter-associated UTIs (CAUTIs) are the principal hospital-acquired infections. Between these, bacterial prostatitis is believed to be the leading cause of recurrent UTIs in men under 50 years of age and occurs both as an acute febrile disease responsive to antibiotics and as a chronic infection that is often unresponsive to antibiotic treatment. *Proteus mirabilis* is more commonly associated with UTIs in these abnormalities, especially in patients undergoing catheterisation. This pathogen is able to colonise the host's tissues and to cause disease thanks to the production of many virulence factors such as fimbriae, flagella, immune avoidance, host-damaging factors, and the ability to form crystalline biofilms. *Lactobacillus* spp. are an important component of the human microbiota and in large quantities occur in foods. Regardless of their origin, those with probiotic properties are proposed as an alternative to antibiotic therapy in the treatment of urinary tract infections. The aim of this work was to test the activity of different *Lactobacillus* strains against two clinical isolates of *P. mirabilis* on bladder and prostatic epithelium. Materials and Methods: T24 (epithelioid carcinoma of human urinary bladder) and PC-3 (prostate adenocarcinoma) cells were cultured respectively in McCoy's 5a and RPMI medium with 10% fetal bovine serum, 100 IU/ml penicillin, 100 mg/ml streptomycin and 2mM glutamine, at 37° C in a 5% CO₂. First, both cell lines were treated with 7 different strains of *Lactobacillus* spp. to select those not cytotoxic and with the main anti-inflammatory activity for each epithelium. Once these parameters have been established, cells were infected with 2 different clinical isolated strains of *P. mirabilis*, named PM1 and PM2, alone or in presence of selected *Lactobacillus* strains, and the bacterial invasiveness ability and flagellar flhDC gene expression were tested. In addition, cellular expression of pro-inflammatory cytokines and antimicrobial peptide HBD-2 was analyzed by Real Time PCR and ELISA assay. Results: the results obtained show that 6 different *Lactobacillus* strains showed anti-inflammatory and invasiveness inhibitory

activity against *P. mirabilis* on bladder epithelium, while only 2 strains were active on prostatic epithelium. Furthermore, the antibacterial action differs according to the clinical isolate of *P. mirabilis* tested. Discussion and Conclusions: it is a misconception that any probiotic strain can be effective. As for the conventional therapeutic approach, also for what concerns the use of probiotics it is important to establish the interactions with the anatomical district and with the pathogen towards which their activity is intended.

169 - Activity of cell-free supernatants from *Lacticaseibacillus rhamnosus* against mono and dual-species biofilms of wound pathogens in a 3D in vitro collagen wound infection model

ESINGÜL KAYA ⁽¹⁾ - MARTA BIANCHI ⁽¹⁾ - GIUSEPPANTONIO MAISETTA ⁽¹⁾ - SEMIH ESIN ⁽¹⁾ - GIOVANNA BATONI ⁽¹⁾

Università Di Pisa, Dipartimento Di Ricerca Traslazionale E Nuove Tecnologie In Medicina E Chirurgia, Pisa, Italia ⁽¹⁾

Activity of cell-free supernatants from *Lacticaseibacillus rhamnosus* against mono and dual-species biofilms of wound pathogens in a 3D in vitro collagen wound infection model

ESINGÜL KAYA, MARTA BIANCHI, GIUSEPPANTONIO MAISETTA, SEMIH ESIN AND GIOVANNA BATONI

Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa, Pisa, Italy

Introduction: It is widely agreed that microbial biofilms play a major role in promoting infection and delaying healing of chronic wounds. In the era of microbial resistance, probiotic strains or their metabolic products, mainly from the lactobacilli group, are emerging as an innovative approach for the treatment of hard-to-heal (chronic) wounds due to their antimicrobial, healing and host immune-modulatory effects. In this study, we aimed to investigate the potential of cell-free supernatants (CFS) of *Lacticaseibacillus rhamnosus* against bacterial biofilms in the context of chronic wound infections by using a 3D in vitro wound infection model.

Materials and Methods: *L. rhamnosus* was grown in the De Man-Rugose-Sharp broth (MRSB) for 48 h. Cultures were then centrifuged and the supernatants passed through 0.22 µm filters. Collagen scaffolds were obtained in simulant wound fluid (SWF) using rat tail collagen type 1 to mimic the wound bed. They were infected with *Staphylococcus aureus* or *Pseudomonas aeruginosa* isolated from chronic wounds and incubated for 24 h at 37°C to obtain mature biofilms. Dual species biofilms were also obtained incubating *S. aureus*:*P. aeruginosa* in 1000:1 ratio. Following incubation, mature mono or dual-species biofilms were treated with CFS at different concentrations and for different time periods. CFU count, and electron microscopy imaging has performed to assess CFS's effect on infected collagen scaffolds. Cytotoxic potential of CFS was evaluated against the HaCaT human keratinocyte cell line following propidium iodide staining and analysis by flow cytometry.

Results: CFS caused a dose-dependent acidification of SWF when added to mature biofilms of both bacterial species. At 1:4 dilution in SWF, CFS exerted a marked antibacterial effect reducing the CFU numbers of both biofilm-embedded bacteria and bacteria released by the biofilm in the supernatant. This effect, observed in mono-species biofilms was also demonstrated for the dual-species biofilms starting from 8 h of incubation. Electron microscopy imaging confirmed these results. 25% CFS exhibited acceptable levels of cytotoxicity at both 4 and 24 h of incubation against HaCaT cells.

Discussion and conclusions: Metabolites derived from probiotics seems a promising therapeutic/preventive strategy in the era of antibiotic resistance. Herein we have demonstrated the anti-biofilm activity of CFS from *L. rhamnosus* in conditions closely resembling the wound bed. Due to the acidic nature of the CFS, their topical use in antimicrobial wound therapy could not only target bacterial cells within the biofilm, but also correct the wound micro-environment whose pH shift from acidic to alkaline in chronic wounds is consistently reported as an obstacle to wound healing.

211 - Probiotic pretreatment protects corneal epithelial cells against *Staphylococcus aureus* infection

IRENE PATERNITI ⁽¹⁾ - SARAH ADRIANA SCUDERI ⁽¹⁾ - LUCIA CAMBRIA ⁽¹⁾ - ANTONIA NOSTRO ⁽¹⁾ - EMANUELA ESPOSITO ⁽¹⁾ - ANDREANA MARINO ⁽¹⁾

Università, Università di Messina/Dipartimento di Scienze Chimiche, Biologiche, Farmaceutiche e Ambientali, Messina, Italia ⁽¹⁾

Probiotic pretreatment protects corneal epithelial cells against *Staphylococcus aureus* infection

IRENE PATERNITI, SARA A. SCUDERI, LUCIA CAMBRIA, ANTONIA NOSTRO, EMANUELA ESPOSITO, ANDREANA MARINO

Department of Chemical, Biological, Pharmaceutical and Environmental Sciences. University of Messina, Messina, Italy.

Introduction. *Staphylococcus aureus* represents a common cause of severe keratitis. Currently, the treatment is based on eye drops containing antibiotics that may cause changes in the microbiome contributing to the increase the resistance, and/or on anti-inflammatory drugs which may bring other side effects. Alternative non-antibiotic strategies such as probiotic therapy need consideration because could help restore the homeostasis of ocular surface and prevent or reduce infections. This study aimed to assess the preventive effect of three probiotics in reducing the infection of human corneal epithelial cells (HCE) caused by *Staphylococcus aureus*. **Materials and Methods.** The human corneal epithelial cells (HCE) were used to assess the extent of protection by probiotics against *S. aureus* infection. Probiotics: *Lactobacillus reuteri* DSM20016, *Bifidobacterium longum* subsp. *infantis* DSM20088 and *S. epidermidis* DSM1798. Pathogen: *S. aureus* ATCC 6538. The following assays were performed: cytotoxicity and adhesion to HCE of probiotics at 24 and 48 h of contact. Antagonism, cytotoxicity, anti-inflammatory and antioxidant properties of probiotics against *S. aureus* infection were evaluated as follows: pretreatment of HCE with each probiotic at 1 h and 24 h prior to *S. aureus* inoculation followed by 1 h and 24 h of growth in combination. **Results.** Probiotics pretreatment significantly increased viability of HCE cells and reduced immuno-inflammatory and nitrosative stress biomarkers respect to the control cells not pretreated and infected. However, it did not counter the adhesion of *S. aureus* to HCE cells. The pretreatment with *L. reuteri*, *B. longum* and *S. epidermidis* protected HCE cells against *S. aureus* challenge. Among all, *S. epidermidis* significantly increased HCE cells viability and demonstrated a greater ability to adhere to them; while *L. reuteri* and *B. longum* significantly showed higher anti-inflammatory and antinitrosative activities, reducing TNF- α level, NO_x amount and re-establishing IL-4 and IL-10 level cytokines. **Discussion and Conclusions.** The results showed that pretreatment with probiotics has not a direct role player in contrasting the adhesion of *S. aureus* to HCE but, is to be highlighted that it protects cells and reduces inflammatory and antinitrosative biomarkers associated with *S. aureus* infection.

224 - In vitro evaluation of the most active probiotic strains able to improve the intestinal barrier functions and to prevent inflammatory diseases of the gastrointestinal system

VITTORIA SAVIO⁽¹⁾ - ALESSANDRA FUSCO⁽¹⁾ - BRUNELLA PERFETTO⁽¹⁾ - ADRIANA CHIAROMONTE⁽¹⁾ - GIOVANNA TORELLI⁽¹⁾ - GIUSY MAVILIO⁽¹⁾ - MARINA ACUNZO⁽¹⁾ - SERGIO D'AMBROSIO⁽²⁾ - DONATELLA CIMINI⁽²⁾ - GIOVANNA DONNARUMMA⁽¹⁾

Università degli Studi della Campania 'Luigi Vanvitelli', Dipartimento di Medicina Sperimentale - Sezione Microbiologia e Microbiologia clinica, Napoli, Italia⁽¹⁾ - Università degli Studi della Campania 'Luigi Vanvitelli', Dipartimento di Medicina Sperimentale - Sezione Biotecnologie e Biologia Molecolare, Napoli, Italia⁽²⁾

In vitro evaluation of the most active probiotic strains able to improve the intestinal barrier functions and to prevent inflammatory diseases of the gastrointestinal system

VITTORIA SAVIO¹, ALESSANDRA FUSCO¹, BRUNELLA PERFETTO¹, ADRIANA CHIAROMONTE¹, GIOVANNA TORELLI¹, GIUSY MAVILIO¹, MARINA ACUNZO¹, SERGIO D'AMBROSIO², DONATELLA CIMINI², GIOVANNA DONNARUMMA¹

¹Department of Experimental Medicine, Section of Microbiology and Clinical Microbiology, University of Campania "Luigi Vanvitelli", Naples, Italy

²Department of Experimental Medicine, Section of Biotechnology and Molecular Biology, University of Campania "Luigi Vanvitelli", Naples, Italy

Introduction: The integrity of the intestinal barrier is fundamental for gut health and homeostasis; its damage can increase intestinal permeability, with translocation of bacteria and/or endotoxins from gut, with the onset of various intestinal diseases. *Lactobacillus* spp. is one of the most common probiotics normally found in fermented foods and dairy products and is known for its anti-inflammatory and immunomodulatory properties and for its ability to protect and enhance intestinal barrier functions. The aim of this work was to evaluate the ability of different strains of *Lactobacillus* spp. to exert anti-inflammatory and immunomodulatory activity and to prevent *Salmonella* Typhimurium and enteroinvasive *Escherichia coli* (EIEC) infections in an intestinal co-cultures cell model. Materials and Methods: adenocarcinoma colorectal Caco-2 and HT29-MTX mucus-secreting epithelial cells were plated in 12-well Transwell[®] with polycarbonate insert, in a 7:3 ratio Caco-2: HT29-MTX and cultured in DMEM medium with 10% fetal bovine serum, 100 IU/mL penicillin, 100 mg/mL streptomycin and 2mM glutamine, at 37° C in a 5% CO₂ for 21 days to obtain their differentiation. Co-cultures were treated with different strains of *Lactobacillus* spp. (10⁸ CFUs/mL) alone or in combination with 20 micrograms/mL of lipopolysaccharide (LPS) of *S. Typhimurium* for 24 h, and then we analyzed, by Real-Time PCR and ELISA assay, the expression of antimicrobial peptide HBD-2 and pro-inflammatory cytokines. In a second set of experiments, we tested the ability of *Lactobacillus* spp. to inhibit *S. Typhimurium* and EIEC adhesion and invasion to intestinal epithelium. Results: *L. brevis*, *L. reuteri* and *L. rhamnosus* proved to be more effective in protecting the intestinal epithelium. Discussion and Conclusions: these in vitro studies can help select strains particularly active on their intended use to obtain consortia formulations that can have as much maximum yield as possible in terms of patient benefit.

239 - Regulatory effects of *Lactiplantibacillus plantarum* on human skin health by counteract *Malassezia furfur* effects.

BRUNELLA PERFETTO ⁽¹⁾ - ALESSANDRA FUSCO ⁽¹⁾ - VITTORIA SAVIO ⁽¹⁾ - ADRIANA CHIAROMONTE ⁽¹⁾ - GIUSY MAVILIO ⁽¹⁾ - GIOVANNA TORELLI ⁽¹⁾ - CARLA CAPASSO ⁽¹⁾ - GIOVANNA DONNARUMMA ⁽¹⁾

Università degli Studi della Campania "Luigi Vanvitelli", Dipartimento di Medicina Sperimentale; Sezione di Microbiologia, Napoli, Italia ⁽¹⁾

Regulatory effects of *Lactiplantibacillus plantarum* on human skin health by counteract *Malassezia furfur* effects.

BRUNELLA PERFETTO, ALESSANDRA FUSCO, VITTORIA SAVIO, ADRIANA CHIAROMONTE, GIOVANNA TORELLI, GIUSY MAVILIO, CARLA CAPASSO, GIOVANNA DONNARUMMA

Department of Experimental Medicine, Section of Microbiology and Clinical Microbiology, University of Campania "Luigi Vanvitelli", Naples, Italy

Introduction: The skin is the first barrier acting in prevention of infections against pathogen attacks, thanks to its multifunctional microbial community ecosystem able to maintain host immune homeostasis and health. *Malassezia furfur* is an opportunistic pathogen which can be found in the normal cutaneous microflora, responsible for dandruff, seborrheic dermatitis, pityriasis versicolor, onychomycosis and which can exacerbate atopic dermatitis and psoriasis. At the basis of its double face commensal vs pathogens probably there are the changes in both the fungus (e.g., variable secretion of AhR agonist) and the host conditions. *M. furfur* acting on different pattern recognition receptors (TLRs, AhR pathway, NLRP3 inflammasome) coordinate the different cellular responses to contact with the microbe lead to cellular damage, barrier impairment and inflammatory cytokines production. Recent studies identify *Lactobacillus* spp. as endogenous inhabitants of healthy skin, and proved that specific lactobacilli strains have a beneficial role in wound healing process, defence against skin inflammation, as well as in resistance to infections by interfering with pathogens. The aim of our study is demonstrate as the presence of live *Lactiplantibacillus plantarum* and/or its metabolites are able to interfere with the harmful effects of *M. furfur* in an in vitro experimental model using human keratinocytes. Materials and Methods: For the evaluation of the barrier integrity and AhR pathway, HaCat cells were treated with *M. furfur* at a ratio of 30:1 yeasts/cell for 24 and 48 h alone or before a pretreatment of 2 h with *L. plantarum* ($\sim 10^8$ CFUs/ml) at a multiplicity of infection (MOI) of 100, at 37°C at 5% CO₂ in DMEM without antibiotics. For investigate inflammasome activation, cells were pre-treated with LPS of *Salmonella Typhimurium* at concentration of 100 ng/ml for 3 h, then infected with *M. furfur* (30:1 yeasts/cell) alone or in presence of *L. plantarum* ($\sim 10^8$ CFUs/ml) for 4 h. *L. plantarum* effects has been tested also on the expression of *M. furfur* phospholipase activity and on wound repair. Results *L. plantarum* well counteract all the harmful strategies of *M. furfur* reducing the phospholipase activity, accelerate wound repair reduce the activation of TJs, MMP2 and MMP9, AhR activation and NLRP3 inflammasome activation with consequently release of inflammatory cytokines. Discussion and Conclusions: Although *Lactobacilli* have a long history of safe use in fermented foods, it can be speculated that they can also have health-promoting activities as topically applied on the skin and whether they could be developed in a skin cream.

319 - Anti-virulence effect of *Lactobacillus rhamnosus* and *Lactobacillus plantarum* cell-free supernatants on *P. aeruginosa* from cystic fibrosis patients

VERONICA LUPETTI ⁽¹⁾ - GIOVANNI DI BONAVENTURA ⁽¹⁾ - GIUSEPPANTONIO MAISETTA ⁽²⁾ - ESINGÜL KAYA ⁽²⁾ - SEMIH ESIN ⁽²⁾ - GIOVANNA BATONI ⁽²⁾ - ARIANNA POMPILIO ⁽¹⁾

Department of Medical, Oral and Biotechnological Sciences, "G. d'Annunzio" University of Chieti-Pescara, Chieti, Italia ⁽¹⁾ - Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa, Pisa, Italia ⁽²⁾

Anti-virulence effect of *Lactobacillus rhamnosus* and *Lactobacillus plantarum* cell-free supernatants on *P. aeruginosa* from cystic fibrosis patients

VERONICA LUPETTI,¹ GIOVANNI DI BONAVENTURA,¹ GIUSEPPANTONIO MAISETTA,² ESINGÜL KAYA,² SEMIH ESIN,² GIOVANNA BATONI,² ARIANNA POMPILIO¹

¹Department of Medical, Oral and Biotechnological Sciences, and Center of Advanced Studies and Technologies (CAST), "G. d'Annunzio" University of Chieti-Pescara, Chieti, Italy. ²Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa, Pisa, Italy.

Introduction. Chronic lung infections caused by *Pseudomonas aeruginosa* play a significant role in the mortality and morbidity of cystic fibrosis (CF) patients. Managing these infections has proven difficult due to the widespread antibiotic resistance. Using probiotics - live microorganisms which, when administered in adequate amounts, confer a health benefit on the host - could represent a new therapeutic strategy to reduce bacterial virulence rather than interfere with bacterial growth. In this frame, here we evaluated the anti-virulence properties of cell-free supernatants (CFS) from *Lactobacillus plantarum* (Lp) and *Lactobacillus rhamnosus* (Lr) probiotic strains against two *P. aeruginosa* strains from CF sputum. **Materials and Methods.** The anti-virulence activity was assessed against mucoid (CF11) and non-mucoid (CF16) *P. aeruginosa* strains. CFS was prepared by centrifuging Lp and Lr cultures 48h-grown in DeMan, Rogosa and Sharpe broth. CF11 and CF16 strains (2×10^5 CFU/mL) grown in Luria-Bertani broth were treated for 48h with different dilutions (1:2, 1:4, 1:8) of Lp and Lr CFS corrected at pH 6. The sample obtained was quantitatively assayed for proteolytic activity, and pyocyanin and pyoverdine production. The total proteolytic activity was determined using a skim milk assay. Pyoverdine was quantified by directly measuring OD₄₀₀, while pyocyanin was extracted with chlorophorm/HCl and quantified as OD₅₂₀. **Results.** Overall, Lp CFS was more effective in reducing protease activity (4 vs. 1 significant reduction, respectively for Lp and Lr), while CFS from Lr was mainly active against pigments production (pyoverdine: 4 vs. 3; pyocyanin: 6 vs. 2, respectively for Lr and Lp). A dose-dependent anti-virulence effect was observed. In more detail, Lp and Lr CFS significantly reduced protease activity and pigment production by both *P. aeruginosa* strains when tested at the highest concentration (1:2), except for Lr vs CF16. The anti-virulence effect observed after exposure to 1:4 and 1:8 depended on the *Lactobacillus* species and *P. aeruginosa* strains tested. **Discussion and Conclusions.** The use of metabolites released in supernatants by *Lactobacillus* strains is proposed today as an attractive therapeutic strategy in the era of antibiotic resistance. Our findings, although preliminary, indicated acellular supernatants of *L. rhamnosus* and *L. plantarum* exhibiting a marked anti-virulence activity against *P. aeruginosa*, thus paving the way to future investigation.

11 Resistenza ai farmaci antimicrobici/Terapia antimicrobica/Sensibilità agli antimicrobici e risvolti terapeutici

10 - Synergistic effects of antivirals and monoclonal antibodies in vitro against SARS-CoV-2 Wild Type B.1 strain and BQ.1.1 Omicron sublineage

LIA FIASCHI ⁽¹⁾ - CAMILLA BIBA ⁽¹⁾ - ILENIA VARASI ⁽¹⁾ - NICCOLÒ BARTOLINI ⁽¹⁾ - CHIARA PALETTI ⁽¹⁾ - FEDERICA GIAMMARINO ⁽¹⁾ - FRANCESCO SALADINI ⁽¹⁾ - MAURIZIO ZAZZI ⁽¹⁾ - ILARIA VICENTI ⁽¹⁾

Dipartimento di Biotecnologie Mediche, Università di Siena, Siena, Italia ⁽¹⁾

Synergistic effects of antivirals and monoclonal antibodies in vitro against SARS-CoV-2 Wild Type B.1 strain and BQ.1.1 Omicron sublineage

LIA FIASCHI¹, CAMILLA BIBA¹, ILENIA VARASI¹, NICCOLÒ BARTOLINI¹, CHIARA PALETTI¹, FEDERICA GIAMMARINO¹, FRANCESCO SALADINI¹, MAURIZIO ZAZZI¹, ILARIA VICENTI¹

¹Department of Medical Biotechnologies, University of Siena, Siena, Italy

Introduction Combination regimens can enhance antiviral potency, limit emergent drug resistance, and lower drug dosage. Aim of this work was to determine, in a live virus cell-based assay, the potential synergistic effects of the combination of approved Directly Acting Antivirals (DAAs) against SARS-COV-2 wild type strain B.1 (WT) and omicron BQ.1.1 and the combination of remdesivir (RDV) with licensed monoclonal antibodies (mAbs) against WT.

Materials and Methods The toxicity of the active form of molnupiravir (EIDD-1931), RDV, nirmatrelvir (NRM), cilgavimab (CIL), tixagevimab (TIX), bebtelovimab (BEB) and sotrovimab (SOT) was determined in VERO-E6 treated with CP-100356 P-gp inhibitor. Scalar dilutions of each DAA were incubated alone or in a 36 pairwise concentration matrix on VERO-E6 infected with WT or BQ.1.1. After 72h cytopathic effect was quantified. The same experiments were performed with RDV plus each mAb. The synergistic score (SC) was calculated by SynergyFinder3.0 using Bliss/Loewe model. SC>10 scores as synergistic, -10≥SC≤10 as additive, SC<-10 as antagonist.

Results Half-maximal inhibitory concentrations (IC₅₀) for EIDD-1931, RDV and NRM were 2.40±0.40/1.59±0.44, 0.06±0.03/0.03±0.01 and 0.10±0.03/0.10±0.01 µM against WT/BQ.1.1, respectively. IC₅₀ of CIL, TIX, BEB and SOT against WT are IC₅₀ of 0.20±0.13, 0.07±0.04, 0.03±0.01, 0.81±0.32 µg/ml, respectively. Global weighted SCs for all DAAs combos showed additivity against WT (-0.96±1.69 EIDD-1931+RDV, -6.71±0.89 EIDD-1931+NRM and 0.02±0.33 RDV+NRM) and BQ.1.1 (-0.33±4.10 EIDD-1931+RDV, -0.47±0.37 EIDD-1931+NRM and -2.12±3.8 RDV+NRM). Additivity was also observed for the following mAbs/RDV combos against W: -5.9±4.4 SOT+RDV, -5.5±1.6 RDV+BEB, and -4.3±1.6 TIX/RDV. SC >10 for a few DAAs combos occurred at specific concentrations including EIDD-1931 0.05-0.19-0.75 µM+RDV 0.4 µM, EIDD-1931 0.075-1.5 µM+NRM 0.05 µM and RDV 0.02 µM+NRM 0.01 µM against both viral strains. Similarly, SC >10 was observed against WT for the following RDV+mAbs combos: RDV 0.06 µM+SOT 0.09-0.04-0.1 µg/ml, RDV 0.016-0.06 µM+BEB 0.04 µg/ml, RDV 0.016-0.06 µM+TIX 0.04-0.009 µg/ml. IC₅₀ potency shifts were confirmed in infected cells treated with 3 fixed NRM concentrations plus scalar EIDD-1931. The IC₅₀ of EIDD-1931 with the addition of 0.1, 0.05,

0.025 μ M NRM was reduced by >61-, 8- and 1-fold against WT and by >41-, 10- and 3-fold against BQ.1.1.

Discussion and Conclusions. Global weighted SCs indicated additivity and each DAAs combos induced synergistic potency shifts against WT and BQ.1.1 at specific concentrations' combination. We observed the same effects for RDV with SOT, BEB or TIX against WT, but it was not possible to evaluate these combos against BQ.1.1 strain due to its resistance to all tested mAbs.

13 - SigH stress response mediates killing of *Mycobacterium tuberculosis* by activating nitronaphthofuran prodrugs

FRANCESCA BOLDRIN ⁽¹⁾

UNIVERSITA' DEGLI STUDI DI PADOVA, DIP. DI MEDICINA MOLECOLARE, PADOVA, Italia ⁽¹⁾

SigH stress response mediates killing of *Mycobacterium tuberculosis* by activating nitronaphthofuran prodrugs

FRANCESCA BOLDRIN ¹, LAURA CIOETTO-MAZZABÒ ¹, CLAIRE BEAUVINEAU ², MARTIN SPETH ³, ALBERTO MARINA ⁴, AMINE NAMOUCHI ^{5 6}, GRETA SEGAFREDDO ¹, MENA CIMINO ⁵, SANDRINE FAVRE-ROCHEX ⁵, SEETHA BALASINGHAM ⁷, BEATRIZ TRASTOY ^{4 8}, HÉLÈNE MUNIER-LEHMANN ⁹, GARETH GRIFFITHS ³, BRIGITTE GICQUEL ^{5 10}, MARCELO E. GUERIN ^{4 8 11}, RICCARDO MANGANELLI ¹, NOELIA ALONSO-RODRÍGUEZ.

¹Department of Molecular Medicine, University of Padova, Padova 35122, Italy; ²Chemical Library Institut Curie/CNRS, CNRS UMR9187, INSERM U1196 and CNRS UMR3666, INSERM U1193, Université Paris-Saclay, Orsay 91405, France; ³Department Biosciences, Faculty of Mathematics and Natural Sciences, University of Oslo, Oslo 0371, Norway; ⁴Center for Cooperative Research in Biosciences (CIC bioGUNE), Basque Research and Technology Alliance (BRTA), Bizkaia Technology Park, Derio 48160 Spain; ⁵Génétique Mycobactérienne, Institut Pasteur, Paris 75015, France; ⁶Centre for Ecological and Evolutionary Synthesis (CEES), Department of Biosciences, University of Oslo, Oslo 0371, Norway; ⁷Department of Microbiology, Oslo University Hospital, Oslo 0450, Norway; ⁸Structural Glycobiology Laboratory, Biocruces Bizkaia Health Research Institute, Cruces University Hospital, Bizkaia 48903, Spain; ⁹Département de Biologie Structurale et Chimie, Institut Pasteur, CNRS UMR3523, Université de Paris, Paris 75015, France; ¹⁰Department of Tuberculosis Control and Prevention, Shenzhen Nanshan Centre for Chronic Disease Control, Shenzhen 518054, China; ¹¹IKERBASQUE, Basque Foundation for Science, Bilbao 48009, Spain.

Introduction

Mycobacterium tuberculosis (Mtb) remains one of the worst scourges of humanity, and TB the leading cause of death from a single infectious agent. The emergence of multidrug drug-resistant (MDR) and extensively drug-resistant (XDR) Mtb strains highlights the need to discover anti-TB drugs with novel mechanisms of action. In this context, nitro heterocyclic compounds have demonstrated promising activity against both replicating and non-replicating Mtb thus representing attractive compounds for the development of future anti-TB drugs. Here we discovered that stress-mediated activation of the ECF sigma factor SigH leads to the activation of nitronaphthofurans prodrugs (nNFs) through the induction of mrx2.

Materials and Methods

MIC90 was determined by Resazurin Microtiter Assay and MBC99 was determined by Charcoal Agar Resazurin Assay. Cytotoxicity was assessed in Vero and HepG2. Intracellular activity of nNFs was

evaluated in bone marrow-derived macrophages infected by Mtb with a MOI of 1; viable intracellular bacteria were enumerated by CFU after 6 days. Mtb resistant mutants to nNFs were obtained and their DNA were sequenced using Illumina MiSeq system. sigH and mrx2 expression level were determined by RT-PCR. Nitric Oxide (NO) release was determined using the 2,3-Diaminonaphthalene (DAN) in nNF-treated *M. bovis* BCG cultures. For the enzymatic kinetics of the different Mrx2 alleles, nNF-C2 reduction was followed by changes in absorbance over time.

Results

Twenty nNFs active against Mtb were selected from a compounds library using a whole-cell screening. All the compound, active against replicating, not replicating and intracellular Mtb, were shown to be prodrugs and to require the nitroreductase Mrx2 for their activation. Whole genome analyses of spontaneous resistant mutants suggested the involvement of the ECF sigma factor SigH in the resistance mechanism. Indeed, SigH was shown to be required for mrx2 induction in stress condition. We showed that small amounts of NO produced by nNFs, induce the SigH response leading to mrx2 induction and more efficient nNFs activation.

Discussion and Conclusions

In this work we characterized a series of nNFs prodrugs active against Mtb and activated by the nitroreductase Mrx2. We discovered that these “smart” compounds, releasing intracellular NO are able to boost their bioactivation by stimulating the induction of mrx2 by the ECF sigma factor SigH.

17 - abcd

SARAH TORELLI ⁽¹⁾

Ciao, Ciao, Roma, Italia ⁽¹⁾

--

Data (da confermare)	Titolo (provvisorio)	
6 marzo 2023	Herpes viruses in the brain: pathogenic mechanisms and challenges for future research INTRODUZIONE: Dr.ssa Giovanna De Chiara (IFT-CNR, Rome), 25 minuti,	Moderatore: prof Roberta Rizzo (UniFe), INTERVENTI (ciascuno di 15 minuti) 1. "Role of Herpesvirus proteins in the control of host immune response." Relatore Daria Bortolotti (UniFe) 2. "Targeting citrullination as a new anti-herpetic strategy" Relatore Selina Pasquero (UniTO)
8 maggio 2023 Ore 16	Dissecting microbial genomes through NGS: from molecular diagnosis to genomic surveillance. The role of national networks INTRODUZIONE: Prof.ssa Anna Teresa Palamara (ISS, Rome), ≈ 25 minutes	Chair: Prof Gian Maria Rossolini, University of Florence (ciascuno di 15 minuti) 1. "Title". Dr. Vincenzo Di Pilato, University of Genoa. 2. "Title" Dr. Antonio Piralla, Fondazione IRCCS Policlinico San Matteo, Pavia
12 Giugno	Human papillomavirus-induced pathogenesis and cancer development	Moderatore: Prof.ssa Marisa Gariglio, Università del Piemonte Orientale (UPO)-Novara INTRODUZIONE: 25 minuti Prof.ssa Arianna Loregian, Università di Padova. "Molecular mechanisms underlying HPV-associated cancer" INTERVENTI (ciascuno di 15 minuti) 1. "Targeted therapies against HPV-associated cancer" Relatore: Dr.ssa Irene Lo Cigno (UPO) 2. "Understanding how HPV infection contributes to skin cancer development" Relatore: Prof.ssa Cinzia Borgogna (UPO)
10 Luglio	Regulation of virulence factors in Group B Streptococcus	Chair: Prof Gianni Pozzi ??, University of Siena

	INTRODUCTION: Prof.ssa Ketty Beninati (University of Messina), » 25 minutes	(ciascuno di 15 minuti) 1. “Update on epidemiology and infection control of Group B Streptococcus in Italy”. Roberta Creti ISS, ROMA. 2. Update on vaccines against Group A Streptococcus Danilo Gomes (GSK-Siena)
Settembre	Phage Therapy	De Luca
Ottobre	Evolution, Molecular and genetic determinants of drug resistance in Gram-negative bacteria	Giani/Migliavacca
Novembre	Pathogenesis of drug resistant fungi	Sanguinetti
Dicembre	Microbes and autoimmunity	Sechi
Gennaio 2024	Evolution, Molecular and genetic determinants of drug resistance Gram-positive	Stefani/Monaco ISS
Febbraio	Breakpoints, PK/PD and the future of susceptibility testing	Rossolini

24 - Strepto Killer, qu'est-ce que c'est?: Identification of compounds inhibiting streptococcal cell division by targeting FtsZ as a novel strategy to fight Gram-positive ovococcal pathogens

BERENICE FURLAN⁽¹⁾ - VALENTINA STRANIERO⁽²⁾ - LORENZO SUIGO⁽²⁾ - ERMANNO VALOTI⁽²⁾ - ORIETTA MASSIDDA⁽¹⁾

Dipartimento di Biologia Computazionale, Cellulare e Integrata, Università di Trento, Trento, Italia

⁽¹⁾ - Department of Pharmaceutical Sciences, Università di Milano, Milano, Italia ⁽²⁾

Strepto Killer, qu'est-ce que c'est?: Identification of compounds inhibiting streptococcal cell division by targeting FtsZ, as a novel strategy to fight Gram-positive ovococcal pathogens

Berenice Furlan^{1*}, Valentina Straniero^{2*}, Lorenzo Suigo², Ermanno Valoti², Orietta Massidda¹

¹Department of Cellular, Computational and Integrative Biology (DiCIBIO), University of Trento, Italy; ²Department of Pharmaceutical Sciences (DiSFarm), University of Milan, Milano, Italy

1. Introduction: Antimicrobial resistance (AMR) is a leading public health emergency at the global level. Despite a few antibacterial drugs have been recently approved or are in clinical development, most of these are analogues of existing ones, making the design of antibiotics with novel mechanisms of action a crucial strategy to combat AMR. Bacterial cell division is an essential process and the proteins involved in this process are among the most attractive targets to develop novel broad-spectrum antibacterials. The cell division filamenting temperature-sensitive Z (FtsZ) protein is a conserved protein that polymerizes at the midcell to form a membrane-attached ring, the Z-ring, a key structure required to recruit the other cell division proteins and to complete cytokinesis. Inactivation or inhibition of FtsZ leads to a division block and, eventually, cell death. Among several FtsZ inhibitors recently reported, the derivatives of 2,6-difluorobenzamides (FZ-benzamides) are considered the most promising class of compounds. However, so far their action seems to be limited to some Gram-positive bacteria, like *Bacillus subtilis* and *Staphylococcus aureus*, and the attempts to broaden their spectrum to other species have not completely succeeded. Here, we show the effectiveness of selected FZ-benzamides against *Streptococcus pneumoniae*, a clinically relevant Gram-positive pathogen for which inhibitors of FtsZ have not been reported. 2. Materials and Methods: The antibacterial activity of FZ-benzamides against *S. pneumoniae* strains was evaluated by determining their Minimal Inhibitory Concentration (MIC) using an agar dilution method. The antibacterial effect at 1x MIC was assessed in exponentially growing *S. pneumoniae* cells by growth and viability assays and the morphological changes observed using phase-contrast and fluorescence microscopy. 3. Results: Four out of twelve FZ-benzamide derivatives tested were active against *S. pneumoniae*. All compounds showed an MIC <100 µg/ml and had a clear effect within 180 minutes of treatment at the 1x MIC concentration, resulting in a bactericidal effect that, in one case, was also rapidly bacteriolytic. Dramatic morphological alterations preceded growth inhibition and death, suggestive of a simultaneous block in sidewall elongation and cell division, as would be expected from a block at the early stages of the pneumococcal cell cycle such as inactivation of FtsZ. 4. Discussion and conclusion: In this study, we report for the first time the identification and the characterization of the antibacterial activity of FZ-benzamides that inhibit *S. pneumoniae* cell division by targeting FtsZ. Their antibacterial activity, besides being potentially effective in the treatment of ovoid-shaped Gram-positive pathogens, like streptococci and

enterococci, suggests that targeting conserved proteins involved in cell division represents a significant advance for a rational development of new antimicrobials with novel mechanism of action for which there is a urgent and compelling need.

33 - Role of transcriptomic and genomic analyses in a step ahead in the comprehension of cefiderocol activity in *A. baumannii*

STEFANO STRACQUADANIO⁽¹⁾ - ALICE NICOLOSI⁽¹⁾ - GRETE PRIVITERA⁽²⁾ -
MARIACRISTINA MASSIMINO⁽³⁾ - ANDREA MARINO⁽⁴⁾ - DAFNE BONGIORNO⁽¹⁾ -
STEFANIA STEFANI⁽¹⁾

Università degli Studi di Catania, Dipartimento di Scienze Biomediche e Biotecnologiche, Catania, Italia⁽¹⁾ - Università degli Studi di Catania, Dipartimento di Medicina Clinica e Sperimentale, Catania, Italia⁽²⁾ - Università di Pisa, Dipartimento di ricerca traslazionale e nuove tecnologie in medicina e chirurgia, Pisa, Italia⁽³⁾ - Università degli Studi di Catania, ARNAS Garibaldi - Unità di malattie infettive, Catania, Italia⁽⁴⁾

Role of transcriptomic and genomic analyses in a step ahead in the comprehension of cefiderocol activity in *A. baumannii*

STEFANO STRACQUADANIO¹, ALICE NICOLOSI¹, GRETE F. PRIVITERA²,
MARIACRISTINA MASSIMINO³, ANDREA MARINO⁴, DAFNE BONGIORNO¹, STEFANIA
STEFANI¹

1. Dipartimento di Scienze Biomediche e Biotecnologiche (BIOMETEC), laboratorio di Microbiologia Medica Molecolare e Antibiotico Resistenza – Università degli Studi di Catania – Catania, Italia; 2. Dipartimento di Medicina Clinica e Sperimentale, Unità di Bioinformatica – Università degli Studi di Catania – Catania, Italia; 3. Dipartimento di ricerca traslazionale e nuove tecnologie in medicina e chirurgia – Università di Pisa – Pisa, Italia; 4. Dipartimento di Medicina Clinica e Sperimentale, Unità di Malattie Infettive – ARNAS Garibaldi – Catania, Italia.

Introduction.

Cefiderocol (FDC) is a siderophore cephalosporin active against Gram-negative bacteria. FDC mechanisms of action and resistance in *A. baumannii* are still not fully elucidated but the iron transport systems have been evoked in the entry of FDC into the cell to reach the PBPs target.

Materials and Methods

In order to capture the dynamic of gene expression related to FDC action in various conditions we report the genomic and transcriptomic features of seven *A. baumannii* strains with different FDC susceptibility focusing on the mutations in the main genes associated to β -lactam resistance as well as on the expression of the two major siderophore biosynthesis and transport systems, i.e. acinetobactin and baumannoferrin. Likewise, we investigated the expression of TonB energy transducer system and of the TonB dependent siderophore receptors *piuA* and *pirA*.

Results

The four clinical samples belonged to the same clonal complex (CC2), and showed some peculiar mutations in *PBP2* gene and in *ampC*, possibly related to two strains highest FDC MICs. Similarly, the two clinical strains with lower MICs shared mutations in a truncated outer membrane protein as well as another amino acid substitution in *ampC*. Gene expression analyses highlighted the up-regulation of the acinetobactin and baumannoferrin genes in response to the iron depletion and, vice versa, a down-regulation of the same genes in the presence of high iron concentrations. In response

to FDC, the gene expression seemed strain-dependant, probably related to other different metabolic features of each strain still under evaluation.

Discussion and Conclusions

Overall, FDC activates the energy transducer machinery, confirming the role of an active import of the drug inside the cells; baumannoferrin more than acinetobactin appeared stimulated by FDC in iron depletion. In conclusion, iron transport systems play a role in FDC activity: this is clear for the drug acquisition, less evident for the antibiotic susceptibility, for which the resistance determinants should be further investigated.

37 - Study of the mechanism of action of the novel antitubercular compound 11826350

DEBORAH RECCHIA⁽¹⁾ - GHERARD BATISTI BIFFIGNANDI⁽²⁾ - ANNA EGOROVA⁽³⁾ - ANTONIO MARINO CERRATO⁽²⁾ - ELENA FRANCHI⁽²⁾ - LAURENT R. CHIARELLI⁽²⁾ - DAVIDE SASSERA⁽²⁾ - VADIM MAKAROV⁽³⁾ - MARIA ROSALIA PASCA⁽²⁾ - GIULIA DEGIACOMI⁽²⁾

Università di Pavia, Dipartimento di Biologia e Biotecnologie "Lazzaro Spallanzani", Pavia, Italia
(¹) - University of Pavia, Department of Biology and Biotechnology "Lazzaro Spallanzani", Pavia, Italia
(²) - Research Center of Biotechnology Russian Academy of Science, a, Mosca, Russia (³)

Study of the mechanism of action of the novel antitubercular compound 11826350

DEBORAH RECCHIA¹, GHERARD BATISTI BIFFIGNANDI¹, ANNA EGOROVA², ANTONIO MARINO CERRATO¹, ELENA FRANCHI¹, LAURENT R. CHIARELLI¹, DAVIDE SASSERA^{1,3}, VADIM MAKAROV², MARIA ROSALIA PASCA^{1,3,*}, GIULIA DEGIACOMI^{1,*}.

1 Department of Biology and Biotechnology "Lazzaro Spallanzani", University of Pavia, Pavia, Italy.

2 Research Center of Biotechnology Russian Academy of Science, Moscow, Russia.

3 Fondazione IRCCS Policlinico San Matteo, Pavia, Italy.

* Co-corresponding authors

1.Introduction

In 2021, an estimated 10.6 million people fell ill with tuberculosis (TB) worldwide, reversing a slow decline that lasted many years due to disruptions in essential TB services during the COVID-19 pandemic. In addition, an increasing number of multidrug-resistant (MDR) *Mycobacterium tuberculosis* (Mtb) strains threaten global health. The search for active anti-TB drugs is still crucial for success in controlling TB. 11826350 compound belonging to izoxazole class, was found to be active against Mtb H37Rv in vitro, leading us to study its mechanism of action (MoA).

2.Materials and Methods

The biological activity of 11826350 was evaluated against Mtb H37Rv and MDR clinical isolates by resazurin microtiter assay (REMA), along with 23 derivatives. To study its MoA, a transcriptomic experiment was performed. Mtb cultures were treated with 11826350 (10-fold or 30-fold MIC) for 4 hours. As a control, untreated Mtb cultures were used. Three biological replicates for each condition were performed.

3.Results

We determined that 11826350 has an MIC of 1.25 μ g/mL by REMA resulting to be the most active compound in this series. After several unsuccessful attempts to isolate Mtb resistant mutants to study its MoA, RNA sequencing of Mtb H37Rv cultures in response to compound exposure was performed. Preliminary data analysis identified several differently expressed genes (DGE) encoding proteins involved in intermediary metabolism and respiration, protein synthesis, as well as regulatory networks and conserved hypotheticals. Overall, 123 and 14 genes were found to be respectively upregulated and repressed in both conditions compared to the control group. Interestingly, one of the most upregulated gene is rv3406, which encodes an iron and α -ketoglutarate (alpha-KG) dependent sulfate ester dioxygenase of broad substrate specificity, already known as a drug modifier. Therefore, we are studying the possible involvement of Rv3406 in the MoA of 11826350. On the other hand,

among the most repressed genes are two essential ones: rpmH, coding for a ribosomal protein, and ndkA, encoding a nucleoside diphosphate kinase.

4. Discussion and Conclusions

We found 11826350 to be an active anti-TB compound. Thanks to transcriptomic analysis, the study of its MoA is in progress. Preliminary results seem to indicate that Rv3406 could act as an activator of the compound.

This research was supported by EU funding within the NextGenerationEU-MUR PNRR Extended Partnership initiative on Emerging Infectious Diseases (Project no. PE00000007, INF-ACT).

38 - In vivo cefiderocol resistance escalation and intra-host evolution of KPC-3 and VIM-1 co-producing *Klebsiella pneumoniae* Sequence Type 512

GABRIELE ARCARI⁽¹⁾ - FEDERICO CECILIA⁽¹⁾ - ALESSANDRA OLIVA⁽²⁾ - RICCARDO POLANI⁽¹⁾ - GIAMMARCO RAPONI⁽²⁾ - ALICE DE FRANCESCO⁽¹⁾ - FEDERICA SACCO⁽²⁾ - GUIDO ANTONELLI⁽¹⁾ - ALESSANDRA CARATTOLI⁽¹⁾

University, Department of Molecular Medicine, Sapienza University of Rome, Rome, Italy, Roma, Italia⁽¹⁾ - University, Department of Public Health and Infectious Diseases, Sapienza University of Rome, Rome, Italy, Roma, Italia⁽²⁾

In vivo cefiderocol resistance escalation and intra-host evolution of KPC-3 and VIM-1 co-producing *Klebsiella pneumoniae* Sequence Type 512

GABRIELE ARCARI¹, FEDERICO CECILIA¹, ALESSANDRA OLIVA², RICCARDO POLANI¹, GIAMMARCO RAPONI², FEDERICA SACCO², ALICE DE FRANCESCO¹, GUIDO ANTONELLI¹ and ALESSANDRA CARATTOLI¹

1 Department of Molecular Medicine, Sapienza University of Rome, Rome, Italy; 2 Department of Public Health and Infectious Diseases, Sapienza University of Rome, Rome, Italy

Introduction

The *Klebsiella pneumoniae* sequence type (ST) 512 is a major carbapenem-resistant clone, but there is limited understanding of its intra-host evolution.

In February 2022 a critical patient colonized by a ST512 *K. pneumoniae*, was admitted to the Policlinico Umberto I hospital of Rome (PUI) for COVID-19 and remained hospitalized for 95 days until exitus.

Here, integrating Whole-Genome Sequencing (WGS), clinical data and molecular microbiology methods, we endeavor to reconstruct the in vivo evolution of these ST512 *K. pneumoniae* isolates, with a focus on cefiderocol (FDC) resistance.

Materials and Methods

WGS was carried out using both Illumina (short reads) and Oxford Nanopore Technologies (long reads), and genomics data were integrated with information on patient conditions and antimicrobial therapies. Moreover, bla_{KPC} gene cloning in *Escherichia coli* was performed to assess the role of KPC variants in the antimicrobial resistance phenotype.

Results

At the entrance in PUI the patient was colonized by *K. pneumoniae* producing both KPC-3 and VIM (strain 1_6379).

In the first month of hospitalization the patient developed a respiratory tract infection (RTI) caused by *Providencia stuartii*, which was treated with ceftazidime-avibactam (CZA).

After one week of treatment, a CZA-resistant and meropenem (MEM)-susceptible *K. pneumoniae* with a dimorphic colony phenotype (resulting in strains 2_1186W and 2_1186T) producing KPC-31 variant, but not VIM, were isolated as colonizers of the patient. Strain 2_1186W presented an integrated copy of the bla_{KPC-31} carrying pKpQIL plasmid, which resulted in lower MIC for MEM. A second RTI was caused by CZA and MEM co-resistant *K. pneumoniae* (strain 3_6099). This strain produced a novel KPC variant (KPC-154) which, after cloning the respective gene in *E. coli* recipient cells, raised the MICs for CZA and MEM.

The patient was treated with meropenem-vaborbactam, and no KPC-154 producing *K. pneumoniae* was identified afterwards. A third RTI caused by carbapenem-resistant *Acinetobacter baumannii* and *K. pneumoniae*, was treated with FDC. A *K. pneumoniae* strain (strain 4_0296) producing both the KPC-3 and VIM-1 was isolated. This strain showed a high MIC for FDC, plausibly ascribable to a nonsense mutation in the *cirA* iron-transporter.

Discussion and conclusions

This study sheds light on the intra-host evolution of carbapenem-resistant ST512 *K. pneumoniae* in a patient during hospitalization. The interplay between plasmids encoding Metallo Beta-lactamases and inhibitor-resistant KPC variants along with disruption of the *cirA* siderophore brought to an extremely-drug resistant phenotype, including all beta-lactams, CZA and FDC resistance.

39 - The Fitness Omic burden associated to new Adaptive Reduced-Glycopeptide-Susceptibility Network in CA-MRSA

ELEONORA CHINES ⁽¹⁾ - MARTINA PAPPALARDO ⁽¹⁾ - CARLOTTA ZIMBONE ⁽¹⁾ - ELISA BELLA ⁽¹⁾ - STEFANIA STEFANI ⁽¹⁾ - VIVIANA CAFISO ⁽¹⁾

Università degli Studi di Catania, Università di Catania/ BIOMETEC, Catania, Italia ⁽¹⁾

The Fitness Omic burden associated to new Adaptive Reduced-Glycopeptide-Susceptibility Network in CA-MRSA

ELEONORA CHINES ¹, MARTINA PAPPALARDO¹, CARLOTTA ZIMBONE¹, ELISA BELLA¹, STEFANIA STEFANI ¹, VIVIANA CAFISO ¹

¹ BIOMETEC Dept - University of Catania - Catania (Italy)

Introduction. Resistance biological costs are crucial issues in antibiotic-resistance and public-health. Multidrug-resistant *Staphylococcus aureus* can show diminished fitness (growth-rate/competitiveness, virulence). Recently, we described a new reduced-glycopeptide-susceptibility adaptive-resistance related to glycopeptide-target biosynthesis and supporter-pathways in an extensively-resistant CA-VISA superbug. New acquired-resistance needs a new Fitness Cost (FC) asset poorly understood at omic-level. Our goal was to investigate FCs in core-genome (cg) and Essential-Genes (EGs) in a DAP-R/CA-VISA emerged under antimicrobial-therapy with high-FCs. Materials and Methods. CA-VISA versus its CA-VSSA parent genomics and transcriptomics was investigated by Illumina-Miseq Whole-Genome and RNA Sequencing. cgSNPs were predicted by Snippy-core, and SNP-effect by snpEff on MW2 RefGen. EG-Transcriptomics were investigated by Rockhopper, DAVID, Gene-Ontology and KEGG. Results. CA-VISA versus its CA-VSSA parent Comparative Genomics evidenced 8 cgSNPs and 4 moderate-impact SNPs in 4 EGs, two in AMR-EGs (*rpoB*, *mprF*) and two in secondary-resistance EGs (*gdpP*, *murG*). 3 SNP-reversions were recovered in EGs for teichoic acid and glutamine synthesis biosynthesis (*tagU*, *glnA*), as well as *recU* for DNA-packaging. Comparative Transcriptomics showed an EG under-expression trend, with 46 under-expressed vs 36 over-expressed EG coding-mRNAs, whilst 56 under-expressed vs 25 over-expressed EG small antisense RNAs (asRNAs). Key differential under-expression was in Protein-Synthesis and Cell-Envelope metabolism EG-clusters, followed by DNA- and RNA-metabolism ones. Contrarily, key dysregulation via asRNAs was progressively in a EG miscellanea, cell-envelope, DNA-metabolism, cofactor, and RNA-metabolism ones. Discussion and Conclusions. Our data find out the EG role as strategic “hot spots” of omic adaptations related to the onset, maintenance, and balancing of the high FCs and acquired-resistance in CA-VISA.

42 - Shift between echinocandins through computerized prescribing software and antifungal resistance profiles

GIACOMO BERTOLINO⁽¹⁾ - RAFFAELE DEIDDA⁽¹⁾ - ROSSELLA M. GHIANI⁽²⁾ - ROBERTO MURA⁽¹⁾ - SILVIA VARGIU⁽¹⁾ - RAFFAELLO POMPEI⁽³⁾ - ELENA POMA⁽⁴⁾ - ELISA CASULA⁽⁴⁾ - PATRIZIA FERRAGUTI⁽⁴⁾ - FERDINANDO COGHE⁽⁴⁾ - ARIANNA CADEDDU⁽¹⁾

Servizio di Farmacia, Azienda Ospedaliero Universitaria di Cagliari, Cagliari, Italia⁽¹⁾ - SOL Group, SOL, Cagliari, Italia⁽²⁾ - RES-S, APE (No Profit Organization), Cagliari, Italia⁽³⁾ - Clinical Analysis Laboratory, Azienda Ospedaliero Universitaria di Cagliari, Cagliari, Italia⁽⁴⁾

Shift between echinocandins through computerized prescribing software and antifungal resistance profiles

GIACOMO BERTOLINO⁽¹⁾, RAFFAELE DEIDDA⁽¹⁾, ROSSELLA M. GHIANI⁽²⁾, ROBERTO MURA⁽¹⁾, SILVIA VARGIU⁽¹⁾, RAFFAELLO POMPEI⁽³⁾, ELENA POMA⁽⁴⁾, CASULA ELISA⁽⁴⁾, PATRIZIA FERRAGUTI⁽⁴⁾, FERDINANDO COGHE⁽⁴⁾, ARIANNA CADEDDU⁽¹⁾

1) Pharmaceutical Department, Azienda Ospedaliero Universitaria di Cagliari, Cagliari, Italy

2) Pharmacist - SOL Group, Cagliari, Italy

3) RES-R, APS No Profit Organization, Capoterra, Italy

4) Clinical Analysis Laboratory – Azienda Ospedaliero Universitaria di Cagliari, Cagliari, Italy

Introduction:

The monitoring of antifungal prescriptions is essential in the hospital context to avoid prescribing inappropriateness which can lead to the unjustified exposure of patients to the risk of Adverse Drug Reactions and the possible increasing resistance of hospital microorganisms. The purpose of this work was to evaluate variations in the antifungal exposure index and *Candida* spp. resistance profiles in Invasive Fungal Infections (IFIs) after a shift between echinocandins in the four-year period 2019–2022.

Materials and Methods:

All the antifungal drugs prescribed in our hospital require physicians to provide a personalized and motivated prescription using BusterMED® software. The antifungal exposure index was extrapolated from the management system and was expressed in DDD/100 days of hospitalization (DGD).

Results:

Antifungal drugs displayed the following trend for DGD value: from 10.71 in 2019 to 14.41 in 2022. Caspofungin's DGD grew from a value of 0.09 in 2019 to 0.25 in 2022 (+181.6%). The related values of fungal resistance in the same period decreased from 3.7% in 2019 to 0.0% in 2022. The correlation between the increase in caspofungin DGD and the relative resistance was not significant ($r=0.859$; $p=0.141$). These data go against the trend for micafungin, whose DGD value decreased progressively from 0.07 in 2019 to 0.03 in 2022 (-61.4%) and related resistance was from 2.4% in 2019 to 0.0% in 2022. The most IFIs isolated species were *Candida non Albicans* (*C. glabrata* 40,4%, 40 isolates in 2022, and *C. parapsilosis* 15,2%, 15 isolates in 2022). Total spending decreased from €93,000 in 2019 to €43,575 in 2022 (-53.2%).

Discussions and Conclusion:

The goal was to curb the consumption of micafungin in favour of caspofungin. Caspofungin appears to have a better cost/effectiveness profile: the average cost of a day of therapy with

casposungin in the period in question is €42, while micafungin costs €368. It was also observed that the drugs DGDs' had no effect on changes in resistance in the period considered, and the two factors are not significantly correlated. Total expenditure in the four-year period decreased. The role of the clinical microbiologist and the hospital pharmacist is fundamental in Antifungal Stewardship programs.

45 - Antimicrobial resistance prediction of *Escherichia coli* strains to Ampicillin via machine learning models on MALDI-TOF mass spectra

ETTORE ROCCHI⁽¹⁾ - MAURO FACCIN⁽¹⁾ - CLAUDIA SALA⁽¹⁾ - DONATELLA LOMBARDO⁽²⁾ - SIMONE AMBRETTI⁽²⁾ - GASTONE CASTELLANI⁽¹⁾

Università di Bologna, Dipartimento di Scienze Mediche e Chirurgiche, Bologna, Italia⁽¹⁾ - IRCCS Azienda Ospedaliero-Universitaria di Bologna, UO Microbiologia, Bologna, Italia⁽²⁾

Antimicrobial resistance prediction of *Escherichia coli* strains to Ampicillin via machine learning models on MALDI-TOF mass spectra

ETTORE ROCCHI¹, MAURO FACCIN¹, CLAUDIA SALA¹, DONATELLA LOMBARDO², SIMONE AMBRETTI², GASTONE CASTELLANI¹

¹Department of Medical and Surgical Sciences, Università di Bologna, Bologna, Italy;

²Microbiology Unit, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy

Introduction Nowadays, antimicrobial resistance (AMR), due to a massive, often uncontrolled use of antibiotics, is a problem of enormous dimensions that carries with itself an enormous burden both in economic and in social and public health terms. In this contribution, we describe a machine-learning-based model capable of predicting the resistance or susceptibility of *Escherichia coli*'s strains to Ampicillin, starting from the MALDI-TOF mass spectra of those strains. This work has been funded by the EU - NextGenerationEU with funds made available by the National Recovery and Resilience Plan (NRRP) - Partenariati Estesi (PE13 - INF-ACT) - CUP J33C22002870005.

Materials and Methods Our work will exploit a publicly available dataset: a part of the DRIAMS database, supplied by Weis et al. (2022). The considered dataset is composed of 213 *Escherichia coli*'s MALDI-TOF mass spectra. Among them 136 are resistant and 77 susceptible to Ampicillin. The raw mass spectra, whose m/z range covers the interval 2k-20k Dalton, have been cleaned up with pre-processing procedures, including de-noising and smoothing filters (Savitzky-Golay), a detrending process, the ION count normalisation, and, finally, data binning (with bin width of 3 Da) to reduce the complexity of the spectra to 6000 features. The pre-processed spectra have then been divided into a training set and a testing set (with proportions 75/25%). The used machine-learning models are a logistic regression classifier, a gradient boosting decision tree, a multi-layer perceptron, a k-nearest neighbours algorithm, a naive Bayes classifier, and a support vector machine. Their hyper-parameters have been optimised via a 5-fold cross validation during the training step. Finally, their predictions have been subjected to the soft voting algorithm to retrieve a unified prediction.

Results The final voting classifier have been evaluated through the most-known metrics, such as the area under the receiver operating characteristic curve (AUROC) and the very major error (VME). The combination of those metrics shows that the voting classifier slightly improves the predictions of the single classifiers. In particular, the voting classifier performed on the testing set (composed of 54 mass spectra) produces an AUROC value equal to 0.74 and a VME equal to 7, meaning that 13% of resistant spectra have been misclassified as susceptible.

Discussion and Conclusions Our work shows that the ensemble learning approach of soft voting classification, and more generally machine-learning models applied to MALDI-TOF mass spectra, may be a

useful and fast tool to predict antimicrobial resistance, thus helping clinicians in the choice of the proper and more effective therapeutical strategy, which in many cases could be of vital importance.

48 - Campylobacter spp. infections in Italy: a 5-year multicenter retrospective study

VERENA ZERBATO ⁽¹⁾ - STEFANO DI BELLA ⁽¹⁾ - RICCARDO POL ⁽¹⁾ - SIMONE AMBRETTI ⁽²⁾ - STEFANO ANDREONI ⁽³⁾ - RICHARD ASCHBACHER ⁽⁴⁾ - MARIANO BERNARDO ⁽⁵⁾ - ALESSANDRA BIELLI ⁽⁶⁾ - GIOCONDA BRIGANTE ⁽⁷⁾ - MARINA Busetti ⁽⁸⁾ - GIULIO CAMARLINGHI ⁽⁹⁾ - DAVIDE CARCIONE ⁽⁷⁾ - ANTONELLA CARDUCCI ⁽⁷⁾ - EDOARDO CARRETTO ⁽¹⁰⁾ - CHIARA CHILLERI ⁽¹¹⁾ - NICOLA CLEMENTI ⁽¹²⁾ - ALESSANDRA CONSONNI ⁽¹³⁾ - VENERE CORTAZZO ⁽¹⁴⁾ - SAVERIA DODARO ⁽¹⁵⁾ - BARBARA FIORI ⁽¹⁴⁾ - AURORA GARCIA FERNANDEZ ⁽¹⁶⁾ - ELISA GOBBATO ⁽³⁾ - FRANCESCA GRECO ⁽¹⁵⁾ - NICASIO MANCINI ⁽¹⁷⁾ - ALBERTO ENRICO MARAOLO ⁽¹⁸⁾ - ROBERTA MARROLLO ⁽¹⁰⁾ - CAROLA MAURI ⁽¹³⁾ - ANNARITA MAZZARIOL ⁽¹⁹⁾ - GIANLUCA MORRONI ⁽²⁰⁾ - ADRIANA MOSCA ⁽²¹⁾ - GIACOMO NIGRISOLI ⁽²⁾ - ELISABETTA PAGANI ⁽⁴⁾ - EVA MARIA PARISIO ⁽⁹⁾ - SIMONA POLLINI ⁽¹¹⁾ - MARIO SARTI ⁽²²⁾ - ANNA MARCHESE ⁽²³⁾ - GIULIA CODDA ⁽²³⁾ - ROBERTO LA RAGIONE ⁽²⁴⁾ - ANNARITA SORRENTINO ⁽¹⁹⁾ - DOMENICO TROTTA ⁽²⁰⁾ - LAURA VILLA ⁽¹⁶⁾ - CHIARA VISMARA ⁽⁶⁾ - GIANFRANCO SANSON ⁽¹⁾ - ROBERTO LUZZATI ⁽¹⁾ - LUIGI PRINCIPE ⁽²⁵⁾

Università di Trieste, Dipartimento di Scienze Mediche, Chirurgiche e della Salute, UOC Malattie Infettive, Trieste, Italia ⁽¹⁾ - Policlinico Universitario Sant'Orsola-Malpighi di Bologna, UOC Microbiologia e Virologia, Bologna, Italia ⁽²⁾ - Azienda Ospedaliero Universitaria Maggiore della Carità di Novara, UOC Microbiologia e Virologia, Novara, Italia ⁽³⁾ - Comprensorio Sanitario di Bolzano, Azienda Sanitaria dell'Alto Adige, Laboratorio Aziendale di Microbiologia e Virologia, Bolzano, Italia ⁽⁴⁾ - Ospedale Monaldi, AORN dei Colli, UOC Microbiologia e Virologia, Napoli, Italia ⁽⁵⁾ - ASST Grande Ospedale Metropolitano Niguarda, UOC Microbiologia e Virologia, Milano, Italia ⁽⁶⁾ - ASST Valle Olona, UOC Medicina di Laboratorio, Busto Arsizio, Italia ⁽⁷⁾ - Ospedale Universitario di Trieste, UOC Microbiologia e Virologia, Trieste, Italia ⁽⁸⁾ - Azienda USL Toscana sud Est, Arezzo, Dipartimento di Medicina di Laboratorio e Trasfusionale, UOSD Microbiologia, Arezzo, Italia ⁽⁹⁾ - IRCCS Arcispedale Santa Maria Nuova, Laboratorio di Microbiologia Clinica, Reggio Emilia, Italia ⁽¹⁰⁾ - Policlinico Universitario Careggi, UOC Microbiologia e Virologia, Firenze, Italia ⁽¹¹⁾ - IRCCS Policlinico Universitario "San Raffaele", Servizio di Medicina di Laboratorio, Unità di Microbiologia e Virologia, Milano, Italia ⁽¹²⁾ - Ospedale "A. Manzoni", UOC Microbiologia e Virologia, Lecco, Italia ⁽¹³⁾ - Policlinico Universitario "A. Gemelli", UOC Microbiologia, Roma, Italia ⁽¹⁴⁾ - Ospedale "Annunziata", UOC Microbiologia e Virologia, Cosenza, Italia ⁽¹⁵⁾ - Istituto Superiore di Sanità, Dipartimento di Malattie Infettive, Roma, Italia ⁽¹⁶⁾ - Azienda Ospedaliero Universitaria Fondazione Macchi, Dipartimento di Medicina ed Innovazione Tecnologica; Laboratorio di Microbiologia e Virologia, Varese, Italia ⁽¹⁷⁾ - Ospedale Cotugno, AORN dei Colli, Prima Divisione di Malattie Infettive, Napoli, Italia ⁽¹⁸⁾ - Azienda Ospedaliero Universitaria Integrata di Verona, UOC Microbiologia e Virologia, Verona, Italia ⁽¹⁹⁾ - Università Politecnica delle Marche, Dipartimento di Scienze Biomediche e Salute Pubblica, Unità di Microbiologia, Ancona, Italia ⁽²⁰⁾ - Policlinico Universitario "Aldo Moro", Dipartimento Interdisciplinare di Medicina, Bari, Italia ⁽²¹⁾ - Azienda Ospedaliero Universitaria Policlinico, UOC Microbiologia e Virologia, Modena, Italia ⁽²²⁾ - Policlinico Universitario San Martino, UOC Microbiologia e Virologia, Genova, Italia ⁽²³⁾ - University of Surrey, Department of Pathology and Infectious Diseases, School of Veterinary Medicine, Guildford, Regno Unito ⁽²⁴⁾ - Azienda Sanitaria Provinciale di Crotone, Ospedale "S. Giovanni di Dio" - UOSD Patologia Clinica e Microbiologia, Crotone, Italia ⁽²⁵⁾

Section: Resistenza ai farmaci antimicrobici/Terapia antimicrobica/Sensibilità agli antimicrobici e risvolti terapeutici

Title - Campylobacter spp. infections in Italy: a 5-year multicenter retrospective study

Authors: Verena Zerbato¹, Stefano Di Bella¹, Riccardo Pol¹, Simone Ambretti², Stefano Andreoni³, Richard Aschbacher⁴, Mariano Bernardo⁵, Alessandra Bielli⁶, Gioconda Brigante⁷, Marina Buseti⁸, Giulio Camarlinghi⁹, Davide Carcione⁷, Antonella Carducci⁷, Edoardo Carretto¹⁰, Chiara Chilleri¹¹, Clementi Nicola¹², Alessandra Consonni¹³, Venere Cortazzo¹⁴, Saveria Dodaro¹⁵, Barbara Fiori¹⁴, Aurora Garcia Fernandez¹⁶, Elisa Gobbato³, Francesca Greco¹⁵, Nicasio Mancini¹⁷, Alberto Enrico Maraolo¹⁸, Roberta Marrollo¹⁰, Carola Mauri¹³, Annarita Mazzariol¹⁹, Gianluca Morroni²⁰, Adriana Mosca²¹, Giacomo Nigrisoli², Elisabetta Pagani⁴, Eva Maria Parisio⁹, Simona Pollini¹¹, Mario Sarti²², Anna Marchese²³, Giulia Codda²³, Roberto La Ragione²⁴, Annarita Sorrentino¹⁹, Domenico Trotta²⁰, Laura Villa¹⁶, Chiara Vismara⁶, Gianfranco Sanson¹, Roberto Luzzati¹, Luigi Principe²⁵

Affiliations:

¹ Dipartimento di Scienze Mediche, Chirurgiche e della Salute, UOC Malattie Infettive, Università di Trieste, Italia;

² UOC Microbiologia e Virologia, Policlinico Universitario Sant'Orsola-Malpighi di Bologna, Italia;

³ UOC Microbiologia e Virologia, Azienda Ospedaliero Universitaria Maggiore della Carità di Novara, Italia;

⁴ Laboratorio Aziendale di Microbiologia e Virologia, Comprensorio Sanitario di Bolzano, Azienda Sanitaria dell'Alto Adige, Bolzano, Italia;

⁵ UOC Microbiologia e Virologia, Ospedale Monaldi, AORN dei Colli, Napoli, Italia;

⁶ UOC Microbiologia e Virologia, ASST Grande Ospedale Metropolitano Niguarda, Milano, Italia;

⁷ UOC Medicina di Laboratorio, ASST Valle Olona, Busto Arsizio, Italia;

⁸ UOC Microbiologia e Virologia, Ospedale Universitario, Trieste, Italia;

⁹ Dipartimento di Medicina di Laboratorio e Trasmfusionale, UOSD Microbiologia, Azienda USL Toscana sud Est, Arezzo, Italia;

¹⁰ Laboratorio di Microbiologia Clinica, IRCCS Arcispedale Santa Maria Nuova, Reggio Emilia, Italia;

¹¹ UOC Microbiologia e Virologia, Policlinico Universitario Careggi, Firenze, Italia;

¹² Servizio di Medicina di Laboratorio, Unità di Microbiologia e Virologia, IRCCS Policlinico Universitario "San Raffaele", Milano, Italia;

¹³ UOC Microbiologia e Virologia, Ospedale "A. Manzoni", Lecco, Italia;

¹⁴ UOC Microbiologia, Policlinico Universitario "A. Gemelli", Roma, Italia;

¹⁵ UOC Microbiologia e Virologia, Ospedale "Annunziata", Cosenza, Italia;

¹⁶ Dipartimento di Malattie Infettive, Istituto Superiore di Sanità, Roma, Italia;

¹⁷ Dipartimento di Medicina ed Innovazione Tecnologica; Laboratorio di Microbiologia e Virologia, Azienda Ospedaliero Universitaria Fondazione Macchi, Varese, Italia;

¹⁸ Prima Divisione di Malattie Infettive, Ospedale Cotugno, AORN dei Colli, Napoli, Italia;

¹⁹ UOC Microbiologia e Virologia, Azienda Ospedaliero Universitaria Integrata di Verona, Verona, Italia;

²⁰ Dipartimento di Scienze Biomediche e Salute Pubblica, Unità di Microbiologia, Università Politecnica delle Marche, Ancona, Italia;

²¹ Dipartimento Interdisciplinare di Medicina, Policlinico Universitario “Aldo Moro”, Bari, Italia;

²² UOC Microbiologia e Virologia, Azienda Ospedaliero Universitaria Policlinico, Modena, Italia;

²³ UOC Microbiologia e Virologia, Policlinico Universitario San Martino, Genova, Italia;

²⁴ Department of Pathology and Infectious Diseases, School of Veterinary Medicine, University of Surrey, Guildford, UK;

²⁵ UOSD Patologia Clinica e Microbiologia, Ospedale “S. Giovanni di Dio”, Crotone, Italia

Introduction: *Campylobacter* spp. is a common cause of enteric infections. Antimicrobial resistance in *Campylobacter* spp. is increasing worldwide. The aim of the study is to provide national epidemiological and microbiological data (Italy) on *Campylobacter* spp. infections in humans and antibiotic resistance trends in the period 2017-2021.

