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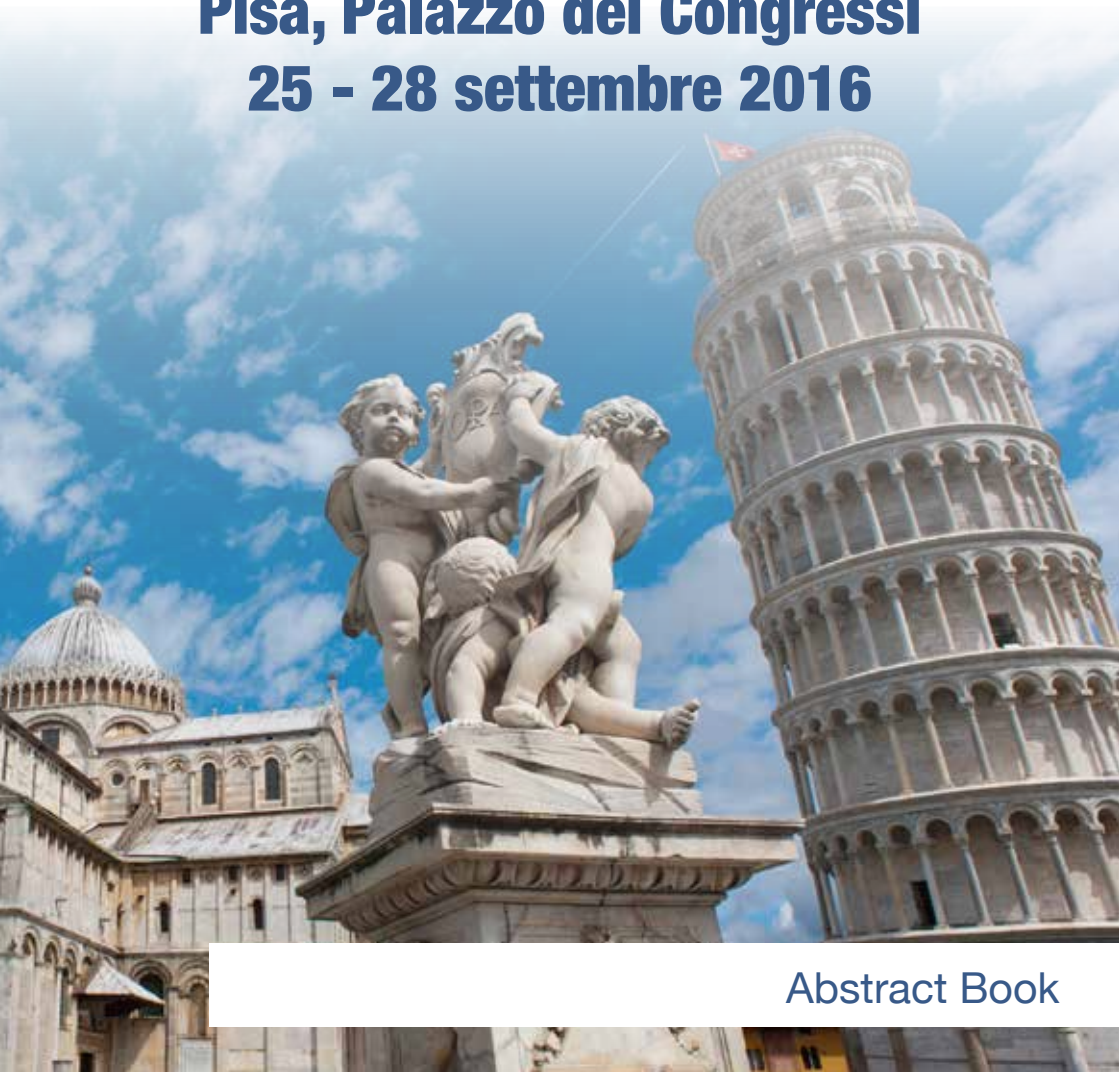

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IMMUNOLOGY OF TUBERCULOSIS

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Mycobacterium tuberculosis infection remains an important cause of morbidity and mortality worldwide, currently accounting for over 8 million new infections and approximately 1.5 million deaths annually. Furthermore, there is a significant spread of drug-resistant organisms, and no broadly effective vaccine strategies are presently available. The continuing global epidemic of tuberculosis emphasizes the urgent need to improve our knowledge of host defense mechanisms and to develop effective strategies for the prevention and treatment of this devastating disease.
A quarter of the world's population is infected with *Mycobacterium tuberculosis* and remains asymptomatic. Of these latently infected individuals, only 5-10% will develop active tuberculosis disease in their lifetime. Both innate and adaptive (T cell-mediated) immune responses are critical in the control of *Mycobacterium tuberculosis* infection, but the host factors that determine why some individuals are protected from infection while others go on to develop disease are unclear. Among the characteristics of adaptive immune responses to *Mycobacterium tuberculosis* is a delay in the onset of detectable T cell responses. Recent studies have revealed mechanisms that contribute to this delay, including pathogen inhibition of apoptosis, delayed migration of dendritic cells from the lungs to the local lymph node, and influence of regulatory T cells. In addition, novel features of *Mycobacterium tuberculosis* antigen-specific T cell differentiation have been discovered, which reveal pathways that limit and promote immune control of infection.
Therefore, continuing development of new strategies and a better understanding of the nature of protective immunity remain important goals for tuberculosis research.

EXOSOMES AS INTERCELLULAR COMMUNICATORS

EXOSOMES IN THE DIAGNOSIS OF TUBERCULOSIS: ROLE OF SERUM MICRORNAS IN THE DIFFERENTIAL DIAGNOSIS BETWEEN ACTIVE AND LATENT TUBERCULOSIS

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Infection with *M. tuberculosis* most commonly leads to persistence of the pathogen (referred as latent tuberculosis infection, LTBI), rather than its killing. Indeed, until the host immunity is effective, mycobacteria proliferation is controlled and sometimes cleared. With the impairment of the host immune system the infection progresses to active disease. Despite the importance of defining LTBI and active TB, clear-cut biological markers separating the two conditions are not yet available, thus limiting the accuracy of current diagnostic tests in determining disease status and progression. Circulating human microRNAs (miRNAs) have been associated to several diseases and physiological status and may have a role as biomarkers. Circulating miRNAs are included in exosomes and have been involved in intercellular communication. In our work we aimed to identify a miRNA signature able to distinguish active TB disease from latent TB infection (LTBI).
Subjects enrolled were assigned to either category according to clinical, microbiological, radiological and immunological features. The study population consisted of 24 active TB and 24 LTBI subjects. After total RNA extraction from serum, the levels of 189 miRNAs were analyzed by qRT-PCR arrays (Qiagen). On the basis of miRNAs associated Importance index, computed by a Random Forest model, an optimal predictive miRNA signature

was found and its diagnostic performance (accuracy) was analyzed using a Support Vector Machine (SVM) model and validated by Leave-One-Out-Cross-Validation (LOOCV) approach.
Among the miRNAs with highest ranking score, we selected the two best ranking miRNAs (hsa-miR-30e-5p and hsa-miR-425-5p) to be used as a diagnostic signature. The signature showed a diagnostic accuracy of 78.3% in the SVM model.
We found a serum miRNA signature able to discriminate between active TB patients and LTBI subjects.

ROLE OF EXTRACELLULAR VESICLES IN FUNGI AS MEDIATORS OF CELLULAR COMMUNICATION

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Fungal extracellular vesicles (EVs) were first described in *Cryptococcus neoformans* in 2007. EV production, which is present in all domains of life, has since been discovered in both filamentous and other unicellular fungi. Evidence suggesting a role for EVs in pathogenesis came from the identification of virulence factors as part of the EV cargo. However, virulence is not the sole function of EVs, since studies on EV proteomes in fungi have revealed a wide array of protein cargoes, including proteins that play a role in cell metabolism, signal transduction, as well as structural scaffold proteins and nuclear proteins. Furthermore, it has been demonstrated that EVs contain RNA of different types, including non-coding RNA (miRNAs, small nuclear RNAs, tRNAs), as well as coding mRNA of different types. This reinforces the view that fungal nucleic acid contained in vesicles may serve as a means to interfere with gene expression in host cells as well as a tool involved in fungal communication, in response to environmental stimuli.
Despite the fact that pieces of evidence on EV characterization are starting to emerge, little is known on the role played by EVs in drug resistance, immune system evasion, host cell invasion and the pathogenesis of *Candida* species, the most common fungal pathogen responsible for human mycoses. The interplay between *Candida* cell and the innate immune system represents a key step influencing the transition from saprophytic/commensal to the pathogenic stage. It is known that the immune response to candidiasis is not exclusively bound to the host-microorganism engagement but it also relies upon secreted components by the pathogen, which are able to challenge pattern recognition receptors eventually modulating the host immune cytokine production.
Moving beyond these considerations, our current project is aimed to shed light on the function of EVs produced by different *Candida* species in both *Candida*-host and *Candida-Candida* interactions. The identification of fungal immunologically active compounds released via EVs could open a new field of study for alternative strategies to control *Candida* invasive infections, which are still associated with a high mortality rates in humans.

microRNA AND EXOSOMES IN HUMAN PAPILLOMAVIRUS-INDUCED TUMORIGENESIS

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Introduction: Many mammalian viruses have evolved strategies counteracting cell antiviral defense, both by viral oncoproteins and by viral microRNA (miRNAs) involved in virus-induced tumorigenesis. Human Papilloma Viruses (HPVs) include more than 100 different genotypes, divided into mucosal or cutaneous HPVs. Mucosal HPVs are the causative agents of cervical cancer and are associated with other cancers. The involvement of cutaneous HPV genotypes in non melanoma skin cancer is still not established. Cancer cells show a specific pattern of miRNA and HPVs modulate miRNAs expressed by infected cells. The production of membrane vesicles, in particular exosomes, is deregulated in cancer, and the cargo delivered by exosomes to the microenvironment can promote tumor growth and progression. Keratinocytes transduced with E6 and E7 oncoproteins from mucosal HPV-16 or cutaneous HPV-38 (K16 and

K38) were analyzed to study the involvement of HPV oncoproteins in miRNA expression in cells and exosomes.
Materials and Methods: MiRNAs were analyzed by TaqMan Array Human MicroRNA Cards, followed by Real Time RT-PCR for specific miRNAs. miRNA targets were analyzed by Western blot and correlated to HPV oncoproteins by specifically silencing E6 and E7 expression. K16 and K38 supernatants underwent differential centrifugations and exosomes quantified through the vesicle-associated acetylcholinesterase activity.
Results: MiRNA profiling led to the identification of miRNAs deregulated in K16 and K38 cells. HPV-16 and/or HPV-38 E6 and E7 single proteins can modify the expression of miRNAs selected on the basis of their role in tumorigenesis, i.e. miR-18a, -19a, -34a, and -590-5p. The exosomes content analysis revealed the presence of E6 and E7 mRNAs and few miRNAs. Interestingly, miR-222, a key miRNA deregulated in many cancers, was identified in exosomes from K16 cells.
Conclusions: HPV infection induces deregulation of some miRNAs through mechanisms involving E6 and/or E7 oncoprotein. Moreover, by production and function of exosomes, HPV oncogenes as well as HPV-deregulated miRNAs can potentiate the virus oncogenic effects in the tumor cell microenvironment.

EXOSOMES AND microRNA IN THE INFECTION OF POLYOMAVIRUSES

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Exosomes, small membrane vesicles present in several body fluids, are involved in intercellular communication also delivering microRNA molecules to neighboring recipient cells. Increasing evidences suggest that the microRNA-mediated gene silencing mechanism, detected during exosomes intercellular communication between cells, may be exploited by the persistent human viruses. Recently, it has been reported that human Polyomaviruses encode microRNAs that down-regulate large T expression and target host factors, helping the virus to escape the immune elimination. Consequently, the microRNAs viral expression and/or their variability could be relevant for the induction of the Polyomaviruses reactivation, the succession of the persistence/replication viral process and the diseases development. In this context, polyomavirus JC (JCPyV) microRNAs expression has been suggested to be relevant for the onset of virus reactivation and development of Progressive multifocal leukoencephalopathy (PML). JCPyV microRNAs expression and variability have been investigated in several clinical samples obtained from patients at risk of PML in order to shed light on the role of viral microRNAs on virus replication. The results reported a variable expression of JCPyV microRNAs in exosomes of blood, urine and cerebrospinal fluid samples from patients at risk of PML and healthy subjects. In this context, the relationship between the JCPyV microRNAs expression and viral DNA load could be taken as indicative factor of viral reactivation. *In vitro* investigation has shown that the expression of JCPyV microRNAs in cells and in exosomes present in their supernatant start from 12 h after the viral infection. Additionally, this study verified that the JCPyV microRNAs in the exosomes present in the supernatants produced by the infected cells might be carried into uninfected cells. These findings suggest that additional in depth studies of the JCPyV and other polyomaviruses microRNAs in exosomes are of warranty to understand their role in viral reactivation and to disclose new antiviral strategies targeting these viruses.

EMERGING PATHOGENS IN THE GLOBALIZED WORLD
SOIPA/SIM SESSION

CHAGAS DISEASE

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Chagas disease, also known as American trypanosomiasis, is caused by the protozoan parasite Trypanosoma cruzi, and was discovered in 1909 by the Brazilian physician Carlos Chagas. Chagas disease is transmitted by bloodsucking bugs of the subfamily Triatominae (Triatoma infestans, Rhodnius prolixus, and Triatoma dimidiata) to domestic animals and wild mammals and to people. The triatomines live in poor housing conditions and people living in rural areas are at greatest risk for acquiring infection.
An infected triatomine takes a blood meal and releases trypomastigotes in its feces near the site of the bite wound. Trypomastigotes enter the host through the wound or through intact mucosal membranes (conjunctiva). Then, the trypomastigotes invade cells near the site of inoculation and then are released into the circulation, infecting cells from a variety of tissues. Also, Chagas disease can be transmitted to man by blood transfusion, organ transplantation and vertically from mother to infant. Several outbreaks of orally transmitted Chagas disease have been reported, probably due to contamination of food with the faeces of infected triatomines or whole infected triatomines.
Chagas disease is endemic in Latin America and an estimated 8 million people are infected.
After an incubation phase (4-28 days), the acute phase is usually asymptomatic. The symptoms include fever (10-20%), malaise, and the signs of entry of T. cruzi through the skin (chagoma) or via the ocular mucous membranes (Romana sign); also ECG abnormality could be present. On the basis of organ involvement, the chronic phase is divided in three forms: cardiac, digestive and cardiodigestive. Benznidazole and nifurtimox are the only drugs with proven efficacy against Chagas disease.
In case of immunosoppression, such as Human immunodeficiency virus infection, organ transplantation, neoplastic diseases and new immunosuppressive therapies, a reactivation of chronic disease may occur, resulting in severe clinical manifestations such as meningitis and encephalitis, and acute myocarditis. Chagas disease can be considered an emerging problem in Europe, where about about 59,000-108,000 cases are estimated. The migration from Latin America promotes the spread of Chagas disease and the asymptomatic people could transmit the infection through blood transfusion, organ transplantation and transplacentally. Also, in Europe, patients with asymptomatic chronic infection may easily access new immunosuppressant drugs and reactivation of infection may occur.
Measures to control transmission have been implemented in some countries in order to mitigate the risk of propagating the disease within Europe. The main concerns are to improve the health of migrants in Europe and to control transmission. In Europe the Chagas disease is underestimated, probably due to the high prevalence of asymptomatic infection, the lack of knowledge of disease and the presence of barriers to access healthcare for migrant populations.

THE FISH-BORNE PARASITIC ZOONOSES

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The parasitic zoonoses linked to fish consumption are mostly helminthic diseases caused by digeneas, cestodes and nematodes. Yet these zoonoses are responsible for large numbers of human infections around the world. The list of potential fish-borne parasitic zoonoses is quite large. However, here emphasis will be given to the species of genus *Anisakis*, considered by EFSA (European Food Safety Authority) as the most important Biological Hazard in seafood products. The parasite's life-cycle involves marine organisms (crustaceans, fish, cephalopods, and marine mammals), being distributed worldwide in oceans and seas. Humans represent accidental host in its life-cycle, acquiring the infection with consumption of parasitized fish products consumed raw or undercooked. The pathology they provoke can be defined as gastric, intestinal, ex-

tra-gastrointestinal, or Gastro Allergic Anisakiasis (GAA). Also, allergy due to antigens of the parasite present in fish has been reported; thus, *Anisakis* has been handled also as a "food allergen".
The application, over the last decades, of the molecular systematics approach to the parasite of genus *Anisakis*, has advanced the recognition of "biological species", with implications for their evolutionary ecology, epidemiology, and relevance to human health. So far, different genetic methodologies provided powerful molecular/genetic markers to discover and identify species of the genus *Anisakis*.
Epidemiological data concerning parasitic infection levels by the zoonotic *Anisakis* spp. occurring in fish hosts, including their differential distribution in organs and tissues of the fish hosts, as well as the use of the methodologies for their detection, will be shown.
The relevance of *Anisakis* spp. to human health, concerning the zoonotic disease (anisakiasis) they provoke, including the "allergic anisakiasis", and its diagnosis will be presented. The occurrence of human cases, on a global scale, will be reported.
On the other hand, despite the zoonotic role of *Anisakis* spp., the possible use of infection levels and genetic variability values of anisakid populations as "ecological indicators" of the "health state" of a marine ecosystem, will be suggested.

MONITORING *AEDES ALBOPICTUS* BY NOVEL NON-CONVENTIONAL APPROACHES: ZANZAMAPP AND CITIZEN SCIENCE

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Introduction: *Aedes albopictus* is an invasive mosquito species which has stably colonized most Italian urban areas and represents a health concerns not only due to its aggressive behaviour, but also to its role as vector of Chikungunya, Dengue and Zika viruses. In Europe, it was responsible of the first outbreak of an exotic arbovirus (> 200 Chikungunya cases in Ravenna Province in 2007). Estimates of the species abundance and biting rates are essential to account for vector-human contact and to predict nuisance threshold and risk of arbovirus autochthonous transmission useful for correct planning of mosquito control interventions. Novel approaches to monitor this daytime and outdoor biting mosquito are deeply needed, as current methods have low effectiveness and/or difficult to be implemented in large scale.
Material and Methods: In 2016, we developed two non-conventional innovative approaches to monitor *Ae. albopictus* abundance and biting rates. The first is represented by a mobile application named ZANZAMAPP which allows users to easily record geo-referenced reports of spotted mosquitoes and basic information on the time and indoor/outdoor location of the record and of the perception of the mosquito nuisance. The second approach is a "citizen science" pilot project in which students of the Faculty of Medicine of Sapienza University were provided with a sticky-trap patented by our research group and asked to: i) report number, species and gender of weekly collected specimens; ii) send us by mobile phone photos of the sticky sheets for double-check identifications; and iii) fill a questionnaire on their perception of mosquito nuisance.
Results: The talk will focus on the description and preliminary results of both these two novel approaches and highlight their potential to complement entomological surveillance of Ae. albopictus.
Discussion: Analysis of the records provided by ZANZAMAPP (> 10,000 in the first 2 months) has the potential to provide a dynamic spatial map of mosquito abundance and of human perception of mosquito nuisance in Italy and to represent a cheap and effective way for public administration to gather information relevant for the planning of mosquito control. Analysis of the information provided by the students will allow to assess whether non-experienced people can effectively contribute after a short training to a capillary monitoring of the species and provide the basis for a larger "citizen science" project.

RECENT DEVELOPMENTS ON ANTIMICROBIAL RESISTANCE

ANTIBIOTIC RESISTANCE AND NEW EMERGENT RESISTANCE MECHANISMS IN GRAM-POSITIVE BACTERIA

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The success of modern medicine is mostly based on the use of drug therapy to control and cure infections that are caused by various pathogens. This legacy is currently threatened by the development and evolution of antibiotic resistance to the drugs on which these therapies rely.
There are multifaceted aspects of drug resistance in Grampos, Gramneg bacteria and fungi, that are being actively studied, such as the molecular nature of resistance mutations, the ability to become resistant through the HGT from a very extensive gene pool, the fitness consequences of resistance (both in the presence or in the absence of drugs) and how these aspects influence the evolutionary trajectories of the resistant microrganisms. But other aspects remained unappreciated until recently, i.e. the additional costs that an antibiotic exerts on an individual's own health via the collateral damage of the drug on bacteria that normally live on or in healthy humans – the microbiota – or through acting on different systems that regulates the life (but not necessarily the molecular target of its action) of the bacteria itself. Examples here are the biofilm life, the SCV, the agr involvement and many other consequences related to the SOS activation.
All these aspects, together with the selective pressure exerted by antibiotics, above all at non -lethal concentrations, can cause different subtle changes in the bacterial genome that, in a stepwise fashion, accumulate, increasing the probability of resistance/tolerance acquisition. Furthermore, another type of interaction involves the effect of resistance to one drug on the susceptibility to other drugs (collateral sensitivity in the case of vancomycin and oxacillin for MRSA). In principle, detailed knowledge of the complex scenario of resistance acquisition could be applicable to clinical settings to reduce resistance development. A more complete understanding of these features will be crucial to predict resistance and to combat it through the optimal use of existing drugs (concentrations, periodic switching, drug combination) and to guide the design of new drugs.

PATHOGENESIS, IMMUNITY AND INNOVATIVE THERAPEUTIC APPROACHES IN VIRAL INFECTIONS

HOST AND VIRAL GENETIC FACTORS AS A RESPONSE TO ANTIVIRAL THERAPY ANTI-HCV: YESTERDAY AND TODAY COMPARED

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The goal of treatment of hepatitis C virus (HCV) infected persons is to reduce all cause mortality and liver related health adverse consequences, including end stage liver disease and hepatocellular carcinoma, by the achievement of individual virologic cure. Hepatitis C is one of the most pressing health emergencies worldwide, with an estimation of 130-170 million people with chronic infection.
A high degree of genetic variability among the endemic HCV strains has been acquired during long-term evolution. HCV genetic variability is higher than what observed in other viruses leading to chronic infections, such as human immunodeficiency virus (HIV) and hepatitis B virus (HBV).
In this presentation, the changes in standard of care for HCV, and improvements in sustained virologic responses will be discussed, as well as the main host and viral genetic factors associated with the response to antiviral therapy.

INTERFERON AND VIRUSES: A DELICATE BALANCE BETWEEN YIN AND YANG

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Interferon (IFN) was discovered in 1957 as an 'activity' that was secreted by influenza virus-infected cells that could protect other cells from infection. All IFNs are named after their ability to stimulate the synthesis of a large set of IFN stimulated gene (ISGs) and therefore to limit viral replication and spread. They are divided into three structurally and functionally distinct types that interact with different cell-surface receptors. While type II and III IFNs are limited to one (IFNγ) or four (IFNλ) members, type I IFNs belongs to a multigene family and includes 12 distinct subtypes of IFNα, and one IFNβ, IFNε, IFNκ and IFNω member. Type I IFN is currently used as a treatment in chronic hepatitis B and C virus infection (HCV), but as yet is not widely utilized for other viral infections. One reason for this restricted clinical use is that the individual antiproliferative, antiviral and immunological properties of the different IFNα subtypes have so far not been investigated in detail to improve IFN therapy against viral infections. However, each time new viruses have been discovered (e. g. severe acute respiratory syndrome coranavirus, middle east respiratory syndrome coronavirus, human metapneumovirus, and pandemic H1N1 influenza virus, etc), the antiviral action of IFNα/β against these new emerging viruses has been immediately evaluated in order to identify effective antiviral agents. It is unclear what advantage the presence of so many different IFNα subtypes provides, but functional differences observed among individual IFNα subtypes suggested that they might play distinct regulatory roles during an immune response. Importantly, loss of IFNα/β signaling in animal models usually leads to uncontrolled viral replication. However, in the context of viral infections, there is now substantial evidence that type I and perhaps type III IFN activity can be both harmful and advantageous for the host organism. For instance, the detrimental effects of type I IFN action are clearly demonstrable during lymphocytic choriomeningitis virus (LCMV), pathogenic simian immunodeficiency virus (SIV) and HIV-1 infections where a direct causal link between type I IFN/ISGs expression and chronic immune activation and dysfunction has been reported. It has been also

observed that the presence of a strong type I IFN and/or ISGs response is associated to impaired spontaneous or treatment induced clearance of HCV infection. More recently, a negative roles of IFNλ and/or genetic variations within type III IFN genes region (i.e IFNL4 polymorphism) has been demonstrated in HCV infection, and in other viral infections. Surely, the timing of IFN production with respect to the course of infection, the type of viral infections (acute versus chronic); and the local or systemic spread of viral infection might be important with regard to the positive or negative effects of IFNs. The ability of viruses to overcome host IFN responses might be yet another factor that determines the efficacy of IFNs in the battle against viruses. The factors that tip this Yin–Yang balance between IFN and viruses will require our future attention.

NEW THERAPEUTIC APPROACHES IN HIV-1 INFECTION

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Introduction: Today, antiretroviral therapy provides potent and durable suppression of HIV replication, reduces HIV-associated morbidity and prevents transmission of infection. The availability of effective drugs in several classes with long term safety and tolerability profiles offers interesting choices for first line treatment and after failure. To maximize safety and resistance profile, combination therapies with improved adherence (single-tablet regimens), molecules with novel mechanisms of action (attachment and maturation inhibitors) and treatment simplification with uncommon dosing (long -acting injectables) are further innovations, essential to achieve the ambitious goals of '90-90-90' target. The actual paradigm is shifting not only to "treat all" HIV positive patients, but to intensified medical prevention efforts focusing on pre-exposure prophylaxis (PrEP). Although the prevention of HIV-1 transmission has been attempted using behavioural style (male circumcision, condom use), these methods seem not sufficient to tackle the sexual transmission of HIV-1. A promising strategy for preventing HIV-1 transmission is represented by microbicides, that, combined in a vehicle gel or film, are used topically on either the vaginal or rectal mucosa. The recent preparation of microbicides in vaginal rings has also provided the ability to achieve sustained long-term release of microbicides on the vaginal mucosa. Hence, microbicides are considered as a form of PrEP.
Material and Methods: We studied the antiviral activity of mini CD4 M48U1 peptide formulated in HEC hydrogel, alone and in association with tenofovir, on activated peripheral blood mononuclear cells (PBMCs) and on a organ-like structure mimicking the human cervicovaginal tissue, infected by R5 and X4–tropic HIV-1 strains.
Results: The results demonstrated that M48U1 prevented HIV-1BaL and HIV-1IIIB infection in activated PBMCs, with half maximal effective concentrations (EC50) of 43.4 and 33.2 nM, respectively, demonstrating a strong antiviral effect without any induction of significant toxicity. In addition, M48U1, tested in association with tenofovir, synergistically inhibited HIV-1 infection sustained by strains isolated from naïve and multi-drug resistance patients, infected with different clades, and from Transmitted/Founder viruses, derived from acute infections and transmitted through mucosal site in vivo.
Discussion: Together, these results indicate that co-treatment with M48U1 plus tenofovir can exert effective antiviral activity, and this association may represent a new topical microbicide to counteract the heterosexual transmission of HIV-1.

INNOVATION AND SPEED IN MICROBIOLOGICAL DIAGNOSIS *AMCLI/SIM SESSION*

MOLECULAR DIAGNOSIS IN EMERGING PARASITIC DISEASES

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Introduction: In parasitology, routine laboratory diagnosis involves conventional methods, such as optical microscopy, used for the morphological identification of parasites. Currently, molecular biology techniques are increasingly used to diagnose parasite structures in order to enhance the identification and characterization of parasites. The objective of the presentation is to review the main current and new diagnostic techniques for confirmation of parasite infections
Materials and Methods: In particular we reported the personal experience concerning the molecular diagnosis of two emerging or re-emerging parasites: free living amoebae in AK (amoebic keratitis) and *Pneumocytis jirovecii* in PcP and colonized patients.
Results: The molecular identification allow us a rapid and sensitive diagnosis and the opportunity to clarify the genotypes involved in the infections.
Discussion: Molecular assays have comprehensively assisted in the diagnosis, treatment and epidemiological studies of parasitic diseases that affect people worldwide, helping to control the morbidity and /or mortality of emerging parasites.

ACCELERATE PHENO: THE NOVEL RAPID PHENOTYPIC ANTIBIOTIC SUSCEPTIBILITY TEST

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Introduction: Antimicrobial resistance (AMR) has become a major threat to public health worldwide due to the excessive and inappropriate use of antimicrobials on humans and animals and inadequate infection control practices. AMR leads to increasing healthcare costs, prolonged hospital stays, treatment failures and a significant number of deaths. The use of diagnostic tools including rapid tests and their uptake in the human and veterinary sector have been indicated as one of the means to improve the prescription of antimicrobials in the fight against AMR under the "One Health" approach.
Materials and Methods: Accelerate Diagnostics has developed a technology based on automated Fluorescence In Situ Hybridization and Morphokinetic Cellular Analysis that delivers microbial identification (ID) and phenotypic antimicrobial susceptibility testing (AST) results with MIC values in less than 7 hours. Notably, the system boasts polymicrobial capabilities. The Accelerate PhenoTest™ BC kit for bloodstream infections (BSIs) is currently available in Europe and other direct specimen kits are in development, including the respiratory kit. Accelerate Diagnostics has recently submitted 140 assays to the FDA. Over 1,800 blood culture samples across 12 trial sites have been analyzed with the Accelerate Pheno™ system using the Accelerate Phenotest™ BC kit and compared with the Standard of Care.
Results: ID results showed an overall sensitivity of 97.2% and an overall specificity of 99.4%. The Accelerate Pheno™ system provided a definitive monomicrobial call with a positive predictive value of 98.3%. Susceptibility results exhibited an overall Essential Agreement of 95.6% and an overall Categorical Agreement (CA) of 95.4% based on FDA/CLSI breakpoints. Resistance Phenotype results for MRSA (Cefoxitin) and MLSb (Erythromycin/Clindamycin) showed a CA of 99.5% and 97.7%, respectively.
Discussion and Conclusions: The Accelerate Pheno system delivers rapid ID and AST results with high sensitivity and specificity, tangibly accelerating targeted antimicrobial therapy by 1-2 days and permitting more rapid de-escalation or escalation. These capabilities have the potential for decreased selective pressures that lead to antimicrobial resistance, dramatically improved patient outcomes including reduced mortality from BSIs, significantly reduced length of stay, broad-spectrum antibiotic use and redundant laboratory tests. In conclusion, the potential exists for clinical, operational, and financial benefits to accrue to institutions using the Accelerate Pheno™ system as part of their antibiotic stewardship initiatives.