Methods: We collected data from 19 Hospitals, located in 13 Italian Regions. Bacterial identification was performed by mass spectrometry. Antimicrobial susceptibility was assessed by Etest or Kirby-Bauer method (EUCAST criteria).

Results: 5420 isolates of *Campylobacter* spp. were reported. The most isolated species was *C. jejuni* (n=4534, 80%), followed by *C. coli* (n=732, 13.5%) and *C. fetus* (n=36, 0.7%). The mean age of patients is 34.54 years (+/- 27.84). 44.1% of cases were found in the age group 0-16 years (n = 1900), 35.2% between 17 and 64 years (n = 2380), 20.7% over 65 years (n = 1122).

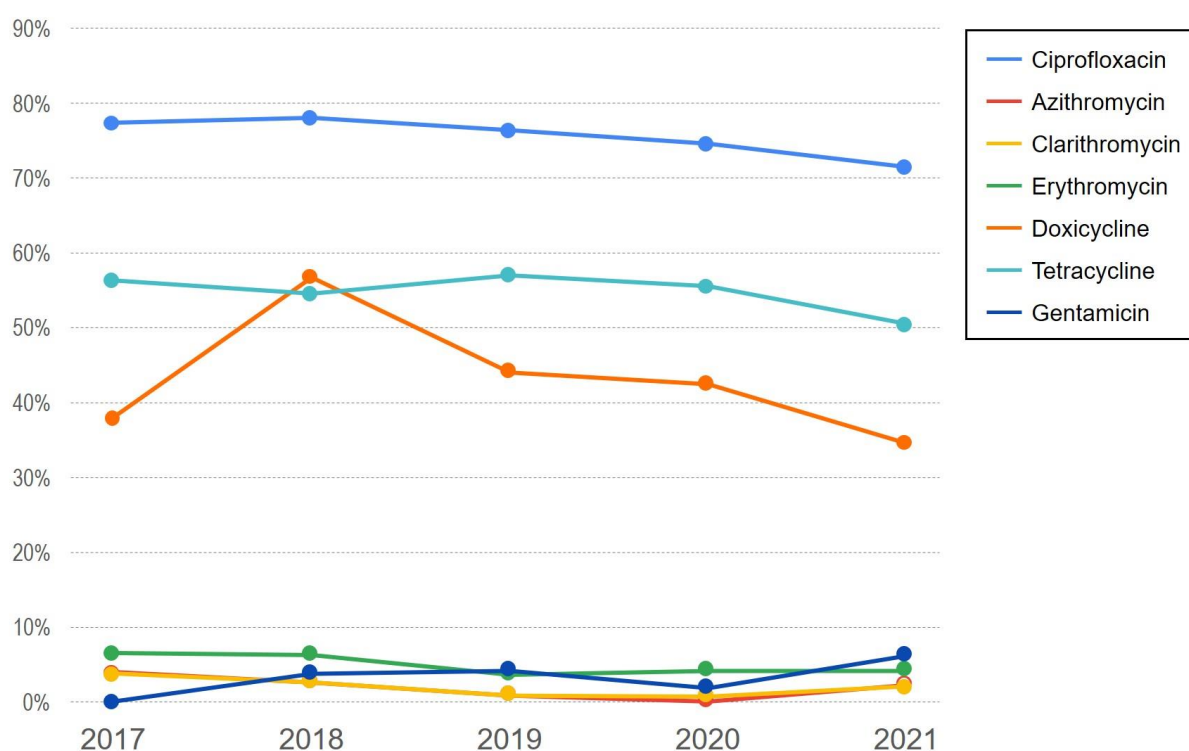
Reported cases were from: outpatient setting (n=2931, 54%), medical wards (n=1734, 32%), emergency departments (n=593, 11%), surgical wards (n=141.3%), intensive care units (n=21, 0.4%).

Campylobacter spp. was isolated from feces (n=5271), blood (n=145) or other sites (n=4). Isolations from blood cultures have been reported in 48% of cases in patients ≥ 65 years.

C. fetus was isolated from blood cultures in 83% of cases. In more than half of the cases (53%) *C. fetus* was isolated in patients aged over 65 years.

4632 strains have been tested (85%). Resistance to ciprofloxacin (CIP) was reported in 74.75% cases, while resistance to tetracycline (TET) in 54%, erythromycin (ERY) in 4.8%, clarithromycin in 2% and azithromycin in 1.9%. All isolates were susceptible to meropenem. Figure 1 shows the antimicrobial resistance trends during the study period.

Figure 1: Antimicrobial resistance trends (2017-2021)



C. jejuni and *C. coli* were commonly resistant to CIP and TET (45.8% and 56.8% of isolates with double resistance, respectively). Double resistance to CIP and ERY, to ERY and TET was mostly reported for *C. coli* (19.1% and 18.1% of isolates), as well as isolates with triple resistance to CIP-ERY-TET (17.1%). No double or triple-resistant isolates of *C. fetus* was reported. All isolates of *C. fetus* were susceptible to TET.

Discussion and Conclusions: *Campylobacter* spp. is a major problem in our country. The percentages of resistance to fluoroquinolones and tetracyclines are high, while macrolides maintain good activity in vitro. In particular, isolates with double or triple resistance to first-line antibiotics represent a serious cause of concern. *C. fetus* causes more invasive infections than *C. jejuni*, and is the most prevalent species in the elderly population. These data are a useful resource to guide clinicians in empiric therapy.

53 - Genome sequence analysis of infertility-associated *Enterococcus faecalis* strains revealed the presence of the novel Tn7086 carrying aminoglycoside resistance genes

LORENZO COLOMBINI ⁽¹⁾ - SUSANNA RICCI ⁽¹⁾ - MARIANA TIRZIU ⁽¹⁾ - ELISA LAZZERI ⁽¹⁾ - ANNA MARIA CUPPONE ⁽¹⁾ - STEFANO DE GIORGI ⁽¹⁾ - DAVID PINZAUTI ⁽¹⁾ - FRANCESCO SANTORO ⁽¹⁾ - FRANCESCO IANNELLI ⁽¹⁾ - GIANNI POZZI ⁽¹⁾

Laboratorio di Microbiologia Molecolare e Biotecnologia (LAMMB), Università degli Studi di Siena/ Dipartimento di Biotecnologie Mediche, Siena, Italia ⁽¹⁾

Genome sequence analysis of infertility-associated *Enterococcus faecalis* strains revealed the presence of the novel Tn7086 carrying aminoglycoside resistance genes

LORENZO COLOMBINI, SUSANNA RICCI, MARIANA TIRZIU, ELISA LAZZERI, ANNA M. CUPPONE, STEFANO DE GIORGI, DAVID PINZAUTI, FRANCESCO SANTORO, FRANCESCO IANNELLI, GIANNI POZZI

Department of Medical Biotechnologies, Laboratory of Molecular Microbiology and Biotechnology (LA.M.M.B.), University of Siena, Siena, Italy

1.Introduction

Enterococcus faecalis is a member of the human gut microbiota, but it is also a pathogen responsible of different types of infections. Furthermore, the presence of *E. faecalis* in genital samples of asymptomatic infertile couples is associated with abnormal semen parameters and reduced levels of vaginal lactobacilli, and it adversely affects in vitro fertilization. Mobile genetic elements contribute to the spread of antimicrobial resistance and virulence traits in *E. faecalis*. The present study aimed at characterizing antibiotic susceptibility and population structure of a collection of 41 *E. faecalis* strains isolated from infertile couples asymptomatic for genital infections.

2.Materials and Methods

Antimicrobial susceptibility was tested using VITEK[®]2 with the AST-P658 card, MIC with the Sensititre GPN3F plate and disk diffusion assays covering the antibiotics recommended for enterococci by EUCAST. Complete genomes of all isolates were obtained combining Nanopore and Illumina sequencing technologies. Population analysis, multilocus sequence typing, antimicrobial resistance genes search and DNA sequence analysis were conducted with PopPUNK, MLST, RGI and Artemis/ACT tool, respectively. PCR and sequencing analysis were used to detect and quantify the circular intermediates of the genetic elements.

3.Results

All the strains resulted susceptible to beta-lactams, glycopeptides, tigecycline, linezolid and nitrofurantoin, whereas 8 of 41 isolates were resistant to at least one antimicrobial. Seventeen sequence types were assigned and the 41 strains were split into 9 partitioning clusters based on core genome sequences. Indeed, 6 out of the 8 resistant isolates belonged to the same cluster. Genome comparison analysis of these isolates revealed a novel family of composite transposons, namely Tn7086 and Tn7086-like elements, carrying the aminoglycoside resistance determinant *aac(6')-aph(2'')* gene and characterized by the (i) presence of identical copies of the insertion sequence

IS1216E flanking the DNA sequence, (ii) insertion in the same chromosomal 2-dehydropantoate 2-reductase panE allele, and (iii) occurrence of similar DNA sequences with additional antimicrobial resistance genes including ermB, ant6-1a'-sat4-aphA-3, acc(6')-aph(2''), lnu(B)-lsa(E), ant9, cat and str. The number of transposon circular intermediates varied with the strain, ranging from 1.3 to 22.4 copies per 10⁶ chromosomes.

4. Discussion and Conclusions

A high level of diversity in this *E. faecalis* population was detected, indicating that association with infertility is not a feature of a specific cluster of *E. faecalis* strains. Furthermore, in this study a novel family of transposons carrying antimicrobial resistance determinants in *E. faecalis* was identified and characterized.

58 - Multiclonal presence of convergent ceftazidime/avibactam resistant and hypervirulent *Klebsiella pneumoniae* clinical isolates: when resistance meets virulence

AURORA PIAZZA⁽¹⁾ - DAVIDE CARCIONE⁽²⁾ - ILARIA PETRIZZI⁽¹⁾ - GHERARD BATISTI BIFFIGNANDI⁽³⁾ - VITTORIA MATTIONI MARCHETTI⁽¹⁾ - GIOCONDA BRIGANTE⁽²⁾ - ROBERTA MIGLIAVACCA⁽¹⁾

Università di Pavia, Dipartimento di Scienze Clinico Chirurgiche Diagnostiche e Pediatriche, Pavia, Italia⁽¹⁾ - ASST Valle Olona, Clinical Pathology Laboratory, Busto Arsizio, Italia⁽²⁾ - Università di Pavia, Dipartimento di Biologia e Biotecnologie "L. Spallanzani", Pavia, Italia⁽³⁾

Multiclonal presence of convergent ceftazidime/avibactam resistant and hypervirulent *Klebsiella pneumoniae* clinical isolates: when resistance meets virulence

Aurora Piazza¹, Davide Carcione², Iliara Petrizzi¹, Gherard Batisti Biffignandi³, Vittoria Mattioni Marchetti¹, Gioconda Brigante², Roberta Migliavacca¹

¹Department of Clinical-Surgical Diagnostic and Pediatric Sciences, Unit of Microbiology and Clinical Microbiology, University of Pavia, Pavia, Italy; ²Clinical Pathology Laboratory, ASST Valle Olona, Busto Arsizio, Italy; ³Department of Biology and Biotechnology "L. Spallanzani", University of Pavia, Pavia, Italy

Introduction: The global drug resistance rate of *Klebsiella pneumoniae* reached 70%. Moreover, hypervirulent *K. pneumoniae* (hvKP) have emerged globally, with recently emerging strains displaying a convergent phenotype of resistance and hypervirulence. Aim of the study was to characterize ceftazidime/avibactam-resistant (CZA-R) KPC-producing *K. pneumoniae* (KPC-Kp) clinical isolates. **Materials and Methods.** In the period February 2020-December 2021, six CZA-R KPC-Kp clinical isolates were obtained from two in- and two out- patients at Busto Arsizio Presidio Hospital of Valle Olona ASST. Species identification and antibiotic susceptibility profiles were carried out by MALDI-TOF MS and Vitek2 system. PCR and sequencing for blaKPC allelic variants were performed. Resistome was investigated by HybriSpot12 system. Molecular typing was assessed by Pulsed-Field Gel Electrophoresis (PFGE) and MultiLocus-Sequence typing (MLST). Three representative strains underwent Whole-Genome Sequencing (WGS) on Illumina MiSeq platform. Reads assembled using Shovill v0.9.0; MLST and resistome were detected using Kleborate v2.1.0. **Results:** Six CZA-R KPC-Kp were collected from Intensive Care Unit (n=3 from the same patient), Emergency and Surgery Departments (n=1, respectively), the last isolate being from a patient transferred from another acute care hospital. Overall, were collected: rectal swabs (n=2), urine/blood/bronchoaspirates (n=1, each) and a sample without available specimen type information. All strains were ciprofloxacin and third-generation cephalosporins resistant, with two strains also colistin resistant. Restoration of carbapenem susceptibility was observed in five out of six cases. Different blaKPC variants have been identified: blaKPC-3*T41A was detected in a strain co-harboring blaCTX-M, sul1, oqxA/B genes, and belonging to the new ST6420, clone A by PFGE; blaKPC-33 in a strain belonging to ST101, pulsotype B, and harboring armA, mphE, msrE, blaTEM-1D genes, plus mutations OmpK36TD, GyrA-83Y, GyrA-87N, ParC-80I. This strain showed a virulence score 1, harboring the ybt9 gene in a ICEKp3 element. A blaKPC-125 was found in a ST86

strain, PFGE clone C, that displayed mutations in *gyrA* (87G) and *mgrB* genes. This strain showed an hypervirulent phenotype with virulent score 4 (*ybt22*, *ICEKp12*, *iuc1*, *rmp 1*, *KpVP-1*, *rmpA2*). Lastly, a *blaKPC-94**C811T, newly assigned as *blaKPC-166*, was detected in a ST307, PFGE clone D, strain. Discussion and Conclusions: In this study, we report a different *blaKPC* variants in CZA-R *Kpn* and in high-risk and *hvKp* clones. These findings highlight the ongoing spread in Italy of strains of relevant concern for global public health.

62 - KPC-166, a novel KPC variant involved in ceftazidime/avibactam resistance

FRANCESCA PISCOPIELLO⁽¹⁾ - ALESSANDRA BIELLI⁽²⁾ - VITTORIA MATTIONI MARCHETTI⁽¹⁾ - RICCARDO GIUDICI⁽³⁾ - AURORA PIAZZA⁽¹⁾ - LIVIA TARTAGLIONE⁽²⁾ - GHERARD BATISTI BIFFIGNANDI⁽⁴⁾ - MARCO MERLI⁽⁵⁾ - ROBERTA MIGLIAVACCA⁽¹⁾ - CHIARA VISMARA⁽²⁾

Università di Pavia, Dipartimento di Scienze Clinico Chirurgiche Diagnostiche e Pediatriche, Pavia, Italia⁽¹⁾ - ASST Grande Ospedale Metropolitano Niguarda, Chemical-clinical and Microbiological Analysis, Milano, Italia⁽²⁾ - ASST Grande Ospedale Metropolitano Niguarda, Department of Anesthesia and Critical Care, Milano, Italia⁽³⁾ - Università di Pavia, Dipartimento di Biologia e Biotecnologie "L. Spallanzani", Pavia, Italia⁽⁴⁾ - ASST Grande Ospedale Metropolitano Niguarda, Infectious Diseases Clinic, Milano, Italia⁽⁵⁾

KPC-166, a novel KPC variant involved in ceftazidime/avibactam resistance

Francesca Piscopiello¹, Alessandra Bielli², Vittoria Mattioni Marchetti¹, Riccardo Giudici³, Aurora Piazza¹, Livia Tartaglione², Gherard Batisti Biffignandi^{1,4}, Marco Merli⁵, Roberta Migliavacca¹, Chiara Vismara²

Department of Clinical-Surgical, Diagnostic and Pediatric Sciences, Unit of Microbiology and Clinical Microbiology, University of Pavia, Pavia, Italy; Chemical-clinical and Microbiological Analysis, ASST Grande Ospedale Metropolitano Niguarda, Milan, Italy; Department of Anesthesia and Critical Care, ASST Grande Ospedale Metropolitano Niguarda, Milan, Italy; Infectious Diseases Clinic, ASST Grande Ospedale Metropolitano Niguarda, Milan, Italy; Department of Biology and Biotechnology "L. Spallanzani", University of Pavia, Pavia, Italy

Introduction: Treatment of carbapenem-resistant Enterobacterales (CRE) is a challenge in hospitalized patients. In recent years, new Beta-lactams-Beta-lactamases inhibitor combinations, as ceftazidime/avibactam (CZA), have been introduced to face severe infections caused by CRE. CZA represents one of the first-line options in the treatment of such infections, nonetheless, episodes of CZA resistance have been already reported. Aim of the study was the molecular characterization of 21 CZA-resistant (CZA-R) *Klebsiella pneumoniae* (Kp) strains, collected in one-year period from an Intensive Care COVID Unit of an Italian hospital. **Materials and Methods:** Antibiograms have been determined for all the strains by two MicroScan semi-automated systems (WalkAway and AutoScan4). The molecular characterization of the resistance genes content was ascertained with different methodologies (PCR, HybriSpot 12, WGS). The clonal relatedness among the isolates was determined by PFGE; the MLST according to the Pasteur scheme was also performed. A SNPs-based phylogenetic tree was obtained comparing representative isolates and global genomes. The evolutionary analysis of the KPC-166 protein and of all the available KPC variants was performed by MEGA 11. The blaKPC-166 horizontal transmission was evaluated with conjugation experiments. **Results:** Sixteen inpatients resulted positive for colonization and/or infection by KPC-producing Kp (KPC-Kp) strains. Twenty-one KPC-Kp isolates showed a MDR phenotype; CZA resistance was displayed while susceptibility to meropenem retained. They showed resistance to piperacillin/tazobactam, cefepime, gentamicin and ciprofloxacin, while susceptibility to amikacin and colistin. All the CZA-R KPC-Kp presented a novel KPC variant, named KPC-166, showing a single point substitution (Y271H) in omega loop. Two different pulsotypes were detected: A in 18/21, B in

1/21 KPC-Kp; two strains from the same patient resulted untypable by PFGE. Interestingly, other three STs namely ST6342, ST6418 and ST22 have been identified in addition to the high-risk ST307 outbreak clone. It is of concern that blaKPC-166 resulted positive for horizontal transfer. Discussion and Conclusions: This novel variant confers CZA-resistance but restoration of susceptibility to carbapenems. blaKPC-166 determinant was found in multiple lineages, suggesting that greater efforts should be made to prevent such MDR strains spread in clinical settings.

64 - Surveillance of Clostridioides difficile infection in the Pisa University Hospital: a correlation between clinical, environmental and pharmaceutical features

MICHELE TOTARO⁽¹⁾ - ANDREA PORRETTA⁽²⁾ - GIULIA GEMIGNANI⁽³⁾ - ANGELO BAGGIANI⁽²⁾ - IELIZZA DESIDERI⁽⁴⁾ - CLAUDIA CARMIGNANI⁽⁴⁾ - EMILIA GHELARDI⁽²⁾

Università di Pisa, Scuola di Specializzazione in Microbiologia e Virologia, Pisa, Italia⁽¹⁾ -
Università di Pisa, Dipartimento di Ricerca Traslationale NTMC, Pisa, Italia⁽²⁾ - Azienda
Ospedaliera Universitaria Pisana, Organizzazione Servizi Ospedalieri (OSO), Pisa, Italia⁽³⁾ -
Azienda Ospedaliera Universitaria Pisana, Farmacia Ospedaliera, Pisa, Italia⁽⁴⁾

Title:

Surveillance of Clostridioides difficile infection in the Pisa University Hospital: a correlation between clinical, environmental and pharmaceutical features.

Authors:

Michele Totaro^{1,2}, Andrea Porretta^{2,3}, Giulia Gemignani³, Angelo Baggiani^{2,3}, Ielizza Desideri³,
Claudia Carmignani³, Emilia Ghelardi^{2,3}

Affiliations:

¹Specialization School in Microbiology and Virology, University of Pisa, Italy

²Department of Translational Research in New Technologies in Medicine and Surgery, University of Pisa, Italy

³The Azienda Ospedaliero-Universitaria Pisana, Pisa, Italy

Introduction

Clostridioides difficile is the main causative agent of antibiotic- and healthcare-associated infective diarrhea. Almost all antibiotic classes may increase the risk of infection, such as the third/second-generation cephalosporin, clindamycin, quinolones, and penicillin combinations. Several institutions recommend specific strategies preventing C. difficile infections (CDI) as part of the healthcare associated infections programs in acute care hospitals. This study represent an epidemiological evaluation of CDI incidence in a tertiary hospital, associated to antibiotics consumption and environmental colonization.

Materials and Methods

CDI data were collected by the hospital infectious risk team with the following information: patient personal data, reason and date of admission, as well as CDI diagnosis references. Data on the mean antibiotics consumption were achieved by the hospital pharmaceutical unit, while microbiological sampling on hospital surfaces of the involved wards were performed by the hospital hygiene unit.

Results

From January to April 2023, 33 patients resulted to be colonized by C. difficile at the Pisa University Hospital. Diagnosis was performed by searching toxins from stool samples with an antigenic test (55%), molecular test (33%) or both methods (12%). Male patients (52%) had a mean age of 65 years and a length stay from 2 to 13 days. Female patients (48%) had a mean age of 71 years and a length stay from 2 to 31 days. The most involved wards were the General Medicine (GM), Gastroenterology (GA), Geriatric (GE), and the Intensive Care Unit (ICU). Environmental microbiological results showed a total aerobic microbial count higher than 100 CFU/cm² in 36% of the sampled surfaces in

the involved wards. Anaerobic microbial counts resulted negative in all samples. In the four considered wards, the difference between the four-monthly consumed antibiotic and the one suggested was: - 9% in GM, +105% in GA, + 172% in GE and -2% in ICU.

Discussion and Conclusions

Our data show the importance of the hospital surveillance for CDI in association with the environmental monitoring in the most involved wards. Despite the lack of anaerobic contamination in GM, GA, GE and ICU, we highlight a inappropriate mean antibiotic consumption in GA and GE, which is due to beta-lactams and fluoroquinolones. Hospitals challenge may be realized in more efforts aimed to reduce these antibiotics to prevent the emergence of CDI and other hospital infections, as recently suggested by the European Medicine Agency.

65 - Study of the role of wild boar (*Sus Scrofa*) as a reservoir of antibiotic resistance

RUBINA PARADISO ⁽¹⁾ - GIORGIA BORRIELLO ⁽¹⁾ - NICOLA D'ALESSIO ⁽¹⁾ - MARCO PAONE ⁽¹⁾ - GIOVANNA DE LUCA ⁽¹⁾ - NUNZIA RICCONI ⁽¹⁾ - FRANCESCA DEL PIANO ⁽¹⁾ - ESTERINA DE CARLO ⁽¹⁾ - GIOVANNA FUSCO ⁽¹⁾

Istituto Zooprofilattico Sperimentale del Mezzogiorno, Dipartimento Coordinamento Sanità Animale, Portici, Italia ⁽¹⁾

Study of the role of wild boar (*Sus Scrofa*) as a reservoir of antibiotic resistance

RUBINA PARADISO, GIORGIA BORRIELLO, NICOLA D'ALESSIO, MARCO PAONE, GIOVANNA DE LUCA, NUNZIA RICCONI, FRANCESCA DEL PIANO, ESTERINA DE CARLO, GIOVANNA FUSCO

Dipartimento Coordinamento Sanità Animale, Istituto Zooprofilattico Sperimentale del Mezzogiorno, Portici (NA), Italy

Introduction

Inappropriate antibiotic treatment and overuse of antibiotics have contributed to the emergence of multi-drug resistance (MDR) among bacteria causing hundreds of thousands of deaths every year. The bacteria developed different mechanisms able to reduce the activity of antibiotic drugs used to treat infection. In addition, the ease of transmission of drug-resistance genes both between bacteria of the same species and different bacterial species promotes the spread of antimicrobial resistance (AMR) in humans and animals. Numerous studies have documented the spread of antimicrobial resistance genes in humans and livestock species, but knowledge of the role of wildlife in dissemination of AMR bacteria is still limited. Therefore, the aim of this study was to assess the role of wild boar (*Sus Scrofa*), which is able to colonize strongly anthropized environments, in the dissemination and persistence of AMR at the human-livestock-wildlife interface.

Materials and Methods

Forty-four wild boar carcasses were collected from the Campania region in South Italy within the Regional plan for surveillance of zoonotic diseases in wildlife, and post mortem examined at the Experimental Zooprophyllactic Institute of Southern Italy. From each animal a sample of faeces was collected to isolate eventual bacterial pathogens by traditional culture method. All the isolated bacterial strains were processed for Whole Genome Sequencing (WGS) on the Ion S5 Gene Studio platform to assess the presence of AMR genes.

Results

Sixteen bacterial strains (twelve *Escherichia coli* and four *Salmonella* spp.) were isolated and sequenced from the examined faeces. Among the “critically important antimicrobials for human medicine”, all the sequenced *E. Coli* strains were resistant to aminoglycosides, carbapenems, macrolides, penicillins, tetracyclines, and quinolones. One of the analyzed strains also harboured the *tem-1* gene, associated with the production of extended spectrum beta-lactamases (ESBL). Similarly, all the sequenced *Salmonella* strains were resistant to aminoglycosides, macrolides, penicillins, tetracyclines, and quinolones.

Discussion and Conclusions

Overall these data highlight that AMR is a wide environmental issue of public health concern, no longer confined only to livestock and human settings. Wildlife can act as an important sentinel for AMR. In particular wild boar, due to its close interaction with humans and livestock, the omnivorous behaviour, the large home range and the possibility to be consumed through hunting harvest, can play a crucial role in the dissemination and persistence of AMR in the wider environment.

76 - Endosymbiosis of *Mycoplasma hominis* in *Trichomonas vaginalis*: influence of intracellular localization on its resistance to antibiotics

VALENTINA MARGARITA⁽¹⁾ - GAVINO CARBONI⁽¹⁾ - NICIA DIAZ⁽¹⁾ - PAOLA RAPPELLI⁽¹⁾ - PIER LUIGI FIORI⁽¹⁾

Universita' di Sassari, Dipartimento di Scienze Biomediche, Sassari, Italia⁽¹⁾

Endosymbiosis of *Mycoplasma hominis* in *Trichomonas vaginalis*: influence of intracellular localization on its resistance to antibiotics

Valentina Margarita¹, Gavino Carboni¹, Nicia Diaz¹, Paola Rappelli^{1;2}, Pier Luigi Fiori^{1;2}

1. Department of Biomedical Sciences, University of Sassari, Viale San Pietro 43/B, 07100 Sassari, Italy

2. Azienda Universitaria Ospedaliera AOU Sassari, 07110 Sassari, Italy

Introduction: *Mycoplasma hominis*, an opportunistic pathogen of the human lower urogenital tract, is correlated with important sequelae in pregnant women such as preterm birth, postpartum infection, and spontaneous abortion. The absence of a rigid cells wall renders the bacterium resistant to beta-lactam antibiotics. Infection is mainly treated with tetracycline, while macrolides are recommended for pregnant women, neonates, and children. *M.hominis* can survive and replicate in the protozoon *Trichomonas vaginalis*, establishing an endosymbiotic relationship. The intracellular location may represent a way for the bacteria to escape the immune system and a protection from antibiomicrobial activities.

Our aim was to investigate on the possible influence of the symbiosis with *T.vaginalis* on the antibiotic susceptibility of *M.hominis*

Materials and Methods: Sensitivity to antibiotics in *M.hominis* isolated in association with *T.vaginalis* was compared with that of mycoplasmas isolated from women without trichomoniasis. Moreover, we assessed the minimal inhibitory (MIC) and lethal concentrations (MLC) of tetracycline on *M.hominis* strains after 8 weeks of in vitro cultivation both alone and in association with *T.vaginalis*, using an experimental model system.

Results: The incidence rate of *M.hominis* resistant to C14 and C15 macrolide members was higher in bacteria isolated from women affected by trichomoniasis, while sensitivity to tetracycline and quinolones was similar in the two groups of clinical strains. In vitro experiments showed higher MIC and MLC values of tetracycline for *M.hominis* strains grown in association with *T.vaginalis*, compared with bacteria cultivated alone.

Discussion and Conclusion: Results obtained suggest that the intracellular lifestyle of *Mycoplasma hominis* within *Trichomonas vaginalis* trophozoites may influence the susceptibility to antibiotics of the bacterium and contribute to the selection of bacterial strains with increased drug resistance.

81 - A strategy to screen compounds against flaviviral infection helps identify a novel compound against Zika virus

ERIKA PLICANTI⁽¹⁾ - GIULIA LOTTINI⁽¹⁾ - SILVIA VEGNI⁽²⁾ - SILVIA NOTTOLI⁽³⁾ - MICHELE LAI⁽²⁾ - ROBERTA IBBA⁽⁴⁾ - SANDRA PIRAS⁽⁴⁾ - ANTONIO CARTA⁽⁴⁾ - GIULIA FREER⁽²⁾

Università di Siena, Dipartimento di Biotecnologie mediche, Siena, Italia⁽¹⁾ - Università di Pisa, Dipartimento di ricerca traslazionale, Pisa, Italia⁽²⁾ - Ospedale Cisanello, Unità di virologia, Pisa, Italia⁽³⁾ - Università di Sassari, Dipartimento di medicina, chirurgia e farmacia, Sassari, Italia⁽⁴⁾

A strategy to screen compounds against flaviviral infection helps identify a novel compound against Zika virus.

ERIKA PLICANTI^{a,c}, GIULIA LOTTINI^{a,c}, SILVIA VEGNI^a, SILVIA NOTTOLI^b, MICHELE LAI^a, ROBERTA IBBA^d, SANDRA PIRAS^d, ANTONIO CARTA^d, GIULIA FREER^a

a: Retrovirus Centre, Department of Translational Research, University of Pisa, Pisa, Italy; b: Virology Unit, Pisa University Hospital, Pisa, Italy; c: Department of Medical Biotechnologies, University of Siena, Siena, Italy; d: Department of Medicine, Surgery and Pharmacy, University of Sassari, Sassari, Italy

1. Introduction

Zika virus (ZIKV) is a positive sense, single stranded RNA Flavivirus that causes Zika fever. It is spread by Aedes mosquitoes. ZIKV can spread from a pregnant woman to her baby; this can result in microcephaly, severe brain malformations, and other birth defects. Usutu virus (USUV) is another Flavivirus that most often causes asymptomatic infection. A panel of molecules of different chemical nature, designed and synthesized at the Department of Medicine, Surgery and Pharmacy, University of Sassari, were selected because they had previously shown antiviral activity against different viruses. The aim of this study was to evaluate the antiviral activity of these compounds against ZIKV and USUV and, possibly, the mechanism of action.

2. Materials and Methods

To evaluate the activity and the cytotoxicity of the compounds, an assay based on the viral cytopathic effect (CPE assay) was developed. HuH7 cells were infected with multiplicity of infection (MOI) of 10 with ZIKV or USUV in the presence of scalar concentrations of drugs. The IC₅₀ of compounds that showed antiviral activity at a minimum of three concentrations was calculated, as well as the corresponding selectivity index (SI). Time of addition experiments were performed by adding the selected drugs before, during and 2 or 6 h after infection and evaluating the change in viral yield.

3. Results

CPE assay showed that a number of the drugs tested effectively inhibited Zika virus infection, but only at specific concentrations, different for each drug. The range of effective concentrations varied between 0.5 and 130 micromolar. This was confirmed by calculating the IC₅₀ and the SI. Time of addition experiments were carried out for the drugs exhibiting the highest SI and helped to understand the mechanism of action.

4. Discussion and conclusion

No drug was active against both ZIKV and USUV. However, several drugs were active against ZIKV, whereas none worked against USUV. Given the encouraging results obtained, we believe that these drugs may be considered good candidates for the treatment of Zika infection. To this purpose, further studies will be needed to determine the mechanism of action of the compounds found. We are using one specific drug, that was active down to nanomolar concentrations against ZIKV, to try to select drug-resistant viral mutants to better understand the site and mode of action of the drug.

84 - Minimum inhibitory concentration distribution of *Candida auris* strains: preliminary data from a large tertiary hospital in Piedmont

LISA PASTRONE (1) - ANTONIO CURTONI (1) - ALESSANDRO BONDI (1) - TERESA ZACCARIA (2) - ROBERTO CASALE (1) - FEDERICA ZULLO (1) - LORENZA CAVALLO (1) - GIORGIA MONTRUCCHIO (3) - SILVIA CORCIONE (4) - ROSSANA CAVALLO (1) - CRISTINA COSTA (1)

SC Microbiologia e Virologia, Dipartimento di Scienze della Sanità Pubblica e Pediatrica, Università di Torino, Torino, Italia (1) - SC Microbiologia e Virologia, Dipartimento di Medicina di Laboratorio, Ospedale Universitario Città della Salute e della Scienza di Torino, Torino, Italia (2) - Anestesia e Rianimazione 1U, Dipartimento di Anestesia, Terapia Intensiva ed Emergenza, Ospedale Universitario Città della Salute e della Scienza di Torino, Torino, Italia (3) - Unità di Malattie Infettive, Dipartimento di Scienze Mediche, Università di Torino, Torino, Italia (4)

Minimum inhibitory concentration distribution of *Candida auris* strains: preliminary data from a large tertiary hospital in Piedmont

LISA PASTRONE¹, ANTONIO CURTONI^{1,2}, ALESSANDRO BONDI^{1,2}, TERESA ZACCARIA², ROBERTO CASALE¹, FEDERICA ZULLO¹, LORENZA CAVALLO¹, GIORGIA MONTRUCCHIO³, SILVIA CORCIONE⁴, ROSSANA CAVALLO^{1,2}, CRISTINA COSTA^{1,2}

¹Department of Sciences of Public Health and Pediatrics, University of Turin, Turin, Italy;

²Department of Laboratory Medicine, Microbiology and Virology Unit, University Hospital Città della Salute e della Scienza di Torino, Turin, Italy;

³Department of Anesthesia, Intensive Care and Emergency, Anestesia e Rianimazione 1U, University Hospital Città della Salute e della Scienza di Torino, Turin, Italy;

⁴Department of Medical Sciences, Infectious Disease Unit, University of Turin, Turin, Italy

Introduction.

Candida auris is an emerging healthcare-associated pathogen isolated for the first time in Japan (2009), responsible for nosocomial infection outbreaks, especially in Intensive Care Units (ICU). Due to its high multidrug resistance, transmissibility, and long persistence in the hospital environments, it is considered a serious global threat. In literature, the percentage of isolates with resistance to azoles, amphotericin B and echinocandins is high but an important geographical variability of the antifungal susceptibility pattern was reported. Herein we provide preliminary data about the antifungal susceptibility of *C. auris* strains isolated in a 10-bed ICU at the University Hospital Città della Salute e della Scienza di Torino, Turin, Italy.

Materials and Methods

From December 2021 to January 2023, 27 *C. auris* strains were isolated from bilateral axillae and groin swabs (according to our active surveillance program), urinary catheters, tracheal aspirates, bronchoalveolar lavages, sputum and blood cultures of different patients. Isolated colonies were identified by MALDI-TOF and MIC determination was performed by broth microdilution with

MICRONAUT-AM EUCAST AFST (Bruker, Germany) kit, following manufacturer instruction and interpreted, when possible, according to the EUCAST Antifungal Clinical Breakpoint v.10.0 (2020).

Results

All the tested strains, referring both to colonization (93%) and invasive infections (7%), resulted resistant to fluconazole with $\text{MIC} \geq 128 \mu\text{g/ml}$, 66.7% showed a 0.5MIC for voriconazole, 74.1% had a MIC distribution between 0.03 and 0.06 for posaconazole and 57.7% had a MIC distribution between 0.06 and 0.125 for itraconazole. 88.9% of the clinical isolates showed a MIC distribution between 0.25 and 1 for amphotericin B, while 77.8% had a $\text{MIC} \leq 0.06$ for 5-fluorocytosin. Finally, as regards echinocandins, 85.2% of the strains showed a MIC distribution between 0.03 and 0.125 for anidulafungin, 81.5% had a MIC distribution between 0.03 and 0.06 for micafungin and 83.3% had a MIC distribution between 0.06 and 0.125 for caspofungin.

Discussion and Conclusions

Preliminary data reaching from the tested strains showed an overall MIC distribution ranging from 0.03 to 0.125 for both echinocandins and azoles, with the exception of fluconazole due to the high resistance of *C. auris* to this antifungal agent. The observed MIC for voriconazole was consistent with that reported by EUCAST MIC distributions. Among the obtained MICs, 5-Fluorocytosine one was the lowest observed. Currently, there are no species-specific antifungal susceptibility breakpoints established for *C. auris*: MIC data from the geographical site of strains isolation are fundamental for patient antifungal treatment and for future species-specific breakpoints definition.

86 - Characterization of a prophage and a defective integrative conjugative element carrying the *optrA* gene in linezolid-resistant *Streptococcus dysgalactiae* subsp. *equisimilis* isolates from pigs, Italy

SONIA NINA COCCITTO⁽¹⁾ - MARZIA CINTHI⁽²⁾ - FRANCESCA ROMANA MASSACCI⁽³⁾ - ELISA ALBINI⁽³⁾ - LUCILLA CUCCO⁽³⁾ - MASSIMILIANO ORSINI⁽⁴⁾ - SERENA SIMONI⁽²⁾ - ELEONORA GIOVANETTI⁽²⁾ - CHIARA FRANCESCA MAGISTRALI⁽³⁾ - ANDREA BRENCIANI⁽¹⁾

Università Politecnica delle Marche, Dipartimento Scienze Biomediche e sanità Pubblica, Ancona, Italia⁽¹⁾ - Università Politecnica delle Marche, Dipartimento Scienze della Vita e dell'Ambiente, Ancona, Italia⁽²⁾ - Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche, Togo Rosati, Dipartimento di Ricerca e Sviluppo, Perugia, Italia⁽³⁾ - Istituto Zooprofilattico Sperimentale delle Venezie, Dipartimento di Microbiologia, Padova, Italia⁽⁴⁾

Characterization of a prophage and a defective integrative conjugative element carrying the *optrA* gene in linezolid-resistant *Streptococcus dysgalactiae* subsp. *equisimilis* isolates from pigs, Italy

SONIA N. COCCITTO³, MARZIA CINTHI¹, FRANCESCA R. MASSACCI², ELISA ALBINI², LUCILLA CUCCO², MASSIMILIANO ORSINI⁴, SERENA SIMONI¹, ELEONORA GIOVANETTI¹, CHIARA F. MAGISTRALI², ANDREA BRENCIANI³

¹Unit of Microbiology, Department of Life and Environmental Sciences, Polytechnic University of Marche, Ancona, Italy; ²Department of Research and Development, Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche 'Togo Rosati', Perugia, Italy; ³Unit of Microbiology, Department of Biomedical Sciences and Public Health, Polytechnic University of Marche Medical School, Ancona, Italy; ⁴Department of Microbiology, Istituto Zooprofilattico Sperimentale delle Venezie, Padova, Italy

Introduction: Oxazolidinones are a synthetic class of last-resort antimicrobials effective against different Gram-positive bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus* spp. (VRE), multi-drug resistant pneumococci and mycobacteria. However, several mechanisms of resistance to this class of antibiotic, due to ribosomal mutations (23S rRNA and/or L3 and/or L4 ribosomal proteins) and to acquisition of transferable resistance genes (*cfr/cfr*-like, *optrA*, *poxtA* and *poxtA2*), have been identified.

The *optrA* gene, initially detected in the linezolid-resistant *Enterococcus faecalis* E349 recovered from a Chinese patient, has proven to be widespread mainly in enterococci of animal origin even if it was also detected in other Gram-positive bacteria due to its frequent localization on several plasmids, prophages, transposons, integrative and conjugative elements (ICEs) and genomic islands. In this study, we investigate the *optrA*-carrying genetic elements and their transferability in two linezolid-resistant *Streptococcus dysgalactiae* subsp. *equisimilis* (SDSE) strains of swine origin.

Materials and Methods: SDSE V220 and V1524 strains were isolated from diseased pigs, originated from two different finishing farms located in Central Italy, in 2019 and 2022, respectively.

The two SDSE isolates were screened for the presence of *cfr/cfr*-like, *optrA* and *poxA* genes by PCR assays. Antimicrobial susceptibility testing was carried out by broth microdilution. Gene transferability was assessed by filter mating experiments using rifampin- and fusidic acid-resistant SDSE 5381-3 and *Streptococcus pyogenes* 12RF isolates as recipients. The *optrA* genetic context and clonal lineage were analyzed by Whole Genome Sequencing (WGS), while excision of the genetic elements was assayed by inverse PCR.

Results: SDSE isolates were resistant to chloramphenicol, florfenicol and linezolid, but susceptible to tedizolid and both carried only the *optrA* gene.

In SDSE V220 *optrA* was located on a novel 72.9-kb ICESdyV220 inserted in the 3' end of the chromosomal *rum* gene. It was 94% - 96% identical (coverage, from 31% to 61%) to other *optrA*-carrying ICEs. In depth ICESdyV220 sequence analysis revealed that *optrA* was carried by an IMESdyV220 (17.9 kb), also containing the *tet(O/W/32/O)* gene. Inverse PCR assays excluded the ICESdyV220 mobility. In SDSE V1524, *optrA* was carried by the novel Φ SdyV1524 prophage, integrated near the 5' end of the chromosomal *had* gene, showing a genetic organization like that of other streptococcal phages. Conjugation and transduction assays failed to demonstrate the *optrA* transferability to streptococcal recipients. V220 and V1524 belonged to two novel sequence types (ST704 and ST634, respectively).

Discussion and Conclusions: To the best of our knowledge, this is the first identification of the *optrA* gene on a prophage and an ICE in SDSE isolates from swine brain.

These findings are consistent with the current belief in the key role of bacteriophages and ICEs in the streptococcal evolution and adaptation.

87 - Outbreak of multi drug-resistant OXA-48 and NDM-5 producing *Klebsiella pneumoniae* in a central Italy regional hospital.

GLORIA D'ACHILLE⁽¹⁾ - ILARIA NUNZI⁽¹⁾ - SONIA NINA COCCITTO⁽¹⁾ - MARZIA CINTHI⁽²⁾ - SIMONA FIORITI⁽¹⁾ - ANDREA BRENCIANI⁽¹⁾ - LUCIA TEODORI⁽³⁾ - MARINA MINGOIA⁽¹⁾ - GIANLUCA MORRONI⁽¹⁾

Università Politecnica delle Marche, Dipartimento di Scienze Biomediche e Sanità Pubblica, Ancona, Italia⁽¹⁾ - Università Politecnica delle Marche, Dipartimento di Scienze della Vita e dell'Ambiente, Ancona, Italia⁽²⁾ - Ospedali Riuniti di Ancona, Laboratorio di Microbiologia, Ancona, Italia⁽³⁾

Outbreak of multi drug-resistant OXA-48 and NDM-5 producing *Klebsiella pneumoniae* in a central Italy regional hospital.

GLORIA D'ACHILLE¹, ILARIA NUNZI¹, SONIA N. COCCITTO¹, MARZIA CINTHI², SIMONA FIORITI¹, ANDREA BRENCIANI¹, LUCIA TEODORI³, MARINA MINGOIA¹, GIANLUCA MORRONI¹

¹Department of Biomedical Sciences and Public Health, Polytechnic University of Marche, Ancona, Italy; ²Department of Life and Environmental Sciences, Unit of Microbiology, Polytechnic University of Marche, Ancona, Italy; ³Microbiology Laboratory, Azienda Ospedaliero Universitaria "Ospedali Riuniti", Ancona, Italy.

Introduction: *Klebsiella pneumoniae* is one of the most important nosocomial pathogens worldwide due to its high mortality rate and resistance determinants. Among these bacteria, carbapenemases production is a crucial mechanism that reduces the effective therapeutic options. Moreover, the co-expression of these enzymes belonging to different classes is even more worrying. In this study 10 OXA-48 and NDM-5 producing *K. pneumoniae* (Kp) were collected from clinical samples at "Ospedali Riuniti" of Ancona, Italy, and phenotypically and genotypically characterized to evaluate their belonging to the same or different clone.

Material and methods: 10 OXA-48 and NDM-5 producing Kp were isolated from different patients between September 2022 and February 2023 at the regional hospital of Ancona. The presence of both genes was confirmed by PCR assay. MIC values for colistin, meropenem, imipenem, aztreonam, ceftazidime-avibactam, gentamycin, and cefiderocol were assessed by broth microdilution method. GenElute bacterial genomic DNA kit (Sigma-Aldrich) was used for total DNA extraction. All strains were typed by XbaI-PFGE and then submitted to whole genome sequencing (WGS) by Oxford Nanopore technology. The reads assembly was performed by Canu v 1.9 and polished with Medaka v 1.7.3 while different softwares (Center for Genomic Epidemiology, BLAST, CARD, BRIG) were used for genome analysis.

Results: All strains were fully resistant to all drugs tested except for cefiderocol which showed different strain-specific MICs. The XbaI digestion revealed identical restriction profiles on 9 out of 10 samples while the last strain was strictly related showing an additional band. WGS analysis highlighted that all Kp belonged to the same sequence type ST383 and brought the carbapenemases genes studied in two different plasmids: bla_{NDM-5} was located on a pNMD-MAR plasmid of 366 kb while a IncL plasmid of 67 kb carried bla_{OXA-48}. Also, a chromosomal copy of the metallo-carbapenemase was found in all samples with identical PFGE.

Discussion and conclusions: This was the first isolation of Kp carrying both carbapenemases genes in our regional hospital. The rapid onset of OXA-48 and NDM-5 producing Kp in a limited time frame suggested a clonal outbreak. Indeed, this suggestion was confirmed by both identical PFGE

and WGS who assigned the ST383 to all samples. This high-risk clone with the same carbapenemases genes was detected in Brescia in 2019, underling his diffusion in different Italian regions. We also noticed a high identity (99,96%) of our plasmids with those of a Qatar 2022 study (NCBI accession number: GCF_025144535.1). This underlines the high spread and global dissemination of these carbapenemases-carrying plasmids, facilitated also by international travel.

88 - Fosfomycin-meropenem synergistic combination against NDM carbapenemase-producing *Klebsiella pneumoniae* strains

Maria Teresa Della Rocca ⁽¹⁾ - Vittorio Panetta ⁽¹⁾ - Filomena Merola ⁽¹⁾ - Giusy Natale ⁽¹⁾ - Giovanni Di Caprio ⁽²⁾ - Francesca Colucci ⁽²⁾ - Emanuele Cordua ⁽²⁾ - Adriana Durante ⁽¹⁾ - Rita Greco ⁽¹⁾

UOSD Microbiology, AORN Sant'Anna and San Sebastiano, Caserta, Italia ⁽¹⁾ - Infectious and Tropical Diseases Clinic, AORN Sant 'Anna and San Sebastiano, caserta, Italia ⁽²⁾

Fosfomycin-meropenem synergistic combination against NDM carbapenemase-producing *Klebsiella pneumoniae* strains

MARIA T. DELLA ROCCA.¹, VITTORIO PANETTA¹, FILOMENA MEROLA¹, GIUSY NATALE¹, GIOVANNI DI CAPRIO², FRANCESCA COLUCCI ², EMANUELE CORDUA², ADRIANA DURANTE¹, RITA GRECO¹

¹UOSD Microbiology - AORN Sant 'Anna and San Sebastiano, Caserta, Italy;

² Infectious and Tropical Diseases Clinic, AORN Sant 'Anna and San Sebastiano, Caserta, Italy
Introduction

Carbapenemase producing Enterobacteriaceae (CPE) has been increasing and threatens global public health. Treatment of CPE isolates, like New Delhi metallo-beta-lactamase (NDM) are limited and often necessitates combination therapies. Although new effective antibiotics against carbapenemase-producing bacteria, therapeutic options currently available for NDM-producing microorganisms are still limited. Aztreonam, ceftazidime-avibactam and cefiderocol has been given with promising responses in patients with severe infections, however aztreonam is not available in Italy and there have been shortages in supplies also for cefiderocol. This situation prompts the search for synergistic combinations against carbapenemase-producing *K.pneumoniae* infections, until new antibiotics will be developed. The aim of this study was to evaluate the synergistic meropenem/fosfomycin combination against *K.pneumoniae*-producing NDM isolates.

Materials and methods

This study was conducted at Microbiology Unit of hospital Sant'Anna and San Sebastiano, Caserta, Italy. A total of 20 clinical isolates of carbapenemase-producing *K. pneumonia* (CPKp NDM) were collected from patients admitted to the Intensive Care Unit (ICU) from September 2022 to December 2022. Isolates susceptibility antimicrobial agents were carried out with broth microdilution method according to European Committee on Antimicrobial Susceptibility Testing (EUCAST,2023). Carbapenemase resistance genes detection was performed with real time PCR methods. Fosfomycin meropenem, fosfomycin/colistin and meropenem/colistin were tested alone and in combination, using e-test and time-kill assay.

Results

K. pneumoniae strains were resistant to meropenem, ceftazidime/avibactam and ceftolozano/tazobactam, 75% and 80% isolates were susceptible to cefiderocol and for colistin respectively. Fosfomycin/meropenem combination was synergic in 95% (n=19) strains. Fosfomycin/colistin and colistin/meropenem combination showed only 10% synergistic combination strains. In 16 isolates (80%) indifference action for fosfomycin/colistin and colistin/meropenem were reported. Only 0,8 % CpKP NDM isolates result antagonism effect for colistin/meropenem and fosfomycin/colistin combination.

Discussion and conclusions

In this study, time kill assay showed combination therapies action versus *K.pneumoniae* metallo beta lactamase producing (NDM) strains and confirmed the synergistic action of fosfomycin/meropenem combination. In vitro synergy testing should be routinely performed in multidrug resistance infections and combo therapies can be used as a possible alternative in targeted patients with the goal of reducing overall antibiotic costs.

92 - The N-acylethanolamine acid amidase is a SARS-CoV-2 host factor

VERONICA LA ROCCA ⁽¹⁾

Scuola Superiore Sant'Anna Pisa, Scuola Superiore Sant'Anna Pisa, Pisa, Italia ⁽¹⁾

The N-acylethanolamine acid amidase is a SARS-CoV-2 host factor

VERONICA LA ROCCA^{1,2}, ROSSELLA FONNESU², CAROLINA FILIPPONI², ELENA IACONO², VIKTORIA DIESENDORF³, KATHERINA SEWALD⁴, HELENA OBERNOLTE⁴, GIULIA FREER², JOCHEN BODEM³, MAURO PISTELLO^{2,5}, MICHELE LAI²

¹Institute of Life Sciences, Sant'Anna School of Advanced Studies, Pisa, Italy; ²Retrovirus Center, Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa, Pisa, Italy; ³Institute of Virology and Immunobiology, Julius-Maximilians-Universität Würzburg, Würzburg, Germany. ⁴Fraunhofer Institute for Toxicology and Experimental Medicine ITEM, Member of Fraunhofer International Consortium for Anti-Infective Research (iCAIR), Member of the German Center for Lung Research (DZL), Biomedical Research in Endstage and Obstructive Lung Disease (BREATH), Hannover, Germany ⁵Virology Unit, Pisa University Hospital, Pisa, Italy.

Introduction: The over-production of pro-inflammatory factors contributes to the high mortality of COVID-19 patients caused by the severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2). As for other positive-stranded-RNA viruses, SARS-CoV-2 modifies the lipid host metabolism to support its replication by boosting lipogenesis and lipid droplet synthesis. In addition, SARS-CoV-2 infection alters bioactive lipids implicated in the inflammatory process. Nuclear factor- κ B (NF- κ B) is a well-characterized transcription factor that regulates the production of pro-inflammatory mediators. Upon activation, NF- κ B translocate to the nucleus and activates the expression of downstream target genes. In SARS-CoV-2 infection, it has been shown that NF- κ B drives robust cytokine production and is also required for viral replication.

Here, we investigate how the enzyme N-Acylethanolamine acid amidase (NAAA) contributes to SARS-CoV-2 replication. NAAA is a lysosome enzyme that catalyzes the hydrolysis of the anti-inflammatory lipid Palmitoylethanolamide (PEA) into ethanolamine and palmitic acid. PEA exerts its anti-inflammatory action by activating Peroxisome Proliferator-Activated Receptor- α (PPAR- α), leading to the downregulation of NF- κ B. In summary, we hypothesize that inhibiting NAAA will lead to NF- κ B suppression that ultimately hinders SARS-CoV-2 replication.

Materials and Methods: We used genetic and pharmacological approaches to suppress NAAA expression in Huh-7 cells, using CRISPR/Cas9 system and selective inhibitors, respectively. SARS-CoV-2 replication was analysed by qRT-PCR and confocal imaging. Then, data were confirmed by pharmacological inhibit NAAA during SARS-CoV-2 replication ex-vivo, using human precision-cut lung slides (PCLS) derived from three donors. To test the activation of the NF- κ B pathway, we used the NF- κ B Luciferase reporter construct in HEK-293T cells treated or not with NAAA inhibitor.

Results and conclusions: NAAA genetic ablation suppress SARS-CoV-2 replication by 2 log₁₀ compared to their wild-type counterparts. Moreover, treatment with the NAAA inhibitor ARN726 resulted in a reduction of SARS-CoV-2 replication by 1 order of magnitude in Huh-7 cells and 4 orders of magnitude in Calu-3 cells, respectively. Furthermore, we observed a 4 order of magnitude

reduction in SARS-CoV-2 replication when administering ARN726 at concentrations above 30 μ M in Human Precision-cut lung slices. We next tested whether the inhibition of NAAA could affect NF- κ B activation through the activation of the transcription factor PPAR α . We found that NAAA^{-/-} cells increase PPAR α expression by 1.5 folds, oppositely, PPAR α expression is kept low in parental-infected cells. As expected, the increased PPAR α expression was accompanied by a 1.6-fold reduction in NF- κ B activation in Huh-7 NAAA^{-/-} cells and HEK-293T cells treated with NAAA inhibitor at 30 μ M. These results show that NAAA is a host factor for SARS-CoV-2 replication and highlights a possible mechanism of action based on the reduction of NF- κ B activation during viral replication.

103 - Time to positivity of blood cultures (Tpos) as surrogate biomarker of in vivo antimicrobial efficacy: in vitro simulations with bacteria and yeasts

IRENE ZAGHI⁽¹⁾ - MARTINA BRANDOLINI⁽¹⁾ - ANNA MARZUCCO⁽²⁾ - SOFIA MONTANARI⁽²⁾ - GIULIA GATTI⁽²⁾ - FRANCESCA TADDEI⁽²⁾ - CLAUDIA COLOSIMO⁽²⁾ - MONICA CRICCA⁽¹⁾ - VITTORIO SAMBRI⁽¹⁾ - JASON ROBERTS⁽³⁾ - RUSSELL E. LEWIS⁽⁴⁾

Department of Medical and Surgical Sciences, University of Bologna, Italy, Department of Medical and Surgical Sciences, University of Bologna, Italy, Bologna, Italia⁽¹⁾ - Laboratorio Unico della AUSL della Romagna, in Pievesestina di Cesena, Italy, Laboratorio Unico della AUSL della Romagna, in Pievesestina di Cesena, Italy, Cesena, Italia⁽²⁾ - The University of Queensland Centre for Clinical Research, Brisbane, Queensland, Australia, The University of Queensland Centre for Clinical Research, Brisbane, Queensland, Australia, Brisbane, Australia⁽³⁾ - Department of Molecular Medicine, University of Padua, Padua, Italy, Department of Molecular Medicine, University of Padua, Padua, Italy, Padova, Italia⁽⁴⁾

Time to positivity of blood cultures (Tpos) as surrogate biomarker of in vivo antimicrobial efficacy: in vitro simulations with bacteria and yeasts

IRENE ZAGHI^{1,2}, MARTINA BRANDOLINI^{1,2}, ANNA MARZUCCO¹, SOFIA MONTANARI¹, GIULIA GATTI¹, FRANCESCA TADDEI¹, CLAUDIA COLOSIMO¹, MONICA CRICCA^{1,2}, VITTORIO SAMBRI^{1,2}, JASON ROBERTS³, RUSSELL E. LEWIS⁴

¹ Laboratorio Unico della AUSL della Romagna, in Pievesestina di Cesena, Italy;

² Department of Medical and Surgical Sciences, University of Bologna, Italy;

³ The University of Queensland Centre for Clinical Research, Brisbane, Queensland, Australia;

⁴ Department of Molecular Medicine, University of Padua, Padua, Italy

Introduction

Time-to-positivity is a commonly reported index in bloodculture systems that can be adapted to measure bactericidal activity in the patient's bloodstream during antibiotic therapy. We

examined how simulated serum concentrations of various antibiotics alone, or in combination, impacted Tpos measured against test inocula of KPC and NDM-

carbapenemase producing *K. pneumoniae* (KP). Some preliminary experiments were also carried out with representative strains of *Candida glabrata*.

Methods

Experiments were carried out with two KPC-2 and one NDM-2 carbapenemase-producing

KP isolate. We first analyzed how Tpos changes with inoculum (3×10^1 - 3×10^8 CFU/ml) prepared in standard BacT/ALERT aerobic bloodculture bottles without antibiotic inactivating matrix. We then inoculated 1 mL of human serum containing antibiotic concentrations/combinations simulating a range of expected human exposures into bottles containing a fixed inoculum (1×10^4 CFU/mL) of test isolates. The relationship of Tpos vs. antibiotic concentrations was fitted to 4-

parameter logistic regression model to estimate EC50/EC90. For combinations, a predicted null-response (Bliss-independence) interaction model was compared to Tpos observed with the antibiotic combination array to identify synergistic or antagonistic interactions.

Results

In the absence of antibiotic-containing serum, all isolates demonstrated a linear relationship between Tpos of 9.4-10.8h to 4.5-5.2h over an inoculum range spanning 3×10^1 to 3×10^8 CFU/mL. A consistent dose-response relationship between Tpos and serum antibiotic exposures was observed for all antibiotics with the EC50 Tpos of ~16 hr evident at 1xMIC. Combination experiments revealed prolongation of Tpos for most antibiotic combinations with the greatest improvement in Tpos observed with CZA + ATM against the NDM-producing KP. For the experiments with yeasts, in the absence of antibiotic-containing serum, a linear relationship was observed between Tpos of 6-6.2h to 19-22h over an inoculum range spanning 3×10^2 to 3×10^8 CFU/mL. A consistent dose-response relationship between Tpos and serum exposures was observed for fluconazole versus both strains with the EC50 Tpos of ~36 hr evident at 1xMIC.

Conclusions

Tpos was a robust and reproducible indicator of antimicrobial activity in serum for both bacteria and yeasts, that is capable of detecting synergistic antibiotic interactions. Further test optimization could support the use of this simple monitoring approach of antimicrobial and antifungal activity in patient serum.

126 - In vitro activity of cefiderocol, alone and in combination with imipenem or bacteriophages, against biofilm-embedded *Pseudomonas aeruginosa*

CATERINA FERRETTI⁽¹⁾ - MARIANO BERNARDO⁽²⁾ - CLAUDIA CAMPOBASSO⁽¹⁾ - SARA BOLOGNINI⁽¹⁾ - LAURA RINDI⁽³⁾ - ARIANNA TAVANTI⁽¹⁾ - CARLO TASCINI⁽⁴⁾ - MARIAGRAZIA DI LUCA⁽¹⁾

Università di Pisa, Dipartimento di Biologia, Pisa, Italia⁽¹⁾ - AORN Ospedali dei Colli-Monaldi, Unità di Microbiologia, Napoli, Italia⁽²⁾ - Università di Pisa, Dipartimento di Ricerca Traslationale e delle Nuove Tecnologie in Medicina e Chirurgia, Pisa, Italia⁽³⁾ - Università di Udine, Dipartimento di Medicina, Udine, Italia⁽⁴⁾

In vitro activity of cefiderocol, alone and in combination with imipenem or bacteriophages, against biofilm-embedded *Pseudomonas aeruginosa*

CATERINA FERRETTI¹, MARIANO BERNARDO², CLAUDIA CAMPOBASSO^{1,3}, SARA BOLOGNINI¹, LAURA RINDI⁴, ARIANNA TAVANTI¹, CARLO TASCINI⁵, MARIAGRAZIA DI LUCA¹

¹Department of Biology, University of Pisa, Pisa, Italy; ²Microbiology Unit, AORN Ospedali dei Colli-Monaldi Hospital, Naples, Italy; ³Department of Biosystems, KU Leuven, Leuven, Belgium;

⁴Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa, Pisa, Italy; ⁵Department of Medicine, University of Udine, Udine, Italy

Introduction

The increased use of implantable medical devices resulted in a higher incidence of biofilm-related infections, in which bacteria, including *Pseudomonas aeruginosa*, grow in an aggregate form embedded in a self-produced extracellular matrix. Given the emergence of the high tolerance to antibiotics of biofilm-embedded cells, the development of novel effective antibacterial strategies is needed. The aim of this study was to evaluate the antibiofilm activity of cefiderocol, a novel siderophore cephalosporin, alone and in combination with either imipenem or bacteriophages versus sessile *P. aeruginosa* isolates.