PNEUMOCOCCI AND MENINGOCOCCI: THE ENDLESS FIGHT BETWEEN VACCINES AND BACTERIA

CHANGES IN PNEUMOCOCCAL DISEASE EPIDEMIOLOGY IN THE ERA OF GLYCOCONJUGATE VACCINES

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Introduction: Following the introduction of glycoconjugate vaccines for children immunization, *Streptococcus pneumoniae* epidemiology has been constantly evolving as for incidence, serotype distribution and antibiotic resistance. Starting from 2001, PCV7 was gradually implemented in the Italian regions with different strategies and was replaced by PCV13 between 2010 and 2011. Since 2007 ISS, with the support of the MoH, is coordinating the national surveillance system of invasive bacterial diseases (MABI Surveillance) that includes pneumococcal invasive diseases (IPD). Serotyping of the isolates is performed at the regional or national levels. The surveillance data are available at the dedicated MABI web site (http://www.iss.it/mabi/).
Materials and Methods: All IPD cases reported to the MABI Surveillance were included in the analysis. Serotyping was also included in the surveillance data. Antibiotic susceptibility was determined by the Etest using the EUCAST breakpoints. Genotyping of selected isolates was obtained by PFGE and MLST.
Results: According to the MABI surveillance, the incidence of IPD in Italy declined in the age group < 1year and 1-4 while in the general population there was an apparent increase, due to an increase in the number of cases in adults and elderly persons, likely due to more complete reporting to the Surveillance. In 5 Italian regions (Emilia-Romagna, Lombardia, Piemonte, P. A. Bolzano, P. A. Trento) where surveillance was more comprehensive and included serotyping of most isolates, the IPD incidence in the age group < 1 year showed a reduction of 64% of the cases, from 12.98 to 4.68 cases/100,000 in three years. PCV13 serotypes represented approximately 78% of the isolates in the years 2008-2011, but decreased to 45% in 2012-2014. Non vaccine serotypes increased from 22% in 2008-2009, to 55% in 2012-2014. In 2014 the most frequent serotypes in children were 12F, 24F, 1, 19A and 3; and in the general population 3, 8, 12F, 19A and 1. The percentage of penicillin non-susceptibility increased; on the contrary, the percentage of ceftriaxone and erythromycin resistance decreased. In the last years resistant isolates were largely represented by non-vaccine serotypes belonging to international antibiotic-resistant clones.
Discussion and Conclusions: The use of glycoconjugate vaccines led to a substantial decrease in IPD incidence in children in Italy. Since new epidemiological changes are expected in the next years, continuous surveillance, including surveillance of pneumococcal colonization of the nasopharynx of children and adults, is relevant for the design and implementation of new vaccines.

NEISSERIA MENINGITIDIS: THE CLUSTER IN TUSCANY REGION

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Introduction: Invasive meningococcal disease (IMD) cases due to serogroup C (MenC) have continuously declined after vaccine introduction with meningococcal C (MCC) vaccine. However, despite the large use of MCC vaccine, outbreaks and smaller clusters of MenC belonging to the ST-11 clonal complex (cc11) (MenC:cc11) have continued to occur in Europe; in particular, the finetype C:P1.5-1,10-8:F3-6:ST-11 (cc11) was responsible of outbreaks in France, in Germany and also in Italy. Since January 2015, an unexpected increase of IMD of MenC belonging to cc11 occurred in Tuscany. To this end, public health measures and the results of the whole genome sequencing (WGS) on isolated meningococci are presented.
Materials and Methods: Surveillance activ-

ities and public health measures were implemented in the Region. Bacterial isolates from IMD cases were characterized by the National reference laboratory of the Istituto Superiore di Sanità (ISS), and WGS was performed on available strains.
Results: The Italian National Surveillance System for IMD coordinated by ISS, with the financial support of Ministry of Health (MoH), reported a total of 51 IMD cases in the period January 2015 - June 2016, compared with 5 cases in the biennium 2013-2014. Chemoprophylaxis of close contacts and vaccination of adolescents and adults, were implemented by Local Authorities to contain the spread of IMD cases. The outbreak was caused by the hypervirulent clone C:P1.5-1,10-8:F3-6:ST-11 (cc11). The core genome MultiLocus Sequence Typing (cgMLST) analysis showed a principal tight clustering group. More exactly, inside this group two subgroups are distinguished. The first one comprised isolates until June 2015; the second one comprised isolates from July 2015. Three meningococci, isolated in February and March 2016, clustered in a different branch. Three genes (Neis0430, penA, fHbp) confirmed the subdivision suggested by cgMLST. As already described by Stefanelli et al. J. of Infection 2016, the target gene Neis0430 allele 398 was found exclusively in meningococci belonging to the main cluster identified in Tuscany.
Conclusions: Our findings show that increased incidence rates of IMD due to hypervirulent meningococcal strains may occur unpredictably also in areas with high vaccination coverage among infants and young children. The WGS analysis permits to define that the strains were closely related to each other, whereas they differed from strains isolated in other Italian Regions which shared the same finetype.

MULTIFACETED ASPECTS OF VIRAL VARIABILITY

VIRUS EVOLUTION AND EPIDEMIOLOGY

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Background: During the last half century, a series of "novel" viral agents have emerged from animal sources, adapting to human hosts.
Methods: The most common form of evolutionary study performed on RNA viruses is molecular epidemiology, in which phylogenetic methods are used to infer the origins, emergence, spread, and dynamics of viral epidemics. Such studies can be i) broad scale, examining the global distribution of viral genetic variation, or ii) localized, in which the precise network of transmission is reconstructed. The high rate of evolutionary change of RNA viruses, and the consequent rapidity of viral evolution, is such that phylogenetic relationships can often be resolved among isolates that have been sampled only a few weeks or even days apart, and the epidemiological processes that shape their genetic diversity act on the same scale as mutations are fixed in viral populations.
Results: Virus epidemiology may be influenced by viral mutation at different stages: A) cross-species passage, through deletion of virus RNS /i.e., SARS), genetic drift and switch (influenza); B) virus spread, through different phylodinamic models, which derive from the different evolutionary characteristics and epidemiological patterns of the viruses. On the basis of the phylodynamic model, we may define at least 3 different groups of viruses: 1) acute viruses, such as measles; 2) long-latency, slow, viruses, such as HIV or HCV; 3) viruses such as flu, which evolve into strains which present partial cross-immunity to each other. Viral mutation patterns differ among these groups of viruses, influencing population immunity/susceptibility and consequent epidemic dynamics.
Conclusions: The combined study of molecular evolution and epidemiology of viruses provides important information on the interpretation of epidemiological events and may help to predict and better control new health threats caused by viruses and other biological agents.

ETIC MECHANISMS DRIVING VIRAL VARIABILITY

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Genetic variability is an essential feature of all living organisms. It provides the resource for natural selection and for the progressive adaptation of the population to a changing environment. Viruses are constantly evolving through a process that sees the alternating emergence of new genetic characteristics and their stabilization in the population by selection or random fluctuation. Viruses use all the possible strategies of the variation to remain a step ahead of their host. Variation creates new genotypes that help virus to adapt quickly to the changing environmental conditions. DNA polymerase, RNA polymerase and reverse transcriptase are the enzymes for copying genetic material and are primarily responsible for creating variation in the genetic makeup of the viruses. Mutation, recombination, reassortment and quasispecies are some of the mechanisms by which viruses creates the variation.

From a genetic perspective, viruses can be classified according to whether the genome comprises RNA or DNA. RNA viruses have the highest rate of mutation among all the viruses followed by single strand-DNA viruses and lastly double strand-DNA viruses. Mutation is the major source of genetic change in viruses. It creates the lethal or advantageous mutations depending on the change they make in amino acid sequence of a gene. Recombination is exploited by many of the viruses. Two viruses infecting same host cell at the same time result in reassortment, and is main feature of the segmented RNA viruses. These three mechanisms provide viruses to with higher rate of evolution as compared to eukaryotic cells. Viral variants generated can be selected on the basis of their effect on viral fitness. The concept of viral quasispecies derives from the observation that, in an individual infected for a long time, there is a continued evolution of the virus, as a result of spontaneous mutations and selective pressures exerted by host. If the mutation rate is too high or too low, the viral population tends to die. This would happen because the genetic information was completely lost or because the population cannot escape by the immune system responses and/or by drug pressure anymore.

Viral evolution can answer some of the questions like why do viruses creates seasonal outbreaks, host change and pandemics. There is still a wide scope of studies in viral evolution and its application in solving problems related to prevention and treatment against viruses.

VIROME: THE NEW PANDORA'S BOX

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Introduction: The extensive use of innovative molecular techniques on a variety of biological human samples has allowed uncovering many novel viruses, extending tissue distribution of known viral types, and rapidly revolutionizing many concepts on viral complexity. It's now evident that many viral agents are present in clinical samples other than the major known pathogenic viruses and that the totality of these agents, defined as human virome, is an integral part of the microbiotic universe that makes us healthy.

Materials and Methods: Next-generation sequencing (NGS) technologies and microarray assays (MA) have recently become available and are being utilized for virome investigation. In NGS, fragmented DNA (and RNA converted into cDNA) is accurately sequenced in millions of reactions. The newly identified strings of bases are aligned and thousands of novel sequences can be obtained in very little time. A DNA MA is a collection of microscopic DNA spots attached to a solid surface. Each spot contains a specific DNA probe that is used to hybridize nucleic acid in the clinical sample. Probe-target hybridization is then read out, allowing the detection of many viruses at a time.

Results: The human virome has been studied in several districts, like the respiratory tract, gut and skin. On the contrary, other districts are still largely unexplored. Circular, replication initiator protein encoding, single stranded DNA viruses have been recently discovered in humans. Studies have revealed they occupy the largest fraction of virome, and that, among these small DNA viruses, anelloviruses (AVs) account for about 70% of total virome. Torquetenovirus (TTV) is the prototype of AVs and is found in almost all body fluids at a very high percentage of normal individuals.

Discussion and Conclusions: To date, the virome largely remains a branch for mere research, with poor clinical applications although a role is hypothesized in determining a state of inflammation/disease or influencing an underlying illness.

An exception is human TTV. Being almost endemic worldwide and insensitive to current antivirals, its monitoring is useful in various conditions: as marker of anthropic pollution, and particularly for assessing functional immune competence in immunosuppressed individuals.

ADVANCES IN BACTERIOPHAGE RESEARCH

PROPHAGES IN THE GUT: FOR GOOD OR FOR BAD? A PHAGE PERSPECTIVE

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Metagenomic approaches applied to viruses have highlighted their prevalence in almost all microbial ecosystems investigated. In those associated with humans, the viral fraction is dominated by bacteriophages (bacterial viruses), and particularly by those having a temperate life style, alternating between dormancy (prophage state) and virulence (lytic cycle). Whether they contribute to dysbiosis, i.e., the departure from microbiota composition in symbiosis at equilibrium, and subsequent entry into a state favoring human or animal disease, is unknown at present.
In theory, bacteriophages can modify bacterial population equilibrium and affect bacterial phenotypes by horizontal transfer. After summarizing briefly what has been learnt on phages associated with human microbiota in cases of diseases, I will present data from our laboratory suggesting that temperate phages represent mostly a cost for their bacterial host in the gastro-intestinal tract of monoxenic mice, and that their contribution to the transfer of antibiotic resistance genes is moderate. These observations lead to the conclusion that in healthy individuals, bacteria control efficiently the spread of bacteriophages, but whether this remains true in situations of diseases is the focus of present investigations.

LYTIC BACTERIOPHAGES AGAINST KPC-KP AND THEIR EFFICACY IN A PRECLINICAL MODEL

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Introduction: The pandemic diffusion of KPC-producing *Klebsiella pneumoniae* (KPC-KP) represents a major public health problem, given their wide spread in nosocomial environments, their extensive multidrug resistance profiles and the associated very high mortality rates. KPC-KP isolates very often exhibit a MDR resistance profile, with very few, if any, antibiotics still active. This is one of the major factors that account for their high mortality rates. In this work we characterized two phages able to specifically lyse isolates of the 2 ST258 clades, and demonstrated their ability in the protection towards death in a *Galleria mellonella* infection model.
Materials and Methods: Two bacteriophages, named ϕBO1E and ϕ207, were isolated from hospital wastewaters obtained from a center located in central Italy. Host specificity was assessed by spot technique. Phages were characterized by electron transmission microscopy (TEM) and by WGS analysis. The ability of the 2 phages to protect towards death was assessed by using a *G. mellonella* infection model.
Results: The isolated phages showed a lytic activity that perfectly correlates with CPS, with ϕ207 that exclusively lysed isolates of ST258a, while ϕBO1E those of ST258b, including isolates of ST512. Results from TEM showed that ϕBO1E belongs to *Podoviridae*, while ϕ207 belongs to the *Myoviridae* family. The 2 phages were able to protect towards death larvae of *G. mellonella* infected by isolates representative of the 2 clades, including one isolate with an hypermucoviscous phenotype ($p < 0.01$).
Discussion and Conclusions: In this work, two bacteriophages lytic for the most widespread KPC-KP clones were characterized. One phage (ϕBO1E) belongs to *Podoviridae*, while ϕ207 to

the *Myoviridae* family. The 2 phages are able to protect against death larvae of *G. mellonella*. The narrow host range displayed by these phages could be advantageous for use as therapeutic agents since it limits the possible adverse effects on the natural microbiota, as compared to conventional antibiotics. These phages could be therefore of interest for the development of the so-called phage or glycosidase therapies.

PHAGE SpSL1 GENE EXPRESSION DURING LYTIC INFECTION IN *STREPTOCOCCUS PNEUMONIAE*

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Introduction: Virus-host interactions are regulated by complex co-evolutionary dynamics including the transcriptional regulation of host genes during lytic bacteriophage infection. We have recently isolated the temperate bacteriophage SpSL1 as a tool to study the phase variable restriction methylation (R-M) systems of its host *Streptococcus pneumoniae.* This phage contains also two phage encoded methylases. In order to generate data of phage-host interaction we have used SpSL1 to test the effects of phage infection on host gene expression and chromosomal methylation.
Materials and Methods: Using RNAseq for gene expression profiling we have performed a time course of infection both in a host with active type I R-M system and in an isogenic deletion mutant. In addition we have tested infection by phages with and without the phage encoded methylases.
Results: SpSL1 shows a classic organisation in separate transcriptional units with early replication related genes and late structural genes. Despite phage gene expression can be detected as soon as 5 minutes after infection, such expression is absent in hosts with active restriction enzymes indicating extremely rapid activity of the R-M systems. No impact on infection could be evidenced by deletion of the phage methylases and no methylation of the chromosome during temperate infection occurred, while only lytic phage DNA showed methylation. The time course experiment of host gene expression sowed reduced gene expression of almost all genes with exception of two nucleotide biosynthesis loci.
Discussion and Conclusion: Data show a predictable gene expression profile of the phage, but a series of unexpected features of the host including absence of chromosomal methylation by the phage methylases and the selective upregulation of few operons associated to the need of the phage for the maintenance of a high level nucleotide biosynthesis during the lytic cycle. The phage regulator involved the regulation of host gene expression is ongoing.

PAPILLOMAVIRUSES: PATHOGENIC MECHANISMS, ONCOGENESIS AND VACCINES

HPV AND CANCER. VACCINE EFFICACY

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Introduction: Human papillomavirus (HPV) infection has been recognized as necessary but not sufficient for cervical cancer development. First HPV prophylactic vaccine was licensed for use 15 years after the discovery of virus like particles (VLP) in 1991 which enabled its production.
Material and Methods: Evidence supporting the high efficacy of the vaccines at time of licensure was derived from mainly two trials enrolling thousands of women: FUTURE for quadrivalent vaccine (Gardasil, Merck) and PATRICIA for bivalent vaccine (Cervarix, GSK). Both vaccines contain VLP from HPV16 and 18, the two most oncogenic types. The quadrivalent vaccine also includes VLP from HPV6 and HPV11, which cause genital warts and recurrent respiratory papillomatosis.
Results: Prophylactic HPV vaccines are highly efficacious (> 90%) at preventing infection with targeted HPV types and related cervical intraepithelial neoplasia (CIN). At present, with over 230 million doses of the vaccines distributed worldwide and national HPV vaccination programs in place in many countries, major achievement of both vaccines are: confirmed high efficacy against HPV infection, CIN, genital warts (quadrivalent vaccine) and vulvar and vaginal intraepithelial neoplasia. Efficacy in mid-adult women has been demonstrated, with licensing now extending up to women aged 45years. Further study of Costa Rica trial cohort has also demonstrated efficacy against targeted HPV infections at other sites, with evidence of protection against anal and oral HPV. the quadrivalent HPV vaccine is efficacious in males against HPV infection of the external genitalia, anus, penile intraepithelial neoplasia, genital warts and anal intraepithelial neoplasia. Modeling has suggested long term protection is likely. The initial vaccine schedule with three doses (0 months, 1-2 months and 6 months) can be replaced by two doses spaced > 6 months apart in those aged < 15 years. HPV vaccines have been repeatedly assessed as safe by the WHO with no increased risk of autoimmune, neurological or thromboembolic disease. A recent meta-analysis of HPV vaccination efficacy in the real world indicates significant effectiveness of HPV vaccination against targeted HPV types, genital warts and high grade CIN.
Discussion and Conclusion: The future is optimistic, with implementation of the nonavalent vaccine on the close horizon and the potential for a reduced need for screening in vaccinated cohorts. New vaccines will be shortly available with higher cross reactivity among types (anti-L2) and different new formulations are under study (i.e. fusion DNA vaccine developed by our group).

INNATE IMMUNITY AGAINST HUMAN PAPILLOMAVIRUS INFECTION

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Upon virus infection, host cells mount a concerted innate immune response involving both type I and III interferon (IFN) and pro-inflammatory cytokines to enable elimination of the pathogen. They initiate innate immune responses upon recognition of pathogen-associated molecular patterns (PAMPs) by diverse pattern recognition receptors (PRRs). Although keratinocytes contain functional PRRs that can recognize and respond to both dsDNA and dsRNA ligands, they do not appear to recognize or initiate a similar response to HPV. Indeed, we failed to detect any induction of the interferon genes in NIKS cells transfected with HPV18 minicircles (NIKS-mcHPV18) and cultured in either methylcellulose or organotypic raft cultures. Recently, the viral E7 protein has been identified as an antagonist of the cGAS-STING signalling pathway in HPV18-transformed cells, such as HeLa, that completely abolished type I IFN production upon transfection of calf thymus DNA. To extend these data, NIKS-mcHPV18 and HeLa cells were challenged with the dsDNA analogue poly(dA:dT) or with salmon sperm DNA and analysed for type I and III IFN production at both mRNA and protein levels. IFN-β induction was largely abolished in HeLa cells and partially inhibited in NIKS-mcHPV18. Of note, IFNλ1/IL29 production (the prototype of type III IFN) was also significantly reduced

in HeLa cells and in NIKS-mcHPV18, while it was highly induced in the parental NIKS cells especially by poly(dA:dT). Since it is known that poly(dA:dT) can induce IFN-β normally in the absence of cGAS or STING through the RNA polymerase III–RIG-I–MAVS pathway, the lack of IFN-β production in HeLa cells upon poly(dA:dT) transfection led us to hypothesize that HPV may also target this pathway. Experiments are on-going to mechanistically explain the inhibition of type I and III IFN induction in HPV18-positive cells upon poly(dA:dT) challenge, beyond the inhibitory activity of E7 on the STING protein. Our results show that modulation of the innate antiviral response by HPV18 in keratinocytes: i) is not restricted to transformed cells with deregulated expression of the viral oncoproteins, but also present in actively HPV replicating cells; and ii) its inhibitory activity affects both type I and III IFN targeting other PRRs in addition to the cGAS/STING DNA sensing complex.

DOES HPV PLAY A ROLE IN THE PATHOGENESIS OF BREAST CANCER?

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Introduction: High-risk Human Papillomavirus (HPV) are oncogenic viruses associated with a variety of human cancers, such as skin, ano-genital and head and neck cancers. In the past decade the association of HPV DNA with non-canonical cancers, e.g. breast cancer, has firmly emerged and a recent meta-analytic study supports the argument that HPV infection increases the risk of breast cancer [1]. Recent literature reports that HPV DNAs positive breast cancer are present in patients with previous cervical neoplasia and that conisation represents a risk factor for breast cancer genesis [2, 3], even though the route of mammary gland infection is still a conundrum, these data suggest a direct link. Since HPV does not yield viremia we tested the hypothesis that HPV DNA, not the virus, may be vehiculated via exosomes, subcellular vesicles capable to carry and transfer information among cells. Furthermore we assessed the prevalence of sixteen high-risk mucosal HPV genotypes in breast cancer tissues and nipple discharges.
Materials and Methods: HPV DNA genotyping in formalin fixed paraffin embedded breast cancer tissues and in serum derived exosomes was assessed by a new developed high throughput mass spectrometry assay [4], able to type sixteen high risk mucosal HPV. Serum and HPV DNA positive cell lines derived exosomes are isolated by differential ultracentrifugation and commercial kits (Life Technologies). The HPV DNA localization in FFPE breast cancer tissues was assessed by ZytoVision Plus CISH Implementation kit (Bio-Optica).
Results: We found that HPV DNA was harbored in about 30% (83 out of 273) of breast cancer (BC) tissues. In particular, HPV-DNA was over-represented in triple negative and Her2+ tumor subtypes and its presence correlates with BC aggressiveness. By in situ hybridization assay, we found that HPV DNA was localized not only in the epithelial compartment but also in the stromal one. To a lesser extent, we found the presence of HPV DNA in para-physiological and pathological nipple discharges. We isolated circulating exosomes from BC patients and we found about 10% of HPV DNA positive samples. Then we performed *in vitro* studies to prove that exosomes isolated from the supernatant of HPV positive cell lines (HeLa and Caski cell lines) may transfer viral DNA to recipient cells (ex vivo isolated human mammary gland fibroblasts, primary human fibroblasts and breast cancer cell lines, e.g MCF-7). We found that exosomes carried viral genetic material to recipient cells triggering a pro-inflammatory phenotype in stromal ones.
Conclusions: In conclusion, HPV DNA positive exosomes transfer viral genetic material to recipient cells and trigger breast cancer aggressiveness.

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THE LINK BETWEEN RESEARCH AND INDUSTRY IN VACCINES DEVELOPMENT

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The commitment of pharmaceutical companies Vaccination was first pioneered in Europe and ever since, Europe has been at the heart of the global vac-

cine industry playing a key role in the research and development of preventive as well as therapeutic vaccines. During the last twenty years, the extraordinary pace of innovative vaccine development has been the result of new knowledge and technology that has emerged from basic research, e.g. use of recombinant DNA and hybridoma technologies.

How to develop a vaccine: Vaccine development is a complex and time-consuming process that differs from the development of conventional drugs. Vaccine clinical trials focus on demonstrating prevention of a disease which implies that a high number of subjects will be required for trials.

Before a vaccine is licensed and brought to the market, it undergoes a long and rigorous process of research, followed by many years of testing. On average, the period for vaccine development is 12 to 15 years and will involve different phases (pre-clinical trials, phase I clinical trials, phase II clinical trials, phase III clinical trials, phase IV or pharmacovigilance).

The production of a vaccine: The production of a vaccine can be divided in the following steps:

1. generation of the antigen: the first step in order to produce a vaccine is generating the antigen that will trigger the immune response;
2. release and isolation of the antigen: the aim of this second step is to release as much virus or bacteria as possible;
3. purification: the antigen will need to be purified in order to produce a high purity/quality product;
4. addition of other components: such as an adjuvant, which is a material that enhances the recipient's immune response to a supplied antigen;
5. packaging.

The role of pharmaceutical companies: new vaccines in development: The vaccine industry is actively engaged in research and development of new vaccines, including use of live viral vector vaccines, DNA plasmid technology and immunotherapy. A number of vaccines in development target diseases that are difficult to treat, such as HIV/AIDS and other viral diseases (e.g. cytomegalovirus, dengue, ebola, zika); cancer; Alzheimer's disease; rheumatic disorders; bacterial diseases (e.g. Clostridium difficile, chlamydia, E. coli) or parasitic diseases (e.g. malaria, hookworm, leishmaniasis) among others. In addition, several combination vaccines offer practical advantages that can boost vaccine uptake, thereby further improving public health.

MICROBIAL BIOFILMS

BIOFILM-ASSOCIATED INFECTIONS: RESISTANCE TO ANTIBIOTICS AND NEW THERAPEUTIC STRATEGIES

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Introduction: Biofilms are complex microbial sessile communities, embedded in a matrix of extracellular polymeric substances (EPS) and exhibiting an altered phenotype with respect to growth rate and gene transcription as compared to their planktonic counterparts. Biofilms are involved in the large majority of infections of the human body and may form on host tissues or on the surface of a variety of medical devices. Such infections represent, so far, one of the major threats of the modern medicine, as they are difficult to prevent, diagnose and treat. Overall biofilms show a severely reduced susceptibility to conventional antibiotics. Beside the classical resistance mechanisms due to the acquisition of mobile genetic elements, several other biofilm-specific resistant mechanisms have been proposed. Numerous innovative antibiofilm therapeutic strategies are under evaluation. Among them antimicrobial peptides (AMPs) have attracted considerable interest over the last few years.

Materials and Methods: The antibiobilm activity of AMPs with different structures and origins was tested against forming or mature biofilms of medically relevant bacterial species/strains. Such activity was evaluated as biofilm biomass reduction after crystal violet staining or number of biofilm-associated viable cells as compared to non-treated control biofilms. Confocal microscopy imaging combined with fluorescence staining gave information on the biofilm architecture following treatment and on the mechanisms of AMP-antibiofilm action.

Results: While some peptides inhibited biofilm formation by a classical microbicidal effect, others exerted their activity at sub-MIC concentrations suggesting specific anti-biofilm mechanisms of action. Combination of AMPs with compounds able to disaggregate biofilm EPS resulted a successful strategy to target mature biofilms. Optimized AMPs showed activity against dormant cells (the so called "persisters") present at high proportion in biofilms,

and prevented the formation of biofilm-like cell aggregates in artificial media mimicking *in vivo* conditions.
Discussion and Conclusions: The management of biofilm-associated infections is a challenging task and requires a multidisciplinary approach. Several new antibiofilm strategies have shown promise but no efficient treatment has so far been identified for biofilm eradication. Success in this respect is likely to involve a combination of different drugs acting against different targets. A growing number of AMPs have been tested in *in vitro* and *in vivo* models against biofilms with promising results.

PROSTHETIC JOINTS AND BIOFILM RELATED INFECTIONS

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Introduction: Replacement of native joints significantly improves quality of life of millions of patients alleviating pain and allowing recovery of joint function. Although affecting a minority of patients, with an incidence of 1-9% after primary total arthroplasty, prosthetic joint infections (PJIs) require long hospital stay, prolonged antibiotic therapy and additional surgery which heavy impact on the health care system. Diagnosis is complicated by the wide spectrum of manifestations of PJIs, depending on the pathogenicity and virulence of the causative organisms, the host conditions (age, comorbidities etc.), the joint involved, the time of infection.
Materials and Methods: PJIs are typically associated to production of biofilm, a microbial community of one or more species embedded in a self-produced matrix composed of exopolysaccharides, proteins, teichoic acids, lipids and extracellular DNA. Biofilms can be found on hardware components, cement, bone and fibrous tissue, and detached clumps of biofilm have been also recovered in the joint fluid and infected tissues. Formation of biofilm on a prosthetic implants begins with adhesion of bacteria through their surface structures such as pili, fimbriae, flagella, and glycocalyx, mediated by non-specific factors like surface tension, hydrophobicity, electrostatic forces and by quorum sensing, a communication system between bacteria dependent on population density. Biofilms may reach a considerable size, up to a thicknesses of 100 mm, but more importantly, biofilm-embedded bacteria have been shown to be up to 1000 times more resistant to most antimicrobial agents when compared to planktonic cell. Therefore, eradication of biofilm with antibiotic therapy alone is often extremely difficult. On the other hand, it notably complicates isolation of bacteria when conventional sampling and culture methods are used, significantly lowering sensitivity of microbiological analysis. To improve detection of microorganisms responsible of PJIs, in the last decades, physical and chemical methods able to favor microbial detachment from prosthetic implants have been proposed. Particularly, treatment of dithiothreitol (DTT) has proved to significantly enhance sensitivity of prosthetic and tissue culture and, more recently, a device for collection, transport and treatment of samples based on DTT has been developed.
Conclusion: In conclusion, presence of biofilm notably hinder diagnosis and therapy of PJI; in the recent past significantly step-forward have been done introducing specific strategies to hamper biofilm formation on prosthetic implants, to improve microbial detachment from prosthesis and to increase sensitivity of PJIs diagnosis.

MULTISPECIES MICROBIAL BIOFILMS; SYNERGIES AND ANTAGONISMS

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Introduction: The biofilm mode of growth is highly relevant in clinical settings where these microbial communities, displaying higher levels of antimicrobial resistance, cause several chronic infections. Even if the majority of the *in vitro* studies on the ability of biofilms to cause difficult-to-treat infections have been carried out on single-species biofilms, it is now beyond doubt that biofilms are often formed by multiple microbial species. Within multi-species biofilms, different microorganisms cooperate or compete with each other in close proximity, by adopting synergistic or antagonistic behaviors that can enhance the protective nature of biofilms and influence biofilm composition, thus leading to the emergence and/or disappearance of some of them. Some interactions among microorganisms have been defined in oral biofilms, but it remains a poor information of such relationships between microorganisms grown as biofilm on epithelial and mucosal tissues, such as

in the intestinal tract, urinary tract and wounds, as well as on the surfaces of indwelling medical devices. In fact, prostheses and catheters, of increasingly common use in hospitals, represent the most common cause of biofilm-related infections, because of their surfaces that favor the rapid colonization by multidrug resistant bacterial strains able to give rise to biofilm, commonly multi-species. This presentation will deal with the most recent advances in the study of multi-species biofilms, including data recently obtained in our lab.

Methods: Quantitative biofilm production assay, Scanning Electron Microscopy and Confocal Microscopy have been used to evaluate the biofilm production and their ability to interact with one another. An *in vitro* gut model, properly adapted to study multi-species intestinal biofilms, has been also experimented in the Mark Wilcox's lab in Leeds (UK) and preliminary results so far obtained together with Caroline Chilton will be here summarized.

Results and Discussion: Data obtained in our lab on the interaction of pathogens, such as *Clostridium difficile, Klebsiella pneumoniae* and *Porphyromonas gengivalis*, with other species within multi-species biofilm as well as the ability of commensal microorganisms, including Lactobacilli, to antagonistically operate against pathogens will be here reported and discussed . Our results also show that some species are able to synergistically interact while others seem to compete. Of course, a better understanding of the mechanisms underlying multi-species biofilm formation can facilitate the development of methods to counteract the growth of bacterial biofilms.

FUNCTIONAL FOODS, PROBIOTICS AND HEALTH

FUNCTIONAL MICROBES FOR FUNCTIONAL FOODS

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Functional microorganisms and health benefits represent a binomial with great potential for fermented functional foods. The health benefits of fermented functional foods are expressed either directly through the interactions of ingested live microorganisms with the host (probiotic effect) or indirectly as the result of the ingestion of microbial metabolites synthesized during fermentation (biogenic effect). This lecture focuses on the use of bacteria, mainly lactic acid bacteria, for the manufacture of foods having functional features. Some of the accumulated literature data are concerning plant matrices wherein lactic acid bacteria enhance the nutritional and/or functional properties of the raw materials, also representing the unique tool to preserve an overall quality similar to that of the fresh products. This is the case, for instance, of tomato, pomegranate, pineapple and mixtures of fruits. Another important field of application are the cereal by products. In particular, the fermentation of wheat germ and bran allows the synthesis of quinones, biogenic peptides, and amino acid derivatives (e.g., γ-amino butyric acid), which have various physiological activities in humans. Functional microbes are even more used in novel strategies for decreasing phenomenon of food intolerance (e.g., gluten intolerance) and allergy. In particular, a recent biotechnology strategy aimed at fully hydrolyze gluten during food processing by using a portfolio of selected sourdough lactic acid bacteria and food-grade fungal proteases.

PROBIOTICS: USE AND ABUSE

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The internationally endorsed definition of probiotics is "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host". Through the years, other more restrictive definitions have been proposed to include mechanisms and site of action, delivery format, safety, persistence, and physiological health effects. Unfortunately, many products exploit the term probiotic without meeting the requisite criteria and misuse of the term has become a major issue. In addition, inaccurate nomenclature or incomplete taxonomic description of probiotic strains still occur.
In 2013, the Italian Ministry of Health regulated the use of the word probiotic for food and food supplements including a minimum number of viable cells administered per day, a full genetic characterization of the probiotic strain and a demonstrable history of safe use in the Italian market. Thus, our Country consider being a core benefit of probiotics to support a healthy gut microbiota and the International Scientific Association for Probiotics and Prebiotics agreed with this approach. Recently, the European Food Safety Authority updated the scientific requirements for probiotics regarding the health claims related to the immune system, the gastrointestinal tract and the defense against pathogenic microorganisms. For future, health claim applications concerning probiotics should include specific statements on what exactly the microorganism affects and a scientific substantiation of the particular health claim.
In general, probiotics are beneficial in the treatment and prevention of gastrointestinal diseases. The underlying mechanisms responsible for the probiotic effects might be widespread among commonly used probiotic genera (e.g. exclusion of pathogens and normalization of altered microbiota), observed among probiotic species (e.g. vitamin synthesis and enzymatic activity), or restricted to only few strains of a given species (e.g. immunological effects). Most probiotic formulations contain lactic acid bacteria and/or bifidobacteria, but yeast and other bacteria (e.g. streptococci, spores of *Bacillus* species) are also frequently included. An overview of commonly used preparations and our experience with spore-based probiotics will be presented.

THERAPEUTIC ACTIVITY OF A *SACCHAROMYCES CEREVISIAE*-BASED PROBIOTIC AND INACTIVATED WHOLE YEAST ON VAGINAL CANDIDIASIS

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Introduction: Vulvovaginal candidiasis (VVC) is the most prevalent vaginal infection worldwide and *Candida albicans* is its major agent. VVC is characterized by disruption of the vaginal microbiota composition, as happens following large spectrum antibiotic usage. Recent studies support the effectiveness of oral and local supplementation for prevention of recurrent VVC. *Saccharomyces cerevisiae* is a safe yeast used as, or for the production of, ingredients for human nutrition and health.
Materials and Methods: The therapeutic effect of probiotic *Saccharomyces cerevisiae* live yeast (GI) and inactivated whole yeast *Saccharomyces cerevisiae* (IY) was evaluated by using a mouse model of VVC. The capacity of GI and IY to: i) induce *Candida* coaggregation and inhibit germ tube and hyphae formation was evaluated by microscopical examination; ii) inhibit the adherence of bioluminescent (BLI) *C. albicans* strain to epithelial cells was evaluated by detection of BLI signal using a luminometer and by CFU count; iii) affect the expression of secreted aspartyl proteinases (Sap) 2 and 6 *in vitro* and *in vivo* by real time PCR; iv) prevent the damage of epithelial cells induced by *Candida* was measured spectrophotometrically by lactate dehydrogenase release.
Results: Vaginal administration of GI and IY, used as post-challenge therapeutics, were able to positively influence the course of VVC by accelerating the clearance of the fungus. This effect was likely due to multiple interactions of *Saccharomyces cerevisiae* with *Candida albicans*. Both live and inactivated yeasts induced coaggregation of *Candida* and consequently inhibited its adherence to epithelial cells. However, only the probiotic yeast was able to suppress some major virulence factors of *Candida albicans* such as the ability to switch from yeast to mycelial form and the capacity to express several Sap. The protection of epithelial cells to *Candida*-induced damage was also observed.
Discussion and Conclusions: The effectiveness of live yeast was higher than that of inactivated

whole yeast suggesting that the synergy between mechanical effects and biological effects were dominant over purely mechanical effects. Overall, our data show for the first time that Saccharomyces cerevisiae-based ingredients, particularly the living cells, can exert beneficial therapeutic effects on a widespread vaginal mucosal infection.

THE FOOD-HUMAN AXIS: EFFECT OF DIET ON STRUCTURE AND FUNCTION OF THE HUMAN MICROBIOME

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Introduction: The important contribution of the gut microbiome to human health and disease is widely recognized. Habitual diet plays a key role in shaping the gut microbial community composition and sudden dietary changes reflect in a prompt response of the gut symbionts. Mediterranean diet (MD), common in the Western Mediterranean culture, can be considered an omnivore diet characterized by a high consumption of fruit, vegetables and legumes, with well-known positive effect on the health.
Materials and Methods: A cross sectional study was set up in order to investigate the effect of the consumption of different diets on the composition and functions of the human microbiome. Healthy vegetarians, vegans and omnivore volunteers were recruited. Daily food and beverage consumption was recorded and fecal and urinary metabolome were analysed by gas-chromatography mass spectrometry-solid-phase microextraction (GC-MS/SPME) and NMR analyses. The microbial diversity was assessed by pyrosequencing of the V1-V3 region of the 16S rRNA gene. Beyond-genus associations of Bacteroidetes *genera* were investigated by oligotyping of the retrieved 16S rRNA gene sequences.
Results: Specific microbial *genera* were associated to vegetable-based diets, as well as to higher adherence to MD: their microbiota was enriched in Bacteroidetes and some fibre-degrading *Firmicutes*, such as *Lachnospira* and *Roseburia*. On the contrary, Streptococcus was linked to O diet. V and VG, as well as subjects with high adherence level to MD, showed higher concentration of fecal short-chain fatty acids (SCFA). The microbiota associated to the intake of fat and animal products was also linked to urinary trimethylamine oxide (TMAO) levels that were higher in omnivores. Beyond-genus associations of *Prevotella* and *Bacteroides* indicated that some oligotypes were more linked to omnivore diets and relative metabolites while the others were associated with plant-based diets.
Discussion and Conclusions: A high-level adherence to a MD and frequent consumption of plant-based foods promotes the development of some beneficial microbes in the gut with consequential production of anti-inflammatory SCFA. The structure of the microbiota is surely diet-dependent; however, the usual association of Bacteroidetes *genera* with agrarian or animal-based dietary patterns may lead to an oversimplification, while different types within the same genus/species show distinctive correlation patterns with dietary components and metabolome.

FUNCTIONAL AND MOLECULAR DIVERSITY OF SOURDOUGH YEASTS

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Introduction: Sourdough is a mixture of wheat flour and water, fermented by a complex microbial community represented by lactic acid bacteria (LAB) and yeasts and used worldwide in bread production. The richness and diversity of sourdough microbiota, which vary with process technologies, types of flour and local environments, may differentially affect organoleptic and technological traits of bread, improving its texture, flavour and nutritional value and prolonging also its shelf life. The sourdough microbial composition of "Pane Toscano DOP" has been analysed in our laboratory, revealing the occurrence of *Lactobacillus sanfranciscensis* in association with *Candida milleri* and Saccharomyces cerevisiae. Here, we carried out

the molecular typization of 70 yeast isolates, and analysed their functionally important traits, such as phytase, amylase and protease activities.
Material and Methods: A preliminary identification of the best performing molecular technique was carried out on 10 isolates, in order to discriminate the different yeasts at strain level. Yeast DNA was analysed using Restriction Fragment Length Polymorphism analysis of mitochondrial DNA (RFLP mtDNA), mitochondrial COX1 gene introns amplification, inter-delta regions amplification, repetitive sequence Polymerase Chain Reaction (rep-PCR) and Random Amplified Polymorphic DNA (RAPD). The 70 yeast isolates were then characterized by rep-PCR analysis. The functional characterization of isolated yeasts was performed for amylase, phytase and protease activities using qualitatively methods on agar plates.
Results: Among the five different fingerprinting techniques, rep-PCR and inter-δ regions amplification showed the best discrimination performance, being able to differentiate 16 and 2 haplotypes out of the 67 *C. milleri* and 3 S. cerevisiae strains, respectively. The qualitative screening of functional abilities of the 70 isolates showed that the three strains identified as S. cerevisiae exhibited phytase, amylase and protease activities. Among *C. milleri* strains, 50% showed protease activity, while only 6% and 2% were able to solubilize phytate and starch, respectively. Only one *C. milleri* strain showed all the activities tested.
Discussion and Conclusions: Our results showed a wide genetic diversity among *C. milleri* strains, as 16 haplotypes out of 67 were discriminated by rep-PCR. It is interesting to note that strains grouped in the same genotypic cluster revealed a high heterogeneity in amylase, phytase and protease activities.

MICROBIOLOGICAL MANAGEMENT OF PATIENTS TREATED WITH BIOLOGIC DRUGS

THE CLINICAL USE OF BIOLOGICAL AGENTS

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Introduction: The development of biological drugs represents a major breakthrough in the therapy of inflammatory disorders and cancer, offering new opportunities to control the disease process. Among biological agents, anti-cytokine and anti-cytokine receptors antibodies, and especially anti-TNFalpha agents, are the most frequently used.
Materials and Methods: Data are available for rheumatoid arthritis patients treated with biological agents in 49 observational studies addressing the issue of drug safety.
Results: Patients treated with anti-TNF had a higher risk of serious infections (HR 1,1-1,8) as compared with patients treated with the traditional disease-modifying drugs. Compared with the general population, tubercolosis occurs more frequently in treated patients. At variance with these findings, a metanalysis of studies conducted on early rheumatoid arthritis patients showed no increased risk of infections.
Discussion and Conclusions: On the whole, patients treated with anti-TNF are at increased risk of infections, including mycobacterial and fungal infections. The other drugs previously or concomitantly used to control the disease (especially steroids) can significantly affect the risk of infections.

RISK OF VIRAL INFECTIONS IN BIOLOGICAL DRUGS TREATED PATIENTS

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Introduction: Biological drugs have been introduced since the late 1990s for the treatment of different immune-mediated diseases as inflammatory bowel disease, rheumatoid arthritis, multiple sclerosis and psoriasis and also for malignant conditions.
Materials and Methods: Among the most commonly used there are the CD-20 antagonists, such as rituximab, the TNF-alpha antagonists, such as infliximab, adalimumab, and certolizumab, and the alpha4 integrins antagonists, such as natalizumab. In general, although biological drugs are highly effective in lowering inflammation, their efficacy must be weighed against the potential for adverse events. In particular, opportunistic infections, due to latent viruses reactivation, represent one of the main concern during the treatment with monoclonal antibodies targeting the cells of the immune system.
Results: Some concerns arose for the well documented evidence regarding the reactivation of hepatitis B virus and cytomegalovirus with drugs such as rituximab, and of Varicella zoster virus during the anti-TNF therapy. The most significant adverse event for natalizumab-treated patients was shown indeed to be the occurrence of Progressive Multifocal Leukoencephalopathy (PML), a fatal demyelinating disease of the Central Nervous System, due to the lytic replication of the human Polyomavirus JC (JCV) in the oligodendrocytes.
Discussion and Conclusion: Consequently, when initiating biological therapy in patients, it should be better for the patients to be risk-stratified for viral screening. However, to date, there are still no strict guidelines for viral screening to guide the clinicians in the monitoring.

DIAGNOSIS OF TUBERCULOSIS IN PATIENTS REQUIRING BIOLOGIC THERAPY

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Patients requiring biological therapy or other immunosuppressive drugs are at increased risk of tuberculosis (TB) disease and for this reason those with Mycobacterium tuberculosis infection must be subjected to chemotherapy with isoniazid to reduce the risk of disease reactivation. Hence, careful assessment of TB infectious status must be performed before starting these therapies. However, diagnosis of TB infection, which is routinely carried out with the Tuberculin Skin Test (TST) or a commercially available Interferon-γ Release Assay (IGRA), poses a number of technical and clinical challenges that must be managed with an appropriate knowledge. In this presentation, I will present and discuss the most recent advancement in terms of Mycobacterium tuberculosis pathogenesis with a special focus on latent infection and the main features that are important to understand the enhanced risk of developing disease in immunocompromised patients and the immunological basis in the diagnosis of TB infection. The performance of TST and IGRAs in these patients will be discussed and an algorithm for the management of Mtb infection in this class of patients will be presented and discussed.

BIOLOGIC THERAPIES AND THE RISK OF FUNGAL INFECTIONS

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In the last decade, new classes, including monoclonal antibodies, protein kinase inhibitors and other biologic response modifiers, have been developed and marketed for the treatment of haematological malignancies, solid tumours and numerous autoimmune and non-oncological diseases. Infections have been reported among frequent side effects of many of these compounds. It is challenging to determined causality between biologic therapy and infection since in many cases, the underlying disease can cause immunosuppression and/or predispose to certain infectious processed. Additionally, in the case of rare infections, including fungal ones, the

association can rarely be proven definitively due to a small number of events, while large trials have been designed to prove drug's efficacy and not safety.

Among available monoclonal antibodies, alemtuzumab has been repeatedly associated with invasive fungal diseases, including pneumocystosis, while the association has been less compelling with rituximab. It is interesting that patients who receive anti-lymphocytic monoclonal antibodies for a relapsing disease present more frequent, and severe complications compared with front line therapies.

Thyrosine kinase inhibitors have been generally regarded as associated with a very low risk of fungal infections, although different compounds within this drug class seem to have different risk profiles.

In non-oncological setting, drugs which interfere with TNF-alfa activity have been associated with the risk of reactivation of granulomatous infections, including endemic mycoses.

The rapidly evolving field of biologic therapies makes mandatory careful and accurate diagnosis of infectious complications, and appropriate collection of data regarding these events is crucial for precisely estimating the risk of fungal diseases associated with these therapies.

ORAL COMMUNICATIONS

PATHOGENESIS, IMMUNITY AND INNOVATIVE THERAPEUTIC APPROACHES IN VIRAL INFECTIONS

C 001

OXYSTEROLS AS POTENT INHIBITORS OF HUMAN ROTAVIRUS INFECTION: MECHANISM OF ACTION AND PUTATIVE TARGET IDENTIFICATION

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Introduction: Oxidized derivatives of cholesterol, namely oxysterols, are 27 carbon molecules playing a pivotal role in regulating cellular lipid homeostasis. Recently, one member of this family, i.e. 25-hydroxycholesterol (25HC), has been indicated as an important modulator of the innate immune responses against enveloped viruses (e.g. human immunodeficiency virus, hepatitis C virus and ebola virus). 25HC is induced by type I interferon (IFN) signaling in macrophages and displays broad antiviral properties. Despite the growing literature investigating the efficacy of oxysterols against enveloped viruses, the antiviral potential and mechanism of action of 25HC and other oxysterols against non-enveloped viruses has yet to be identified. In the light of these emerging evidences, we tested the antiviral activity of oxysterols against human rotavirus (HRoV), a non-enveloped virus causing severe gastroenteritis in infants.

Materials and methods: In a first set of experiments, we tested the antiviral efficacy of 25HC and of a panel of oxysterols, i.e. 27-hydroxycholesterol (27HC), 7α-hydroxycholesterol (7αHC), 7β-hydroxycholesterol (7βHC) and 7κ-cholesterol (7κC) by focus reduction assay. Oxysterols with the highest selectivity indexes (SIs) were selected and their antiviral activity was further confirmed against several strains of HRoV and by virus yield reduction assay. The step of viral replication inhibited by oxysterol treatment and the putative cellular target of these molecules were investigated by in vitro assays.

Results: 25HC and 27HC can block the infectivity of different HRoV strains at 50% inhibitory concentrations (EC_{50}) in the low micromolar range, and were characterized by SIs above 100. Both oxysterols can block the earliest steps of HRoV infection: while virus-cell attachment is not impaired, virus-cell penetration (i.e. virus endocytosis and HRoV excape from endocytic vescicles) is totally inhibited. Preliminary experiments suggest that the antiviral activity of 25HC and 27HC could be ascribable to their ability to interact with the oxysterol binding protein (OSBP) thus disturbing the recycling of cholesterol between late endosomes and endoplasmic reticulum.

Conclusions: These findings suggest that oxysterols of endogenous origin might be a primary host strategy to counteract HRoV infection. Moreover, 25HC and 27HC could be considered for new therapeutic strategies against HRoV.

C 002

GLUTATHIONYLATION OF VIRAL PROTEINS: A NEW MECHANISM FOR INFLUENZA VIRUS PATHOGENICITY?

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Introduction: Glutathionylation, i.e. formation of mixed disulfides between protein cysteines and glutathione (GSH) cysteine, is a mechanism by which the redox state of the cell can modulate the function of several targets, including proteins involved in inflammation. Using a redox proteomic technique, we identified a set of cellular proteins released in glutathionylated form in inflammation and influenza virus infection. So far, there are no evidences on the possibility for influenza virus proteins to undergo glutathionylation and about the potential relationship of this modification with viral replication and inflammatory response.

Materials and Methods: Eight influenza A virus strains, considered with low and high pathogenicity (LP and HP) from the literature, were selected for a predictive study respect to the possibility for viral proteins to undergo glutathionylation. Such an approach took advantage of multiple aminoacidic sequence alignment, with particular focus on cysteines position and the chemical nature of their flanking regions. In order to verify whether and

which proteins were actually glutathionylated, immunoprecipitation assay of viral proteins followed by Western blot with anti-GSH antibody was performed from both epithelial and macrophage infected cells.
Results: Among the eleven influenza A virus proteins, nuclear export protein (NEP) has no cysteines in its sequence while all of the cysteines in both matrix proteins (M1 and M2), in nucleoprotein (NP) and in polymerase protein PB1 are highly conserved respect position and flanking regions in all the strains analysed. Differences in cysteines content, position and chemical contour of the glycoproteins hemagglutinin (HA), neuraminidase (NA), nonstructural protein NS, proteins PB1 F2, PB2 and PA-X were found. Of particular interest, the cysteine in position 387 of the HA protein codified by the HP strain A/Hong Kong/156/97 H5N1, that is not present in any other strain analysed. Similarly, the cysteine in position 53 in the NA protein seemed a unique feature of the HP strain A/Vietnam/1194/2004 H5N1. The Western blot performed in cell lysates from epithelial and macrophage cell lines infected with Influenza A/Puerto Rico/8/34 H1N1 virus showed that HA, NP and M1 proteins were glutathionylated.
Conclusion: It could be of interest to study these proteins in the selected LP and HP strains to establish a possible correlation of the process with the replication ability of viruses. The study could be useful to characterize the combined role of cellular and viral mechanisms underlying the different outcomes produced by different viral strains in different cells. It could be also useful for the identification of new biological markers to predict the severity of disease.

C 003

EPSTEIN-BARR VIRUS INTERLEUKIN-10 EXPRESSION ENHANCES HUMAN WHARTON'S JELLY-DERIVED MESENCHYMAL STEM CELLS ENGINEERED IMMUNOSUPPRESSIVE PROPERTIES

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Introduction: Herpesviruses express cytokine-like molecules that potently inhibit the immune response during infection. Interleukin-10 (IL-10) is a pivotal cytokine that quenches immune responses, but may also have stimulatory effects depending on environmental conditions. Epstein-Barr virus-derived interleukin-10 (vIL-10) shows extensive homology with hIL-10 and shares immunosuppressive properties with human IL-10, but lacks its immunostimulatory activity. Mesenchymal stem cells (MSCs) are used in clinical transplantation thanks to their low immunogenicity and potent capacity of regulating immune responses. They are considered promising tools for cell therapy of several chronic conditions. We asked whether engineered Wharton Jelly-derived human MSCs (WJ-hMSCs) expressing vIL-10 increased their intrinsic immunomodulatory properties without altering their native features.
Materials and Methods: WJ-hMSCs were transduced with a self-inactivating feline immunodeficiency virus-derived vector co-expressing vIL-10 and herpes simplex virus type-1 thymidine kinase (TK). TK was added to allow future tracking of WJ-hMSC in vivo by positron electron tomography (PET). Their phenotype was then compared to naïve WJ-hMSCs and evaluated to assess their ability to inhibit adaptive and innate cellular responses by ELISPOT and flow cytometry.
Results: The results show that: (i) Expression of vIL-10 and/or TK does not change WJ-hMSC phenotypic and functional properties; (ii) Engineered

WJ-hMSCs are able to inhibit release of both TNFα by activated monocytes and interferon (IFN)γ by activated T cells.
Conclusions: WJ-hMSCs engineered to secrete vIL-10 could be a powerful tool for adoptive cell therapy of immune-mediated diseases, therefore further studies are warranted to confirm their efficacy in suitable animal disease models.

C 004

HERPESVIRUSES AND NK CELLS: CHARACTERIZATION OF KIR2DL2 ANTIVIRAL ACTIVITY

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Introduction: Natural killer (NK) cells are fundamental in the control of viral infections and their activation status is controlled by killer-cell inhibitory receptor (KIR). We recently showed that patients with Multiple Sclerosis (MS) or type 2 diabetes with NK cells expressing the inhibitory KIR2DL2 and its ligand HLA-C1 are more susceptible to herpesvirus (HHV) infections. Here we evaluate the possible role of HHV infection in modulating KIR2DL2 expression.
Materials and Methods: We used an in vitro system with NK92 cell line and NK cells from control subjects and HSV-1, HHV-6A, HHV-6B, EBV infected cells. We evaluated NK cell activation and HHV infection by flow cytometry and Real Time PCR. We evaluated the transcription factor expression pattern by Real Time PCR.
Results: NK92 cells were engineered to switch on and off KIR2DL2 expression by transfection. The turning on of KIR2DL2 resulted in NK cell inability to control HHV (HSV-1, HHV-6A and 6B, EBV) infection, with a 3 log increase in viral production. KIR2DL2+ NK cells from control subjects increased KIR2DL2 expression after co-culture with HHV infected cells, that coincided with an increased expression of two transcription factors, known to up-regulate KIR2DL2 expression (Sp1, Runx). The addition of Sp1 inhibitor (WP631) resulted in the decrease of KIR2DL2 expression, even in the presence of HHV infected cells.
Conclusions: Our results suggest that KIR2DL2 might be an important determinant of susceptibility to HHV infection for all 3 Herpesvirinae (-α, -ß, -ɣ) subfamilies, affecting NK cell activation. Interesting, the presence of HHV infection induces a specific pattern of transcription factors resulting in an increased expression of KIR2DL2 on NK cells. These results could have an important implication in HHV disease pathogenesis.

ORAL COMMUNICATIONS

RECENT DEVELOPMENTS ON ANTIMICROBIAL RESISTANCE

C 005

ANALYSIS OF THE RESISTOME OF THE MICROBIOTA CONTAMINATING HOSPITAL SURFACES: MODULATION BY A SANITIZING PROBIOTIC-BASED INTERVENTION

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Introduction: The growing drug resistance (R) of pathogens is a global concern, thus it is important to set up methods able to detect R onset in a sensitive and fast way. Recently, we were involved in the study of a new approach for sanitization, based on cleansers containing spores of some probiotic *Bacillus* strains,which is now used by several hospitals in Italy and Europe, as it was shown able to induce a drop of several pathogens on hospital surfaces [1]. Here, we wanted to analyze the R features of the microbial population persistently contaminating hospital surfaces, in order to evidence any variation in its resistome upon use of probiotic cleansers. In parallel, we evaluated the genetic stability of the probiotic *Bacilli* contained in the cleansers, and their eventual potential to infect hospitalized patients.
Materials and Methods: Both surface microbial population and cleanser *Bacilli* were analyzed by a real-time PCR microarray, capable of evidencing and quantifying simultaneously 84 different R genes (sensitivity ~ 10 copies/reaction). Each molecularly evidenced R was confirmed by conventional antibiograms. In parallel, all the clinical samples derived from the patients admitted to the hospitals enrolled in the study were analyzed for the presence of *Bacillus* strains, by conventional microbiology or by specific real time PCR.
Results: Results showed that: 1) microarray analyses allow to detect small traces of R genes in a contaminating population, otherwise not detectable; 2) detected R genes correspond to actual drug R when tested by antibiograms; 3) *Bacilli*-based cleansers did not select R species, but conversely induced a strong decrease of R genes in the resistome of the original contaminating population; 4) probiotic-*Bacilli* did not acquire/transfer R genes to surface pathogens, even after prolonged continuous contact on treated surfaces. Furthermore, probiotic *Bacilli* were never detected in biological fluids of patients hosted in the treated areas, and no HAIs attributable to *Bacilli* were detected in 4 years of continuous use of probiotic cleansers.
Conclusions: The results suggest that molecular assays can represent a very fast, precise and reliable tool to monitor the drug R features of a microbial population. By this method, we were able to evidence that probiotic-based sanitation can modulate the resistome of the contaminating population, decreasing the original drug R, without acquiring or transferring new R genes. Also, both molecular and microbiological analyses performed on clinical samples suggest that probiotic *Bacilli* rarely or at all can cause infections, even following continuous contact with subjects at higher risk for adverse events, such as hospitalized patients.

1. Vandini A, et al., Hard surface biocontrol in hospitals using microbial-based cleaning products. PLoS One 2014; 9: p. e108598.

C 006

ANALYSIS IN VITRO OF THE SPECTRUM AND ANTIBIOTIC RESISTANCE OF GRAM-NEGATIVE BACTERIA ISOLATED FROM CATS WITH UTI: RESULTS BASED ON A STUDY FROM A VETERINARY HOSPITAL IN ITALY

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Introduction: The aim of the present study was to examine the antimicrobial susceptibility pattern of 93 Gram negative bacteria (71 Escherichia coli, 8 *Klebsiella* spp., 5 *Pseudomonas aeruginosa*, 5 *Proteus mirabilis* and 4 *Enterobacter cloacae*) isolated from urine samples of cats with urinary tract infections (UTIs) in Italy over a 4-year time period. The

epidemiologic and clinical utility of resistance rates was discussed.
Materials and Methods: An automated antimicrobial susceptibility system was used to detect antimicrobial sensitivity to a panel of 21 antimicrobial drugs and to identify ESBL-*E. coli* and ESBL-*Klebsiella* spp. The presence of beta-lactamases among *Proteus*, *Pseudomonas* and *Enterobacter* isolates was detected through double-disk test. ESBL blaCTX-, bla_{SHV} and bla_{TEM} were determined by PCR amplification.
Results: As concerns first-line antibiotics used for UTI treatment in veterinary medicine (amoxycillin + beta-lactamase-inhibitors, fluoroquinolones, trimethoprim-sulfamethoxazole), percentages of susceptibility > 60% were observed for *E. coli* (amoxicillin-clavulanic acid 76%, ciprofloxacin 63% and trimethoprim-sulfamethoxazole 69%), *Klebsiella* spp. (amoxicillin-clavulanic acid 62%), P. mirabilis (amoxicillin-clavulanic acid 80%), *P. aeruginosa* (ciprofloxacin 80%). Our results outline a high prevalence of MDR strains (63,4%) among Gram negative bacteria responsible of feline UTIs and 27 strains (32% *E. coli* and 50% *Klebsiella* spp.) were found to be ESBL-producers.
Conclusions: All bacterial species isolated in the present study exhibit high level of multidrug resistance; this phenomenon, that in recent years is becoming a serious problem for therapy of feline patients and an important public health issue, can be the result of excessive and incorrect use of antibiotics. About 30% of feline strains were resistant to more than 5 classes of antibiotics, implying serious risks both from a therapeutic point of view and for transmission of these bacteria to humans. Although in Europe increasing rates of *E. coli* resistance to potentiated beta-lactams has been recently documented in cats our data suggest that in Italy the majority of Gram negative strains, except intrinsically resistant species, involved in UTIs are still susceptible to amoxicillin-clavulanate. Therefore, this drug combination still represents an excellent first choice antimicrobial agent. Finally, the high percentage of MDR isolates and the presence of strains resistant to last-line antimicrobials for human found in this study is an outcome should not be underestimated, as bacteria isolated from companion animals may play an important role in the dissemination of antimicrobial resistance to humans, particularly to owners.

C 007

MOLECULAR BASIS OF AZOLE RESISTANCE IN *CANDIDA ORTHOPSILOSIS*

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Introduction: *Candida orthopsilosis* is a human fungal pathogen belonging to the *Candida parapsilosis* complex. This species is able to colonise virtually all body sites and is responsible for a wide spectrum of symptomatic infections in humans, ranging from mucosal to life-threatening mycoses. Although *C. parapsilosis sensu lato* species are not considered particularly prone to develop drug resistance, epidemiological investigations have highlighted a reduced susceptibility to azole and echinocandins, mainly for *C. parapsilosis sensu stricto*. So far, a very limited number of *C. orthopsilosis* fluconazole resistant clinical isolates have been reported. The aim of this study was to investigate the molecular mechanisms of fluconazole resistance in *C. orthopsilosis*.
Materials and Methods: We selected 13 *C. orthopsilosis* clinical isolates characterized by a fluconazole resistant (MIC = 4-64 μg/ml) and in some isolates a reduced susceptibility to itraconazole and voriconazole. Two *C. orthopsilosis* clinical isolates with a fluconazole susceptible phenotype (MIC = 0.125-2 μg/ml) were also included in the study. We evaluated the involvement of efflux multidrug transporters in the development of fluconazole resistance by gene expression analysis as well as the presence of amino acid substitutions in the cytochrome P-450 lanosterol 14alpha-demethylase gene.
Results: The fluconazole target enzyme is encoded in *C. orthopsilosis* by CORT0E05900, an orthologue of C. albicans ERG11. Interestingly, a non-synonymous amino acid substitution was found in the predicted protein of fluconazole resistant *C. orthopsilosis* isolates only. The same polymorphism was previously associated with azole resistance in other Candida species. Transcriptional analysis did not indicate a significant increased expression of demethylase gene in absence of the drug, while preliminary data suggest overexpression of efflux pump genes may also play a role in

azole resistance.
Conclusions: These findings provide the first insight on fluconazole resistance in this species. Considering the lack of information on the natural reservoir of this cryptic species, and its ability to cause superficial as well as disseminated infection, further surveillance for *C. orthopsilosis* isolates harboring these mutations is highly recommended.