Materials and Methods

Ten *P. aeruginosa* clinical isolates from infected implants and bloodstream were included in the study. Cefiderocol was tested alone and in combination with either imipenem or phages on 24 hour-old *P. aeruginosa* biofilm formed on porous glass beads. For each antibiotic/phage formulation minimum bactericidal biofilm concentration (MBBC), defined as the lowest concentration that determined a reduction of at least 3-log₁₀ CFU/mL in comparison to the untreated control, was evaluated. Scanning electron microscopy (SEM) was used to investigate the colonisation of *P. aeruginosa* on beads treated and untreated with either cefiderocol, imipenem or their combination.

Result

The MBBCs of cefiderocol and imipenem tested alone versus *P. aeruginosa* biofilms ranged from 2 to >512 µg/mL and from 8 to 512 µg/mL, respectively. The combination of the two drugs achieved MBBC values lower of at least 2-fold serial dilutions with respect to the MBBCs of antibiotics alone,

suggesting a synergistic bactericidal activity between cefiderocol and imipenem. The activity of the lytic phage 2L Pa3iii in combination with cefiderocol also determined a synergistic bactericidal effect against one selected strain isolated from an infected thoracic prosthesis. Tested phage titers were not able to reduce the bacterial concentration, but through the combination of a selected phage (2L Pa3iii) with cefiderocol, it was possible to achieve a synergistic bactericidal effect. SEM analysis of *P. aeruginosa* biofilm showed differences in the shape of bacterial cells and in the amount of biofilm matrix in drug-treated samples compared to the untreated control.

Discussion and Conclusions

Cefiderocol exhibited an encouraging antimicrobial activity against tested strains, resulting as a valid option for the treatment of *P. aeruginosa* biofilm-associated infections, in particular when administered in combination with imipenem. The bactericidal effect of cefiderocol and phage 2L Pa3iii in combination suggests the possibility of using this phage as adjuvant in the antibiotic treatment of biofilm-related infections, preventing the selection of cefiderocol resistant strains.

127 - K. pneumoniae-KPC: Molecular tools to improve epidemiological surveillance of resistance genes.

CLAUDIA CICINO⁽¹⁾ - GRAZIA PAVIA⁽¹⁾ - GIUSEPPE GUIDO MARIA SCARLATA⁽¹⁾ - LICIA ELVIRA PRESTAGIACOMO⁽¹⁾ - LUIGIA GALLO⁽¹⁾ - CAROLINA DI CELLO⁽¹⁾ - VALENTINA ESPOSITO⁽¹⁾ - NADIA MARASCIO⁽¹⁾ - GIOVANNI MATERA⁽¹⁾ - ANGELA QUIRINO⁽¹⁾

Università degli Studi "Magna Graecia" di Catanzaro, Azienda Ospedaliero-Universitaria "R. Dulbecco" Presidio "Mater Domini", Catanzaro, Dipartimento Scienze della Salute, Catanzaro, Italia⁽¹⁾

K. pneumoniae-KPC: Molecular tools to improve epidemiological surveillance of resistance genes.

CLAUDIA CICINO¹, GRAZIA PAVIA¹, GIUSEPPE G.M. SCARLATA¹, LICIA E. PRESTAGIACOMO¹, LUIGIA GALLO¹, CAROLINA DI CELLO¹, VALENTINA ESPOSITO¹, NADIA MARASCIO¹, GIOVANNI MATERA¹ AND ANGELA QUIRINO¹.

¹Department of Health Sciences, Institute of Clinical Microbiology, "Magna Graecia" University of Catanzaro, Italy.

Introduction: An emerging clinical problem is related to the isolation of carbapenemase-producing *Klebsiella pneumoniae* (KPC). To date, this pathogen, is a therapeutic problem especially in the intensive care unit. The emergence of resistance to ceftazidime-avibactam has also been reported in recent years, and it is important to investigate which genes are involved in the mechanisms of resistance due to mutations in bla_{KPC} in *K. pneumoniae*. The gene encoded by *K. pneumoniae* (bla_{KPC}) is a gene carried on a plasmid of the Enterobacteriaceae family. This study aims to detect the presence of the bla_{KPC} gene and to evaluate the circulation of different sequencing typing by molecular characterization of ceftazidime-avibactam-resistant *K. pneumoniae*. Materials and Methods: *K. pneumoniae* ceftazidime-avibactam resistant clinical isolates were collected from different clinical blood cultures and respiratory samples. Antimicrobial sensitivity test was carried out by VITEK-2 system and by broth microdilution method. Bacterial DNA extraction from pure culture was performed using QIAamp® DNA Mini Kit (Qiagen) followed by polymerase chain reaction (PCR) amplification. Genomic regions were sequenced by Sanger method (ABI PRISM 3500 Genetic Analyzer). Sequences were edited by SeqScape Software Version 2.5 (Applied Biosystems), then compared to the reference sequence available in the public database using the Basic Local Alignment Search Tool nucleotide (BLASTn) algorithm. Results: The results of this study showed that all explored isolates were resistant to Meropenem and Imipenem (100%). Phenotypically, all the isolates had carbapenemase which hydrolyzed the carbapenem antibiotics. However, the most effective antibiotic was Tigecycline (90%) and 40% of isolate showed resistance to colistin. Clinical Carbapenem-Resistant-*K. pneumoniae* (CR-KP) isolates are mainly attributed to the bla_{KPC-3} gene carried on plasmids. The results of conventional PCR technique for the detection of bla_{KPC} gene showed that 70% of isolates of carbapenem-resistant *K. pneumoniae* harboured bla_{KPC} gene KPC3 and 30% bla_{KPC} gene KPC55 while none carried other carbapenemase genes including bla_{NDM1}, bla_{VIM} and bla_{IMP} genes. Molecular analysis showed only two circulating Sequencing typing: ST307 and ST512. Discussions and Conclusions. The ST307 is one of the epidemiologically successful clones of *K. pneumoniae*, associated with multidrug resistance and nosocomial outbreaks worldwide. Our results underline the importance of continuous surveillance of carbapenem-resistant Enterobacterales in health facilities. Novel STs carrying resistance, support the importance of

implementation bacterial genomics in clinical practice to improve traditional methods and overcome their limited resolution.

142 - Linezolid-induced mitochondrial damage impairs wound closure after MRSA infection

GIANLUCA MORRONI⁽¹⁾ - CATERINA LICINI⁽²⁾ - GUENDALINA LUCARINI⁽²⁾ - GLORIA D'ACHILLE⁽¹⁾ - FIORENZA ORLANDO⁽³⁾ - MAURO PROVINCIALI⁽³⁾ - SIMONA FIORITI⁽¹⁾ - ILARIA NUNZI⁽²⁾ - ANDREA GIACOMETTI⁽¹⁾ - ORIANA SIMONETTI⁽²⁾ - OSCAR CIRIONI⁽¹⁾ - SAVERIO MARCHI⁽²⁾

Università Politecnica delle Marche, Dipartimento di Scienze Biomediche e Sanità Pubblica, Ancona, Italia⁽¹⁾ - Università Politecnica delle Marche, Dipartimento di Scienze Cliniche e Molecolari, Ancona, Italia⁽²⁾ - IRCCS - INRCA, Experimental Animal Models for Aging Unit, Ancona, Italia⁽³⁾

Linezolid-induced mitochondrial damage impairs wound closure after MRSA infection

GIANLUCA MORRONI¹, CATERINA LICINI², GUENDALINA LUCARINI², GLORIA D'ACHILLE¹, FIORENZA ORLANDO³, MAURO PROVINCIALI³, SIMONA FIORITI⁴, ILARIA NUNZI², ANDREA GIACOMETTI⁴, ORIANA SIMONETTI⁵, OSCAR CIRIONI⁴, SAVERIO MARCHI²

1 Dipartimento di Scienze Biomediche e Sanità Pubblica, sezione Microbiologia, Università Politecnica delle Marche, Ancona, Italia; 2 Dipartimento di Scienze Cliniche e Molecolari, sezione Istologia, Università Politecnica delle Marche, Ancona, Italia; 3 Experimental Animal Models for Aging Unit, IRCCS INRCA, Ancona, Italia; 4 Dipartimento di Scienze Biomediche e Sanità Pubblica, sezione Malattie Infettive, Università Politecnica delle Marche, Ancona, Italia; 5 Dipartimento di Scienze Cliniche e Molecolari, sezione Dermatologia, Università Politecnica delle Marche, Ancona, Italia

Introduction: Linezolid (LZD) is an effective antibiotic approved for skin and skin structure infections and widely used against Gram-positive bacteria. Despite its microbiological activity, LZD displays some side effects related to its interference with the eukaryotic mitochondria. In this study, we found that LZD treatment after methicillin-resistant *Staphylococcus aureus* (MRSA) infection in delays wound closure and increases inflammation through mitochondrial damage and consequent aberrant release of interleukin 1 beta (IL-1B).

Materials and Methods: LZD treatment was compared to vancomycin in a wound infection In vivo mouse protocol using BALB/c mice and MRSA ATCC4300. Immunohistochemistry was performed to assess markers related to healing and inflammation. Simultaneously, in vitro experiments on HaCaT and nHEK cells infected with MRSA ATCC 43300 were established to identify the inflammasome activation mediated by infection or LZD treatment. Isolation of mitochondria and assessment of mitochondrial perturbations (respiratory capacity, morphological modifications, changes in membrane potential, production of reactive oxygen species, and mitochondrial DNA release) were performed to evaluate the influence of MRSA infection and LZD administration.

Results: Despite a good reduction in microbial burden compared to vancomycin, LZD delayed wound closure, reduced the vascular endothelial growth factor level, and promotes inflammation through the production of metalloproteinase and IL-1B. Caspase 1, a precursor of IL-1B production by NLRP3 inflammasome, was detected both in MRSA-infected mice and in LZD-treated mice. Inhibition of NLRP3 blocked the production of IL-1B and restored wound healing in LZD-treated mice. LZD alone was not able to induce IL-1B production, which was consistently produced after MRSA infection and sustained by LZD treatment. LZD induced mitochondrial damage and increased the NLRP3 mitochondrial localization.

Discussion and Conclusions: LZD-treated wounds showed decreased healing with a consistent upregulation of IL-1B. The inflammation was mediated by NLRP3 activation, which required a “priming” stimulation by MRSA infection and was sustained by LZD administration. MRSA damage included mitochondrial remodeling and perturbation, with the activation of NLRP3 inflammasome through ROS production. Conversely, LZD activates NLRP3 by a ROS-independent non-canonical pathway that requires further investigation.

152 - Molecular epidemiology of carbapenemase-producing Enterobacteriaceae in the Piacenza area

MELISSA SPALLA⁽¹⁾ - FRANCESCA PISCOPIELLO⁽¹⁾ - AURORA PIAZZA⁽¹⁾ - GABRIELLA TOCCI⁽²⁾ - ANDREA ZAPPAVIGNA⁽²⁾ - VALENTINA LEPERA⁽²⁾ - VITTORIA MATTIONI MARCHETTI⁽¹⁾ - GIULIANA LO CASCIO⁽²⁾ - ROBERTA MIGLIAVACCA⁽¹⁾

Università di Pavia, Dipartimento di Scienze Clinico Chirurgiche Diagnostiche e Pediatriche, Pavia, Italia⁽¹⁾ - AUSL “Guglielmo da Saliceto” ospedale di Piacenza, Dipartimento di Patologia Clinica, Unità di Microbiologia, Piacenza, Italia⁽²⁾

Molecular epidemiology of carbapenemase-producing Enterobacteriaceae in the Piacenza area

Melissa Spalla¹, Francesca Piscopiello¹, Aurora Piazza¹, Gabriella Tocci², Andrea Zappavigna², Valentina Lepera², Vittoria Mattioni Marchetti¹, Giuliana Lo Cascio², Roberta Migliavacca¹

¹University of Pavia, Dep. SCCDP, Microbiology and Clinical Microbiology Unit, Pavia (PV);

²AUSL “Guglielmo da Saliceto” hospital of Piacenza, Dep. Clinical Pathology, Microbiology Unit, Piacenza (PC)

Introduction: The dissemination of MDR and carbapenemase-producing Enterobacteriaceae (CPE) is emerging in acute care and territorial facilities. Aim of the study was the molecular characterisation of CPE isolates collected from seven facilities (acute/long-term care) in the Piacenza area. **Materials and methods:** From January 2020 to June 2021, 31 CPE from 20 patients have been isolated. Identification and antibiotic susceptibility profiles were obtained by MicroscanAutoscan4. The characterisation of carbapenems/aminoglycosides/fluoroquinolones resistance genes was performed by PCR and sequencing. The molecular typing of *Klebsiella pneumoniae* (KP) and *Escherichia coli* (EC) isolates was accomplished by MLST, PFGE, and EC phylogroup determination. **Results:** Of the 31 CPE collected, 12 were from 10 patients admitted to the Piacenza hospital, and 19 from rehabilitation facilities/long-term care. Twenty-three/31 (74.1%) isolates were from rectal swabs, 8/31 (25.9%) from urine (4/8), 1/8 blood, and 1/8 sputum. The CPE identified were: 17/31 (54.8%) *Klebsiella* spp. (15/17 KP and 2/17 *K. oxytoca*), 8/31 (26%) EC, 4/31 (12.9%) *Enterobacter* spp. (3/4 *E. cloacae* and 1/4 *E. hormaechei*), 1/31 (3%) *Serratia marcescens* and 1/31 (3%) *Citrobacter freundii*. Susceptibility to amikacin, gentamicin, colistin and chloramphenicol was maintained in 50% of the strains. All CPE showed resistance to amoxicillin/clavulanate, piperacillin/tazobactam, and tetracycline, >80% to aztreonam, cephalosporins, carbapenems; >50% to tobramycin, trimethoprim/sulphamethoxazole and fluoroquinolones. In 22/23 cases the *aac(6')-Ib-cr* gene was present, in 4/23 in association with *qnrS*; the latter identified in three NDM-producing isolates. *blaKPC-3/2* genes were detected in 11 isolates each. This latter variant was *blaVIM-1* co-present in 5/22 (*C. freundii*, *E. cloacae* (n=2), *E. hormaechei*, *S. marcescens*). KP, *K. oxytoca*, *E. cloacae* harboured *blaVIM-1*. Five *blaNDM-5* genes have been detected in EC. *blaOXA-48* (n=2) was detected in EC and KP, in this latter in association with a *blaCTX-M-15*. PFGE showed KP multi-clonality (clones A-F), regardless of carbapenemase type (*blaKPC-2-3/OXA-48*). The most represented lineage was B (8/15 KP; ST554), spread inter-hospitally from November 2020 to June 2021. Two isolates were of clones A (ST395) and F (ST2355) each; clones C (ST661), D (ST45) and E (ST323) were sporadic. Five pulsotypes were detected for EC (A-E): A (n=3; phylogroup F; ST648), B (n=2; phylogroup B2;

ST131), and the singletons C (phylogroup A; ST1125), D (phylogroup D; ST405), and E (phylogroup D; ST69). Discussion and conclusions: Although KPC is still prevalent in the Piacenza area, we report an increase in the presence of NDM/VIM/OXA-48 CPE.

153 - Next Generation Sequencing assay for HIV genotyping and resistance testing: the sicilian experience

CELESTINO BONURA ⁽¹⁾ - CHIARA MASCARELLA ⁽¹⁾ - SARA CANNELLA ⁽¹⁾ - GIUSEPPA SCIORTINO ⁽¹⁾ - GIOVANNI GIAMMANCO ⁽¹⁾

Università degli Studi di Palermo, Dipartimento di Promozione della Salute, Materno Infantile, Medicina Interna e Specialistica di eccellenza (PROMISE) “G. D’Alessandro”, Palermo, Italia ⁽¹⁾

Next Generation Sequencing assay for HIV genotyping and resistance testing: the sicilian experience

*Chiara Mascarella^{1,2}, *Sara Cannella^{1,2}, Celestino Bonura^{1,2}, Giuseppa Sciortino^{1,2} and Giovanni Giammanco^{1,2}, on behalf of the Sicilian GRT-HIV Network

*These authors contributed equally

¹Dipartimento di Promozione della Salute, Materno Infantile, Medicina Interna e Specialistica di eccellenza (PROMISE) “G. D’Alessandro” – Università degli Studi di Palermo

²Azienda Ospedaliera Universitaria Policlinico Paolo Giaccone di Palermo - U.O.C Microbiologia e Virologia

³The Sicilian GRT-HIV Network: Antonio Cascio (Azienda Ospedaliera Universitaria Policlinico “Paolo Giaccone” di Palermo); Chiara Iaria (ARNAS Ospedale Civico Di Cristina di Palermo); Pietro Colletti (Ospedale Paolo Borsellino di Marsala, TP); Giovanni Mazzola (Presidio Ospedaliero Sant’Elia, Caltanissetta); Maria Antonietta Di Rosolini (Ospedale Giovanni Paolo II, Ragusa); Antonella Franco (Presidio Ospedaliero Umberto I, Siracusa); Rosa Boncoraglio (Ospedale Maggiore di Modica, RG); Luigi Guarneri (Ospedale Umberto I di Enna); Rosa Fontana Del Vecchio (Ospedale Umberto I di Enna); Giovanni Francesco Pellicanò (Università degli Studi di Messina, AOU Policlinico “G. Martino” di Messina); Antonio Albanese (Azienda Ospedaliera Papardo di Messina)

Introduction

Next-Generation Sequencing (NGS) technology has revealed the way forward for Genotypic Resistance Test (GRT) in HIV to be performed at the time of diagnosis of infection before the start of antiretroviral therapy (ART) in naïve patients or to check for resistance-associated mutations upon treatment failure.

Materials and Methods

Genotyping and drug resistance testing was performed by using the HIV-1 Solution V2 Kit (Arrow diagnostics) with the MiSeq Illumina NGS platform on 198 patients admitted from March 2021 to December 2022 to twelve acute general hospitals in Sicily, Italy. Before genotyping, routine viral load assessment was performed for each plasma sample by the dual-target COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test (v.2.0, Roche Diagnostics S.p.A., Monza, Italy).

Results

All samples passed the quality control of the HIV-1 Solution V2 Kit (Arrow diagnostics), but 16/198 samples had viral loads <1000 cp/mL and have been excluded from the study following the recommendations of Arrow Diagnostics.

39/182 sample shared at least one resistance associated mutation when the 20% variant frequency was used as a threshold. In particular: 16 samples were resistant to NRTI drugs, 21 to NNRTIs, 4 to PIs and 10 to INSTIs. Furthermore, an increase in the number of samples sharing resistance associated mutations was observed when the 5% variant frequency was used as a threshold: 26 samples to NRTIs, 30 to NNRTIs, 4 samples to PIs and 32 to INSTIs. The most common mutations detected within the tested samples were: M184I/V in NRTIs (with an increase of M41L, L210V, T215D when the 5% variant frequency was used as a threshold); K103T/N in NNRTIs; Q148H/K/R in INSTIs (with an increase in S147G and Y143S/C/H when the 5% variant frequency was used as a threshold). No single mutation conferring resistance to PIs has been observed in any of the tested samples.

In addition, it is important to emphasize that 36% (14) of the patients showing a resistant genotype were newly diagnosed and had never been treated before, 51% were under treatment (20); while for about 13% (5) of the patients this information was missing.

Discussion and Conclusion

The evaluation of the genotyping and drug resistance testing data obtained with the highly sensitive NGS technology allows to guide the choice of the most effective antiretroviral therapy, with a consequent impact on the maintenance of viraemia levels below the detection thresholds (undetected) in newly diagnosed patients and in patients under ART treatment and with beneficial effects on the reduction of new infections.

155 - ST2502 KPC-Klebsiella pneumoniae and OXA-23-Acinetobacter baumannii from LTCFs in Piacenza area: results of a Point Prevalence Study of colonization

GABRIELLA TOCCI⁽¹⁾ - FRANCESCA PISCOPIELLO⁽²⁾ - MELISSA SPALLA⁽²⁾ - ROBERTA SCHIAVO⁽¹⁾ - MARIA G. SILVOTTI⁽³⁾ - FRANCO FEDERICI⁽³⁾ - AURORA PIAZZA⁽²⁾ - GIULIANA LO CASCIO⁽¹⁾ - ROBERTA MIGLIAVACCA⁽²⁾

AUSL “Guglielmo da Saliceto” ospedale di Piacenza, Dipartimento di Patologia Clinica, Unità di Microbiologia, Piacenza, Italia⁽¹⁾ - Università di Pavia, Dipartimento di Scienze Clinico Chirurgiche Diagnostiche e Pediatriche, Pavia, Italia⁽²⁾ - AUSL “Guglielmo da Saliceto” ospedale di Piacenza, Dipartimento di Igiene, Piacenza, Italia⁽³⁾

ST2502 KPC-Klebsiella pneumoniae and OXA-23-Acinetobacter baumannii from LTCFs in Piacenza area: results of a Point Prevalence Study of colonization

Gabriella Tocci¹, Francesca Piscopiello², Melissa Spalla², Roberta Schiavo¹, Maria G. Silvotti³, Franco Federici³, Aurora Piazza², Giuliana Lo Cascio¹, Roberta Migliavacca²

AUSL “Guglielmo da Saliceto” hospital of Piacenza, Dep. Clinical Pathology, Microbiology Unit, Piacenza (PC); Department of Clinical-Surgical, Diagnostic and Pediatric Sciences, Unit of Microbiology and Clinical Microbiology, University of Pavia, Pavia, Italy; AUSL of Piacenza, Hospital Hygiene Piacenza (PC)

Introduction: Infections due to MDR organisms (MDRO), may arise from a prior asymptomatic colonization. Such infections are associated with higher health-care costs, morbidity and mortality. Aim of the Point Prevalence Study (PPS) undertaken in 2022-23 at three LTCFs of Piacenza area was to determine the prevalence of the resident's colonization by Extended Spectrum Beta-Lactamases (ESBLs)/AmpC/carbapenemase-producing Enterobacterales, Carbapenem-Resistant Acinetobacter baumannii (CRAB) and Pseudomonas aeruginosa (CRPA), Meticillin-Resistant Staphylococcus aureus (MRSA) and Vancomycin-Resistant Enterococcus spp. (VRE). Materials and Methods: rectal, inguinal and nasal swabs were collected from 118 residents and plated on selective agar. MDRO resistance genes content was investigated using the 56 target HybriSpot system. MultiLocusSequence Typing (MLST) and PFGE were performed. Demographic and clinical data were collected. Results: The 2.5% (3/118) of the residents were infected on the day of PPS, 24.5% (29/118) were recently hospitalized and 37.2% (44/118) was subjected to antibiotic therapy (45.4%, cephalosporins; 29.5%, penicillins) during the last three months. Incontinence (76.2%), cardiovascular disease (50.8%), and reduced alertness (48.3%) were common risk-factors. Among LTCFs residents 71/118 were colonized by a MDRO: 90.1%, 38.0%, and 85.9% of the rectal, nasal and inguinal swabs were positive, respectively. The 57.6% of Enterobacterales were ESBL positive 8.5% CPE, 1.7% CRPA, 6.8% CRAB. 4.2%VRE (vanA) and 9.3% MRSA were detected. N=55 Escherichia coli (EC), n=31 Klebsiella pneumoniae (KP) and n=7 AB were further investigated. blaCTX-M was found in 53/55 (96.4%) EC and 25/31 (80.6%) KP. Seven out of 31 (22.6%) KP were blaKPC and oqxA/B positive, while all AB harboured blaOXA-23 and armA. ST2502, ST17, ST2648 and ST512 KP-KPC were detected. Discussion and conclusions: colonization with MDRO in subjects admitted to long-term institutions represent a serious concern with high risk of MDR infections and spread of emerging high-risk clones.

166 - Antibiotic resistance of *Streptococcus pneumoniae* causing invasive diseases during the 13-valent conjugate vaccine era in Italy.

SARA GIANCRISTOFARO (1) - FABIO D'AMBROSIO (1) - GIULIA ERRICO (1) - MICHELA PAGNOTTA (1) - MONICA MONACO (1) - MARIA DEL GROSSO (1) - ROMINA CAMILLI (1)

Istituto Superiore di Sanità, Malattie Infettive, Roma, Italia (1)

Antibiotic resistance of *Streptococcus pneumoniae* causing invasive diseases during the 13-valent conjugate vaccine era in Italy

SARA GIANCRISTOFARO, FABIO D'AMBROSIO, GIULIA ERRICO, MICHELA PAGNOTTA, MONICA MONACO, MARIA DEL GROSSO, ROMINA CAMILLI

Department of Infectious Diseases, Antibiotic-Resistance and Special Pathogens Unit, Istituto Superiore di Sanità, Rome, Italy

Introduction. Despite the efficacy of conjugate vaccines, *Streptococcus pneumoniae* remains a leading cause of morbidity and mortality worldwide and antibiotic resistance is a concern. Aim of this study was to describe antibiotic resistance trends in Invasive Pneumococcal Diseases (IPD) along ten years of 13-valent conjugate vaccine (PCV13) use in Italy.

Materials and Methods. All pneumococcal isolates received at the National Reference Laboratory from 2010 to 2019, in the framework of the nationwide surveillance of invasive bacterial diseases, were serotyped by the Quellung reaction and tested for antibiotic susceptibility to penicillin (PEN), ceftriaxone (TX), erythromycin (ERY), clindamycin (CLI), and tetracycline (TET), by gradient strip test following the EUCAST breakpoints. For PEN and TX, meningitis breakpoints were used. **Results.** Among a total of 1917 pneumococcal isolates examined, PEN-resistance (R) declined from 14.4% in 2010 to 10.47% in 2019, TX-R from 5.15% to 2.09%, ERY-R from 32.99% to 21.46%, CLI-R from 26.80% to 21.47%, and TET-R from 27.84% to 16.23%. In 2010, 85.72% of PEN-R was due to PCV13 serotypes, while it declined to 44.98% in 2019, showing persistence of serotypes 19F, 14, and 19A. Similarly, ERY-R due to PCV13 serotypes decreased from 81.23% in 2010 to 53.63% in 2019, with persistence of serotypes 3, 19A, 19F, and 14. Overall, among non-PCV13 serotypes, PEN-R was mainly driven by serotypes 15A, 23B, and 24F whereas ERY-R by serotypes 6C, 11A, 15A, 15B/C, 24F, and 33F. Multidrug resistance (MDR), defined as non-susceptibility to at least 3 antimicrobial classes, showed a decreasing trend from 26.8% in 2010 to 18.32% in 2019. MDR was due to the above-mentioned PCV13 serotypes and to non-PCV13 serotypes, e.g. 15A, 15B/C, 24F, and 33F.

Discussion and Conclusions. In Italy, the antibiotic resistance rate in IPD showed an overall downward trend from the early- to the late-PCV13 period as a result of a decrease of resistant isolates belonging to PCV13 serotypes, although vaccine serotypes 3, 14, 19A, and 19F still contribute to antibiotic resistance. Concurrently, in the latest years new emergent antibiotic resistant non-PCV13 serotypes began to spread such as serotypes 15A, 15B/C, 23B, 24F, and 33F, thus leading to a new alert. A continuous surveillance is warranted to monitor the evolution of IPD and antibiotic resistance in the next years also in view of the new upcoming higher-valent conjugate vaccines.

173 - Molecular investigation to monitor the spreading of fluoroquinolone and tetracycline resistance among *Campylobacter jejuni* clinical isolates

ALICE TOSADORI⁽¹⁾ - ANNARITA SORRENTINO⁽²⁾ - ELENA ADDIS⁽¹⁾ - MICHELE BORDINI⁽¹⁾ - DAVIDE GIBELLINI⁽¹⁾ - ANNARITA MAZZARIOL⁽¹⁾

Università di Verona, Dipartimento di Diagnostica e Sanità Pubblica, Verona, Italia⁽¹⁾ - Azienda Ospedaliera Universitaria Integrata Verona, UOC Microbiologia e Virologia, Verona, Italia⁽²⁾

Molecular investigation to monitor the spreading of fluoroquinolone and tetracycline resistance among *Campylobacter jejuni* clinical isolates

ALICE TOSADORI¹, ANNARITA SORRENTINO², ELENA ADDIS¹, MICHELE BORDINI¹, DAVIDE GIBELLINI¹, ANNARITA MAZZARIOL¹

¹Department of Diagnostics and Public Health, University of Verona, Verona, Italy

²UOC Microbiologia e Virologia, Azienda Ospedaliera Universitaria Integrata di Verona, Verona, Italy

Introduction. *Campylobacteriosis* continues to be a major public health problem. Center for Disease Control and Prevention (CDC) reports that *Campylobacter* is one of the most common cause of bacterial diarrheal illness worldwide: it is estimated 1.5 million illnesses each year in USA. CDC's measures to control the spread of antibiotic resistant *Campylobacter* include the tracking changes in antibiotic resistance among bacterial isolates. The aim of this study is to investigate the mechanisms of fluoroquinolone and tetracycline resistance in 45 *Campylobacter jejuni* selected resistant strains isolated at the University Hospital of Verona.

Materials and Methods. *C. jejuni* isolation was carried out streaking the fecal swab (Copan, Italy) on CAMPY agar. After incubation, the plates were examined for characteristic morphology and the identification was carried out by MALDI-TOF with VITEK MS (BioMérieux, France). Antimicrobial susceptibility testing was performed by E-test. 45 *C. jejuni* strains ciprofloxacin resistant and/or tetracycline resistant, were chosen for the study. *GyrA* quinolone resistance determining region (QRDR) were amplified by PCR and sequenced for mutations analysis, to determine the fluoroquinolone mechanism of resistance. Three efflux pumps genes, *tet(O)*, *tet(A)* and *tet(B)* were analyzed through PCR amplification in order to evaluate the tetracycline mechanism of resistance.

Results. All 45-selected *C. jejuni* were ciprofloxacin resistant and 28 out 45 resulted tetracycline resistant so 62.2% of strains could be defined as multidrug resistant. Analysis of the *gyrA* sequence data permits the determination of the presence or absence of amino acid mutations in the QRDR. 39/45 ciprofloxacin-resistant strains showed a substitution at the amino acid position 86 of the *gyrA* protein due to mutation of the DNA codon from ACA (threonine) to ATA (isoleucine). Silent mutation was noted at amino acid position 81 in 30/45 ciprofloxacin-resistant isolates and at amino acid position 92 in 2/45. Six ciprofloxacin resistant strains did not show QRDR mutation and need further investigation. 20 out of 28 tetracycline-resistant isolates were positive for *tet(O)* gene. No *tet(A)* or *tet(B)* gene were detected. Eight strains need further investigation.

Discussion and conclusions. Fluoroquinolones and tetracycline are considered as wide-use drugs for campylobacteriosis treatment. The T86I mutation is the main mechanism of *C. jejuni* ciprofloxacin resistance in our strains while the presence of Tet(O) gene is the main mechanism involved in the tetracycline resistance. Monitoring *Campylobacter* spp mechanism of resistance is of great importance in order to tackle antimicrobial resistance and understand the epidemiology of antimicrobial resistance gene.

176 - *Clostridioides difficile* over the years: evaluation of resistance profile in clinical isolates at Padua University Hospital

SHIRIN ASA'AD ⁽¹⁾ - GIULIA BERNABÈ ⁽¹⁾ - IOANNIS BEKAS ⁽¹⁾ - CLAUDIA DEL VECCHIO ⁽¹⁾ - IGNAZIO CASTAGLIUOLO ⁽¹⁾ - VALERIA BESUTTI ⁽²⁾

Università di Padova, Dipartimento di Medicina Molecolare, Padova, Italia ⁽¹⁾ - Unità di Microbiologia, Ospedale Università di Padova, Padova, Italia ⁽²⁾

Clostridioides difficile over the years: evaluation of resistance profile in clinical isolates at Padua University Hospital

SHIRIN ASA'AD 1, GIULIA BERNABÈ 1, IOANNIS BEKAS 1, CLAUDIA DEL VECCHIO 1;2, IGNAZIO CASTAGLIUOLO 1;2, VALERIA BESUTTI 2

1 Department of Molecular Medicine, University of Padua, Padua, Italy; 2 Microbiology Unit of Padua University Hospital, Padua, Italy

Introduction

Clostridioides difficile, a spore-forming anaerobe bacterium, is responsible for infections in hospitalized patients treated with broad-spectrum antibiotics. The incidence of *C. difficile* associated diarrhea (CDAD) has showed a worldwide significant increase in recent decades. Since pathogens' antibiotic resistance is rapidly changing under the selective pressure of therapies, we evaluated whether the antibiotic susceptibility profile of *C. difficile* strains circulating at Padua University Hospital has modified over a 13-years period.

Materials and methods

C. difficile strains were isolated from fresh stool samples of CDAD patients between 2010 and 2018 and between January and May 2023 at the Microbiology Unit of Padua University Hospital. Isolates were identified by MALDI-TOF analysis (99.9% confidence). Susceptibility to vancomycin, clindamycin, moxifloxacin, metronidazole, ertapenem, imipenem, meropenem, piperacillin/tazobactam, amoxicillin/clavulanic acid, ampicillin and penicillin was determined by broth microdilution method with a commercial kit (MICRONAUTS-S Anaerobes MIC, Bruker). Resistances were confirmed with the E-test method. Moreover, fidaxomicin sensitivity was tested by agar diffusion method (20 micrograms/disk). CLSI and EUCAST Breakpoint were used to define susceptibility/resistance profile.

Results

Twentythree *C. difficile* strains from 2010-2018 and 30 strains from 2023 were analyzed. All strains resulted susceptible to amoxicillin/clavulanic acid, ertapenem, meropenem, piperacillin/tazobactam, metronidazole and vancomycin in both groups. Resistance rate significantly decreased for moxifloxacin (60.9% vs 16.7%), while resistance to ampicillin and penicillin significantly increased (0% vs 6.7%, 8.7% vs 23.3% respectively) over the 13-years period. Resistance incidence to clindamycin remained stable (13% vs 13.3%) whereas slightly decreased for imipenem (13% vs 10%). Fidaxomicin was tested on 20 strains from the 2010-2018 group, and on 25 strains from 2023. The inhibition zones ranged between 15 mm and 25 mm in the 2010-2018 strains, whereas in the 2023 group inhibition zone varied between 15 and 31 mm (20.60±0.56 versus 21.28±0.75, respectively, $p = n.s.$).

Discussion and Conclusions

Our data show a modification in the sensitivity/resistance profile in *C. difficile* clinical isolates at Padua University Hospital, although strains remain sensitive to metronidazole and vancomycin. No changes in fidaxomicin sensitivity were detected between the two groups. A comparison method with agar dilution (the gold standard method) is in progress to correlated inhibition zone with fidaxomicin MIC. These data support the need of continuous monitoring of antibiotic susceptibility for this nosocomial acquired pathogen.

182 - In vitro antimicrobial susceptibility testing and biofilm characterization of *Borrelia burgdorferi* sensu lato isolated from patients with erythema migrans

GIORGIA FABRIZIO⁽¹⁾ - ILARIA CAVALLO⁽¹⁾ - FRANCESCA SIVORI⁽¹⁾ - MARTINA PONTONE⁽¹⁾ - MAURO TRUGLIO⁽¹⁾ - FULVIA PIMPINELLI⁽¹⁾ - ENEA GINO DI DOMENICO⁽²⁾

Microbiology and Virology, San Gallicano Dermatological Institute, IRCCS, ROMA, Italia⁽¹⁾ - Department of Biology and Biotechnology "C. Darwin", Sapienza University of Rome, ROMA, Italia⁽²⁾

In vitro antimicrobial susceptibility testing and biofilm characterization of *Borrelia burgdorferi* sensu lato isolated from patients with erythema migrans

Giorgia FABRIZIO¹, Iliaria CAVALLO¹, Francesca SIVORI¹, Martina PONTONE¹, Mauro TRUGLIO¹, Fulvia PIMPINELLI¹, Enea G. DI DOMENICO²

¹ Microbiology and Virology, San Gallicano Dermatological Institute, IRCCS, Rome, Italy;

² Department of Biology and Biotechnology "C. Darwin" Sapienza University of Rome, 00185 Rome, Italy.

Introduction. A skin lesion known as erythema migrans is often the earliest sign of Lyme borreliosis (LB) observed in more than 70% of early cases. *Borrelia afzelii* and *B. garinii* are the most common cause of LB in Europe. The early treatment with oral antimicrobials is effective in the majority of patients with LB. Nevertheless, persistent forms are reported and the reoccurrence of LB can be associated with biofilm-like aggregates.

Materials and methods. We evaluated the efficacy of 4 different antibiotics in planktonic and biofilm-growing *Borrelia* isolates. In particular, broth microdilution assay was performed to determine minimum inhibitory concentration (MIC) of amoxicillin, azithromycin, ceftriaxone and doxycycline against 8 strains of *B. afzelii* and 4 strains of *B. garinii*, from skin culture biopsies. Biofilm was characterized by measuring biomass, curli amyloid fibers, extracellular DNA (eDNA) production and by confocal microscopy analysis.

Results. Our results showed that *B. afzelii* and *B. garinii* isolates exhibited comparable antibiotic susceptibility profiles. Indeed, MIC for β -lactam antibiotics (amoxicillin, and ceftriaxone) was 0.25 μ g/mL (0.032-0.5 μ g/mL) for both *B. afzelii* and *B. garinii*. MIC for azithromycin was 0.125 μ g/mL (0.032-0.125 μ g/mL) and 0.125 μ g/mL (0.032-0.25 μ g/mL) for *B. afzelii* and *B. garinii* respectively. Lastly, MIC for doxycycline was 0.5 μ g/mL (0.1–1 μ g/mL) and 0.5 μ g/mL (0.1–1 μ g/mL) for *B. afzelii* and *B. garinii* respectively. Quantitative analysis demonstrated that *B. afzelii* produced a higher level of biomass ($p=0.003$) compared with *B. garinii*, and that biofilm structure were composed by amyloid fibers and eDNA. Specifically, biomass was significantly ($p=0.003$) correlated with eDNA production in *Borrelia* isolates. Whereas, an inverse correlation ($p=0.035$) was observed between biomass production and the presence of amyloid fibers suggesting that curli are probably produced only during the initial stage of attachment and of biofilm formation.

Confocal microscopy analysis of 7-day old biofilms demonstrated the presence of thick and structurally complex biofilms of approximately 20 μ m in height, with a marked predominance of live spirochetes.

Discussion and Conclusions. Clinical isolates of *B. afzelii* and *B. garinii* are highly susceptible to amoxicillin azithromycin, ceftriaxone and doxycycline, in vitro. However, substantial evidence

suggests that *B. afzelii* and *B. garinii* are capable to form biofilm-like aggregates in vitro largely composed of eDNA and curli amyloid fibers.

183 - Prevalence and drug resistance for invasive candidiasis in Pediatric and adult Hospitalized Patients

SILVIA MENEGHELLO ⁽¹⁾ - GIUSEPPE DI PIETRA ⁽¹⁾ - IGNAZIO CASTAGLIUOLO ⁽¹⁾ -
PAOLA BRUN ⁽²⁾

Ospedale di Padova, Unità di Microbiologia, Padova, Italia ⁽¹⁾ - Università di Padova, Dip Medicina Molecolare, Padova, Italia ⁽²⁾

Prevalence and drug resistance for invasive candidiasis in Pediatric and adult Hospitalized Patients

Silvia Meneghello, Giuseppe Di Pietra, Ignazio Castagliuolo, Paola Brun

Invasive candidiasis (IC) are life-threatening complications in hospitalized patients. Clinical practice guideline recommends primary antifungal prophylaxis for pediatric patients with cancer or hematopoietic stem cell transplant recipients. Systemic antifungal prophylaxis can be an effective approach to reduce invasive mycotic infections even if benefits and risks, such as antimycotic resistance onset, need to be considered according to the patient's clinical condition and the prevalent microorganisms in the clinic. In this study, we aimed to identify causative yeasts or molds and their susceptibility to antimycotic drugs in hospitalized pediatric and adult patients admitted at the Padova University Hospital between January 2019 and December 2022.

The diagnosis of IC was defined on the basis of criteria concerning the basic clinical conditions of the patient (patient at risk), the clinical signs, and the evidence of microbiological tests. Normally, 2 or 3 sets of blood cultures were analyzed. When for the same patient, there were repeatedly positive cultures with the same strain within 10 days, the sample was considered as a single case. We identified the fungal strains by mass spectrometry (MALDI - TOFF) and their susceptibility to antifungal drugs was tested by the semi-automatic method of broth microdilution with the SensititreY010 kit. Resistance was defined using revised CLSI breakpoints/epidemiological cutoff values. Patient's clinical data were retrieved retrospectively from the hospital's digitalized information systems and microbiology laboratory records.

Totally 34 pediatric and 557 adult patients who had fungal positive emoculture were included in this study. In the adult population, the incidence of IC was higher in 2019 and has stabilized at slightly lower levels in the last three years of the study. On the contrary, in the pediatric population, the incidence of IC has been stable over the years but reduced in 2021, probably because COVID-19 pandemic. *Candida albicans*, *C. parapsilosis*, and *C. glabrata* were the predominant isolates both in adult and pediatric patients. *C. parapsilosis* reported a significant resistance to fluconazole with an increasing trend of multiresistance. At the moment of mycotic infections, adult patients were not hospitalized at the intensive care unit but children did.

Pediatric and adult patients admitted to the intensive care unit with catheters are particularly exposed to systemic mycotic infection by *C. parapsilosis* and might require specific primary antifungal prophylaxis recommendations.

185 - Insight into *Acinetobacter baumannii*'s phenotypic and genotypic traits in oncological patients

ILARIA CAVALLO ⁽¹⁾ - FRANCESCA SIVORI ⁽¹⁾ - MAURO TRUGLIO ⁽¹⁾ - REBECCA PAGES ⁽¹⁾ - FRANCESCO MARCHESI ⁽²⁾ - LUIGI TOMA ⁽³⁾ - ELVA ABRIL ⁽¹⁾ - ANDREA MENGARELLI ⁽²⁾ - FULVIA PIMPINELLI ⁽¹⁾ - ENEA GINO DI DOMENICO ⁽⁴⁾

Microbiology and Virology, San Gallicano Dermatological Institute, IRCCS, ROMA, Italia ⁽¹⁾ - Hematology and Stem Cell Transplant Unit, IRCCS Regina Elena National Cancer Institute, Roma, Italia ⁽²⁾ - Department of Research, Advanced Diagnostics, and Technological Innovation, Translational Research Area, IRCCS Regina Elena National Cancer Institute, Roma, Italia ⁽³⁾ - Department of Biology and Biotechnology, Sapienza University of Rome, ROMA, Italia ⁽⁴⁾

Insight into *Acinetobacter baumannii*'s phenotypic and genotypic traits in oncological patients

Ilaria CAVALLO^a, Francesca SIVORI^a, Mauro TRUGLIO^a, Rebecca PAGES^a, Francesco MARCHESI^b, Luigi TOMA^c, Elva ABRIL^a, Andrea MENGARELLI^b, Fulvia PIMPINELLI^a, Enea G. DI DOMENICO^c

^a Microbiology and Virology, San Gallicano Dermatological Institute, IRCCS, Rome, Italy;

^b Hematology and Stem Cell Transplant Unit, IRCCS Regina Elena National Cancer Institute, Rome, Italy; ^c Department of Research, Advanced Diagnostics, and Technological Innovation, Translational Research Area, IRCCS Regina Elena National Cancer Institute, Rome, Italy; ^d Scientific Direction, IRCCS San Gallicano Institute, Rome, Italy; ^e Department of Biology and Biotechnology "C. Darwin" Sapienza University of Rome, Rome, Italy.

Introduction. *Acinetobacter baumannii* is a leading nosocomial pathogen and a significant threat worldwide. The therapeutic options are limited due to high rates of antibiotic resistance. In addition, the ability to form biofilm allows *A. baumannii* to persist in the hospital environment, making treatment even more complicated. This study investigates the clinical impact of *A. baumannii* isolates in oncological patients.

Materials and Methods. Antibiotic resistance, a comprehensive bioinformatic analysis, and different virulence factors of 25 *A. baumannii* isolates were examined.

Results. The most common infectious manifestations were pneumonia (51.8%), followed by surgical site infection (29.6%) and sepsis (18.6%). Most of the isolates were resistant to fluoroquinolones (65%), aminoglycosides (50.5%), and carbapenems (48.6). In addition, many genes encoding virulence factors, such as the *adeF*, *bfmS*, and *bfmR*, and *bau* and *bas* genes involved in iron uptake, were highly prevalent in *A. baumannii* isolates. Moreover, genes responsible for fimbriae production (*csu*), early adhesion (*ompA*), and biofilm formation (*pgaB*, *pgaC*, *pgaD*, *ageG*, *adeH*, and *bap*) were reported in more than 95% of the strains. Sequence type (ST) 2 was the most prevalent (52%).

Accordingly, samples were divided into two subgroups: the dominant ST2 and the non-dominant ST (NST). ST2 was associated with high rates of antimicrobial resistance, low biomass production, and poor adhesion than the NST and *A. baumannii* ATCC 19606. The median MIC₉₀ values for these isolates were 0.5 µg/mL (0.25-32 µg/mL) for colistin and 1.0 µg/mL (0.125–4 µg/mL) for cefiderocol. In contrast, the minimal biofilm eradication concentration (MBEC₉₀) for cefiderocol and colistin was 2 µg/mL (0.25-64 µg/mL) and 2 µg/mL (0.25-128), respectively. The overall mortality rate was 32.0% (N=8). ST2 was associated with increased infection-related mortality. The lowest attributable 30-day mortality was found in patients infected with NST (18.2%), while the highest was found in those with ST2 (42.9%). These findings imply a 24.7% excess mortality risk in the presence of *A. baumannii* ST2 infections compared to NST subpopulation infections.

Discussion and Conclusions. This study provides significant information about the genetic determinants and virulence factors of *A. baumannii* isolates that could assist the development of strategies for improved control and treatment of these infections among oncological patients.

187 - Exploring the Interaction between Ceftobiprole and High Molecular Mass (HMM) PBPs

PAOLA CONTI⁽¹⁾ - LORENZO MATTIA LAZZARO⁽¹⁾ - FEDERICA LENZO⁽¹⁾ -
SEBASTIANO ALBERTO FORTUNA⁽¹⁾ - FLORIANA CAMPANILE⁽¹⁾

Università degli Studi di Catania, BIOMETEC, Catania, Italia⁽¹⁾

Exploring the Interaction between Ceftobiprole and High Molecular Mass (HMM) PBPs

PAOLA CONTI, LORENZO M. LAZZARO, FEDERICA LENZO, SEBASTIANO A. FORTUNA,
FLORIANA CAMPANILE.

Department of Biomedical and Biotechnological Sciences (BIOMETEC), University of Catania,
Catania, Italy.

Introduction Penicillin-resistance among *Enterococcus faecalis* has been associated with overexpression and aminoacidic substitutions in low-affinity PBP4. In *E. faecalis*, ceftobiprole (BPR) is frequently use as therapeutic option due to its potential higher affinity for PBP4, even if mutations in *pbp4* may interfere with BPR/PBP4 binding, leading to remarkable multi-drug-resistance phenotypes. This study aims to investigate the relationship between *pbp4* expression levels and the interactions of BPR and High-Molecular-Mass (HMM) low-reactive PBPs, with a specific focus on PBP4, in clinical isolates of Penicillin-Resistant-Ampicillin-Susceptible/ceftobiprole non susceptible (PRAS/BPR-NS) *E. faecalis*, leading to multi-drug resistant phenotypes. **Materials and methods** Class A and B HMM-PBPs of 7 previously characterized PRAS/BPR-NS *E. faecalis* strains were analyzed by competition assays, by using purified membrane proteins and fluorescent penicillin (Bocillin FL), in treated (1/2- 1- 2- 4-fold the BPR-MIC value) and untreated conditions. BPR/HMM PBPs interactions were analyzed by the determination of the 50% inhibitory concentration (IC₅₀) values for ceftobiprole, evaluating the intensity of fluorescence as indicator (decrease of fluorescence). **Results** Treatment with ceftobiprole resulted in a variable acylation of all PBPs, at diverse concentrations, except for PonA (PBP1a), PBP1b and PBP4. BPR had lower affinity for PBP4 in all isolates, demonstrating its leading role in beta-lactam resistance. Similar unresponsiveness was detected for PonA (PBP1a) in almost all strains, whereas PBP1b was not acylated in 3 strains. Sensitivity of the other PBPs to ceftobiprole was variable, with IC₅₀ values ranging from 1/2-fold to 4-fold MICs. In only one strains, no PBP was significantly acylated by BPR. Of note, in 2 strains all PBPs were strongly inhibited already at 1/2-fold the MIC concentration, except for PBP4, that remains the only PBP able to confer resistance to ceftobiprole. Unexpectedly, Efs7 did not show PBP1b. **Discussion and conclusion** Our results shift the paradigm for enterococcal cephalosporin resistance, by demonstrating that the PBP4 is necessary but not sufficient for BPR resistance. BPR exposure involves the cooperation between low-affinity class B PBP4 and at least one (PonA/PBP1a) or two (PBPF/2a) bifunctional class A PBPs. This cooperation could be necessary to synthesize peptidoglycan and promote growth.

223 - Impact of Interdisciplinary Antimicrobial Diagnostic Stewardship on Bloodstream infections and antibiotic resistance patterns.

FRANCESCO FOGLIA (1) - GIUSEPPE GRECO (2) - MARIA TERESA DELLA ROCCA (2) - COLOMBINA MELARDO (2) - MARIA VASCO (2) - ELENA GRIMALDI (2) - CONCETTA BENTIVOGLIO (2) - FORTUNATO MONTELLA (2) - VALERIA CRUDELE (2) - EMILIANA FINAMORE (2) - ANNA DE FILIPPIS (3) - MASSIMILIANO GALDIERO (1)

Dipartimento di Medicina Sperimentale, Università degli Studi della Campania "Luigi Vanvitelli", Sezione di Microbiologia e Microbiologia Clinica, Azienda Ospedaliera Universitaria "Luigi Vanvitelli" di Napoli, U.O.C. di Virologia e Microbiologia, Sant'Andrea delle Dame, 80138 Napoli, Italia., Napoli, Italia (1) - Azienda Ospedaliera Universitaria "Luigi Vanvitelli" di Napoli, U.O.C. di Virologia e Microbiologia, Sant'Andrea delle Dame, 80138 Napoli, Italia., Napoli, Italia (2) - Dipartimento di Medicina Sperimentale, Università degli Studi della Campania "Luigi Vanvitelli", Sezione di Microbiologia e Microbiologia Clinica, Via Santa Maria di Costantinopoli 16, 80138 Napoli, Italia., Napoli, Italia (3)

Impact of Interdisciplinary Antimicrobial Diagnostic Stewardship on Bloodstream infections and antibiotic resistance patterns.

Foglia Francesco^{1,2}, Giuseppe Greco², Maria Teresa Della Rocca², Colombina Melardo², Maria Vasco², Elena Grimaldi², Concetta Bentivoglio², Fortunato Montella², Valeria Crudele², Emiliana Finamore², Anna De Filippis¹, Massimiliano Galdiero^{1,2}

¹Dipartimento di Medicina Sperimentale, Università degli Studi della Campania "Luigi Vanvitelli", Sezione di Microbiologia e Microbiologia Clinica, Via Santa Maria di Costantinopoli 16, 80138 Napoli, Italia.

²Azienda Ospedaliera Universitaria "Luigi Vanvitelli" di Napoli, U.O.C. di Virologia e Microbiologia, Sant'Andrea delle Dame, 80138 Napoli, Italia.

Introduction: Bloodstream infections (BSI) are associated with high morbidity and mortality. This study aimed to describe the epidemiology of BSI and antimicrobial resistance patterns amongst its bacterial causes. Gram-positive bacteria were the most predominant pathogens followed by gram-negative bacteria. Among all isolates, bacterial pathogens most frequently identified included coagulase-negative Staphylococci (CoNS), *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and enterococci.

Materials and Methods: We conducted a record review of blood culture results of patients hospitalized at University Hospital 'L. Vanvitelli' from 2016 to 2022. For each patient records were obtained from the database using microbiological information. Gram-positive bacteria were the most predominant pathogens followed by Gram-negative bacteria.

Results: We observed a decrease in antimicrobial resistance amongst BSI pathogens in the latter years of the study. High levels of macrolide and aminoglycoside resistance amongst CoNS were reported. Carbapenem resistance rates amongst *K. pneumoniae* declined considerably between 2018 and 2022. The prevalence of methicillin-resistant *S. aureus* decreased during the study period while that of methicillin-resistant CoNS remained relatively high throughout. The prevalence of extended spectrum β -lactamase – producing *E. coli* increased considerably between 2016 and 2018 but showed

a decrease thereafter. There was a decline in the resistant rates of extended spectrum β -lactamase – producing *K. pneumoniae* between 2016 and 2018 with a similar trend being noted for carbapenem resistance in *K. Pneumoniae*.

Discussion and Conclusions: The declining antibiotic resistance trend in our hospital could be attributed to the effectiveness of the interdisciplinary antimicrobial diagnostic stewardship program in our facility and highlight the need to continue to improve these infection control strategies.

Antimicrobial diagnostic stewardship program was coordinated interventions designed to improve and measure the appropriate use of antimicrobials by promoting the selection of the optimal antimicrobial drug regimen, dose, duration of therapy, and route of administration. The results of implementing this program could improve the antibiotic prescribing behavior, reduce antibiotic use with consequent reduced costs, shorter hospitalizations and reduced spread of MDR strains.

252 - In vivo transfer of linezolid resistance among enterococci in *Mytilus galloprovincialis*

SERENA SIMONI⁽¹⁾ - CARLA VIGNAROLI⁽²⁾ - ANDREA BRENCIANI⁽³⁾ - ELEONORA GIOVANETTI⁽²⁾ - LUIGI VEZZULLI⁽⁴⁾ - ELISA TAVIANI⁽⁵⁾ - MANON AUGUSTE⁽⁵⁾ - ALESSANDRA DI GREGORIO⁽²⁾

UNIVERSITA POLITECNICA DELLE MARCHE, Department of Life and Environmental Sciences, ANCONA, Italia⁽¹⁾ - UNIVERSITA POLITECNICA DELLE MARCHE, DEPARTMENT OF LIFE AND ENVIRONMENTAL SCIENCES, ANCONA, Italia⁽²⁾ - UNIVERSITA POLITECNICA DELLE MARCHE, Department of Biomedical Sciences and Public Health, ANCONA, Italia⁽³⁾ - UNIVERSITA DI GENOVA, Department of Earth, Environment, and Life Sciences, ANCONA, Italia⁽⁴⁾ - UNIVERSITA DI GENOVA, Department of Earth, Environment, and Life Sciences, GENOVA, Italia⁽⁵⁾

In vivo transfer of linezolid resistance among enterococci in *Mytilus galloprovincialis*

SERENA SIMONI¹, ALESSANDRA DI GREGORIO¹, MANON AUGUSTE², ELISA TAVIANI², LUIGI VEZZULLI², ANDREA BRENCIANI³, ELEONORA GIOVANETTI², CARLA VIGNAROLI¹

¹Department of Life and Environmental Sciences, Polytechnic University of Marche, Ancona, Italy;

²DISTAV, Department of Earth, Environment, and Life Sciences, University of Genoa, Genoa, Italy;

³Department of Biomedical Sciences and Public Health, Polytechnic University of Marche, Ancona, Italy

Introduction. The exchange of genetic material, such as plasmids and mobile genetic elements, among bacteria plays a crucial role in the evolution and adaptation of microbial communities. Molluscs during their filter-feeding activity concentrate complex bacterial communities in their tissues where bacteria could interact each other creating opportunities for genetic exchange, contributing to bacterial genomes evolution, and spread of antimicrobial resistances. For this reason, in this study, we explored the potential of marine bivalve molluscs to serve as an ideal hot-spot for conjugation exploiting their ability to concentrate microorganisms from surrounding water. **Materials and Methods.** The laboratory strain *Enterococcus faecium* 64/3 and the marine sediment isolate *E. faecium* ES2 were used in mating experiments. *E. faecium* 64/3 (recipient) was rifampin and fusidic acid resistant while *E. faecium* ES2 (donor) was rifampin and fusidic acid susceptible but linezolid resistant due to a ~30-kb plasmid carrying the *poxtA* linezolid resistance gene. In vitro conjugations were performed by the filter-mating method and by resuspending strains in haemolymph serum (HS) and haemolymph extracts. In the in vivo experiments donor and recipient were inoculated simultaneously (1:1 ratio) into aquaria containing 6 *Mytilus galloprovincialis* individuals. The transconjugants were periodically detected in mussels and in water samples by plating on selective agar and subsequently phenotypically, and genotypically, analysed to confirm plasmid acquiring.

Results. *E. faecium* ES2 was able to transfer linezolid resistance to *E. faecium* 64/3 both in in vitro and in vivo experiments with frequencies of $\sim 10^{-1}$ and $\sim 10^{-7}$, respectively. In vivo, the highest conjugation frequency was obtained at a water temperature of 20°C, while no conjugation occurs at lower temperatures. The transconjugants were obtained within the first 24h, both from the digestive gland and the haemolymph, at similar frequencies ($\sim 10^{-7}$); no conjugation seemed to have occurred in the gills. Transconjugants were obtained also from water but at a lower frequency ($\sim 10^{-8}$). *E. faecium* ES2 was able to transfer linezolid resistance in vitro even using HS and haemolymph but at a lower frequency ($\sim 10^{-8}$) compared to in vivo haemolymph.

Discussion and Conclusions. In this study we demonstrated that mussels could serve as an effective niche for promoting genetic exchange between antibiotic-resistant bacteria. Mussels might contribute to the dissemination of antibiotic resistance genes in aquatic environments, as well as to the reintroduction of resistant bacteria into humans through their consumption.

254 - Evaluation of Ceftazidime/Avibactam resistance in clinical isolates of Enterobacterales from a large tertiary-care hospital, Italy, 2019-2023

LUCA CALABRESE⁽¹⁾ - EDWARD WILLISON⁽¹⁾ - GIULIA CODDA⁽¹⁾ - ELISA COSTA⁽¹⁾ - VINCENZO DI PILATO⁽¹⁾ - ANNA MARCHESE⁽¹⁾

DISC, Università di Genova, Genova, Italia⁽¹⁾

Evaluation of Ceftazidime/Avibactam resistance in clinical isolates of Enterobacterales from a large tertiary-care hospital, Italy, 2019-2023

LUCA CALABRESE^a, EDWARD WILLISON^a, GIULIA CODDA^a, ELISA COSTA^a, VINCENZO DI PILATO^a, ANNA MARCHESE^a

^aDISC, Microbiology Unit, University of Genoa, Genoa, Italy

Introduction

Over the past decades, carbapenemase-resistant Enterobacterales (CRE), notably KPC-producing *K. pneumoniae*, have spread in health care facilities and have become a serious global threat. Among the new β -lactam/ β -lactamase-inhibitors combinations, ceftazidime-avibactam (CZA) was the first approved for the treatment of infections caused KPC-producing strains. Despite its recent commercialization, an increasing number of cases of CZA-resistant (CZAr) CRE have been reported. The aim of this study is to investigate the molecular bases of CZA resistance in a collection of CRE of clinical origin collected at the San Martino Hospital (Genoa) from 2019 to 2023.

Materials and methods

Identification and antibiotic susceptibility testing were performed with Vitek MS MALDI-TOF and VITEK2 system, respectively. Carbapenemases production (KPC, VIM, NDM and OXA-48 like enzymes) was assessed with immunochromatographic assays and qualitative RT-PCR. Resistance to CZA and meropenem (MEM) was confirmed by reference broth microdilution method and MICs were interpreted according to EUCAST v13.0 clinical breakpoints. The *bla*_{KPC} gene copy-number was determined by relative RT-PCR, and putative novel gene variants were investigated by Sanger sequencing of PCR products.

Results

We studied 71 CZAr isolates (31 *K. pneumoniae*, 21 *E. cloacae* cpx, 2 *S. marcescens*, 5 *K. oxytoca*, 4 *E. coli* e 8 *C. freundii*), of which 66% (47/71) tested positive for VIM, 20% (14/71) for KPC, 8% (6/71) for KPC and VIM and 6% (4/71) for NDM. In CZAr-KPC producing strains (13 *K. pneumoniae*, 1 *C. freundii*), MIC values ranged from 16 to 128 mg/L for CZA and from 0,5 to 512 mg/L for MEM. Among these, 71% (10/14 *K. pneumoniae*) were resistant to MEM, while 29% (4/14) (3 *K. pneumoniae*, 1 *C. freundii*) tested susceptible. According to RT-PCR, 64% (9/14) of KPC-producing isolates showed an increased *bla*_{KPC} copy number, while 21% (3/14) produced mutated KPC enzymes (KPC-49, KPC-66, KPC-109). In a single case, it was not possible to determine the *bla*_{KPC} allelic variant.

Discussion

Overall, our results show that: i) CZAr was largely associated with production of metallo- β -lactamase (mostly of VIM type); ii) among KPC-producing strains, CZAr was primarily associated with an increased *bla*_{KPC} copy number, leading to overproduction of KPC, and to emergence of novel KPC

variants, to a lesser extent. Present results highlight that overproduction of KPC could represent an important cause of CZAr. This genotype circumvents rapid molecular diagnostic tests, jeopardizing the inference of the putative resistance phenotype and application of appropriate antibiotic therapy.

262 - ESKAPE pathogens in the wounds of stray dogs and cats and their antimicrobial resistance profile

FRANCESCA PIZZANO ⁽¹⁾ - ANGELO MASULLO ⁽¹⁾ - CRISTINA DI PALMA ⁽¹⁾ - SILVIA CAPPIELLO ⁽²⁾ - MARINA POMPAMEO ⁽²⁾ - BARBARA LAMAGNA ⁽¹⁾ - LUISA DE MARTINO ⁽¹⁾ - FRANCESCA PAOLA NOCERA ⁽¹⁾

Università degli Studi di Napoli "Federico II", Dipartimento di Medicina Veterinaria e Produzioni Animali, Napoli, Italia ⁽¹⁾ - ASL Napoli 1, Ospedale Veterinario Frullone, Napoli, Italia ⁽²⁾

ESKAPE pathogens in the wounds of stray dogs and cats and their antimicrobial resistance profile
FRANCESCA PIZZANO¹, ANGELO MASULLO¹, CRISTINA DI PALMA¹, SILVIA CAPPIELLO², MARINA POMPAMEO², BARBARA LAMAGNA¹, LUISA DE MARTINO^{1,3}
AND FRANCESCA PAOLA NOCERA¹

¹Department of Veterinary Medicine and Animal Production, University of Naples Federico II, Via F. Delpino 1, 80137 Naples, Italy; ²Veterinary Hospital, ASL Napoli 1 Centro, via M. Rocco di Torrepadula 13, 80145 Naples, Italy; ³Task Force on Microbiome Studies, University of Naples "Federico II", Naples, Italy.

Introduction The ESKAPE group of pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species) represents the leading cause of nosocomial infections in human all over the world, and all the six ESKAPE pathogens cause infections in animals. Contaminated and infected wounds occur very frequently in veterinary medicine, especially for the stray animals. There are few reports concerning the risks related to infections with ESKAPE bacteria in the stray dogs and cats presenting traumatic or polytraumatic injuries. The purpose of this report was to describe the ESKAPE bacteria associated with the site of traumatic wounds in stray dogs and cats. **Materials and Methods** Wounds swabs were collected from stray dogs and cats presenting to Veterinary Hospital (ASL Napoli 1) during the period between May 2022 - May 2023. Precisely, swabs, performed on injured skin, were first incubated in enrichment broth (BHI) for 24 hours at 37°C and then plated using different culture agar media for the isolation of Gram-positive and Gram-negative bacteria. Then, the identification of species was carried out by proteomic analysis (MALDI TOF MS). The antimicrobial susceptibility testing, by using 18 and 17 antibiotics, for Gram-positive and Gram-negative bacteria, respectively, was performed. **Results** Out of 29 wound samples, (12 coming from stray dogs and 17 from stray cats), n.9 *S. aureus*; n.1 *K. pneumoniae*; n.2 *P. aeruginosa*; and n.2 *Enterobacter cloacae* were isolated. *S. aureus* was the most frequently identified ESKAPE bacterium in wounds of stray cats, with 7 strains isolated (70%), while *S. aureus* and *P. aeruginosa* were found with same frequency from wounds of stray dogs (50%). All the isolated strains showed phenotypic resistance to penicillin 14/14 (100%), 12/14 (85.7%) to amoxicillin + clavulanic acid, while 88% of Gram-positive isolates showing resistance to cefoxitin. For the remaining antimicrobial classes, the strains generally presented a similar antimicrobial resistance pattern. Furthermore, 9 out of 14 clones (64.3%) were multidrug resistant (MDR) bacteria. **Discussion and Conclusions** The acquisition of antimicrobial resistance genes by ESKAPE pathogens has reduced the treatment options for serious infections and requires a careful and coordinated global response for antimicrobial resistance surveillance. Antimicrobial-resistant

ESKAPE pathogens represent a global threat to human health which, and as is well known, is closely linked to animal and environmental health. Our results show that stray dogs and cats are potential reservoirs of antimicrobial resistant ESKAPE bacteria and that they could be involved in the dissemination of those bacteria in the environment, with resulting public health concerns. This work will help raise awareness of the potential zoonotic risks posed by involving stray dogs and cats in the environment.