C 008

MOLECULAR CHARACTERIZATION OF PLASMID HARBORING MCR-1 GENE CIRCULATING IN ITALY

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Introduction: Polymyxins are old antibiotics that have recently regained popularity for treatment of severe infections caused by extensively-drug-resistant (XDR) Gram-negatives. Acquired resistance to polymyxins is generally associated with chromosomal mutations, but recently a new plasmid-mediated transferable resistance determinant, the mcr-1 gene, encoding a phosphoethanolamine transferase, has been described. The mcr-1 gene, firstly described in China, has been now reported worldwide in Enterobacteriaceae (mostly Escherichia coli) of animal and human origin. To date different mcr-1 plasmids types have been described and belonged to the IncI2, IncHI2, IncP, IncF and IncX4 incompatibility groups. Here we describe the genetic features of the first mcr-1 plasmids reported in Italy.
Materials and Methods: Plasmid transfer was attempted by transformation using E. coli DH10B (F-, recA1, endA1, rpsl, StrR) or by conjugation using E. coli J53 (F-,met, pro, AziR) as recipients and the previously described 8 mcr-1 positive E. coli as donors.The complete sequence of the transferred plasmids was obtained through a 2x250 paired-end approach using the Illumina MiSeq system. Sequence assembly and annotation were performed using SPAdes and RAST, respectively. Sequence alignments and comparisons were performed using Mauve.
Results: Plasmid transfer was achieved from 6 out of 8 strains (five transconjugants and one transformant). Sequenced plasmids belonged only to two incompatibility groups, namely IncX4 (n = 4) and IncHI2 (n = 2) and showed a variable length ranging from 40kb to 230kb. IncX4 plasmids carried mainly mcr-1 as unique antibiotic resistance determinant and in a single case a blaTEM-1 gene was present. Conversely, IncHI2 plasmids carried multiple resistance determinants including blaTEM-1, sul2, tetA and dfrA1 genes. According to the plasmid type, mcr-1 showed different genetic background, being flanked by ISApl1 insertion sequence in IncHI2 plasmids only.
Conclusions: The mcr-1 gene, in Italy, was carried by IncHI2 and IncX4 plasmid types with a structure similar to other plasmids described worldwide, confirming the stability of the genetic support of this transferable mechanism of resistance to colistin.

ORAL COMMUNICATIONS

MOBILE GENETIC ELEMENTS

C 009

GENETIC CHARACTERIZATION OF BLPU-LIKE BACTERIOCIN CASSETTE IN ORAL PROBIOTIC 24 SMBC *S. SALIVARIUS*

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Introduction: Human commensal *Streptococcus salivarius* plays an essential role in the balance of the human oral microbiome, in part controlled by bacteriocin activity finding a wide application as an oral probiotic in the prevention of several upper respiratory tract infections such as otitis media. In this study, we show for the first time the genetic organization of the *blpU*-like bacteriocin cassette in 24SMBc *S. salivarius*, previously characterized by us for its remarkable ability against AOM pathogens and its use as an oral probiotic. The secondary aims were to determine: i) genomic strain target identification, ii) the expression of all *orfs* by Real-time qPCR.
Materials and Methods: The 24SMBc *S. salivarius* genome was sequenced by NGS, obtaining 375 contigs covering 1,893,903 bp; and finding the bacteriocin locus. The preliminary sequence analysis was performed by PRODIGAL and MyRast server. RNA extraction was performed by RNeasy kit (Qiagen) in stationary and exponential phases. RT-qPCR assays were performed. TaqMan primers and probes were designed by Beacon DesignerTM 8.0. The strain target identification was performed by qPCR. Standard curves were generated by dilution series of purified DNA from 24SMBc *S. salivarius* (107–10 genome copies). The cross reaction was evaluated with 20 strains belonging to different streptococcal species.
Results: The *blpU*-like bacteriocin cassette is 8,023 bp inserted adjacent to the *pep*X gene at 1,863,862 bp referred to *S. salivarius* NCTC 8618 genome (GenBank NZ_CP009913.1). The bacteriocin cassette is organized in 11*orfs* of which *orf*1 and *orf2* are involved in the immunity system encoding blpx_2-like and EntA_Immun protein, respectively; *orf*8 encoding *blpU*-like (59 aa), a pore forming peptide belonging to bacteriocin class II showing the consensus and conserved region relative to the *com*C family; and *orf*9-10 encoding ABC transporter involved in peptide export. We found the strain-target sequence between *pep*X and *orf*1 that shows high sensitivity and specificity able to identify and quantify the presence of our strain from other *S. salivarius* strains. The expression of all *orfs* present in the bacteriocin cassette shows their expression in both stationary and exponential phases and only *orf* 1 and 5 appear to be upregulated in the stationary phase.
Conclusion: We describe for the first time the genetic characterization of *blpU*-like bacteriocin region in 24SMBc *S. salivarius*, to date reported only for *S. pneumoniae* and *S. thermophilus*. The *blpU*-like could be involved in bacteriocin production since our strain lacks salivaricins, bacteriocins commonly produced by *S. salivarius*. The identification of the strain specific target distinguishing 24SMBc from other strains could find use in clinical applications.

C 010

A NOVEL METHOD TO SCREEN FOR MACROLIDE EFFLUX PUMP INHIBITION IN *STREPTOCOCCUS PYOGENES*

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Introduction: Antibiotic resistance is a global concern mining the effective treatment of microbial infections. One of the new strategies to overcome resistance is to search for molecules interfering with its molecular mechanism to restore the original susceptibility. In the present study we tried to set up a fast and effective method to screen for the activity of novel efflux pump inhibitors (EPI). We focused on the efflux pump system *MefA-msr*D, which mediates efflux of erythromycin (ERY) in *Streptococcus pyogenes*.
Materials and Methods: One ERY-susceptible *S. pyogenes* strain (a24) and two ERY-resistant strains were used (m46 and a24T). The ERY-resistant strains carried the Φm46.1 element harboring *mefA-msr*D locus. We basically exploited the double disk methodology. Two disks, one containing the antibiotic erythromycin (15 µg/disk) and the oth-

er spotted with the reference (Carbonyl Cyanide m-Chloro-Phenylhydrazone - CCCP) or putative EPI-containing extract were put 15-19 mm apart on a solid medium inoculated with the *S. pyogenes* strain following the EUCAST recommendations. After 16-20 hours at 35-37°C we measured changes in the circularity of the inhibition zone (IZ) around the erythromycin disk. We evaluated the method using three different growth media. Wherever required, the Minimum Inhibitory Concentration (MIC) was determined following EUCAST guidelines. Ethanolic extracts of 15 different plants to be tested for possible EPI activity were prepared following standard protocols.

Results: CCCP induced a reproducible change in the flattening of the IZ around the erythromycin disk (mean f value = 0.04-0.05) in both macrolide resistant strains, namely m46 and a24T. This effect was maximal at the lowest amount of CCCP (0.5 μg), which was below the minimal tested amount of CCCP not affecting the growth (1 μg). The same double disk interference test was applied to plant extracts. Two out of 15 plant ethanol extracts showed an interference with the macrolide efflux phenotype (f = 0.03 ÷ 0.07). Susceptibility analysis was extended by the microdilution method using at sub-inhibitory concentrations of CCCP or plant extracts (1/2, 1/4, and 1/8 of the MIC). CCCP reduced the MIC of erythromycin from 8 to 4 mg/L, while the second plant extract was able to decrease the MIC of erythromycin down to 0.5 ÷ 1 mg/L. Observed effects were dependent upon the growth medium used.

Conclusions: We developed a simple and affordable plate method to screen for macrolide efflux pump inhibiting activity of new lead compounds or complex mixtures. By the application of the method to plant extracts we found two positive records, by which the MIC of erythromycin towards *S. pyogenes* strains expressing *mefA-msr*D mediated resistance was significantly decreased.

C 011

WHOLE GENOME SEQUENCING FOR RESISTOME, VIRULOME AND CLONAL LINEAGE PROFILING CHARACTERIZATION OF CARBAPENEM-RESISTANT *KLEBSIELLA PNEUMONIAE* STRAINS ISOLATED IN SICILY, 2014-2016

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Introduction: Carbapenem-resistant *Klebsiella pneumoniae* (CRKP) strains represent the majority of carbapenem-resistant *Enterobacteriaceae* (CRE) in clinical settings. CRKP diffusion is often sustained by the expansion of successful "high-risk" carbapenemase-producing clones, mostly sequence type 258 (ST258). The most common carbapenems-resistance mechanism identified is the *K. pneumoniae* carbapenemase (KPCs) production largely due to the *blaKPC* gene. In this work, we analyzed by whole genome sequencing (WGS) the resistome, virulome and clonal lineage profiling of 25 CRKP strains isolated at Palermo (Sicily) between 2014-2016.

Materials and Methods: Twenty-five CRKP strains, collected between 2014-2016, from patients admitted in different wards of A.O.U.P "P. Giaccone" in Palermo, were investigated. Paired-end Illumina sequencing was performed on a NextSeq 500 system using the mid output v1 kit with 300 cycles. *In silico* Multilocus sequence typing (MLST), antimicrobial resistance (resistome) and virulence (virulome) profiles were defined using online bioinformatics tools (http://www.pasteur. fr/recherche/genopole/PF8/mlst/Kpneumoniae.html; http://www.genomicepidemiology.org). SNPs discovery was performed using the program kSNP v2.1.2 and the core SNPs based tree was visualized with the

Dendroscope v3.2.10 software.
Results: All the *K. pneumoniae* carbapenemase producers resulted as multi-drug resistant isolates and high resistance frequency was observed for most of the tested antibiotics. The *In silico* resistome analysis revealed the presence of *blaKPC* genes (mostly KPC-3) and less frequently *blaSHV, blaTEM, blaCTX-M, blaOXA* and genes conferring resistance to other antibiotic (aminoglycoside, fluoroquinolone, phenicol, sulphonamide, tetracycline, trimethoprim). The virulome profile revealed the presence of genes encoding for virulence factors, including pili and fimbriae, iron uptake systems and receptors, capsular polysaccharides and lipopolysaccharides. *In silico* MLST revealed 8 different STs: 512 (32%), ST258 (20%), 307 and 395 (12%), 348 and 392 (8%), 405 and 101 (4%). The core SNPs analysis was very coherent with MLST results but SNPs identification allowed us to obtain a higher resolution strains tracking and discrimination.
Conclusions: Our results support the importance of WGS in medical clinic to accurately reconstruct the molecular characterization of isolates, including resistome, virulome and fine resolution of bacterial typing. Our data confirmed that most CRKP isolates belonged to CC258 (ST258 and ST512) and emphasized the circulation of a more complex polyclonal spread among *K. pneumoniae* carbapenemase producers.

C 012

A NEW MOSAIC INTEGRATIVE AND CONJUGATIVE ELEMENT FROM *STREPTOCOCCUS AGALACTIAE* CARRYING RESISTANCE GENES FOR CHLORAMPHENICOL (CATQ) AND MACROLIDES [MEF(I) AND ERM(TR)]

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Introduction: In streptococci, the macrolide resistance gene *mef*(I) and the chloramphenicol resistance gene *catQ*, are typically linked in a genetic structure designated IQ module. Subsequent to the first description of the prototype in *Streptococcus pneumoniae*, other IQ module variants have been detected in *Streptococcus pyogenes* and in different species of viridans group streptococci. The IQ module is carried by integrative and conjugative elements (ICEs) like Tn5253. This is the first description of the IQ module in two clinical isolates of *Streptococcus agalactiae*. Both strains show a new mosaic ICE resulting from the recombination of two previously characterized ICEs.
Materials and Methods: Several molecular methods (PCR mapping, restriction assays, Southern blotting, sequencing and sequence analysis, conjugal transfer assays) were used to determine the genetic context of *catQ* and c haracterise the genetic element in two clinical isolates (Sag236 and Sag403) of *catQ*-positive, chloramphenicol-resistant *S. agalactiae*.
Results: The two isolates shared the same sequence type (ST19) but exhibited a different capsular type (III and V, respectively) and pulsotype. Both harboured the macrolide resistance genes *mef*(I) and *erm*(TR) and the tetracycline resistance gene *tet*(M). Accordingly, they were resistant to chloramphenicol, erythromycin, and tetracycline. *catQ* and *mef*(I) were associated in an IQ module that was undistinguishable in Sag236 and Sag403. In mating assays, chloramphenicol and erythromycin resistance proved transferable, at low frequency, only from Sag236. Transconjugants carried not only *catQ* and *mef*(I), but also *erm*(TR), suggesting a linkage of the three resistance genes in a mobile element which, though seemingly non-mobile, was also detected in Sag403. The new element (designated ICESag236, ~110 kb) results from recombination of two ICEs originally described in different streptococcal species: *S. agalactiae* ICESagTR7, carrying *erm*(TR), and *Streptococcus pneumoniae* ICE*Spn*529IQ, carrying the prototype IQ module.
Discussion and Conclusions: These findings strengthen the notion that widespread streptococcal ICEs may form mosaics that enhance their diversity and spread, broaden their host range, and carry new cargo genes.

C 013

BACTERIOPHAGES: AN ALTERNATIVE STRATEGY FOR BIOFILM CONTROL IN THE POST-ANTIBIOTIC ERA?

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Introduction: Biofilms on both soft tissue and implants are causing persistent infections that are often difficult to eradicate, also due to the fact that microbial cells in biofilm are more resistant to antibiotics than their planktonic counterparts. As a result, new strategies to fight biofilm infections need to be considered. Bacteriophages have emerged as a promising alternative because of their high selectivity and rapid bactericidal activity, which they also exert on multi-resistant bacteria. In this study we investigated the activity of lytic bacteriophages against methicillin resistant *Staphylococcus aureus* (MRSA) in planktonic and biofilm form by taking advantage of the high sensitivity of isothermal microcalorimetry.
Materials and Methods: *S. aureus* specific phage (Sb-1) and Pyo-bacteriophage cocktail, were tested against methicillin-resistant MRSA ATCC 43300 and different clinical strains isolated by prosthetic joint infections. For eradication studies, MRSA was co-incubated for 24h at 37°C in BHI broth (BHIB) with porous glass beads in order to allow biofilm formation. The beads were then washed and, exposed to different titers of bacteriophages. After a second wash, treated beads were placed in sealed ampoules containing BHIB. The heat flow (μW) and total heat (J) were measured in real-time for 48h by isothermal microcalorimetry. In biofilm prevention experiments, MRSA was directly co-incubated with different bacteriophage titers and glass beads for 24h at 37°C, and contextually monitored by microcalorimetry analysis.
Results: Both Sb-1 and Pyo-phage bacteriophages exerted a dose-dependent anti-biofilm activity on MRSA, as attested by the inhibition of the heat production observed within the first 24h. However, MRSA biofilm was eradicated only by co-incubation with the highest phage titer tested (107 PFUs/ml for Sb-1 and 106 PFUs/ml for Pyo-phage). Notably, after a 48h-exposure, also the lowest titers tested resulted in a strong reduction of biofilm viability. By contrast, a significant reduction of MRSA heat production was already achieved at a lower titer (102 PFUs/ml) for both bacteriophages in prevention experiments, and the presence of 104 PFUs/ml completely abolished the heat production. Hence, bacteriophages might interfere with the early stages of MRSA biofilm formation (e.g. adhesion on the glass beads).
Conclusions: Our results showed that Sb-1 and Pyo-phage are able to both eradicate MRSA biofilm *in vitro* and prevent its formation, suggesting that phage therapy may be a promising approach for preventing device colonization and controlling biofilms on surface. Novel strategies for direct coating of biomaterials with bacteriophages and/or local release of phages at the site of the infection might also be further investigated.

C 014

FIRST DETECTION OF THE PLASMID MEDIATED IMI-2 CARBAPENEMASE IN *E. CLOACAE* COMPLEX, ITALY

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Introduction: The spread of carbapenemase-producing *Enterobacteriaceae* (CPE) represents a worldwide threat. Carbapenemases of the class A β-lactamases, mostly represented by bla_{KPC}, are the most frequently detected in many countries including Italy. NMC-A and IMI carbapenemases, quite uncommon in clinical setting, represent a separate lineage from the other class A enzymes showing an extended hydrolytic profile that includes carbapenems. In Italy, only a single case of NMC-A-producing *Enterobacter ludwigii* was described in 2014. In this study, we report on a *E. ludwigii* isolate producing a plasmid-borne IMI-2 β-lactamase.

Materials and Methods: *E. ludwigii* AOUC-07/15 was isolated in July 2015 during a routine screening for colonization by CPE from an Italian inpatient, and was firstly identified by MALDI-TOF mass spectrometry. Antimicrobial susceptibility testing was carried out by reference broth microdilution methods. Plasmid transfer experiments were performed using Escherichia coli DH10B as host, and plasmid size was estimated by S1 nuclease mapping. The isolate was tested with a homebrew multiplex-Real-Time PCR (m-RT-PCR) for the detection of bla_{KPC}, bla_{NDM}, blaOXA-48 and blaVIM genes. Hydrolysis of carbapenems was measured on crude cellular extracts using imipenem as substrate. *E. ludwigii* AOUC-07/15 was finally subjected to whole-genome-sequencing (WGS) using the Illumina MiSeq platform.

Results: *E. ludwigii* AOUC-07/15 was grown on a chromogenic selective media (for CPE) and firstly identified as E. cloacae complex by MALDI-TOF. *E. ludwigii* AOUC-07/15 was resistant to penicillins, aztreonam, carbapenems, but susceptible to expanded-spectrum cephalosporins, aminoglycosides, trimethoprim/sulfamethoxazole and colistin. The isolate tested negative with a m-RT-PCR for the detection of bla_{KPC}, bla_{NDM}, bla_{OXA}-48 and blaVIM genes, but resulted positive for an enzymatic activity assay on imipenem, thus suggesting the presence of a carbapenemase. Carbapenems resistance could be transferred to an E. coli host by electrotransformation. WGS data of AOUC-07/15 allowed the species identification as *E. ludwigii*, and revealed the presence of a bla_{IMI}-2 gene. S1 nuclease mapping on a randomly selected transformant revealed that the carbapenemase gene was located on a plasmid of around 170 kb (pEL-IMI-2) and was embedded in an original genetic context.

Conclusions: This is the first report of an IMI-2 producing *E. ludwigii* in Italy. Current surveillance protocols based on molecular methods such as Real-Time PCR and species identification by MALDI-TOF are not always sufficient for a correct detection and characterization of rare carbapenemase producers. Whole genome sequencing represents a fast and convenient tool for the complete characterization of less common CPE.

ORAL COMMUNICATIONS

MICROORGANISM / HOST INTERACTIONS

C 015

EVALUATION OF INTERFERON LAMBDA 4 NUCLEOTIDE POLYMORPHISM IN RSV-INFECTED INFANTS

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Introduction: The clinical spectrum of respiratory syncytial virus (RSV) -associated bronchiolitis in infants is variable, ranging from a mild disease to a severe respiratory distress causing hospitalization. Genetic heterogeneity of the host in immune response and viral factors may together contribute to disease severity. The importance of Interferon Lambda (IFNL) in the protection against viral respiratory infections is acquired. Recently, ss469415590[ΔG] frameshift variant was described, that creates a novel gene encoding IFNL4 protein; it is more strongly associated with spontaneous Hepatitis C Virus clearance and treatment-induced response than the IFNL3/IL28B single nucleotide polymorphism (SNP) rs12979860. Given the importance of the IFNLs in respiratory infections, we evaluated whether IFNL4 SNP could be associated with bronchiolitis severity.
Materials and Methods: In the course of ongoing studies of respiratory virus molecular characterization, infants admitted for bronchiolitis to the Paediatric Department of Sapienza University Hospital, are tested for 14 respiratory viruses on nasopharyngeal aspirates. RSV positive samples were selected for this study and tested for the presence of the ss469415590 SNP. DNA for the haplotype analysis was obtained from a buccal swab, or from respiratory cells. TT/ΔG genotyping was performed with the "StepOne Real-Time PCR System" method (Express program and Genotyping assay service Applied Biosystem).
Results: The presence of at least one ΔG allele (homo- or heterozygous) was significantly associated with disease severity (score 5-8), and respiratory parameters (but not with length of hospital stay, age or weight and other clinical parameters).
Conclusions: This preliminary data suggest that the course of RSV-infection may be worsened by the presence of IFNL4 protein, but they have to be validated in a larger group of infants affected with bronchiolitis. We previously reported that the IL28B rs12979860 TT allele, that is in linkage disequilibrium with the IFNL4 ΔG allele, was not associated with clinical severity in bronchiolitis cases. That study did not analyze the RSV-infected children separately, because of a smaller number of samples. Further studies are needed also to understand the protective or detrimental effects of IFNL4 production during respiratory virus infections

C 016

AN EMERGING CLUSTER OF CUTANEOUS LEISHMANIASIS IN NORTH-EASTERN ITALY: IS A NOVEL STRAIN CIRCULATING IN THIS AREA?

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Introduction: *Leishmania*sis is a phlebotomine-transmitted infection caused by protozoan belonging to the genus *Leishmania*. A broad spectrum of clinical manifestation, ranging from tegumentary to visceral *Leishmania*sis is recognized, the latter being fatal if untreated. Human *Leishmania*sis may vary from asymptomatic to clinically evident disease, which can remain localized to the skin (cutaneous *Leishmania*sis, CL) or extend to the respiratory mucous membranes or throughout the reticulo-endothelial system (visceral *Leishmania*sis).
Human *Leishmania*sis is on increase in the Mediter-

ranean Europe. Nevertheless, the exact prevalence of cutaneous CL cases is largely unknown as underdiagnosis and underreporting are common. In this study, we evaluated epidemiological, clinicopathological and microbiological aspects of CL cases occurring in the Bologna province, north-eastern Italy.
Materials and Methods: We performed a retrospective study on CL cases diagnosed in the Bologna province between January 2013 and December 2015.
Results: During 2013-2015, 30 cases of CL were identified in the Bologna province with an average incidence of 1.00/100,000, with a 4- up to 12-fold increase as compared to previous years. Sixteen out of 30 (53%) CL cases presented as single, typical lesions. CL diagnosis was carried out by histological and molecular techniques, nevertheless in 6 out of 29 (21%) PCR-positive cases amastigotes were not visible on histology. Molecular identification of *Leishmania* species by *hsp*70-based PCR showed signatures of both *L. infantum* and *L. donovani*. Further molecular evaluation of strain characteristics is ongoing.
Conclusions: We report an increased number of cases of CL in a focal area of north-eastern Italy in 2013-2015. Our study highlights the importance of CL surveillance in the Mediterranean basin and emphasize the need of molecular laboratory surveillance for CL in endemic areas.

C 017

EPIGENETIC DRUG UVI5008 ACTIVITY AGAINST METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS*

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Introduction: Global health emergency is represented by microorganism acquired resistance against old and recent drugs. Last World Health Organization (WHO) epidemiology reports shown that over than 450.000 person die each year due to multi-resistant microorganism1. For example multi-resistant *Staphylococcus aureus* (MRSA) is the responsible of thousands of septic infections and deceases in hospitals throughout the world. Private and public companies are looking for new antibiotics therapies in order to face the bacteria multi-resistant problem. In this scenario we screened a panel of drugs previously characterized as epigenetic modulators. We found several drugs able to modify the Gram-positive bacteria growth. Among them, we selected, Ellagic Acid2 and UVI50083, a derivate of natural compound Psammaplin A.
Methods: In vitro, antibacterial activity of selected compounds was evaluated by broth micro-dilution method. Relative absorbance recorded at 600nm was normalized and T-test statistical filter was applied. The inhibitory activity was evaluated against four pathogenic bacterial strains including Gram-positive (*S. aureus* and *Enterococcus faecalis*) and Gram-negative bacteria (*Escherichia col*i and *Klebsiella pneumonia*).
Results: The UVI5008 results to be active growth inhibitory against Gram-positive bacteria in a specific manner as well as in MRSA. The UVI5008 minimum inhibit concentration MIC in multi-sensible *S. aureus* (MSSA) was registered at 7 μM; meanwhile the minimum bactericidal concentration (MBC) was enriched at 50μM. Synergic effect was evaluated in combination with ampicillin on MRSA. Our results shown a strong synergistic effect on MRSA mediated by the UVI5008 and Ampicillin treatment. In-silico structural studies predicted the potential interaction of UVI5008 with *S. aureus* DNA gyrase protein in a similar manner of Psammaplin A. Finally we used the SEM electro microscopy that shown the structural *S. aureus* modification post stimulation with UVI5008.
Conclusions: Among the tested drugs, UVI5008 could be an alternative approach to fight the MRSA diffusion. Futures studies should implement the UVI5008 anti-bacterial effet and its stability. Detailed studies on the synergic effect with ampicillin should be accomplished.

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C 018

TRANSGENERATIONAL MODIFICATION OF ENDOGENOUS RETROVIRUSES EXPRESSION IN THE VALPROIC ACID-INDUCED MOUSE MODEL OF AUTISM

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Introduction: Endogenous Retroviruses (ERVs) have originated during primate evolution from the ancient infections of germ cells by exogenous retroviruses and are inserted as proviruses in chromosomal DNA. These sequences were wrongly defined as "junk DNA" but nowadays, accumulating evidences support their involvement in many multifactorial diseases such as neurodevelopmental disorders, schizophrenia and autism, resulting by the interaction of genetic and environmental factors. It's known that prenatal exposure to stressful insults contributes to the etiology of offspring neuro-disorders and, recently, the transgenerational transmission of autistic-like traits, probably due to epigenetic mechanisms, has been demonstrated. In our trial we used the valproic acid (VPA)-induced mouse model of autism, with the aim to assess the ERVs and pro-inflammatory cytokines profile expression in the direct descendants of VPA treated mice and in the subsequent generations.
Materials and Methods: We used a CD-1 outbred mice prenatally exposed to VPA. VPA is recognized to increase autism risk in humans and to trigger an autistic-like phenotype in mice. The treatment was done at gestational day 10.5 and the transcriptional activity of several ERVs and pro-inflammatory cytokines in blood and brain samples from the first-, second- and third-generation of VPA-treated mice in comparison to controls was evaluated. Neonatal and adult behavioural assessment was also done.
Results: VPA treatment induces transgenerational transmission of autistic-like behavioural traits and modifies the expression levels of ERVS and pro-inflammatory cytokines in blood and brain samples across generations in comparison to untreated mice, without any further exposure to VPA.
Conclusions: The transgenerational ERVs modification suggests a novel pathological aspect of the early-life adversity in shaping disease across generations. Early epigenetic changes could contribute to the mechanism of VPA-induced neurobehavioral alterations and candidate ERVs as downstream effectors able to target, direct or indirect, pathways involved in the etiology of these complex diseases, such as the inflammatory component.

C 019

PEDIATRIC OBESITY IS ASSOCIATED WITH AN ALTERED GUT MICROBIOTA AND DISCORDANT SHIFTS IN *FIRMICUTES* POPULATIONS

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Introduction: The gut microbiota co-develops with its host from birth and has an important role in gut homeostasis and various diseases such as obesity. Although it has been suggested that the microbiota reaches a relatively stable adult-like state in the first three years of life, other evidence indicates that it continues to develop through adolescence. The goal of the present study was to compare the gut microbiota of obese and normal-weight children with short chain fatty acids production (SCFAs) and body mass index z-scores (BMI z-score) to gain insights into the structure and activity of the microbiota in pediatric obesity.
Materials and Methods: Seventy-eight children (36 males/42 females, 9-16 y) were enrolled in the study at the Pediatric Department of San Paolo Hospital in Milan and grouped in 36 normal-weight and 42 obese. Children's BMI was calculated by reported weight/height2 (kg/m^2) and transformed to age and sex-specific z-scores. Fecal samples were collected, total bacterial DNA were extracted and

SCFAs were quantified by capillary electrophoresis.16S rRNA gene sequencing was performed using Illumina MiSeq platform.
Results: The intestinal microbiota of obese children was enriched in *Firmicutes* (N: 60.9 ± 14.1, O: 72.1 ± 12.1) and depleted in *Bacteroidetes* (N: 30 ± 12.6, O: 16.6 ± 11.8). Accordingly, the *Firmicutes/Bacteroidetes* ratio was significantly elevated in obese children ($p < 0.0001$).We observed significantly higher concentrations of acetate, propionate, and butyrate, as well as total SCFAs, in the stool of obese subjects. Both BMI z-score and SCFAs were significantly correlated with microbiota composition at every taxonomic level (operational taxonomic unit [OTU] to phylum; $p < 0.05$) especially with *Bacteroidetes* and *Firmicutes*. Interestingly the correlation network analysis described an increased correlation density and clustering of OTUs in obese children.
Conclusion: Obesity was associated with an altered gut microbiota, characterized by a more complex correlation structure in the obese gut microbiota. Members of the *Bacteroidetes* were generally better predictors of BMI z-score and obesity than *Firmicutes*, which was likely due to discordant responses of *Firmicutes* OTUs, with some positively and some negatively correlated with BMI z-score. In accordance with these observations, the main metabolites produced by gut bacteria, SCFAs, were significantly higher in obese children, suggesting elevated substrate utilization. Multiple taxa were correlated with SCFA levels and BMIz-score, reinforcing the tight link between the microbiota, SCFAs, and obesity. Our results suggest that gut microbiota dysbiosis and elevated fermentation activity may be involved in the etiology of childhood obesity.

C 020

IMMUNE-ESCAPE MUTATIONS AND STOP-CODONS IN HBSAG CIRCULATING AMONG EUROPEAN PATIENTS WITH CHRONIC HBV INFECTION CAN IMPACT ON HBV TRANSMISSION AND DISEASE PROGRESSION

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Introduction: In HBsAg, immune-escape mutations hamper HBsAg recognition from antibodies, and stop-codons can increase HBV oncogenic potential. Due to HBV genome organization, some drug-resistance RT mutations correspond to immune-escape mutations or stop-codons in HBsAg. No information is available on the circulation of these mutations in patients with chronic HBV- infection (CHB) exposed to nucleos(t)ide analogues (NA) in Europe.

Methods: This study includes 828 patients (255 genotype-A; 573 genotype-D) enrolled from 18 European Countries from 1997 to 2012. Inclusion criteria are: CHB with detectable HBV-DNA, exposure to > 1 NA, availability of a HBsAg sequence. We analyze the immune-associated escape mutations retrieved from http://hbv.geno2pheno.org, and the NA-induced immune-escape mutations I195M, I196S, and E164D (resulting from drug-resistance mutation M204V, M204I, and V173L) (Torresi,2002). Mutations analyzed are those resulting from an amino acid substitution according to the reference sequence of genotype A or D.

Results: Most patients were exposed to LAM (85%) followed by ETV/ADV/TDF (15%). We found > 1 immune-associated escape mutation in 33% of patients with an increasing trend over time (from 21.9% in 1997-2002 to 36.5% in 2009-2012, $p = 0.04$). Gen-D presented a higher number of patients with > 1 immune-associated escape mutation (gen-A: 14.9% vs gen-D: 40.5%, $p < 0.001$). Among them, T118A is present more frequently in gen-D than gen-A (19.7% vs 0.4%, $p \leq 0.001$). Of note, in gen-D, the selection of specific immune-associated escape mutations occurs predominantly during nucleoside- than nucleotide-analogues treatment (A128V:6.4% vs 1.6%, $p = 0.02$; T126S:1.8% vs 0%, $p = 0.001$; T118A: 18.6% vs 8.6%, $p \leq 0.001$). > 1 NA-induced immune-escape mutation occurs in 29% of patients (gen-A:39.6% vs gen-D:23.7%, $p < 0.001$), with a stable temporal trend. Among them, the vaccine-escape pattern I195M+E164D occurs more frequently in gen-A than D (7.1% vs 3.7%, $p = 0.03$). Vaccine-escape mutations occur in 14.9% of patients (gen-A: 7.1% vs gen-D: 18.3, $p \leq 0.001$). Among them, P120S is present more frequently in gen-D than A (5.1% vs 0.8%, $p = 0.003$). Finally, stop-codons are observed in 8.5% of patients (gen-A: 9.8% vs gen-D: 8%) at 20 HBsAg positions including 172 (corresponding to drug-resistance mutation A181T) and 182, known to increase HBV oncogenic potential.