263 - Strains Of Staphylococcus Epidermidis Isolated In The Milking Parlor Of Buffalo Farms In The Campania Region

ANGELO MASULLO (1) - FRANCESCA PAOLA NOCERA (1) - VALENTINA IOVANE (2) -
FRANCESCA PIZZANO (1) - LUISA DE MARTINO (1)

Università degli Studi di Napoli "Federico II", Dipartimento di Medicina Veterinaria e Produzioni
Animali, Napoli, Italia (1) - Università degli Studi di Napoli "Federico II", Dipartimento di Agraria,
Portici (Napoli), Italia (2)

STRAINS OF STAPHYLOCOCCUS EPIDERMIDIS ISOLATED IN THE MILKING PARLOR OF BUFFALO FARMS IN THE CAMPANIA REGION

ANGELO MASULLO¹, FRANCESCA PAOLA NOCERA¹, VALENTINA IOVANE²,
FRANCESCA PIZZANO¹, LUISA DE MARTINO^{1,3}

¹Department of Veterinary Medicine and Animal Production, University of Naples "Federico II",
Naples, Italy; ²Department of Agricultural Sciences, University of Naples Federico II, 80055
Naples, Italy; ³Task Force on Microbiome Studies, University of Naples "Federico II", Naples,
Italy.

Introduction Contamination of the milking parlor environment with multi-resistant (MDR) *Staphylococcus* spp. increases the risk of infection. Antibiotic resistant staphylococci can affect humans, animals and many natural environments, and this represents a major public health problem. *Staphylococcus epidermidis*, an opportunistic pathogen, is the most clinically significant of the coagulase-negative staphylococci and may prevent the colonization of several pathogenic microorganisms such as the coagulase-positive *Staphylococcus aureus*. The aim of this study is to identify the MDR *Staphylococcus epidermidis* isolated in buffalo milking parlor from different types of samples. **Materials and methods** Eight Mediterranean buffalo farms have been identified within the Salerno district, and they were characterized by approximately 200 animals in each one. A total of 240 samples were analysed and included surface, animals, milk and farm staff specimens. In the period December 2022 - April 2023, each farm was visited three times for collecting samples of bulk milk, nipples swabs, surface samples (milking parlor equipment, wall and floor of parlor), and nasal swabs of farm staff. Samples were streaked onto Mannitol Salt Agar (Oxoid, Italy) and bacterial identification was performed by Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF-MS) considering Brukers' cut-off value for reliability (LogScore > 1.80). A panel of 15 antimicrobials representing different classes was selected, all of them with a background history of antimicrobial resistance in the *Staphylococcus* genus, to define the phenotypic characterization of *S. epidermidis* strains isolated. **Results** The occurrence of *S. epidermidis* in milk of the Italian buffalo farms was not detected as well as in nipples swabs, whereas a strain was recovered from 144 surface swabs (0.7%), and 41 *S. epidermidis* strains were obtained from 48 nasal swabs (85.4%) of the farm staff. The isolated strains that showed phenotypic resistance to oxacillin/cefoxitin/penicillin were 27/42 (64%), while those showing phenotypic resistance to cefoxitin/penicillin were 10/42 (23.8%) and those with penicillin resistance 4/42 (9.5%). Only one strain, the one isolated from the surfaces, showed susceptibility to oxacillin/cefoxitin/penicillin. **Discussion and conclusion** It is known that CLSI recommends usage of cefoxitin when using the disk diffusion method to determine resistance against methicillin mediated by the *mecA* gene, therefore,

we suppose that 37/42 (88.1%) are *mecA*-positive *S. epidermidis* isolates which we will confirm, in subsequent studies, using the gold standard *mecA* PCR. The isolated clones generally presented a similar antimicrobial resistance pattern, and more than 69% of them were MDR demonstrating the importance of the farm staff as reservoirs. Thus, the results obtained indicate that MDR *S. epidermidis* strains are relatively widespread in farm workers but not in milk, teats or parlor surfaces. The microbiological surveillance of the milking parlor is the keystone to ensure the safety of both operators and animals and keeps the possibility of biological risk under control.

270 - Antimicrobial activity of gemini quaternary ammonium compounds against multidrug-resistant bacteria isolated from central venous catheters.

MARIANGELA PIZZO ⁽¹⁾ - ANTONIO PALUMBO PICCIONELLO ⁽²⁾ - SARA AMATA ⁽²⁾ - RITA IMMORDINO ⁽³⁾ - CARLA RIZZO ⁽²⁾ - IVANA PIBIRI ⁽²⁾ - GIUSEPPA SCIORTINO ⁽³⁾ - GIOVANNI M. GIAMMANCO ⁽¹⁾ - CINZIA CALÀ ⁽¹⁾

Università degli studi di Palermo, Promozione della Salute, Materno-Infantile, di Medicina Interna e Specialistica di Eccellenza "G. D'Alessandro" (PROMISE), Palermo, Italia ⁽¹⁾ - Università degli studi di Palermo, Dipartimento di Scienze e Tecnologie Biologiche Chimiche e Farmaceutiche (STEBICEF), Palermo, Italia ⁽²⁾ - A.O.U.P. "Paolo Giaccone", Palermo, U.O.C di Microbiologia, Virologia e Parassitologia, Palermo, Italia ⁽³⁾

Antimicrobial activity of gemini quaternary ammonium compounds against multidrug-resistant bacteria isolated from central venous catheters.

MARIANGELA PIZZO*, ANTONIO PALUMBO PICCIONELLO**, SARA AMATA**, RITA IMMORDINO***, CARLA RIZZO**, IVANA PIBIRI**, GIUSEPPA SCIORTINO***, GIOVANNI M. GIAMMANCO*, CINZIA CALÀ*

*Department of Health Promotion, Mother and Child Care, Internal Medicine and Medical Specialties (PROMISE), University of Palermo

** Department of Biological, Chemical and Pharmaceutical Sciences and Technologies (STEBICEF), University of Palermo

*** U.O.C. of Microbiology, Virology and Parassitology, A.O.U.P. "Paolo Giaccone", Palermo, Italy

Introduction

The emergence of resistance to multiple antimicrobial agents in pathogenic bacteria today represents a significant threat to public health. Unfortunately, there are fewer and fewer antimicrobial agents still effective for infections caused by these bacteria. In addition, antimicrobial resistance is often associated with the ability of bacteria to form biofilms on the surfaces of medical devices. Therefore, new antimicrobial agents are needed to keep pace with and counteract microbial resistance. Quaternary ammonium compounds are frequently used as antibacterial agents that disrupts cell membranes through the binding of their ammonium cations to anionic sites in the outer layer of bacteria. To assess the in vitro antimicrobial activities of two di-cationic salts as NV1582 and NV2542, characterized by two pyridine heads connected through the same 1,2,4-oxadiazole spacer and differing only for the length of the long alkyl side chains, we tested clinical isolates from central venous catheters exhibiting antimicrobial resistance.

Materials and Methods

The antibacterial activity of NV1582 and NV2542 were evaluated in vitro against reference strains of *S. aureus* (ATCC 26923) and *E. coli* (ATCC 25922) and ten clinical strains isolated from central venous catheters, including seven Gram-negative bacteria (5 *Klebsiella pneumoniae*, 1 *Acinetobacter baumannii*, 1 *Pseudomonas aeruginosa*) and three methicillin resistant *Staphylococcus aureus* (MRSA). Minimum Inhibitory Concentrations (MICs) were defined by broth microdilution method, according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines. The inhibition of biofilm formation was assessed using crystal violet method and spectrophotometer biomass quantification (at a wavelength of 540 nm).

Results

Both biocides produced a MIC of 8 mg/liter in two *K. pneumoniae*, in *A. baumannii* and *P. aeruginosa* and a MIC of 16 mg/liter in the remaining *K. pneumoniae* strain. For the three MRSA, NV1582 and NV2542 MICs were 1mg/liter in two strains, but 2 mg/liter in the third. Both reference strains had the lowest MIC levels among the strains tested for the two di-cationic salts. All clinical strains were biofilm producers and biofilm inhibition was obtained with sub-MIC concentrations of the compounds.

Discussion and Conclusions

Increasing emergence of antimicrobial resistance affects both Gram-positive and Gram-negative bacteria and is often related to infections by biofilm-producer strains. New synthetic compounds may represent an interesting option to contain the phenomenon. In our preliminary study two quaternary ammonium compounds, NV1582 and NV2542, showed both antimicrobial activity against clinical and reference strains and ability to inhibit biofilm.

274 - Molnupiravir imprinting of SARS-CoV-2 genome: a case-control study

EMANUELA GIOMBINI ⁽¹⁾ - FABIO TUCCI ⁽¹⁾ - MARTINA RUECA ⁽¹⁾ - LAVINIA FABENI ⁽¹⁾ - CESARE GRUBER ⁽¹⁾ - VALENTINA MAZZOTTA ⁽²⁾ - GIULIA GRAMIGNA ⁽¹⁾ - ALESSANDRA VERGORI ⁽²⁾ - ORNELLA BUTERA ⁽³⁾ - PIETRO SPEZIA ⁽⁴⁾ - DANIELE FOCOSI ⁽⁵⁾ - EMANUELE NICASTRI ⁽²⁾ - ENRICO GIRARDI ⁽⁶⁾ - FRANCESCO VAIA ⁽⁷⁾ - ANDREA ANTINORI ⁽²⁾ - FABRIZIO MAGGI ⁽¹⁾

INMI Lazzaro Spallanzani IRCCS, Laboratorio di Virologia, Roma, Italia ⁽¹⁾ - INMI Lazzaro Spallanzani IRCCS, Clinical and Research Infectious Diseases Department, Roma, Italia ⁽²⁾ - INMI Lazzaro Spallanzani IRCCS, Laboratorio di Microbiologia, Roma, Italia ⁽³⁾ - University of Pisa, Department of Translational Research, Retrovirus Center, Pisa, Italia ⁽⁴⁾ - Pisa University Hospital, North-Western Tuscany Blood Bank, Pisa, Italia ⁽⁵⁾ - INMI Lazzaro Spallanzani IRCCS, Direzione Scientifica, Roma, Italia ⁽⁶⁾ - INMI Lazzaro Spallanzani IRCCS, Direzione Generale, Roma, Italia ⁽⁷⁾

Molnupiravir imprinting of SARS-CoV-2 genome: a case-control study

Emanuela Giombini¹, Fabio G. Tucci¹, Martina Rueca¹, Lavinia Fabeni¹, Cesare E.M. Gruber¹, Valentina Mazzotta², Giulia Gramigna¹, Alessandra Vergori², Ornella Butera³, Piergiorgio Spezia⁴, Daniele Focosi⁵, Emanuele Nicastrì², Enrico Girardi⁶, Francesco Vaia⁷, Andrea Antinori², Fabrizio Maggi¹

¹Laboratory of Virology, National Institute for Infectious Diseases “L. Spallanzani” - IRCCS, Rome, Italy

²Clinical and Research Infectious Diseases Department, National Institute for Infectious Diseases “L. Spallanzani” - IRCCS, Rome, Italy

³Laboratory of Microbiology, National Institute for Infectious Diseases “L. Spallanzani” - IRCCS, Rome, Italy

⁴Department of Translational Research, Retrovirus Center - University of Pisa, Pisa, Italy

⁵North-Western Tuscany Blood Bank, Pisa University Hospital, Pisa, Italy

⁶Scientific Direction, National Institute for Infectious Diseases “L. Spallanzani” - IRCCS, Rome, Italy

⁷General Direction, National Institute for Infectious Diseases “L. Spallanzani” - IRCCS, Rome, Italy

Abstract

1. Introduction

Molnupiravir is one of the oral direct-acting antivirals against SARS-CoV-2, largely deployed during the COVID-19 pandemic since the 2022 Omicron wave. While efficacy has been evaluated in post-marketing clinical trials, recently growing concerns have mounted regarding its mutagenicity for humans and the virus itself. In fact, during the trials, it was observed that the host viral load was cleared by the drug, or that the generated variants had mutations that were not fit enough to survive. However, in some countries, after the introduction of molnupiravir treatment, the emergence of several lineages with a high transition/transversion ratio, a characteristic signature of molnupiravir action, has been observed. Here, we assess the drug action using whole viral genome sequencing (WGS) on 38 COVID-19 outpatients receiving molnupiravir monotherapy and their follow-up after 7 days.

2. Materials and Methods

We retrospectively reviewed 38 immunocompetent patients at high risk for disease progression who received intramuscular molnupiravir as early COVID-19 treatment and who presented a prolonged high viral load. The prescription of drugs was based on clinical judgment. None of them had received molnupiravir for PrEP, nor had they received previous or concurrent direct acting antivirals. Nasopharyngeal swabs (NPS) were collected at baseline (T0) and at day 7 after treatment (T7). WGS was performed using the Ion Torrent Gene Studio S5 Prime platform, and complete SARS-CoV-2 genomes and quasispecies analysis were analyzed with ESCA software for a deep investigation of mutation frequencies in Spike protein before and during treatment. Moreover, to better characterize the mutational signature of molnupiravir treatment, we also compare SARS-CoV-2 quasispecies characterization in 17 Evusheld-treated patients.

3. Results

All patients had Ct<27 at T0 and showed a decrease in viral load at T7, except in two patients in whom the Ct decreased from 18.1 to 17.0 and from 17.0 to 9.0, respectively. Mutation analysis revealed that after 7 days of treatment, a higher transition ratio and G>A ratio was observed in all molnupiravir subjects compared to the Evusheld-treated patients. Comparing intra-host diversity and complexity before and after therapy in the two groups, we observed that the molnupiravir administration increased the variability compared to control samples.

4. Discussion and Conclusions

Our study confirms that during molnupiravir treatment, a significant increase of mutations is generated in outpatients. Therefore, in light of this mechanism of action, monitoring of SARS-CoV-2 genome sequence variation could be advisable during the administration of molnupiravir, where authorized, in both immunocompetent and immunocompromised patients.

281 - Drugs porins-permeability coefficient correlate with the antimicrobial effectiveness

SILVIA PUXEDDU ⁽¹⁾ - SARA BOI ⁽²⁾ - M. ANDREA SCORCIAPINO ⁽²⁾ - MATTEO CECCARELLI ⁽³⁾ - STEFAN MILENKOVIC ⁽³⁾ - ALDO MANZIN ⁽¹⁾ - FABRIZIO ANGIUS ⁽¹⁾

Università degli studi di Cagliari, Dipartimento di Scienze Biomediche, Cagliari, Italia ⁽¹⁾ -
Università degli studi di Cagliari, Dipartimenti di Scienze Chimiche e Geologiche, Cagliari, Italia ⁽²⁾
- Università degli studi di Cagliari, Dipartimento di fisica, Cagliari, Italia ⁽³⁾

Drugs porins-permeability coefficient correlate with the antimicrobial effectiveness

SILVIA PUXEDDU ¹, SARA BOI ², M. ANDREA SCORCIAPINO ², MATTEO CECCARELLI ³,
STEFAN MILENKOVIC ³, ALDO MANZIN ¹, FABRIZIO ANGIUS ¹

¹ Department of Biomedical Sciences, Unit of Microbiology and Virology, University of Cagliari, Cagliari, Italy

² Department of Chemical and Geological Sciences, University of Cagliari, Cagliari, Italy

³ Department of Physics, University of Cagliari, Cagliari, Italy

Introduction

Development of antibiotics against Gram-negative bacteria requires further optimization compared to other drugs due to the presence of the outer membrane. The porin channels control permeability to small water-soluble molecules as several antibiotics and inhibitors. No matter how potent a drug is against the isolated target, its effectiveness will be low if it fails to reach it inside the cell. Nevertheless, permeation is overlooked in drug development campaigns. Here we focused on the OmpF (one of the most expressed porins in Enterobacteriaceae) permeability to antibiotics from three different classes, by performing a correlation study of their relative permeability coefficient (RPC) with their effectiveness in the biologic system.

Materials and methods

Liposomes swelling assays were used to obtain the RPC. Wild-type Escherichia coli (BL21) and a mutant expressing only OmpF have been challenged with selected drugs to determine the minimum inhibitory (MIC) and the non-inhibitory (NIC) concentrations. Chemoinformatics tools were used in combination with molecular dynamics simulations to characterize the drugs in-silico. The first version of our predictive function (PF) has been used to predict the RPC. The PF considers the chemico-physical features of the drug and its interactions with the characteristics of the channel: cross-section area, electrostatic potential and electric field. In addition, also a new parametrization of the formula has been tested.

Results

Selected drugs among carbapenem and sulfonamide showed a good correlation between liposome swelling and microbiological data. In particular, our data indicated that the higher the RPC, the lower the MIC/NIC, both in the WT and OmpF mutant. However, cephalosporins did not show correlation with the same OmpF mutant, likely due to its ampicillin-resistance mediated by the plasmid for OmpF expression. On the other hand, the BL21 WT confirmed the correlation observed for the other classes. Depending on the specific molecular class, the first FP correlation with

experimental permeability data is either good or rather poor, while a generalized improvement was observed with the new parametrization.

Discussion and conclusion

The goal of the project is the PoC of the PF for two main applications: i) comparison of different analogues of a lead antibiotic to guide the optimization phase of drug development; ii) identification of non-antibiotic molecular classes with good permeation to be used as vectors to facilitate the absorption by bacteria of potent drugs affected by low permeation. The results obtained so far are encouraging and further development of the FP is possible by including additional terms to the function itself, and by including more experimental data in the parametrization process.

283 - Sulfonamide treatments inhibit gamma-Herpesvirus persistent infection in vivo

FABRIZIO ANGIUS ⁽¹⁾ - TANIA RODRIGUES ⁽²⁾ - SILVIA PUXEDDU ⁽¹⁾ - ILENIA DELOGU ⁽¹⁾
- RAFFAELLO POMPEI ⁽¹⁾ - J. PEDRO SIMAS ⁽²⁾ - ALDO MANZIN ⁽¹⁾

Università di Cagliari, Dipartimento di Scienze Biomediche, Cagliari, Italia ⁽¹⁾ - Universidade de Lisboa, Instituto de Medicina Molecular, Faculdade de Medicina, Lisbona, Portugal ⁽²⁾

Sulfonamide treatments inhibit gamma-Herpesvirus persistent infection in vivo

FABRIZIO ANGIUS ¹, TÂNIA RODRIGUES ², SILVIA PUXEDDU ¹, ILENIA DELOGU ¹,
RAFFAELLO POMPEI ¹, J. PEDRO SIMAS ^{2,3}, ALDO MANZIN ¹

¹ Department of Biomedical Sciences, Unit of Microbiology and Virology, University of Cagliari, Cagliari, Italy;

² Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Lisboa, Portugal;

³ Católica Biomedical Research and Católica Medical School, Universidade Católica Portuguesa, Lisboa, Portugal.

Introduction

The Kaposi's sarcoma-associated Herpesvirus (KSHV), a gamma-herpesvirus etiologically associated to Kaposi's sarcoma (KS), is also correlated to lympho-proliferative diseases such as primary effusion lymphoma and multicentric Castleman's disease. KSHV shows specific tropism for endothelial and B-cells, and is highly oncogenic in immunosuppressed adults and AIDS patients. To date, KS is treated as a malignant tumor with conventional chemotherapeutic agents that often control disease progression, but do not cure KSHV infection. KSHV establishes latent infection in permissive cells and persists as a nuclear episome through the action of latency factor (LANA) that ensures viral genome persistency for the host's lifespan. The latent state allows the virus to survive and control cellular pathways in order to promote its persistence. This causes important alterations that may lead to disease, which suggests that viral persistence is the main cause of tumor recurrence/progression. We recently found that sulfonamide antibiotics have a strong and specific antiviral activity on KSHV latent infection in vitro. In fact, they are able to disengage the KSHV genome from cellular DNA, likely by interfering with the binding of LANA that normally guarantees viral genome segregation to daughter nuclei. Here we assessed a panel of in vitro effective compounds as suppressors of the KSHV-latency in infected cells to validate their efficacy in vivo.

Materials and methods

We use a small animal model of gamma-herpesvirus latent infection based on a chimeric MuHV4-kLANA recombinant virus, which is genetically related to KSHV encoding for the human kLANA in place of the murine mLANA. This recombinant virus establishes LANA-dependent latent infection in mice, allowing assessment of the efficacy of several compounds in clearing viral persistency in vivo.

Results

We corroborated our previous findings that some of the most common sulfonamide-derivative drugs possess antiviral activity against latently KSHV-infected BC3 cells. New evidences showed that

sulfonamide treatments decreased viral latent load in vivo compared to untreated controls, supporting the hypothesis that these compounds are potentially able to clear latent virus.

Discussion and conclusion

So far, no drugs have been found able to clear KSHV infection in vivo. Our findings indicate that the widely used sulfonamide antibiotics are effective in decreasing gamma-herpesvirus latent infection in vivo, hence being potentially useful in preventing disease in immunosuppressed patients. Our study reveals a new potential target and treatment to tackle or prevent diseases related to KSHV-latency by the eradication of the virus.

297 - Antiviral activity of three residues peptides against SARS-CoV-2 Infection

GIANLUIGI FRANCI ⁽¹⁾ - AVINASH MALI ⁽²⁾ - CARLA ZANNELLA ⁽²⁾ - ANNALISA CHIANESE ⁽²⁾ - GIUSEPPINA SANNA ⁽³⁾ - SHUBAASH ANTHIYA ⁽⁴⁾ - ANA M. LÓPEZ-ESTÉVEZ ⁽⁴⁾ - ALESSANDRA MONTI ⁽⁵⁾ - GIUSEPPE SQUILLACI ⁽⁶⁾ - BIAGIO SANTELLA ⁽²⁾ - GIUSEPPE GRECO ⁽²⁾ - ANNA DE FILIPPIS ⁽²⁾ - FRANCESCO PALMIERI ⁽¹⁾ - GIANNI D'ANGELO ⁽⁷⁾ - ALESSANDRA MORANA ⁽⁶⁾ - NUNZIANNA DOTI ⁽⁵⁾ - MARÍA JOSÉ ALONSO ⁽⁴⁾ - ALDO MANZIN ⁽³⁾ - MASSIMILIANO GALDIERO ⁽⁸⁾

University of Salerno, Department of Medicine, Surgery and Dentistry, Scuola Medica Salernitana, Salerno, Italia ⁽¹⁾ - University of Campania "Luigi Vanvitelli", Department of Experimental Medicine, Napoli, Italia ⁽²⁾ - University of Cagliari, Cittadella Universitaria, Department of Biomedical Sciences, Cagliari, Italia ⁽³⁾ - University of Santiago de Compostela, Department of Pharmacy and Pharmaceutical Technology, School of Pharmacy, Santiago de Compostela, Spagna ⁽⁴⁾ - National Research Council (CNR), Institute of Biostructures and Bioimaging (IBB), Napoli, Italia ⁽⁵⁾ - National Research Council (CNR), Research Institute on Terrestrial Ecosystems, Napoli, Italia ⁽⁶⁾ - University of Salerno, Department of Computer Science, Salerno, Italia ⁽⁷⁾ - University of Campania "Luigi Vanvitelli", Department of Experimental Medicine, Napoli, Italia ⁽⁸⁾

Antiviral activity of three residues peptides against SARS-CoV-2 Infection

Gianluigi Franci¹, Avinash Mali², Carla Zannella², Annalisa Chianese², Giuseppina Sanna³, Shubaash Anthiya⁴, Ana M. López-Estévez⁴, Alessandra Monti⁵, Giuseppe Squillaci⁶, Biagio Santella², Giuseppe Greco², Anna De Filippis², Francesco Palmieri¹, Gianni D'Angelo⁷, Alessandra Morana⁶, Nunzianna Doti⁵, María José Alonso⁴, Aldo Manzin³, Massimiliano Galdiero².

1. Department of Medicine, Surgery and Dentistry, "Scuola Medica Salernitana", University of Salerno, Salerno, Italy;
2. Department of Experimental Medicine, University of Campania "Luigi Vanvitelli", Naples, Italy;
3. Department of Biomedical Sciences, University of Cagliari, Cittadella Universitaria, Cagliari, Italy;
4. Center for Research in Molecular Medicine and Chronic Diseases (CIMUS), Health Research Institute of Santiago de Compostela (IDIS), Department of Pharmacy and Pharmaceutical Technology, School of Pharmacy, University of Santiago de Compostela, Santiago de Compostela, Spain;
5. Institute of Biostructures and Bioimaging (IBB), National Research Council (CNR), Naples, Italy;
6. Research Institute on Terrestrial Ecosystems, National Research Council (CNR), Naples, Italy;
7. Department of Computer Science, University of Salerno, Salerno, Italy;

Introduction: The global spread of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) has posed significant challenges to public health. The urgent need for innovative therapies has prompted research into peptide antivirals, which offer advantages such as low toxicity and high efficacy. Recently, two small nucleotide signatures encoding tripeptide sequences were identified in the genomes of pathogenic microorganisms within the Coronaviridae family. These sequences are not part of the coding reading frame of the S gene. However, one limitation of peptide drugs is their high proteolytic instability in vivo. In this study, we conjugated these tripeptide sequences to nanoparticles

(NPs) for intranasal administration and evaluated their antiviral efficacy. **Materials and Methods:** Recurring genomic sequences were obtained from the National Center for Biotechnology Information database. Peptides were synthesized using solid-phase synthesis on Rink-4-methylbenzhydrylamine (MBHA) resin with a loading of 0.4-0.8 mmol/g. The identity of the peptides was confirmed using liquid chromatography-mass spectrometry (LC-MS) coupled with electrospray ionization time-of-flight mass spectrometry (ESI-TOF-MS). The peptides were then conjugated to HA and DS biopolymers and incorporated into small and large NPs. Encapsulation efficiency was determined by LC/MS analysis of unencapsulated peptides. Cytotoxicity of naked and conjugated peptides was evaluated using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide assay on Vero cells. Antiviral efficiency was assessed against SARS-CoV-2 through co-treatment and virus pre-treatment experiments. **Results:** The identified nucleotide sequences were ACATTACAC and GTGTTTATT, which encoded the tripeptide sequences TLH and VFI, respectively. Cytotoxicity analysis demonstrated that both naked and conjugated peptides were safe at concentrations greater than 200 μ M. The VFI peptide exhibited better antiviral efficacy than TLH, with IC₅₀ values of 40 μ M and 75 μ M in the co-treatment and virus pre-treatment assays, respectively. The antiviral activity of the peptides was enhanced when conjugated to NPs. Specifically, the combination of large NPs and VFI showed the highest antiviral potential, with IC₅₀ values of 30 μ M and 20 μ M in the co-treatment and virus pre-treatment assays, respectively. **Discussion and Conclusions:** This study demonstrates the high antiviral efficacy of TLH and VFI peptides against SARS-CoV-2. The use of NPs preserves the antiviral activity of the peptides and enhances their potential for in vivo applications. NP-peptide complexes represent promising therapeutic candidates for the treatment of SARS-CoV-2 infections.

298 - Genome-wide DNA methylome and transcriptome identify epigenetically based drug-resistance mechanisms in *Acinetobacter baumannii*

VERONICA FOLLIERO ⁽¹⁾ - ROSARIO BRANCACCIO ⁽¹⁾ - DOMENICO DI ROSA ⁽¹⁾ -
FEDERICA DELL'ANNUNZIATA ⁽¹⁾ - RITA GRECO ⁽²⁾ - ALESSANDRO WEISZ ⁽¹⁾ -
FRANCESCA RIZZO ⁽¹⁾ - GIANLUIGI FRANCI ⁽¹⁾

University of Salerno, Department of Medicine, Surgery and Dentistry "Scuola Medica Salernitana, Salerno, Italia ⁽¹⁾ - AORN Sant 'Anna and San Sebastiano, UOSD Microbiology, Caserta, Italia ⁽²⁾

Genome-wide DNA methylome and transcriptome identify epigenetically based drug-resistance mechanisms in *Acinetobacter baumannii*

Veronica Folliero¹, Rosario Brancaccio¹, Domenico Di Rosa¹, Federica Dell'Annunziata¹, Rita Greco², Alessandro Weisz¹, Francesca Rizzo¹, Gianluigi Franci¹

1. Department of Medicine, Surgery and Dentistry "Scuola Medica Salernitana", University of Salerno, Salerno, Italy;

2. UOSD Microbiology - AORN Sant 'Anna and San Sebastiano, Caserta, Italy

Introduction. Antibiotic resistance (AR) represents one of the most concerning public health threats. The World Health Organization has placed *Acinetobacter baumannii* (*A. baumannii*) among the priority strains against which new drugs are urgently needed. Not all currently known biochemical mechanisms and genetic modifications account for multidrug resistance in *A. baumannii*. Recent evidence suggests the role of DNA methylation in the development of antibiotic resistance in bacteria. This study aimed to provide the first description of the whole *A. baumannii* methylome and transcriptome with different antibiotic resistance profiles, leading to the identification of new epigenetically based resistance mechanisms, not known for this microorganism. **Materials and Methods.** Six strains of *A. baumannii* were studied: 5 field strains isolated from blood samples (strains 1, 2, 3, 4 and 6) of patients hospitalized at the "San Giovanni di Dio e Ruggi d'Aragona" University Hospital (Salerno, Italy). The reference strain *A. baumannii* (BAA-747, strain 5) was purchased from the American Type Culture Collection (ATCC). Identification of bacterial species and their antibacterial resistance profile was assessed by matrix-assisted laser desorption flight mass spectrometry (MALDI-TOF MS) and VITEK2 (bioMérieux, Nürtingen, Germany), respectively. DNA and RNA were extracted from bacterial cultures using the QIAGEN® Genomic DNA Handbook and TRIzol protocol and quantitatively and qualitatively evaluated by Nanodrop and Bioanalyzer. Oxford Nanopore Technologies sequencing and RNA-seq were exploited to identify the methylated bases and the transcriptome of *A. baumannii* strains. ClueGO plugin from Cytoscape was used for pathways identification. **Results.** Bacterial strains 1, 3 and 4 are defined as multiresistant, while strains 2, 5 and 6 are multisensitive. Two types of methylations were analyzed in *A. Baumannii*: 5-methyl-cytosine (5mC) and N6-methyl-adenosine (m6A). Through transcriptomic and epigenetic approaches, we demonstrate that m5C and m6A modifications are significantly correlated with drug resistance events. In detail, we identified 3835 5mC and 382 6mA methylation motifs differently present between sensitive and resistant strains. Combining methylation and gene expression data, the major pathway (22.2%) epigenetically regulated and implicated in drug resistance was associated with homoserine metabolism. **Discussion and Conclusions.** Our results illustrate the complete map of the *A. baumannii* methylome and transcriptome, highlighting epigenetic-based mechanisms of antibiotic resistance. This could provide novel diagnostic markers and drug targets, improving the management of multidrug-resistant *A. baumannii* infections.

299 - Broad-spectrum antimicrobial potential of Oftasecur and Visuprime ophthalmic solutions

ILARIA COSIMATO⁽¹⁾ - FEDERICA DELL'ANNUNZIATA⁽¹⁾ - MARIA VITTORIA MORONE⁽²⁾ - MARCO GIOIA⁽¹⁾ - FERDINANDO CIONE⁽¹⁾ - MASSIMILIANO GALDIERO⁽²⁾ - NICOLA ROSA⁽¹⁾ - MADDALENA DE BERNARDO⁽¹⁾ - VERONICA FOLLIERO⁽¹⁾ - GIANLUIGI FRANCI⁽¹⁾

Department of Medicine, Surgery and Dentistry "Scuola Medica Salernitana", University Salerno, Salerno, Italia⁽¹⁾ - University of Campania Luigi Vanvitelli, Department of Experimental Medicine, Napoli, Italia⁽²⁾

Broad-spectrum antimicrobial potential of Oftasecur and Visuprime ophthalmic solutions

Federica Dell'Annunziata^{1,2}, Maria Vittoria Morone², Marco Gioia¹, Ferdinando Cione¹, Iaria Cosimato^{1,2}, Massimiliano Galdiero^{2,3}, Nicola Rosa¹, Maddalena De Bernardo¹, Veronica Folliero¹, Gianluigi Franci^{1,4}

¹ Department of Medicine, Surgery and Dentistry "Scuola Medica Salernitana", University of Salerno, 84081 Baronissi Salerno, Italy

² Department of Experimental Medicine, University of Campania Luigi Vanvitelli, 80138 Naples, Italy

³ Complex Operative Unity of Virology and Microbiology, University Hospital of Campania "Luigi Vanvitelli", 80138 Naples, Italy

⁴ Clinical Pathology and Microbiology Unit, San Giovanni di Dio e Ruggi D'Aragona University Hospital, 84126 Salerno, Italy

Introduction: Conjunctivitis is a common disorder seen in ophthalmology clinics worldwide, with approximately 80% of cases being of infectious origin. Viruses are the primary cause of infectious conjunctivitis, followed by bacteria, although fungi and parasites can also contribute to the infection. The microorganisms responsible for conjunctivitis can be acquired through direct contact with contaminated hands or objects, aerosol transmission, and vertical transmission from mother to child. The diverse etiology of conjunctivitis, along with the costly and time-consuming diagnostic process, necessitates the development of new therapeutic strategies with broad-spectrum antimicrobial activity and non-selective mechanisms of action. In this study, we compare the antibacterial and antiviral activities of two commercially available ophthalmic solutions, Oftasecur and Visuprime.

Materials and Methods: Cytotoxicity assays were conducted on Vero cells using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay. The antibacterial efficacy of Oftasecur and Visuprime was evaluated against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Klebsiella pneumoniae* using the disc diffusion method, broth microdilution method, and time-killing assay. Additionally, the antiviral activity against herpes simplex virus type 1 (HSV-1) was assessed through co-treatment, viral and cellular pre-treatment, and post-treatment using plaque reduction assays, fluorescence assessment (using GFP-engineered HSV-1), and real-time PCR analysis of viral genes UL27 and UL54 expression.

Results: After 24 hours of exposure, Oftasecur and Visuprime exhibited 50% cytotoxicity at concentrations of 125 μ L and 15.8 μ L, respectively. Oftasecur and Visuprime demonstrated 90% antibacterial activity at average volumes of 10.0 μ L and 4.4 μ L against Gram-positive and Gram-negative strains, respectively. Oftasecur exhibited bactericidal activity against both bacterial populations, while Visuprime displayed bacteriostatic effects on Gram-negative strains and slight bactericidal effects on Gram-positive bacteria. The ophthalmic solutions also demonstrated a

significant impact on viral infectivity, with 50% inhibition observed at exposures of 3.12 μ L and 0.84 μ L of Oftasecur and Visuprime, respectively, for 1 hour. The antiviral activity was further confirmed by the reduction in fluorescence and expression levels of viral genes.

Discussion and Conclusions: The study evaluated the antibacterial and antiviral activities of Oftasecur and Visuprime, commercially available ophthalmic solutions, for conjunctivitis treatment. Both solutions showed acceptable cytotoxicity profiles and demonstrated significant antibacterial activity against a range of bacteria. They also exhibited effective inhibition of herpes simplex virus type 1 (HSV-1) in various treatment scenarios. These findings suggest that Oftasecur and Visuprime hold potential as safe and broad-spectrum treatment options for conjunctivitis of viral and bacterial origins. Further clinical trials are needed to confirm their efficacy and safety.

308 - Genotypic characterization of linezolid-resistant *Staphylococcus epidermidis* strains isolated from invasive infections collected in the Careggi University Hospital of Florence

ILARIA BACCANI⁽¹⁾ - MARCO COPPI⁽¹⁾ - LORENZO PACI⁽²⁾ - NICLA GIOVACCHINI⁽²⁾ - ALBERTO ANTONELLI⁽¹⁾ - TOMMASO GIANI⁽¹⁾ - ORIETTA MASSIDDA⁽³⁾ - GIAN MARIA ROSSOLINI⁽¹⁾

University of Florence, Department of Experimental and Clinical Medicine, University of Florence; Microbiology and Virology Unit, Careggi University Hospital, Florence, Italia⁽¹⁾ - University of Florence, Department of Experimental and Clinical Medicine, University of Florence, Florence, Italia⁽²⁾ - University of Trento, Department of Cellular, Computational and Integrative Biology (CIBIO), Trento, Italia⁽³⁾

Genotypic characterization of linezolid-resistant *Staphylococcus epidermidis* strains isolated from invasive infections collected in the Careggi University Hospital of Florence

ILARIA BACCANI^{1,2}, MARCO COPPI^{1,2}, LORENZO PACI¹, NICLA GIOVACCHINI¹, ALBERTO ANTONELLI^{1,2}, TOMMASO GIANI^{1,2}, ORIETTA MASSIDDA³, GIAN MARIA ROSSOLINI^{1,2}

1 Department of Experimental and Clinical Medicine, University of Florence, Florence, Italy; 2 Microbiology and Virology Unit, Careggi University Hospital, Florence, Italy; 3 Department of Cellular, Computational and Integrative Biology (CIBIO), University of Trento, Trento, Italy

Introduction. *Staphylococcus epidermidis* is a common commensal of human epidermis which may be responsible for invasive nosocomial infections due to its ability to colonize medical devices. Of particular concern is the capacity of resisting to several class of antibiotics including linezolid. Linezolid resistance was associated to the acquisition of *cfr*, *cfr(B)* *poxtA* and *optrA* resistance genes or mutations on the L3, L4 and L22 ribosomal proteins and on the rRNA 23S. The aim of this study is to characterize the linezolid resistance mechanism in a collection of *S. epidermidis* clinical isolates. **Materials and Methods.** Strains were collected at the AOU-Careggi Hospital during the period 2014-2022. Linezolid resistance was confirmed by broth microdilution assay. All strains were subjected to Whole Genome Sequencing by Illumina Novaseq 6000 platform using a 2x150 bp approach and raw reads were assembled using SPAdes software. Bioinformatic analysis was performed using specific tools: RESfinder, BLAST and Pathogenwatch platform. **Results.** Study collection was composed of 104 strains from bloodstream infections (n=103) and cerebrospinal fluid (n=1). 92.3% (96/104) of isolates showed a highly resistant linezolid phenotype with a MIC $\geq$ 32 mg/L. The *cfr* gene was detected in 14/104 isolates (13.5%), while no other acquired resistance determinants were present. On the other hand, sequencing analysis detected mutations in the 23S rDNA in all but one resistant isolate which harbored the *cfr* gene. The most frequent mutation in the rDNA 23S was G2603T (87/104), while the L3 protein mostly showed a M156T mutation alone (86/104) or in combination with Q136L (20/104) and 13 isolates presented different mutations (H146Q and A157R), previously associated to linezolid resistance. In L4 a glycine insertion at aminoacidic position 72 was detected in 33 strains, while no mutations were observed in L22. Most of the isolates belonged to sequence type (ST) 2 (85/104) while few strains belonged to ST5 (n=12), ST32 (n=2) and ST242 (n=1) and 4 isolates were not attributed to any ST. **Discussion and**

Conclusions. This study shows that chromosomal mutations on the 23S ribosomal subunit and on ribosomal proteins L3 and L4 represent a common mechanism of linezolid resistance. On the contrary, the impact of transferrable resistance mechanism seems to be less frequent in our setting and mainly associated with the *cfr* gene acquisition. Different STs were also detected highlighting the lack of correlation between the diffusion of specific clones and linezolid resistance in *S. epidermidis*. Further studies using a WGS approach will be necessary to monitor the spread of this phenomenon.

311 - Phenotypic and genotypic characterization of a clinical isolate of IMP-19 producing *Enterobacter hormaechei*

CHIARA CHILLERI⁽¹⁾ - ALBERTO ANTONELLI⁽¹⁾ - NOEMI AIEZZA⁽¹⁾ - MARCO COPPI⁽¹⁾ - GUENDALINA VAGGELLI⁽²⁾ - TOMMASO GIANI⁽¹⁾ - GIAN MARIA ROSSOLINI⁽¹⁾

University of Florence, Department of Experimental and Clinical Medicine; Clinical Microbiology and Virology Unit, Careggi University Hospital, Firenze, Italia⁽¹⁾ - Clinical Microbiology and Virology Unit, Careggi University Hospital, Firenze, Italia⁽²⁾

Phenotypic and genotypic characterization of a clinical isolate of IMP-19 producing *Enterobacter hormaechei*

CHIARA CHILLERI^{1,2}, ALBERTO ANTONELLI^{1,2}, NOEMI AIEZZA^{1,2}, MARCO COPPI^{1,2}, GUENDALINA VAGGELLI², TOMMASO GIANI^{1,2}, GIAN MARIA ROSSOLINI^{1,2}

¹Department of Experimental and Clinical Medicine, University of Florence, Italy

²Clinical Microbiology and Virology Unit, Careggi University Hospital, Florence, Italy

Introduction: Carbapenemase producing Enterobacterales (CPE) are among the most problematic causes of nosocomial infections due to the limited therapeutic options available. The most common carbapenemases in Europe among enterobacteria are KPC, NDM, OXA-48 and VIM, while only rare cases of IMP producers have been detected. The aim of this study is the characterization of an IMP-producing *Enterobacter hormaechei* (KN α 19) recovered from a patient during a routine screening for intestinal carriage of CPE in a long-term rehabilitation center.

Materials and Methods: KN α 19 was isolated from a rectal swab plated on ChromID™ CARBA SMART selective plate (bioMérieux). Antimicrobial susceptibility testing (AST) was carried out by reference broth microdilution and results were interpreted according to the EUCAST v. 13.0 2023 clinical breakpoints. Bacterial DNA was extracted with DNeasy PowerLyzer PowerSoil (Qiagen®). Whole genome sequencing (WGS) was carried out with Illumina® and Oxford Nanopore platforms. Electrotransformation experiments on MHA supplemented with ceftazidime (4 µg/ml) were carried out using *E. coli* DH10B as host strain.

Results: WGS analysis revealed that KN α 19 strain was an *Enterobacter hormaechei* subsp. *hoffmannii* belonging to the high-risk clone ST78. The bla_{IMP-19} gene was harbored on an original IncFIB plasmid of 140094 bp and was located in a novel class I integron, flanked downstream by aacA4, aadA1 and catB2 gene cassettes. KN α 19 strain showed a resistant phenotype to ceftazidime (MIC >64 mg/L), cefepime (MIC >16 mg/L), ertapenem (MIC= 1 mg/L), ceftazidime–avibactam (MIC >64/4 mg/L), ciprofloxacin (MIC >1 mg/L), gentamicin (MIC >8 mg/L) and co-trimoxazole (MIC >8/152 mg/L) while retained susceptibility to piperacillin-tazobactam (MIC= 8/4 mg/L), meropenem (MIC 0.5 mg/L), amikacin (MIC ≤4mg/L), tigecycline (MIC= 0.5 mg/L) and colistin (MIC ≤0.5 mg/L). The plasmid pKN α 19 was successfully mobilized in *E. coli* DH10B conferring a resistance phenotype similar to KN α 19 clinical strain.

Discussion and Conclusions: CPE screening of is an important approach to promote tracking of common and uncommon carbapenemases and implement infection control practices. The detection of a high-risk clone of IMP-19-producing *E. hormaechei* in Italy is of concern and further studies will be necessary to assess the possible transmission source.

320 - IS26-mediated chromosomal integration of a blaKPC-3-harboring IncFII-derived plasmid in a clinical isolate of *Pseudomonas aeruginosa*

ALBERTO ANTONELLI⁽¹⁾ - BENEDETTA CASCIATO⁽¹⁾ - MARIA NARDONE⁽²⁾ - GRAZIA LOMBARDI⁽²⁾ - CHIARA BIANCHIMANI⁽²⁾ - FEDERICO POSTERARO⁽³⁾ - STEFANO RAPI⁽²⁾ - TOMMASO GIANI⁽¹⁾ - GIAN MARIA ROSSOLINI⁽¹⁾

Department of Experimental and Clinical Medicine; Microbiology and Virology Unit, University of Florence; Careggi University Hospital, Firenze, Italia⁽¹⁾ - Clinical Chemistry and Microbiology Analysis Unit, San Luca Hospital, AUSL Toscana Nord Ovest, Lucca, Italia⁽²⁾ - Rehabilitation Department, Versilia Hospital, AUSL Toscana Nord Overst, Camaiore, Italia⁽³⁾

IS26-mediated chromosomal integration of a bla_{KPC-3}-harboring IncFII-derived plasmid in a clinical isolate of *Pseudomonas aeruginosa*

Alberto Antonelli^{1,2}, Benedetta Casciato^{1,2}, Maria Nardone³, Grazia Lombardi³, Chiara Bianchimani³, Federico Posteraro⁴, Stefano Rapi³, Tommaso Giani^{1,2}, Gian Maria Rossolini^{1,2}

¹ Department of Experimental and Clinical Medicine, University of Florence, Florence, Italy

² Microbiology and Virology Unit, Careggi University Hospital, Florence, Italy

³ Clinical Chemistry and Microbiology Analysis Unit, San Luca Hospital, AUSL Toscana Nord Ovest, Lucca, Italy

⁴ Rehabilitation Department, Versilia Hospital, AUSL Toscana Nord Overst, Camaiore, Lucca, Italy.

Introduction. Carbapenem-resistant *Pseudomonas aeruginosa* is an important nosocomial pathogen for which new antimicrobials are urgently needed as reported by the World Health Organization. Carbapenem resistance in *P. aeruginosa* is mediated by permeability defects, overexpression of AmpC beta-lactamase or by the acquisition of carbapenemase genes. The most common acquired carbapenemases in *P. aeruginosa* are VIM- and IMP-type metallo-enzymes and class A GES, while other less frequent carbapenemases NDM, KPC and FIM were only sporadically detected. In this report we characterized a clinical isolate of KPC-producing *P. aeruginosa*.

Materials and Methods. LUMN-PSA *P. aeruginosa* was isolated on ChromID™ CARBA SMART selective plate (bioMérieux). Antibiotic susceptibility testing (AST) was carried out by reference broth microdilution and results were interpreted according to the EUCAST v. 13.0 2023 clinical breakpoints. Whole genome sequencing (WGS) was carried out with an Illumina® platform.

Results. LUMN-PSA *P. aeruginosa* strain was isolated in March 2023 during a routine molecular screening for carbapenemase producing Gram-negatives from an inpatient of Versilia Hospital, previously hospitalized in different structures over a period of 9 months for an aortic aneurism and multiple comorbidities. The strain showed a resistant phenotype to piperacillin-tazobactam (MIC >128/4 mg/L), ceftazidime (MIC >512 mg/L), cefepime (MIC >16 mg/L), ertapenem (MIC >2 mg/L), meropenem (MIC= 128 mg/L), meropenem-vaborbactam (MIC 64/8 mg/L), imipenem-relebactam (MIC= 16 mg/L), ceftazidime-avibactam (MIC >64/4 mg/L) and ceftolozane-tazobactam (MIC >64/4 mg/L), while retained susceptibility to gentamicin (MIC= 2 mg/L), co-trimoxazole (MIC= 8/152 mg/L), ciprofloxacin (MIC 0.25 mg/L), amikacin (MIC ≤4mg/L), colistin (MIC= 4 mg/L) and cefiderocol (MIC= 2 mg/L). WGS analysis revealed that the strain belonged to ST260 (a globally disseminated clone) and carried a bla_{KPC-3} carbapenemase gene inserted a Tn4401 transposon and in turn located in a 24398 bp composite transposon derived from an IncFII_k plasmid integrated in *P.*

aeruginosa chromosome through an IS26-mediated transposition event. The patient was not reported to have been colonized by a KPC-producing *Klebsiella pneumoniae*, suggesting that the recombination event likely happened in another patient.

Discussion and Conclusions: The detection of a KPC-producing *P. aeruginosa* in Italy is of concern, and further studies will be needed to investigate its possible diffusion in clinical settings in order to prevent its spread.

12 Risposta dell'ospite alle infezioni/Correlati immunologici

6 - Role of Epstein-Barr virus and Mycobacterium avium paratuberculosis in CNS demyelinating disorders

DAVIDE COSSU ⁽¹⁾ - NOBUTAKA HATTORI ⁽²⁾ - LEONARDO ANTONIO SECHI ⁽¹⁾

Universita di Sassari, universita, dipartimento di scienze biomediche, Sassari, Italia ⁽¹⁾ -
Juntendo University, dipartimento neurologia, Tokyo, Giappone ⁽²⁾

Role of Epstein-Barr virus and Mycobacterium avium paratuberculosis in CNS
demyelinating disorders

Davide Cossu ^{a,b}, Nobutaka Hattori ^b, Leonardo Antonio Sechi ^a

^a Sassari University, Department of Biomedical Sciences, Sassari, Italy

^b Juntendo University, Department of Neurology, Tokyo, Japan

Abstract

Objective. To characterized the subclasses of IgG against Epstein-Barr virus (EBV) nuclear antigen 1 (EBNA1), Mycobacterium avium subsp. paratuberculosis (MAP) heat shock protein (HSP) 70, and homologues peptides of human central nervous system proteins in blood samples from Italian and Japanese patients with multiple sclerosis (MS), neuromyelitis optica spectrum disorder (NMOSD), and myelin oligodendrocyte glycoprotein antibody-associated disease (MOGAD). Furthermore, we tested the effects of these peptide in MOG-induced experimental autoimmune encephalomyelitis (EAE). Methods. A total of 164 Japanese subjects (MS=65, NMOSD=14, MOGAD=20; healthy controls=65) and 130 Italian subjects (MS=65, healthy controls =65), were retrospectively analyzed by indirect ELISA. A group of C57BL/6J mice was immunized with EBNA1₃₈₆₋₄₀₅ and MAPHSP70₅₃₃₋₅₄₅ peptides two weeks prior to induction of active MOG₃₅₋₅₅ EAE. Results. A significantly strong antibody response against all peptides was detected in the serum of MS and NMOSD patients compared to MOGAD patients and controls. Inhibition immunoassay demonstrated the presence of cross-reactive antibodies against EBV and MAP. Furthermore, after immunization with the peptides EBNA1₃₈₆₋₄₀₅ and MAPHSP70₅₃₃₋₅₄₅ peptides, EAE mice showed more severe disease and increased MOG₃₅₋₅₅-specific T-cell reactivity compared to untreated mice. Conclusions. These data provide evidence for an association between EBV, MAP and the development or exacerbation of MS and NMOSD, at least in a subgroup of patients with relapsing remitting MS and in patients with double seronegative NMOSD.

8 - Neutralizing antibodies response to novel SARS-CoV-2 omicron sublineages in long-term care facility residents after the fourth dose of monovalent BNT162b2 Covid-19 vaccination

ILENIA VARASI ⁽¹⁾ - ALESSIA LAI ⁽²⁾ - ANNALISA BERGNA ⁽²⁾ - LIA FIASCHI ⁽¹⁾ - ANTONELLA GATTI ⁽³⁾ - BARBARA CAIMI ⁽³⁾ - CLAUDIA BALOTTA ⁽³⁾ - CAMILLA BIBA ⁽¹⁾ - AGOSTINO RIVA ⁽²⁾ - GIANGUGLIELMO ZEHENDER ⁽²⁾ - MAURIZIO ZAZZI ⁽¹⁾ - ILARIA VICENTI ⁽¹⁾

Università degli Studi di Siena, Dipartimento Biotecnologie Mediche/AOUS Siena, Siena, Italia ⁽¹⁾ - University of Milan, Department of Biomedical and Clinical Sciences, Milano, Italia ⁽²⁾ - Istituto Milanese Martini e Stelline e Pio Albergo Trivulzio, Azienda di Servizi alla Persona, Milano, Italia ⁽³⁾

Neutralizing antibodies response to novel SARS-CoV-2 omicron sublineages in long-term care facility residents after the fourth dose of monovalent BNT162b2 Covid-19 vaccination

ILENIA VARASI¹, ALESSIA LAI², ANNALISA BERGNA², LIA FIASCHI¹, ANTONELLA GATTI³, BARBARA CAIMI³, CLAUDIA BALOTTA³, CAMILLA BIBA¹, AGOSTINO RIVA², GIANGUGLIELMO ZEHENDER², MAURIZIO ZAZZI¹, ILARIA VICENTI¹

¹ Department of Medical Biotechnologies, University of Siena, Siena, Italy; ² Department of Biomedical and Clinical Sciences, University of Milan, Milan, Italy; ³ Azienda di Servizi alla Persona, Istituto Milanese Martini e Stelline e Pio Albergo Trivulzio, Milan, Italy

INTRODUCTION: The dynamics of SARS-CoV-2 variants has transitioned from long-term dominance of a successful variant to a dynamic swarm of genetically related sublineages, carrying convergent aminoacidic mutations in the spike region. Aim of this work was to quantify the neutralizing antibodies (NtAb) titre against the recently predominant omicron sublineages in a fragile population naïve for SARS-COV-2 infection and vaccinated with four doses of monovalent BNT162b2 COVID-19 mRNA vaccine.

MATERIALS AND METHODS: Plasma samples were collected from 40 residents at the largest Italian long-term care facility, 71 [68-75] and 89 [80-91] median [IQR] days after the third (T3) and fourth (T4) BNT162b2 vaccine dose. The study group had median age of 91 [84-94] years, included 5 males and no subject with positive anti-nucleocapsid serology at first vaccine dose. Patients were weekly screened by swab analysis to exclude subsequently SARS-CoV-2 infection. NtAb titers were measured at T3 and T4 against the wild type (WT) lineage B.1 and at T4 against five Omicron sublineages (BA.5, BQ.1.1, BA.2.75.2, XBB.1 and CH.1.1). Live virus microneutralization was performed in VERO E6 cells quantifying the cell viability by luminescence. The NtAb titer was defined as the reciprocal value of the sample dilution showing 50% protection of virus-induced cytopathic effect (ID₅₀). Antibodies with ID₅₀ titers < 10 were defined as negative and scored as 5 for statistical analysis. SARS-CoV-2 IgG II Quant assay (Abbott) was used to quantify the anti-spike protein Ab at T3, T4 and after the first and the second vaccine dose administration (T1, T2).

RESULTS: Thirty-four and 13 patients had at least 1 and 3 comorbidities respectively, and polypharmacy was common. As expected, NtAb titers to WT variant significantly increased ($p < 0.001$) at T4 (1094 [612-3252] ID₅₀) with respect to T3 (518 [60-1515] ID₅₀). A significant increase was also observed when comparing the anti-spike Ab median titres at T3 and T4 (2724 [35-4542] vs. 9939 [4168-12,500]; $p < 0.001$). Overall, at T4 median NtAb titres to the WT strain correlated with those to each omicron variant ($p < 0.001$ for all comparisons) but absolute values expressed as ID₅₀

were significantly lower: BA.5, 287 (38-533); BA.2.75.2, 30 (15-67); BQ.1.1, 22 (11-58), XBB.1, 13 (5-24) and CH.1.1, 13 (5-19) ($p < 0.001$). At each time point analyzed the anti-spike Ab and NtAb titres against WT and different variants, were not correlated to the different comorbidities ($p > 0.05$).

CONCLUSIONS: In this fragile elderly population, recently circulating omicron sublineages BQ.1.1, BA.2.75.2, XBB.1 and CH.1.1 showed greater escape from monovalent BNT162b2 COVID-19 mRNA vaccine with respect to the previously dominant BA.5 variant. It remains to be established whether the reduced NtAb titers still protect from incident infection with these and future variants.

12 - Prevalence of polyomaviruses and papillomaviruses related to lymphocyte immunophenotyping in Actinic Keratoses and Squamous Cell Carcinoma: one-year data from clinical practice

TERENZIO COSIO⁽¹⁾ - MARCO CIOTTI⁽²⁾ - CARLA PREZIOSO⁽³⁾ - GABRIELE BRAZZINI⁽³⁾ - SARA PASSERINI⁽³⁾ - SERGIO BERNANRDINI⁽¹⁾ - UGO MOENS⁽⁴⁾ - VALERIA PIETROPAOLO⁽³⁾ - ROBERTA GAZIANO⁽¹⁾ - ELENA CAMPIONE⁽⁵⁾

Università di Roma Tor Vergata, Department of Experimental Medicine, Roma, Italia⁽¹⁾ - University of Rome Tor Vergata, Virology Unit, Polyclinic Tor Vergata Foundation, Viale Oxford 81, 00133 Rome, Italy., Roma, Italia⁽²⁾ - "Sapienza" University of Rome, 00185 Rome, Italy, Department of Public Health and Infectious Diseases, "Sapienza" University of Rome, 00185 Rome, Italy., Roma, Italia⁽³⁾ - University of Tromsø-The Arctic University of Norway, Department of Medical Biology, Faculty of Health Sciences, University of Tromsø-The Arctic University of Norway, 9037 Tromsø, Norway, Tromsø, Norvegia⁽⁴⁾ - Tor Vergata University of Rome, Department of Systems Medicine, Dermatology Unit, Tor Vergata University Hospital, 00133 Rome, Italy, Roma, Italia⁽⁵⁾

Prevalence of polyomaviruses and papillomaviruses related to lymphocyte immunophenotyping in Actinic Keratoses and Squamous Cell Carcinoma: one-year data from clinical practice

Terenzio COSIO^{1,2}, Marco CIOTTI³, Carla PREZIOSO^{4,5}, Gabriele BRAZZINI⁵, Sara PASSERINI⁵, Sergio BERNARDINI¹, Ugo MOENS⁶, Valeria PIETROPAOLO⁵, Roberta GAZIANO¹, Elena CAMPIONE²

¹Department of Experimental Medicine, Tor Vergata University of Rome, 00133 Rome, Italy;

²Department of Systems Medicine, Dermatology Unit, Tor Vergata University Hospital, 00133 Rome, Italy;

³Virology Unit, Polyclinic Tor Vergata Foundation, Viale Oxford 81, 00133 Rome, Italy.

⁴IRCSS San Raffaele Roma, Microbiology of Chronic Neuro-Degenerative Pathologies, 00163 Rome, Italy;

⁵Department of Public Health and Infectious Diseases, "Sapienza" University of Rome, 00185 Rome, Italy;

⁶Department of Medical Biology, Faculty of Health Sciences, University of Tromsø-The Arctic University of Norway, 9037 Tromsø, Norway;

Introduction: To date, human polyomaviruses (HpyVs) and papillomaviruses (HPV) have a controversial role in non-melanoma skin cancer (NMSC). Among them, Merkel cell polyomaviruses (MCPyV), human polyomaviruses (HpyV6, HpyV7) and Trichodysplasia Spinulosa-associated polyomaviruses (TSPyV) present a skin tropism, but a causal role in skin diseases has been established only for MCPyV as causative agent of Merkel cell carcinoma (MCC) and TSPyV as etiological agent of Trichodysplasia Spinulosa (TS). In this study, we investigated the prevalence of beta-HPV, MCPyV, HpyV6, HpyV7 and TSPyV in Actinic Keratoses (AKs) and squamous cell carcinoma (SCC) and lymphocyte immunophenotyping in enrolled patients.

Materials & Methods: Thirty-seven consecutive patients with clinical diagnosis of AKs or SCC have been recruited in our center. A lesional and a non-lesional skin biopsy have been performed in each patient to confirm clinical diagnosis and to investigate the prevalence of the viruses parallel to lymphocyte immunophenotyping. Positive HPyVs DNA samples were subjected to qualitative PCR with different specific primer pairs mapping VP1 and LT regions of the genome and subsequently sequenced.

Results: Among thirty-seven patients enrolled, 3 are women (8.1%) and 34 men (91.9%). According to positive histology for SCC (11/37) or AK alone (23/37), MCPyV was present in 4 SCC samples and 7 AKs while HPyV6 was detected exclusively in AKs. Considering 19 patients without SCC, MCPyV was found to be present in both AKs and unaffected skin. HPyV6 was found to be more present in unaffected skin than in AKs. MCPyV DNA was found in 11 lesional tissue samples and 11 non-lesional biopsy samples. Considering beta-HPV, 2 patients showed the presence of oncogenic genotypes both on healthy and damaged skin. Also, there were 6 patients who had no oncogenic genotypes. All patients who presented oncogenic genotypes of β -HPV on lesion biopsy received a histological diagnosis of SCC. Moreover, subjects with SCC had more oncogenic genotypes of beta-HPV than subjects with only AKs ($p < 0.017$). Lymphocyte immunophenotyping highlines a decrease in the absolute number of lymphocytes in patients with SCC and increased in those with AK. CD4⁺ cell counts increased in patients with SCC, whereas NK cells decreased compared with AK patients.

Discussion and Conclusions: HPyV6 is, like MCPyV, part of the skin microbiota whereas, the absence of HPyV6 and HPyV7 in AKs and SCC samples could confirm that are not involved in cutaneous malignancies. Moreover, subjects with SCC had more oncogenic genotypes of beta-HPV than subjects with only AKs. Lymphocyte immunophenotyping presents peculiar features in patients with AKs respect to SCC and could play a possible role in the control of cutaneous virome.

30 - Caspase-8 controls a spontaneous pro-inflammatory program that can be exploited therapeutically to control bacterial infections

GERMANA LENTINI⁽¹⁾ - AGATA FAMA'⁽¹⁾ - GIUSEPPE VALERIO DE GAETANO⁽¹⁾ - FRANCESCO COPPOLINO⁽²⁾ - AHLEM MAHJOUB⁽¹⁾ - LIV RYAN⁽³⁾ - EGIL LIEN⁽⁴⁾ - TERJE ESPEVIK⁽³⁾ - CONCETTA BENINATI⁽¹⁾ - GIUSEPPE TETI⁽⁵⁾

Universita' Degli Studi Di Messina, Dipartimento Di Patologia Umana E Dell'eta' Evolutiva G. Barresi, Messina, Italia⁽¹⁾ - Universita' Degli Studi Di Messina, Dipartimento Di Scienze Biomediche, Odontoiatriche E Delle Immagini Morfologiche E Funzionali, Messina, Italia⁽²⁾ - Centre Of Molecular Inflammation Research(Cemir), Department Of Cancer Research And Molecular Medicine, Norwegian University Of Science And Technology, Trondheim, Norvegia⁽³⁾ - Division Of Infectious Diseases And Immunology Program In Innate Immunity, Department Of Medicine, University Of Massachusetts Chan Medical School, Worcester, Stati Uniti D'america⁽⁴⁾ - Scylla Biotech Srl, Messina, Italy, Scylla Biotech Srl, Messina, Italy, Messina, Italia⁽⁵⁾

Caspase-8 controls a spontaneous pro-inflammatory program that can be exploited therapeutically to control bacterial infections

GERMANA LENTINI¹, AGATA FAMÀ¹, GIUSEPPE V. DE GAETANO¹, FRANCESCO COPPOLINO², AHLEM KHACHROUB MAHJOUB¹, LIV RYAN³, EGIL LIEN^{3,4}, TERJE ESPEVIK³, CONCETTA BENINATI^{1,5} AND GIUSEPPE TETI⁵

¹Department of Human Pathology of Adult and Developmental Age "Gaetano Barresi", University of Messina, Messina, Italy; ²Department of Biomedical, Dental and Imaging Sciences, University of Messina, Messina, Italy; ³Centre of Molecular Inflammation Research, Department of Cancer Research and Molecular Medicine, Norwegian University of Science and Technology, Trondheim, Norway; ⁴Division of Infectious Diseases and Immunology, Program in Innate Immunity, Department of Medicine, University of Massachusetts Chan Medical School, Worcester, MA, USA; ⁵Scylla Biotech Srl, Messina, Italy

Introduction: Caspase-8 has a prominent role in controlling inflammation in various host tissues, but the underlying mechanisms are unclear. We find here that caspase-8 controls a novel pathway in neutrophils that can be exploited therapeutically.

Materials and Methods: In vivo and in vitro caspase-8 inhibition was accomplished with z-IETD-fmk. Inflammatory responses were measured in the peritoneal cavity of mice injected with IETD or well characterized inflammatory stimuli. Results were compared in genetically defective mice and in wild type controls.

Results: In vivo, intraperitoneal administration of the caspase-8 inhibitor z-IETD-fmk was sufficient to unleash the production of pro-inflammatory cytokines and neutrophil influx in the peritoneal cavity in the absence of cell death. These effects are due to selective inhibition of caspase-8 and require tonic interferon (IFN)-beta production and receptor-interacting serine/threonine-protein kinase 3 (RIPK3), but not Mixed lineage kinase domain like pseudokinase (MLKL), the essential downstream executioner of necroptotic cell death, as observed in mice lacking these signaling molecules. In vitro, stimulation with z-IETD-fmk is sufficient to induce significant cytokine

production in murine neutrophils, but not in macrophages. Therapeutic administration of z-IETD-fmk improves clinical outcome in models of lethal bacterial peritonitis and pneumonia by augmenting cytokine release, neutrophil influx and bacterial clearance. Moreover, the inhibitor protects mice against high-dose endotoxin shock.