Conclusions: Immune-escape mutations and stop-codons circulate in a relevant proportion of patients exposed to nucleos(t)ide analogues in Europe. Genetic backbone of genotypes and use of specific drugs can influence their emergence. These mutations could favor HBV transmission (potentially including vaccinated persons with inadequate anti-HBs titer) and predispose to a faster progression to liver cancer.

C 021

PERSISTENT HIGH-RISK HPV INFECTION AND VAGINAL MICROBIOTA

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Introduction: Changes in the composition of cervicovaginal microbiota and the modulation of immune-inflammatory environment could favour the colonization of pathogens, including Human Papilloma Virus (HPV). Persistent infection with high-risk (HR)- HPV is cause for the development of cervical cancer. Few data are available about interactions between the cervical microbiota and the clearance or persistence of HPV infection. In order to identify metagenomic markers predictive of HR-HPV persistence, we characterized the vaginal bacterial microbiota from women subjected to screening for HR-HPV and compared the microbiota profiles with respect to HR-HPV status (clearance or persistence), assessed after one year follow-up.
Materials and Methods: We enrolled 70 women (age range 24-64 years) screened for HR-HPV infection with Hybrid Capture 2 (HC2) test at ISPO (Florence Italy). Pyrosequencing of hypervariable V3-V5 region of 16S rDNA gene was performed on bacterial genomic DNA purified from cervicovaginal mucus samples. According with HC2 assay results after one year of diagnosis, we identified three groups: 23 HPV+ women who developed persistent infection (namely HPV+ unhealed-women); 24 HPV+ women who cleared the infection (HPV+ healed-women); 21 control HPV- women. Linear Discriminant analysis, effect size methods (LEfSe) was used to identify enriched taxa in HPV infection and persistence.
Results: Metataxonomic analysis showed differential microbiota profiles between HPV- and HPV+; an increased biodiversity was revealed in HPV+ unhealed-women compared to control and to HPV+ healed women. LEfSe analysis showed enrichment of *Sneathia*, *Megasphera*, *Pedicoccus* and *Pseudomonas* in cervicovaginal mucus of HPV+ women compared to HPV-women. A significant enrichment in *Atopobium* and *Faecalibacterium* was evident in the group of HPV+ unhealed women, compared with HPV+ healed and it was significantly associated with the reduction in *Lactobacillus* sp and increase in *Gardnerella, Prevotella* spp. Stratifying for age range, we observed richness of bacteria belonging to Chloroflexi phylum in women aged 50-64 years, and Lachnospiraceae family in HPV+ women. In women aged 20-49 years, we observed enrichment of Lactobacillaceae, Mycoplasmataceae and Burkholderiales incerte sedis.
Conclusions: The observed differential cervico-vaginal microbiota profiles in women with HPV infection suggest important insight on the role of bacterial vaginal microbiota in HPV infection. Apart the reduction in *Lactobacillus* spp we found that the enrichment in *Sneathia*, *Megasphera*, *Pedicoccus* and *Pseudomonas* may be an overall feature of HPV infection, while the enrichment in *Atopobium* and *Faecalibacterium* is more associated with the persistence of viral infection.

C 022

REGULATORY INTERACTION BETWEEN THE CELLULAR RESTRICTION FACTOR IFI16 AND VIRAL PP65 (PUL83) MODULATES VIRAL GENE EXPRESSION AND IFI16 PROTEIN STABILITY

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Introduction: A key player in the intrinsic resistance against human cytomegalovirus (HCMV) is the interferon-γ-inducible protein 16 (IFI16), which behaves as a viral DNA sensor in the first hours post infection and as a repressor of viral gene transcription in the later stages. Previous studies on HCMV replication demonstrated that IFI16 binds to the viral protein kinase pUL97, undergoes phosphorylation and relocalizes to the cytoplasm of infected cells.

Materials and Methods: To assess the interplay between IFI16 and pUL83 we employed human foreskin fibroblasts (HFF) infected with the wild type HCMV strain (v65Rev), the HCMV v65Stop lacking pUL83 expression, or the HCMV mutant virus (RV-VM1) expressing a pUL83 lacking the nuclear egression signal (NES).

Results: In this study, we demonstrate that the tegument protein pp65 (pUL83) recruits IFI16 to the promoter of the UL54 gene and downregulates viral replication as shown by use of the HCMV mutant v65Stop, which lacks pp65 expression. Interestingly, at late time-points of HCMV infection, IFI16 is stabilized by its interaction with pp65, which stood in contrast to IFI16 degradation, observed in herpes simplex virus (HSV-1)-infected cells. Moreover, we found that its translocation to the cytoplasm, in addition to pUL97, strictly depends on pp65, as demonstrated with the HCMV mutant RV-VM1, which expresses a form of pp65 unable to translocate into the cytoplasm.

Discussion and Conclusions: These data reveal a dual role for pp65: during early infection, it modulates IFI16 activity at the promoter of immediate-early and early genes; subsequently, it delocalizes IFI16 from the nucleus into the cytoplasm, thereby stabilizing and protecting it from degradation. Overall, these data identify a novel activity of the pp65/IFI16 interactome involved in the regulation of UL54 gene expression and IFI16 stability during early and late phases of HCMV replication.

ORAL COMMUNICATIONS

MICROBIAL BIOFILMS

C 023

HELICOBACTER PYLORI BIOFILM DEVELOPMENT AND OUTER MEMBRANE VESICLES (OMVS) PRODUCTION: WHERE IS THE LINK?

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Introduction: *Helicobacter pylori* produces outer membrane vesicles (OMVs) during its growth. Such structures are involved in direct cell-cell binding suggesting that OMVs serve as a potentially novel gastric cell colonization factor of this microorganism. Recently it has also been demonstrated that extracellular DNA (eDNA) is associated with the OMVs surface, suggesting a possible structural role. The aim of the present study was to demonstrate the role of the eDNA associated with OMVs in "bridging" OMV-OMV and OMV-cell interactions as well as in the biofilm development.

Materials and Methods: *H. pylori* ATCC 43629 biofilm formation was evaluated at 2, 6 and 10 days of incubation using live/dead staining and Confocal Laser Scanning Microscopy (CLSM) followed by COMSTAT image analysis. The OMVs production generated from the biofilm and planktonic phenotypes (bOMV & pOMV) was detected using PicoGreen and PKH staining followed by flow cytometry (FACSVerse). The quantification of eDNA over time was assessed using Quant-iT™-PicoGreendsDNA and a NanoDrop2000cUV-VIS spectrophotometer. Finally, an aggregation assay was performed by adding exogenous DNA followed by DNase I digestion and Dynamic Light Scattering (DLS) analysis.

Results: COMSTAT analysis showed no significant changes in thickness, biomass and roughness on each of the days, suggesting the biofilm had reached maturity after 2 days. The biofilm had a well-developed 3D-structure with an equal amount of live and dead (or membrane compromised) cells. Flow cytometry data demonstrated an increase of bOMVs associated with eDNA over time. The number of vesicles associated with eDNA was significantly higher for the biofilm, compared to the planktonic phenotype. Picogreen staining showed the eDNA associated with the isolated bOMVs corroborating the flow cytometry data. The eDNA-OMVs association protected eDNA from enzymatic digestion from DNase I. Moreover the DLS aggregation assay suggested that eDNA may play a role in the aggregation of OMVs in the biofilm phenotype.

Conclusions: The results obtained suggest a key role for eDNA and OMVs in *H. pylori* biofilm formation and highlight the structural role these play.

C 024

MODULATION OF BIOFILM FORMATION BY *STENOTROPHOMONAS MALTOPHILIA* DURING CHRONIC LUNG INFECTION IN CYSTIC FIBROSIS PATIENTS: A LONGITUDINAL POPULATION STUDY

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Introduction: The isolation of the multi-drug resistant *Stenotrophomonas maltophilia* from the lungs of people with cystic fibrosis (CF) is increasing. In the diseased CF lung, pathogens are exposed to a complex range of selection pressures (including host physiological factors, therapeutic antimicro-

bials, and competing microorganisms) that are thought to drive genetic and phenotypic diversity in the pathogen over time. In a series of studies, we found that CF *S. maltophilia* isolates can grow as biofilms - sessile communities inherently resistant to antibiotics and host immune response - not only on abiotic surfaces, but also on CF-derived epithelial monolayer, probably because of a selective adaptation to CF airways. The present study was carried out to understand how biofilm forming ability evolves during chronic colonization of CF lung. For this purpose, temporally isolated strains from several CF patients were comparatively evaluated for biofilm formation ability according to their clonal relatedness. Variations in biofilm formation were also related to growth rate, motility, and *In vivo* virulence.

Materials and Methods: Ninety-three *S. maltophilia* isolates were collected, over a 11-year period, from 10 chronically infected CF patients. A comparative genomic analysis based on Whole Genome Sequencing was performed. Pooled DNA sequencing libraries were 300 bp paired-end sequenced on the Illumina MiSeq platform. Biofilm formation onto polystyrene and growth rate were spectrophotometrically evaluated, whereas bacterial motility (swimming, swarming, and twitching) were assessed using dedicated agar media. *In vivo* virulence was evaluated in a *Galleria mellonella* model of systemic infection.

Results: Genome-wide SNPs analysis revealed 20 sequence types (STs), 11 of which "unknown" for a total of 47 isolates. Strains belonging to 5 STs were shared by at least two patients. Most of strains (91 out of 93, 97.8%) were able to form biofilm, although with striking differences. STs were comparable for biofilm biomass formed. In ST1002 only, biofilm amount was negatively correlated with growth rate, but positively with both swimming and twitching motilities ($p < 0.05$, Spearman r). Biofilm formation was significantly modulated over time towards a direction (increased or decreased) depending on the patient and ST considered. Principal Component Analysis showed a positive association between biofilm biomass formed and virulence as measured in wax moth model.

Conclusions: Our results showed, for the first time, that the highly stressful environment such as CF lung significantly modulates the ability of *S. maltophilia* to form biofilm over chronic infection, depending on allelic profile and lung considered. The improved understanding of the "biological cost" this bacterium has to pay will be helpful to design new therapeutic strategies in the future.

C 025

NEW ANTI-BIOFILM COMPOUNDS FROM DIFFERENT NATURAL SOURCES ACTIVE AGAINST STAPHYLOCOCCI

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Introduction: Implantable medical devices associate with a high risk of microbial infections mainly sustained by Staphylococci due to their strong ability to form biofilm. Antibiotics often fail in eradicating due to the antibiotic resistance of biofilm-growing microorganisms. A viable approach should target adhesive properties of bacterial cells without affecting their vitality in order to avoid escape mutants. Marine bacteria, and in particular Polar bacteria, represent an untapped reservoir of biodiversity able to synthesize a broad range of bioactive compounds, including anti-biofilm molecules. Furthermore, surface coating compounds that prevent bacterial adhesion and biofilm formation are of increased importance. Hydrophobins (HFBs) are small proteins of about 100 amino acid residues, produced by fungi, able to self-assemble into an amphipathic membrane at interfaces such as water–air or solid–water. Recently HFBs emerged as interesting candidates for the prevention of bacterial adhesion and biofilm formation.

Materials and Methods: Anti-biofilm activity of cell-free supernatants from sessile and planktonic cultures of Polar bacteria were tested against the biofilm formation of staphylococci. Biofilm formation was quantified using crystal violet assay and a preliminary physico-chemical characterization was performed. Their activity was evaluated also in dynamic condition by BioFlux system. The anti-biofilm molecule produced by *Pseudoalteromonas haloplanktis* TAC125 was purified using an activity-guided purification strategy with chromatographic techniques. The structural characterization was obtained by NMR and mass spectrometry. Two different HFBs, from the edible fungus *Pleurotus ostreatus* and from a marine microrganism, were

used to pre-coat the wells, then biofilm formation was performed.

Results: Reported results demonstrate that we have selected supernatants containing non-biocidal agents that destabilize biofilm matrix without killing cells. The physico-chemical characterization of the supernatants suggests the presence of molecules of different nature that inhibit biofilm formation (proteins, surfactants or polysaccharides). The anti-biofilm activity of *P. haloplanktis* cell free supernatant is due to hydrophobic molecule that likely works as signal. The reported results demonstrate that the anti-biofilm molecule is a long-chain fatty aldehyde. Both HFBs tested showed good capability in impeding biofilm formation.

Conclusions: Several molecules with different chemical features have been identified as potential anti-biofilm activity. Some of them are also able to impair the initial attachment to the surface, acting as surfactant molecules. They could be combined with a conventional antibiotic to eradicate biofilm infection.

POSTERS

BACTERIOLOGY / MYCOLOGY / PARASITOLOGY

P 001

WHOLE GENOME RE-SEQUENCING AND TRANSCRIPT ANALYSIS OF COLISTIN-RESISTANT *ACINETOBACTER BAUMANNII*

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Introduction: Despite the extensive use of colistin in the treatment of Multi-Drug-Resistant (MDR) Gram-negative infections, colistin resistance is rare in *Acinetobacter baumannii* (Ab) and the mechanisms that underpin this phenomenon are still unclear.

Materials and Methods: As part of this analysis, one Extensively Drug Resistant (XDR) colistin-resistant (COL-R) Turkish strain and two Italian XDR colistin-susceptible/resistant (COL-S/R) strain pairs were studied. The COL-R strains were isolated following the use of colistin during combination-therapy. The kinetic-growth patterns and resistance mechanisms of the strains were monitored using high-throughput-sequence-technologies. Expression analyses were also conducted on these strains.

Results: The analyzed strains were resistant to imipenem (IMP), meropenem (MEM), ampicillin/sulbactam (SAM), ciprofloxacin (CIP), gentamicin (GEN), amikacin (AK), rifampicin (RIF), and trimethoprim/sulfamethoxazole (SXT). Conversely, these strains were susceptible to tigecycline. Relative fitness costs, exerted independently of COL-R acquisition, were observed in all of the XDR organisms. All strains were categorized as PFGE profile A, ST2 (with only one ST187 strain) and OXA-23 producers. Furthermore, a resistome analysis confirmed the presence of the XDR genotype in the analyzed strains. Despite this homogeneity, the generation of an SNP phylogenetic-tree demonstrated there to be genome variability in all strains. Whole Genome Sequencing (WGS) of all COL-R strains showed that the occurrence of *pmrB* SNPs led to different amino acid changes, which resulted in a predicted decrease in PmrB stability. We also observed a transversion event that culminated in trans-membrane domain modification. Interestingly, we did not detect any *lpx*A-D-C SNPs in the analyzed strains. Additional common SNPs were observed among the COL-R isolates. We also observed that a statistically significant constitutive *pmr*BCA over-expression pattern resulted in a greater positive net cell-charge in COL-R isolates compared with COL-S isolates. Moreover, the data demonstrated that down-regulation of lpxA-D-C correlated with low LPS levels. Furthermore, this study is the first to demonstrate proB up-regulation in COL-R isolates.

Discussion and Conclusion: Colistin resistance is a multifactorial phenomenon, and is predominantly caused by: i) increased phosphoethanolamine lipid A modification leading to a reduction in target drug affinity; ii) down-regulation of the lpx cluster (which is colistin-inducible) resulting in reduced drug targeting; and iii) proB up-regulation, in strains presenting a novel glutamate-5-kinase SNP, implicated in proline biosynthesis, osmotolerance and intrinsic COL-R.

P 002

GENOMIC DIVERSITY AND MOLECULAR EPIDEMIOLOGY OF *MYCOBACTERIUM TUBERCULOSIS* STRAINS OF BEIJING GENOTYPE ISOLATED IN TUSCANY, ITALY

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Introduction: The Beijing genotype of *Mycobacterium tuberculosis* (MTB) is cause of global concern as it is rapidly spreading worldwide, is considered hypervirulent, and is most often associated to MDR/XDR tuberculosis, although these properties have not been confirmed for all strains and in all geographic settings. In this study we report the molecular characterization of a collection of Beijing strains isolated in Tuscany, Italy, a region where the ethnic diversity of patients provides an opportunity to study a global sample of Beijing strains and gain new insights into the heterogeneity and phylogeny of the Beijing genotype.

Materials and Methods: A total of 80 MTB Beijing strains isolated in the years 2002-2010 from 18

Italian- and 62 foreign-born patients were studied. Spoligotype patterns, deletions of large genomic sequences RD105, RD181, RD150 and RD142, missense mutations in putative DNA repair genes *mutT*4 and *mutT*2, and MIRU-VNTR profiles, based on 11 discriminative loci, were determined according to standard procedures.
Results: Molecular analysis of MTB Beijing isolates revealed 56 MIRU-VNTR profiles, 6 spoligotype patterns, 3 deletions of large genomic sequences and polymorphic codons in mutT genes. Based on these polymorphisms, a phylogenetic reconstruction of the Beijing lineage was drawn. A minimun spanning tree (MST), constructed by the MIRU-VNTR profiles, showed that strains with deletions RD105 and RD181 and mutated *mutT*4/*mutT*2 genes form a large clonal complex of strains linked together, representing the prevalent expanding strain population and that RD105/RD181-deleted strains with mutated mutT4 and wild-type *mutT*2 form two star-like clonal complexes typical of expanding strain populations. Moreover, MIRU-VNTR analysis revealed 47 unique profiles and 9 clusters including 33 (41%) isolates; active transmission rate of Beijing strains (30.0%) was two-fold higher than non-Beijing strains previously reported in our setting.
Conclusions: Our study shows a considerable genomic heterogeneity of MTB Beijing isolates, yielding both ancient and recent phylogenetic sublineages, and confirms the high transmissibility of Beijing strains also in our setting. Notably, as the prevalent Beijing sublineages harbour missense mutations in one or both putative DNA repair mutT genes, it can be speculated that a defective DNA repair system may confer selective advantages and contribute to the successful spreading of the Beijing family.

P 003

OCCURRENCE OF ESBLS, KPC AND MCR-1 IN GRAM-NEGATIVE MICROORGANISMS FROM OLTREPÒ PAVESE ENVIRONMENT

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Introduction: Water represents a way of dissemination of antibiotic-resistant organisms and antibiotic resistance genes in natural ecosystems. In this study water samples from wells, rivers and treatment plants of the Oltrepò Pavese area were evaluated for the presence of ESBLs, carbapenemases and MCR-1 in Enterobacteriaceae.
Materials and Methods: A total of 246 water samples of which 43,9% from 11 wells, 48% from 5 streams and 8,1% from 4 treatment plants were collected, once a month, during the period 12/01/'14-12/01/'15. A water volume of 100ml and 1ml from wells and streams/treatment plants respectively, were filtered and the 0.45µm membranes were placed on Plate Count Agar, McConkey Agar (MCA) and selective MCA containing 8mg/L of cefotaxime (CTX). Identification and antimicrobial susceptibility profiles were obtained by MicroScan/A4 System (Beckman Coulter) and interpreted according to EUCAST 2015 guidelines. Chromogenic media ESβL-agar (Biolife), double disc (DD) tests, CT103XL microarray (Check-Points), PCR and sequencing were performed. PFGE, PCR-based Replicon typing and conjugation transferability were also accomplished.
Results: 132 (50%) *Enterobacteriaceae*, 49 (19%) *Pseudomonas spp.*, 29 (11%) *Acinetobacter spp*., 18 (7%) *Vibrio fluvialis*, 25 (9%) *Aeromonas hydrophila* and 11 (4%) other Gram-negative were overall identified. On the bases of antibiotic susceptibility tests, all *Yersinia enterocolitica* (100%), 14/22 (81.25%) *Escherichia coli* and 8/11 (63,6%) *Klebsiella spp.* resulted MDR.
A total of 50 *Enterobacteriaceae*: 22 *E. coli*, 13 *Y. enterocolitica*, 11 *Klebsiella spp.*, 2 *Enterobacter spp.* and 2 *Leminorella spp.* were ESBL-pos-

itive by DD test. In 23/50 *Enterobacteriaceae* an ESβL determinant was detected: 15 *E. coli* and 6 *Klebsiella spp.* harboured a *bla*CTX-M-type (blaCTX-M-15/-1/-14 by sequencing), 1 *E. coli* a *bla*TEM-type and a single strain of both species a *bla*SHV-type gene. Worryingly, *bla*KPC-type and mcr-1 genes were identified in 2 different *E. coli* strains from a well (9P; June 2015) and a stream (7T; November 2015), respectively. PFGE analysis on 13 representative ESBL-positive strains (10 E. coli and 3 *K. pneumoniae* isolates) showed clonal diversity within the two species. Interestingly an *E. coli* sampled in January 2015 from a stream resulted clonally related with a community acquired *E. coli* collected 2011in the same area; both were IncF plasmid-CTX-M-1-type positive.

Conclusions: Here we report an alarming occurrence MDR *Enterobacteriaceae* in surface and ground waters of the Pianura Padana area. The presence of ESBL-, KPC-, MCR-1-producers in the environment poses potential risks for human health and highlights the importance to improve both surveillance and remediation of local surface and ground waters.

P 004

IDENTIFICATION, ANTIMICROBIAL RESISTANCE AND MOLECULAR CHARACTERIZATION OF THE HUMAN EMERGING PATHOGEN *STREPTOCOCCUS GALLOLYTICUS* SUBSP PASTEURIANUS

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Introduction: *Streptococcus bovis* group taxonomy has progressively changed and the most common species encountered in human diseases is *S. gallolyticus*, with the subspecies *gallolyticus* (SGSG; formerly biotype I) and pasteurianus (SGSP; formerly biotype II/2). Due to specific disease association, an accurate identification of *S. bovis* isolates is mandatory. This study aimed to retrospectively identify 22 *S. bovis* clinical isolates based on the new taxonomy, as well as to investigate their phenotypic and genotypic resistance profiles and clonal relationship.

Materials and Methods: Identification was performed biochemically by Phoenix100 system, by 16S rRNA gene PCR and sequencing, and by two different MALDI TOF MS platforms, Bruker Biotyper software 3.0 and Vitek MS v2.0 system. Antibiotic susceptibility/resistance was determined by conventional methods and PCR [*erm*(A), *erm*(B), *mef*(A), *tet*(M) and *tet*(O)]. D-tests were performed to determine the phenotypes of macrolide-lincosamide-streptogramin resistance. The clonal relationship was determined by PFGE after *Sma*I-enzyme DNA digestion.

Results: Sixteen *S. bovis* strains were isolated in 14 patients from urine (of which 10 from patients with urinary tract infections), 3 from bile of patients with biliary tract malignancy, 2 from blood of patients with endocarditis and one from a diabetic ulcer. The most common patients' underlying disease was diabetes (31.8%). The automated Phoenix system revealed all isolates belonged to *S. bovis* biotype II; 16S rRNA gene sequencing and MALDI Biotyper properly identified isolates as SGSP. Six isolates were correctly identified to the subspecies level

(SGSP) by MALDI Vitek MS, while the remaining 16 isolates showed identification to the species level (*S. gallolyticus*) with low discrimination at the subspecies level. All isolates were susceptible to penicillin, cefotaxime, vancomycin, meropenem, and chloramphenicol. Erythromycin resistance rate was 31.8%, all erythromycin-resistant isolates being also clindamycin-resistant (phenotype cMLSB) and carrying *erm*(B). Tetracycline resistance rate was 68.2%; most strains carried *tet*(O). All erythromycin-resistant isolates were also tetracycline-resistant. Overall, 17 different PFGE types were identified, of which 14 were unique types.
Conclusions: Both MALDI TOF systems correctly identified the isolates to the species level (*S. gallolyticus*), although only MALDI Biotyper accurately identified all isolates to the subspecies level (SGSP). SGSP was most frequently associated with urinary tract infections. An association between SGSP infection/colonization and diabetes as underlying disease was detected. High erythromycin and tetracycline resistance rates were observed. Finally, SGSP isolates showed a high genetic variability.

P 005

ANTI-BIOFILM ACTIVITY OF A LONG-CHAIN FATTY ALDEHYDE FROM ANTARCTIC *PSEUDOALTEROMONAS HALOPLANKTIS* TAC125 AGAINST *STAPHYLOCOCCUS EPIDERMIDIS* BIOFILM

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Introduction: *Staphylococcus epidermidis* is a harmless human skin colonizer responsible for approximately 20% of orthopaedic device-related infections. This latter is due to its capability to form biofilms. Nowadays there is an interest it developing of anti-biofilm molecules. In this field the interest in the development of new approaches for the prevention and treatment of bacterial adhesion and biofilm formation has increased. A possible approach should target adhesive properties without affecting bacterial viability in order to avoid the rapid appearance of escape mutants. Marine bacteria represent an untapped reservoir of biodiversity able to synthesize a broad range of bioactive compounds, including anti-biofilm molecules. Previously reported results showed that *S. epidermidis* biofilm is impaired by the supernatant of *Antarctic Pseudoalteromonas haloplanktis* TAC125. The anti-biofilm activity of P. haloplanktis cell free supernatant seems to be due to hydrophobic molecule that likely works as signal. In this paper we purified and characterised the anti-biofilm molecule strengthening our hypothesis on its mode of action.
Materials and Methods: The anti-biofilm molecule purification protocol was developed using an activity-guided purification strategy. Classical chromatographic techniques like gel-filtration LC and HPLC were applied to purify the anti-biofilm compound. The structural characterization of the molecule was obtained by NMR and mass spectrometry. The anti-biofilm activity was evaluated using either static or dynamic biofilm assay by Bioflux. *Vibrio harveyi* reporter strain for the detection of autoinducers AI2-like, was used to investigate the anti-biofilm molecule mode of action.
Results: The reported results demonstrated that the anti-biofilm molecule is a long-chain fatty aldehyde. The specificity of its activity on *S. epidermidis* biofilm was demonstrated testing chemical analogues differing in length of aliphatic chain and in functional group properties. The aldehyde works as an AI2 signal. Moreover, the aldehyde metabolic role in Antarctic source strain was investigated.
Conclusions: *P. haloplanktis* TAC125 produces an a long-chain fatty aldehyde active against *S. epidermidis* biofilm. The aldehyde works as an AI2 signal. This is the first report on action of a long-chain fatty aldehyde as anti-biofilm molecule acting through AI2-mediated quorum sensing system.

P 006

THE SEMI-SYNTHETIC ANTIMICROBIAL PEPTIDE LIN-SB056-1, IN COMBINATION WITH EDTA, KILLS *PSEUDOMONAS AERUGINOSA* AND PREVENT BIOFILM FORMATION IN EXPERIMENTAL CONDITIONS RESEMBLING THE SPUTUM OF CYSTIC FIBROSIS PATIENTS

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Introduction: Chronic pulmonary infections sustained by *Pseudomonas aeruginosa* represent the most common cause of death in patients suffering from cystic fibrosis (CF). Eradication of *P. aeruginosa* from CF lung is particularly challenging due to the ability of the bacterium to form biofilms, bacterial communities highly recalcitrant to antimicrobial treatments and host immune system, and to the production and accumulation of a thick and stagnant mucus within the alveoli that favors bacterial colonization and persistence. It has been reported that in chronically infected lungs *P. aeruginosa* forms non-attached cell aggregates, referred to as biofilm-like structures (BLS), that float in the viscous airway mucus. The present study investigates the bactericidal and antibiofilm activity of lin-SB056-1, a recently optimized semi-synthetic antimicrobial peptide (AMP), in experimental conditions resembling the complex environment found in CF lungs.
Methods: The antibacterial activity of lin-SB056-1, used alone and in combination with disodium-EDTA, was investigated against *P. aeruginosa* planktonic cells and BLS in an artificial sputum medium (ASM) containing, among other components, high concentrations of DNA, mucin and salts. The bactericidal activity against planktonic cells was evaluated as reduction in CFU number as compared to the inoculum. The antibiofilm activity was evaluated in terms of BLS number and dimensions through optical microscopy and/or number of BLS-associated viable cells.
Results: When assayed alone, the peptide was highly inhibited in ASM. In contrast, it showed a striking ability to kill *P. aeruginosa* planktonic cells at relatively low concentrations (10 μM), when used in combination with host-compatible concentrations of disodium-EDTA (1.25 mM). Interestingly, the lin-SB056-1/EDTA combination completely inhibited the formation of *P. aeruginosa* BLS at non-haemolytic concentrations. In addition, the combination was able to reduce to a certain extent the number of BLS-associated viable bacteria.
Conclusions: The combination of lin-SB056-1 with EDTA displayed a marked bactericidal and antibiofilm activity against *P. aeruginosa* in an artificial medium mimicking the mucus found in CF lung during the chronic stage of the infection. Overall, these results highlight the potential of the lin-SB056-1/EDTA combination for the treatment of *P. aeruginosa* pulmonary infections in CF patients.

P 007

DEVELOPMENT OF A NOVEL METHOD TO GENERATE "PERSISTER CELLS" OF MEDICALLY RELEVANT BACTERIA AND TO EVALUATE THEIR SUSCEPTIBILITY TO ANTIMICROBIAL AGENTS

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Introduction: Bacterial "persister cells" (PC) are defined as non-growing, dormant, phenotypic variants of regular cells with high tolerance towards antibiotics. Generation of PC *in vivo* may account for the recalcitrance of most chronic infections to antimicrobial treatment and demands for the identification of new antimicrobial agents able to target such cells. Traditionally, PC are selected *in vitro* by exposing stationary phase cultures to high concentrations of antibiotics (up to 100 x MIC) that kill metabolically active bacteria, but are ineffec-

tive against PC. Aim of this study was to develop a method to generate *in vitro* populations of PC at high efficiency and with low proportion of dead cells, and to test their susceptibility to structurally different antimicrobial peptides (AMPs). Indeed, the main mechanism of action of these molecules (i.e. membrane-perturbing activity) render them potential candidates to act against dormant cells.
Methods: The condition of persistence in bacteria is due to a disturbance of the membrane proton gradient that leads to a lower level of ATP generation. To dissipate the proton-motive force and generate PC, we used the membrane uncoupler carbonyl cyanide 3-chlorophenylhydrazone (CCCP). Briefly, stationary cultures of Staphylococcus aureus ATCC 33591 and *Pseudomonas aeruginosa* ATCC 27853 were exposed to CCCP for 3h. To verify the condition of persistence, bacterial suspensions were exposed to different antibiotics at 10 x MIC concentration and the number of CFU was compared with that of CCCP un-treated cultures. Activity of AMPs against PC was evaluated exposing CCCP-pre-treated bacteria to three synthetic AMPs characterized by different structures and origins: i) TB-L1FK, an α-helix peptide analogue of the frog skin-derived temporin 1Tb; ii) C5, a human β-defensin 2 and β-defensin 3 chimeric β-sheet peptide; iii) SB056, a semi-synthetic peptide with a dimeric dendrimer scaffold.
Results: Exposure of bacterial cultures to optimized concentrations of CCCP was able to generate PC at high efficiency without causing significant killing effect. CCCP pre-treated bacteria of both bacterial species resulted resistant to antibiotics acting on different cellular targets such as ciprofloxacin, rifampin or meropenem. In contrast, PC generated by CCCP treatment resulted sensitive to all the three AMPs tested at levels comparable to those of CCCP un-treated bacteria.
Conclusions: CCCP treatment is a suitable method to generate *in vitro* PC of medically important bacterial species at high efficiency. Importantly, unlike many conventional antibiotics, structurally different AMPs were able to kill PC highlighting that such molecules might represent valid templates for the development of new antimicrobials active against persisters.