Discussion and Conclusions: Permanent deletion or inactivation of caspase 8 can produce detrimental effects due to severe necroptosis or pyroptosis. However, we show here that temporary inhibition of this protease had no obvious detrimental effects and markedly ameliorates, instead, the outcome of bacterial infections by promoting neutrophil-mediated bacterial clearance and preventing the toxic effects of toll like receptor agonists such as lipopolysaccharide component of the Gram negative bacterial cell wall (LPS). In summary, the paper unveils a molecular mechanism of activation of neutrophils that is IFN-beta and RIPK3 dependent and that might provide a solution to the need for effective host-directed therapies against infections.

46 - SARS-CoV-2 evolution during persistent infection in a CAR-T recipient shows an escape to both sotrovimab and T cell response

MARIA SIDOTI⁽¹⁾ - PAOLA MAZZETTI⁽¹⁾ - PIETRO GIORGIO SPEZIA⁽²⁾ - ANNALISA CAPRIA⁽¹⁾ - GIULIA FREER⁽²⁾ - SILVIA COSTARELLI⁽³⁾ - ALICE CARA⁽¹⁾ - ALFREDO ROSELLINI⁽¹⁾ - SUSI FRATESCHI⁽¹⁾ - GIOVANNA MOSCATO⁽¹⁾ - IACOPO FRANCONI⁽¹⁾ - MICHELE CURCIO⁽⁴⁾ - MARIA LINDA VATTERONI⁽¹⁾ - MICHELE LAI⁽²⁾ - PAOLA QUARANTA⁽²⁾ - DANIELE FOCOSI⁽⁵⁾ - SPARTACO SANI⁽³⁾ - ALESSIA CALÒ⁽²⁾ - MAURO PISTELLO⁽¹⁾

Azienda ospedaliera universitaria pisana, U.O. Virologia, Ospedale Cisanello, Pisa, Italia⁽¹⁾ - Centro Retrovirus, Università di Pisa, Pisa, Italia⁽²⁾ - Azienda Usl Toscana Nord Ovest, Dipartimento di malattie infettive e tropicali, ospedale di Livorno, Livorno, Italia⁽³⁾ - Azienda ospedaliera universitaria pisana, U.O. Immunogenetica, Ospedale di Cisanello, Pisa, Italia⁽⁴⁾ - Azienda Ospedaliera Universitaria Pisana, Banca del Sangue, Ospedale di Cisanello, Pisa, Italia⁽⁵⁾

SARS-CoV-2 evolution during persistent infection in a CAR-T recipient shows an escape to both sotrovimab and T-cell response

MARIA SIDOTI^{a,b}, PAOLA MAZZETTI^a, PIETRO G. SPEZIA^b, ANNALISA CAPRIA^a, GIULIA FREER^b, SILVIA COSTARELLI^c, ALICE CARA^a, ALFREDO ROSELLINI^a, SUSI FRATESCHI^a, GIOVANNA MOSCATO^a, IACOPO FRANCONI^a, MICHELE CURCIO^d, MARIA L. VATTERONI^a, MICHELE LAI^b, PAOLA QUARANTA^b, DANIELE FOCOSI^e, SPARTACO SANI^c, ALESSIA CALÒ^b, MAURO PISTELLO^{a,b}.

a) Department of Laboratory Medicine, Virology Unit, Pisa University Hospital, Pisa, Italy; b) Department of Translational Research, Retrovirus Center and Virology Section, University of Pisa, Pisa, Italy; c) Department of Infectious and Tropical Diseases, Infectious Diseases Unit, Leghorn Hospital, Azienda USL Toscana Nord-Ovest, Leghorn, Italy; d) Department of Laboratory Medicine, Immunogenetic Unit, Pisa University Hospital, Pisa, Italy; e) Department of Laboratory Medicine, North-Western Tuscany Blood Bank, Pisa University Hospital, Pisa, Italy.

1. Introduction

Immunocompromised patients are at higher risk for severe COVID-19. We studied a group of immunocompromised patients with persistent SARS-CoV-2 infection as determined by repeated positive nasopharyngeal swab (NPS) results. From this cohort, we isolated SARS-CoV-2 from NPS samples and assessed serological and cell-mediated immune responses. Here, we present the case of a 58-year-old male with high-grade B cell lymphoma and persistent positive NPS.

2. Materials and Methods

NPS samples were inoculated into Vero/TMPRSS2 cells and monitored for cytopathic effects. Virus presence was tested using RT-PCR and antigenic assays. Serum neutralizing antibodies were measured by a microtiter plate assay. T-cell response was evaluated using ELISPOT and Ficoll-purified peripheral blood mononuclear cells. Whole-genome sequencing (WGS) and HLA typing were performed using Illumina COVID Seq Assay kit and LinkSeq™ Typing Kit, One Lambda, respectively.

3. Results

The 58-year-old male had high-grade B cell lymphoma and achieved remission after CAR-T treatment. He had mild COVID-19 in February 2021 and received three doses of BNT162b2 vaccine. In 2022, his NPS scored SARS-CoV-2 positive again, he developed severe pneumonia and was treated with Sotrovimab. The patient remained positive until August 2022. Over time, serum anti-S IgG increased, while anti-N IgG remained negative. Similarly, the T-cell response was observed for S protein but not N protein. WGS revealed Spike protein mutations, including E340A associated with Sotrovimab resistance. HLA typing showed a reduced number of S epitopes presented by the patient's HLA alleles.

4. Discussion and Conclusions

Our findings suggest that the patient had an immune response primarily to vaccination rather than natural infection. Despite the presence of anti-S antibodies and monoclonal antibodies, the virus mutated. Tailoring treatment based on individual sensitivity and monitoring resistant variants is crucial for improved outcomes.

59 - The *Propionibacterium* spp. extract reduces *Candida albicans*-induced damage to vaginal epithelial cells and increases mitochondrial response to *Candida albicans* infection in vitro

FRANCESCO RICCHI⁽¹⁾ - ANDREA ARDIZZONI⁽²⁾ - ELISABETTA BLASI⁽²⁾ - FRANCESCO DE SETA⁽³⁾ - EVA PERICOLINI⁽²⁾

Università degli studi di Modena e Reggio Emilia, Dipartimento di Scienze Biomediche, Metaboliche e Neuroscienze, Modena, Italia⁽¹⁾ - Università degli studi di Modena e Reggio Emilia, Dipartimento Chirurgico, Medico, Odontoiatrico e di Scienze Morfologiche con Interesse Trapiantologico, Oncologico e di Medicina Rigenerativa, Modena, Italia⁽²⁾ - Università degli studi di Trieste, Dipartimento di Scienze Mediche, Trieste, Italia⁽³⁾

The *Propionibacterium* spp. extract reduces *Candida albicans*-induced damage to vaginal epithelial cells and increases mitochondrial response to *Candida albicans* infection in vitro

FRANCESCO RICCHI¹, ANDREA ARDIZZONI², ELISABETTA BLASI², FRANCESCO DE SETA^{3,4}, EVA PERICOLINI²

¹Clinical and Experimental Medicine Ph.D. Program, University of Modena and Reggio Emilia, Modena, Italy;

²Department of Surgical, Medical, Dental and Morphological Sciences with interest in Transplant, Oncological and Regenerative Medicine, University of Modena and Reggio Emilia, Modena, Italy

³Department of Medical Sciences, University of Trieste, Trieste, Italy;

⁴Institute for Maternal and Child Health IRCCS "Burlo Garofolo", Trieste, Italy.

Introduction. Bacterial lysates are prepared by inactivated microorganisms and are extensively employed in clinical settings as immunomodulants and to improve mucosal immunity. However, despite their extensive clinical use, their effects on the host are only partially known. The *Propionibacterium* spp. extract (PE) is a bacterial lysate included as an active compound in a gel formulation used to treat the symptoms of vulvovaginal candidiasis. Here, we analyzed its possible beneficial effects in an in vitro model of vaginal epithelial cells infected with *Candida*.

Materials and Methods. Initially, we analyzed the PE effects on *C. albicans* and *C. parapsilosis* growth by the microdilution method. We then assessed the capacity of PE to reduce *C. albicans*-induced damage of vaginal epithelial cells through the quantification of lactate-dehydrogenase released by damaged cells in the growth medium. Moreover, in order to test the capacity of the PE to modulate epithelial mitochondrial activity, we evaluated Reactive-Oxygen-Species (ROS) production by the infected epithelial cells, stimulated or not with PE. This was kinetically monitored through the analysis of emitted fluorescence, after addition of the MitoSOX Red probe.

Results. Our results show that PE did not affect directly microbial growth. In addition, the epithelial cells stimulation with PE reduced *C. albicans*-induced cell damage. Moreover, the treatment with PE increased the epithelial cells mitochondrial activity in response to *C. albicans* infection in vitro.

Discussion and Conclusions. Taken together, our results show that PE increases ROS production by epithelial cells in response to *C. albicans* infection. Therefore, our results suggest that the increased

mitochondrial activity induced by PE, could protect epithelial cells against the damage induced by *C. albicans* infection.

83 - The interferon-beta/MLKL axis promotes anti-bacterial defenses by enhancing neutrophil-mediated pathogen killing

Agata Famà⁽¹⁾ - GERMANA LENTINI⁽¹⁾ - GIUSEPPE VALERIO DE GAETANO⁽¹⁾ - FRANCESCO COPPOLINO⁽²⁾ - GIUSEPPE TETI⁽³⁾ - CONCETTA BENINATI⁽¹⁾

Università degli Studi di Messina, Dipartimento di Patologia Umana, Messina, Italia⁽¹⁾ - UNIVERSITA' DEGLI STUDI DI MESSINA, DIPARTIMENTO DELLE SCIENZE BIOMEDICHE, ODONTOIATRICHE E DELLE IMMAGINI MORFOLOGICHE E FUNZIONALI, MESSINA, Italia⁽²⁾ - Scylla Biotech, srl, Scylla Biotech, srl, MESSINA, Italia⁽³⁾

The interferon-beta/MLKL axis promotes anti-bacterial defenses by enhancing neutrophil-mediated pathogen killing

AGATA FAMA¹, GERMANA LENTINI¹, GIUSEPPE V. DE GAETANO¹, FRANCESCO COPPOLINO², GIUSEPPE TETI³ AND CONCETTA BENINATI^{1,3}

1. Department of Human Pathology, University of Messina, Messina, Italy;

2. Department of Biomedical, Dental and Imaging Sciences, University of Messina, Messina, Italy;

3. Scylla Biotech Srl, Messina, Italy

Introduction. The type I interferon (IFN I) system, which has long been recognized as the mainstem of anti-viral defenses, plays a crucial role also in bacterial infections, but the underlying mechanisms are complex and ill understood. We have previously reported that IFN-beta is produced early during bacterial infection and potently limits in vivo pathogen outgrowth. We analyze here the interrelationships between IFN-beta and necroptosis, an important form of programmed cell death. We find that IFN- beta promotes bacterial killing in neutrophils by mechanisms requiring the pseudokinase MLKL, the essential executioner of necroptotic cell death, but not its upstream activator RIPK3. **Material and Methods.** Wild type C57Bl6 mice or knock-out (KO) animals lacking IFN-beta, the RIPK3 kinase or the MLKL pseudokinase were challenged subcutaneously with *Staphylococcus aureus* USA 300. Biopsies were taken from the diseased skin and analyzed for bacterial and inflammatory cell numbers and for cytokine concentrations. Neutrophils were isolated from the bone marrow of these mice and tested for their ability to kill *S. aureus* in vitro. **Results.** Bacterial numbers were considerably higher in skin lesions from mice lacking IFN-beta or MLKL, than in those of wild type mice. The susceptibility to infection of IFN-beta or MLKL KO animals approached that of severely immunodeficient MyD88 ko mice used as positive controls. These changes were associated with reduced neutrophil infiltration and cytokine levels at the infection sites in IFN-beta, but not MLKL, ko mice. In contrast, RIPK3 ko mice were resistant to staphylococcal infection. Immunodeficiency was completely rescued by administration of recombinant IFN-beta in IFN-beta ko animals, but only partially in MLKL ko mice. MLKL expression was considerably decreased in IFN-beta KO macrophages and neutrophils, which was reversed by treatment with recombinant IFN-beta. Both IFN-beta and MLKL ko neutrophils showed a markedly decreased ability to kill *S. aureus* in vitro. **Discussion and Conclusions.** These data unveil an unexpected role of MLKL in promoting neutrophil-dependent bacterial killing. Since IFN-beta production upregulates MLKL expression, it is likely that the antibacterial activities of the cytokine are at least in part mediated by MLKL. In addition, IFN-beta promotes cytokine/chemokine production and neutrophil recruitment in a MLKL-independent fashion. Surprisingly, no effects on neutrophil bactericidal activity or resistance to

infection were detected in animals lacking RIPK3, the classical upstream MLKL activator in the necroptotic pathway. The latter data can be explained by effects of RIPK3 that are independent from its ability to activate MLKL, but further studies will be needed to clarify this point.

95 - Kinetics and quality of humoral immune response against SARS-CoV-2 after vaccination and natural infection

SARA CAUCCI⁽¹⁾ - ROBERTA LONGO⁽¹⁾ - IRENE CORI⁽¹⁾ - BENEDETTA CORVARO⁽¹⁾ - ANNA VALENZA⁽²⁾ - KATIA MARINELLI⁽²⁾ - STEFANO MENZO⁽¹⁾

Dipartimento di scienze biomediche e sanità pubblica, Università politecnica delle Marche, Ancona, Italia⁽¹⁾ - Laboratorio di virologia, Azienda ospedaliero universitaria delle Marche, Ancona, Italia⁽²⁾

Kinetics and quality of humoral immune response against SARS-CoV-2 after vaccination and natural infection.

SARA CAUCCI¹, ROBERTA LONGO¹, CORI IRENE¹, BENEDETTA CORVARO¹, ANNA VALENZA², KATIA MARINELLI², AND STEFANO MENZO^{1,2}

¹ Department of Biomedical Sciences and Public Health, Università Politecnica delle Marche, Ancona, Italy;

² Virology Laboratory, Azienda Ospedaliero Universitaria delle Marche, Ancona, Italy;

Introduction Immunity developed through natural infection and/or vaccination is essential for combating COVID-19 at both personal and population level. It has been demonstrated that repeated antigenic stimulus improves both quantity and quality of the specific response. However, the duration and kinetics of neutralizing activity through natural and vaccine-acquired immunity remain unknown. We analyzed the data obtained in the context of the recent pandemic through the Omicron wave in Italy.

Materials and Methods The isolation of 10 different lineages of SARS-CoV-2 and neutralization assays were performed on Vero E6 cells, as well as plate microneutralization assays. 4 groups of subjects were compared: subjects receiving 3 doses of vaccine and naïve for the infection (NV3); subjects receiving 3 vaccine doses and subsequently infected with different Omicron variants (V3I_BA.1, V3I_BA.2, V3I_BA.5). Sera were collected 20, 120 and 180 days after the last immunization (vaccine or infection).

Results Sera from V3I displayed a significantly enhanced neutralizing power against all lineages compared to NV3. This difference is statistically significant against lineages most refractory to neutralization, notably beta, delta, or omicron. By contrast, no significant differences were observed in terms of antibody binding activity (BAU/ml). To assess the role of antibody affinity maturation in the efficacy of neutralization, we divided the neutralizing titer by BAU/ml as a measure of specific Neutralizing Efficacy (NE). This index was greater in V3I compared to NV3 independently of the omicron variant infecting patient. To understand the role of the anamnestic response underpinning this improvement, we compared the binding and neutralizing activity of V3I as a function of time elapsed between the last vaccine dose and natural infection. Interestingly, the titers rose as a function of time elapsed between the last vaccination and infection. In addition, results demonstrated a steeper reduction in neutralizing and binding activity in NV3 than V3I in a 180-day follow-up.

Discussion and Conclusions Mutations accumulating in the spike protein confer the potential to evade humoral immunity from prior infections or vaccinations in vitro and in vivo. The affinity maturation, enhanced by repeated immunological stimuli over an extended period of time, is a fundamental contributor to the increased breadth of neutralizing power. The rapidly decaying trend of neutralization and binding activity of SARS-CoV-2 anti-spike antibodies from subjects vaccinated with 3 doses in the absence of natural infection underlines the importance of periodical vaccination,

especially in immunocompromised patients. Future studies should concentrate also on cell-mediated immune responses.

101 - A novel subset of IFN γ -producing Th17 cells is selectively associated with intestinal inflammation in Crohn's Disease and specifically activated by adherent-invasive Escherichia coli (AIEC)

MOIRA PARONI⁽¹⁾ - GABRIELLA LECCESE⁽¹⁾ - VALERIA RANZANI⁽²⁾ - DANIELE NOVIELLO⁽³⁾ - MAURIZIO VECCHI⁽³⁾ - FEDERICA FACCIOTTI⁽⁴⁾ - JENS GEGINAT⁽²⁾ - FLAVIO CAPRIOLI⁽³⁾

Università degli studi di Milano, Dipartimento di Bioscienze, Milano, Italia⁽¹⁾ - INGM, Istituto Nazionale di Genetica Molecolare, milano, Italia⁽²⁾ - Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Gastroenterology and Endoscopy Unit, Milano, Italia⁽³⁾ - University of Milano-Bicocca, Italy, Department of Biotechnology and Bioscience, Milano, Italia⁽⁴⁾

Maira Paroni^{1*}, Gabriella Leccese¹, Valeria Ranzani², Daniele Noviello³, Maurizio Vecchi³, Federica Facciotti⁴, Jens Geginat² and Flavio Caprioli³.

¹ Department of Biosciences, Università degli Studi di Milano, Milan, Italy

² INGM-National Institute of Molecular Genetics "Romeo ed Enrica Invernizzi", Milan, Italy

³ Gastroenterology and Endoscopy Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy Department of Experimental Oncology, European Institute of Oncology, Milan, Italy

⁴ Department of Biotechnology and Bioscience, University of Milano-Bicocca, [Milan,Italy

A novel subset of IFN γ -producing Th17 cells is selectively associated with intestinal inflammation in Crohn's Disease and specifically activated by adherent-invasive Escherichia coli (AIEC)

INTRODUCTION: Th17 cells are found enriched in inflamed gut lesions of Crohn's disease (CD) patients. Several evidences indicate that Th17 cells may have both a protective role in the gut but also a more pro-inflammatory function. Indeed, in response to specific polarizing cytokines and pathogenic stimuli, Th17 cells can transdifferentiate into IFN γ -producing Th17 cells acquiring a more pathogenic profile. Here, we aimed to identify the specific immunological and transcriptomic profile of gut-resident IFN γ -producing Th17 cells, as well as the role in the CD pathogenesis and specific gut microbiota components that induce their activation.

METHODS: IFN γ -producing Th17 cells as well conventional Th17 cells from CD patients and controls were phenotypically and functionally analyzed by RNA sequencing and flow cytometry. They were analyzed for responses to CD-relevant antigens with the antigen-specificity assay and monitored in CD patients before and after treatment with antibodies that neutralize TNF α (Infliximab), integrin $\alpha4\beta7$ (Vedolizumab), or IL-23p19 (Risankizumab).

RESULTS: Th17 cells expressing CCR5 (pTh17) co-produced the highest levels of IFN γ /IL-17 but were distinct from established Th1/17 cells. pTh17 cells were associated with intestinal inflammation in CD, but not with UC. Conversely, Th1/17 cells were not increased in inflamed compared to non-inflamed lamina propria of CD or non-IBD patients. Biological therapies differentially affected intestinal pTh17 cell frequency: whether Infliximab significantly reduced gut-infiltrating pTh17 in CD responder patients, Vedolizumab only impaired gut migration of cTh17 cells but did not reduce intestinal-resident pTh17 cells. Notably, pTh17 cells transdifferentiated in-vitro from conventional

cTh17 in the presence of IL-23. Consistently, intestinal pTh17 cells were specifically and strongly reduced after Risankizumab therapy in CD patients. Importantly, intestinal pTh17 cells were selectively activated by adherent-invasive *Escherichia coli* (AIEC) strains, but not by a commensal *E. coli* strain. In contrast, Th1/17 cells were selectively activated by Cytomegalovirus.

CONCLUSIONS: We identified a previously uncharacterized subset of human intestinal IFN γ -producing Th17 cells, whose generation was promoted by IL-23 in vitro and in vivo. These pTh17 cells produced high levels of pro-inflammatory cytokines, were selectively associated with intestinal inflammation in CD, and responded to CD-associated AIEC strains, suggesting a key pathogenic role in CD. Finally, besides the possibility to use pTh17 cells as a diagnostic or predictive biomarker, they also represent an important novel target for the development of new therapeutic strategies aimed at selectively blocking the harmful signaling pathways driving intestinal chronic inflammation in CD.

109 - Anti-HBc loss in multiple sclerosis patients treated with ocrelizumab

PATRIZIA PASCULLI⁽¹⁾ - MARIA ANTONELLA ZINGAROPOLI⁽¹⁾ - FEDERICA DOMINELLI⁽¹⁾ - EEEVA TORTELLINI⁽¹⁾ - MATTEO TARTAGLIA⁽²⁾ - GINA FERRAZZANO⁽³⁾ - ANTONELLA CONTE⁽³⁾ - CLAUDIO MARIA MASTROIANNI⁽¹⁾ - MARIA ROSA CIARDI⁽¹⁾

Policlinico Umberto I, Department of Public Health and Infectious Diseases, Sapienza University of Rome, Roma, Italia⁽¹⁾ - Policlinico Umberto I, Department of Human Neuroscience, Sapienza University of Rome, Viale dell'Università, Italia, Italia⁽²⁾ - Policlinico Umberto I, Department of Human Neuroscience, Sapienza University of Rome, Viale dell'Università, Roma, Italia⁽³⁾

Anti-HBc loss in multiple sclerosis patients treated with ocrelizumab

Patrizia Pasculli¹, Maria Antonella Zingaropoli¹, Federica Dominelli¹, Eeva Tortellini¹, Matteo Tartaglia², Gina Ferrazzano², Antonella Conte², Claudio Maria Mastroianni¹, Maria Rosa Ciardi¹

¹Department of Public Health and Infectious Diseases, Sapienza University of Rome, Viale del Policlinico 155, 00185, Rome, Italy.

²Department of Human Neuroscience, Sapienza University of Rome, Viale dell'Università 30, 00185, Rome, Italy.

Background

Hepatitis B virus (HBV) reactivation during immunosuppressive/immunomodulatory therapy is still a hot topic worldwide. Both hepatitis B surface antigen (HBsAg) patients and subjects with serological signs of previous resolved exposure to the virus (HBsAg negative/anti-core antibody positive patients) are at risk of HBV reactivation. In case of serological signs of current or past HBV infection, appropriate therapeutic or preventive strategies are suggested, according to the risk of HBV reactivation by immunosuppressive/immunomodulatory drugs and liver status.

Anti-HBc antibodies, together with HBsAg, play an important role in identifying patients with a need for a more intensive follow-up or a prophylaxis against HBV reactivation, especially as anti-HBc antibodies are considered to persist lifelong. Although the usefulness and dependability of anti-HBc antibodies as a marker for previous or ongoing HBV infection has been well documented, cases of HBV reactivation lacking detectable anti-HBc antibodies have been described. Furthermore, a few cases with HBV viremia and the absence of anti-HBc antibodies have been described in blood donors.

Objective: In a 4-years follow-up, people with MS were longitudinally evaluated each 6 months during DMTs, focusing on HBV reactivation with HBV-DNA and HBV surface antigen periodic assessment.

Methods: Serological parameters such as HBsAg, anti-HBs, and anti-HBc (IgM and IgG), were periodically measured by chemiluminescence immunoassays. HBV-DNA was investigated by real-time PCR in each subject showing previous resolved HBV exposure. A pre-emptive strategy was chosen to manage people with previous resolved exposure to the virus

Results: In this four-year observational cohort study, we collected real-world data on the infectious risk before starting or switching or during DMT in people with MS patients. Two hundred and twenty-seven people with MS were enrolled (135 women, 92 men) with a median age of 49 [41-59]. Among them, 80 patients were ocrelizumab-treated. At baseline 3 patients candidates to ocrelizumab treatment, were HBsAg+ with HBV-DNA detectable and started antiviral therapy before starting DMTs; 18 patients were anti-HBc+. No patients with anti-HBc+ showed a detectable HBV-DNA and all started DMT. During DMTs, two ocrelizumab-treated patients with HBsAg- and anti-HBc+ developed HBV reactivation.

After 4 years of follow-up, 2 of 18 patients with anti-HBc+ treated with ocrelizumab, lost Hbc antibodies.

Conclusion: Screening of infectious diseases in DMT candidate MS patients helps to mitigate the infectious risk and choose the most appropriate treatment in MS management. HBV screening should be performed at the beginning to know patient's serological status to decide the most appropriate therapy and/or follow-up for MS patient. Moreover, as in solid organ transplant recipients, MS patients treated with anti-CD20 can lose anti-HBc maybe due to an inefficient immune response.

Permanent or intermittent anti-HBc loss is common in immunocompromised hosts (i.e hematologic/oncologic patients), few data on its clinical impact are available. To date, no data on people with MS and permanent or intermittent anti-HBc loss were reported.

119 - Infection of macrophages by SARS-CoV-2: implications for viral spreading and immune response

LUCIA SIGNORINI⁽¹⁾ - STEFANO GIANOLI⁽²⁾ - MARIA DOLCI⁽¹⁾ - KELVIN KAMAU MAINA⁽¹⁾ - MASSIMO LOCATI⁽²⁾ - SERENA DELBUE⁽¹⁾

University of Milan, Department of Biomedical, Surgical and Dental Sciences, University of Milan, Milan, Italy, Milano, Italia⁽¹⁾ - University of Milan, Milan, Italy, Department of Medical Biotechnologies and Translational Medicine, Milano, Italia⁽²⁾

Infection of macrophages by SARS-CoV-2: implications for viral spreading and immune response

LUCIA SIGNORINI¹, STEFANO GIANOLI², MARIA DOLCI¹, KELVIN KAMAU MAINA¹, MASSIMO LOCATI^{2,3}, SERENA DELBUE¹

¹Department of Biomedical, Surgical and Dental Sciences, University of Milan, Milan, Italy;

²Department of Medical Biotechnologies and Translational Medicine, University of Milan, Milan, Italy;

³IRCCS Human Research Hospital, Rozzano, Italy.

Introduction: Macrophages are crucial components of the innate immunity, which represent the first line of defense to pathogens. In case of infection, they are responsible for early pathogen recognition, initiation and resolution of inflammation, as well as repair of tissue damage. Considering their ability to regulate the production of cytokines and growth factors in inflammatory conditions, they are likely involved in the cytokine storm characterizing the severe COVID-19. A clear understanding on how macrophages directly sense and how react to SARS-CoV-2 is crucial to better decipher COVID-19 pathogenesis.

Materials and Methods: Monocytes were isolated from buffy coats of healthy donors and differentiated to macrophages (MDMs) ex vivo. ACE2 transcript and protein levels were assessed in resting MDMs and in MDMs directly exposed in vitro to SARS-CoV-2. Quantitative real time PCR for genomic and subgenomic viral sequences, plaque assay and immunofluorescence analysis were performed to define viral entry and replication kinetics. An automated multiplex immunoassay system was employed to analyze pro-inflammatory and anti-inflammatory mediators. An ACE2 inhibition assay was performed using a blocking anti-ACE2 monoclonal antibody on MDMs and conventional epithelial cells.

Results: Analysis of ACE2 expression demonstrated a low level of transcript corresponding to detection at the membrane level in only a fraction (40%) of MDMs. Infection assays demonstrated that MDMs are susceptible to infection but not permissive for viral replication. Immunoassay analysis evidenced an active and direct response of MDMs exposed to SARS-CoV-2. Interestingly, this response included the release of both pro-inflammatory and anti-inflammatory mediators, but not interferon-inducing cytokines neither type-1 nor type-2 interferons. ACE2 blockade assays had marginal effect on the infection process as anti-ACE2 monoclonal antibodies efficiently blocked viral infection in conventional epithelial cells but had no effect in MDMs.

Discussion and Conclusions: We demonstrated that MDMs express low levels of the conventional viral entry protein ACE2, with evidence for an ACE2-independent entry pathway. Though macrophages cannot be exploited as a spreading platform by the virus, still after SARS-CoV-2 engagement they proved to be promptly activated, with the production of cytokines known to be involved in the cytokine storm that characterizes the most severe clinical manifestations of COVID-19. On the opposite, macrophages were not supportive to the prototypical interferon-mediated antiviral response as neither type-I nor type-II interferon responses were evident.

122 - Questioning the role of fibroblast growth factor-2 during Zika virus infection and infection by other viruses.

GIULIA LOTTINI⁽¹⁾ - ERIKA PLICANTI⁽¹⁾ - MATTEO MATTEUCCI⁽²⁾ - MICHELE LAI⁽²⁾ - MAURO PISTELLO⁽²⁾ - GIULIA FREER⁽²⁾

University of Siena, Department of Medical biotechnologies, Siena, Italia⁽¹⁾ - University of Pisa, Department of Translational Research, Pisa, Italia⁽²⁾

Questioning the role of fibroblast growth factor-2 during Zika virus infection and infection by other viruses.

GIULIA LOTTINI^{1, 2}, ERIKA PLICANTI^{1, 2}, MATTEO MATTEUCCI¹, MICHELE LAI¹, MAURO PISTELLO¹, GIULIA FREER¹,

¹Dept. Translational Research, Retrovirus Center, University of Pisa, Pisa, Italy;

²Dept. Medical Biotechnologies, University of Siena, Siena, Italy.

1.Introduction

Zika virus (ZIKV) is a mosquito-borne viral infection that was first identified in the Zika Forest of Uganda in 1947. It belongs to the Flaviviridae family and is primarily transmitted by Aedes mosquitoes, which are also responsible for the spread of dengue and chikungunya viruses. ZIKV gained international attention in recent years due to outbreaks in various regions, including South and Central America, the Caribbean, and Southeast Asia. We recently showed that the presence of fibroblast growth factor (FGF)-2 affects defined consequences of ZIKV infection, such as the exit of FOXG1, a nuclear transcription factor, from the nucleus early in infection. FGFs are a group of proteins that play a vital role in cellular growth, development, and tissue regeneration. They are part of the signaling pathway responsible for regulating cell proliferation, differentiation, and survival. FGFs are produced by a wide range of cell types, including fibroblasts, endothelial cells, and immune cells, and they bind to specific receptors on the surface of target cells to trigger downstream signaling events. In this work, we shed light into the relationship between FGF-2 and ZIKV infection, as compared to other viruses.

2.Materials and Methods

A549 were infected with ZIKV, Usutu, Chikungunya and Herpes simplex-2, or transfected with 200 ng/ml of poly I:C, a synthetic analog of double-stranded RNA as immunostimulant and, as a control, with an expression plasmid. Both supernatant and cell lysates were collected 24, 48, 72 and 96 hours following infection. Western blot analysis was performed to confirm viral infection and to analyse FGF-2 and RIG-1 expression, in a time-dependent manner. Moreover, FGF-2 concentration in infected cells supernatants were quantified using ELISA assay.

3.Results

We questioned whether FGF-2 is secreted differentially following different insults and viral infection. In addition, we investigated whether the presence of FGF-2 in cell medium impinges on viral yield and replication. Lastly, we performed experiments to clarify if a correlation of FGF-2 secretion in the medium with the expression of RIG-1 during infection exists by detection of RIG-1 in infected cell lysates and FGF-2 in supernatants by ELISA.

4. Discussion and Conclusions

FGF-2 is secreted differentially following different insults and viral infection. Differently from what reported in the literature, our data do not point to a role of FGF-2 in promoting viral replication.

Future experiments will clarify if FGF-2 secretion occurs as consequence of specific infections or stimulation of cell innate immunity.

134 - HERV-K Antibodies and their Potential Protective Effect in Amyotrophic Lateral Sclerosis

ELENA RITA SIMULA⁽¹⁾ - MARTA GARCIA-MONTOJO⁽²⁾ - AVINDRA NATH⁽²⁾ -
LEONARDO ANTONIO SECHI⁽¹⁾

Università degli Studi di Sassari, Dipartimento di Scienze Biomediche, Sassari, Italia⁽¹⁾ - National Institutes of Health, National Institute of Neurological Disorders and Stroke, Bethesda, Stati Uniti D'america⁽²⁾

HERV-K Antibodies and their Potential Protective Effect in Amyotrophic Lateral Sclerosis

ELENA R. SIMULA¹, MARTA GARCIA-MONTOJO³, AVINDRA NATH³, LEONARDO A. SECHI^{1,2}

¹Department of Biomedical Sciences, Division of Microbiology and Virology, University of Sassari, Sassari, Italy;

²Struttura Complessa Microbiologia e Virologia, Azienda Ospedaliera Universitaria Sassari, Sassari, Italy;

³National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, Maryland, USA.

Introduction. Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disease characterized by the loss of upper and lower motor neurons. Numerous factors have emerged as potential contributors to the development of ALS. Recent studies, backed by mounting supporting evidence, have substantiated the role of Human Endogenous Retroviruses (HERVs) in the pathogenesis of ALS. HERVs constitute about 8% of the human genome and consist of retroviral genomic sequences integrated into the germ line million years ago. The investigation of the role of HERVs in the pathogenesis of ALS originated with the evidence of reverse transcriptase activity in the brain, blood, and cerebrospinal fluid of individuals with ALS. Increased levels of HERV-K expression have been detected in postmortem brain samples from ALS. In a Sardinian cohort of ALS patients, higher levels of antibodies (Abs) directed against specific epitopes of the envelope (env) protein were observed compared with control populations. These Abs showed a positive correlation with Abs against TDP-43, a protein identified as a player in ALS pathophysiology.

Materials and Methods. The analysis of Abs targeting the HERV-K-env involved two methods: epitope mapping using a peptide array and an ELISA assay. A total of 242 healthy donors (HD), 243 ALS patients, and 85 multiple sclerosis (MS) individuals were included in the study. Additionally, the extracellular levels of HERV-K were assessed using dPCR.

Results. Compared with HD, ALS subjects exhibited significantly higher recognition of HERV-K env peptides by Abs in their sera. ALS patients also showed increased levels of HERV-K than HD, along with higher levels of Abs to a specific HERV-K-env peptide (HERV-K₍₁₉₋₃₇₎), exceeding those of HD and individuals with MS. Specifically, 55.14% of ALS patients, compared with 21.16% of HD and 13.10% of individuals with MS, had Abs against the HERV-K peptide. Serum HERV-K extracellular DNA levels ($p = 0.02$) and the number of HERV-K env peptides recognized by ALS sera ($p = 0.02$) were correlated with disease duration. Among ALS patients, lower levels of HERV-

K Abs were associated with a definitive diagnosis based on EL Escorial criteria ($p = 0.03$), as well as reduced predicted ($p = 0.02$) and observed ($p = 0.03$) survival.

Discussion and Conclusion. These results suggest a distinct Ab response targeting env-specific epitopes of HERV-K in ALS patients and HD, indicating epitope spreading. In addition, reduced levels of Abs to HERV-K env in ALS are linked to a worse prognosis and a lower likelihood of survival. Ongoing studies aim to elucidate the molecular mechanisms involved in the regulation of HERV-K and the potential impact of its reactivation on key proteins associated with the pathology.

136 - Cerebrospinal fluid and plasma biomarkers in patients with central nervous system infections: a retrospective study

MARIA ANTONELLA ZINGAROPOLI ⁽¹⁾ - PATRIZIA PASCULLI ⁽¹⁾ - FEDERICA DOMINELLI ⁽¹⁾ - FEDERICA CICCONE ⁽¹⁾ - MATTEO TARTAGLIA ⁽²⁾ - GINA FERRAZZANO ⁽²⁾ - TIZIANA LATRONICO ⁽³⁾ - MIRIAM LICHTNER ⁽⁴⁾ - ANTONELLA CONTE ⁽²⁾ - GIOACCHINO GALARDO ⁽⁵⁾ - GRAZIA MARIA LIUZZI ⁽³⁾ - CLAUDIO MARIA MASTROIANNI ⁽¹⁾ - GUIDO ANTONELLI ⁽⁶⁾ - OMBRETTA TURRIZIANI ⁽⁶⁾ - MARIA ROSA CIARDI ⁽¹⁾

Rome University of Rome, Department of Public Health and Infectious Diseases, Rome, Italia ⁽¹⁾ - Sapienza University of Rome, Department of Human Neurosciences, Rome, Italia ⁽²⁾ - University of Bari Aldo Moro, Department of Biosciences, Biotechnologies and Biopharmaceutics, Bari, Italia ⁽³⁾ - Sapienza University of Rome, Department of Neurosciences Mental Health and Sensory Organs, Rome, Italia ⁽⁴⁾ - Sapienza University of Rome, Medical Emergency Unit, Policlinico Umberto I, Rome, Italia ⁽⁵⁾ - Sapienza University of Rome, Department of Molecular Medicine, Rome, Italia ⁽⁶⁾

Cerebrospinal fluid and plasma biomarkers in patients with central nervous system infections: a retrospective study

Maria Antonella Zingaropoli¹, Patrizia Pasculli¹, Federica Dominelli¹, Federica Ciccone¹, Matteo Tartaglia², Gina Ferrazzano², Tiziana Latronico³, Miriam Lichtner⁴, Antonella Conte², Gioacchino Galardo⁵, Grazia Maria Liuzzi³, Claudio Maria Mastroianni¹, Guido Antonelli⁶, Ombretta Turriziani⁶, Maria Rosa Ciardi¹

¹Department of Public Health and Infectious Diseases, Sapienza University of Rome – Rome

²Department of Human Neurosciences, Sapienza University of Rome - Rome

³Department of Biosciences, Biotechnologies and Biopharmaceutics, University of Bari Aldo Moro - Bari

⁴Department of Neurosciences Mental Health and Sensory Organs, Sapienza University of Rome - Rome

⁵Medical Emergency Unit, Policlinico Umberto I - Sapienza University of Rome- Rome

⁶Department of Molecular Medicine - Sapienza University of Rome - Rome

Background: Analysis of CSF and plasma biomarkers could be helpful to differentiate specific CNS condition and setting an appropriate therapy. A major advance in the field of neurology has been the development of blood-based biomarkers.

Materials and Methods: Patients presenting with signs and symptoms were enrolled if, before receiving a diagnostic lumbar puncture, signed a written informed consent. We retrospective analyzed CSF and plasma levels of several biomarkers of neuronal damage (NfL), astrocyte damage (GFAP), monocyte/macrophage activation (sCD163 and sCD14) and blood-brain barrier permeability (MMP-9 and TIMP-1).

Results: Eight-four subjects were included. Patients were affected by herpesvirus encephalitis (21/84), neuro-COVID (17/84), progressive multifocal leukoencephalopathy (PML, 14/84), HIV-associated neurocognitive disorders (13/84), bacterial meningitis (7/84), infection by unknown pathogens (6/84) and subjects without CNS involvement (control group, 6/84).

Overall, positive correlation between CSF and plasma levels of NfL was found ($\rho=0.8290$, $p<0.0001$) as well as between CSF and plasma levels of GFAP ($\rho=0.5752$, $p<0.0001$). Both CSF NfL and GFAP levels were positive correlated with CSF levels of sCD163 ($\rho=0.5730$, $p<0.0001$ and $\rho=0.4177$, $p=0.0007$) as well as with CSF TIMP-1 levels ($\rho=0.6286$, $p<0.0001$ and $\rho=0.5952$, $p<0.0001$). In CSF, levels of monocyte/macrophage activation markers were positive correlated to plasma NfL levels (sCD163: $\rho=0.5213$, $p=0.0026$; sCD14: $\rho=0.4992$, $p=0.0250$). Finally, CSF TIMP-1 levels were positive correlated with plasma NfL levels ($\rho=0.4075$, $p=0.0282$) and plasma GFAP levels ($\rho=0.4180$, $p=0.0300$).

Compared to control group, bacterial meningitis and PML groups showed higher CSF NfL levels ($p=0.0091$ and $p=0.0003$, respectively). Higher CSF GFAP levels in PML and HIV-associated neurocognitive disorder groups compared to control group were observed ($p=0.0003$ and $p=0.0390$, respectively). CSF GFAP levels were higher in herpesvirus encephalitis ($p=0.0071$), neuro-COVID ($p=0.0155$), HIV-associated neurocognitive disorder ($p=0.0003$) and bacterial meningitis ($p=0.0005$) compared to control group. Otherwise, CSF sCD14 levels were higher only in bacterial meningitis compared to control group ($p=0.0172$). CSF MMP-9 levels were higher in bacterial meningitis and PML groups ($p=0.0145$ and $p=0.0321$, respectively) compared to control group. Finally, in bacterial meningitis group higher levels of CSF TIMP-1 were found ($p=0.0005$).

Conclusion: Our data showed that CSF and plasma biomarkers of neuronal and astrocyte damage or inflammation may vary during CNS infections according to different causative agents. The observed correlations between CSF and plasma samples underscore the possibility of measuring CNS markers in an easily accessible source such as blood. The prognostic value of these biomarkers needs to be assessed in prospective studies.

139 - Evaluation of LIAISON® MeMed BV® to discriminate bacterial and viral infections in patients with Chronic Obstructive Pulmonary Disease

ALESSANDRO GRAZIANI⁽¹⁾ - MARIA CRISTINA BITONTE⁽¹⁾ - ERICA GIOMBETTI⁽²⁾ - ALFREDO VILLA⁽²⁾ - ANTONELLA MENCACCI⁽¹⁾ - BARBARA CAMILLONI⁽¹⁾

Università degli Studi di Perugia, Dipartimento di Medicina e Chirurgia, Perugia, Italia⁽¹⁾ - Azienda Ospedaliera di Perugia, Laboratorio di Patologia Clinica, Perugia, Italia⁽²⁾

Evaluation of LIAISON® MeMed BV® to discriminate bacterial and viral infections in patients with Chronic Obstructive Pulmonary Disease

ALESSANDRO GRAZIANI¹, MARIA C. BITONTE¹, ERICA GIOMBETTI², ALFREDO VILLA², ANTONELLA MENCACCI¹, BARBARA CAMILLONI¹

¹ Department of Medicine and Surgery, Microbiology and Clinical Microbiology Section, "Santa Maria Della Misericordia" Hospital, University of Perugia, 06129 Perugia, Italy;

² Clinical Pathology and Haematology Laboratory, "Santa Maria Della Misericordia" Hospital, 06129 Perugia, Italy

Introduction

Chronic obstructive pulmonary disease (COPD), the third leading cause of death, is a lung progressive inflammatory disease causing restriction airflow and breathing problems. The acute episodes are treated with corticosteroids and antibiotics. Microbiological tests could take hours or days before giving a result and in this period, antibiotics can be used improperly favoring the phenomenon of antibiotic resistance. In this study we want to investigate the use of the kit LIAISON® MeMed-BV® (DiaSorin, Saluggia, Italy) in patient with COPD to discriminate bacterial and viral infection to treat it properly and promptly.

Materials and Methods

Serum samples were collected from hospitalized patients with and without COPD and selected based on a confirmed bacterial or viral infection. MeMed-BV is an automated in vitro diagnostic semi-quantitative assay that uses chemiluminescent immunoassay (CLIA) technology to measure three non-microbial host proteins (TRAIL, IP-10, and CRP) to differentiate bacterial from viral infections. The test provides a score from 0 (viral infection) to 100 (bacterial infection) calculated through an algorithm after the dosage of the three proteins.

Results

A total of 99 samples collected from 60 males and 39 females have been analyzed. The mean age of study population is 75.7 years old (range 36-96). The average time from symptoms onset to collection was 2.9 days. The study population was divided in two groups: 48 patients with COPD and 51 without COPD. Gold standard methods provide a diagnosis of viral infection in 46 patients (47%), bacterial infection in 42 patients (42%) and 11 (11%) co-infections. The samples from 53 patients with bacterial or co-infections have been classified as HighBACT or ModBACT in 85% of cases, 5 samples were indeterminate (9%) and 3 were classified as ModVIR (6%). The samples of 46 patients with viral infection have been classified as HighVIR or ModVIR in 43% of cases, indeterminate in 9 samples (20%) and 17 samples (37%) resulted as ModBACT or HighBACT. Excluding the 14 samples with an "indeterminate" result, the overall agreement was 76%. Comparing the two groups, the agreement was 74% and 78% for group with or without COPD, respectively.

Discussion and Conclusions

Our data show that MeMed-BV immunoassay has demonstrated acceptable analytical performance and is reliable for distinguishing viral and bacterial infections or co-infections in COPD patients, typically characterized by overprescribing antibiotics when bacterial and viral pathogens are difficult to differentiate. The test in just 40 minutes provides a result which guides the clinician in choosing the most appropriate therapy, a fundamental approach in modern antibiotic stewardship projects that are currently being developed.

143 - Association of human endogenous retroviruses with immune dysfunction and development, progression and long-term sequelae of COVID-19.

VITA PETRONE⁽¹⁾ - ANTONELLA MINUTOLO⁽¹⁾ - MARIALAURA FANELLI⁽¹⁾ - CHRISTIAN MARACCHIONI⁽¹⁾ - MARTINA GIUDICE⁽¹⁾ - ROSSELLA CHIRICO⁽¹⁾ - CHIARA CIPRIANI⁽¹⁾ - LUIGI COPPOLA⁽²⁾ - ELISABETTA TETI⁽²⁾ - CHIARA SORACE⁽²⁾ - MARTA ZORDAN⁽²⁾ - PIETRO VITALE⁽¹⁾ - VINCENZO MALAGNINO⁽²⁾ - MARCO IANNETTA⁽²⁾ - HERVE PERRON⁽³⁾ - SANDRO GRELLI⁽⁴⁾ - EMANUELA BALESTRIERI⁽¹⁾ - LOREDANA SARMATI⁽²⁾ - CLAUDIA MATTEUCCI⁽¹⁾

Università degli studi di Roma "Tor Vergata", Dipartimento di Medicina Sperimentale, ROMA, Italia⁽¹⁾ - POLICLINO TOR VERGATA, Malattie Infettive, Roma, Italia⁽²⁾ - GENEURO, Geneuro Innovation, Lyon, Francia⁽³⁾ - POLICLINO TOR VERGATA, Unità di Virologia, Roma, Italia⁽⁴⁾

Association of human endogenous retroviruses with immune dysfunction and development, progression and long-term sequelae of COVID-19.

VITA PETRONE¹, ANTONELLA MINUTOLO¹, MARIALAURA FANELLI¹, CHRISTIAN MARACCHIONI¹, MARTINA GIUDICE¹, ROSSELLA CHIRICO¹, CHIARA CIPRIANI¹, LUIGI COPPOLA², ELISABETTA TETI², CHIARA SORACE², MARTA ZORDAN^{2,3}, PIETRO VITALE^{2,3}, VINCENZO MALAGNINO^{2,3}, MARCO IANNETTA^{2,3}, HERVÈ PERRON⁴, SANDRO GRELLI^{1,5}, EMANUELA BALESTRIERI¹, LOREDANA SARMATI^{2,3}, CLAUDIA MATTEUCCI¹.

1. Department of Experimental Medicine, University of Rome Tor Vergata, Rome, 00133, Italy; 2. Infectious Diseases Clinic, Policlinic of Tor Vergata, Rome, 00133, Italy; 3. Department of Systems Medicine, University of Rome Tor Vergata, Rome, 00133, Italy; 4. Geneuro – Innovation, Lyon-France 69008; 5. Virology Unit, Policlinic of Tor Vergata, Rome, 00133, Italy.

Introduction: The activation of Human Endogenous Retroviruses (HERVs) in response to infectious agents has been described. In particular, we already demonstrated HERVs implications during SARS-CoV-2 infection and in COVID-19. Considering the complex alterations of the immune response in the pathogenesis of COVID-19 and the persistence of this dysregulation in individuals with post-acute sequelae of SARS-CoV-2 infection (PASC), the aim of this study was the evaluation of HERVs in the immune-pathogenesis of COVID-19 and in post infection alterations. **Materials and Methods:** Blood samples were collected at Tor Vergata University Hospital of Rome from Acute COVID-19 patients (COV) and Healthy Donors (HD). Blood from Post-Acute Sequelae of SARS- CoV-2 individuals (PASC) was also collected (range: 7-48 weeks post-infection). The expression of the envelope (ENV) of HERV-K and HERV-W have been analyzed by flow cytometry and correlated with clinical signs, immunophenotyping, inflammatory markers, and disease progression. The T cell differentiation markers were also analysed. **Results:** HERV-W and HERV-K ENV proteins have been found expressed in blood samples from COV but not in HDs. Despite the percentage of Lymphocytes recovery, even after several weeks' post-infection the expression of HERVs remained elevated in PASC. Among leukocytes, lymphocytes displayed the highest percentage of HERV-W ENV positive cells in COV, which correlated with T cell differentiation, exhaustion, and senescence markers. Instead, HERV-K ENV resulted in highly expressed in granulocytes and directly correlates with

senescence markers CD57. HERV-W ENV positive CD4⁺ T cells significantly correlated with coagulopathy and biochemical parameters associated with COVID-19 severity. In PASC, HERVs protein expression remained high, especially in granulocytes. Notably, despite a restore of the normal distribution of leukocytes subpopulation in PASC, the percentage of CD4 naïve cells and CD8 terminal effector memory resulted altered suggesting a persistent immune dysfunction in PASC. To date, long-term health problems, including neurological symptoms such as headache, fatigue, dizziness, memory loss, confusion, and difficulty focusing, are associated with post-COVID-19 infection. Notably, HERVs expression was found modified in patients with specific neurological symptoms such as paraesthesia and tremors, suggesting their involvement in neurological alterations related to COVID-19. Discussion and Conclusion: These data suggest HERVs as contributing factors in the immune dysfunction and development, progression, and long-term complications of COVID-19 describing disease evolution and opening avenues for novel therapeutic strategies for personalized medicine.

Funded by HERVCOV project (GA101057302) HORIZON-HLTH-2021-DISEASE-04 Personalised medicine and infectious diseases: understanding the individual host response to viruses.

156 - Differential type I interferon expression between children and adolescents with long covid

MATTEO FRACELLA⁽¹⁾ - FEDERICA FRASCA⁽¹⁾ - LEONARDO SORRENTINO⁽¹⁾ - ALESSANDRA D'AURIA⁽¹⁾ - LETIZIA SANTINELLI⁽²⁾ - LUCA MADDALONI⁽²⁾ - GINEVRA BUGANI⁽²⁾ - CHIARA VIRIGILLITO⁽²⁾ - ENRICA MANCINO⁽³⁾ - GRETA DI MATTIA⁽³⁾ - MARIA GIULIA CONTI⁽³⁾ - LUIGI MATERA⁽³⁾ - BENIAMINO CAPUTO⁽²⁾ - GABRIELLA D'ETTORRE⁽²⁾ - FABIO MIDULLA⁽³⁾ - GUIDO ANTONELLI⁽¹⁾ - ALESSANDRA PIERANGELI⁽¹⁾ - CAROLINA SCAGNOLARI⁽¹⁾

Sapienza - University Of Rome, Virology Laboratory, Department Of Molecular Medicine, Rome, Italia⁽¹⁾ - Sapienza - University Of Rome, Department Of Public Health And Infectious Diseases, Rome, Italia⁽²⁾ - Sapienza - University Of Rome, Department Of Pediatrics And Infantile Neuropsychiatry, Rome, Italia⁽³⁾

Differential Type I Interferon Expression Between Children And Adolescents With Long Covid

MATTEO FRACELLA¹, FEDERICA FRASCA¹, LEONARDO SORRENTINO¹, ALESSANDRA D'AURIA¹, LETIZIA SANTINELLI², LUCA MADDALONI², GINEVRA BUGANI², CHIARA VIRIGILLITO², ENRICA MANCINO³, GRETA DI MATTIA³, MARIA GIULIA CONTI³, LUIGI MATERA³, BENIAMINO CAPUTO², GABRIELLA D'ETTORRE², FABIO MIDULLA³, GUIDO ANTONELLI¹, ALESSANDRA PIERANGELI¹, CAROLINA SCAGNOLARI¹.

1department Of Molecular Medicine, Virology Laboratory, "Sapienza" University, Rome, Italy;

2department Of Public Health And Infectious Diseases, "Sapienza University", Rome, Italy;

3department Of Pediatrics And Infantile Neuropsychiatry, "Sapienza" University, Rome, Italy.

Introduction

SARS-CoV-2 mostly causes mild or no symptoms in children. Nevertheless, increasing evidence show that distinct immune-pathological changes remain weeks or months after the infection, leading to long COVID (LC). To gain insight on the interplay between LC manifestations and innate immunity, we evaluated type I Interferon (IFN-I) mRNA expression in peripheral blood mononuclear cells (PBMCs) collected 3-6 months after SARS-CoV-2 infection from children and adolescents who have exhibited LC symptoms (LC), compared to age- and gender-matched SARS-CoV-2 recovered subjects without LC (MC) and healthy controls (HC).

Materials and Methods

Blood samples were collected from children/adolescents attending Umberto I hospital of Rome, within 3-6 months after a SARS-CoV-2 positive test and from control children. From PBMCs, RNA was extracted for determining cellular gene expression (IFN-Alpha, IFN-Beta, IFN-Epsilon, IFN-Omega, IFN-AR1/-2) by RealTime-PCR assays, normalized to the housekeeping GUS gene. To account for multiple and potentially confounding variables the analysis was done using multivariate regression models.

Results

28 patients (M 12.5y SD 3.0) with LC symptoms, 28 patients (M 11.8y SD 3.0) without LC symptoms and 18 children/adolescents never SARS-CoV-2 infected (M 10.5y SD 3.1) were enrolled. Among adolescents (13-17 years of age), increased amount of IFN-Alpha, IFN-Beta and IFN-Omega mRNAs

in LC cases were found compared to MC, while the opposite was observed for children aged 6-12 years ($p<0.05$). Of note, LC adolescents who experienced neurological symptoms showed significantly higher levels of IFN-Alpha/-Beta with respect to age-matched MC ($p<0.05$). Further, LC adolescents had significantly higher IFN-Beta, IFN-Epsilon and IFN-Omega values compared to HC ($p<0.05$); this scenario was once more reversed in children.

Discussion and Conclusions

Our findings lead to the hypothesis that the IFN-I pathway is involved to immune-pathological changes seen in children and adolescents 3-6 months following recovery from symptomatic SARS-CoV-2 infection. We also found age-related differences in IFN-I gene expression, with upregulation of IFN-I genes in adolescents and down regulation of expression in children. LC adolescents have an immune landscape similar to that reported in adults, where IFN-I activation seems to play a role in developing LC manifestations, especially for neurological symptoms. On the other hand, the IFN-I signatures in LC children, characterized by downregulation, operates through still unknown mechanisms. Overall, these investigations provide novel insights into the pathogenesis of pediatric long COVID and may help in devising immunotherapeutic strategies to reduce the impact of COVID-19 pandemic on children's health.

163 - Phosphatidylserine liposomes as a possible candidate for the therapeutic management of Mycobacterium abscessus infection

NOEMI POERIO⁽¹⁾ - TOMMASO OLIMPIERI⁽¹⁾ - GRETA PONSECCHI⁽¹⁾ - FABIANA CICIRIELLO⁽²⁾ - VINCENZINA LUCIDI⁽²⁾ - MARCO MARIA D'ANDREA⁽¹⁾ - MAURIZIO FRAZIANO⁽¹⁾

University of Rome "Tor Vergata", Department of Biology, Rome, Italia⁽¹⁾ - Bambino Gesù Pediatric Hospital, Department of Pediatric Medicine, Cystic Fibrosis Complex Operating Unit, Rome, Italia⁽²⁾

Phosphatidylserine liposomes as a possible candidate for the therapeutic management of Mycobacterium abscessus infection

NOEMI POERIO¹, TOMMASO OLIMPIERI¹, GRETA PONSECCHI^{1,3}, FABIANA CICIRIELLO², VINCENZINA LUCIDI², MARCO M. D'ANDREA¹, MAURIZIO FRAZIANO¹

¹Dept. of Biology, University of Rome "Tor Vergata", Rome, Italy;

²Department of Pediatric Medicine, Cystic Fibrosis Complex Operating Unit, Bambino Gesù Pediatric Hospital, Rome, Italy.

³PhD Program in Evolutionary Biology and Ecology, Dept. of Biology, University of Rome "Tor Vergata", Rome, Italy.

Introduction: we have previously reported that phosphatidylserine liposomes (PS-L) were capable of inducing a significant antimycobacterial response in Mycobacterium tuberculosis (MTB)-infected and MTB/HIV-1-coinfected macrophages. Moreover, the treatment was associated with a reduction of both proinflammatory response and HIV replication in coinfecting cells. Here, we have in vitro assessed the therapeutic value of PS-L against Mycobacterium abscessus (Mab), a non-tubercular mycobacterium which represents a relevant cause of morbidity and mortality in vulnerable patients such as those affected with cystic fibrosis (CF).

Materials and methods: differentiated THP-1 cells (dTHP-1), used as a model of human macrophages, and primary macrophages derived from CF patients, were in vitro infected with Mab and then stimulated or not with PS-L. The therapeutic value of the treatment was assessed in terms of intracellular mycobacterial viability, by CFU assay, and NF-κB activation (calculated as the ratio between total NF-κB and phosphorylated NF-κB) by NF-κB p65 (Total/Phospho) Human InstantOne™ ELISA Kit (Invitrogen).

Results: results show that in vitro stimulation with PS-L of both dTHP-1 cells and CF macrophages, in vitro infected with Mab, significantly reduces intracellular mycobacterial viability. Additionally, PS-L treatment on dTHP-1 cells infected with Mab resulted in a significant drop in NF-κB activation.

Discussion and conclusions: although preliminary, these results extend our previously reported results obtained on MTB-infected and MTB/HIV-coinfected cells, also against a relevant difficult-to-treat non tubercular mycobacterium, such as Mab, and support the therapeutic exploitation of PS-L as host directed therapy to simultaneously limit potentially pathogenetic proinflammatory response and intracellular Mab viability in CF patients.

184 - Yet another potential microbial player in autoimmunity: multicentric analysis of the association between *Mycoplasma hominis* serostatus and rheumatoid arthritis

ANTONELLA CONGIARGIU (1) - NICIA DIAZ (1) - NGOC DAN THANH PHAN (2) - CIRIACO CARRU (1) - LEONARDO ANTONIO SECHI (1) - PAOLA RAPPELLI (1) - PIER LUIGI FIORI (1) - ALBERTO ALBERTI (3) - GIANLUCA ERRE (4) - DANIELE DESSI (1)

Università degli Studi di Sassari, Dipartimento di Scienze Biomediche, Sassari, Italia (1) - Hue University of Medicine and Pharmacy, Department of Parasitology Hue University of Medicine and Pharmacy, Hue, Vietnam (2) - Università degli Studi di Sassari, Dipartimento di Medicina Veterinaria, Sassari, Italia (3) - Università degli Studi di Sassari, Dipartimento di Medicina, Chirurgia e Farmacia, Sassari, Italia (4)

Yet another potential microbial player in autoimmunity: multicentric analysis of the association between *Mycoplasma hominis* serostatus and rheumatoid arthritis

ANTONELLA CONGIARGIU 1, NICIA DIAZ 1, NGOC DAN THANH PHAN 2, CIRIACO CARRU 1, LEONARDO A. SECHI 1, PAOLA RAPPELLI 1, PIER L. FIORI 1, ALBERTO ALBERTI 3, GIANLUCA ERRE 4, DANIELE DESSI 1

1 Department of Biomedical Sciences, University of Sassari, Sassari, Italy;

2 Department of Parasitology Hue University of Medicine and Pharmacy, Hue, Vietnam;

3 Department of Veterinary Medicine, University of Sassari, Sassari, Italy;

4 Department of Medicine, Surgery and Pharmacy, School of Medicine, University of Sassari, Sassari, Italy

Introduction

Due to their minimal genomes, mycoplasmas evolved an obligate parasitic lifestyle. This strict dependence on the host has led mycoplasmas to develop mechanisms promoting the establishment of chronic infections, including host immunity modulation. *Mycoplasma hominis* is a commensal bacterium of humans which can be associated with a number of urogenital conditions. While many aspects of *M. hominis* host-parasite relationship have been elucidated, its potential involvement as a hypothetical trigger of autoimmune processes has been seldom addressed. Since *M. hominis* is a potent inducer of Neutrophil Extracellular Traps, an innate immune mechanism of response to infections which has been recently associated with rheumatoid arthritis (RA) onset and progression, we designed a serological multicentric study aimed at assessing the prevalence of anti-*M. hominis* antibodies (Abs) in a group of RA patients as compared to a matched healthy control.

Materials and Methods

A total of 108 sera from RA patients from 2 different settings (42 sera from Hue University Hospital, Vietnam; 66 from AOU, Sassari, Italy) were tested by ELISA for the presence of anti-*M. hominis* Abs, as compared with a healthy control population (HC). Specificity of ELISA immunoreactivity was confirmed with immunoblot experiments.

Results

Anti-*M. hominis* antibodies were detected in RA patients' sera as compared to a HC population, with significant differences in both optical densities and Ab detection between the two groups (overall 75% vs 41%; p value $<0,0001$). The specificity of the immune reaction was shown by means of

western blot experiments, showing the binding of RA sera-derived Abs to *M. hominis* antigens. No immune reactivity was detected in ELISA-negative sera.

Discussion and Conclusions

RA is a systemic inflammatory disease of autoimmune origin, in which genetic background of patients is of paramount importance. However, genetic factors alone do not fully explain RA pathogenesis, and the contribution of several environmental factors including mucosal microbial infections has gained growing attention. In light of this, we investigated the potential role of *M. hominis* by assessing the prevalence of anti-*M. hominis* Abs in a group of RA patients from 2 distinct settings. ELISA experiments showed that positivity to *M. hominis* antigens clustered significantly with RA patients' sera (75% vs 41%). Interestingly, we couldn't observe any significant differences in *M. hominis* serostatus between italian and vietnamese groups. These data suggest a hypothetical role of *M. hominis* as a trigger in RA pathogenesis. Whether RA and *M. hominis* serostatus are actually linked by a cause-effect relationship need more research aimed at elucidating the potential contribution of *M. hominis* to RA.

199 - Evaluation of the immunomodulatory activity of Cellulophaga species LPSs.

GIUSEPPE MANTOVA⁽¹⁾ - ELENA SCAGLIONE⁽²⁾ - MARTINA DI ROSARIO⁽¹⁾ - CIRO VECE⁽¹⁾ - ROBERTA CIRELLA⁽³⁾ - MOLLY D. PITHER⁽³⁾ - CHIARA PAGLIUCA⁽¹⁾ - FLAVIANA DI LORENZO⁽⁴⁾ - ALBA SILIPO⁽⁴⁾ - ANTONIO MOLINARO⁽⁴⁾ - MARIA TERESA VITILIELLO⁽²⁾ - ROBERTA COLICCHIO⁽²⁾ - PAOLA SALVATORE⁽⁵⁾

University of Naples Federico II, Department of Molecular Medicine and Medical Biotechnology, Naples, Italia⁽¹⁾ - University of Naples Federico II, Dpt of Mol Med and Med Bio; Dpt of Int Act of Lab Med and Transf, Complex Oper Unit of Clinical Microbi, Naples, Italia⁽²⁾ - University of Naples Federico II, Department of Chemical Sciences, Naples, Italia⁽³⁾ - University of Naples Federico II, Department of Chemical Sciences; Task Force on Microbiome Studies, Naples, Italia⁽⁴⁾ - University of Naples Federico II, Dpt of Mol Med and Med Bio; Dpt of Int Act of Lab Med and Transf, Unit of Clinical Microbi; Task Force Microbiome; CEINGE Franco Salvatore, Naples, Italia⁽⁵⁾

Evaluation of the immunomodulatory activity of Cellulophaga species LPSs.