P 008

DIAGNOSTIC ACCURACY OF XPERT MTB/RIF VERSUS SMEAR MICROSCOPY IN THE EARLY DIAGNOSIS OF TUBERCULOSIS IN THE REAL LIFE OF THE "UMBERTO I" HOSPITAL IN ROME

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Introduction: Early diagnosis of tuberculosis (TB) is one of the primary challenges in curtailing the spread of TB. The aim was to evaluate in the real life of the "Umberto I" Hospital (Rome), the diagnostic accuracy of Xpert MTB/RIF for the identification of *Mycobacterium tuberculosis* (MT) in clinical specimens, and compare this to routinely diagnostic methods.
Materials and Methods: For this study, 433 patients with suspected TB were assessed prospectively with three protocol-specified tests (smear microscopy, culture and Xpert MTB/RIF) specimens of individuals with suspected TB between September 2013 and June 2015.
Results: Overall, by culture testing, 58 of the 433 samples (13.4%) were positive for MT, 15 (3.46%) were positive for growth of non-tuberculosis mycobacteria, 354 (81.7%) were negative for any growth, and 6 (1.38%) were contaminated and excluded from the study. By smear microscopy testing, 46 of the 433 samples (10.6%) were acid fast bacilli positive and 387 (89.3%) were negative. In particular, smear microscopy was positive only in 37 of 58 specimens (63.7%) that were positive for MT growth, and in 9 of 15 specimens (60%) that had growth of a mycobacterium determined not to be MT. By Xpert MTB/RIF testing, 58 of the 433 samples (13.4%) were positive for MT and 369 (85.2%) were negative.
Conclusions: Xpert MTB/RIF was positive in all specimens with culture-confirmed TB, giving a higher sensitivity than the smear microscopy (100% versus 63%). The use of the Xpert MTB/RIF, as part of routine assay, permits rapid diagnosis of TB and enables clinicians to start an effective treatment.

P 009

APPLICATION OF WHOLE-GENOME ENRICHMENT AND NEXT GENERATION SEQUENCING FOR DIRECT DETECTION AND GENOTYPING OF *VIBRIO CHOLERAE* IN FRESHWATER

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Introduction: As a response to unfavorable growth conditions *V. cholerae* is often present in the aquatic environment in a VBNC state thus being no longer detectable by culture-dependent methods. Furthermore, in environmental samples, *V. cholerae* cells might be present at very low abundance and this may also hamper their detection by PCR or shotgun metagenomic techniques. To overcome these issues, we applied for the first time a whole-genome enrichment (WGE) and next generation sequencing approach for the direct detection and genotyping of low abundance *V. cholerae* cells (< 10 cell/L) from a natural water sample collected in the Morogoro river (Tanzania), a cholera endemic area since 1976 with cases reported each year. The applied protocol is based on the use of biotinylated RNA baits for the target enrichment of *V. cholerae* genomes from complex DNA samples.
Materials and Methods: A water sample (U3D8) was collected from Morogoro river at urban location. A synthetic Mock community sample was also prepared by mixing equal DNA concentration (100 µg/ml) of 8 different strains (*V. cholerae* ATCC 39315, E. coli MG1655, *S. typhirium*, *P. fluorescens* VET67, *K. pneumoniae* E12, *S. marcescens* E19, *P. stuartii* NE35, *C. freundii* NE111). DNA from U3D8 and Mock samples were enzymatically fragmented and used for the production of an indexed libraries for next-generation sequencing on the Illumina platform (Illumina, Inc). About 200 ng of the produced library were used for *V. cholerae* DNA target capturing using MYbaits protocol (MYcroarray, Ann Arbor, MI, USA). Post-capture PCR amplification was finally carried out. Sequencing was performed on a MiSeq Illumina™ platform.
Results: The success of the enrichment was evident for the Mock sample where a total of 1.70x109bp out of 1.74x109bp mapped against the *V. cholerae* N16961 reference genome. In the Morogoro sample a total of 2.2X107bp (around 5.6x mean read depth) mapped against a *V. cholerae* N16961 reference genome with > 99% coverage. Search against the genome-specific markers (GSMs) database suggests that a single strain is present in the sample whose genotype is phylogenetically closed to *V. cholerae* environmental strains TM 11079-80 and VL426. Mapping of the reads against selected genomic regions showed that the strain lacks the two major virulence-related genomic islands, i.e., CTX prophage and Vibrio pathogenicity island-1, whilst possess the MARTX gene region encoding for the RTX toxins.
Conclusions: This study provides a proof of concept for the use of WGE for direct genotyping of V. cholerae in natural water sample which can be highly relevant to the study of cholera epidemiology.

P 010

ANTIBIOTIC CONSUMPTION AND ANTIMICROBIAL RESISTANCE IN MICROORGANISM OF NOSOCOMIAL INTEREST: A SNAPSHOT OF A NEUROMUSCULAR REHABILITATION CENTER

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Introduction: Antibiotic resistance is a serious threat to public health above all in Europe. Particularly, Italy is placed among the first European countries with the highest rate of antibiotic-resistant microorganisms and with the highest consumption of antibiotics for systemic use. The aim of the study was to take a snapshot of the situation related to incidence of antimicrobial resistant microorganisms and to systemic antimicrobial consumption in an Italian rehabilitation center during a two-year period (2014-2015) in order to provide an internal and external benchmark.

Materials And Methods: Data about microorganisms isolated from patients admitted to neurorehabilitation hospital were retrospectively obtained from the diagnostic laboratory of the hospital. Microorganisms identification and antimicrobial susceptibility testing were performed using the automatic system Vitek®2 Compact (Biomerieux). For each identified species it has been calculated: the isolation density, the antibiotic resistance rates, and the incidence density of resistant isolates per 1000 patient-days (IDRI). Data on antimicrobial consumption were obtained from the hospital pharmacy on six-monthly basis. We have expressed antibiotic consumption as Defined Daily Dose (DDD) per 1000 patient-days to conform us to the use of the ATC/DDD system, recognised by WHO as an international standard for drug utilization studies since 1996.
Results: The most frequently isolated microorganism was Klebsiella pneumoniae (19.3%), followed by Proteus mirabilis (18.2%) and Escherichia coli (9.5%).Particularly we observed a significant increasing trend for piperacillin/tazobactam-resistant *K. pneumoniae* from 1.47 to 3.32 in 2015 (p-value = 0.04). Among all antimicrobials used, carbapenems have always been the most prescribed antibiotic class (31% of the total antimicrobial usage density), followed by penicillins (13%), fluoroquinolones (11.3%), glycopeptides (10.6%), azoles (8.3%) and colistin (8.2%). Particularly, the consumption of piperacillin/tazobactam and teicoplanin were significantly increased (p-value = 0.01 and p-value = 0.02, respectively).
Conclusion: The increased IDRI to carbapenems reflects the dramatic situation in the Mediterranean area, in which the strains of Enterobacteriaceae have spread in endemic form. By DDD comparison, our data seem to have, in all classes of antibiotics, lower values than the intensive care units, (except for glycopeptides), and higher values than long-term care facilities. Results suggest the need for a continuous monitoring of antimicrobial consumption and bacterial resistance trend, since the adaptability of bacteria accelerates while searching for new molecules is currently stopped.

P 011

THE SECRETED *VIBRIO CHOLERAE* COLONIZATION FACTOR GBPA PROMOTES BINDING TO SURFACES OF DIFFERENT VIBRIOS

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Introduction: N-acetyl glucosamine (GlcNAc)-binding protein A (GbpA) is involved in V. cholerae O1 attachment to chitin and adhesion to cultured intestinal epithelial cells, thus providing a direct link between *V. cholerae* environmental lifestyle and human infection. GbpA is present in the outer membrane of the bacteria and is secreted in the extracellular medium. In this work, we provided more insights into the mechanism involved in the capability of secreted GbpA to promote interactions between V. cholerae bacteria and GlcNAc containing substrates (chitin particles and human intestinal cells).
Materials and Methods: Wild type *V. cholerae* strains, *V. cholerae* ΔgbpA and TnphoA mutants, as well as adhesion assays to chitin beads and CaCo2 cultured cells are described in Zampini et al. (2005) and Stauder et al. (2012). His-tagged GbpA protein (rGbpA) was produced in E. coli and purified as described by Stauder et al. (2012). Binding of rGbpA to bacterial surface was visualized by immunofluorescence with anti-histidine Tag (D3L10) XP rabbit monoclonal antibodies conjugated to Alexa Fluor 488 fluorescent dye; images were acquired using a Leica TCS SP5 AOBS confocal laser scanning microscope.
Results. Capability of secreted rGbpA to act as a bridge between bacteria and chitin particles (or CaCo2 cultured cells) was verified using a *V. cholerae* N16961 deletion mutant that does not produce the adhesin. When the purified rGbpA was added to the adhesion mixture, ΔgbpA strain adhesion efficiency increased by about 5 fold; similar results were obtained when rGbpA was used to pre-treat either bacteria or the substrate. Capability of rGbpA to bind *V. cholerae* strains of different biotypes and serotypes and *V. cholerae* O1 TnphoA mutants was investigated by immunofluorescence. The results showed that rGbpA interacts with O1 (El Tor and classical biotypes) and non O1/O139 bacteria, and

all but one TnphoA mutants. This suggests that a membrane spanning protein, and not the O-specific polysaccharide, is likely involved in V. cholerae receptor for rGbpA. This was also supported by the observation that pronase E treatment of *V. cholerae* N16961 and its ΔgbpA derivative drastically decreased rGbpA binding to bacteria. Interestingly, it was also found that rGbpA binds the surface of other vibrios (*V. tasmaniensis*, *V. celticus*) and promotes their interactions with chitin beads.
Conclusion: These results show that *V. cholerae* receptor for GbpA is present regardless of the ability to synthesize the protein, and includes protein components. Our results also show that GbpA mediates adhesion to chitin beads of bacteria different from V. cholerae thus exhibiting a general role in shaping the microbial community associated to environmental surfaces.

P 012
ANTIMICROBIAL ACTIVITY OF TWO FOOD-INTAKE REGULATORY NEUROPEPTIDES: GHRELIN AND OREXIN B

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Introduction: Ghrelin is a 28-amino acid peptide expressed mainly in the stomach, pancreas and also present in plasma and saliva. It binds to the growth hormone receptor (GHS-R1a) in pituitary cells, inducing the secretion of growth hormone and an appetite-stimulating effect. Orexins are hypothalamic neuropeptides that have been implicated in the regulation of feeding. They are cleaved from a common precursor molecule, preopro-orexin, forming orexin A (33 amino-acids) and orexin B (28 amino-acids). Interestingly, the chemical properties of orexin B and ghrelin, such as amino-acid composition, net charge and isoelectric point, are very similar to those of vasoactive intestinal polypeptide (VIP) reported to have antimicrobial activities. Since antimicrobial action of a peptide can be predicted by its chemical properties, we hypothesized that the two neuropeptides may function as anti-infective molecules.
Materials and Methods: In the present work we have analysed their antibacterial activity against Gram-negative (Escherichia coli and Salmonella *Typhimurium*) and Gram-positive (*Staphylococcus aureus*) ATCC bacteria using the microdiluition method. In particular we have used a range of concentrations between 100 and 0.78 μM. Moreover, we have also evaluated the cytotoxicity of the two neuropeptides. We also evaluated the antiviral activity of these compounds in a co-treatment assay.
Results: Ghrelin and orexin B have a strong antibacterial activity against Gram-negative bacteria with a minimal inhibitory concentration at 25 μg/ml, while the effects against Gram-positive *S. aureus* are absent. They don't show toxicity effects until highest concentrations tested. On the other hand, orexin B shows a high antiviral activity at the concentration of 50 μg/ml.
Conclusions: We demonstrated that ghrelin and orexin B possess similar antibacterial activity against Gram-negative, while only orexin B shows a high antiviral effect in a co-treatment assay . In future investigations, we will aim to elucidate its biological role in the context of innate immune responses.

P 013
MALDI-TOF MS VS VITEK2: COMPARISON OF SYSTEMS FOR THE IDENTIFICATION OF MICROORGANISMS RESPONSIBLE OF BACTEREMIA

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Introduction: To date, bloodstream infection is still a major cause of mortality and morbidity and it is a medical emergency. Patients outcomes are improved by rapid identification of the responsible microorganisms since the timely initiation of antimicrobial therapy is crucial for patients prognosis and it is associated with higher survival rates and decreased healthcare costs.
Materials and Methods: We evaluated the reli-

ability and accuracy of the combined use of MALDI-TOF MS and classical ID VITEK2 to identify mono-microbial infection in blood culture bottles. In total, 70 consecutive positive blood cultures were included in this study. Positive blood culture bottles were subjected to Gram staining and sub-cultured on solid media. Isolates grown from such culture media were used for classical ID using VITEK2 System. In parallel, an aliquot was subjected to a Lysing-Centrifugation Method (LCM) and used for the identification with the MALDI-TOF System.
Results: Results evidenced the correct genus and species identification of 91.4% of microorganisms responsible of the bacteremia with an agreement to the species and the genus level. If compared with the standard method Vitek2, our simple and cost-effective sample preparation method would be very useful for rapid identification of microorganisms using blood culture bottles. In fact, the direct method showed rapid and reliable results, especially for the gram-negative group.
Conclusions: In summary, our simple and cost-effective sample preparation method would be very useful for rapid identification of microorganisms from positive blood culture bottles. However, for identification of gram positive, further improvement in the method is needed.

P 014

ANTIMICROBIAL ACTIVITY OF METHANOLIC EXTRACT FROM MICROALGA *GALDIERIA SULPHURARIA*

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Introduction: antibiotic resistance in bacteria is one of the major emerging health care related problems, it is becoming a continuously problem raising the treatment against resistant pathogenic bacteria. One approach to overcome antibiotic resistance is the discovery of novel antimicrobial compounds for clinical applications. Algal organisms are a rich source of biological active secondary and primary metabolites which may be bioactive compounds of interest in the pharmaceutical industry. Several biogenic compounds belonging to different classes of microalgae have been identified over the last few decades and their pharmacological activities against Gram-negative and Gram-positive bacteria have been studied in details. In this study a methanolic extract from the thermophyla microalga *Galdieria sulphuraria* was screened against *Escherichia coli* ATCC, Staphylococcus aureus ATCC, Methicillin - Resistant *S. aureus* (MRSA) and Macrolid - Resistant *S. aureus.*
Materials and Methods: *G. sulphuraria* strain was gently provided from the Biological Science Department of Federico II University of Naples. The strain was cultured in Allen medium, both in autotrophy and heterotrophy condition, maintaining the pH at 1,5 and the temperature at 37°C. The cultures growth was monitored by measuring their optical density at 550 nm and the ammonium and glycerol levels. Afterward, the methanolic extract was tested against Gram-positive and Gram-negative bacteria, using the microdiluition method; in particular we have used a range of concentrations between 12.5 µg/µl to 0.39 µg/µl (5 different concentrations). Likewise, the antiviral activity was assayed against HSV-1 using a co-treatment assay at the same concentrations as above. *In vitro* cytotoxicity assays (MTT) have been also performed.
Results: the methanolic extract of *G. sulphuraria* has a strong antimicrobial activity against Gram-positive bacteria using minimal inhibitory concentrations, while the effects against Gram-negatives are minimal. The extract does not show toxicity effects until highest concentrations tested; instead the antiviral activity has revealed that the extract was not so interesting as an antiviral agent.
Conclusions: we demonstrated the strong effect of methanolic extract of *G. sulphuraria* on Gram-positive bacteria. Future investigations will aim at elucidation of the mechanism responsible for the inhibition of bacterial growth and to study the effects as antifungal of *G. sulphuraria* extracts.

P 015

EMERGENCE OF KPC-PRODUCING *CITROBACTER FREUNDII* IN AN ITALIAN HOSPITAL

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Introduction: The rapid identification of patients colonized or infected with carbapenemase-producing enterobacteria (CPE) is recognized as a basic tool to control and prevent the global spread of these multi-drug resistant (MDR) pathogens. In Italy few studies have reported the isolation of carbapenemase-producing Citrobacter freundii strains. The aim of the study was to characterize two isolates of C. freundii supected for the carbapenemase-production.
Materials and Methods: In the period February-March 2016, two *C. freundii* strains with reduced susceptibility to carbapenems were isolated from a rectal swab and an urine specimen of two patients, only one been hospitalized. Species identification and antimicrobial susceptibility testing were carried out by Vitek2 (bioMérieux, France) and Autoscan4 Microscan (Beckman Coulter) semi-automated systems. Carbapenems MIC values were confirmed by E-test. The isolates were analyzed by phenotypic tests, PCR, sequencing and PFGE (XbaI). Conjugation assay, extraction, replicon typing and sequencing of plasmids were also performed.
Results: The isolates were resistant to third generation cephalosporins and carbapenems, being still susceptible to gentamycin, colistin, tigecycline and trimethoprim-sulfamethoxazole. The synergy test gave positive results for the meropenem disk plus boronic acid. PCR and sequencing revealed the presence of two different KPC variants: *bla*KPC-2 gene harbored on an IncN (conjugative) plasmid; and *bla*KPC-3 on an *Inc*X3 plasmid. The KPC-2-producing *C. freundii* isolates presented also a co-resident helper plasmid of IncA/C. The two isolates resulted clonally unrelated by PFGE and belonged to different sequence types.
Discussion and Conclusions: Our findings show that different clones of KPC *C. freundii* (KPC-Cf) are emerging in Italy. Risk factors for the KPC-Cf carriage of the hospitalized patient could be the gastroenterology prolonged stay under imipenem therapy due to the rapid identification of KPC-Cf avoid the spread of this unusual type of CPE among hospitalized patients.

P 016

IN VITRO EFFECT OF CLARITHROMYCIN AND ALGINATE LYASE AGAINST *HELICOBACTER PYLORI* BIOFILM

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Introduction: It is now established that the gastric pathogen *Helicobacter pylori* has the ability to form biofilms *in vitro* as well as on the human gastric mucosa. The aim of this study is to evaluate the antimicrobial effects of Clarithromycin on *H. pylori* biofilm and to enhance the effects of this antibiotic by combining it with Alginate Lyase, an enzyme degrading the polysaccharides produced as extracellular polymeric substances within the biofilm matrix.
Materials and Methods: We evaluated the Clarithromycin Minimum Inhibition Concentration on *in vitro* preformed biofilm of a *H. pylori* clinical isolate. Then the synergic effect of Clarithromycin and Alginate Lyase treatment has been quantified by using the Fractional Inhibitory Concentration index, measured by checkerboard microdilution assay. To clarify the mechanisms behind the effectiveness of this antibiofilm therapeutic combination, we used Atomic Force Microscopy to analyze modifications of bacterial morphology, percentage of bacillary or coccoid shaped bacteria cells and to quantify biofilm properties.
Results: The combined treatment of AlgL and CLR

doesn't show difference in planktonic MIC values in respect to the treatment with AlgL or CLR alone, while a synergistic activity has been measured for the combination of CLR and AlgL against the biofilm grown *H. pylori* with sessile MICs values for AlgL and CLR reduced when the two drugs are combined. CLR-AlgL treatment is besides related to a response of H. pylori in terms of cellular morphology and biofilm structure. Untreated cells display an equilibrated number between coccoid and bacillary cells with a little prevalence of the coccoid form (55%). After AlgL treatment, the result is overturned, with a decrease of the percentage of bacteria in coccoid shape. Hence, the AlgL alone affects the *H. pylori* shape in favor of the less resistant bacillary shape (52%). On the other hand, the CLR causes a more pronounced reduction of cell height, with a maximized effect in combination with AlgL. These findings, obtained on the single cell analysis, are in good agreement with the results obtained with the checkerboard analysis where AlgL did not show any antibacterial activity and the combined effect showed the maximum antibacterial effects.
Conclusions: In conclusion, the synergistic activity measured by checkerboard microdilution assay emphasizes the potential clinical importance of combining traditional antibacterial agents with EPS-degrading enzymes, reinforcing the biofilm matrix as a valuable antibiofilm drug target to improve the management of biofilm-associated infections.

P 017

A CLONE OF LINEZOLID-RESISTANT STAPHYLOCOCCUS EPIDERMIDIS BEARING THE G2576T MUTATION IS ENDEMIC IN AN ITALIAN HOSPITAL

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Introduction: Linezolid has broad activity against important multidrug-resistant Gram-positive pathogens, including methicillin-resistant staphylococci and vancomycin-resistant enterococci. Linezolid inhibits protein synthesis by binding to the peptidyl transferase centre of the bacterial ribosome. Although resistance may be mediated by transferable genes such as *cfr* and *optr*A, mutations in the central loop of domain V of 23S rDNA and in rplC and rplD genes, which encode the ribosomal proteins L3 and L4, respectively, constitute the main mechanism associated with linezolid resistance among clinical staphylococci.
Methods: 26 linezolid- and methicillin-resistant Staphylococcus epidermidis (LR-MRSE) strains were isolated from three hospital: 24 from the Ancona regional hospital and 1 each from the nearby hospitals of Jesi and Senigallia. All were phenotypically studied and genotypically characterised for their linezolid resistance mechanisms, and typed by PFGE and MLST.
Results: All isolates exhibited full resistance to linezolid, with MICs ranging from 16 to 64 mg/L. PCR experiments were negative for *cfr* and *optrA*. With the exception of three, which bore a wild-type 23S rRNA gene, all the other isolates carried the G2576T mutation in domain V of 23S rDNA. Mutations in *rplC* and *rplD* genes were found in 8 isolates: F147L/A157R in *rplC* and K68Q in *rplD* (n = 2); H146Q/V154L/A157R in *rplC* and ins-71GGR72 in *rplD* (n = 1); and M156T in *rplC* (n = 5). 23/26 isolates exhibited the same PFGE pat-

tern (pulsotype A) and shared clonal lineage ST2. Three strains had different pulsotypes (B, B1, and C, respectively) and sequence types (ST23 for two isolates, and ST5 for an isolate).
Conclusions: We report a LR-MRSE clone bearing the G2576T mutation in 23S rDNA that is endemic in a regional hospital in central Italy. In retrospective, the original isolate, detected in Ancona in November 2004, is the first linezolid-resistant staphylococcus reported in Italy.

P 018

REQUIREMENT OF FLHF FOR SWARMING MOTILITY AND VIRULENCE OF *BACILLUS CEREUS*

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Introduction: Besides sporulation, *Bacillus cereus* can also undergo a periodic process of differentiation in which short swimmer cells become elongated hyperflagellated swarmer cells during migration across surfaces. Swarming differentiation of *B. cereus* was previously associated with increased secretion of virulence proteins and pathogenicity. Since swarming requires the integrity and functionality of flagella, in this study we evaluated the role of the enigmatic flagellar protein FlhF, the third paralog of the signal recognition particle (SRP) GTPases, Ffh and FtsY, in swarming and pathogenicity of *B. cereus*.
Materials and Methods: *B. cereus* wt, its Δ*flhf* mutant, and Δ*flhf*-comp (complemented) strains were used. *In silico* analysis was performed using the ProDom program and the Raptor X Structure Prediction Server. Swarming migration was analyzed measuring colony diameters and looking for differentiated swarmer cells. Proteomic analysis was performed by two-dimensional electrophoresis of bacterial supernatants and protein spots were identified and quantified. Bacterial pathogenicity was evaluated in the *Galleria mellonella* larvae model of infection.
Results: *In silico* analysis of *B. cereus* FlhF revealed that the protein presents conserved domains that are typical of SRPs in many organisms. Conserved motifs required for SRP conformational assembly and binding to the signal peptide of nascent proteins were found in the *B. cereus* protein. Proteomic analysis of the Δ*flhf* mutant showed a significant effect of the FlhF depletion on protein secretion. The amount of some proteins was increased (e.g. B component of non-hemolytic enterotoxin, cereolysin O, enolase) and other proteins were reduced (e.g. flagellin, L2 component of hemolysin BL, bacillolysin, sphingomyelinase, PC-PLC, PI-PLC, cytotoxin K) in the extracellular proteome of the mutant. *B. cereus* Δ*flhf* cells also resulted to be less pathogenic in *G. mellonella* larvae.
Conclusions: In this study, we demonstrate that B. cereus FlhF is required for swarming motility and that loss of this protein results in defective export of flagellin and important virulence factors, thus significantly reducing bacterial pathogenicity.

P 019

TRENDS OF BLOODSTREAM INFECTIONS DUE TO *ESCHERICHIA COLI* AND *KLEBSIELLA PNEUMONIAE*: A NINE-YEAR SURVEY AT A LARGE ITALIAN TEACHING HOSPITAL

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Introduction: Monitoring the local ecology of pathogens responsible of bloodstream infections (BSIs) is extremely important to start adequate empirical therapy, especially in the era of multi-drug-resistance (MDR). Over the last years the resistance to antibiotics among *Escherichia coli* and *Klebsiella pneumoniae* is of particular concern. The aim of this retrospective study was to analyze the incidence and trends of *Escherichia coli* and *Klebsiella pneumoniae* in patients with hospital onset (HO) and community onset (CO) BSI in a single tertiary care university hospital over a 9-year period (January 2007-December 2015).
Matherials and Methods: All de-duplicated blood cultures positive for *E.coli* and *K. pneumoniae* were

recorded through laboratory databases. Antibiotic susceptibility was interpreted according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints. A linear regression analysis was performed to describe the trend of incidence over time.
Results: Of 160482 collected blood cultures, 1991 were positive for *E. coli* and 1001 for *K. pneumoniae*. Of these, 1807 fulfilled the criteria for HO (60.4%), and the remaining 1185 were CO. Among HO BSIs, only *K. pneumoniae* significantly increased (+2.2/10,000 patient-days, p = 0.001), whereas BSIs caused by *E. coli* increased over time although not significantly (+1.7/10000 patient-days). We observed an increasing trend of CO BSIs in both groups: *E. coli* +12.6/10000 admissions (p = 0.004), and *K. pneumoniae* +3.69/10000 admissions (p = 0.02). Resistance rates of tested antibiotics were similar between HO and CO strains of EC. On the other hand, resistance to carbapenems (17.4% versus 45.9%), aminoglycosides (31.9% v. 51.6%), fluoroquinolones (41.2% v. 61.2%) and colistin (4.6% v. 15.5%) was markedly different between CO and HO strains. Incidence of CO BSIs due to extended-spectrum β-lactamase (ESBL) producing *E. coli* increased from 3.2 to 9.1 BSIs x 10000 admissions (p = 0.03). HO episodes did not significantly increased. Concerning HO *K. pneumoniae* a significant increasing trend of carbapenem and colistin resistant strains was observed (+1.55, p < 0.001 and +0.62, p = 0.002, respectively).
Conclusions: This study underlines the increase of ESBL phenotype among *E. coli* BSI within the first 48 hours of hospitalization and the rise of resistance to carbapenem and colistin among *K. pneumoniae* BSIs. Continuing to monitor the incidence of BSI is very important for establishing the burden of invasive infection and to drive proper use of antibiotics.

P 020

BACTERIAL BIOFILM FORMATION ON ANTIBIOTIC-LOADED BONE CEMENT. A PRELIMINARY *IN VITRO* STUDY

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Introduction: The use of antibiotic-loaded polymethylmethacrylate bone-cement spacers (ALBCs) during two-stage exchange procedures is the standard in the treatment of patients with delayed prosthetic joint infection. The real antimicrobial activity of these spacers is unclear because the adherence of bacteria to cement might result in clinical recurrence of infection. The purpose of the study is to evaluate the *in vitro* formation of *Pseudomonas aeruginosa* (PA) and methicillin-resistant *Staphylococcus aureus* (MRSA) biofilm on ALBCs.
Materials and Methods: Cement disks (diameter = 6 mm) impregnated with different classes of antibiotics (gentamicin, colistin, linezolid, clindamycin, tobramycin, vancomycin), individually or with two molecules in combination, were submerged in bacterial suspensions of PA and MRSA for different times of incubation (maximum 96h). Negative controls (specimen disks without antibiotic) were similarly prepared. Biofilm formation was visualized by confocal scanning laser microscopy (CSLM), after staining the discs with the Live/Dead BacLight Viability Kit. Sequential optical sections of 1 μm were collected in sequence along the z-axis over the complete thickness of the sample. The resulting stacks of images were analyzed, quantified and rendered into three-dimensional (3D).
Results: CSLM shown living bacteria grown in biofilm on the surface of all cement disks controls, but not on the surface of all ALBCs. The 3D rendering showed total inhibition of PA biofilm formation as compared with the controls on gentamicin-, tobramycin-impregnated disks until 96h incubation period, and on colistin- impregnated disks until 48h of incubation. A total inhibition of MRSA biofilm formation was observed until 72h of incubation on linezolid, vancomycin, tobramycin-vancomycin disks, while an adhesion phase and/or biofilm

formation already was observed at 72h incubation on clindamycin, tobramycin, gentamycin, gentamycin-clindamycin disks.
Conclusion: The ability to deliver high local concentrations of antimicrobial agents has made ALBCs one of the standards of treatment for patients with chronic infection of the skeletal system. The PA and MRSA viability and biofilm formation are reduced by adding antibiotics to bone cement but not all tested ALBCs completely inhibit the formation of an infectious biofilm *in vitro*. The efficiency of the release of antibiotics from bone cement is a critical factor which determines the antibacterial activity of ALBCs. Our study provide relevant informations about the ability of ALBCs to inhibit biofilm formation after different times from the implant.