GIUSEPPE MANTOVA¹, ELENA SCAGLIONE^{1,2}, MARTINA DI ROSARIO¹, CIRO VECE¹, ROBERTA CIRELLA³ MOLLY D. PITHER³, CHIARA PAGLIUCA¹, FLAVIANA DI LORENZO^{3,4}, ALBA SILIPO^{3,4}, ANTONIO MOLINARO^{3,4}, MARIA TERESA VITIELLO^{1,2}, ROBERTA COLICCHIO^{1,2}, PAOLA SALVATORE^{1,2,4,5}

¹Department of Molecular Medicine and Medical Biotechnology, University of Naples Federico II, Naples, Italy; ²Department of Integrated Activity of Laboratory Medicine and Transfusion, Complex Operative Unit of Clinical Microbiology, University Hospital Federico II, Naples, Italy; ³Department of Chemical Sciences, University of Naples Federico II, Naples, Italy; ⁴Task Force on Microbiome Studies, University of Naples Federico II, Naples, Italy, ⁵CEINGE, Advanced Biotechnologies Franco Salvatore s.c.ar.l., Naples, Italy

Introduction: Gram-negative bacteria are widely distributed in marine environments, as free-living entities or in symbiotic relationships. To thrive in these diverse habitats, marine Gram-negative bacteria have developed adaptive strategies, including the modification in the composition of their cell envelope components, such as Lipopolysaccharides (LPSs). LPSs are significant constituents of the outer membrane in Gram-negative bacteria. The glycolipid component of LPS, known as lipid A, activates the mammalian innate immune receptor complex myeloid differentiation protein-2 (MD-2)/Toll-like receptor 4 (TLR4) and triggers an inflammatory response. This study aimed to evaluate the immunomodulatory activity of LPSs from three bacterial species belonging to Cellulophaga genus (*C. algicola* ACAM 630, *C. baltica* NNO 15840 and *C. tyrosinoydans* EM41) using engineered human TLR4 and TLR2 reporter cells (HEK-Blue hTLR4; HEK-Blue hTLR2). **Materials and Methods:** HEK-Blue hTLR4 cells were seeded in 96-well plates (3×10^5 cells per well) and stimulated with different concentrations (1, 10, and 100 ng/mL) of LPS from *C. algicola*, *C. baltica*, *C. tyrosinoydans*, or *S. typhimurium* SH 2201 LPS for 18 hours. The evaluation of NF- κ B activation served as the readout for this assay. HEK-Blue hTLR4 cells express secreted embryonic alkaline phosphatase (SEAP) upon NF- κ B activation, which can be detected using an alkaline phosphatase substrate (QUANTI-Blue). Similarly, HEK-Blue hTLR2 cells were exposed to

the same concentrations of *Cellulophaga* species and *S. typhimurium* LPSs for 18 hours. Additionally, Pam3CSK4 (500 ng/mL) was included as the positive control for HEK-Blue hTLR2 cells. Results: LPSs from *C. baltica* and *C. tyrosinioxidans* showed significantly lower NF- κ B activation compared to *S. typhimurium* LPS, while no significant difference was observed between *C. algicola* LPS and *S. typhimurium* LPS. HEK-Blue hTLR2 cells did not show NF- κ B activation with both *Cellulophaga* LPSs and *S. typhimurium* LPS. Discussion and Conclusions: The immunomodulatory impact of *Cellulophaga* species on the human innate immune system in vitro indicated a weaker engagement of TLR4/MD-2/CD14 by *C. tyrosinioxidans* and *C. baltica* LPSs compared to *S. typhimurium* LPS. However, no statistical significance was observed between *S. typhimurium* LPS and *C. algicola* LPS. In the future, it would be intriguing to assess the ability of *Cellulophaga* LPS to inhibit the anti-inflammatory action of *Salmonella* LPS through co-treatment and evaluate the subsequent release of pro-inflammatory factors. This research could provide insights into the potential role of *Cellulophaga* species in modulating the inflammatory response, offering new perspectives in manipulating bacterial interactions for therapeutic purposes.

245 - Regulation of permissiveness/restriction to herpes simplex virus 1 infection in different cell types by cellular microRNAs

AGNESE RAGNO (1) - DOMENICO GIOSA (1) - VALERIA STEFANIZZI (2) - ANTONIO MASTINO (3) - FRANCESCA MARINO-MERLO (1)

Department of Chemical, Biological, Pharmaceutical, and Environmental Sciences,, University of Messina, MESSINA, Italia (1) - Department of Chemical Science and Technology, University of i Rome “Tor Vergata”, ROMA, Italia (2) - The Institute of Translational Pharmacology, CNR, ROMA, Italia (3)

Regulation of permissiveness/restriction to herpes simplex virus 1 infection in different cell types by cellular microRNAs

AGNESE RAGNO¹, DOMENICO GIOSA¹, VALERIA STEFANIZZI², ANTONIO MASTINO³, FRANCESCA MARINO-MERLO¹

¹Department of Chemical, Biological, Pharmaceutical, and Environmental Sciences, University of Messina, Messina, Italy; ²Department of Chemical Science and Technology, University of Rome “Tor Vergata”, Rome, Italy; ³The Institute of Translational Pharmacology, CNR, Rome, Italy.

1. Introduction. MicroRNAs (miRNAs) are noncoding RNAs that block translation or promote degradation of target messenger RNAs in approximately 30% of the protein-coding genes in humans. Based on virus-ligand/cell-receptor interactions, a wide range of cell types, including those of the adaptive and innate immunity, can be infected by herpes simplex viruses 1 (HSV-1). However, a fully permissive replication cycle can be, normally, completed by HSV-1 only in epithelial or neural cells. Complex mechanisms controlling the balance permissiveness/restriction of HSV-1 infection in different cell types, have not been fully elucidated.

2. Materials and Methods. A preliminarily in silico analysis for selecting cellular miRNAs with high score of predictability to regulate permissiveness/restriction to HSV-1, was performed using two tools. One software analysis matched data from a miRNAs array performed in two U937 cell lines with a different restriction state to HSV-1 due to functional or non-functional NF-kB activation and data on miRNA targeting innate-response-related genes from databases. The other software analysis utilized data from databases to predict the interaction of cellular miRNAs with HSV-1 transcription products. To validate the in silico generated data, expressions levels of the selected miRNAs were detected by qRT-PCR in cells infected with HSV-1 under different permissiveness/restriction conditions.

3. Results. Based on the arbitrary scores obtained from in silico analysis miR-99a-5p and miR-762 were selected for validation by qRT-PCR. The miR-99a-5p was highly expressed in HSV-1-infected U937 cells with non-functional NF-kB activation, with respect to mock-infected cells, while quite equally in mock-infected and HSV-1-infected U937 cells with functional NF-kB activation. This indicate an NF-kB-dependent involvement of miR-99a-5p in regulation of restriction to HSV-1 in monocytic cells. Regarding miR-762, expression following HSV-1 infection with respect to mock-infected cells was inversely correlated with the cell permissiveness state in different cell types, thus indicating a possible involvement of this miRNA in regulation of permissiveness/restriction to HSV-1 infection.

4. Discussion and Conclusions.

HSV-1 diverged from its ancestor at the same time as humans diverged from their primate ancestor. Consequently, an extremely efficient balance between HSV-1 and the host innate response has been established during the human/virus coevolution to limit virus replication in cell types where the virus could cause irreparable damage. Results of this study account for the possible involvement of two, previously unconsidered, cellular miRNAs in the regulation of the permissiveness/ restriction of HSV-1 infection in different cell types.

250 - The viral sensor IFI16 at the crossroads between damage and tolerance during human Coronavirus infection

CINZIA BORGOGNA⁽¹⁾ - ILARIA CISLAGHI⁽¹⁾ - SARAH TURATI⁽¹⁾ - GLORIA GRIFFANTE⁽¹⁾ - SHIKA CHANDEL⁽¹⁾ - IRENE LO CIGNO⁽¹⁾ - MARCO DE ANDREA⁽²⁾ - LUCIA SIGNORINI⁽³⁾ - SERENA DELBUE⁽³⁾ - MARISA GARIGLIO⁽¹⁾

Università Piemonte Orientale, Dip. Medicina Traslazionale, Novara, Italia⁽¹⁾ - Università Studi di Torino, Dip. Salute Pubblica e Scienze Pediatriche, Torino, Italia⁽²⁾ - Università di Milano, Dip. Scienze Biomediche, Chirurgiche ed Odontoiatriche, Milano, Italia⁽³⁾

The viral sensor IFI16 at the crossroads between damage and tolerance during human Coronavirus infection

Cinzia BORGOGNA¹, Ilaria CISLAGHI¹, Sarah TURATI¹, Gloria GRIFFANTE¹, Shika CHANDEL¹, Irene LO CIGNO¹, Marco DE ANDREA^{2,3}, Lucia SIGNORINI⁴, Serena DELBUE⁴, Marisa GARIGLIO^{1,2}.

¹ Virology Unit, Department of Translational Medicine, University of Piemonte Orientale, Novara, Italy.

² CAAD Center for Translational Research on Autoimmune and Allergic Disease, Novara, Italy.

³ Viral Pathogenesis Unit, Department of Public Health and Pediatric Sciences, University of Turin, Turin, Italy.

⁴ Biomedical, Surgical and Dental Sciences, University of Milan, Milano, Italy.

Introduction. Bats possess a unique ability to asymptotically host zoonotic viruses like SARS-CoVs, thanks to their unique ability to efficiently regulate host immune responses to viral infections. Intriguingly, bats harbour genetic or functional loss of several proteins involved in innate immunity, including the PHYN gene family that comprises the interferon-inducible gene IFI16. The evolutionary loss of the PYHIN family in bats highlights that an impaired innate immune system might contribute to their resilience against pathogenic viruses. Thus, we propose that IFI16 might have a role in the human coronavirus (hCoV) replication and host innate response.

Materials and methods. Our infection model consists of two bat-derived coronaviruses NL63 (low pathogenic) and SARS-CoV-2 (highly pathogenic). As cell model, we use the monkey epithelial cell line LLC-MK2 because it is naturally infected by both NL63 and SARS-CoV-2 with a good infection efficiency and it displays a functional antiviral response upon exogenous RNA sensing. Through the CRISPR-Cas9 gene editing technology, we successfully generated LLC-MK2 cells with the IFI16 gene knocked out (LLC-MK2 IFI16 KO), as well as a transfected control LLC-MK2 cell line (LLC-MK2 TC). Viral replication in these cells have been assessed by measuring the levels of genomic and subgenomic viral RNAs (vRNA) by qPCR. IFI16 downstream signalling in hCoV-infected cells have been dissected using qPCR, immunoblot, immunoprecipitation, immunofluorescence, and ELISA.

Results. Immunofluorescence analyses have revealed the translocation of the IFI16 protein from the nucleus to the cytoplasm in infected cells and its colocalization with the viral nucleoprotein (NP). Additionally, through immunoprecipitation assays, we have also observed that IFI16 can directly interact with both NP and vRNA. We have also found that IFI16 restricts hCoV replication as determined by plaque assays and quantification of viral gene expression by qPCR. Surprisingly, we

have also observed an enhancement of the innate immune response in IFI16KO, including IL-6 production, that we are currently investigating in more details.

Discussion and Conclusions. We show that the IFI16 protein is able to restrict hCoV replication and it is involved in host-virus interaction and control of inflammation.

255 - Effects of Ropivacaine and Propofol on Human endogenous retroviruses activity, embryonic genes, CD-133 and PI3K-AKT-mTOR signaling in head-neck carcinoma cell lines: a proof-of-concept study providing

MARTINA GIUDICE⁽¹⁾ - VITA PETRONE⁽¹⁾ - MARIALAURA FANELLI⁽¹⁾ - ERMAL PASHAJ⁽²⁾ - ROSSELLA CHIRICO⁽¹⁾ - FILADELFO CONIGLIONE⁽³⁾ - ANTONELLA MINUTOLO⁽¹⁾ - PAOLA SINIBALDI-VALLEBONA⁽¹⁾ - CLAUDIA MATTEUCCI⁽¹⁾ - CHIARA CIPRIANI⁽¹⁾ - EMANUELA BALESTRIERI⁽¹⁾

University of Rome Tor Vergata, Department of Experimental Medicine, Rome, Italia⁽¹⁾ - Catholic University'Our Lady of Good Council', Department of Surgery, Tirana, Albania⁽²⁾ - Catholic University'Our Lady of Good Council', Department of Surgical Science, Tirana, Albania⁽³⁾

Effects of Ropivacaine and Propofol on Human endogenous retroviruses activity, embryonic genes, CD-133 and PI3K-AKT-mTOR signaling in head-neck carcinoma cell lines: a proof-of-concept study providing potential implications in the clinical setting.

Martina Giudice¹, Vita Petrone¹, Marialaura Fanelli¹, Ermal Pashaj², Rossella Chirico¹, Filadelfo Coniglione^{3,4}, Antonella Minutolo¹, Paola Sinibaldi-Vallebona¹, Claudia Matteucci¹, Chiara Cipriani¹, Emanuela Balestrieri¹.

1. Department of Experimental Medicine, University of Rome Tor Vergata, Rome, 00133, Italy.

2. Department of Surgery at Catholic University'Our Lady of Good Council', Tirana, 1000, Albania.

3. Department of Surgical Science, Catholic University'Our Lady of Good Council', Tirana, 1000, Albania.

4. Department of Clinical Science and Translational Medicine, University of Rome Tor Vergata, Rome, 00133, Italy.

Introduction: In our previous studies, an aberrant activation of Human Endogenous Retroviruses (HERVs) closely related to stemness and aggressiveness features of cancer cells has been described in different tumour cell lines. HERVs are genetic elements originating from ancestral infection within the germ line cells by exogenous retroviruses, currently making up about 8% of the human genome. Although HERVs are generally silenced, peculiar HERV copies can be activated by different environmental stimuli, leading to the expression of immunopathogenic proteins. Based on the evidence that the use of anaesthetic agents in the surgical management of head and neck cancer (HNC) can influence the risk of cancer recurrence in either a beneficial or detrimental way, the present study aimed to figure out any effects of in vitro treatment with two anaesthetics (Propofol and Ropivacaine) on the activity of HERVs, embryonic genes, and PI3K/AKT/mTOR pathway in HNC cell lines.

Materials and Methods: The head-neck hypopharyngeal squamous carcinoma cell line (FaDu), the squamous carcinoma cell line (SCC-25) and the oral adenosquamous carcinoma cell line (CAL-27) were treated for 48 hours with different concentrations of Propofol and Ropivacaine. The relative

expression of HERV-K, HERV-H, OCT4, NANOG, SOX2, KFL4 CD133 and PI3K/AKT/mTOR pathway was assessed by RT-Real time PCR.

Results: The gene expression analysis indicates that Ropivacaine induced the expression of HERV-K in FaDu and SCC-25 cell lines, whereas Propofol reduced its expression. An opposite expression pattern was observed in CAL-27 cells, since Propofol induced the expression of HERV-K. HERV-H expression was reduced only in FaDu cell line by Propofol. Concerning the embryonic genes, in FaDu cells, the expression of OCT4, NANOG and CD133 was induced by Ropivacaine and a decreasing trend in the expression of the same genes was observed upon treatment with Propofol, while in CAL-27, both anaesthetics induced the expression of SOX-2. Concerning PI3K/AKT/mTOR pathway, in FaDu cells the exposure to anaesthetics resulted in the modulation of PTEN expression that was reduced by Ropivacaine and increased by Propofol, while TSC1 was reduced by exposure to Propofol. In CAL-27 cell line, the in vitro treatments with Propofol induced the expression of PIK3CB, AKT, mTOR, RPS6KB1, EIF4EBP1 and EIF4E while reduced PTEN expression levels.

Discussion and Conclusions: This in vitro study provides new knowledge concerning the expression profile of HERVs, embryonic genes and PI3K/AKT/mTOR pathway in head-neck carcinoma cell lines indicating a cell- and concentration-dependent effect of anaesthetics, that could be useful to identify potential implications in the clinical setting.

256 - Preliminary evidence of the differential expression of Human endogenous retroviruses in Kawasaki disease and SARS-CoV-2-associated multisystem inflammatory syndrome.

CHIARA CIPRIANI⁽¹⁾ - MARTINA GIUDICE⁽¹⁾ - ELENA CORINALDESI⁽²⁾ - VITA PETRONE⁽¹⁾ - MARIALAURA FANELLI⁽¹⁾ - MARIANNA FABI⁽³⁾ - MARCELLO LANARI⁽³⁾ - ANTONELLA MINUTOLO⁽¹⁾ - SANDRO GRELLI⁽¹⁾ - PAOLA SINIBALDI-VALLEBONA⁽¹⁾ - CLAUDIA MATTEUCCI⁽¹⁾ - EMANUELA BALESTRIERI⁽¹⁾

University of Rome Tor Vergata, Department of Experimental Medicine, Rome, Italia⁽¹⁾ - Ramazzini Hospital, Pediatric Unit, Carpi, Italia⁽²⁾ - IRCCS University Hospital of Bologna, Pediatric Emergency Unit, Bologna, Italia⁽³⁾

Preliminary evidence of the differential expression of Human endogenous retroviruses in Kawasaki disease and SARS-CoV-2-associated multisystem inflammatory syndrome.

Chiara Cipriani¹, Martina Giudice¹, Elena Corinaldesi², Vita Petrone¹, Marialaura Fanelli¹, Marianna Fabi³, Marcello Lanari³, Antonella Minutolo¹, Sandro Grelli^{1,4}, Paola Sinibaldi-Vallebona¹, Claudia Matteucci¹, Emanuela Balestrieri¹

1. Department of Experimental Medicine, University of Rome Tor Vergata, Rome, 00133 Italy. 2. Pediatric Unit, Ramazzini Hospital, Carpi, Mo, 41121 Italy. 3. Pediatric Emergency Unit, IRCCS University Hospital of Bologna, Bologna, 40138 Italy. 4. Virology Unit, Policlinic of Tor Vergata, Rome, 00133, Italy;

Introduction: Some severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) positive children are at risk for developing a post-infectious complication called multisystem inflammatory syndrome in children (MIS-C) with some clinical features overlap with Kawasaki disease (KD). Our research group and others have highlighted that the spike protein of SARS-CoV-2 can trigger the activation of Human Endogenous retroviruses (HERVs), which in turn can induce inflammatory and immune reactions, suggesting HERVs as contributing factors in COVID-19 immunopathology. HERVs are genetic elements originating from ancestral infection within the germ line cells by exogenous retroviruses. Although HERVs are generally silenced, peculiar HERV copies can be activated by different environmental stimuli, leading to the expression of immunopathogenic proteins. With the aim to identify new factors that may contribute to the immunopathogenic processes underlying KD and MIS-C, we analysed the expression of HERVs, HERV-related genes and immune mediators in children during acute and subacute phases compared with COVID paediatric patients and healthy controls (HC).

Materials and Methods: We conducted a multicenter prospective study (October 2020-June 2021) including children with KD, MIS-C and acute COVID-19 (COV) at the IRCCS- St. Orsola University Hospital (Bologna, Italy) and Ramazzini Hospital (Carpi, Italy). The RT-Real time PCR was performed to evaluate the relative expression of HERV-W ENV, HERV-K, Syn-1, Syn-2, ASCT-1/2, MFSD2A, IL-1beta, IL-6, IL-10, TNF- α , INF- γ , MCP-1, TLR-3, TLR-4 and TLR-9 in blood samples from all patients.

Results: The results showed higher levels of HERV-W, HERV-K, Syn-1, and ASCT-1/2 in KD, MIS-C and COV patients, regardless of the disease phase, while higher levels of Syn-2 and MFSD2A were found only in MIS-C patients, regardless the disease phase. Inflammatory and regulatory cytokines expression is higher in the three diseases considered than in HC, and showed a phase- and disease-dependent trend. Interestingly, in MIS-C patients during the acute phase, negative correlations have been found between HERV-W and IL-10 and between Syn-2 and IL-10, while positive between HERV-K and IL-10. In addition, HERV-W expression positively correlated with CRP, a marker of inflammation.

Discussion and Conclusions: This pilot study supports the role of HERVs in inflammatory diseases and suggesting their interplay with the immune system in this setting. The elevated expression of Syn-2 and MFSD2A seems to be a distinctive trait of MIS-C patients allowing to distinguish them from KD ones. The understanding of pathological mechanisms can lead to the best available treatment for these two diseases limiting complications and serious outcomes.

257 - "REDUCED CLEARANCE OF SARS-COV-2 INFECTION IN IMMUNOCOMPROMISED INDIVIDUALS DESPITE VACCINATION-INDUCED RESPONSE"

PAOLA MAZZETTI⁽¹⁾ - MARIA SIDOTI⁽¹⁾

Università di Pisa, Azienda Ospedaliero-Universitaria Pisana, pisa, Italia⁽¹⁾

Reduced Clearance of SARS-CoV-2 Infection in Immunocompromised Individuals Despite Vaccination-Induced Response

MARIA SIDOTI^{a,b}, PAOLA MAZZETTI^a, PIETRO G. SPEZIA^b, ALESSIA CALÓ^b, ANNALISA CAPRIA^a, GIULIA FREER^b, SILVIA COSTARELLI^c, ALICE CARA^a, ALFREDO ROSELLINI^a, SUSI FRATESCHI^a, GIOVANNA MOSCATO^a, IACOPO FRANCONI^a, MARIA L. VATTERONI^a, MICHELE LAI^b, PAOLA QUARANTA^b, SPARTACO SANI^c, ENRICO TAGLIAFERRI^d, MAURO PISTELLO^{a,b}.

^a Department of Laboratory Medicine, Virology Unit, Pisa University Hospital, Pisa, Italy;

^b Department of Translational Research, Retrovirus Center and Virology Section, University of Pisa, Pisa, Italy;

^c Department of Infectious and Tropical Diseases, Infectious Diseases Unit, Leghorn Hospital, Azienda USL Toscana Nord-Ovest, Leghorn, Italy;

^d Infectious Diseases Unit, Department of Clinical and Experimental Medicine, Infectious Disease Unit, Pisa University Hospital, Pisa, Italy

1. Introduction

Immunocompromised patients are at higher risk for severe COVID -19 and have prolonged shedding of viable SARS-CoV-2 virus from the upper respiratory tract. To investigate whether prior vaccination reduces persistence of infection, a group of immunocompromised patients was examined for the presence of infectious SARS-CoV-2 in nasopharyngeal swabs (NPS) and serologic and cell-mediated immune responses in blood samples.

2. Materials and Methods

NPS samples were inoculated into Vero/TMPRSS2 cells and then monitored for cytopathic effects thereafter. The presence of virus was tested using RT-PCR and antigen assays. Neutralizing antibodies in serum were measured by microtiter plate assay. Serum anti-S RBD and anti-N IgG levels were measured using Abbott Architect® SARS-CoV-2 Ig assays. T-cell responses were measured by ELISPOT on Ficoll-purified peripheral blood mononuclear cells. Whole genome sequencing (WGS) was performed using the Illumina COVIDSeq Assay Kit.

3. Results

In our cohort, which consisted predominantly of immunocompromised patients with oncohematologic diseases, it was observed that, while they showed significant response to vaccination with high serum levels of anti-S IgG and a good T-cell response, they did not appear to respond to natural SARS-CoV-2 infection since no anti-N IgG could be detected in their sera. Furthermore, the patients from whom the virus could be isolated were, in most cases, patients with a poor anti-N T-cell response.

4. Discussion and Conclusions

Our findings highlight that immunocompromised patients exhibit a predominant immune response to vaccination rather than to natural infection. Additionally, the isolation of the virus from NPS holds potential as a valuable marker for assessing clinical outcomes and guiding their clinical management. The characterization of the immunological status of these patients could provide valuable insights for customizing pharmacological treatments aimed at clearing the infection.

273 - Type I and III IFN signature in cells from skin lesions, anal canal brushing and nasopharyngeal swabs of MPXV infected patients

ALESSANDRA D'AURIA⁽¹⁾ - MATTEO FRACELLA⁽¹⁾ - FEDERICA FRASCA⁽¹⁾ - LEONARDO SORRENTINO⁽¹⁾ - LAURA RAIMONDO⁽¹⁾ - LUCA MADDALONI⁽²⁾ - GINEVRA BUGANI⁽²⁾ - LETIZIA SANTINELLI⁽²⁾ - ALESSANDRA PIERANGELI⁽¹⁾ - GABRIELLA D'ETTORRE⁽²⁾ - GUIDO ANTONELLI⁽¹⁾ - MAURO PISTELLO⁽³⁾ - CAROLINA SCAGNOLARI⁽¹⁾

Sapienza University of Rome, Department of Molecular Medicine, Roma, Italia⁽¹⁾ - Sapienza University of Rome, Department of Public Health and Infectious Diseases, Roma, Italia⁽²⁾ - University of Pisa, Department of Biomedicine, Pisa, Italia⁽³⁾

Type I and III IFN signature in cells from skin lesions, anal canal brushing and nasopharyngeal swabs of MPXV infected patients

Alessandra D'Auria1, Matteo Fracella1, Federica Federica1, Leonardo Sorrentino1, Laura Raimondo1, Luca Maddaloni2, Ginevra Bugani2, Letizia Santinelli2, Alessandra Pierangeli1, Gabriella D'Ettore2, Guido Antonelli1, Mauro Pistello3, Carolina Scagnolari1

¹ Department of Molecular Medicine, Laboratory of Virology, Sapienza University of Rome, Rome, Italy; ² Department of Public Health and Infectious Diseases, Sapienza University of Rome, Rome, Italy; ³ Department of Biomedicine, Virology Section and Retrovirus Centre, University of Pisa, Pisa, Italy

1. Introduction

Human-to-human transmission of Monkeypox virus (MPXV) can occur through contact with lesions, body fluids and respiratory droplets of infected people. Several in vitro studies reported that MPXV can replicate in the presence of Interferon (IFN) and antagonize the IFN-mediated antiviral immunity. In order to provide additional insight concerning the viral and host processes that control in vivo the innate immunity to MPXV infection, we investigated the viral load of MPXV and the IFN signature in cells from skin lesions (SL), anal canal brushing (ACB) and nasopharyngeal swabs (NSP) of MPXV-infected male patients.

2. Materials and Methods

Eighteen samples belonging to different anatomical sites were collected from MPXV-infected male patients: SL, ACB and NSP. MPXV-DNA levels were evaluated by Bioperfectus Monkeypox Virus Real Time PCR kit. Gene expression of type I (alpha and beta), type III IFNs (lambda1, lambda2 and lambda3), interferon stimulated genes (ISGs: ISG15, ISG56 and PKR) was measured by quantitative RT-PCR assays.

3. Results

Analysis of the association between IFNs transcript levels and MPXV DNA raw threshold cycle (Ct) values (a low Ct value reflects a high target DNA concentration, i.e. viral load) in SL indicated negative correlation between MPXV-DNA raw Ct and IFN-alpha ($r = -0.471$; $p = 0.049$) and IFN-beta ($r = -0.571$; $p = 0.013$). Furthermore, SL samples were stratified in two groups depending on MPXV-DNA Ct median value (SL with Ct <21, n=4 vs SL with Ct ≥21, n=5). A trend toward increased levels of IFN-I (IFN-alpha and IFN-beta) and PKR, but not those of ISG15 and ISG56, were observed in SL with higher MPXV viral load in comparison to those with the lower one. We also found that MPXV-DNA Ct median value was lower in ACB (CT median value=19.00; IQR=17.00-21.00) in

comparison to SL and NSP (SL=21.00 and NSP=23.50). Examination of IFN-related genes according to the anatomical sites revealed that IFN-alpha, IFN-lambda1 and PKR were highly expressed in ACB compared to the other ones, while IFN-beta levels increase in SL ($p<0.05$). No significant differences were observed for IFN-lambda2, IFN-lambda3, ISG15 and ISG56.

4. Discussion

We showed that the activation of IFN response is associated to higher levels of MPXV viral load. Compared to the other respiratory and skin districts, anal mucosa showed a higher levels of MPXV-DNA and increased expression of IFN genes, suggesting that MPXV may interfere with IFN antiviral activities.

284 - IL-6-mediated paracrine senescence drives the intercellular communication within the HCMV microenvironment in renal epithelial cells

STEFANO RAVIOLA⁽¹⁾ - ANDREA IANNUCI⁽¹⁾ - VALERIA CANEPARO⁽¹⁾ - DAVIDE LACARBONARA⁽¹⁾ - FEDERICA CASTIGLIONI⁽¹⁾ - IRENE LO CIGNO⁽²⁾ - FRANCESCO FAVERO⁽³⁾ - DAVIDE CORÀ⁽³⁾ - VINCENZO CANTALUPPI⁽⁴⁾ - SANTO LANDOLFO⁽⁵⁾ - MARISA GARIGLIO⁽²⁾ - MARCO DE ANDREA⁽⁵⁾

Università del Piemonte orientale, CAAD - Center for Translational Research on Autoimmune and Allergic Disease, Intrinsic Immunity Unit, University of Piemonte Orientale, Novara, Italia⁽¹⁾ - Università del Piemonte orientale, Department of Translational Medicine, Virology Unit, University of Piemonte Orientale, Novara, Italia⁽²⁾ - Università del Piemonte orientale, CAAD - Center for Translational Research on Autoimmune and Allergic Disease, Bioinformatics Unit, University of Piemonte Orientale, Novara, Italia⁽³⁾ - Università del Piemonte orientale, Department of Translational Medicine, Nephrology and Transplantation Unit, University of Piemonte Orientale, Novara, Italia⁽⁴⁾ - Università degli Studi di Torino, Department of Public Health and Pediatric Sciences, Viral Pathogenesis Unit, University of Turin, Torino, Italia⁽⁵⁾

IL-6-mediated paracrine senescence drives the intercellular communication within the HCMV microenvironment in renal epithelial cells

STEFANO RAVIOLA¹, ANDREA IANNUCCI¹, VALERIA CANEPARO¹, DAVIDE LACARBONARA¹, FEDERICA CASTIGLIONI¹, IRENE LO CIGNO², FRANCESCO FAVERO⁴, DAVIDE CORÀ⁴, VINCENZO CANTALUPPI³, SANTO LANDOLFO⁵, MARISA GARIGLIO^{1,2} AND MARCO DE ANDREA^{1,5}

¹CAAD - Center for Translational Research on Autoimmune and Allergic Disease, Intrinsic Immunity Unit, University of Piemonte Orientale, Novara, Italy;

²Department of Translational Medicine, Virology Unit, University of Piemonte Orientale, Novara, Italy;

³Department of Translational Medicine, Nephrology and Transplantation Unit, University of Piemonte Orientale, Novara, Italy;

⁴CAAD - Center for Translational Research on Autoimmune and Allergic Disease, Bioinformatics Unit, University of Piemonte Orientale, Novara, Italy;

⁵Department of Public Health and Pediatric Sciences, Viral Pathogenesis Unit, University of Turin, Turin, Italy.

INTRODUCTION. Human cytomegalovirus (HCMV) is an opportunistic pathogen that can cause severe diseases in immunosuppressed individuals, such as transplant recipients and AIDS patients. To enable replication of its long double-stranded DNA genome, HCMV induces profound changes in cellular homeostasis that may resemble senescence. Senescent cells produce and secrete a complex combination of factors, collectively referred to as the senescence-associated secretory phenotype (SASP), that mediate most of their immune-modulatory effects along with the induction of paracrine senescence. Here, by using renal proximal tubular epithelial cells (RPTECs), a natural setting of HCMV infection and disease, we characterized the occurrence of IL-6-driven paracrine changes conducive to viral-induced senescence (VIS) and ensuing organ damage.

MATERIAL AND METHODS. RPTECs were infected with the HCMV strain TR. The fate of infection was evaluated through time-course morphological analysis, western blotting for viral protein expression, and cytokine release analysis. A transcriptome analysis using SenMayo-enriched terms was also performed to determine the presence of senescence induction, and the senescent profile confirmed using a panel of senescent hallmarks. Finally, IL-6 activity inhibition was performed using the IL-6R inhibitor tocilizumab (TCZ).

RESULTS. We have experimentally demonstrated that RPTECs fully support HCMV replication and undergo a senescence program upon infection that triggers a harmful secretory phenotype with the ensuing induction of paracrine senescence in uninfected surrounding cells, mainly driven by IL-6. Consistently, our transcriptome analysis showed a specific enrichment of the IL-6/JAK-STAT3 signaling pathway only in HCMV-infected RPTECs. When the IL-6R inhibitor TCZ was added in the course of infection, a dramatic decrease in the total number of cells expressing nuclear NF- κ B and the DNA-damage marker γ -H2AX was observed, mainly in the bystander uninfected cells. Moreover, the addition of TCZ to the UVB-inactivated conditioned medium from infected RPTECs significantly affected its capability to trigger senescence when applied to fresh target cells.

DISCUSSION AND CONCLUSION. Altogether, our findings demonstrate that HCMV infection of RPTECs, which are naturally susceptible to infection and associated with virus-related disease in vivo, elicits a senescence-like program characterized by stable growth arrest and secretion of senescence-associated inflammatory cytokines. Intriguingly, this senescence phenotype, mainly driven by IL-6, is passed on to neighboring uninfected kidney tubular cells indicating a paracrine mechanism of disease amplification.

285 - The PYRIN domain of IFI16 drives LPS-induced and sterile toll-like receptor 4-mediated inflammation

DAVIDE LACARBONARA⁽¹⁾ - ANDREA IANNUCCI⁽¹⁾ - VALERIA CANEPARO⁽¹⁾ - STEFANO RAVIOLA⁽¹⁾ - GIORGIA MARTINIELLO⁽¹⁾ - RICCARDO MIGGIANO⁽²⁾ - MARISA GARIGLIO⁽³⁾ - MARCO DE ANDREA⁽⁴⁾

Università del Piemonte Orientale, CAAD - Center for Translational Research on Autoimmune and Allergic Disease, Intrinsic Immunity Unit, University of Piemonte Orientale, Novara, Italia⁽¹⁾ - Università del Piemonte Orientale, Department of Pharmaceutical Sciences, University of Piemonte Orientale, Novara, Italia⁽²⁾ - Università del Piemonte Orientale, Department of Translational Medicine, Virology Unit, University of Piemonte Orientale, Novara, Italia⁽³⁾ - Università di Torino, Department of Public Health and Pediatric Sciences, Viral Pathogenesis Unit, University of Turin, Torino, Italia⁽⁴⁾

The PYRIN domain of IFI16 drives LPS-induced and sterile toll-like receptor 4-mediated inflammation

DAVIDE LACARBONARA¹, ANDREA IANNUCCI¹, VALERIA CANEPARO¹, STEFANO RAVIOLA¹, GIORGIA MARTINIELLO¹, RICCARDO MIGGIANO², MARISA GARIGLIO^{1,3} AND MARCO DE ANDREA^{1,4}

¹CAAD - Center for Translational Research on Autoimmune and Allergic Disease, Intrinsic Immunity Unit, University of Piemonte Orientale, Novara, Italy;

²Department of Pharmaceutical Sciences, University of Piemonte Orientale, Novara, Italy;

³Department of Translational Medicine, Virology Unit, University of Piemonte Orientale, Novara, Italy;

⁴Department of Public Health and Pediatric Sciences, Viral Pathogenesis Unit, University of Turin, Turin, Italy.

INTRODUCTION. The interferon- γ -inducible protein 16 (IFI16) is a nuclear phosphoprotein belonging to the PYHIN family of proteins which acts as DNA sensor and antiviral restriction factor. In addition, IFI16 can be released in the extracellular space upon a variety of stimuli and be found in the sera of patients with autoimmune diseases. We have previously shown that the IFI16 protein, per se or upon binding to LPS from *E. coli* O111:B4 (EB-LPS), acts like DAMP (damage-associated molecular pattern) propagating danger signals and amplifying the secretion of IL-6 and IL-8. However, the exact domain of IFI16 involved in LPS- and sterile toll-like receptor 4-mediated inflammation, as well as the mechanisms of IFI16 release have yet to be identified.

MATERIALS AND METHODS. To define the region of IFI16 responsible for TLR4 activation, we took advantage of antibodies directed against the N- or the C-terminal region of IFI16, along with a panel of IFI16 recombinant domains spanning the whole protein from the N-terminus to the C-terminus that comprises the PYRIN, HINA or HINB domains as well as truncated IFI16 proteins that lack either the PYRIN or HINB domains (IFI16 Δ PYRIN or IFI16 Δ HINB, respectively). Then, human macrophages were stimulated with 10 μ g/ml of IFI16, equimolar concentrations of IFI16 domains or truncated proteins, in presence or absence of the previously described antibodies, and the inflammatory response was evaluated through qPCR or ELISA.

Moreover, pull-down assay and surface plasmon resonance (SPR) experiments were used to confirm the interaction of the single domains to TLR4. Finally, to reveal the nature of the interaction between the PYRIN domain and TLR4, specific point mutations of highly conserved amino acids, as determined by *in silico* analysis, have been performed to assess their involvement in the IFI16/TLR4.

RESULTS. We have demonstrated that IFI16 interacts with TLR4 and LPS through the PYRIN and HINB domains, respectively. Moreover, we have observed that the PYRIN domain of IFI16 is sufficient to induce inflammation in human macrophages. Finally, we have shown that specific point-mutations in the PYRIN domain, involving amino acids highly conserved across the different PYHIN members and absent in other PYRIN-carrying proteins not belonging to the PYHIN family, inhibit the IFI16-induced inflammatory activity.

DISCUSSION AND CONCLUSIONS. Collectively, our findings point to an unprecedented role of the PYRIN domain, which is conserved among the different PYHIN family members, as a new class of endogenous ligands of TLR4. These results could lead to the generation of new drug candidates to be exploited for dampening the inflammatory response in autoimmune and autoinflammatory diseases.

302 - Comorbidities and clinical complications associated with SARS-CoV-2 variants.

ROBERTA MANENTE ⁽¹⁾ - VERONICA FOLLIERO ⁽¹⁾ - AMALIA MATTIELLO ⁽²⁾ - BIAGIO SANTELLA ⁽¹⁾ - CAROLINA VITALE ⁽¹⁾ - ANGELANTONIO MAGLIO ⁽¹⁾ - ALESSANDRO VATRELLA ⁽¹⁾ - GIANLUIGI FRANCI ⁽¹⁾ - ROBERTO PARRELLA ⁽³⁾ - ALBERTO MARRA ⁽³⁾

Dipartimento di Medicina, Chirurgia e Odontoiatria "Scuola Medica Salernitana", Università degli studi di Salerno, Salerno, Italia ⁽¹⁾ - Dipartimento di Medicina e Chirurgia, Università di Napoli Federico II, Napoli, Italia ⁽²⁾ - UOC Malattie infettive ad indirizzo respiratorio, AORN Ospedali dei Colli-Monaldi-Cotugno-CTO, Napoli, Italia ⁽³⁾

Comorbidities and clinical complications associated with SARS-CoV-2 variants.

Roberta Manente¹, Veronica Folliero¹, Amalia Mattiello², Biagio Santella¹, Carolina Vitale¹, Angelantonio Maglio¹, Alessandro Vatrella¹, Gianluigi Franci¹, Roberto Parrella³, Alberto Marra³

1. Department of Medicine, Surgery and Dentistry "Scuola Medica Salernitana", University of Salerno, Salerno, Italy

2. Department of Medicine and Surgery, University "Federico II", Naples, Italy

3. UOC Respiratory Infectious Diseases, AORN Ospedali dei Colli-Monaldi-Cotugno-CTO, Naples, Italy.

Introduction The outbreak of the coronavirus disease 2019 (COVID-19), caused by the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), remains an ongoing global health crisis. Since the rapid spread of SARS-CoV-2, numerous genotypic variants have emerged, including Alpha (B.1.1.7), Gamma (P1), Delta (B.1.617.2), Delta plus (B.1.617.2), and Omicron (BA.1). Among these variants, our focus was on Delta plus and Omicron due to their potential for increased transmission, pathogenicity, and immune evasion compared to previously circulating strains. **Material and Methods.** A retrospective cohort study was conducted at the "Ospedale dei Colli" hospital in Naples, involving adult patients with laboratory-confirmed COVID-19 and symptom onset from March 2020 to February 2022. The enrolled subjects were stratified on the molecular SARS-CoV-2 virus identification: Wild type, Delta, Delta plus, Gamma, and Omicron. Epidemiological, clinical, and outcome data were collected, analyzed, and retrieved. **Results.** During the study period, a total of 1300 laboratory-confirmed COVID-19 cases were admitted to Ospedale dei Colli hospital in Naples from March 2020 to February 2022. Of these cases, 842 (66.9%) were male, and the median age was 68 years (range 57-78 years). Variant determination was made for all patients, with 459 classified as Wild type, 207 as Alpha, 199 as Delta, 150 as Delta plus, 43 as Gamma, and 242 as Omicron. The most common comorbidities observed among the patients were cardiovascular disease (55.4%), diabetes (23.8%), renal disease (15.7%), respiratory disease (11.8%), and malignant tumors (11.8%). There were statistically significant differences in comorbidities. Cardiovascular disease was the most prevalent comorbidity in the population, with the highest rate observed in the Wild type group (63.8%) and the Omicron group (52.1%). Diabetes showed the highest rate only in Delta plus infected patients (27.3%). Kidney disease mostly affected patients with Wild type (13.9%) and those with Omicron (29.8%). Lung disease was more common in patients with Wild type (13.9%) and Omicron (18.2%), as well as neoplasm (malignant tumor) patients infected with Wild type (11.5%) and Omicron (21.5%). The highest rates of severe disease or fatality were observed in the Omicron group (59.9%), followed by the Delta plus group (57.3%). **Discussion and Conclusions.** According to the World Health Organization's classification of Variants of Concern (VOCs), Omicron and Delta are

considered the most problematic variants, with Delta plus being a sub-lineage of Delta. Both Omicron and Delta plus variants have demonstrated increased transmissibility, and Delta plus has shown a greater ability to bind to lung epithelial cells compared to other VOCs. Our study indicates a higher involvement of Omicron and Delta plus variants in adverse disease outcomes.

13 Vaccini

125 - Heterologous boost immunisation with Spike protein after mucosal priming with recombinant *Streptococcus gordonii* expressing the receptor binding domain of SARS-CoV-2 spike protein on its surface

MARIANA TIRZIU ⁽¹⁾ - ELENA PETTINI ⁽¹⁾ - FABIO FIORINO ⁽¹⁾ - LORENZO COLOMBINI ⁽¹⁾ - ELISA LAZZERI ⁽¹⁾ - ANNA MARIA CUPPONE ⁽¹⁾ - FRANCESCO SANTORO ⁽¹⁾ - FRANCESCO IANNELLI ⁽¹⁾ - GIANNI POZZI ⁽¹⁾

Laboratorio di Microbiologia Molecolare e Biotecnologia (LAMMB), Università degli Studi di Siena/ Dipartimento di Biotecnologie Mediche, Siena, Italia ⁽¹⁾

Heterologous boost immunisation with Spike protein after mucosal priming with recombinant *Streptococcus gordonii* expressing the receptor binding domain of SARS-CoV-2 spike protein on its surface

MARIANA TIRZIU, ELENA PETTINI, FABIO FIORINO, LORENZO COLOMBINI, ELISA LAZZERI, ANNA MARIA CUPPONE, FRANCESCO SANTORO, FRANCESCO IANNELLI, GIANNI POZZI

Department of Medical Biotechnologies, Laboratory of Molecular Microbiology and Biotechnology (LAMMB), University of Siena, Siena, Italy

1. Introduction

The emergence of SARS-CoV2 in late 2019 led to the COVID-19 pandemic, which has caused millions of deaths and affected many more. The Spike glycoprotein is the major immunogenic component, and the receptor-binding domain (RBD) is the most commonly used platform for the development of COVID-19 vaccines. The aim of our research was to investigate the immune response to a mucosal vaccine against COVID-19 using a live bacterial vector expressing the RBD of Spike glycoprotein.

2. Materials and Methods

The plasmid vector pMBT-20 was constructed by standard molecular cloning procedures and designed to contain a 1350-bp synthetic DNA encoding the SARS-CoV2 Spike Receptor Binding Domain, cloned in frame with the emm6.1 gene encoding the M protein of *Streptococcus pyogenes* present in the chromosome of our *Streptococcus gordonii* live vaccine vector. Recipient *S. gordonii* competent cells were transformed with pMBT-20, resulting in recombinant strain FR168, which was sequenced to confirm chromosomal integration of the emm6.1::rbd fusion gene. Analysis of M6-RBD protein expression was performed on FR168 bacterial cultures by Western blot and Flow cytometry. C57BL/6 mouse groups were primed orally or intranasally with FR168, and subcutaneously boosted with three doses of purified Spike protein at 2, 3 and 9 months. Serum anti-Spike specific IgG levels were assayed by ELISA.

3. Results

We demonstrated that the *S. gordonii* FR168 recombinant strain induced a humoral immune response in mice immunized via oral and intranasal routes, with preliminary data indicating similar anti-Spike

IgG levels in both groups. Two months after priming, both groups were subcutaneously boosted one month apart with two doses of purified Spike protein, and anti-Spike serum IgG levels were assayed. The results showed that intranasal administration induced a humoral immune response more efficiently than oral administration. A third dose of purified Spike protein was administered six months after the second one and results confirmed once again the higher anti-Spike IgG titers in the intranasally primed group compared to the oral and non-primed groups.

4. Discussion and Conclusions

Heterologous prime-boost approach proved to effectively induce a strong humoral response in both mice groups, with remarkably increased IgG titers in mice primed intranasally compared with the oral group. Our research demonstrates the potential of using live bacterial vectors as a delivery system for mucosal vaccines against COVID-19, focusing now on the cellular immune response.

154 - Humoral and T-cell response to SARS-CoV-2 vaccination one year after the booster dose in oncology patients

ROBERTA CAMPAGNA ⁽¹⁾ - FEDERICA DOMINELLI ⁽²⁾ - MARIA ANTONELLA ZINGAROPOLI ⁽²⁾ - FABIO CIURLUINI ⁽³⁾ - VINCENZO PICONE ⁽⁴⁾ - ENRICO CORTESI ⁽⁴⁾ - GUIDO ANTONELLI ⁽¹⁾ - OMBRETTA TURRIZIANI ⁽¹⁾

Università La Sapienza, Dipartimento di Medicina Molecolare, Roma, Italia ⁽¹⁾ - Università La Sapienza, Dipartimento di Sanità Pubblica e Malattie Infettive, Roma, Italia ⁽²⁾ - Università La Sapienza, Dipartimento di Scienze Radiologiche, Oncologiche e Anatomo-patologiche, Roma, Italia ⁽³⁾ - Policlinico Umberto I, Dipartimento di Oncologia Medica, Roma, Italia ⁽⁴⁾

Humoral and T-cell response to SARS-CoV-2 vaccination one year after the booster dose in oncology patients

Authors

ROBERTA CAMPAGNA 1, FEDERICA DOMINELLI 2, MARIA A. ZINGAROPOLI 2, FABIO CIURLUINI 3, VINCENZO PICONE 4, ENRICO CORTESI 4, GUIDO ANTONELLI 1, OMBRETTA TURRIZIANI 1

Affiliation

1 Department of Molecular Medicine, Sapienza University of Rome, Italy; 2 Department of Public Health and Infectious diseases, Sapienza University of Rome, Italy; 3 Department of Radiological, Oncological and Pathological Science, Sapienza University of Rome, Italy; 4 Medical Oncology Department, Policlinico Umberto I, Sapienza University of Rome, Italy

Introduction

In this study we evaluated both humoral and T-cell response in patients with solid tumors undergoing chemotherapy and/or immunotherapy and compared to healthy donors (HDs).

Materials and methods

Whole blood samples were collected after one year from the booster dose of anti-SARS-CoV-2 vaccine. Anti-Spike serum antibodies were quantified and expressed in Binding Antibody Units/ml (BAU/ml). After overnight PBMC stimulation with SARS-CoV-2 peptide libraries, T-cell specific response was assessed by multiparametric flow cytometry. All possible combinations of intracellular IFN γ , IL2 and TNF α T-cell production were evaluated, and T-cells were labelled “responding T-cells”, those cells that produced at least one of the three cytokines of interest, and “triple positive T-cells”, those cells that produced simultaneously all three cytokines.

Results

Sixty-seven oncology patients (OPs, 29 females/28 males, median age [interquartile range, IQR] of 60 [53-68] years) and 33 HDs (27 females/6 males, median age [IQR] of 54 [39-59] years) were enrolled. Among OPs, the most frequent primary tumor was lung cancer (33%) followed by breast

cancer (28%) and colon cancer (7%). Overall, 26/67 patients were in treatment with immunotherapy. Forty-one OPs and 14 HDs experienced COVID-19 before specimen collection.

OPs group showed a lower antibodies production (1640 [823-4820] BAU/ml) compared to HDs (2810 [1880-6650] BAU/ml, $p=0.026$). No significant difference was found between patients receiving immunotherapy and those receiving chemotherapy. A higher antibody level in patients who experienced SARS-CoV-2 infection (OPs+, 2440 [1247-6510]) compared to those without prior infection was observed (OPs-, 1043 [314-1781], $p<0.001$). No significant difference between HDs and OPs with previous infection was observed, while a higher antibody titer in HDs without previous SARS-CoV-2 infection (HDs-, 2470 [661-6650]) compared to OPs- was found ($p=0.031$). In OPs, lower percentages of responding T-cells (CD4: $p=0.0078$; CD8: $p=0.0022$) and triple positive T-cells compared to HDs were observed (CD4: $p=0.0067$ and CD8: $p=0.0003$).

Discussion and Conclusions

One year after the third dose of anti-SARS-CoV-2 vaccine we found that the anti-Spike IgG titer was lower in OPs than HDs. In our study population the treatment does not seem to influence the antibody production after vaccination. To improve our results, we intend to evaluate the neutralizing capacity of the detected antibodies. Moreover, in OPs, a lower T-cell response compared to HDs was observed. Further studies are needed to complement our results and determine the implication of low T-cell response on clinical protection and durability of OPs against SARS-CoV-2 infection.

161 - Enhancing influenza prevention in the paediatric population: pre-school located vaccination program and saliva-based molecular surveillance in hospitalized children in Milan municipality

ANTONELLA AMENDOLA⁽¹⁾ - CLARA FAPPANI⁽¹⁾ - MARIA GORI⁽¹⁾ - SILVIA BIANCHI⁽¹⁾ - ELISA BORGHI⁽¹⁾ - EMERENZIANA OTTAVIANO⁽¹⁾ - MIRIANA MORELLI⁽¹⁾ - ERIKA CHIERA⁽¹⁾ - CHIARA GASPARINI⁽²⁾ - PAOLO BALDASSARRE⁽²⁾ - FRANCESCA BARILLI⁽²⁾ - ALICE BIANCHI⁽²⁾ - ANDREA BIUSO⁽²⁾ - CLAUDIA CHILLEMI⁽²⁾ - GABRIELLA GUIDA⁽²⁾ - FRANCESCA RIPOLI⁽²⁾ - STEFANIA RONCAGLIA⁽²⁾ - LAURA SGAMBETTERRA⁽²⁾ - DANIELA COLZANI⁽¹⁾ - ELISABETTA TANZI⁽¹⁾ - GIANVINCENZO ZUCCOTTI⁽²⁾

Università degli Studi di Milano, Dipartimento di Scienze della Salute, Milano, Italia⁽¹⁾ - Ospedale Buzzi, Dipartimento di Pediatria, Milano, Italia⁽²⁾

Enhancing influenza prevention in the paediatric population: pre-school located vaccination program and saliva-based molecular surveillance in hospitalized children in Milan municipality

ANTONELLA AMENDOLA¹, CLARA FAPPANI¹, MARIA GORI¹, SILVIA BIANCHI¹, ELISA BORGHI¹, EMERENZIANA OTTAVIANO¹, MIRIANA MORELLI¹, ERIKA CHIERA², CHIARA GASPARINI³, PAOLO BALDASSARRE³, FRANCESCA BARILLI³, ALICE BIANCHI³, ANDREA BIUSO³, CLAUDIA CHILLEMI³, GABRIELLA GUIDA³, FRANCESCA RIPOLI³, STEFANIA RONCAGLIA³, LAURA SGAMBETTERRA³, DANIELA COLZANI¹, ELISABETTA TANZI¹, GIANVINCENZO ZUCCOTTI³

¹Department of Health Sciences, Università degli Studi di Milano, Milan, Italy; ²Università degli Studi di Milano, Milan, Italy; ³Department of Pediatrics, V. Buzzi Children's Hospital, Università degli Studi di Milano, Milan, Italy

Introduction

Following the World Health Organization (WHO) recommendation to implement influenza vaccination coverage and to strengthen influenza surveillance strategies in the paediatric population, the objectives of this study were: a) to set-up a preschool-located influenza vaccination campaign in the Milan municipality (Northern Italy) during the 2022/2023 influenza season; b) to conduct a molecular surveillance in children hospitalized at the largest children's hospital in Milan using a self-sampling non-invasive saliva collection method.

Materials and Methods

Twelve preschools (1513 children aged 3-6 years) took part in the project. Parents were informed through tailor-made information campaigns. Healthy children were immunized with nasal-spray live-attenuated vaccine Fluenz™ Tetra (AstraZeneca) between November 2022 and January 2023, directly in school settings. Influenza surveillance in hospitalized children with respiratory symptoms was conducted starting from January 2023 by collecting saliva specimens. Influenza A/B

viruses RNA was detected using Real-Time RT-PCR. Positive samples were further characterized by sequence analysis of the hemagglutinin (HA) gene, according to the WHO protocol.

Results

Overall, 286 children (18.9%; mean age 4.3 years) received vaccination at school. The percentage of adherence in the twelve schools was very heterogeneous, ranging from 4.5% in a school located in downtown Milan to 47.6% in a small suburban school attended only by children with an immigrant background. No children experienced adverse reactions after vaccination. During surveillance activity, a total of 75 saliva samples were collected from as many hospitalized children (mean age 4 years); 8% tested positive for influenza (1 for A(H1N1)pdm09, 4 for A(H3N2), and 1 for influenza B). Notably, the sample positive for A(H1N1)pdm09 fell into the 6B.1A.5a.2a.1 clade. None of the influenza positive children were vaccinated, whereas 36.4% of influenza negative children were immunized.

Discussion and conclusions

Importantly, 33% of pre-school children involved in the study were vaccinated for the first time during the vaccination campaign described here. School-located vaccine administration was well accepted, helping abolish barriers to vaccine access despite the socioeconomic status. Vaccine hesitancy was mainly observed in pre-schools located in downtown Milan. Importantly, surveillance activity results suggested the circulation of a genetically drifted variant of A(H1N1)pdm09 virus with respect to the 2023/2024 vaccine strain. In this perspective, the implementation of a saliva-based virological surveillance could allow us to conduct future studies on the vaccine effectiveness that are lacking in Italy.

210 - Oral potentially malignant disorders (OPMDs): HPV prevalence and vaccines

MICHELA BUTTÀ⁽¹⁾ - NICOLA SERRA⁽²⁾ - ARIANNA SUCATO⁽¹⁾ - VERA PANZARELLA⁽³⁾
- ERIKA MANNINO⁽¹⁾ - DANIELA CABIBI⁽¹⁾ - DANIELA PISTOIA⁽⁴⁾ - GIUSEPPINA CAPRA⁽¹⁾

Università degli Studi di Palermo, Dipartimento di Promozione della Salute, Materno-Infantile, di Medicina Interna e Specialistica di Eccellenza “G. D’Alessandro”, Palermo, Italia⁽¹⁾ - Università Federico II di Napoli, Dipartimento di Sanità Pubblica, Napoli, Italia⁽²⁾ - Università degli Studi di Palermo, Dipartimento di Discipline Chirurgiche, Oncologiche e Stomatologiche, Palermo, Italia⁽³⁾ - Azienda Ospedaliera Universitaria Policlinico P. Giaccone, Unità di Microbiologia e Virologia, Palermo, Italia⁽⁴⁾

Oral potentially malignant disorders (OPMDs): HPV prevalence and vaccines

MICHELA BUTTÀ¹, NICOLA SERRA², ARIANNA SUCATO¹, VERA PANZARELLA³, ERIKA MANNINO¹, DANIELA CABIBI^{1,4}, DANIELA PISTOIA⁵, AND GIUSEPPINA CAPRA^{1,5}

¹Department of Health Promotion, Mother and Child Care, Internal Medicine and Medical Specialties (PROMISE) “G. D’Alessandro”, University of Palermo, Palermo, Italy;

²Department of Public Health, University Federico II of Naples, Naples, Italy;

³Department of Surgical, Oncological, and Oral Sciences (DICHIRONS), University of Palermo, Palermo, Italy;

⁴Anatomic Pathology Unit, Polyclinic Hospital “P. Giaccone”, Palermo, Italy;

⁵Microbiology and Virology Unit, Polyclinic Hospital “P. Giaccone”, Palermo, Italy

Introduction

Oral potentially malignant disorders (OPMDs) are a heterogeneous group of oral conditions (e.g., leukoplakia, lichen planus, and erythroplakia), which can lead to the onset of oral squamous cell carcinoma (OSCC). Oro-genital transmission of human papillomavirus (HPV), one of the most common sexually transmitted pathogens, appears to play a role in the development of these pathologies. Furthermore, the influence of HPV vaccines on oral HPV infection in general and OPMDs prevention specifically is still unclear. The study aims to evaluate the prevalence of HPV and the potential impact of vaccines in a cohort of OPMD patients.

Materials and methods

The study examined 313 oral rinses from patients with OPMDs. The clinical diagnosis confirmed by histological examination was carried out at the Oral Medicine and Anatomic Pathology Units, Polyclinic Hospital of Palermo. HPV detection and genotyping were performed by INNO-LiPA HPV Genotyping Extra II kit (Fujirebio) at the Microbiology and Virology Unit, Polyclinic Hospital of Palermo. The statistical analysis made it possible to calculate impact estimates respectively as the presence of vaccine genotypes, alone or in association, but excluding the presence of another type of HPV (low impact estimate), or in the presence of another type of HPV (high impact estimate).

Results

The patients’ cohort exhibited leukoplakia and lichen planus as the most common diagnoses, with rates of 49.2% (154/313) and 46.3% (145/313), respectively. The overall HPV prevalence was 11.2% (35/313), mainly represented by single infections, detected in 91.4% (32/35). HrHPVs were found in 71.4% (25/35), while the most common types were HPV16 (40%), HPV6 (25.7%), HPV18 (11.4%) and HPV51 (8.6%). The two groups showed HPV prevalence rates of 13.6% (21/154) in leukoplakia patients and 9% (13/145) in lichen planus subjects. Vaccine impact evaluation showed no significant

differences between vaccines' high impact estimates. Conversely, the comparison of low-impact estimates showed how quadrivalent and nonavalent ones were significantly higher than the bivalent one (bivalent vs quadrivalent: 48.6% vs 68.6%, bivalent vs nonavalent: 48.6% vs 77.1%).

Discussion and conclusions

The prevalence found in our study falls in the wide range from 0% and 85% described in the literature, but it is lower than the pooled prevalence of 22.5% shown by de la Cour et al. The identification of HPV16 as the prevalent type is explained by the high numbers of lichen planus and leukoplakia diagnoses in our group, which Shang et al. described as strongly correlated with HPV16/18 infections. Concerning vaccines' impact, quadrivalent and nonavalent vaccines have proven to be more effective than the bivalent ones in potentially counteracting HPV oral infection.

227 - Characterization of T-cell response to recombinant VZV vaccine in pwMS under DMTs

FEDERICA DOMINELLI⁽¹⁾ - MARIA ANTONELLA ZINGAROPOLI⁽¹⁾ - PATRIZIA PASCULLI⁽¹⁾ - MATTEO TARTAGLIA⁽²⁾ - FEDERICA CICCONE⁽¹⁾ - LEONARDO MALIMPENSA⁽²⁾ - GINA FERRAZZANO⁽²⁾ - EEVA TORTELLINI⁽¹⁾ - MARIASILVIA GUARDIANI⁽¹⁾ - MIRIAM LICHTNER⁽³⁾ - CLAUDIO MARIA MASTROIANNI⁽¹⁾ - ANTONELLA CONTE⁽²⁾ - MARIA ROSA CIARDI⁽¹⁾

Sapienza, University of Rome, Department of Public health and Infectious diseases, Roma, Italia⁽¹⁾ - Sapienza, University of Rome, Department of Human Neurosciences, Roma, Italia⁽²⁾ - Sapienza, University of Rome, Infectious Diseases Unit, Santa Maria Goretti Hospital, Latina, Italia⁽³⁾

ABSTRACT SIM 2023

Title: Characterization of T-cell response to recombinant VZV vaccine in pwMS under DMTs.

FEDERICA DOMINELLI¹, MARIA A. ZINGAROPOLI¹, PATRIZIA PASCULLI¹, MATTEO TARTAGLIA², FEDERICA CICCONE¹, LEONARDO MALIMPENSA², GINA FERRAZZANO², EEVA TORTELLINI¹, MARIASILVIA GUARDIANI¹, MIRIAM LICHTNER^{3,4}, CLAUDIO M. MASTROIANNI¹, ANTONELLA CONTE^{2,5}, MARIA R. CIARDI¹

¹Department of Public Health and Infectious Diseases, Sapienza University of Rome, Rome, Italy;

²Department of Human Neurosciences, Sapienza University of Rome, Rome, Italy; ³Infectious Diseases Unit, Santa Maria Goretti Hospital, Sapienza, University of Rome, Latina, Italy;

⁴Department of Neurosciences Mental Health and Sensory Organs, Sapienza University of Rome, Rome, Italy; ⁵IRCCS Neuromed, Pozzilli, Italy.

Introduction: People living with Multiple sclerosis (pwMS) are at increased risk of Herpes zoster (HZ), due to Disease modifying therapies (DMTs) mechanism of action. Because of HZ neurological sequelae, like post herpetic neuralgia, the recombinant Varicella-zoster virus gE vaccine (RZV) has been approved for pwMS without interruption of current DMT. However, among different DMTs, reduced humoral and cellular responses to vaccination might be observed. The study aimed to characterize T-cell specific response to RZV in pwMS under different DMTs. Materials and methods: At the Neuroinfectious Unit, during the assessment of infectious risk, RZV has been proposed to pwMS since February 2023 and pwMS at increased risk of HZ have been considered. Before (T0) and after 2 months (T1) from first dose, pwMS were enrolled. The production of IFN γ , IL2 and TNF α by T-cells upon VZV gE peptide library stimulation was performed, defining “activated” T-cells those producing any of IFN γ , IL2 and TNF α and “triple-positive” T-cells those simultaneously producing all 3 cytokines.

Results: 12 pwMS underwent RZV schedule (10 females/2 males, median age[IQR] 50[40-60] years). Common DMTs taken were: 50% ocrelizumab, 8% fingolimod, 17% dimethyl fumarate, 8% natalizumab, 8% cladribine and 8% peginterferon beta1a. At T1 an evaluation for 3 pwMS has been performed. No differences in percentages of CD4 and CD8 activated T-cells was observed between T0 and T1 (CD4: 0.0[0.0-0.0] and 0.18[0.17-0.18], respectively, p=ns; CD8: 0.0[0.0-0.0] and 0.0[0.0-0.0], respectively, p=ns). The same results were observed for percentages of triple-positive T-cells between T0 and T1 (CD4: 0.0[0.0-0.0] and 0.02[0.01-0.02], respectively, p=ns; CD8: 0.0[0.0-0.0] and 0.0[0.0-0.01], respectively, p=ns).

Conclusions: In line with current literature on other vaccine responses, such as influenza and SARS-CoV-2, our preliminary data showed that T-cell responses to vaccination in pwMS seems to be significantly influenced by DMTs.

234 - Transcriptomic analysis after mRNA BNT162b2 vaccination revealed a specific gene expression profile in low-responders hemodialysis patients

SIMONE LUCCHESI⁽¹⁾ - GIORGIO MONTESI⁽¹⁾ - ANNALISA CIABATTINI⁽¹⁾ - JACOPO POLVERE⁽¹⁾ - GABIRIA PASTORE⁽¹⁾ - FRANCESCA MONTAGNANI⁽²⁾ - GUIDO GAROSI⁽³⁾ - FRANCESCO SANTORO⁽¹⁾ - DONATA MEDAGLINI⁽¹⁾

Università di Siena, Dipartimento di Biotecnologie mediche, Siena, Italia⁽¹⁾ - Azienda ospedaliero-universitaria Senese, Malattie infettive e tropicali, Siena, Italia⁽²⁾ - Azienda ospedaliero-universitaria Senese, Nefrologia, Siena, Italia⁽³⁾

Transcriptomic analysis after mRNA BNT162b2 vaccination revealed a specific gene expression profile in low-responders hemodialysis patients

Simone Lucchesi¹, Giorgio Montesi¹, Jacopo Polvere¹, Elena Pettini¹, Francesca Montagnani^{2,3}, Guido Garosi⁴, Annalisa Ciabattini¹, Francesco Santoro¹, Donata Medaglini¹

¹ Laboratory of Molecular Microbiology and Biotechnology, Department of Medical Biotechnologies, University of Siena, Italy

² Department of Medical Sciences, Infectious and Tropical Diseases Unit, Siena University Hospital, Italy

³ Department of Medical Biotechnologies, University of Siena, Italy

⁴ Nephrology, Dialysis, and Transplantation Unit, Siena University Hospital, Italy

Introduction: The COVID-19 pandemic has had a significant global impact, resulting in a high number of confirmed cases and fatalities. Elderly individuals with comorbidities, such as chronic kidney disease and specifically those undergoing hemodialysis (hemodialysis patients; HDP), are particularly susceptible to COVID-19 complications. Furthermore, their immune response to vaccines is often impaired, necessitating tailored vaccination strategies.

In this study, we aimed to investigate the immune response of HDP vaccinated with the mRNA BNT162b2 vaccine through transcriptomic profiling, to uncover the molecular mechanisms underlying immune response heterogeneity.

Materials and Methods: Peripheral blood mononuclear cells (PBMC) were collected from cohorts of HDP and healthy controls (HC) vaccinated with two doses of mRNA BNT162b2 vaccine at baseline (day 0), at days 7, 21 (before the second dose) and 28 (7 days after the second dose). The HDP were stratified into high- and low-responders based on their humoral response after second dose. RNA was extracted from PBMC and libraries prepared using “Illumina Stranded Total RNA Prep, Ligation with Ribo-Zero Plus” kit and sequenced on NovaSeq 6000 System. Data were analyzed using different approaches comprising: differential gene expression analysis, gene co-expression analyses, and feature selection methods.