P 021

MOLECULAR EPIDEMIOLOGY OF HETEROGENEOUS VANCOMYCIN-INTERMEDIATE *STAPHYLOCOCCUS AUREUS* (HVISA), IN ITALY

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Introduction: During a three-month *Staphylococcus aureus* epidemiological survey performed in Italy during 2012, collecting 640 methicillin-resistant *S. aureus* isolates from 52 centers throughout Italy, 160 MRSA were confirmed to be hVISA, with a prevalence of 25% among all clinically relevant isolates.
Material and Methods: hVISA subpopulations were detected by GRD and δ hemolysis tests, and confirmed by the gold standard population analysis (PAP/AUC) . Antimicrobial susceptibility testing to the main anti-Gram-positive drugs was performed by broth-microdilution-method (BDM), following EUCAST guidelines. All confirmed hVISA were characterized at the molecular level by MLST/SCCmec/spa-typing. The presence of Panton-Valentine leukocidin was also evaluated.
Results: Vancomycin and teicoplanin MIC distribution showed that the subgroups of hVISA and VSSA strains were almost indistinguishable with only one dilution difference from the two phenotypes. hVISA MICs were in the range of 0.5-2 mg/L for vancomycin and 0.25-4 mg/L for teicoplanin, while VSSA MICs were in the range of 0.25-2 mg/L for both drugs. GRD and δ hemolysis tests, confirmed by PAP/AUC, were able to capture heteroresistant subpopulations with good sensitivity and specificity. In fact, our results showed the following: δ hemolysis test (86.36% sensitivity and 77.27% specificity; accuracy of 79.54% and a negative predictive value - NPV - of 44.11%) with respect to the GRD test (90.90 sensitivity and 65.9% specificity; accuracy of 72.16% and an NPV of 47%). Among hVISA isolates, 57% belonged to ST228/SCCmecI; 16% were ST5/105 SCCmec II; 13.5% were ST8-USA500-like-SCCmecIVC/I and 9% belonged to sporadic clones. hVISA clones and their prevalence are differently represented with respect to VSSA strains, in which more than 50% of isolates belonged to the E-MRSA15-ST22-SCCmecIVh and USA500-like-ST8-SCCmecIVc. This difference in the prevalence and heterogeneity of their STs was greater looking at the spa-type distribution: some spa-types (t041; t1686) are typically represented only in the hVISA group with respect to the vancomycin susceptible clones.
Conclusion: These data suggest that glycopeptide heterogeneous subpopulations are present among MRSA strains with a frequency of about 25%; these strains can be found even at MICs lower than 1 mg/L and are unrecognizable only when using the BMD method. δ hemolysis and GRD tests seemed to be able to predict the presence of these subpopulations and their proclivity to persist in many severe infections (above all endovascular) despite glycopeptide therapy. The deep molecular characterization of hVISA and VSSA clones demonstrated a sort of clonality among the hVISA subgroup, different from the main VSSA clones circulating in our country.

P 022

ANTIMICROBIAL ACTIVITY OF ESSENTIAL OILS AGAINST FOOD-BORNE PATHOGENS IN READY-TO-EAT VEGETABLE

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Introduction: The Mediterranean diet is known worldwide for its balanced content of proteins, carbohydrates, vitamins, minerals and fiber. Fruits and vegetables are essential for the well-being and health of the organism and are recommended in a healthy and balanced diet. However, 25% of foodborne diseases are linked to the consumption of vegetable, in particular the ready-to-eat (RTE) vegetable. The foodborne pathogens associated at RTE vegetable are Enterobacteriaceae and *Listeria monocytogenes*. In recent time, many studies have been directed to the use of natural antimicrobial substances, like essential oils (EOs), in order to inhibit pathogens growth in food products.
Methods: *Rosmarinus officinalis L.* and *Thymus vulgaris L.* EOs have been submitted to qualitative and semi-quantitative analysis by using GC-MS and GC-FID techniques, respectively, in order to obtain their phytochemical composition. The antibacterial effects against food-borne pathogens bacteria (*Listeria monocytogenes, Salmonella Enteritidis , Yersinia enterocolitica* and *Escherichia coli*) were tested using disk diffusion assay, followed by determination of minimum inhibitory concentration (MIC) and the fractional inhibitory concentration index (FICI). Also, the antimicrobial activity of EOs was evaluated with direct contact with artificially contaminated RTE vegetable stored at refrigeration temperature.
Results: *R. officinalis* EO major constituents were 1,8-cineole (45.27%), borneol (12.94%), α-pinene (11.39%), β-pinene (6.55%), camphene (5.34%) and camphor (4.08%). *T. vulgaris* EO, showed a high amount of p-cymene (39.18%), followed by thymol (25.05%) and γ-terpinene (5.25%). The lowest minimal inhibitory concentrations (MIC) were obtained with *R. officinalis* against *E. coli* (3.9 μL/ml),while against *S. Enteriditis* and *Y. enterocolitica* the *R. officinalis* EO MIC was 7.8 μL/ml and 31.5 μL/ml for *L. monocytogenes*. *T. vulgaris* EO showed a MIC of 31.5 μL/ml for *S. Enteriditis* and *Y. enterocolitica*, 7.8 μL/ml against E. *coli* and a highest minimum inhibitory concentration (250 μL/ml) against *L. monocytogenes*. The FICI of the combined EOs suggested a synergic interaction. The incorporation of EOs alone or combined in RTE vegetable reduced the viable cell counts of all test strains already to 24 hours and up to the end of the experiment (8 days). Moreover, according to the GC profiles the high concentration of oxigenated monoterpenes was peculiar for the bioactivity, in particular 1,8-cineole in *R. officinalis* EO and thymol in *T. vulgaris* EO.
Conclusion: The results confirm that EOs could be used as natural alternatives to prevent bacterial growth in food products to extend their shelf life when combined with the protective functions of pre-existing packaging technologies.

P 023

EXTENDED SPECTRUM β-LACTAMASE AND AMPC-PRODUCING *ENTEROBACTERIACEAE* IN RAW VEGETABLES

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Introduction: Antibiotic resistance in bacteria is a global problem exacerbated by the dissemination of resistant bacteria via uncooked food, such as green leafy vegetables. The attribution of fresh produce to the overall community-associated exposure of humans to ESBL- or AmpC- producing. The aim of the current study was carried out to assess the of extended spectrum β-lactamase and AmpC type β-lactamase in *Enterobacteriaceae* isolated from fresh vegetables sold in the local markets of Modena town.
Methods: A total of 80 samples comprising carrot, spring onion, tomatoes, arugula, chicory, endive and frisee salad were processed microbiologically to isolate Enterobacteriaceae. The vegetables were mostly obtained from supermarkets. Species identification and antimicrobial susceptibility testing were carried out using the Vitek 2 system and the

AST-GN041 card (bioMérieux). Phenotypic testing by using the Double Synergy Differential Test (DSDT) for ESBL/AmpC-positive strains was confirmed by PCR and DNA sequence analysis. The localization of β-lactamase genes was established by conjugation experiments.
Results: Overall, of the 160 analyzed strains ESBLs/AmpC were detected in 20 isolates (12,5%) for the double-disk synergy test. The major ESBL determinant was the CTX-M type (12 isolates) and two SHV (2 isolates). DHA and ACC (4 isolates) were AmpC-β lactamases type. Additionally, we identified newly recognized ESBL blaRAHN-2 sequences from *Rahnella aquatilis* (2 isolate). The conjugation has demonstrated the transmissibility of this resistance determinant.
Conclusion: In conclusion, we report a highter and a low prevalence of ESBL and AmpC β-lactamase determinants respectively mostly in arugula and frisee salad. Public health risks associated with exposure to ESBL- and AmpC-producing bacteria through consumption of uncooked fresh produce are diverse. They range from occasional ingestion of 3GC-resistant opportunistic pathogens which may result in difficult-to-treat infections, to frequent ingestion of relatively harmless ESBL producing environmental bacteria that may therewith constitute a continuously replenished intestinal reservoir facilitating dissemination of ESBL genes to (opportunistic) pathogens.

P 024

RIFABUTIN CONTAINING TRIPLE THERAPY AND RIFABUTIN WITH BISMUTH CONTAINING QUADRUPLE THERAPY FOR THIRD-LINE TREATMENT OF *HELICOBACTER PYLORI* INFECTION: TWO PILOT STUDIES

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Introduction: *Helicobacter pylori* expresses in time an increased resistance in respect to antimicrobial agents currently used in therapy also related to different geographic areas. Therefore, the selection of therapeutic regimes needs to be adapted to local resistance patterns. The aim of the study, was to evaluate the therapeutic gain of the addition of bismuth to a rifabutin containing triple therapy with amoxicillin and pantoprazole at standard dosages for the treatment of third-line *H. pylori* infection after a preliminary susceptibility test.
Materials and Methods: Two groups of patients in two pilot studies which were carried out. One group was treated with rifabutin 150 mg b.i.d., pantoprazole 20 mg b.i.d., and amoxicillin 1g b.i.d. for 10 days and the other group with rifabutin 150 mg b.i.d., pantoprazole 20 mg b.i.d., amoxicillin 1g b.i.d. and bismuth subcitrate 240mg b.i.d. for 10 days. All patients underwent to culture and susceptibility testing prior to their inclusion in the study. A successful outcome was confirmed with an Urea Breath test performed 8 weeks after the end of treatment.
Results: Twenty-nine patients were recruited in the pantoprazole, amoxicillin, rifabutin group and 30 in the pantoprazole, amoxicillin, rifabutin, and bismuth subcitrate group. All patients had a positive *H. pylori* culture and the susceptibility test used showed *H. pylori* sensitivity to rifabutin and amoxicillin. *H. pylori* eradication during follow-up was 18/27 (66.7%, 95% CI: 47.7-85.7%) in the pantoprazole, amoxicillin, rifabutin group and 28/29 (96.6%, 95% CI: 89.5-100.0%) in the pantoprazole, amoxicillin, rifabutin, and bismuth subcitrate

group. Both treatments were well-tolerated with no reported side effects.
Conclusion: The addition of bismuth subcitrate to a triple therapy that includes proton pump inhibitors, amoxicillin, and rifabutin in patients who are treated for the third time for *H. pylori* infection resulted in a 30% therapeutic gain. In summary, this study suggests that rifabutin is another drug that appears to benefit from the addition of bismuth.

P 025

EVALUATION OF TEDIZOLID MIC TEST STRIP COMPARED TO BROTH MICRODILUTION METHOD FOR GRAM-POSITIVE ISOLATES

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Introduction: Tedizolid phosphate is a novel oxazolidinone prodrug that is rapidly converted *in vivo* by phosphatases to the microbiologically active antibiotic tedizolid which has potent activity against Gram-positive pathogens. In the EU, it is indicated for the treatment of ABSSSI caused by susceptible isolates of the following Gram-positive microorganisms: *Staphylococcus aureus* (including methicillin-resistant strains (MRSA) and methicicllin-susceptible strains) and *Enterococcus faecalis*.
This study was performed to evaluate the performance of a newly developed gradient strip, the Tedizolid MIC Test Strip (MTS) from Liofilchem, Roseto degli Abruzzi, Italy compared to the broth microdilution (BMD) reference method against indicated Gram-positive isolates.

Materials and Methods: The study isolates (51 Staphylococci: 11 Methicillin Sensitive *S. aureus*, 40 Methicillin Resistant *S. aureus* and 26 *E. faecalis*) were chosen to include a wide range of TZD MIC results. Each isolate was tested for Tedizolid MIC by BMD (Liofilchem prepared panels) and MTS (Becton Dickinson Mueller Hinton II Agar) methods. Comparisons between the two methods were made by rounding-up the MTS MIC to the next equivalent BMD doubling-dilution (DD) MIC and calculating the DD difference. Performance and reproducibility of Tedizolid MTS was also evaluated by using the CLSI QC strains. *S. aureus* ATCC® 29213 and *E. faecalis* ATCC® 29212 were tested with MHA from 2 suppliers (Becton Dickinson and Oxoid) while *S. pneumoniae* ATCC® 49619 was tested with both Mueller Hinton Fastidious Agar and Mueller Hinton Sheep Blood. MIC results were compared to the relevant CLSI expected ranges. Influence of inoculum density was evaluated as well.
Results: For *Staphyloccci* and *E. faecalis* the essential agreement between the two MIC methods for susceptibility testing of tedizolid was 98.0% and 97.0%, respectively. QC results were within the CLSI established ranges. Use of Mueller Hinton II Agar from different manufactures as well as use of the medium recommended by either CLSI or EUCAST for susceptibility testing of fastidious organisms produce comparable results.
Conclusions: This first evaluation of Tedizolid MTS showed good correlation to BMD method. Further testing with additional isolates at multiple sites and with media from multiple manufacturers is warranted.

P 026

DETECTION OF QNR GENES IN ENTEROBACTERIACEAE STRAINS FROM A HOSPITAL OF MODENA CONFERRING HIGH LEVEL OF RESISTANCE TO CIPROFLOXACIN

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Introduction: The *qnr* genes have been described to confer a low level of resistance to fluoroquinolones producing an increase in ciprofloxacin MIC of about 16-32 fold in a recipient strain. It has been also described that an increased expression of *qnr*A in a plasmid transferred from a clinical isolate to J53 recipient strain, conferred a 128-fold increase in ciprofloxacin MIC. We studied ciprofloxacin resistance conferred by plasmids carrying qnr genes from *Enterobactericae* strains isolated from urinary tract of patients in a Sant'Agostino Civil Hospital of Modena.
Materials and Methods: Ninety *Enterobactericae* strains resistant to ciprofloxacin were studied

that included: 61 E. coli and 29 P. mirabilis strains. The presence of qnr-like genes (A, B, C, D, S) was assessed by PCR and sequencing. Antibiotic MICs were determined by microdilution method. The strains *qnr*-like positive were subjected to the search of aac(6')-Ib-cr, a variant aminoglycoside acetyltransferase capable of reducing ciprofloxacin activity also carried on plasmids. Resistance conferred by plasmids carrying qnr genes (one *qnr*S, one *qnr*B, one both *qnr*S and *qnr*B genes and another both *qnr*S and *qnr*D genes) was evaluated by conjugation assays using as recipient strains J53 E. coli rifampicin resistant.

Results: For ninety strains MIC50 for Ciprofloxacin was 2 µg/mL and MIC90 32 µg/mL in the range of 0.625 - 128 µg /mL. Fifteen strains were positive for qnr-like genes, all belonging to *E. coli* species. In particular 9 with qnrS-positive, 2 with *qnr*B-positive, 3 with *qnr*B-positive and contemporary *qnr*S-positive and 1 with *qnr*S-positive and contemporary *qnr*D-positive. The increase in ciprofloxacin MICs in the recipient strain ranged 16- to 32-fold, with the exception of the plasmid 27-IA that only carried *qnr*S1 and increased 128-fold the ciprofloxacin MIC in J53 strain (from 1 to 128 µg/ml).

Conclusions: Our results indicate like the acquisition of a qnr-like plasmid, in particular carrying a *qnr*S, *qnr*B, both *qnr*S and *qnr*B and both *qnr*S and qnrD genes, influence the MIC, increasing them. In this study is also reported the discovery of *qnr*D gene (present in contemporary with a *qnr*S gene), that is one of the few found in Europe.

P 027

ANTIBACTERIAL ACTIVITY OF 50:50 TI/AG BI-ELEMENT NANOPARTICLE FILMS OBTAINED BY SUPERSONIC CLUSTER BEAM DEPOSITION

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Introduction: The dissemination of bacterial pathogens by indirect transmission via contaminated surfaces is a major global public health threat, especially in the hospital settings. To counteract this phenomenon, the implementation of effective antimicrobial coatings has been advocated as a promising approach. Here we investigated the activity of 50:50 Ti/Ag bi-element nanoparticle films (Ti/Ag NPs), synthesized by Supersonic Cluster Beam Deposition (SCBD), against a panel of clinically relevant pathogens (including multidrug-resistant Gram-positive and Gram-negative strains).

Materials and Methods: The antibacterial activity of the Ti/Ag NPs was investigated against 7 strains (including *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Acinetobacter baumannii, Staphylococcus aureus* and Enterococcus faecalis). A standardized bacterial suspension (about 5 × 106 CFU) was distributed on the supports with and without Ti/Ag NPs films. Each support was incubated at 25°C in damp environment for 3 hours. After the incubation time, bacteria were resuspended in PBS and appropriately diluted for viable cell count (VCC). The kinetics of bacterial killing was investigated for *A. baumannii*, by determining the VCC after 15, 45, 90 and 180 minutes of incubation. Results were presented as VCC log reduction compared to controls and represented the mean of three independent experiments.

Results: Ti/Ag NPs obtained by SCBD showed a relevant bactericidal activity against all the Gram-negative strains tested, for which the reduction of VCC by more than 4 log was observed. On the contrary, the Ti/Ag NPs films did not affect

viability of Gram-positive strains. For *A. baumannii* a VCC log reduction of 0.6, 2.3, 3.8 and 6.0 was observed after 15, 45, 90 and 180 minutes of incubation, respectively.
Conclusions: Ti/Ag NPs showed a relevant bactericidal activity against major Gram-negative pathogens. The lack of activity against Gram-positive bacteria could reflect a slower bactericidal activity against those pathogens, as reported for silver NPs films in previous studies. The obtained results emphasize the potential role of SCBD as an alternative method for the synthesis of antibacterial coatings by combining elements to into NPs.

P 028

EVALUATION OF ANTIBACTERIAL AND ANTIBIOFILM ACTIVITY OF TURKISH PLANT EXTRACTS

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Introduction: The acceptance of traditional medicine as an alternative form of health care has led to an increased use of medicinal plants. Turkey is known as one of the richest country of plants in the world. However, only a small proportion of these have been thoroughly studied and investigated for their antimicrobial activity and very few studies on biofilm production have been published. In this context, the study contributes to enrich the literature data exploring the activity of five Turkish plant extracts.
Materials and Methods: *Ficus carica L., Juglans regia L., Olea europaea L., Punica granatum L.* and *Rhus coriaria L.* methanol extracts were evaluated for polyphenols content, antibacterial, antibiofilm and antioxidant activities. The most active extracts were tested by High Performance Thin Layer Chromatography (HP-TLC) bioautographic assay in order to determine the active fractions and characterize the compounds. The genotoxic and antigenotoxic potential was also estimated.
Results: Among the extracts, *P. granatum L.* and *R. coriaria L.* leaves showed the highest polyphenols content (17.63 ± 0.43 and 25.38 ± 1.03 ggallic acid /100 gplant extract, respectively) and the best antibacterial activity with minimum inhibitory concentrations (MIC) of 78-625 μg/ml for Listeria monocytogenes and *Staphylococcus aureus* and 312-1250 μg/ml for *Escherichia coli* and *Pseudomonas aeruginosa*. Moreover, the extracts were able to reduce biofilm formation more readily than other extracts. SubMICs of *P. granatum L.* and *R. coriaria L.* extract poorly interfered with the planktonic growth while produced a significant inhibition of biofilm formation equal to 80-60% and 90-80% for *L. monocytogenes* and *S. aureus*, respectively. Also for the antioxidant activity tested by spectrophotometric 2,2-diphenylpicrylhydrazyl (DPPH) assay, the most relevant results were exhibited by *R. coriaria L.* extract, which showed an IC50 value better than positive control Trolox, followed by *P. granatum L.* extract. Bioassay-guided and HPLC procedures demonstrated the presence of apigenin 4′-O-β-glucoside in *P. granatum L.* and myricetrin and quercitrin in *R. coriaria L.* SOS-Chromotest showed the antigenotoxicity of plant extracts.
Conclusion: To the best of our knowledge, this is the first study reporting the antibiofilm activity of *P. granatum L.* and *R. coriaria L.* leaves. Overall, the present findings provide scientific basis to promote the value-adding of *P. granatum L.* and *R. coriaria L.* leaves as sources of biologically active promising compounds.

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P 029

INORGANIC PHOSPHATE STARVATION INDUCES PE_PGRS3 EXPRESSION AND ITS POST TRANSLATIONAL MODIFICATION IN *M. SMEGMATIS*

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Background: PE_PGRS proteins are unique to *Mycobacterium tuberculosis* (Mtb) complex and few other pathogenic mycobacteria. Although many studies have demonstrated a cell wall localization and an involvement during Mtb pathogenesis, their role and function remain elusive. We investigated environmental stress effect on the expression pattern of two closely related PE_PGRSs. Our attenction was then focused on phosphate starvation.
Material / Methods: PE_PGRS3 and PE_PGRS4 were amplified from Mtb H37Rv genome and cloned, with their putative promoters, in pMV vector tagged with green fluorescent protein (GFP) or with hemagglutinin epitope (HA). Four recombinant *M. smegmatis* (Ms), expressing PE_PGRS3GFP, PE_PGRS4GFP, PE_PGRS3GFP/4HA, PE_PGRS4GFP/3HA, were created. Recombinant strains were grown in different conditions to study different expression pattern. Confocal microscope and ImageJ software were used to acquire and analyze images. Fluorescence analysis was supported by immunoblotting assay and flowcytometer (FACS-canto) quantification after 15 days of growing in low phosphate medium.
Results: PE_PGRS3 and PE_PGRS4 are localized in the same gene locus, each with its own putative promoter. PE_PGRS3 and PE_PGRS4 showed a diverse expression pattern depending on the time of incubation and phosphate concentration. Particularly, recombinant strains expressing PE_PGRS3GFP or PE_PGRS3GFP/4HA, showed a significant fluorescence after 15 days of incubation in phosphate depletion condition. Stable fluorescence was observed for PE_PGRS4 chimeras in both phosphate rich and poor medium. No fluorescence was observed during other stress conditions (acid pH, oxygen, magnesium or iron starvation). Ms expressing GFP was used like positive growth control. Finally, immunoblotting had shown that PE_PGRS3 C-terminal domain was cleaved and secreted in low phosphate condition.
Conclusions: PE_PGRS3 and PE_PGRS4 genome localization proposed a possible duplication-insertion event and therefore a common function. Actually, our results showed a different expression pattern in phosphate depletion environment, suggesting a different function of PE_PGRS3 and PE_PGRS4. Phosphate starvation is related with mycobacterial latency phase so as to suppose a role of PE_PGRS3 during nonreplicating persistence.

P 030

EVALUATION OF DALBAVANCIN MIC TEST STRIP COMPARED TO BROTH MICRODILUTION METHOD FOR STAPHYLOCOCCI

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Introduction: Dalbavancin is an antibiotic used to treat acute bacterial skin and skin structure infections (ABSSSI) in adults caused by susceptible Gram-positive organisms.This lipoglycopeptide interferes with cell wall synthesis having strong activity against many Gram-positive bacteria, including methicillin-sensitive and methicillin-resistant Staphylococcus aureus. This study was performed to evaluate the performance of a newly developed gradient strip, the Dalbavancin MIC Test Strip (MTS) from Liofilchem, Roseto degli Abruzzi, Italy compared to the broth microdilution (BMD) reference method against indicated Staphylococci isolates.
Materials and Methods: The study organism collection consisted in 100 Gram-positive isolates including 50 *Staphylococcus aureus* (Methicillin-Sensitive *Staphylococcus aureus*), 10 *Staphylococcus aureus* VISA, 10 *Staphylococcus aureus* hVISA, 25 *Staphyloccus aureus* (Methicillin-Resistant *Staphylococcus aureus*). Each isolate was tested for Dalbavancin MIC by BMD (Liofilchem prepared panels) and MTS (Becton Dickinson Mueller Hinton II Agar) methods. Comparisons between the two methods were made by rounding-up the MTS MIC to the next equivalent BMD doubling-dilution (DD) MIC and calculating the DD difference. Performance and reproducibility of Dalbavancin MTS

was also evaluated by using the CLSI QC strains. *S. aureus* ATCC® 29213 and *E. faecalis* ATCC® 29212 were tested with MHA from 2 suppliers (Becton Dickinson and Oxoid) MIC results were compared to the relevant CLSI expected ranges.
Results: Dalbavancin MIC results were within ± one doubling dilution of broth microdiluiton MIC results for all isolates. The essential agreement between the two MIC methods was 100% and the categorical agrement is 99%. QC results were within the CLSI established ranges. Use of Mueller Hinton II Agar from different manufactures produce comparable results.
Conclusions: The performance of Dalbavancin MTS shows an accurate alternative procedure to the broth microdilution assay when susceptibility tests are performed on isolates of Staphylococcus aureus.

P 031

WHOLE-GENOME SEQUENCE OF A *STENOTROPHOMONAS MALTOPHILIA* CLINICAL ISOLATE SUSCEPTIBLE TO CARBAPENEMS

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Introduction: *Stenotrophomonas maltophilia* is one of the most common emerging multi-drug resistant organisms found in the lungs of people with cystic fibrosis and its prevalence is increasing. *S. maltophilia* is an important cause of persistent airway colonization and chronic infection in cystic fibrosis (CF) patients. Eradication of *S. maltophilia* colonization is complicated by the propensity of this opportunistic pathogen to grow as biofilm and to its intrinsic low susceptibility to many antibiotics. In particular, intrinsic carbapenems resistance is related to the expression of the chromosomally encoded metallo-beta-lactamase L1. Here we report on the characterization of a *S. maltophilia* clinical isolate susceptible to carbapenems, responsible for ten-year airway colonization in a CF patient.
Materials and Methods: Hydrolytic activity toward carbapenems was investigated by a spectrophotometric assay at 300 nm, using 150 μM imipenem as substrate (in naive condition and after induction of L1 expression by cefuroxime). blaL1 amplification was attempted by PCR. The complete genome sequence was obtained through a 2x300 paired-end approach using the Illumina MiSeq system. Sequence assembly and annotation were performed using SPAdes and RAST, respectively. Sequence alignments and whole genome comparisons were performed using Mauve.
Results: Consistently with the carbapenem susceptible phenotype, no hydrolytic activity toward imipenem was observed, nor the blaL1 could be amplified by PCR. Whole-genome sequencing revealed a genome length of about 4.20 Mb, a size overall smaller than the other publicly available genomes of *S. maltophilia* (median total length: 4.76 Mb). Species identification was confirmed in silico using the Species Finder tool available at Center for genomic epidemiology. Genome comparison with the *S. maltophilia* reference strain D457 revealed that a large deletion (~255 kb) occurred in the chromosomal region comprising the blaL1 locus.
Conclusions: A large deletion comprising the blaL1 locus was documented in the genome of a S. maltophilia clinical isolate responsible for long-term colonization in a CF patient. Further studies will be performed to clarify the relationship between this strain and a *S. maltophilia* isolate resistant to carbapenems previously colonizing the same patient.

P 032

MULTICENTRIC EVALUATION OF RAPIDEC® CARBA NP TEST FOR THE RAPID SCREENING OF CARBAPENEMASE-PRODUCING ENTEROBACTERIACEAE, *PSEUDOMONAS AERUGINOSA* AND *ACINETOBACTER BAUMANNII* ISOLATED FROM BLOOD CULTURE, URINE AND OTHER MATERIALS

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Introduction: The global spread of carbapenemase-producing (CP) Gram-negative bacteria is worrisome and their rapid diagnosis is necessary in order to limit their diffusion and to adopt a targeted therapy. The aim of this multicenter study, which involved five centers (A. Manzoni Hospital, Careggi University Hospital, A. Gemelli Hospital, Tor Vergata Polyclinic and S. Agostino-Estense Hospital) was the evaluation of a new colorimetric test, RAPIDEC® CARBA NP, for the screening of CP Gram-negative bacteria isolated from blood culture and other materials.
Materials and Methods: Overall, 250 consecutive, non-replicate clinical isolates of Enterobacteriaceae, *Acinetobacter baumannii* and *Pseudomonas aeruginosa* were tested with RAPIDEC® CARBA NP. Among them, 125 isolates were from blood cultures (that were processed 6 hours after seeding), and 125 from other materials (mostly urine and respiratory, processed 24 hours after seeding). Carbapenemase production has been confirmed, on the isolates which showed reduced susceptibility to meropenem, by multiplex real-time PCRs targeting blaKPC-type, blaNDM-type, blaOXA-48-like, blaOXA-23-like, blaOXA-24-like, blaOXA-58-like, ISAba1+blaOXA-51-like, blaIMP-type, blaVIM-type, blaGES-type and blaFIM carbapenemase genes. Finally each center evaluated 20 well-characterized strains with a blind-test.
Results: Of the 125 isolates from blood cultures, RAPIDEC® CARBA NP correctly identified 27 isolates as CP, but missed 9 class D carbapenemase-producing *A. baumannii* isolates while four non-CP were classified as positive (one *Klebsiella pneumoniae* and three *P. aeruginosa* isolates). A sensitivity (SE) of 75%, a specificity (SP) of 95%, a positive and negative predictive value (PPV and NPV) of 87%, and 90%, respectively, were estimated with blood cultures isolates. Among isolates from other materials, 12 CP Enterobacteriaceae were correctly detected, but 11 out of 19 CP A. baumannii tested negative, while among non CP one *K. pneumoniae* and five P. aeruginosa tested positive with a consequent SE, SP, PPV and PNV of 65%, 94%, 77%, and 89%, respectively. Overall, RAPIDEC® CARBA NP test showed a SE, a SP, a PPV, and a NPV of 70%, 94%, 82%, and 89%, respectively. However, considering Enterobacteriaceae isolates only, these percentages showed a remarkable increase (100%, 98%, 94%, 100%, respectively). Finally, 4 of 20 well-characterized strains yielded discordant results in the blind test.
Conclusions: Although the test showed a low sensitivity with CP A. baumannii, the use of RAPIDEC® CARBA NP reduced significatively the time required for identification of CP Enterobacteriaceae and P. aeruginosa. In particular, CP isolates could be detected in less than 8.5 hours since the positivization of blood cultures.

P 033

FLOW CYTOMETRY APPLIED IN ENVIRONMENTAL SURVEILLANCE OF *L. PNEUMOPHILA* DURING DISINFECTION TREATMENT

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Introduction: Legionnaires' disease is a serious pneumonic infection mainly caused by inhaling *L. pneumophila*, widespread bacteria in natural and manmade water systems. The culture on Legionella selective agar (GVPC) is the reference method required by current norms for environmental surveillance in order to prevent outbreaks or supervise disinfection treatments. Anyway the concentration of *L. pneumophila* is largely underestimated by culture-based assays for the presence of viable but nonculturable cells (VBNC). In this study we compared the traditional culture results with flow cytometry (FCM), a rapid method for total cell count and live/dead enumeration.
Materials and Methods: We monitored the viability of 8 cultures of wild and reference *L. pneumophila* strains before and after thermal (70°C for 10 and 60 minutes) and chemical (50 mg/L sodium hypochlorite for 10 and 120 minutes) shock treatments. We plated all the samples on GVPC medium and we obtained the number of colony-forming units (CFU). At the same time we quantified viable, dead and intermediate bacteria by Sysmex Partec CyFlow® Cube 6 Flow Cytometer (50mW 488nm laser) using 2 different staining combinations: SYBR®Green (SG) or Fluorescein diacetate (FDA) combined with Propidium Iodide (PI).
Results: We expressed the all strains mean value as Log-data/mL. Before treatments, the viable cells from both SG/PI and FDA/PI are 5.0 (4.8-5.1) whereas dead and intermediate cells lie close to the set baseline; the CFU is 3.1 (2.5-3.4). After treatments CFU hasn't been detected on GVPC. During thermal shock, with SG/PI we observed that viable cells gradually fall down to 4.4 after 120 minutes; intermediate and dead cells increase, instead. Anyway after 10 minutes we didn't observe any viable cells with FDA/PI. For chemical shock, we found that the hyperchlorination leads immediately the cells count (viable, dead and intermediate) to baseline in both SG/PI and FDA/PI.
Conclusions: FCM opens new perspectives for environmental studies and permits to: have real-time results with high sensitivity; discriminate among viable, dead and intermediate cells; evaluate the cell metabolic activity (FDA staining). For thermal shock, the FDA/PI results seem to better reflect what observed with traditional method due to the inactivation of the metabolic activity. According to CFU, we didn't find any viable cells after hyperchlorination with both SG/PI and FDA/PI. The cell destruction effect is confirmed by the cytogram generated on FCM by side and forward scatter light. Therefore FCM is suitable for the rapid environmental surveillance of *L. pneumophila* before and during water system decontamination. Further studies will be performed in environmental samples, evaluating the content of VBNC with L. pneumophila specific staining.