Results: We detected statistically significant differences in gene expression between HDP and HC, in particular at baseline and at early time points. The key differences were primarily detected in genes related to B cell abundance/survival, CD4 T cell proliferation, and inflammation pathways. Notably, differences observed between HDP and HC were more evident when HC cohort was compared with HDP low responders, while the HDP high-responder cohort displayed an intermediate expression profile for these genes.

Discussion and Conclusions: We unveiled significant and distinct differences in gene expression between HDP low-responders and HC already at the baseline, and identified a different pattern of

transcriptomic response at day 7 after vaccination. These differences correlated with the number of antigen-specific B cells and with the production of neutralizing antibody. The main alterations, related to B cell lymphopenia, CD4 T cell proliferation, and inflammation, were also consistent with the known immunologic alteration occurring in HDP cohorts. These results not only underscore the critical importance of conducting a comprehensive characterization of frail cohorts, but also shed light on potential correlations between gene expression and the diminished immune response in HDP low-responders.

235 - Two years after COVID-19 vaccination: persistence of spike-specific memory B cells and antibodies after homologous and heterologous vaccine combinations

ANNALISA CIABATTINI⁽¹⁾ - CHIARA COPPOLA⁽¹⁾ - SIMONE COSTAGLI⁽¹⁾ - GABIRIA PASTORE⁽¹⁾ - FABIO FIORINO⁽¹⁾ - JACOPO POLVERE⁽¹⁾ - SIMONE LUCCHESI⁽¹⁾ - MASSIMILIANO FABBIANI⁽²⁾ - FRANCESCA MONTAGNANI⁽²⁾ - MARIO TUMBARELLO⁽²⁾ - DONATA MEDAGLINI⁽¹⁾

University of Siena, Department of of Medical Biotechnology, Siena, Italia⁽¹⁾ - University Hospital of Siena, Department of Medical Sciences, Infectious and Tropical Diseases Unit, Siena, Italia⁽²⁾

Two years after COVID-19 vaccination: persistence of spike-specific memory B cells and antibodies after homologous and heterologous vaccine combinations

ANNALISA CIABATTINI¹, CHIARA COPPOLA¹, SIMONE COSTAGLI¹, GABIRIA PASTORE¹, FABIO FIORINO¹, JACOPO POLVERE¹, SIMONE LUCCHESI¹, MASSIMILIANO FABBIANI³, FRANCESCA MONTAGNANI^{2,3}, MARIO TUMBARELLO^{2,3}, DONATA MEDAGLINI¹

¹Laboratory of Molecular Microbiology and Biotechnology, Department of Medical Biotechnologies, University of Siena; Siena, Italy; ²Department of Medical Biotechnologies, University of Siena; Siena, Italy ; ³Department of Medical Sciences, Infectious and Tropical Diseases Unit, University Hospital of Siena; Siena, Italy

Abstract

Introduction. The mRNA vaccines for SARS-CoV-2 have demonstrated efficacy and immunogenicity in a real-world setting. Previous studies, also from our group, have shown the persistence of the immune response up to 9 months after two vaccine doses. Here we characterized the spike-specific antibody and memory B cell response, directed against the spike vaccine antigen and the recent circulating variants, in healthy individuals up to two years after the first cycle of vaccination, with a special focus on spike-specific memory B cells.

Materials and Methods. 180 healthy subjects, enrolled in the IMMUNO_COV study (Siena University Hospital) at the beginning of the COVID-19 vaccination campaign, were recalled 18 and 24 months after the first vaccination cycle. Many of them had received the third or the fourth vaccination dose, with mRNA vaccines. The homologous or heterologous vaccine combination received, and the possible SARS-CoV-2 infection were reported by each subject in a written survey. According to this information, subjects have been stratified into different groups, to characterize not only the persistence of the immune response elicited by different vaccine strategies, but also the impact of the hybrid immunity, i.e the interplay between immunity from natural infection and the one elicited by vaccination. To evaluate the spike-specific antibody response, IgG were analysed by ELISA, and their ability of blocking the RBD-ACE2 binding was assessed using a surrogate of neutralization assay. B cells recognizing the spike RBD from the wild type strain or variants, were identified by multiparametric flow cytometry with computational analysis of data.

Results. High levels of spike-specific IgG titres (> 100,000) were maintained in all groups 18 months after vaccination, that corresponded to 6 months after the third booster dose, without significant

differences among the vaccine combinations. Antibodies were capable of binding not only the wild type but also the Delta and the Omicron RBD proteins, thus inhibiting the interaction with the ACE-2 receptor. Circulating spike-specific B cells peaked 1 month after the third dose and persisted 6 months later. Serological analysis, together with the deep characterization of the spike-specific memory B cells at month 24 are under evaluation.

Discussion and Conclusion. The present research gains insights into the long-term immunogenicity of SARS-CoV-2 vaccination and infection, and it can help in guiding future decisions and vaccination schedules.

240 - Characterization of the Immune Responsiveness to COVID-19 Vaccination in Rett Syndrome Patients

ANNALISA CIABATTINI ⁽¹⁾ - FABIO FIORINO ⁽¹⁾ - SILVIA LEONCINI ⁽²⁾ - LIDIA BOASLAKO ⁽²⁾ - LUDOVICA SOLDATESCHI ⁽¹⁾ - GABIRIA PASTORE ⁽¹⁾ - JACOPO POLVERE ⁽¹⁾ - CLAUDIO DE FELICE ⁽²⁾ - DONATA MEADGLINI ⁽¹⁾

University of Siena, Department of of Medical Biotechnology, Siena, Italia ⁽¹⁾ - Azienda Ospedaliera Universitaria Senese, Neonatal Intensive Care Unit, Department of Women& Children Health, Siena, Italia ⁽²⁾

Characterization of the Immune Responsiveness to COVID-19 Vaccination in Rett Syndrome Patients

ANNALISA CIABATTINI¹, FABIO FIORINO¹, SILVIA LEONCINI^{2,3}, LIDIA BOASLAKO^{2,3}, LUDOVICA SOLDATESCHI¹, GABIRIA PASTORE¹, JACOPO POLVERE¹, CLAUDIO DE FELICE, DONATA MEDAGLINI¹

¹Laboratory of Molecular Microbiology and Biotechnology, Department of Medical Biotechnologies, University of Siena; Siena, Italy; ²Neonatal Intensive Care Unit, Department of Women& Children Health, Policlinico “S. Maria alle Scotte” Azienda Ospedaliera Universitaria Senese, Siena, Italy; ³ Rett Syndrome Trial Center, Child Neuropsychiatry Unit, Department of Mental Health, Policlinico “S. Maria alle Scotte” AOUS, Siena, Italy.

Abstract

Introduction. In the context of the current SARS-CoV-2 pandemic, limited data are available on the immune response in Rett syndrome (RTT) patients to SARS-CoV-2 infection and/or vaccination. RTT is a severe neurodevelopmental disorder, mainly caused by loss-of-function mutations in the X-linked MECP2 gene, a multifunctional epigenetic regulator. The research on RTT has mainly focused on studying its neurological aspects, and only a few studies to date have investigated its immunological features. As a general rule, fragile patients mount a reduced immune response to infection and vaccination due to immunological disorders and pharmacological treatments, often coupled with a detrimental inflammatory response. The dissection of the immune mechanisms activated by infection and/or by vaccination are therefore of key relevance to understand mechanisms of immune responsiveness and the biology of the vaccine response in these particular rare vulnerable patients. In this study we profiled the immune responsiveness of RTT patients to antigenic stimuli, and specifically characterized the immune response developed in the context of the COVID-19 infection and/or vaccination.

Materials and Methods. The study was performed in a cohort of 35 Rett patients, grouped into unvaccinated, or vaccinated with two or three doses of mRNA vaccines, with or without a natural infection with SARS-CoV-2, and compared them to age and sex matched healthy controls. PBMC were tested for the cytokine/chemokine production following mitogens restimulation, by using a 27-plex multiplex assay. Another aliquot of PBMC was stained for identifying spike-specific B cells by multiparametric flow cytometry. Spike-specific IgG were analysed by ELISA, while the ability of blocking the RBD-ACE2 binding was assessed using a surrogate of neutralization assay

Results. For most of the cytokine/chemokines assessed Rett patients reacted similar to healthy subjects, even though a lower production was observed for some pro-inflammatory cytokines. Rett

patients developed a significant spike-specific IgG response already after the second vaccine dose with antibody titers comparable to healthy subjects, that increased after vaccine booster. The analysis of B cell frequency and B cell subsets showed a very similar profile between Rett patients and healthy controls. Both antibody and memory B cell responses were observed to persist overtime similarly to what reported in healthy subjects.

Discussion and Conclusion. The immune responsiveness observed in RTT patients was generally comparable to healthy controls. Such insights are crucial for better understanding the immune in RTT patients, and contributing to the definition of the guidelines of COVID-19 vaccination tailored for this cohort of patients.

259 - B cell response after SARS-CoV-2 mRNA vaccination in people living with HIV

JACOPO POLVERE⁽¹⁾ - MASSIMILIANO FABBIANI⁽²⁾ - GABIRIA PASTORE⁽¹⁾ - ILARIA RANCAN⁽²⁾ - BARBARA ROSSETTI⁽²⁾ - MIRIAM DURANTE⁽³⁾ - ELENA PETTINI⁽¹⁾ - SIMONE LUCCHESI⁽¹⁾ - SARA ZIRPOLI⁽¹⁾ - ENRICO MORELLI⁽³⁾ - GIORGIO MONTESI⁽¹⁾ - FABIO FIORINO⁽¹⁾ - MARIO TUMBARELLO⁽²⁾ - ANNALISA CIABATTINI⁽¹⁾ - FRANCESCA MONTAGNANI⁽²⁾ - DONATA MEDAGLINI⁽¹⁾

Laboratorio di Microbiologia Molecolare e Biotecnologia, Dipartimento di Biotecnologie Mediche, Università degli studi di Siena, Siena, Italia⁽¹⁾ - UOC Malattie infettive e tropicali, Dipartimento di Scienze mediche, chirurgiche e neuroscienze, Azienda Ospedaliera Universitaria Senese, Siena, Italia⁽²⁾ - Dipartimento di Biotecnologie Mediche, Università degli studi di Siena, Siena, Italia⁽³⁾

B cell response after SARS-CoV-2 mRNA vaccination in people living with HIV

JACOPO POLVERE¹, MASSIMILIANO FABBIANI², GABIRIA PASTORE¹, ILARIA RANCAN^{2,3}, BARBARA ROSSETTI², MIRIAM DURANTE³, ELENA PETTINI¹, SIMONE LUCCHESI¹, SARA ZIRPOLI¹, ENRICO MORELLI², GIORGIO MONTESI¹, FABIO FIORINO¹, MARIO TUMBARELLO^{2,3}, ANNALISA CIABATTINI¹, FRANCESCA MONTAGNANI^{2,3} AND DONATA MEDAGLINI¹

1 Laboratory of Molecular Microbiology and Biotechnology, Department of Medical Biotechnologies, University of Siena; Siena, Italy; 2 Department of Medical Sciences, Infectious and Tropical Diseases Unit, University Hospital of Siena; Siena, Italy; 3 Department of Medical Biotechnologies, University of Siena; Siena, Italy

Introduction. SARS-CoV-2 mRNA vaccines proved to be highly immunogenic in most people living with HIV (PLWHIV) under antiretroviral therapy. However, limited longitudinal data are available for PLWHIV and the effect of chronic immune impairment on spike-specific memory B response has not yet been clarified.

Materials and Methods. The persistence of spike-specific memory B cells and circulating anti-spike antibodies up to six months after mRNA SARS-CoV-2 vaccination were investigated in PLWHIV and compared to healthy controls (HCs). Spike-specific B cells were characterized for surface markers expression with a flow cytometry approach. The humoral response was assessed with enzyme-linked immunosorbent assay and a surrogate virus neutralization assay, measuring the inhibition of binding between angiotensin-converting enzyme 2 (ACE2) and receptor-binding domain (RBD).

Results. Spike-specific IgG persisted six months in PLWHIV with no significant differences compared to HCs, even though a significantly lower IgG response was observed in patients with CD4⁺ T cells < 350/mm³. The frequency of subjects with antibodies capable of inhibiting ACE2/RBD binding was comparable between PLWHIV and HCs one month after the second vaccine dose, then a higher drop was observed in PLWHIV. A comparable percentage of spike-specific memory B cells was observed at month six in PLWHIV and HCs. However, PLWHIV showed a higher frequency of spike-specific IgD⁻ CD27⁻ double-negative memory B cells and a significantly lower rate of IgD⁻ CD27⁺ Ig-switched memory B cells compared to HCs, suggesting a reduced functionality of the antigen-specific memory B cell population.

Discussion and Conclusions. Humoral and B cell responses induced by mRNA SARS-CoV-2 vaccination are quantitatively similar between PLWHIV and HCs. Nevertheless, there are differences in terms of antibody functionality and phenotypes of memory B cells, reinforcing the notion that tailored vaccination policies should be considered for these patients. New analyses are currently underway to characterize the immune response to additional vaccine doses.

265 - Persistence of spike-specific immune responses in solid organ transplant recipients up to two years following mRNA vaccination for SARS-CoV-2

SIMONE COSTAGLI⁽¹⁾ - CHIARA COPPOLA⁽¹⁾ - JACOPO POLVERE⁽¹⁾ - MASSIMILIANO FABBIANI⁽²⁾ - MARIO TUMBARELLO⁽³⁾ - FRANCESCA MONTAGNANI⁽³⁾ - SONIA BERNAZZALI⁽⁴⁾ - DAVID BENNETT⁽⁵⁾ - ANNALISA CIABATTINI⁽¹⁾ - DONATA MEDAGLINI⁽¹⁾

Università degli Studi di Siena, Dipartimento di Biotecnologie Mediche, Laboratorio di Microbiologia Molecolare e Biotecnologie, Siena, Italia⁽¹⁾ - Ospedale Universitario di Siena, Dipartimento di Scienze Mediche, Unità di Malattie Infettive e Tropicali, Siena, Italia⁽²⁾ - Università degli Studi di Siena; Ospedale Universitario di Siena, Dipartimento di Biotecnologie Mediche; Dipartimento di Scienze Mediche, Unità di Malattie Infettive e Tropicali, Siena, Italia⁽³⁾ - Ospedale Universitario di Siena, Dipartimento Cardio-Toraco-Vascolare, Unità di Chirurgia Cardiaca, Siena, Italia⁽⁴⁾ - Ospedale Universitario di Siena, Dipartimento di Scienze Mediche, Unità di Malattie Respiratorie, Siena, Italia⁽⁵⁾

Persistence of spike-specific immune responses in solid organ transplant recipients up to two years following mRNA vaccination for SARS-CoV-2

SIMONE COSTAGLI¹, CHIARA COPPOLA¹, JACOPO POLVERE¹, MASSIMILIANO FABBIANI³, MARIO TUMBARELLO^{2,3}, FRANCESCA MONTAGNANI^{2,3}, SONIA BERNAZZALI⁴, DAVID BENNETT⁵, DONATA MEDAGLINI¹, ANNALISA CIABATTINI¹

¹Laboratory of Molecular Microbiology and Biotechnology, Department of Medical Biotechnologies, University of Siena; Siena, Italy; ²Department of Medical Biotechnologies, University of Siena; Siena, Italy; ³Department of Medical Sciences, Infectious and Tropical Diseases Unit, University Hospital of Siena; Siena, Italy; ⁴Cardio-Thoracic and Vascular Department, Cardiac Surgery Unit, University Hospital of Siena; Siena, Italy; ⁵Department of Medical Sciences, Respiratory Disease Unit, University Hospital of Siena; Siena, Italy

Abstract

Introduction. Solid organ transplant (SOT) recipients have shown a reduced immune responsiveness to mRNA vaccines for SARS-CoV-2 compared to healthy vaccinated individuals, mainly due to the immunosuppressive pharmacological treatments. Here, we longitudinally characterized the spike-specific antibody and memory B cell responses in SOT recipients up to two years after the first cycle of vaccination, targeting the vaccine antigen and the recent circulating variants.

Materials and Methods. 157 heart transplant recipients and 59 lung transplant recipients, enrolled in the context of the PATOVAC study (n. 19479, approved by University Siena Hospital local Ethical Committee), were evaluated for their humoral and cellular responses up to two years after the first dose of SARS-CoV-2 vaccination. Spike-specific IgG were analyzed by ELISA, while B cells recognizing the spike protein from the Wuhan wild type or variant strains were identified by multiparametric flow cytometry, and data analyzed employing computational algorithms.

Results Subjects were stratified according to the number and type of vaccine doses received, the natural infection and the number of years since transplantation. Spike-specific IgG were elicited by two vaccine doses, with a drop observed after 2 months. However, the third dose elicited a recall effect of the immune response, with a subsequent increase in the concentration of spike-specific IgG, that persisted one year after vaccination, especially in subjects transplanted for more than 10 years which showed no significant differences compared to healthy controls. Serological analysis to evaluate the persistence of the spike-specific IgG response up to two years following vaccination is ongoing. Furthermore, a deep characterization of spike-specific memory B cells, in terms of quantity and quality, is in progress. This is particularly important due to our previous observations in which altered phenotypes, such as atypical CD21- CD27- B cells, have been identified in immunocompromised individuals.

Discussion and Conclusion. These data contribute to better understanding the immune mechanisms in SOT recipients and could help in making decisions on future vaccination schedules in this population.

278 - Recombinant BCG strains as innovative treatment of non-muscle invasive bladder cancer

Alessandro Salustri⁽¹⁾ - Flavio De Maio⁽²⁾ - Ivana Palucci⁽²⁾ - Giulia Santarelli⁽¹⁾ - Valentina Palmieri⁽³⁾ - Massimiliano Papi⁽⁴⁾ - Michela Sali⁽¹⁾ - Giovanni Delogu⁽⁵⁾

Dipartimento di Scienze biotecnologiche di base, cliniche intensivologiche e perioperatorie., Università Cattolica del Sacro Cuore, Roma, Italia⁽¹⁾ - Dipartimento di Scienze di Laboratorio e Infettivologiche, Facoltà di Medicina e Chirurgia., Fondazione Policlinico A. Gemelli IRCCS, Università Cattolica del Sacro Cuore., Roma, Italia⁽²⁾ - Istituto dei Sistemi Complessi, Centro Nazionale delle Ricerche (CNR), Roma, Italia⁽³⁾ - Dipartimento di Neuroscienze, Università Cattolica del Sacro Cuore, Roma, Italia⁽⁴⁾ - Dipartimento di Medicina di Laboratorio, Mater Olbia Hospital, Olbia, Italia⁽⁵⁾

Recombinant BCG strains as innovative treatment of non-muscle invasive bladder cancer

Alessandro Salustri¹, Flavio De Maio², Ivana Palucci^{1,2}, Giulia Santarelli¹, Valentina Palmieri^{4,5}, Massimiliano Papi^{3,4}, Michela Sali^{1,2}, Giovanni Delogu^{2,6}

¹Department of Sciences of Basic Biotechnologies, Intensive and Perioperative Clinics, Section of Microbiology, Università Cattolica del Sacro Cuore, Rome, Italy;

²Department of Laboratory and Infective Sciences, Faculty of Medicine, Fondazione Policlinico A. Gemelli IRCCS, Università Cattolica del Sacro Cuore, Rome, Italy;

³Fondazione Policlinico Universitario “A. Gemelli”, IRCSS, Rome, Italy;

⁴Dipartimento di Neuroscienze, Università Cattolica del Sacro Cuore, 00168 Rome, Italy;

⁵Istituto dei Sistemi Complessi, CNR, Rome, Italy;

⁶Mater Olbia Hospital, Olbia, Italy;

Introduction. Bladder cancer (BC) is the second most common malignant tumor of the urinary tract. A standard treatment of non-muscle-invasive BC consists in multiple instillations of *Mycobacterium bovis* BCG (BCG), a live attenuated vaccines routinely used to prevent TB diseases in infants. However, therapeutic activity can be achieved only following administration of high doses of BCG in the bladder that cause extreme pain and toxicity, leading to poor patient adherence. We reasoned that enhancing BCG immunogenicity and vaccine persistence in the bladder, may lead to the administration of fewer bacteria, prompting improved activity and reduced toxicity. To this aim, we developed a series of BCG recombinant strains expressing chimeric proteins generated by fusing different *Mycobacterium tuberculosis* (MTB) antigens (PE_PGRS33, PE_PGRS3, MPT64) with the aim to enhance BCG adhesion, internalization and an overall more efficient and long lasting activation of the anti-tumor immune response.

Material and methods. We generated two vectors expressing the PE domain from PE_PGRS33, responsible of PE_PGRS33 surface localization, fused either with MPT64, an immuno-dominant antigen, or C-terminal domain from PE_PGRS3, that promotes bacterial adhesion. A final vector was generated with all antigens to constitute the chimera PE^{PE_PGRS33}-Mpt64-CT^{PE_PGRS3}. The plasmids were electroporated in BCG Pasteur and protein expression was assessed through immunoblot assays using the HA epitope included in each recombinant protein. Cell adhesion and intracellular viability of these BCG strains was tested through confocal microscopy and in in vitro infection models using both murine urothelial carcinoma cells (MB49) and murine macrophages (RAW264.7).

Results. Immunoblot analysis showed proper expression and cellular localization of the chimeric proteins. Confocal microscopy experiments and in vitro infection with the recombinant BCG in human MB49 cells did not show any appreciable difference in terms of adhesion or persistence between the strains. Conversely, in macrophages, BCG expressing the full-length chimera (PE^{PE_PGRS33}-Mpt64-CT^{PE_PGRS33}) showed an increased internalization and reduced intracellular persistence at 7 days post-infection.

Discussion. BCG recombinant strains overexpressing selected mycobacterial antigens may warrant enhanced adhesion and reduced intracellular survival in macrophages, thus opening the possibility to improve immunogenicity while reducing BCG pathogenicity.

304 - Moving beyond the titer paradigm: rational design of a next-generation glycoconjugate vaccine inducing highly functional antibodies

GIUSEPPE STEFANETTI⁽¹⁾ - DENNIS LEE KASPER⁽²⁾

Universita' degli Studi di Urbino "Carlo Bo", Dipartimento di Scienze Biomolecolari (DISB), Urbino, Italia⁽¹⁾ - Harvard Medical School, Immunology, Boston, Stati Uniti D'america⁽²⁾

Moving beyond the titer paradigm: rational design of a next-generation glycoconjugate vaccine inducing highly functional antibodies

GIUSEPPE STEFANETTI¹, DENNIS L. KASPER²

¹ Department of Biomolecular Sciences, University of Urbino Carlo Bo, Urbino, Italy

² Department of Immunology, Blavatnik Institute, Harvard Medical School, Boston, MA, USA

Introduction: Glycoconjugate vaccines provide enormous global health benefits, but their efficacy in high-risk populations remains a challenge. Conjugation of sugar antigens to carrier proteins has often been empirical, overlooking immune response variables. New insights suggest that carbohydrate presentation to T cells by antigen-presenting cells enhances antibody response efficacy, encouraging the use of peptides as carriers in glycoconjugate vaccines as alternative to whole proteins.

In this study, we present a platform for optimizing glycopeptide vaccine construction, including determining the ideal polysaccharide chain length, peptide composition, and conjugation site. Animal studies were conducted to compare the immune response induced by these optimized glycopeptide vaccines with conventional glycoprotein conjugate vaccines.

Materials and Methods: Female Balb/c mice or rabbits were immunized with Group B Streptococcus type III (GBSIII) and Francisella tularensis (Ft) glycoprotein or glycopeptide conjugate vaccines, given intraperitoneally three times at two-week intervals using Alum or Complete Freund Adjuvant (CFA). Antigen-specific antibody responses were monitored during the experiment by ELISA. In the GBSIII model, the functional activity of vaccine-induced antibodies was evaluated using opsonophagocytic killing assays, as well as by transferring immunized rabbit sera to mice and assessing protection against lethal bacterial challenge. Furthermore, challenge studies were conducted in both the GBSIII and Ft models to determine the level of protection conferred by the different glycoconjugate vaccines against lethal bacterial challenge.

Results: Our findings demonstrate that using a full-length polysaccharide induces optimal immunogenicity for glycopeptide conjugate vaccines. We also demonstrated the critical role of the peptide linker and the conjugation site in enhancing both the conjugation efficiency and the immune response to vaccines. In mouse models of GBS and Ft infection, glycopeptide conjugate vaccines demonstrate superior protection against bacterial challenges when compared to standard glycoprotein conjugate vaccines, despite inducing significantly lower levels of anti-carbohydrate specific IgG antibodies.

Discussion and Conclusions: Our observation challenges the paradigm of a direct correlation between the amount of IgG induced by a glycoconjugate vaccine and the level of conferred protection. Ongoing research aims to comprehensively evaluate the humoral and cellular response elicited by both glycopeptide and glycoprotein conjugate vaccines. The knowledge gained from this evaluation

will be instrumental in the development of glycoconjugate vaccines that provide enhanced efficacy and longer-lasting immunity.

315 - Humoral And Cellular Immune Response after breakthrough infection in Orthotopic Liver Transplant patients who received 3 or 4 doses of SARS-CoV-2 mRNA BNT162b2 Vaccine.

GIUSEPPINA SANNA ⁽¹⁾ - DAVIDE FIRINU ⁽²⁾ - PAOLA CARIA ⁽¹⁾ - VALERIA MANCA ⁽¹⁾ - DANIELA VIRGINIA FRAU ⁽¹⁾ - VANESSA PALMAS ⁽¹⁾ - MASSIMILIANO GALDIERO ⁽³⁾ - CARLA MARIA DEIANA ⁽²⁾ - FRANCESCO PES ⁽²⁾ - LUCHINO CHESSA ⁽²⁾ - ALDO MANZIN ⁽¹⁾

Università di Cagliari, Scienze Biomediche, Cagliari, Italia ⁽¹⁾ - Università di Cagliari, Scienze Mediche e sanità pubblica, Cagliari, Italia ⁽²⁾ - Università Vanvitelli Napoli, Medicina sperimentale, Napoli, Italia ⁽³⁾

Humoral And Cellular Immune Response after breakthrough infection in Orthotopic Liver Transplant patients who received 3 or 4 doses of SARS-CoV-2 mRNA BNT162b2 Vaccine.

GIUSEPPINA SANNA¹, DAVIDE FIRINU², PAOLA CARIA¹, VALERIA MANCA¹, DANIELA VIRGINIA FRAU¹, VANESSA PALMAS¹, MASSIMILIANO GALDIERO³, CARLA MARIA DEIANA², FRANCESCO PES², LUCHINO CHESSA², ALDO MANZIN¹.

¹Department of Biomedical Sciences, Microbiology and Virology Unit, University of Cagliari, Cittadella Universitaria, Sp. 8, 09042 Monserrato (CA), Italy;

²Department of Medical Sciences and Public Health, University of Cagliari, Cittadella Universitaria, 09042, Monserrato, Cagliari, Italy;

³Department of Experimental Medicine, University of Campania Luigi Vanvitelli, 80138 Naples, Italy.

Abstract

Introduction: Solid organ transplant subjects elicit attenuated immune responses to SARS-CoV-2 vaccines. In this study, we report on immune responses after breakthrough infection in Orthotopic Liver Transplant (OLT) subjects who received 3rd- (V3) or 4th- (V4) doses of mRNA BNT162b2 vaccine.

Materials and Methods: Our case-control study included 14 OLT subjects (19 male, aged 47–83 years; 1 female, age 63), and 10 healthy controls (HC) (5 male, aged 32–65 years; 5 females, aged 28–55). Humoral [neutralizing Abs (nAbs)], cellular [interferon-gamma release assay (IGRA), and T cell activation induced markers (AIM)] of immune response were assessed 6–8 months after 3rd- or 4th doses of BNT162b2 mRNA vaccine.

Results: Among OLT patients, 9 out of 14 (64,2%) were simultaneously positive for neutralizing antibodies, IGRA, and T Cell AIM. Eleven out of 14 (79%) tested positive for nAbs, 11 (79%) for IGRA, and 13 (93%) for T-cell AIM. Moreover, after 3rd- or 4th doses of mRNA BNT162b2 vaccine, 1 out of 14 (7%) was simultaneously negative for both neutralizing antibodies and T Cell AIM.

The rate of IGRA response in the healthy control group was 100%, as well as for T cell activation-induced markers. Among HC, only 9% resulted negative for neutralizing antibodies (<1:10, established cut-off value).

Discussion and Conclusions: Taken together, these preliminary data suggest that all subjects analyzed are able to mount an effective immune response after vaccination and infection.

The humoral and cellular responses to SARS-CoV-2 mRNA BNT162b2 vaccine were lower in the OLT cohort than in healthy controls.

Further studies are required to determine the extent and efficacy of responses to vaccines in immunocompromised (transplanted recipients) individuals.

Indice degli autori

A

- A. Jolley Keith 144
 Abate Rosamaria 254
 Abbondio Marcello 89; 90; 98; 101
 Abdelmalek Nader 238
 ABRIL ELVA 428
 Acciarri Carla 143
 Acunzo Marina 216
 ACUNZO MARINA 363
 Addis Elena 130; 423
 AGRILLO CHIARA 92
 Aiezza Noemi 54
 AIEZZA NOEMI 456
 AIROLA CARLO 92
 Airola Sabina 239
 Akbari Nasim 146
 Albano Camilla 260
 Alberti Alberto 250; 300; 492
 Albini Elisa 402
 Aldegheri Luana 67; 87
 Alexandrova Elena 113; 115
 Alfano Alberto 358
 alfarone giovanna 135
 Alifano Pietro 296
 Allizond Valeria 158; 161; 323
 Alobaidallah Mosaed Saleh 238
 Alonso María José 448
 Alteri Claudia 123; 317
 Alviano Francesco 298
 Alvisi Gualtiero 146
 Amata Sara 440
 Amato Caterina 52
 Ambretti Simone 133; 382; 383
 Ambrogi Federico 277
 Ambrosi Cecilia 264; 285; 305
 Ambrosino Annalisa 214; 222; 351
 AMBROSIO LUIGINA 286
 Amendola Antonella 22; 516
 Ammendola Serena 83
 Ammoni Emanuela 73
 Ancona Angelica 36
 Ancona Silvia 26; 111; 219; 290
 Andreoni Stefano 383
 Angeli Andrea 160
 ANGELLOTTI GIUSEPPE 218
 Angius Fabrizio 60; 227; 444; 446
 ANGIUS FABRIZIO 225
 Anthiya Shubaash 448
 Antinori Andrea 56; 347; 442
 Antonelli Alberto 54; 78; 179; 454; 457
 ANTONELLI ALBERTO 456
 Antonelli Guido . 58; 104; 376; 482; 488; 504;
 514
 Antonioli Paola 69
 Antonioti Valentina 108
 Appelberg Sofia 333
 Arcari Gabriele 281; 376
 Ardizzoni Andrea 469
 Arena Fabio 313
 ARGENZIANAO MONICA 189
 Ariawan Daryl 146
 Armillis Alessandra 42
 Arnoldo Luca 69
 ARRIGO IGNAZIO 71; 218
 Artesani Lorenza 177
 ARTESANI LORENZA 184
 Artini Marco 152; 153
 Asa'ad Shirin 425
 Aschbacher Richard 383
 Ashraf Hajra 109
 Assoni Claudia 30
 Atripaldi Luigi 254
 Attili Anna-Rita 242
 AUGUSTE MANON 432
 Auricchio Rosa 196
 Aversa Martina 197
 Azzellino Arianna 73
 B
 Babini Giulia 272
 Baccani Ilaria 454
 BACCI CRISTINA 184
 Baggiani Angelo 393
 Baj Andreina 349
 Bajetto Greta 260
 Baldanti Fausto 131
 Baldassarre Paolo 516
 Baldoni Teresa 254
 Balestrieri Emanuela 18; 498; 500
 BALESTRIERI EMANUELA 327; 486
 Balotta Claudia 461

Balzani Marcello	65	Bianchi Silvia.....	22; 26; 290; 516
Banche Giuliana.....	158; 161; 323	Bianchimani Chiara	457
Bancheri Selenia	204	BIANCO GABRIELE	189
Banchiero Alessandro	181	Biba Camilla	3; 147; 366; 461
Bandera Alessandra.....	123; 317	bichicchi federica.....	275
BANKS LAWRENCE	163	Bielli Alessandra.....	42; 123; 383; 391
Barbieri Francesca.....	191	Bilardi Erica.....	22
Barbieri Giulia.....	268; 283	Bimbati Matteo	273
Bargellini Annalisa.....	154	Bini Francesca	63; 65; 69; 96
Barilli Francesca	516	Biolatti Matteo	260
Barnini Simona	204	Bisi Matteo.....	63; 65; 69
Barreca Giorgio S.....	335	Bitonte Maria Cristina	484
Barreca Giorgio Settimo	20; 129	Biuso Andrea	516
Barski Piotr	170	Bivona Dalida Angela.....	209; 244; 256
Bartolini Niccolò.....	366	Blanda Valeria.....	250
BATANI GIAMPIERO	156	Blasi Elisabetta	469
Batisti Biffignandi Gherard.....	389; 391	Blonna Davide	323
BATISTI BIFFIGNANDI GHERARD.....	374	Boaslako Lidia.....	525
batoni giovanna.....	360	Boccia Giovanni	113; 115
Batoni Giovanna	187; 356; 357; 365	boetius Antje	81
Battistoni Andrea.....	83	BOI CARLA.....	7
Bekas Ioannis	425	Boi Sara	444
Bella Elisa	378	BOLDRIN FRANCESCA.....	368
Bellato Enrico	323	Bolognini Sara	150; 198; 412
Belli Manuel.....	285	BOLOGNINI SARA	38
Bellone Simonetta.....	108	Bolzoni Luca.....	248
Beninati Concetta.....	270; 278	Bona Elisa.....	108
BENINATI CONCETTA	465; 471	Bonacci Paolo Giuseppe	209
Bennett David	529	Bonaccorso Carmela.....	209
Benteley Charlotte.....	250	Bonanno Ferraro Giusy.....	246
Bentivoglio Concetta.....	431	Bondi Alessandro.....	9; 319; 323; 400
Bergna Annalisa	461	BONDI ALESSANDRO	125
Bernabè Giulia	425	Bonfanti Paolo	123
Bernanrdini Sergio	463	Bongiorno Dafne	244; 246; 256; 373
BERNARDINI SERGIO.....	327	Bongiorno Dafne Samantha Irene	44; 209
Bernardo Mariano	383; 412	BONINI ANDREA.....	38
Bernazzali Sonia	529	Bonomo Carmelo.....	44; 209; 246; 256
Berni Melissa	248	Bonsi Laura.....	298
Bertagnin Chiara	165	Bonura Celestino	36; 419
BERTAGNIN CHIARA.....	163	Bonura Floriana	40; 139
Bertoldi Alessia.....	133	bonvicini francesca	275
BERTOLI ADA.....	327	Bordini Michele	423
Bertolino Giacomo.....	380	Borghi Elisa	26; 111; 219; 290; 516
Bertoncelli Anna	130; 196	Borghi Vanni	3
Besutti Valeria.....	425	Borgogna Cinzia	497
Bettini Aurora.....	347	Borriello Giorgia.....	395
Biancalana Edoardo	97	Boru Cristian E.	99
Bianchetti Giada.....	177	BOSCH SORAYA SOLEDAD	156
Bianchi Alice.....	516	Bossi Paolo	30
bianchi marta.....	360	Bottai Daria.....	204
Bianchi Marta.....	356; 357	BOTTAI DARIA	38

BOTTALICO NICOLA	327
Bottazzi Barbara.....	123
Botti Beatrice	200
Bottino Paolo.....	319
Braca Alessandra.....	252
Bracchi Chiara.....	248
Bracci Luisa	147
Brancaccio Rosario	113; 115; 450
Brandolini Martina.....	5; 16; 250; 329
BRANDOLINI Martina	410
Bray James	144
Brazzini Gabriele	463
Brenciani Andrea.....	402; 404
BRENCIANI ANDREA	432
Brescia Brunella.....	20
Briancesco Rossella	246
Brigante Gioconda	383; 389
Brisse Sylvain	144
Brun Paola.....	427
Brunetti Jlenia	147
Bruno Erika	28; 73
bua gloria.....	275
Bua Gloria.....	146; 298
Bucci Cecilia.....	296
Bugani Ginevra	104; 488; 504
Buono frencesco.....	240
Buonocore Carmine	214
Buonocore Francesco	152
Buseti Marina.....	383
Busni Andrea.....	42
Butera Ornella	442
Buttà Michela.....	299; 518
C	

Cabibi Daniela.....	518
Cabiddu Gianluigi.....	185
Cacchiarelli Davide.....	266
Cacciotto Carla.....	250; 300
Cadeddu Arianna.....	380
Cafiso Viviana.....	378
CAFISO VIVIANA	189
Cagliani Rachele	265
Caimi Barbara	461
Cairns Dana.....	260
Calà Cinzia	48; 50; 440
Calabrese Luca.....	137; 434
Calò Alessia.....	279; 467
Calovi Massimo	62
Calvigioni Marco	97; 169
Calvo Maddalena	1
Camarlinghi Giulio	383

CAMBARAU GIULIA	94
Cambria Lucia	361
CAMERA EMANUELA	106
Camilli Romina.....	102; 132; 422
Camilloni Barbara.....	484
Campagna Roberta.....	58; 514
Campagnoli Massimiliano Sergio Francesco	26
Campanella Salvatore	36
Campanile Floriana.....	430
Campioli Edoardo	277
Campione Elena.....	148; 272; 463
Campisciano Giuseppina	67; 85; 87
Campobasso Claudia	150; 198; 204; 412
CAMPOBASSO CLAUDIA	38
campolo morena.....	331
Candela Marco.....	103
Caneparo Valeria.....	506; 508
Cannella Sara	419
CANNOVA LUCIA.....	71
Cantaluppi Vincenzo	506
Canuti Marta	22
Capaldo Brunella	99
Capasso Carla	223; 364
Capitani Valerio	281
Capoccia Danila.....	99
Capozzi Loredana	321
Cappiello Silvia	436
Capra Giuseppina.....	299; 518
Capria Annalisa.....	467
Capria Anna-Lisa	294
caprioli Flavio	474
Capuano Riccardo.....	349
Caputo Beniamino	488
Caputo Marta	36
Cara Alice	467
Caramaschi Alice	108
CARANNANTE ANNA	286
CARAPELLESE CLAUDIO.....	327
Carattoli Alessandra.....	281; 376
Carbognin Elena	266
Carboni Gavino.....	397
Carcione Davide	383; 389
CARDINALI GIULIA.....	156
Cardone Franco.....	60
Carducci Antonella	383
Careddu Fabrizio	158
Caria Paola.....	533
Carmignani Claudia.....	393
Carradori Simone.....	160
Carraturo Francesca	32

Carretto Edoardo	383	Chianese Annalisa....	212; 216; 223; 351; 353; 448
Carru Ciriaco.....	492	Chiarelli Laurent R.	374
Carta Antonio	194; 341; 398	Chiaromonte Adriana.....	358; 364
CARTA FRANCO.....	7	CHIAROMONTE ADRIANA.....	363
Caruccio Ilaria.....	32	Chiera Erika	516
Casale Roberto	400	Chillemi Claudia.....	516
Casana Maddalena	123	Chilleri Chiara	383
Casciato Benedetta.....	457	CHILLERI CHIARA.....	456
Cascio Antonio.....	48	Chimaliro Laston	250
Caselli Elisabetta.....	63; 65; 67; 69; 96	Chines Eleonora.....	378
Cason Carolina.....	67; 85; 87	Chirico Rossella.....	18; 498
Cassani Barbara.....	123	CHIRICO ROSSELLA.....	486
Cassani Gianluca.....	349	Chisu Valentina	316
Castagliuolo Ignazio	425; 427	Ciabattini Annalisa....	522; 523; 525; 527; 529
Castaldo Elisa.....	240	Ciaglia Elena.....	307
CASTANGIA INES	225	CIAMMARUCONI ANDREA.....	286
Castellana Stefano.....	321	Ciardi Maria Rosa.....	476; 482; 521
Castellani Gastone.....	133; 382	Ciccone Federica	482; 521
Castiglioni Federica	506	Cicino Claudia	20; 129; 335; 414
Castoldi Filippo.....	323	Ciciriello Fabiana.....	490
Casu Enrico	150	CIMINI DONATELLA	363
Casula Elisa.....	380	Cinthi Marzia	402; 404
Catamo Eulalia.....	87	Ciociola Tecla	177
Catania Maria Rosaria.....	197	CIOCIOLA TECLA.....	184
Cattaneo Antonino.....	273	Cione Ferdinando.....	452
Caucci Sara	143; 473	Ciotti Marco.....	34; 272; 463
CAUCCI SARA.....	127	CIOTTI MARCO.....	327
Caughey Byron	60	Cipriani Chiara.....	498; 500
CAVALLI ROBERTA	189	CIPRIANI CHIARA.....	486
CAVALLO ALESSIA	106	Cirella Roberta.....	494
CAVALLO ILARIA	106; 426; 428	Cirioni Oscar.....	415
Cavallo Lorenza	158; 161; 319; 323; 400	Cislaghi Ilaria.....	497
CAVALLO LORENZA.....	125	Ciurluini Fabio.....	514
Cavallo Rossana.....	9; 319; 400	clementi nicola.....	310
Ceccarani Camilla.....	111	Clementi Nicola	383
Ceccarelli Matteo	444	Clerici Pierangelo	47
CECCHETTO RICCARDO.....	141; 354	Coccia Anna Maria	246
Cecilia Federico	376	Coccitto Sonia Nina.....	402; 404
Celandroni Francesco.....	97; 169	COCOLIN LUCA.....	125
Celegato Marta	165	Cocuzza Clementina	292
CELEGATO MARTA	163	Cocuzza Clementina Elvezia	28; 30; 73
Cellini Luigina	208; 230	Codda Giulia.....	137; 383; 434
Cereda Danilo	73	Coen Sabrina.....	347
Cermelli Claudio	154	Coghe Ferdinando.....	380
Cerquetti Marina	102	Colacicco Annamaria.....	14
Cerracchio Claudia.....	242	Colavita Francesca.....	347
CERRATO ANTONIO MARINO	374	Colella Marica	14
Cervo Adriana	3	Colicchio Roberta	32; 200; 202; 296; 494
Chandel Shika	497	Colico Caterina	277
Cheri Elena.....	285	Collura Antonina.....	40; 139
Chessa Luchino	533		

Colombarolli Stella Garcia.....	177
Colombini Lorenzo	387
COLOMBINI LORENZO	512
Colombo Alberto.....	349
COLOSIMO CLAUDIA.....	410
Colosimo Elena.....	129
Colucci Francesca	406
Colzani Daniela.....	22; 516
Comar Manola.....	67; 85; 87; 104; 262
Comelli Agnese.....	123
Comini Sara.....	161
Compagnino Daniele Emanuele.....	58
Cona Andrea.....	244
Congiargiu Antonella	492
Coniglione Filadelfo	498
Consoli Grazia M.L.....	206
Consonni Alessandra.....	383
Consortium Micro-RER.....	248
Conte Antonella.....	476; 482; 521
Conti Andrea	87
Conti Maria Giulia	488
Conti Paola.....	430
Conti Stefania.....	177
CONTI STEFANIA	184
Coppi Marco	54; 78; 179; 454
COPPI MARCO.....	456
Coppola Bartolomeo	161
Coppola Chiara	523; 529
Coppola Luigi	18
COPPOLA LUIGI.....	486
Coppola Maria Grazia.....	254
Coppolino Francesco.....	270; 278
COPPOLINO FRANCESCO.....	465; 471
Corà Davide	506
Corcione Silvia.....	319; 400
Cordini Claudia.....	339
Cordua Emanuele.....	406
Cori Irene	473
Corinaldesi Elena	500
Coroneo Valentina.....	176; 181
Corradini Corrado Maria.....	242
Corraini Alessio.....	196
Cortazzo Venere	383
Cortegiani Andrea	36
Cortesi Enrico	514
Corvaro Benedetta.....	143; 473
Corvino Giusy.....	254
Cosco Cristina.....	331
Cosimato Ilaria.....	307; 452
Cosio Terenzio	148; 272; 463
Cossentino Luigi	76

Cossu Davide	459
Cossu Giovanni.....	60
Costa Cristina.....	9; 319; 323; 400
COSTA CRISTINA	125
Costa Elisa	137; 434
Costa Mario	273
Costagli Simone.....	523; 529
Costanza Gaetana.....	148
COSTANZA GAETANA.....	327
Costarelli Silvia	467
Crescenzo Laura	254
creti roberta.....	135
Cricca Monica.....	5; 16; 250; 329
CRICCA MONICA	410
Criscuolo Anna	34
CRISTAUDO ANTONIO.....	106
Cristina Mario	285
Cross Emily, M.	146
Crovara Pesce Cristina Maria	133
CRUCIANI GABRIELE	163
Crudele Valeria	113; 431
Crudele Veleria	115
CUBEDDU MARINA.....	7
Cucco Lucilla.....	402
Cuffini Anna Maria.....	158; 161; 323
Cuomo Maria Concetta.....	32
Cuppone Anna Maria.....	387
CUPPONE ANNA MARIA.....	512
Curcio Michele	467
Curtoni Antonio	9; 319; 323; 400
CURTONI ANTONIO	125
Cuteri Vincenzo	242
D	

D. Pither Molly	494
D'Accolti Maria	63; 65; 69; 96
D'Achille Gloria.....	404; 415
D'Agostini Cartesio.....	305
Dal Piaz Fabrizio	232
D'Alessandro Gaetano	48; 52
D'alessio Nicola	240
D'Alessio Nicola	395
Dalla Pietà Anna	266
D'Ambrosio Fabio.....	132; 422
D'Ambrosio Pasquale	14
D'AMBROSIO SERGIO	363
D'Amico Emira	208
D'Andrea Marco Maria.....	179; 490
D'ANGELI TOMMASO.....	163
D'Angelo Caterina	153
D'Angelo Gianni	448

D'Arcangelo Sara	208; 230	Del Piano Francesca	395
D'Argenio Annamaria	254	Del Prete Raffaele	14; 24; 321
D'Auria Alessandra	488; 504	Del Vecchio Claudia	316; 425
D'Auria Rosa	32	Delbue Serena	277; 478; 497
De Andrea Marco	260; 497; 506; 508	DELEDDA ANDREA	94
De Angelis Marta	191	Deledda Maria Antonietta	89; 101
De Bernardo Maddalena	452	Dell' Annunziata Federica	212
de carli alessandro	310	Della Marca Roberta	174
De Carli Alessandro	170; 289	Della Paolera Sara	85
De Carlo Carmela	24; 321	Della Rocca Maria Teresa	12; 254; 406; 431
De Carlo Esterina	395	Della Sala Gerardo	214
DE CARO VIVIANA	218	Dell'Annunziata Federica	113; 115; 232; 307; 450; 452
De Chiara Giovanna	303	Dell'Oste Valentina	260
De Diego Laura	89; 90; 98; 101	Delogu Giovanni	99; 167; 236; 531
De Felice Claudio	525	Delogu Ilenia	227; 446
De filippis Anna	448	DELOGU ILENIA	225
De Filippis Anna	212; 214; 216; 222; 351; 353; 431	Demuru Laura	185
De Filippis Anna	223	Depau Lorenzo	147
De Filippis Barbara	230	D'Ercole Simonetta	208; 230
De Francesco Alice	376	DESANTIS JENNY	163
De Gaetano Giuseppe V.	270	Desideri Ielizza	393
De Gaetano Giuseppe Valerio	278	Dessi Daniele	492
DE GAETANO GIUSEPPE VALERIO	465; 471	d'Ettorre Gabriella	104
De Giorgi Stefano	387	D'Ettorre Gabriella	488; 504
De Grazia Simona	40; 52; 139	Di Bella Stefano	383
De Gregorio Eliana	234	Di Bernardo Francesca	40; 139
De Gregorio Rebecca	191	Di Bonaventura Giovanni	285; 365
De Iaco Giulia	337	Di Campi Emanuela	230
De Laurentiis Vittoriana	24; 321	Di Caprio Giovanni	406
De Leo Marinella	252	Di Cello Carolina	414
De Leonardis Francesco	24; 321	DI DOMENICO ENEA GINO	106; 426; 428
De Logu Alessandro	185	Di Fermo Paola	208; 230
De Luca Giovanna	395	DI FRANCESCO FABIO	38
De Luca Nicoletta	50	Di Gennaro Francesco	337
De Maio Flavio	99; 167; 236; 531	Di Giulio Mara	208; 230
DE MAIO FLAVIO	92; 106	DI GREGORIO ALESSANDRA	432
De Martino Luisa	242; 436; 438	Di Lodovico Silvia	208; 230
De Nittis Rosella	103; 313	Di Lorenzo Flaviana	494
De Palma Angela	337	Di Luca Mariagrazia	150; 198; 204; 412
de Pascale Donatella	214	DI LUCA MARIAGRAZIA	38
De Pascali Alessandra Mistral.	5; 16; 250; 329	di Marzo Carlo	252
De Seta Francesco	469	Di Marzo Carlo	194; 229
De Siena Massimo	335	Di Mattia Greta	488
Degiacomi Giulia	374	Di Meo Maria Letizia	28; 292
Dei Cas Michele	219	Di Palma Cristina	436
Deiana Carla maria	533	Di Pietra Giuseppe	427
Deidda Raffaele	380	Di Pilato Vincenzo	54; 137; 434
Del Giudice Annalisa	254	Di Primio Cristina	309
Del Grosso Maria	132; 422	Di Rosa Domenico	113; 115; 450
		Di Rosario Martina	200; 202; 494

Di Salvo Sebastiano	335
Di Sopra Sarah	316
Diamante Linda.....	266
DIANI ERICA	141; 354
Diaz Nicia	397; 492
Diban Firas.....	208; 230
Diprimio Cristina	273
Dirani Giorgio	5; 16; 329
Distefano Salvatore Antonino	36
Divenuto Francesca.....	331
Dizanzo Maria Paula.....	266
Dodaro Saveria.....	383
Dodi Alessandra	248
Dolci Maria	277; 478
Doldo Patrizia	331
Dominelli Federica.....	476; 482; 514; 521
doneddu sonia	7
Donnarumma Giovanna	216; 358; 364
DONNARUMMA GIOVANNA.....	363
Dore Maria Pina	98
Doti Nunzianna	216; 223; 351; 353; 448
Douce Gillian	84
Drago Ferrante Francesca	349
Dreassi Elena.....	229
Durante Adriana	12; 406
Durante Miriam.....	527
E	

Egorova Anna.....	343
EGOROVA ANNA	374
Ehrhardt Carsten	187
Ennas Guido.....	227
Erre Gianluca	492
Errico Giulia.....	132; 422
esin semih.....	360
Esin Semih	187; 356; 357
ESIN SEMIH	365
ESPEVIK TERJE.....	465
Esposito Anna	234
Esposito Assunta	222
Esposito Emanuela.....	361
Esposito Valentina.....	414
F	

Fabbiani Massimiliano.....	523; 527; 529
Fabbri Chiara.....	96
Fabeni Lavinia.....	442
Fabi Marianna	500
Fabi Silvia	316
FABRIZIO GIORGIA.....	106; 426
Faccin Mauro	133; 382
Facciotti Federica.....	474

Falanga Annarita.....	202
Falciani Chiara.....	147
FALCONE MARCO	156
FAMA' AGATA	465
Famà Agata	270; 278; 471
FANCELLO GIOVANNI.....	92
Fanelli Marialaura.....	18; 498; 500
FANELLI MARIALAURA.....	327; 486
Fanti Diana.....	42
Fappani Clara.....	22; 516
Farabegoli Patrizia	16
Faraone Sara	58
Farina Roberto	96
fasano erika	275
FASCIANA TERESA.....	71; 218
Fasciana Teresa M.A.....	50
Fasciana Teresa Maria Assunta.....	48
Fausti Elisa.....	204
FAVARATO MOSè.....	141; 354
Favaro Annagiulia.....	165
favaro domenico	310
Favaro Domenico.....	170
Favero Francesco	506
Favi Evaldo	277
FAZIO CECILIA	286
Federici Franco	421
Ferlosio Amedeo	272
Ferraguti Patrizia	380
Ferrante Pasquale.....	277
Ferraresso Mariano	277
Ferrari Guglielmo	131
Ferraro Donatella.....	40
Ferravante Carlo	113; 115; 254
Ferrazzano Gina.....	476; 482; 521
Ferreri Loredana	206
Ferretti Caterina.....	150; 412
FERRETTI CATERINA.....	38
FERROCINO ILARIO	189
FIAMMA MAURA	7
Fiaschi Lia	3; 147; 366; 461
Filice Maria Elena.....	294
filippini fabio	310
Filippini Fabio	150
Filipponi Carolina.....	289
Filizzolo Chiara	40; 139
FILLO SILVIA	286
Finamore Emiliana.....	254; 431
Fioretti Anna	197
Fiori Barbara.....	383
Fiori Pier Luigi	397; 492
Fiorilla Carlotta.....	305

Fiorino Fabio..... 301; 523; 525; 527
 FIORINO FABIO.....512
 Fioriti Simona 404; 415
 Fiorito Filomena.....242
 Firinu Davide 533
 Fiume Elisa 161
 Floresta Giuseppe.....221
 Focosi Daniele..... 442; 467
 Foglia Francesco 222; 431
 FOIS MARCO 7
 Folliero Veronica..... 113; 115; 212; 232; 254;
 307; 341; 450; 452; 510
 Forcella Matilde E.....265
 Forni Diego 265
 Fortuna Sebastiano Alberto.....430
 FORTUNATO ANTONELLA 286
 Forwood Jade 146
 FOSCI MICHELE.....94
 Fox Valeria 123; 317
 Foxi Cipriano 316
 Fracella Matteo 104; 488; 504
 Frallicciardi Francesco 32
 Francati Santolo 160
 Franchi Elena 374
 Franci Gianluigi 113; 115; 212; 214; 232; 254;
 307; 343; 448; 450; 452; 510
 Franconi Iacopo..... 10; 172; 467
 Franzetti Andrea 73
 Frasca Federica 104; 488; 504
 Frasson Ilaria.....266
 Frateschi Susi 467
 Frau Daniela Virginia.....533
 Fraziano Maurizio 179; 490
 freer giulia 310
 Freer Giulia 150; 194; 229; 252; 279; 289;
 309; 398; 467; 480
 Fruscio Robert..... 28; 292
 Fuochi Virginia..... 182; 221
 Furlan Berenice 62; 80; 372
 Furnari Salvatore..... 182; 221
 Furneri Pio Maria 182; 221
 Fusco Alessandra..... 216; 358; 364
 FUSCO ALESSANDRA.....363
 Fusco Giovanna..... 76; 395
 Fusi Paola.....265
 G
 Galardo Gioacchino 482
 Galdiero massimiliano 214

Galdiero Massimiliano..... 174; 212; 216; 222;
 223; 232; 254; 307; 351; 353; 431; 448;
 452; 533
 Galdiero Stefania 202
 Galdiero Umberto 197
 GALIA ELENA 71
 GALLINA GIUSEPPE 71
 gallinella giorgio 275
 Gallinella Giorgio 146; 298
 Gallo Luigia 129; 414
 gambardella erika.....331
 Gamberini Chiara.....298
 GARAU MARIA CRISTINA.....7
 Garbero Olga Valentina 158
 Garcia Fernandez Aurora.....383
 Garcia-Montojo Marta 481
 Garg Ritu.....262
 Gariglio Marisa.....497; 506; 508
 Garosi Guido.....522
 Garzone Stefania.....24
 Gasparini Chiara 516
 Gatti Antonella.....461
 Gatti Giulia 5; 16; 250; 329
 GATTI GIULIA 410
 Gaudino Giulia.....232
 Gaziano Roberta 148; 463
 Geginat Jens.....474
 Gemignani Giulia.....393
 Genco Mattia 319
 GENCO MATTIA 125
 Gennenzi Benedetta 58
 Genoni Angelo Paolo.....349
 Geoghegan Joan.....268
 Ghelardi Emilia.....97; 169; 393
 gherardi giovanni 135
 Ghiani Rossella M. 380
 Giacca Mauro.....262
 Giacometti Andrea 415
 GIAMMANCO ANNA 71; 218
 Giammanco Giovanni 36; 419
 Giammanco Giovanni M. 40; 48; 50; 440
 Giammanco Giovanni Maria 52
 Giammanco Giovanni Maurizio 139
 Giammarino Federica 366
 Giancotti Aida.....20; 129; 335
 Giancristofaro Sara 132; 422
 Giani Tommaso 54; 454; 457
 GIANI TOMMASO.....456
 Gianoli Stefano 478
 Giardina Federica Anna Maria.....131
 Gibellini Davide.....130; 423

GIBELLINI DAVIDE	141; 354
Gigante Paolo	349
Gigli Francesca	242
Gigliotti Simona	20; 331
Ginestra Giovanna	206
Gioia Marco	452
Giombetti Erica	484
Giombini Emanuela	442
Giordano Cesira	204
GIOSA DOMENICO	496
Giovacchini Nicla	454
Giovanetti Eleonora	402
GIOVANETTI ELEONORA	432
Giovati Laura	177
GIOVATI LAURA	184
Girardi Enrico	56; 347; 442
Girardi Santa	76
Giubbi Chiara	28; 30; 73; 292
Giudice Martina	18; 498; 500
GIUDICE MARTINA	486
Giudici Riccardo	391
Giufre' Maria	102
Giugliano Rosa	214; 351; 353
Giurato Giorgio	254
Gobbato Elisa	383
GORACCI LAURA	163
Gori Andrea	123; 317
Gori Maria	22; 290; 516
Gorrini Chiara	339
Graceffa Domenico	36
Gramigna Giulia	442
Granata Giuseppe	206
Grande Rossella	160
Grassi Massimo	321
Grassiri Brunella	187; 357
Gravina Claudia	222
Gravina Tiziana	335
Graziani Alessandro	484
Greco Francesca	383
Greco Giuseppe	254; 431; 448
Greco Miani Vittorio	24
Greco Rita	12; 113; 115; 254; 406; 450
Grelli Sandro	18; 500
GRELLI SANDRO	327; 486
Gribaudo Giorgio	333
Griffante Gloria	497
Griffith Darren M.	187
Grimaldi Elena	431
Gruber Cesare	442
Grumiro Laura	5; 16; 329
Guaragna Annalisa	234

Guardiani Mariasilvia	521
GUARNACCIA ALESSANDRA	92
Guarnelli Maria Elena	96
Guarrasi Luisa	319
Guerra Massimiliano	5
Guerrini Alessandro	242
Gugliesi Francesca	260
Guida Gabriella	516
Gurizzan Cristina	30
H	

Hallam Jennifer	84
Harder Olivia	174
Hattori Nobutaka	459
Healy Anne Marie	187
HEIDRICH VITOR	94
HEWELT-BELKA Weronika	260
Hoad Mikayla	146

I

Iacono Elena	252; 289; 310
Iacovelli Federico	34
IANNARELLI FEDERICA	92
Iannarelli Silvia	305
Iannelli Francesco	387
IANNELLI FRANCESCO	512
Iannetta Marco	18
IANNETTA MARCO	486
Iannucci Andrea	508
Iannuci Andrea	506
Iannuzzi Filomena	303
Iatta Roberta	240; 337
Ibba Roberta	194; 341; 398
Iebba Valerio	104; 119; 121
Ijaz Umer Zeeshan	109
Immordino Rita	50; 299; 440
imperi monica	135
Imperlini Esther	152
Indraccolo Francesca	240; 321
Intoccia Michele	144
Iovane Giuseppe	254
Iovane Valentina	223; 254; 438
Izzo Viviana	232

K

KABANOVA ANNA	156
KALENZI ATUHAIRE DAVID	250
Kaplan David	260
Kasper Dennis Lee	532
Kavanagh Kevin	187
kaya esingul	360
Kaya Esingul	356; 357

KAYA ESINGÜL	365
Kazakova Elena.....	343
L	
La Costa Giorgia	24
La Frazia Simone	193; 317
La Ragione Roberto	383
La rocca Veronica.....	310
La Rocca Veronica	289; 408
La Rosa Giuseppina	73; 76; 246
La Vergata Lorena	339
Labella Stefano	12
Lacarbonara Davide	506; 508
Laganà Pasqualina.....	270
LAGNI ANNA.....	141; 354
Lai Alessia.....	461
LAI ALESSIO.....	94
Lai Michele	170; 194; 252; 279; 289; 294; 309; 310; 398; 467; 480
Lamagna Barbara	436
Lamberti Angelo G.....	335
Lamberti Angelo Giuseppe	129
Lamberti Jessica.....	113; 115
Lanar Marcello	500
Landolfo Santo.....	260; 506
Landoni Fabio	28; 292
Lanfredi Caterina	239
Laratta Emanuela	20
Latronico Tiziana	482
Laurenti Patrizia.....	69
Lavigne Rob.....	198
Lazzari Elisabetta.....	58
Lazzaro Lorenzo Mattia	430
Lazzarotto Tiziana.....	325; 345
Lazzeri Elisa.....	387
LAZZERI ELISA.....	512
Lazzi Stefano.....	301
leccese gabriella	474
Lentini Germana	270; 278
LENTINI GERMANA.....	465; 471
Lenzo Federica.....	430
Leonardi Giovanna.....	3
Leoncini Silvia	525
Leone Alessio.....	9
Leone Maria Gemma	58
Leonetti Frida.....	99
Lepera Valentina.....	339; 417
Lepioshkin Alexander	343
LEWIS RUSSELL E.....	410
Li Puma Domenica D.....	303
Lia Riccardo P.	337

Lichtner Miriam.....	482; 521
Licini Caterina	415
LIEN EGIL	465
Limongi Dolores.....	305
Lipani Francesco.....	154
LISTA FLORIGIO ROMANO	286
Liuzzi Grazia Maria.....	482
Lo Cascio Giuliana	339; 417; 421
Lo Cigno Irene.....	497; 506
Lo Porto Davide.....	244
Locantore Francesco	240
Locati Massimo	478
LODDO FRANCESCO MARCO	7
LOI ELISABETTA.....	94
Lombardi Annalisa.....	76
Lombardi Donatella	133
Lombardi Grazia	457
Lombardo Donatella	382
Longhitano Lucia.....	182
Longo Fabio.....	158; 161
LONGO FABIO.....	323
Longo Roberta	143; 473
Lopardo Valentina.....	307
López-Estévez Ana M.....	448
Loregian Arianna	165
LOREGIAN ARIANNA.....	163
Lorini Luigi.....	30
Lorusso Mara	24
LOSCHI STEFANIA.....	327
Lotti Tommaso.....	78
LOTTI VIRGINIA.....	141; 354
Lottini Giulia	279; 309; 398; 480
LOVISELLI ANDREA	94
Lovreglio Piero	240
Lubello Claudio	78
Lucarini Guendalina	415
Lucas Alexandre	18
Lucchesi Simone.....	522; 523; 527
Lucidi Vincenzina	490
Luganini Anna	333
Luongo Marianna.....	204
Lupetti Antonella	10; 172
Lupetti Pietro	179
Lupetti Veronica.....	365
Luzzati Roberto	383
M	
MACCARI GIUSEPPE.....	156
Maccioni Elias	185
Maciag Anna	307
Maddaloni Luca	104; 488; 504

Maffei Massimo Emilio	333	Marchesi Isabella	154
Maggi Fabrizio	56; 347; 442	Marchi Saverio	415
Magistrali Chiara Francesca	402	Marcocci Maria E.	303
Maglio Angelantonio	510	MARCUCCILLI FABBIO	327
MAHJOUB AHLEM	465	Margaglione Maurizio	103; 313
maida chiara	331	Margarita Valentina	397
Maina Kelvin Kamau	478	Marinacci Beatrice	160
Maina Kevin Kamau	277	Marinelli Katia	473
maisetta giuseppantonio	360	MARINELLI KATIA	127
Maisetta Giuseppantonio	356; 357; 365	Marino Andrea	373
Makarov Vadim	343; 374	Marino Andreana	206; 361
MALAGNINO VINCENZO	486	MARINO-MERLO FRANCESCA	496
Mali Avinash	448	Marongiu Alessandra	343
Malimpensa Leonardo	521	Marra Alberto	510
Malinowski Michal	357	Marras Luisa	176; 181
Malpei Francesca	73	Marrazzo Pasquale	298
manaresi elisabetta	275	Marrollo Roberta	383
Manaresi Elisabetta	146	Marsiglia Matteo	290
MANCA MARIA LETIZIA	225	Marsiglia Matteo Domenico	26
Manca Valeria	341; 533	Martello Graziano	266
Mancini Nicasio	383	Martin Kristina Nicole	244
Mancini Nicasio	349	Martina Maria Grazia	191
Mancini Pamela	246	Martinelli Marianna	28; 30; 73; 292
Mancino Enrica	488	Martiniello Giorgia	508
Mancioppi Valentina	108	Martins Queiroz-Junior Celso	165
Mancusi Andrea	76	Martins Teixeira Mauro	165
Mancuso Elettra	331	Martorana Alessandra	80
Mandalà Edoardo	52	Marulli Giuseppe	337
Mandas Antonella	60	Marzucco Anna	5; 16; 329
Mandras Narcisa	158; 161; 319	MARZUCCO ANNA	410
MANDRAS NARCISA	125	Marzuillo Carolina	281
Manente Roberta	113; 115; 510	Masala Giovanna	316
Mangioni Davide	123	Mascarella Chiara	139; 419
Maniscalco Eleonora	158; 161; 323	Massacci Francesca Romana	402
MANISCALCO ELEONORA	189	Massaro Chiara	48
Mannavola Cataldo Maria	236	Massidda Orietta	62; 80; 372; 454
Mannino Erika	518	Massimino Mariacristina	169; 373
Manno Michele	129; 331	MASTINO ANTONIO	496
Mansi Antonella	154	Mastria Michele	24
Mantova Giuseppe	200; 202; 296; 494	Mastroianni Claudio Maria	104; 476; 482; 521
Manzin Aldo	60; 227; 341; 343; 444; 446; 448; 533	MASUCCI LUCA	92
MANZIN ALDO	94; 225	Masullo Angelo	436; 438
Manzoni Mariateresa	197	Matarazzo Elisa	42
Maracchioni Christian	18	Matera Giovanni	20; 129; 331; 335; 414
MARACCHIONI CHRISTIAN	486	Matera Luigi	488
Marangi Marianna	103; 239; 313	Matteucci Claudia	18; 498; 500
Maraolo Alberto Enrico	383	MATTEUCCI CLAUDIA	327; 486
Marascio Nadia	20; 129; 331; 335; 414	Matteucci Matteo	279; 480
Marchese Anna	137; 383; 434	Mattiello Amalia	510
MARCHESI FRANCESCO	428	Mattioni Marchetti Vittoria	389; 391; 417
		Matusali Giulia	56; 347

Maulucci Giuseppe	177	Minutolo Antonella.....	18; 34; 498; 500
Mauri Carola	383	MINUTOLO ANTONELLA	327; 486
Mavilio Giusy	358; 364	Mirabile Alessia	44; 244
MAVILIO GIUSY.....	363	Mirandola Mattia	333
Mazzacane Sante.....	63; 65; 69	Mirasola Pietro.....	36
Mazzantini Diletta.....	97; 169	Mirazimi Ali.....	333
Mazzariol Annarita.....	130; 196; 383; 423	Miteva Mariya T.	303
mazzetti paola	502	Mocci Valentina	301
Mazzetti Paola.....	229; 294; 467	Moens Ugo.....	272; 463
Mazziga Eleonora	63; 65; 69; 96	Mola Beatrice.....	325; 345
Mazzotta Valentina.....	347; 442	Molari Massimiliano.....	81
Meadglini Donata.....	525	Molinaro Antonio.....	494
Medaglini Donata.....	301; 522; 523; 527; 529	Monaco Francesco	244
MEDAINA NICOLETTA.....	141; 354	Monaco Monica	132; 422
Mela Ludovica	123	Monakhova Natalia.....	343
Melardo Colombina	431	Mongiardo Fabio	335
Meleddu Rita.....	185	Montagna Maria Teresa	24; 321
MELIS MARIETTA	94	Montagnani Francesca	522; 523; 527; 529
Mencacci Antonella.....	484	Montagnaro Serena.....	223
Mencarelli Noemi	160	Montagner Marco	266
Meneghello Silvia	427	Montanari Maria Sofia.....	5; 16; 329
MENGARELLI ANDREA	428	MONTANARI SOFIA.....	410
MENICHETTI FRANCESCO.....	156	Montella Fortunato	431
Menotti Francesca	158; 161; 323	Montesano Carla	34
MENOTTI FRANCESCA	125; 189	Montesi Giorgio.....	522; 527
Menozzi Ilaria	248	Monti Alessandra	216; 223; 351; 353; 448
Mensi Magda.....	96	Montrucchio Giorgia.....	400
Mensitieri Francesca	232	Morana Alessandra	448
Menzo Stefano	143; 473	Morea Anna	240; 337
MENZO STEFANO.....	127	Morecchiato Fabio	78
Merakou Christina.....	132	Morelli Enrico.....	527
Mercorelli Beatrice	165	Morelli Miriana.....	516
MERCORELLI BEATRICE.....	163	Morelli Paola	123
Merli Marco	391	Morganti Marina	248
Merola Filomena.....	406	Moro Stefano	165
Messa Lorenzo	165	MORO STEFANO.....	163
MESSA LORENZO.....	163	Morone Antonio.....	212
Mezzatesta Maria Lina.....	186	Morone Maria Vittoria.....	212; 452
Michetti Emma.....	83	Morone Mariavittoria.....	222
Miciakova Zuzana.....	305	Morrone Gianluca.....	383; 404; 415
Midulla Fabio.....	488	Mosca Adriana	24; 321; 383
Miggiano Riccardo.....	508	Moscato Giovanna	467
Migliaccio Antonella.....	144; 234	Motta Chiara	268; 283
Migliavacca Roberta	389; 391; 417; 421	Mozzi Alessandra.....	265
MIGLIORISI GIUSEPPE	1	Mularoni Alessandra.....	244
Mignanelli Marianna.....	273; 309	Mullis Adam	260
MILENKOVIC Stefan	444	Mura Roberto.....	380
Milito Cinzia	102	Muresu Narcisa	292
Mina Erika.....	260	Murgia Marcello	176; 181
Mingoia Marina.....	404	MURGIONI MARIA TERESA.....	7
Minì Giuseppe.....	48	Muscatello Antonio.....	123

Musella Mario 99
 Mussini Cristina 3
 Musso Nicolò 44; 209; 246; 256
 MUSSO TIZIANA 189
 Musumeci Rosario 28; 73; 292

N

Nardini Vincenzo 294
 Nardone Maria 457
 Nassa Giovanni 254
 Nastri Bianca Maria 223
 Natale Giusy 406
 Nath Avindra 481
 Nencioni Lucia 191
 NERI ARIANNA 286
 Nicastri Emanuele 56; 442
 Niccolai Claudia 78
 Nicitra Emanuele 44; 209; 256
 Nicolini Elena 42
 Nicolosi Alice 314; 373
 Niewiesk Stefan 174
 Nigrisoli Giacomo 383
 Nisi Lucia 24
 Njoku Ruth 292
 Njoku Ruth Chinyere 28; 30
 Nocera Francesca Paola 242; 436; 438
 Nonne Chiara 58
 Nostro Antonia 206; 361
 Nottoli Silvia 194; 229; 398
 Novazzi Federica 349
 Novello Giorgia 108
 Noviello Daniele 474
 Nunnari Giuseppe 256
 Nunzi Ilaria 404; 415

O

O'Ferrall Lewis M. 187
 Olandese Chiara 202
 Oldani Monica 265
 Olimpieri Tommaso 179; 490
 Oliva Alessandra 376
 Olivares Renato 76
 OLLA GIACOMO 7
 Olsen John Emerdahl 238
 OMBRES DOMENICO 327
 Orlando Fiorenza 415
 ORRÙ CHRISTINA D. 60
 Orsini Massimiliano 402
 Otranto Domenico 240; 337
 ottaviano emerenziana 111
 Ottaviano Emerenziana 26; 219; 290; 516

P

PABA PIERPAOLO 327
 Paccagnella Michele 316
 Paccagnini Eugenio 179
 Pacello Francesca 83
 Pacheco Daniela 117
 Paci Lorenzo 78; 454
 PACIELLO IDA 156
 Pacifico Severina 222
 PADDEU ANTONELLO 7
 Paduano Consiglia 30
 Pagani Elisabetta 383
 Pagano Claudia 158; 161; 323
 PAGES REBECCA 428
 Pagliano Pasquale 113; 115
 Pagliarulo Caterina 200; 296
 Paglietti Bianca 238
 Paglione Francesco 236
 Pagliuca Chiara 32; 200; 202; 296; 494
 Pagnini Ugo 223; 254
 Pagnotta Michela 132; 422
 Pala Giuseppe 227
 Palamara Anna T. 303
 Palamara Anna Teresa 104; 264; 285; 305
 PALERMO MARIO 71
 Paletti Chiara 366
 Palladino Giorgia 103
 Palma Francesca 216; 353
 Palmas Vanessa 341; 343; 533
 PALMAS VANESSA 94
 Palmero Paola 161
 Palmieri Francesco 448
 Palmieri Valentina 167; 531
 Palucci Ivana 167; 236; 531
 Palumbo Domenico 113; 115
 Palumbo Piccionello Antonio 440
 Panetta Vittorio 12; 254; 406
 Panico Erica 216
 Pantanella Marta 20; 331; 335
 Pantosti Annalisa 102
 Panzarella Vera 518
 Paone Marco 395
 PAPA ROSANNA 152; 153
 Papi Massimiliano 167; 531
 Pappalardo Martina 378
 Paradiso Rubina 395
 Pariani Elena 317
 Paris Irene 152; 153
 Parisi Antonio 321
 Parisi Michela 321

Parisio Eva Maria.....383
 Parolini Ornella.....236
 Paroni Moira.....474
 Parrella Roberto.....510
 Parrilli Ermenegilda.....153
 Pasca Maria Rosalia.....374
 Pasciuta Renée.....349
 pasculli patrizia.....476
 Pasculli Patrizia.....482; 521
 Pasetto Carlotta.....250
 Pashaj Ermal.....498
 Pasqua Martina.....264
 Pasqual Denis.....333
 Pasquero Selina.....260
 Passerini Sara.....272; 463
 Pastore Gabiria.....522; 523; 525; 527
 Pastori Elena.....42
 Pastrone Lisa.....400
 Pasutto Priscilla.....26
 Patamia Ildebrando.....314
 Patamia Vincenzo.....221
 Paterniti Irene.....361
 Pauciullo Silvia.....193
 PAVAN MATTEO.....163
 Pavan Silvia.....146
 Pavia Grazia.....20; 129; 335; 414
 Pellegrini Angelica.....268; 283
 Pellegrini Benedetta.....160
 PELUSO EMANUELA.....117
 Penna Alessandro.....266
 Pennino Francesca.....76
 Pentimalli Tancredi M.....102
 Perdoni Federica.....28; 30; 73; 292
 Perego Marta.....277
 Perfetto Brunella.....358; 363; 364
 Pericolini Eva.....469
 Perini Giordano.....167
 Perna Rossella.....254
 Perno Carlo F.....123
 Perno Carlo Federico.....3
 Perrera Paola.....273; 288; 309
 PERRON HERVE.....486
 PERUGINI IOLANDA.....125
 Pes Francesco.....533
 Pes Giovanni Mario.....98
 Petersen Gayle, F.....146
 Petitta Marco.....65
 Petrini Morena.....208
 Petrini Paola.....117
 Petrizzi Ilaria.....389
 Petrone Vita.....18; 498; 500

PETRONE VITA.....327; 486
 PETRUCCELLI ROSALBA.....327
 Pettini Elena.....301; 527
 PETTINI ELENA.....512
 PHAN NGOC DAN THANH.....492
 Piana Andrea.....292
 Piatek Magdalena.....187
 Piazza Aurora.....389; 391; 417; 421
 Piazza Carmen Rita.....273; 309
 Pibiri Ivana.....440
 Piccolella Simona.....222
 Picone Vincenzo.....514
 Piemontese Simona.....3
 Pierangeli Alessandra.....104; 488; 504
 Pierri Biancamaria.....76
 Pietrocola Giampiero.....268; 278; 283
 Pietropaolo Valeria.....272; 463
 PILLONI SILVIA.....7
 PIMPINELLI FULVIA.....106; 426; 428
 Pinzauti David.....387
 Pipitò Luca.....48
 Piralla Antonio.....131; 317
 Piras Anna M.....187
 Piras Anna Maria.....357
 Piras Sandra.....194; 341; 398
 PIREDDA LUCIA.....327
 Piscopiello Francesca.....391; 417; 421
 Piselli Pierluca.....347
 pistello mauro.....310
 Pistello Mauro.. 170; 229; 252; 273; 289; 294;
 309; 467; 480; 504
 Pistoia Daniela.....518
 Pistoia Enrico Salvatore.....148
 Pitrolo Antonino Maria Guglielmo.....131
 Pizzano Francesca.....436; 438
 Pizzo Mariangela.....40; 48; 139; 440
 Pizzolante Antonio.....76
 Plicanti Erika.....279; 398; 480
 Poerio Noemi.....179; 490
 Pol Riccardo.....383
 Polani Riccardo.....376
 Poleggi Anna.....60
 Polissi Alessandra.....80
 Pollini Simona.....78; 383
 Polvere Jacopo.....522; 523; 525; 527; 529
 Poma Elena.....380
 POMA NOEMI.....38
 Pompameo Marina.....436
 Pompei Raffaello.....380; 446
 Pompilio Arianna.....285; 365
 Pongolini Stefano.....248; 339

Ponsecchi Greta.....	179; 490
PONTONE MARTINA.....	426
PONZIANI FRANCESCA ROMANA.....	92
Porotto Matteo.....	174
Porporato Paolo.....	260
Porretta Andrea	393
Porta Amalia.....	76
Posteraro Federico.....	457
Potestà Marina.....	34
Pozzi Gianni	301; 387
POZZI GIANNI	512
Precisi o Prociassi Arianna	294
Prestagiacomo Licia Elvira	414
Prezioso Carla	272; 463
PRIGIONE VALERIA	125
Principe Luigi.....	383
Privitera Grete.....	373
Prodani Flavia	108
Proroga Yolande Thérèse Rose	76
Protto Virginia.....	303
Provinciali Mauro	415
Puca Annibale Alessandro.....	307
Puca Valentina.....	160
PUGLIA LORENZO.....	94
Puglisi Vincenzo.....	10
Pulvirenti Federica	102
Puxeddu Silvia	227; 444; 446
PUXEDDU SILVIA.....	225

Q

QUARANTA GIANLUCA.....	92
Quaranta Miriana	281
Quaranta Paola .. 194; 229; 252; 273; 309; 467	
Quinti Isabella.....	102
Quirino Angela.....	20; 129; 331; 335; 414

R

Radi Marco.....	191
Radocchia Giulia.....	104; 191
Radu Claudia Maria	18
Raffa Vittoria.....	170
Raffaelli Marco	99
RAGA ELISA	225
RAGNO AGNESE.....	496
Raimondi Francesco.....	32
Raimondo Laura.....	504
Rajena More Pragati.....	222
Ramazzotti Matteo	78
Rampelli Simone.....	103
Rancan Ilaria	527
Ranieri Enrica M.	24; 321
Ranieri Enrica Maddalena.....	14

ranzani valeria.....	474
Rapi Stefano.....	457
Raponi Giammarco	376
Rappelli Paola	397; 492
RAPPUOLI RINO	156
raso nora noemi.....	331
Raspanti Calogero.....	50
Rauch Ciro	197
Raviola Stefano.....	506; 508
Razete Walter	50; 52
Reale Stefano	314
Reboli Camilla.....	339
Recchia Deborah.....	374
recchia simona	135
Renica Silvia.....	123
Rescifina Antonio	221
Resta Silvia Caterina.....	296
Riabova Olga	343
Ricchi Francesco.....	154; 469
Ricci Susanna.....	301; 387
Ricciardi Walter	69
Riccio Anna	193
Riccone Nunzia.....	395
Richter Sara N.....	266
Ricotti Roberta.....	108
Rindi Laura	10; 150; 198; 412
RINDI LAURA	38
Ripoli Francesca	516
Riva Agostino	461
Rizzato Cosmeri.....	172
Rizzo Carla	440
Rizzo Francesca	113; 115; 450
Roana Janira.....	158
ROANA JANIRA	125
Roberta Creti.....	47
ROBERTS JASON	410
Robino Antonietta.....	87
Rocchi Ettore	133; 382
Rodrigues Tania	446
Roe Andrew	84
Roglia Valentina.....	34
Romanelli Federica.....	24; 321
Romano Adriano.....	32
Roncaglia Stefania.....	516
Ronga Luigi	24; 321
Rosa Nicola.....	452
Rosato Antonio	266
Rosato Roberto	167
Roschetto Emanuela	197
ROSCIOLI EMANUELE.....	156
Rosellini Alfredo.....	467

Rossetti Barbara	527
Rossi Agostino	349
Rossi Marianna	123
Rossi Silvia	65
Rossi Stefano.....	62
Rossini Giada	325; 345
Rossolini Gian Maria	54; 78; 179; 454; 457
ROSSOLINI GIAN MARIA	456
Rubino Salvatore.....	238
Rueca Martina	56; 442
Rufolo Sandra	232
Ruotolo Luca.....	325; 345
Russo Andrea	314
Russo Assunta	32
Russo Francesca.....	62
Russo Sharon.....	52
RYAN LIV	465
S	

Sabbatucci Michela	47
Sacco Federica	376
Sake Johannes A.....	187
Sala Claudia	133; 382
SALA CLAUDIA	156
Saladini Francesco	3; 366
Salata Cristiano	266; 316; 333
Salb AMANDA L.....	250
Sali Michela	167; 531
Salomè Serena.....	32
Salpini Romina.....	317
Salustri Alessandro.....	167; 236; 531
Salvati Annamaria	254
Salvatore Paola.....	32; 200; 202; 296; 494
Sambri Vittorio.....	5; 16; 250; 329
SAMBRI VITTORIO.....	410
Samer Lallo Dias Angelica	165
Sandalli Sofia	84
Sanfilippo Giuseppa Luisa	139
Sanguinetti Maurizio.....	99
SANGUINETTI MAURIZIO	92; 106
Sani Spartaco.....	467
Sanna Giuseppina.....	341; 343; 448; 533
SANNA GIUSEPPINA.....	94
Sanna Laura.....	341
Sanson Gianfranco	383
Sansone Luigi.....	285
Santacroce Luigi	14; 321
Santagati Maria	186
Santarelli Giulia	99; 167; 531
Santella Biagio	448; 510
Santinelli Letizia	104; 488; 504

SANTO PAOLO FRANCESCO.....	92
Santoro Francesco.....	387; 522
SANTORO FRANCESCO	512
Santoro Maria Gabriella	193
SARACINO Annalisa.....	337
Sarmati Loredana.....	18
SARMATI LOREDANA.....	486
Sarnelli Bruno	254
Sarshar Meysam.....	264; 285; 305
Sarti Mario	383
Sassera Davide.....	374
Sateriale Daniela.....	200
Sau Rosangela.....	98
Savio Vittoria	358; 364
SAVIO VITTORIA	363
Scabini Silvia.....	319
Scagliarini Alessandra	250
Scaglione Elena	32; 200; 202; 296; 494
Scagnolari Carolina	104; 488; 504
Scala Giovanni.....	144
SCALAS DANIELA	125
SCALIA GUIDO	1
Scaltriti Erika.....	248; 339
Scano Alessandra	227
Scarasciulli Marialuisa.....	14
Scarazzai Mattia.....	130
Scarcelli Stefano	240
Scarfone Laura.....	179
Scarlata Giuseppe Guido Maria.....	129; 414
Schiavo Roberta.....	339; 421
Schino Valentina	240
Schippa Serena.....	104; 191
Sciandrone Giulia	289
Sciortino Giuseppa.....	419; 440
Scolz Sabrina	67
Scorciapino M. Andrea	444
Scribano Daniela.....	264; 285; 305
Scuderi Sarah Adriana	361
Scutari Rossana.....	317
Scutera Sara	161
SCUTERA SARA.....	189
SECCHI MARIA FRANCESCA	7
Secco Ilaria	262
Sechi Illari.....	292
Sechi Leonardo Antonio	109; 459; 481; 492
Secli Valerio.....	83
Selan Laura	152; 153
Semprini Simona	16; 329
Serra Nicola	299; 518
Sestito Simona	194
Sfara Germana	58

Sgambetterra Laura	516	Stufano Angela.....	240
Sgroi Giovanni	240	Sturlese Mattia	165
Siano Giacomo	273	STURLESE MATTIA	163
Sibille Giulia	333	Sucato Arianna.....	40; 139; 299; 518
sidoti maria.....	467; 502	Suffredini Elisabetta	73; 76
Sidoti Maria.....	229	Suigo Lorenzo.....	372
Signorini Lucia.....	277; 478; 497	Supuran Claudiu T.	160
Silecchia Gianfranco	99	Svicher Valentina	317
Silenzi Silvia	325; 345	T	
Silini Antonietta Rosa	236	Taddei Francesca.....	5; 16; 329
Silipo Alba.....	494	TADDEI FRANCESCA	410
Silvotti Maria G.	421	Taddio Andrea.....	85
Silvotti Maria Grazia.....	339	Tagueha Astri Dwyanti	305
Simas J. Pedro	446	Tambassi Martina.....	248
Simonetti Oriana	415	Tanca Alessandro	89; 90; 98; 101; 238
Simoni Serena	402	Tancrè Valentina	20
SIMONI SERENA.....	432	Tanzi Elisabetta.....	22; 290; 516
Simula Elena Rita.....	481	Tartaglia Matteo.....	476; 482; 521
Sinibaldi-Vallebona Paola	498; 500	Tartaglione Livia.....	42; 391
Sinopoli Raffaella	129	Tascini Carlo	412
Sironi Manuela.....	265	Tateo Fabio	154
SIVORI FRANCESCA.....	106; 426; 428	Tavanti Arianna.....	150; 172; 198; 204; 412
Smussi Davide.....	30	TAVANTI ARIANNA.....	38
Soffritti Irene.....	63; 65; 69; 96	TAVIANI ELISA	432
Soldateschi Ludovica	525	Tedesco Pietro	214
Solini Anna.....	97	Teodori Lucia.....	404
Sorace Chiara	18	Terraneo Mara.....	22
SORACE CHIARA.....	486	Teti Elisabetta	18
Sorrentino Annarita	383; 423	TETI ELISABETTA.....	486
Sorrentino Leonardo	104; 488; 504	Teti Giuseppe	270; 278
Souza Passos Ingredy Beatriz	165	TETI GIUSEPPE	465; 471
Spagnuolo Angela	32	Tibullo Daniele	182
Spalla Melissa	417; 421	Tiralongo Francesco	182
Sparapano Eleonora	24; 321	Tirziu Mariana	387
Sparti Rosaria.....	161	TIRZIU MARIANA	512
Spezia Pietro	442	TISEO GIUSY	156
spezia pietro giorgio.....	310	Tocci Gabriella.....	339; 417; 421
Spezia Pietro Giorgio	273; 294; 309; 467	Tognini Paola	111
Spuria Noele.....	50; 52	TOMA LUIGI	428
Squillaci Giuseppe	448	TONON EMIL.....	141; 354
Stabile Maria	32; 144; 234	Torelli Giovanna	358; 364
STEFANELLI PAOLA	286	TORELLI GIOVANNA.....	363
Stefanetti Giuseppe	532	TORELLI SARAH	370
Stefani Stefania ..	44; 186; 209; 244; 246; 256; 373; 378	Torina Alessandra	250
STEFANI STEFANIA	1	Tornese Gianluca	87
STEFANIZZI VALERIA	496	Torrigiani Filippo.....	266
Stillitano Maria Giuseppina	129	Tortellini Eeva	476; 521
Stolfa Stefania.....	24; 321	Tosadori Alice.....	423
Stracquadanio Stefano.....	373	Tosco Alessandra	76
Straniero Valentina	372	totaro michele	393

Tramontozzi Caterina.....	193
Tranfa Alessandro	254
Trecca Marika	152; 153
Trentadue Angela	14
Trevisan Marta	165
Triassi Maria	76
TRICOLI MARIA RITA.....	71; 218
Trifirò Linda.....	260
Trimarco Simona.....	32
Trimboli Francesca.....	20; 335
Triva Francesca	26
Trombelli Leonardo	96
Trotta Domenico	383
TRUGLIO MAURO	106; 426; 428
Tucci Fabio.....	442
Tucci Giuseppina	12
TULLIO VIVIAN	125
Tumbarello Mario	523; 527; 529
Turati Sarah.....	497
Turolla Andrea	73
TURRI GIONA.....	141; 354
Turriziani Ombretta.....	58; 482; 514
Turroni Silvia	103
U	

Ur Rehman Hamza.....	62
USAI ALEXANDRA.....	7
Uzzau Sergio	89; 90; 98; 101
V	

VACCA PAOLA	286
Vaccaro Lorenzo	266
VAGGELLI GUENDALINA	456
Vaia Francesco	347; 442
Valenza Anna.....	473
Valli Maria Beatrice	56
Valoti Ermanno	372
van Uden Sebastiao.....	117
Vantaggiato Chiara.....	265
Varani Stefania	345
Varasi Ilenia.....	3; 147; 366; 461
VARESE CRISTINA	125
Vargiu Silvia.....	380
Vascellari Sarah.....	60
Vaschellati Sarah	227
Vasco Maria.....	431
Vasconcelos Costa Vivian	165
Vatrella Alessandro	510
Vatteroni Maria Linda	467
Vecchi Maurizio	474
Vece Ciro.....	494
Vegni Silvia.....	398

VELLUZZI FERNANDA	94
Velotti Nunzio	99
Venanzi Rullo Emmanuele	256
Venditti Sofia	191
Veneri Carolina	246
Veneziano Vincenzo.....	240
Vergori Alessandra.....	442
Verin Ranieri	266
Verrastro Ornella.....	99
VERTILLO ALUISIO Gaia.....	186
VEZZULLI LUIGI	432
Vicenti Ilaria	3; 147; 366; 461
Vidmar Martina.....	262
VIGNAROLI CARLA.....	432
Vignoli Aglaia	111
Villa Alfredo	484
Villa Chiara	292
Villa Laura	383
Vincenti Sara.....	69
Virigillito Chiara	488
Viroli Francesco	65
Virusso Roberta.....	48; 50
Visai Livia.....	117
Visentin Sonja	117; 158
Vismara Chiara	383; 391
Vismara Chiara S.	42; 123
VISMARRA ALICE	184
Vitale Carolina	510
Vitale Pietro	18
VITALE PIETRO	486
Vitali Alberto	177
Vitiello Mariateresa	32; 200; 202
Vitiliello Maria Teresa	494
Voli Antonia	76
Volta Antonella	63; 65; 69
W	

Wagemans Jeroen	198
Wegener Gunter	81
Weisz Alessandro	113; 115; 254; 450
Whalen Michael.....	80
Willison Edward	137; 434
X	

Xynomilakis Ornella.....	111
Y	

Yonekura Satoru.....	119; 121
YOUSEFNIAJEJAHROMI	
ZAHRAALSADAT	38
Yousief Sally Waheed	238

Z

Zaccaria Teresa.....	9; 400
Zagaglia Carlo.....	264; 285
Zaghi Irene	5; 16; 329; 410
Zagni Chiara.....	221
Zangari Concetta	20
Zangrossi Manuela.....	266
Zannella Carla..	212; 214; 216; 222; 223; 351; 353; 448
Zannoli Silvia.....	5; 329
Zanotta Nunzia.....	67; 85; 87
Zanusso Gianluigi	60
Zappavigna Andrea	339; 417
Zarrilli Raffaele.....	144; 234
Zazzi Maurizio.....	3; 147; 366; 461
Zehender Gianguglielmo	461
Zerbato Verena.....	383
Zerbi Beatrice	339
Zimbone Carlotta	378
Zingaropoli Maria Antonella	58; 476; 482; 514; 521
Zirpoli Sara	527
Zobba Rosanna	300
Zordan Marta	18
ZORDAN MARTA.....	486
ZUCCONI GALLI FONSECA VITTORIA	156
Zuccotti Gianvincenzo.....	516
Zullo Federica	9; 400

Sommario

7 – The application of liquid colony in the sepsis diagnostic workflow: a preliminary overview on paediatric patients	554
9 - Extensive virologic characterization in an HIV-infected individual with apparent HIV remission for 2 years after allogeneic stem cell transplantation with CCR5 wild-type cells: a case study.	3
16 - Characterization and classification of diagnostically unidentified mycobacteria by de novo sequencing	5
34 - Nocardia otitidiscavarium infection in an immunocompromised host: Importance of proper microbiological testing	7
63 - Molecular surveillance of Candida auris colonization: our experience.	9
74 - Evaluation of STANDARD M10 MDR-TB assay for rapid diagnosis of tuberculosis	10
89 - "Fast" viral respiratory diagnosis in Pediatric Patients	12
100 - Preliminary results of valacyclovir efficacy in HCMV positive pregnant women	14
102 - Analytical evaluation of a novel Point Of Care (POCT) Loop Mediated Isothermal Amplification (LAMP) test for the detection of Sexually Transmitted Infections	16
123 - Dynamic expression of CD169 in blood cells and circulating microvesicles as a potential diagnostic marker of immune response in COVID-19 and PASC individuals	18
133 - Fecal calprotectin values in pediatric patients with bacterial and viral diarrhoea: An observational study.	20
151 - Differential diagnosis for fever and rash as a key strategy to achieve the goal of eliminating Measles and Rubella	22
174 - Fusarium Solani fungemia in pediatric patient with acute B-lymphoblastic leukemia	24
177 - SARS-CoV-2 infection in healthy school-age children	26
188 - Self-sampling as alternative strategy to detect Human Papillomavirus (HPV) and other sexually transmitted pathogens in women referred to colposcopy	28
200 - Comparison of two detection methods for the quantitative detection of circulating Human Papillomavirus (HPV) from plasma and saliva of patients with head and neck cancer.	30

205 - Analysis of antibody titre decay in the mother-infant dyad exposed to congenital <i>Toxoplasma gondii</i> infection	32
209 - Comparative Analysis of miRNA Expression Profiles in HPV-Negative and HPV-Positive Cancer Cell Lines: Implications for Tumorigenesis	34
216 - Contribution of T2 Magnetic Resonance (T2MR ®) technology for the early detection of ESKAPE pathogens in ICU patients: a two years experience in Palermo, Italy	36
217 - Innovative phage-based sensor for the rapid detection of <i>staphylococcus aureus</i>	38
225 - Evaluation of syndromic SeeGene Allplex GI panels for detection of pathogens associated with acute gastroenteritis.	40
246 - Multidrug-resistant bacteria surveillance in intensive care unit: the importance of a molecular approach	42
251 - Advanced Bacterial Detection in a Simulated Bloodstream Infections through Digital PCR: A Proof of Concept for Enhanced Diagnostic Accuracy	44
266 - Preventing early-onset group B streptococcus (GBS-EOD) invasive infection in the new-born: a national survey, Italy, 15 December 2022 to 12 February 2023	47
269 - Laboratory strategy for the rapid diagnosis of leishmaniasis: experience in Palermo University hospital.	48
294 - Screening of carriage of Carbapenem-resistant Enterobacterales (CRE), <i>Acinetobacter baumannii</i> (CRAB), and <i>Pseudomonas aeruginosa</i> (CRPsA) in a hospital setting by culture and molecular tests.	50
300 - Extraction-free RT-qPCR for the detection of SARS-CoV-2: modification of a commercial assay protocol reduces turnaround time.	554
307 - Assessment of increased blaKPC gene dosage by digital PCR for the rapid prediction of meropenem/vaborbactam resistance in <i>Klebsiella pneumoniae</i> clinical strains	554
314 - Concomitant syndromic diagnosis of monkeypox and other vesicular viruses in patients with skin and genital lesions	56
324 - Relationship between Cycle threshold value and N antigen amount in SARS-CoV-2 variants	58
326 - RT-QuIC detection prion seeding activity in spiked human tears	554
26 - Antimicrobial activities of functionalized enamelled-coated surfaces	62

66 - Shaping the subway microbiome by a probiotic-based sanitation during the COVID-19 emergency	63
79 - Characterization of the pathogenic potential of the beach sand microbiome and assessment of quicklime as a remediation tool.	65
82 - Molecular surveillance of the pediatric hospital microbiome: strategies for infection prevention and control	554
90 - Potential Use of a Combined Bacteriophage-Probiotic Sanitation System to Control Microbial Contamination and AMR in Healthcare Settings: A Pre-Post Intervention Study	69
131 - EVALUATION OF LEGIONELLA PNEUMOPHILA IN WATER SAMPLES AND COMPARISON WITH LEGIOLERT/REAL-TIME PCR	71
230 - Tracking SARS-CoV-2 RNA in urban wastewater through diverse biomarkers	73
318 - Investigation of SARS-CoV-2 RNA in wastewater and bivalve mollusks from the Campania Region	76
321 - Detection and characterization of carbapenemase producing Enterobacterales from wastewater samples	78
25 - Characterization of Mutants Involved in the Regulation of Cell Wall Synthesis and Remodeling in <i>Acinetobacter baumannii</i>	80
195 - Culture-independent methods contributed to elucidate mechanisms for O ₂ tolerance in the bacterial genus <i>Sulfurimonas</i>	81
329 - <i>Pseudomonas aeruginosa</i> exploits zinc-binding metallophores to cope with host nutritional immunity strategies.	83
20 - Repression of a potent genotoxin via amino acid supplementation	84
35 - Characterization of the fecal microbiome in pediatric patients affected with chronic recurrent multifocal osteomyelitis: a pilot study	85
36 - Exploring the interplay between oral microbiome, inflammatory factors and glycemic control in Type 1 Diabetes Mellitus.	87
44 - Fecal sample collection and room-temperature storage using swabs with preservation medium enables deep characterization of the human gut metaproteome	89
54 - Effects of <i>Clostridioides difficile</i> infection on the structure and functions of the human gut microbiota	90
56 - Preliminary evaluation of fecal microbiota transplantation by oral capsules in patients with liver cirrhosis and hepatic encephalopathy refractory to conventional treatment	92

57 - Dynamics of Gut Microbiota and Clinical Variables after Medium (3 months) and Long-Term (6-12 months) Ketogenic and Mediterranean Diets in Drug-Naïve Patients with Type 2 Diabetes Mellitus and Obesity	94
67 - Effect on sub-gingival microbiome of diverse subgingival air-polishing in the treatment of advanced periodontitis: a randomized controlled clinical trial.	554
69 - Urinary microbiota and type II diabetes: a randomized clinical trial	97
70 - Metaproteomic changes in the human gut microbiota following an antibiotic treatment for the eradication of <i>Helicobacter pylori</i>	98
73 - Profiling gut microbiota in patients undergoing laparoscopic R-en-Y vs One Anastomosis Gastric Bypass	99
75 - Comparison of methods for processing fecal protein extracts on a multiwell plate for metaproteomic analysis	101
147 - Altered oropharyngeal microbiota in common variable immunodeficiency (CVID) patients	102
159 - Preliminary insights in the gut microbiome profile of immigrant patients from African countries	103
179 - Oral and blood microbiota relate to interferon and endothelial genes expression during COVID-19	104
186 - Phenotypic characterization and whole genome analysis of <i>Staphylococcus aureus</i> isolates colonizing the skin microbiota of patients with severe atopic dermatitis.	106
196 - Effect of food supplements on gut microbiota in obese patients: preliminary results of zimba trial	108
207 - <i>Mycobacterium avium</i> subspecies paratuberculosis (MAP) infection, and its impact on gut microbiome of individuals with Multiple Sclerosis	109
243 - Gut microbiota profile in CDKL5 deficiency disorder patients	111
303 - Dynamics of nasopharyngeal tract mycobiota in COVID-19 patients	113
305 - Dynamics of nasopharyngeal tract mycobiota in COVID-19 patients	115
316 - In vitro study of various bacterial strains from human microbiota cultured in different types of advanced 3D substrates.	117
323 - Bacterial, fungal and viral gut microbiota signatures predict immunotherapy response in cutaneous and mucosal melanoma patients	554
325 - A metagenomic cross-cohort analysis of bacterial virulence factors in colorectal cancer	121
15 - Genomic epidemiology of <i>K. pneumoniae</i> carbapenemase-producing <i>Klebsiella pneumoniae</i> (KPC-Kp) strains from three Northern-Italy hospitals and investigation of intra- and inter- hospitals genetic diver	123

93 - Microorganisms from human clinical samples as a tool for technological innovation: creation of a shared platform of the UNITO collections.	125
106 - Human enterovirus surveillance in the Marche Region (Italy)	127
114 - Frequency and Variation of Viral Respiratory Infections in Pre- and During -COVID-19 Pandemic: A 7-year experience in a teaching hospital of Southern Italy	129
137 - A novel epidemiological tool: Fourier Transform infrared spectroscopy (FT-IR) for real time tracing of <i>Klebsiella pneumoniae</i> producing NDM-5 and NDM-1/OXA-48-like enzymes	130
150 - The mosaic of Adenovirus variants circulating in 2022-2023 in hospitalized patients.	131
172 - Genomic analysis of methicillin-resistant <i>Staphylococcus aureus</i> (MRSA) isolates from bloodstream infections in Italy: a pilot study	132
190 - Machine learning model based on MALDI-TOF mass spectra for prediction of ceftazidime resistance in <i>Escherichia coli</i> strains isolated from bloodstream infections	554
214 - Emergence of high-level gentamicin resistance in <i>Streptococcus agalactiae</i> hypervirulent serotype IV strains	135
248 - Retrospective epidemiological study of candidemia at San Martino Hospital (HSM), Genoa, Italy, 2019-2022	137
287 - IMPACT OF THE SARS-COV-2 PANDEMIC ON THE CIRCULATION OF ENTERIC VIRUSES IN CHILDREN IN PALERMO	139
301 - Epidemiological surveillance of SARS-CoV-2 variants using NGS during pandemic waves in Verona	141
322 - SARS-CoV-2 variants in Marche region: evolution, mutations, and variability.	143
330 - Characterization of a genome database of 837 isolates assigned to 72 distinct species in the <i>Acinetobacter</i> genus using the Pasteur Multi-locus sequencing type (MLST) scheme.	144
1 - Importin alpha/beta-dependent nuclear transport of human parvovirus B19 nonstructural protein 1 is essential for viral replication	146
11 - A heparan sulfate proteoglycan binding tetrapeptide successfully inhibits SARS-CoV-2 Omicron replication	147
27 - All-Trans Retinoic Acid Effect on <i>Candida albicans</i> growth and biofilm formation	148
28 - Isolation and characterization of novel mycobacteriophages and evaluation of their lytic activity against <i>Mycobacterium abscessus</i>	150

31 - A promising antimicrobial peptide against some virulence features of <i>Acinetobacter baumannii</i> clinical strains	152
32 - Escape pathogens: looking for alternative strategies from an exotic source	153
41 - Claudio	154
43 - Isolation of Human Monoclonal Antibodies Against Pan-Drug Resistant <i>Klebsiella pneumoniae</i>	156
49 - Azole-loaded mucosomes as multi drug delivery platform to counteract infections caused by <i>Candida albicans</i> .	158
50 - New inhibitors of Carbonic Anhydrases from <i>Pseudomonas aeruginosa</i> : antimicrobial and antibiofilm activities with respect to ciprofloxacin	160
52 - Silver-doped composite modified scaffolds to impart both anti-microbial features and enhanced biological properties in bone regenerative medicine.	161
55 - Small molecules and PROTACs to inhibit and degrade HPV oncoproteins	163
60 - A small-molecule inhibitor of flaviviral NS3-NS5 interaction with broad-spectrum activity and efficacy in vivo	165
71 - Graphene Quantum Dots as promising nanomaterials against <i>Mycobacterium tuberculosis</i> infection	167
78 - Citrus fruit polyphenols for fighting antibiotic resistance	169
94 - Improving Gene-Editing delivery to fight viral infections	170
97 - Effect of the Human Lactoferricin-Derived Peptide hLF 1-11 on <i>Candida albicans</i> and <i>Staphylococcus</i> spp. mixed biofilms.	172
99 - Humanized monoclonal antibody targeting a pre-fusion domain of the viral fusion protein stabilizes the extended state of the measles viral fusion process	174
110 - <i>Pseudomonas</i> spp.: a new ally to fight drug resistance?	176
111 - Antimicrobial activity of novel arginine- and proline-rich peptides obtained through a bioinformatic approach	177
112 - Characterization of four novel bacteriophages targeting multi-drug resistant <i>Klebsiella pneumoniae</i> strains of sequence type 147 and 307	179
113 - Evaluation of the antibiotic activity of grapefruit seed extract in edible mushrooms contaminations	181
121 - Effect of fish skin mucous derived from bathypelagic fish <i>Centrolophus niger</i> (Gmelin, 1789) on <i>Escherichia coli</i> strains	182
124 - Antimicrobial activity of in silico designed synthetic peptides against biofilms of antibiotic-resistant <i>Escherichia coli</i> isolates	184
128 - Novel fungicidal thiazole derivatives inhibit the growth of <i>Candida albicans</i> by ergosterol depletion.	185

138 - The inhibitory effects of <i>Lactobacillus gasseri</i> 1A-TV cell-free supernatant against ESBL and carbapenemase producing <i>Klebsiella pneumoniae</i>	186
141 - Activity of a newly designed gallium-siderophore complex (GaS1) against lung isolates of <i>A. fumigatus</i> in an in vitro air-liquid interface lung infection model	187
158 - Synergistic antibacterial effect of colistin in combination with human albumin nanoparticles on multidrug resistant <i>Acinetobacter baumannii</i> isolates	189
160 - Bithiazole compounds in the treatment of virus/bacteria co-infections	191
165 - Antiviral activity of the non-steroidal anti-inflammatory drug indomethacin against Respiratory Syncytial Virus	554
167 - Screening of a panel of compounds as anti-Sars-CoV-2 antiviral drugs	194
189 - Activity evaluation of phage SAU1593 with levofloxacin or erythromycin against <i>Staphylococcus aureus</i> strains isolated from diabetic foot infections	196
192 - Antibiotic resistance, a global health problem: search for alternative antimicrobial molecules and Superbug Awareness For Education (SAFE) activities.	197
203 - Zeno_omega: a novel therapeutic phage-based formulation evolved for the treatment of multi-drug resistant <i>Staphylococcus aureus</i>	198
204 - In vitro antibacterial effects of Thyme essential oil against <i>Salmonella enterica</i> group B	200
206 - Antimicrobial activity of WMR-K and WMR-K+gH625 against <i>Salmonella enterica</i> group B	202
208 - Isolation and characterization of novel bacteriophages active against clinical strains of <i>Klebsiella pneumoniae</i>	204
212 - Synergistic effects of carvacrol in combination with antibiotics against <i>Acinetobacter baumannii</i> , <i>Escherichia coli</i> and <i>Pseudomonas aeruginosa</i>	206
221 - Complex Electromagnetic Fields: eco-sustainable strategy to counteract the resistant <i>Candida albicans</i> infections	208
232 - A preliminary study on bacterial control strains to assess the synergistic effect of new heteroaryl-ethylene molecules in combination with antibiotics.	209
236 - Ligand-free AgNPs: an innovative antimicrobial strategy	212
237 - New imidazolium alkaloids from the marine bacterium <i>Shewanella aquimarina</i> as innovative antimicrobial strategies.	214
238 - Anti-staphylococcal and anti-biofilm properties of the cationic peptide Orechromicin-1 from <i>Oreochromis niloticus</i>	216
242 - Effect of resveratrol on antibiofilm action against <i>Pseudomonas aeruginosa</i> clinical strains	218

244 - Pilocarpine hydrochloride promotes <i>Candida albicans</i> cell membrane damage leading to reactive species production and apoptosis	219
249 - Cryogel based on β -cyclodextrin and a maltol derivative for antimicrobial applications	221
258 - Antiviral activity of lipophilic extracts derived from <i>Lavandula austroapennina</i> organs	222
264 - Exploring the Inhibitory Potential of oreochromicin peptide against viruses with different Classes of Fusion Proteins	223
277 - Antimicrobial and antioxidant properties of vesicles loaded with an extract obtained from olive leaves	225
280 - Ionic liquids as a new effective antimicrobial approach	227
282 - Screening of novel anti-HIV drugs as inhibitors against Sars-CoV-2 and HSV-2 viruses	229
286 - Resveratrol derivatives, promising strategy for innovative therapeutic schemes to tackle the <i>Helicobacter pylori</i> persistent infections	230
306 - Outer membrane vesicles derived from Gram-Negative bacteria: biological vectors for innovative antiviral therapies	232
317 - PYED-1: new molecule able to re-sensitize colistin-resistant <i>Acinetobacter baumannii</i> strains	234
327 - Potential of Mesenchymal Stromal Cells secretome as host-directed therapy for mycobacterial infection	554
331 - The Secondary Resistome to Oxacillin and Tetracycline in MRSA ST398	238
2 - Zoonotic pathogenic microorganisms from the marine environment: A One Health perspective	239
162 - <i>Echinococcus granulosus sensu lato</i> in hunters and hunting dogs from southern Italy: a One Health approach	240
178 - Occurrence of ESKAPE bacteria in healthy cattle population and the related zoonotic risk	242
241 - Rising threat: the prevalence of <i>K. pneumoniae</i> Ceftazidime/Avibactam resistant strains in healthcare facilities	244
253 - Uncovering Antibiotic Resistance in Italian Wastewater: Exploring ARB, ARGs, and Microbial Diversity in WWTPs	246
275 - Following <i>mcr</i> genes: from one-health surveillance to functional genomics of bacterial mobile elements	248
296 - Ticks and tick-borne pathogens at human-domestic-wildlife interfaces in Malawi through a One Health approach	250

310 - Evaluation of Antiviral Properties of Grape Pomace Extracts: A Sustainable Approach for Broad-Spectrum Viral Inhibition	252
312 - Distribution of Mycobacterium tuberculosis lineages and drug resistance in Campania Region	254
313 - Methicillin-Susceptible Staphylococcus aureus wild-type and small colony variant (SCVs) isolated from osteomyelitis: genomic and expression analysis of selected genes	256
4 - EXPLOITING LIPID METABOLISM BY HSV-1: A CHALLENGE TO RETHINK NEW THERAPIES FOR ALZHEIMER'S DISEASE.	260
19 - A novel role of TMEM16A and TMEM16F in SARS-CoV-2 pathogenesis	262
22 - The protein HslJ boosts Acinetobacter baumannii survival against oxidative stress	264
40 - SARS-CoV-2 ORF3c impairs mitochondrial respiratory metabolism, oxidative stress, and autophagic flux	265
61 - Identification of druggable host dependency factors shared by multiple SARS-CoV-2 variants of concern	266
72 - A study on cross-link formation between fibrinogen and region A of FnBPA from Staphylococcus aureus promoted by von Willebrand Factor-binding protein (vWbp) and tissue Transglutaminase 2 (TG2)	268
85 - The SaeRS two-component system regulates the expression of group B streptococcal genes that are highly expressed in vivo	270
91 - Investigation of Merkel Cell Polyomavirus (MCPyV) status in Merkel Cell Carcinoma (MCC)	272
98 - SARS-CoV-2 infection induces Tau pathological signature	273
107 - Transcriptome analysis of Parvovirus B19 infected erythroid progenitor cells	275
117 - Longitudinal study of Human Polyomaviruses viruria in kidney transplant recipients	277
118 - The BM 110, a hypervirulent strain of group B streptococci, exploits $\alpha 2\beta 1$ and $\alpha 3\beta 1$ integrins to invade human enterocytes	278
120 - Pseudotyped lentiviral particles may help shed light on HIV maturation	279
132 - Fimbriae and Adhesins in clinical, Extremely Drug-Resistant Providencia stuartii Isolates producing NDM-1	281
144 - The serine-rich repeat glycoprotein Srr2 from Streptococcus agalactiae acts as an adhesin for the human fibronectin	283
149 - Acinetobacter baumannii OmpA-like porins: functional characterization in bacterial physiology, antibiotic-resistance, and virulence	285

168 - Invasive meningococcal disease caused by rare capsular serogroups in Italy: genomic investigation of virulence genes associated with pathogenesis	286
170 - The study of the Unfolded Protein Response and Herpes Simplex Virus infection in neuronal cell line	288
175 - Unveiling the role of NAAA enzyme and peroxisome regulation in HSV-1 infection	289
181 - In vivo virulence study of <i>Listeria monocytogenes</i> with truncated Internalin A in <i>Galleria mellonella</i> larvae	290
194 - Analysis of viral variants associated with high-risk Human Papillomavirus (HPV) persisting infection	292
202 - Sudden and unexplained death of an 18-month-old healthy boy due to interstitial pneumonia with multiple superinfections	294
213 - <i>Neisseria meningitidis</i> activates pyroptotic pathways in a mouse model of meningitis: role of a two-partner secretion system.	296
222 - Parvovirus B19 infection in a co-culture model of erythroid progenitor cells and mesenchymal stromal cells	298
229 - Cervical lesions and HPV oral infection: is there a relation?	299
231 - Eating the enemy: <i>Mycoplasma</i> strategies to evade Neutrophil Extracellular Traps (NETs) promoting bacterial nucleotides uptake and inflammatory damage.	300
247 - Development and characterisation of a mouse model of invasive Non-Typhoidal <i>Salmonella</i> (iNTS) disease	301
268 - HSV-1 exploits extracellular vesicles to disseminate neurotoxic proteins within the brain	303
271 - Phenotypic and genotypic characterization of <i>Acinetobacter baumannii</i> clinical isolates: yesterday and today	305
292 - <i>Klebsiella pneumoniae</i> -OMVs activate death-signaling pathways in human bronchial epithelial host cells (BEAS-2B)	307
309 - The role of human DDX3 helicase in HSV-2 infection	309
328 - Inhibiting immunoregulatory amidase NAAA blocks ZIKV maturation in Human Neural Stem Cells	310
3 - Prevalence of gastrointestinal pathogens and COVID-19 in patients with diarrhoea referring to a University Hospital	313
14 - <i>Dirofilaria</i> in humans: a new insight into an old knowledge	314
18 - Screening for tick-borne and tick-associated viruses in ticks collected in Sardinia, Italy	316

23 - Amino acid variability of small-molecule inhibitors and mAbs target sites in globally circulating RSV strains	317
29 - First case of <i>Chryseobacterium gallinarum</i> bloodstream infection: a diagnostic challenge for an emerging pathogen	319
47 - New putative <i>Pannonibacter</i> species isolated from nosocomial bacteraemia in two oncological pediatric patients	321
68 - <i>Cutibacterium acnes</i> in shoulder arthroplasty: preliminary results on the efficacy of an intraoperative iodopovidone irrigation in reducing its contamination	323
96 - Genomic dissection of SARS-CoV-2 evolution in immunocompromised patient with prolonged viral shedding	325
104 - The infection rate of respiratory viruses in pandemic SARS-CoV2 period (2019-2023): an observational study.	327
105 - Prevalence of <i>Schistosoma mansoni</i> monoinfection and co-infections in the Romagna region	329
108 - Presence of alive <i>Ascaris lumbricoides</i> in a symptomatic patient repeatedly negative for worm eggs in fecal samples	331
129 - The cranberry (<i>Vaccinium macrocarpon</i>) extract blocks Ebola virus and Crimean-Congo hemorrhagic fever virus infection by interfering with viral entry into target cells.	333
130 - Hepatitis E virus acute infection in an immunocompetent subject with non-alcoholic fatty liver disease.	335
164 - Case report of pulmonary echinococcosis in a young patient from southern Italy.	337
171 - The role of whole-genome sequencing in cluster investigation of Carbapenem-Resistant <i>K. pneumoniae</i> isolated in a northern Italy hospital	554
218 - Novel benzimidazole derivatives as potent and selective anti Poxvirus agents	341
219 - Efficacy of dispirotripiperazine PDSTP in a golden Syrian hamster model of SARS-CoV-2 pneumonia	343
220 - Elevated plasma level of IP-10 /CXCL10 is correlated with severity of human West Nile virus (WNV) infection	345
233 - Asymptomatic Mpox infection in subjects presenting for MVA-BN vaccine	347
260 - Federica	349
267 - Frog-derived antiviral peptides (AVPs): pan-inhibitors against respiratory viruses	351
272 - Design of Mopeia virus fusion inhibitors on GP2 glycoprotein	353

295 - SARS-CoV-2 large ORF8 deletion cluster in routine NGS screening for epidemiological surveillance	354
116 - Targeting persister cells of <i>Pseudomonas aeruginosa</i> under conditions relevant to cystic fibrosis using culture filtrate supernatants of lactobacilli	356
135 - Exploring the antibacterial effect of <i>Lactocaseibacillus rhamnosus</i> cell-free culture supernatants against <i>Pseudomonas aeruginosa</i> in an air-liquid interface lung infection model	357
146 - Different activity of <i>Lactobacillus</i> spp. against two <i>Proteus mirabilis</i> isolated clinical strains in different anatomical sites: an explorative study to improve the therapeutic approach	358
169 - Activity of cell-free supernatants from <i>Lactocaseibacillus rhamnosus</i> against mono and dual-species biofilms of wound pathogens in a 3D in vitro collagen wound infection model	360
211 - Probiotic pretreatment protects corneal epithelial cells against <i>Staphylococcus aureus</i> infection	361
224 - In vitro evaluation of the most active probiotic strains able to improve the intestinal barrier functions and to prevent inflammatory diseases of the gastrointestinal system	363
239 - Regulatory effects of <i>Lactiplantibacillus plantarum</i> on human skin health by counteract <i>Malassezia furfur</i> effects.	554
319 - Anti-virulence effect of <i>Lactobacillus rhamnosus</i> and <i>Lactobacillus plantarum</i> cell-free supernatants on <i>P. aeruginosa</i> from cystic fibrosis patients	365
10 - Synergistic effects of antivirals and monoclonal antibodies in vitro against SARS-CoV-2 Wild Type B.1 strain and BQ.1.1 Omicron sublineage	366
13 - SigH stress response mediates killing of <i>Mycobacterium tuberculosis</i> by activating nitronaphthofuran prodrugs	368
17 - abcd	370
24 - Strepto Killer, qu'est-ce que c'est?: Identification of compounds inhibiting streptococcal cell division by targeting FtsZ as a novel strategy to fight Gram-positive ovococcal pathogens	372
33 - Role of transcriptomic and genomic analyses in a step ahead in the comprehension of cefiderocol activity in <i>A. baumannii</i>	373
37 - Study of the mechanism of action of the novel antitubercular compound 11826350	374
38 - In vivo cefiderocol resistance escalation and intra-host evolution of KPC-3 and VIM-1 co-producing <i>Klebsiella pneumoniae</i> Sequence Type 512	376
39 - The Fitness Omic burden associated to new Adaptive Reduced-Glycopeptide-Susceptibility Network in CA-MRSA	378

42 - Shift between echinocandins through computerized prescribing software and antifungal resistance profiles	380
45 - Antimicrobial resistance prediction of <i>Escherichia coli</i> strains to Ampicillin via machine learning models on MALDI-TOF mass spectra	382
48 - <i>Campylobacter</i> spp. infections in Italy: a 5-year multicenter retrospective study	383
53 - Genome sequence analysis of infertility-associated <i>Enterococcus faecalis</i> strains revealed the presence of the novel Tn7086 carrying aminoglycoside resistance genes	387
58 - Multiclonal presence of convergent ceftazidime/avibactam resistant and hypervirulent <i>Klebsiella pneumoniae</i> clinical isolates: when resistance meets virulence	389
62 - KPC-166, a novel KPC variant involved in ceftazidime/avibactam resistance	391
64 - Surveillance of <i>Clostridioides difficile</i> infection in the Pisa University Hospital: a correlation between clinical, environmental and pharmaceutical features	393
65 - Study of the role of wild boar (<i>Sus Scrofa</i>) as a reservoir of antibiotic resistance	395
76 - Endosymbiosis of <i>Mycoplasma hominis</i> in <i>Trichomonas vaginalis</i> : influence of intracellular localization on its resistance to antibiotics	397
81 - A strategy to screen compounds against flaviviral infection helps identify a novel compound against Zika virus	398
84 - Minimum inhibitory concentration distribution of <i>Candida auris</i> strains: preliminary data from a large tertiary hospital in Piedmont	400
86 - Characterization of a prophage and a defective integrative conjugative element carrying the <i>optrA</i> gene in linezolid-resistant <i>Streptococcus dysgalactiae</i> subsp. <i>equisimilis</i> isolates from pigs, Italy	402
87 - Outbreak of multi drug-resistant OXA-48 and NDM-5 producing <i>Klebsiella pneumoniae</i> in a central Italy regional hospital.	404
88 - Fosfomycin-meropenem synergistic combination against NDM carbapenemase-producing <i>Klebsiella pneumoniae</i> strains	406
92 - The N-acyl ethanolamine acid amidase is a SARS-CoV-2 host factor	408
103 - Time to positivity of blood cultures (Tpos) as surrogate biomarker of in vivo antimicrobial efficacy: in vitro simulations with bacteria and yeasts	554
126 - In vitro activity of cefiderocol, alone and in combination with imipenem or bacteriophages, against biofilm-embedded <i>Pseudomonas aeruginosa</i>	412

127 - K. pneumoniae-KPC: Molecular tools to improve epidemiological surveillance of resistance genes.	414
142 - Linezolid-induced mitochondrial damage impairs wound closure after MRSA infection	415
152 - Molecular epidemiology of carbapenemase-producing Enterobacteriaceae in the Piacenza area	417
153 - Next Generation Sequencing assay for HIV genotyping and resistance testing: the sicilian experience	419
155 - ST2502 KPC-Klebsiella pneumoniae and OXA-23-Acinetobacter baumannii from LTCFs in Piacenza area: results of a Point Prevalence Study of colonization	421
166 - Antibiotic resistance of Streptococcus pneumoniae causing invasive diseases during the 13-valent conjugate vaccine era in Italy.	422
173 - Molecular investigation to monitor the spreading of fluoroquinolone and tetracycline resistance among Campylobacter jejuni clinical isolates	423
176 - Clostridioides difficile over the years: evaluation of resistance profile in clinical isolates at Padua University Hospital	425
182 - In vitro antimicrobial susceptibility testing and biofilm characterization of Borrelia burgdorferi sensu lato isolated from patients with erythema migrans	426
183 - Prevalence and drug resistance for invasive candidiasis in Pediatric and adult Hospitalized Patients	427
185 - Insight into Acinetobacter baumannii's phenotypic and genotypic traits in oncological patients	428
187 - Exploring the Interaction between Ceftobiprole and High Molecular Mass (HMM) PBPs	430
223 - Impact of Interdisciplinary Antimicrobial Diagnostic Stewardship on Bloodstream infections and antibiotic resistance patterns.	431
252 - In vivo transfer of linezolid resistance among enterococci in Mytilus galloprovincialis	432
254 - Evaluation of Ceftazidime/Avibactam resistance in clinical isolates of Enterobacterales from a large tertiary-care hospital, Italy, 2019-2023	434
262 - ESKAPE pathogens in the wounds of stray dogs and cats and their antimicrobial resistance profile	436
263 - STRAINS OF STAPHYLOCOCCUS EPIDERMIDIS ISOLATED IN THE MILKING PARLOR OF BUFFALO FARMS IN THE CAMPANIA REGION	438
270 - Antimicrobial activity of gemini quaternary ammonium compounds against multidrug-resistant bacteria isolated from central venous catheters.	440

274 - Molnupiravir imprinting of SARS-CoV-2 genome: a case-control study	442
281 - Drugs porins-permeability coefficient correlate with the antimicrobial effectiveness	444
283 - Sulfonamide treatments inhibit gamma-Herpesvirus persistent infection in vivo	446
297 - Antiviral activity of three residues peptides against SARS-CoV-2 Infection	448
298 - Genome-wide DNA methylome and transcriptome identify epigenetically based drug-resistance mechanisms in <i>Acinetobacter baumannii</i>	450
299 - Broad-spectrum antimicrobial potential of Oftasecur and Visuprime ophthalmic solutions	452
308 - Genotypic characterization of linezolid-resistant <i>Staphylococcus epidermidis</i> strains isolated from invasive infections collected in the Careggi University Hospital of Florence	454
311 - Phenotypic and genotypic characterization of a clinical isolate of IMP-19 producing <i>Enterobacter hormaechei</i>	456
320 - IS26-mediated chromosomal integration of a blaKPC-3-harboring InFII-derived plasmid in a clinical isolate of <i>Pseudomonas aeruginosa</i>	457
6 - Role of Epstein-Barr virus and <i>Mycobacterium avium</i> paratuberculosis in CNS demyelinating disorders	459
8 - Neutralizing antibodies response to novel SARS-CoV-2 omicron sublineages in long-term care facility residents after the fourth dose of monovalent BNT162b2 Covid-19 vaccination	461
12 - Prevalence of polyomaviruses and papillomaviruses related to lymphocyte immunophenotyping in Actinic Keratoses and Squamous Cell Carcinoma: one-year data from clinical practice	463
30 - Caspase-8 controls a spontaneous pro-inflammatory program that can be exploited therapeutically to control bacterial infections	465
46 - SARS-CoV-2 evolution during persistent infection in a CAR-T recipient shows an escape to both sotrovimab and T cell response	467
59 - The <i>Propionibacterium</i> spp. extract reduces <i>Candida albicans</i> -induced damage to vaginal epithelial cells and increases mitochondrial response to <i>Candida albicans</i> infection in vitro	469
83 - The interferon-beta/MLKL axis promotes anti-bacterial defenses by enhancing neutrophil-mediated pathogen killing	471
95 - Kinetics and quality of humoral immune response against SARS-CoV-2 after vaccination and natural infection	473

101 - A novel subset of IFN γ -producing Th17 cells is selectively associated with intestinal inflammation in Crohn's Disease and specifically activated by adherent-invasive Escherichia coli (AIEC)	474
109 - Anti-HBc loss in multiple sclerosis patients treated with ocrelizumab	476
119 - Infection of macrophages by SARS-CoV-2: implications for viral spreading and immune response	478
122 - Questioning the role of fibroblast growth factor-2 during Zika virus infection and infection by other viruses.	480
134 - HERV-K Antibodies and their Potential Protective Effect in Amyotrophic Lateral Sclerosis	481
136 - Cerebrospinal fluid and plasma biomarkers in patients with central nervous system infections: a retrospective study	482
139 - Evaluation of LIAISON® MeMed BV® to discriminate bacterial and viral infections in patients with Chronic Obstructive Pulmonary Disease	484
143 - Association of human endogenous retroviruses with immune dysfunction and development, progression and long-term sequelae of COVID-19.	554
156 - Differential type I interferon expression between children and adolescents with long covid	488
163 - Phosphatidylserine liposomes as a possible candidate for the therapeutic management of Mycobacterium abscessus infection	490
184 - Yet another potential microbial player in autoimmunity: multicentric analysis of the association between Mycoplasma hominis serostatus and rheumatoid arthritis	492
199 - Evaluation of the immunomodulatory activity of Cellulophaga species LPSs.	494
245 - Regulation of permissiveness/restriction to herpes simplex virus 1 infection in different cell types by cellular microRNAs	496
250 - The viral sensor IFI16 at the crossroads between damage and tolerance during human Coronavirus infection	497
255 - Effects of Ropivacaine and Propofol on Human endogenous retroviruses activity, embryonic genes, CD-133 and PI3K-AKT-mTOR signaling in head-neck carcinoma cell lines: a proof-of-concept study providing	498
256 - Preliminary evidence of the differential expression of Human endogenous retroviruses in Kawasaki disease and SARS-CoV-2-associated multisystem inflammatory syndrome.	500
257 - "REDUCED CLEARANCE OF SARS-COV-2 INFECTION IN IMMUNOCOMPROMISED INDIVIDUALS DESPITE VACCINATION-INDUCED RESPONSE"	502

273 - Type I and III IFN signature in cells from skin lesions, anal canal brushing and nasopharyngeal swabs of MPXV infected patients	504
284 - IL-6-mediated paracrine senescence drives the intercellular communication within the HCMV microenvironment in renal epithelial cells	506
285 - The PYRIN domain of IFI16 drives LPS-induced and sterile toll-like receptor 4-mediated inflammation	508
302 - Comorbidities and clinical complications associated with SARS-CoV-2 variants.	510
125 - Heterologous boost immunisation with Spike protein after mucosal priming with recombinant Streptococcus gordonii expressing the receptor binding domain of SARS-CoV-2 spike protein on its surface	512
154 - Humoral and T-cell response to SARS-CoV-2 vaccination one year after the booster dose in oncology patients	514
161 - Enhancing influenza prevention in the paediatric population: pre-school located vaccination program and saliva-based molecular surveillance in hospitalized children in Milan municipality	516
210 - Oral potentially malignant disorders (OPMDs): HPV prevalence and vaccines	518
227 - Characterization of T-cell response to recombinant VZV vaccine in pwMS under DMTs	521
234 - Transcriptomic analysis after mRNA BNT162b2 vaccination revealed a specific gene expression profile in low-responders hemodialysis patients	522
235 - Two years after COVID-19 vaccination: persistence of spike-specific memory B cells and antibodies after homologous and heterologous vaccine combinations	523
240 - Characterization of the Immune Responsiveness to COVID-19 Vaccination in Rett Syndrome Patients	525
259 - B cell response after SARS-CoV-2 mRNA vaccination in people living with HIV	527
265 - Persistence of spike-specific immune responses in solid organ transplant recipients up to two years following mRNA vaccination for SARS-CoV-2	529
278 - Recombinant BCG strains as innovative treatment of non-muscle invasive bladder cancer	531
304 - Moving beyond the titer paradigm: rational design of a next-generation glycoconjugate vaccine inducing highly functional antibodies	532
315 - Humoral And Cellular Immune Response after breakthrough infection in Orthotopic Liver Transplant patients who received 3 or 4 doses of SARS-CoV-2 mRNA BNT162b2 Vaccine.	554