P 034

"AGARIZED" WELL DIFFUSION TEST: AN ALTERNATIVE EXPERIMENTAL MODEL FOR THE ANTIMICROBIAL SUSCEPTIBILITY TESTING OF COMPLEX MATRICES

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Introduction: Antibiotic susceptibility testing allows the evaluation of the sensitivity profile of a microbial strain towards compounds with known or unknown antimicrobial activity. The reference methods are the disk diffusion, the agar diffusion and the dilution tests. This work had the purpose to improve the agar well diffusion to overcome some of the limits of the reference methods.
Materials and Methods: Cylindrical wells were pre-formed in semisolid growth medium contained in Petri dishes and filled with the compound to be tested embedded in the same growth medium containing 1.6% of agar. Reference antibiotics used to validate the technique were gentamicin, erythromycin, levofloxacin, norfloxacin, oxacillin, clindamycin, and rifampicin while the bacterial species included in this study were *Staphylococcus*

aureus, Streptococcus pyogenes, Streptococcus mutans, Escherichia coli and *Pseudomonas aeruginosa*. The reference test was the disk diffusion method according to EUCAST guidelines. Ethanolic plant extracts and essential oils were prepared according to standard procedures.
Results: At first the technical validation was accomplished by using different amounts of classical antibiotics, which, with the exception of fluoroquinolones and the aminoglycoside, were comparatively active both by disk diffusion and "agarized" well diffusion. Subsequently, the new test was applied to evaluate the antimicrobial activity of plant extracts and essential oils. Results from these experiments were confirming the applicability of the new method in this area of research.
Conclusion: "Agarized" well diffusion method may prove advantageous by: 1) limiting the superficial evaporation of solvent and volatile components compared to the disk diffusion test and to the agar well diffusion method; 2) overcoming the limitations imposed by the disk-based methods regarding the amount of compound to be tested; 3) ensuring a better handling due to the semi-solid consistency of the sample in the well; 4) setting itself as an more appropriate experimental model for preliminary screening of new compounds to be included in complex high density matrices such as creams, pastes, gels, and ointments.

P 035

EFFECTIVENESS OF INNOVATIVE ANTIMICROBIAL MOLECULES AGAINST BACTERIAL SPECIES OF CLINICAL INTEREST

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Introduction: Fluoroquinolone antibiotics are commonly used for treatment of several community and nosocomial bacterial infections. Fluoroquinolone resistance typically arises as result of alterations in the target enzymes DNA gyrase (mostly in GyrA subunit) and topoisomerase IV (mostly in ParC subunit). The development of novel compounds with dual target activity would have the advantage to reduce the emergence of resistance. A series of compounds has been synthesized and selected by Angelini Laboratories for their ability to interact to a similar extent with subunits GyrA and ParC on a site different from the classical quinolone site. This work investigated the effectiveness of these novel bacterial topoisomerase inhibitors (NBTIs) against bacterial species of clinical interest.
Materials and Methods: Susceptibility of control strains (*Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213 and methicillin resistant *S. aureus* (MRSA) ATCC BAA1720) to ciprofloxacin, levofloxacin and 10 NBTIs was evaluated by MIC and MBC assays according to M07-A9 CLSI guidelines. Some compounds were also investigated by time-kill curves: bacterial cultures were incubated with 1x, 2x and 4x MIC of each compound. At several times, viable counts were performed and the decrease of the initial inoculum of 3log10 CFU/ml was assessed.
Results: The antibacterial activity of 10 NBTIs assessed against *S. aureus* and MRSA strains was higher (0.125 to 8 μg/ml) than those against *E. coli* (0.5 to > 8 μg/ml). For all strains, MBCs were mostly one- or two-fold the MICs. Ciprofloxacin, levofloxacin and the 5 most active compounds (73007, 73009, 73011, 73012, 73013, MIC < 2 μg/ml) were investigated for killing kinetics: the greatest bactericidal activity against all control strains was displayed by 73013. Indeed 0.5 μg/ml was bactericidal against *E. coli* and *S. aureus* (after 8 h and 24 h of incubation, respectively) and even against MRSA (after 24 h). Ciprofloxacin showed no bactericidal effect against MRSA whereas levofloxacin exhibited a bactericidal kinetic comparable to that of 73013 but at higher concentration (64 μg/ml).
Conclusions: The NBTIs tested showed high bacterial growth inhibition against *S. aureus*, while the bactericidal activity was more evident against E. coli. The MRSA strain, highly resistant to 2nd generation quinolones, was susceptible to several molecules. 73013 proved to be the most active compound against all control strains. These preliminary but very promising results prompted the study of 73013 chemical stability, currently on going, to better elucidate its potential as a new broad spectrum antimicrobial agent.

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P 036

ANTIMICROBIAL ACTIVITY OF BIOACTIVE COMPOUNDS FROM PLANTS AGAINST A MULTI-RESISTANT *MYCOBACTERIUM ABSCESSUS* CLINICAL ISOLATE

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Introduction: Rapid-growing mycobacteria of the *Mycobacterium abscessus* complex are now recognized as important emerging human pathogens causing serious lung infections in patients with chronic pulmonary diseases and are also associated with infections following surgical interventions or tattooing. Recently, whole genome sequencing data analysis supported the differentiation of the *M. abscessus* complex (*M. abscessus*) into three subspecies, i.e. *abscessus, bolletii,* and *massiliense*. *M. abscessus* shows innate antimicrobial resistance to all major anti-tuberculous drugs in addition to resistance to broad-spectrum antibiotics such as β-lactams and tetracyclines. In consequence, infections with *M. abscessus* respond poorly to antibiotic chemotherapy. The global burden of antibiotic resistance has revived the interest in plant compounds as alternative/adjunct antimicrobial agents to control pathogenic microorganisms. Recent studies have also demonstrated that plant compounds can act in synergy with antibiotics against antibiotic-resistant pathogens. The aim of the present study was to investigate the antimicrobial activity and the synergy with antibiotics of plant compounds against a M. abscessus clinical isolate.

Materials and Methods: *M. abscessus* strain 29904, isolated from a bronchoaspirate of a 70-year old woman with a long history of pulmonary disease, was used throughout the study. Essential oils from *Allium sativum, Lavandula angustifolia, Melaleuca alternifolia, Mentha piperita, Origanum vulgare, Rosmarinus officinalis, Salvia officinalis,* and *Thymus vulgaris* and other plant compounds as carvacrol, thymol, α- and β- pinene, capsaicin, and curcumin were tested. MICs were determined by the microdilution method according to the CLSI guidelines. The synergy with antibiotics was evaluated by the checkerboard assay and by calculating the Fractional Inhibitory Concentration Index (FICI).

Results: The *M. abscessus* isolate was found to be resistant/intermediate to ciprofloxacin (MIC, 8-16 μg/ml), sulfamethoxazole (MIC, > 128 μg/ml), meropenem (MIC, 32 μg/ml), clarithromycin (MIC, 64-128 μg/ml), tigecycline (MIC, 8 μg/ml), amikacin (MIC, 32 μg/ml), and cefoxitin (MIC, 32 μg/ml). The MICs of essential oils ranged from 32 to 128 μg/mL, while those of other plant compounds ranged from 128 to >2048 μg/mL. In the checkerboard assay, a synergy was detected between curcumin and amikacin, clarithromycin, and ciprofloxacin (FICI values ≤ 0.5). Antagonism was never observed.

Conclusions: Overall, results of this study demonstrate an antimicrobial activity of some plant compounds against multi-resistant *M. abscessus*. Further evaluation is required to determine whether the present findings can be exploited in treating *M. abscessus* infections.

P 037

FIRST DETECTION OF LINEZOLID-RESISTANT OPTRA-POSITIVE CLINICAL ISOLATE OF *ENTEROCOCCUS FAECALIS* IN ITALY

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Introduction: *Enterococcus spp* are common members of the human gastrointestinal tract, but they are also able to cause serious opportunistic infections in immunocompromised patients. In recent years the number of multiresistant enterococcal infections has increased; in particular, the recent discovery of oxazolidinone resistance (considered as last resort antibiotics) in *Enterococcus spp.* is worrisome. Oxazolidinone resistance is caused by mutations in 23S rDNA, or by acquired resistance mechanisms: the cfr and cfr(B) genes, producing ribosome-modifying enzymes, and optrA, coding for an efflux pump. optrA was discovered in China in 2015 and since then only few isolates were reported worldwide, including two optrA- and cfr-positive *Enterococcus faecium* in Italy. In this work we studied the mechanism of acquired oxazolidinone resistance in a linezolid-resistant *Enterococcus faecalis* clinical isolate collected in January 2015 in Careggi University Hospital, Florence.
Materials and Methods: *E. faecalis* Ef2353 was analyzed by PCR to detect the presence of cfr, cfr(B), and optrA. Genotyping was performed by MLST. S1-PFGE/Southern blot, and hybridization assays were performed to investigate the optrA genetic environment. DNA transfer experiments were carried out by electrotransformation and conjugation, using *Enterococcus faecalis* JH2-2 as recipient strain. Linezolid and tedizolid MICs were determined by standard broth microdilution and E- test, respectively. Plasmid was sequenced by using Illumina Miseq and a 2X250 bp paired end approach.
Results: *E. faecalis* Ef-2353 was resistant to both linezolid and tedizolid with MICs of 8 and 4 mg/L, respectively. The isolate was positive for the optrA gene and was assigned to ST585 by MLST. S1-PFGE mapping and hybridization assays demonstrated that optrA was located on a plasmid of ~60 kb. Conjugation and electrotransformation experiments were unsuccessful. Analysis of data from NGS showed also the presence of the phenicol resistance gene fexA, and of the macrolide-lincosamide-streptogramin B (MLSB) resistance gene erm(A), located upstream and downstream of the optrA gene, respectively.
Conclusion: The emergence of oxazolidinone-resistant, optrA-positive *E. faecalis* in Italy is alarming. Remarkably, a clinical isolate of E. faecalis harboring optrA belonging to the same sequence type was previously described in China. This finding suggests the possible diffusion between two different countries of a successful clone of optrA-carrying linezolid-resistant *E. faecalis*. Surveillance studies need to be undertaken in order to limit the diffusion of these multi-resistance determinants.

P 038

ANTIBACTERIAL ACTIVITY OF EXTRACTS FROM COMMERCIAL OENOLOGICAL TANNINS

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Introduction: In recent years, plant polyphenols have received renewed attention, and a growing number of studies deals with the beneficial health properties of phenolic compounds found in food and beverages. Among plant polyphenols, tannins found in medicinal and food plants have various biological properties; in particular, hydrolyzable tannins have been cited for their antioxidant and antimicrobial activity, as well as for chemoprevention of degenerative diseases. However, commercial tannins are complex mixtures and their chemical composition is not always clearly defined. In the present work, we planned to evaluate antibacterial activity of extracts obtained from some commercial oenological tannins, produced by Silvateam Spa (http://en.silvateam.com/). More specifically, we considered the Tan'Activ R (obtained from Quercus robur oak wood); Tan'Activ FNG/A (obtained from turkish Quercus infectoria gallnuts); Tan'Activ GC (obtained from chinese Rhus semialata gallnuts) and Tan'Activ QS-SOL (obtained from Schinopsis lorentzii wood).
Materials and Methods: The four commercial tannin powders were subjected to a simple and scalable extraction procedure employing Ethyl acetate (EtOAC) and Ethanol (EtOH) as solvents. The extracts were tested for their antibacterial activity against Gram-positive (*Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus saprophyticus, Enterococcus faecium, Enterococcus faecalis*) and Gram-negative (*Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Enterobacter aerogenes, Proteus mirabilis*) pathogenic bacteria. Assays were performed by 96-well microplates method, according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI M100-S24, 2014).
Results: Tan'Activ FNG/A and Tan'Activ GC extracts inhibited the growth of all the tested Gram-positive pathogenic bacteria. Tan'Activ R and Tan'Activ QS-SOL resulted inactive against *Enterococcus faecium* Gram-positive clinical strain. The four tannin extracts showed no significant activity against Gram-negative bacteria.
Conclusions: For isolation of biological components, the need for selection of most appropriate extraction methodology is evident from the fact that when different methods are applied on same plant material with same solvent, extraction efficiency can vary significantly. In addition, the method selected as the most appropriate one also needs to be standardized in order to achieve acceptable degree of reproducibility. The results obtained suggest that the four tannin extracts represent an important source of pharmacologically active natural products. However further research is still needed for the isolation and purification of the active compounds and the determination of the mechanisms involved in the antibacterial activity.

P 039

FILTER JUGS AS SUPPORT FOR MICROBIAL BIOFILM DEVELOPMENT: POSSIBLE HEALTH IMPLICATIONS

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Introduction: There are a number of methods available to improve or enhance drinking water quality. Thus, in recent years, filtering jugs have had a remarkable growth in the European market and they have been used to improve the quality of water intended for human consumption. The advertising campaigns promise the highest water quality and excellent taste. Nevertheless, due to the lack of specific studies, the scientific community has not expressed an informed opinion on the quality and safety of filtered water. Jug filters employ a replaceable filter cartridge that typically contains a mixture of weak base ion-exchange resin, silver and granular activated carbon. The resin determines partial softening, while the activated carbon removes chlorine and some other organic chemicals. The aim of the study was to verify a possible bacterial colonization of pitchers equipped with filtering devices.
Materials and Methods: Nine different jug fil-

ters were supplied with tap water and challenged separately with *Pseudomonas aeruginosa* and *Enterobacter aerogenes* to simulate a contamination incident. For each filter tested over a five-week period, the water added with bacteria was filtered once a week. Then, drinking water was filtered more than once daily and filter jugs were maintained at ambient temperature. The filtrates were examined for the presence of these bacteria and the total aerobic bacteria at 25 °C. *Pseudomonas aeruginosa* and *Enterobacter aerogenes* were detected by miniaturized MPN techniques (Pseudalert - Colilert 18, Idexx), while pour plating culture method was used to detect aerobic bacteria.

Results: The obtained data showed a different trend in the colonization of the filters from the two bacterial strains tested. *P. aeruginosa*, typical species of the biofilm, was found in most filter cartridges (6/9), whereas *E. aerogenes* concentration decreased rapidly, compared to post-inoculum levels, in all filtrates. The aerobic bacteria were detected from the first week in all filtered water in variable concentrations (from 102 to 107 cfu/100 mL).

Conclusions: *P. aeruginosa* showed a good ability to colonize the jug filters, as opposed to *E. aerogenes*. According to the manufactures' instructions, filter cartridges should be replaced after 30 days of use and the jugs can be maintained also at ambient temperature. Nevertheless these results suggest that filters can be easily colonized at least by *P. aeruginosa*, as even by heterotrophic bacteria. Consequently, filtering jugs can be potential vehicles of infection in case of misuse/abuse by the consumer or in the event of microbial contamination.

P 040

IN VITRO ANTIBACTERIAL ACTIVITY OF HYDROLATES OF CORIDOTHYMUS CAPITATUS *(L.) RCHB. F.* AND *MENTHA SUAVEOLENS* ALONE AND IN COMBINATION

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Introduction: Hydrolates, also referred to as hydrosols or aromatic waters, are obtained during the extraction of essential oils by steam distillation. They are used in foods and cosmetics because less toxic then their corresponding essential oil, while maintaining good antimicrobial and antioxidant properties. Due to the emergence of multidrug resistance, alternatives to conventional antimicrobial therapy are needed. The growing interest in plant-derived components makes it necessary to determine their antimicrobial properties. The aim of this study was to evaluate the susceptibility of bacteria to the hydrolates of *Coridothymus capitatus (L.) Rchb.f.* and *Mentha suaveolens* alone or in combination.

Materials and Methods: Hydrolates of *C. capitatus (L.) Rchb.f.* and *M. suaveolens* (Sicilian variants) each contained 0.6% essential oil (Exentiae S.r.l., Catania). The chemical components were analyzed by Gas Chromatography-Mass Spectrometry (GC/MS). The antibacterial screening was tested against *Staphylococcus aureus* ATCC 6538, S. aureus ATCC 43300 and three clinical isolates (including methicillin-resistant *S. aureus* strains - MRSA), *Staphylococcus epidermidis* ATCC 35984, *Pseudomonas aeruginosa* ATCC 9027 and *P. aeruginosa* ATCC 14552. The checkerboard method was used to evaluate the efficacy of hydrolates in combination with each other, and in combination with tetracycline or gemifloxacin by means of the fractional inhibitory concentration index (FICI) against *S. aureus* strains. Propidium iodide staining was used to gauge alterations in membrane permeability.

Results: C. capitatus hydrolate showed a good antibacterial activity against *S. aureus* ATCC strains and clinical isolates (including MRSA), S. epidermidis ATCC 35984 and *P. aeruginosa* ATCC 14552 (MIC: 0.075-0.15% v/v). *M. suaveolens* hydrolate

activity was slightly lower against *S. aureus* ATCC 6538 (MIC: 0.3% v/v) than *C. capitatus* hydrolate, while it was the same vs *P. aeruginosa* ATCC 14552 (MIC: 0.15% v/v). A synergistic effect was observed when the hydrolates were in combination with each other (FICI: 0.5), while an additive effect when they were combined with antibiotics (FICI: 0.74-1.25). Change in cell membrane permeability was also observed.
Conclusion: These findings lay the ground for further more extensive investigations to find new organic and natural antimicrobial agents.

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P 041

EXTRAINTESTINAL PATHOGENIC *ESCHERICHIA COLI* SEQUENCE TYPE 131 SUBCLONE H30-R IN RETAIL CHICKEN MEAT, ITALY

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Introduction: Extraintestinal pathogenic *Escherichia coli* sequence type 131 (ST131), typically fluoroquinolone-resistant (FQ-R) and/or extended-spectrum β-lactamase (ESBL)-producing, has emerged globally. We assessed prevalence of subclone H30-R and its characteristics among raw chicken meat samples in Palermo, Italy.
Materials and Methods: A collection of 237 fluoroquinolones resistant and ESBL/AmpC producing *E. coli* isolates, which had been isolated from processed retail chicken meat in the period May 2013-April 2015, was analyzed. Established PCR methods were used to define ST131 and its H30 subclone, extended-spectrum β-lactamase (ESBL) and AmpC production, and plasmid-mediated quinolone resistance determinants. Amplified Fragment Length Polymorphism (AFLP) and Raman spectroscopy were performed to analyze the relatedness among ST131 isolates.
Results: 13 isolates (5.5%) belonged to the phylogenetic group B2. Based on the molecular definition of ExPEC, all these isolates were attributed with the status of ExPEC. The most prevalent virulence factors were kpsMT II and iutA. SNP-PCRs results confirmed that nine out of the 13 isolates were ST131 and, among these isolates, five isolates were positive for H30 subclone. The results of both AFLP and Raman Spectroscopy showed that, except for two isolates which proved to be indistinguishable, the isolates under study were genetically heterogeneous.
Conclusions: Among human clinical isolates, ST131, primarily its H30 subclone, accounts for most antimicrobial-resistant E. coli and is the dominant E. coli strain overall. Detecting this subclone in broiler chicken meat can strengthen the hypothesis of a food animal source for transmission to humans.

P 042

MICROBIAL AIR AND SURFACES CONTAMINATION ASSESSMENT: A FIVE-YEAR EXPERIENCE OF MICROBIOLOGICAL RISK MANAGEMENT

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Introduction: The rise of nosocomial infections has drawn attention in the last decades. "The guidelines for the definition of security and environmental hygiene standards in operating rooms" were the reference of this work that tried to make a contribution for the development of thresholdcriteria to prevent nosocomial infections, given the lack of regulations for the biological risk management in healthcare sector. The aim of this work was to assess air quality and microbial contamination on surfaces in operating rooms in order to highlight the importance of microbiological environmental monitoring in the collection of data and to minimize the risks, in a sequence of actions connected to the application of the HACCP system. Furthermore, this work intended to contribute to identify critical control points for a future harmonization of "Hospital security" based on a systematic organization of preventive operating procedures with the application of the HACCP system.

Materials and Methods: The programme involved 24 operating rooms of 6 Southern Sardinia Hospitals in agreement with the Local Health Authority. Sampling was performed every six months (2009-2013) with a total of 240 active air samples before the beginning of the activity in the operating room, 148 during the activity, 480 active air samples at the air conditioning vents and 2160 active samples on various surfaces.
Results: 47% of the examinated operating rooms proved to be out of acceptable values for air quality at least once a year over the five-year study period. Three operating rooms resulted constantly outside the limits at every sampling "at rest" over the five-year period. Over 240 active air samples "at rest", in 53% of cases mesophilic total colony units were within the threshold limits indicated by the Guidelines ISPESL. The 480 air samples collected at the air conditioning vents showed microbial contamination values that only in 10% cases proved to be within the limits indicated by the Guidelines ISPESL. Over the five- year study period, in operational conditions, 30% of the operating rooms were not compliant at least once a year. Out of a total of 148 active air samples collected during the activity, in 29% of cases mesophilic total colony units exceeded the threshold values.
Conclusions: This work's results reinforce the convinction that the organization of hospital security urgently requires a systematic model of organization based on HACCP system so that common sense or a few people's dedication will not prevail in hospital infective risk management.

P 043

BIOSURFACTANT-BASED COATINGS FOR THE INHIBITION OF BIOFILM FORMATION ON MEDICAL-GRADE SILICONE

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Introduction: Biofilms play a pivotal role in healthcare-associated infections related with medical devices use. Once a mature biofilm is formed, the microorganisms embedded within this complex structure become highly resistant to antimicrobial agents and host immune response. Nowadays, the use of medical surfaces coated with antimicrobial agents is a common biofilm preventive strategy and biosurfactants have recently emerged as a potential new generation of anti-adhesive and anti-biofilm agents with enhanced biocompatibility.
Materials and Methods: The ability of the biosurfactants AC7 and R89 (AC7BS, R89BS) to inhibit biofilm formation of *Candida albicans, Staphylococcus aureus* and *Staphylococcus epidermidis* on medical-grade silicone disks was investigated by crystal violet staining and MTT assays. The selected biomaterial was functionalized with biosurfactant (BS) solutions, at the concentration of 2 mg/mL, following three different surface modification strategies: direct BS physical absorption and plasma treatment or 3,4-Dihydroxy-L-phenylalanine (L-DOPA) treatment followed by BS absorption. The composition of the crude extracts was evaluated by ESI-MS analysis.
Results: Chemical characterization revealed that AC7BS is a mixture of surfactin and fengycin and R89BS is a mixture of mono- and di-rhamnolipids. In general, the highest inhibition of adhesion and biofilm growth were observed on disks treated by direct BS physical adsorption and on those treated with plasma followed by BS absorption. For these coating strategies, the mean inhibition of *C. albicans* adhesion after 90 min incubation was 71%, whereas the mean reduction of biofilm biomass after 24 h was 50%. The highest inhibition of S. aureus biofilm formation was observed on R89BS

treated disks (69%) rather than on AC7BS treated disks (43%). Similar results were observed for *S. epidermidis* (64% biofilm inhibition on R89BS treated disks and 21% on AC7BS treated disks). On the other hand, L-DOPA treatment combined with AC7BS or R89BS was not useful to inhibit *C. albicans* and *S. aureus* biofilm growth but reduced *S. epidermidis* biofilm formation of 48% and 63%, respectively. Finally, AC7BS did not inhibit fungal and bacterial planktonic cells, indicating anti-adhesive but no antimicrobial activity. Similarly, R89BS had no activity against *C. albicans* and *S. epidermidis* planktonic cells, whereas for S. aureus a Minimum Inhibitory Concentration of 0.06 mg/mL was observed.

Conclusions: It can be concluded that AC7BS and R89BS are able to significantly inhibit fungal and bacterial biofilm formation on silicone and plasma treatment prior to BS adsorption is a promising method for BS functionalization of medical-grade silicone.

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P 044

PHENOTYPIC COMPARISON OF VIRULENCE-ASSOCIATED TRAITS BETWEEN A NEW SEQUENCE TYPE AND ITALIAN ENDEMIC INTERNATIONAL CLONES OF *ACINETOBACTER BAUMANNII*

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Introduction: *Acinetobacter baumannii* is an opportunistic Gram-negative pathogen that has emerged in recent decades as a worldwide cause of nosocomial infections associated with elevated morbidity and mortality. The sequence type (ST)2 and the ST78 are commonly isolated in Italian hospitals; ST2 belongs to the international clonal (IC) 2 and has a wide geographical distribution, while ST78 belongs to the IC 6 and is known as the 'Italian clone' being mainly found in Italy and Mediterranean countries. In a previous study on Extensively Drug-Resistant *A. baumannii* clinical isolates from respiratory specimens, we have identified an isolate belonging to ST632. This new ST seemed to be related to ST2, although exhibited differences both in the rpoB allele and in the pulse field macrorestriction pattern. The aim of present study was to compare phenotypic virulence-associated traits of isolate #237 belonging to ST632 to those of isolates belonging to ST2 and ST78.

Material and Methods: Motility on semi-solid LB agar plates, biofilm-forming ability on polystyrene microtiter plates, lipopolysaccharide (LPS) and outer membrane protein (OMP) extractions were studied by standard techniques. The number of bacteria that adhered to, invaded and persisted within human lung epithelial cells A549 was determined for each isolate. The reference strain ATCC 17978 was included as control. Bacterial invasion was examined by fluorescence microscopy, following DAPI and rhodamine phalloidin staining.

Results: The clinical isolate #237 (ST632) was capable of surface-associated motility although limited with respect to the reference strain. Moreover, #237 showed a low biofilm-forming activity, low adhesion to and invasion of A549 cells. Conversely, both isolates #36 (ST78) and #150 (ST72) invaded and persisted within A549 cells. Interestingly, the non-motile isolate #36 was the higher biofilm producer but displayed a low adhesiveness to cells. Instead, a remarkable adhesion to cells was detected in the non-biofilm-forming isolate #150. Since bacterial-surface components are involved in virulence, LPS types, and OMP profiles were compared among isolates highlighting several differences. How and whether these differences are involved in virulence, it remains to be investigated.

Conclusions: Despite the genetic similarities, ST632 seemed to be less virulent in terms of adherence to, invasion and persistence within epithelial cells as biofilm forming activity, and motility. Since it is known that both LPS and the OMPs are associated with bacterial virulence, we hypothesize that these differences might explain the variation in the phenotypic traits observed among isolates (i.e. motility, adhesion to biotic and abiotic surfaces, invasion and persistence within cells).

P 045

NOSOCOMIAL OUTBREAK OF COLISTIN-RESISTANT OXA-48-PRODUCING *KLEBSIELLA PNEUMONIAE* FROM BLOODSTREAM INFECTIONS, GREECE

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Introduction: OXA-48 is a class D carbapenemase originally identified in isolates from Turkey and subsequently detected in several European and North African countries. This enzyme is mostly found in isolates of *Klebsiella pneumoniae* and *Escherichia coli* and shows a peculiar spectrum of activity, being able to hydrolyze carbapenems (although weakly), penicillins, and narrow-spectrum cephalosporins but not expanded-spectrum cephalosporins. In Greece a hospital outbreak of OXA-48-producing ST11 *K. pneumoniae* has been described in 2012, even though nosocomial outbreaks caused by KPC- and VIM-producing *K. pneumoniae* are most commonly reported. In the present study, we investigate an ongoing nosocomial outbreak of colistin-resistant OXA-48-producing *K. pneumoniae* isolates from bloodstream infections that started in June 2014.
Materials and Methods: Eighteen isolates of OXA-48-producing *K. pneumoniae* from blood cultures were collected from different patients during the period June 2014-March 2016 at the Tzaneio General Hospital of Piraeus (Greece), the same hospital that experienced the outbreak in 2012. Species identification was performed with the Vitek 2 automated identification system (bioMérieux, Marcy l'Étoile, France) and MICs were determined by broth microdilution according to CLSI guidelines. Fosfomycin susceptibility testing was performed by agar dilution according to CLSI. EUCAST clinical breakpoints were used for the interpretation. MLST was carried out following the Pasteur Institute scheme and a PCR-sequencing approach was used for the analysis of blaOXA-48 and mgrB genes. The clonal relationship was examined by pulsed-field gel electrophoresis (PFGE).
Results: Four of the 18 OXA-48-producing isolates exhibited a pandrug-resistant (PDR) phenotype being non-susceptible to all tested antibiotics, including colistin, while the remaining isolates showed variable susceptibility to gentamicin, fosfomycin and tigecycline (extensive drug-resistant, XDR, phenotype). In addition to OXA-48 all PDR strains produced CTX-M type ESBL. In all isolates, colistin resistance was due to insertional inactivation of the mgrB gene. Results from PFGE showed two different pulsotypes (A and B). MLST assigned all PDR isolates to ST147 and XDR to ST101, pulsotype A and B, respectively.
Conclusions: In the present study, we describe an ongoing nosocomial outbreak of OXA-48-producing *K. pneumoniae* in Greece caused by the spread of two different clones belonging to ST147, isolated in the first six months of the outbreak, and ST101, collected from 2015 to now. Of note, all ST147 strains showed a PDR phenotype, which seriously limits the therapeutic options for the treatment of such severe infections.

P 046

ANTIBACTERIAL PROPERTIES AND CYTOTOXICITY OF *SATUREJA MONTANA L.* AND *CORIANDRUM SATIVUM L.*

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Introduction: Essential oils (EOs) are used in traditional medicine due to recognized therapeutic properties, namely anti-microbial and cytotoxic

activities. Coriander (*Coriandrum sativum L., Apiaceae*) (CDO) and *Satureja montana, L. Lamiaceae* (SGG), are herb widely used as a spice, in food and pharmacy industries and in folk medicine. This study aims to analyze the antibacterial properties of CDO and SGG towards multidrug resistant (MDR) strains. Toxicity in eukaryotic cell lines it has been also valuated.

Materials and Methods: The CDO and SGG (Talia, Rome) compositional analysis was evaluated by gas chromatography and mass spectrometry (GC-MS). MDR uropathogenic *Escherichia coli* ECP19 and ECP32 were isolated at Hospital "Umberto I" Rome. Identification and susceptibility tests were performed by automated VITEK-2 System. *E. coli* ATCC 25922 was used as reference strain. The combined antimicrobial activity of EOs and gentamicin was reported as the fractional inhibitory concentration index (FICI). Bacterial cell modifications was evaluated by propidium iodide staining and electron microscopy. The *in vitro* cytotoxicity of CDO and SGG against human tumor cell lines (Hep-2) was determined by the MTT assay.

Results: The chemical analysis of essential oils showed that the main component of CDO was Linalool (74,7%) whereas in the SGG Carvacrol (51%), Tymol (11%), Terpinene (5,5%) and Cymene (13%). MICs and MBCs of SGG showed a better inhibitory activity respect to CDO values. The FICI values showed a positive interaction between EOs and gentamicin: additive for CDO and synergic for SGG. This effect can be explained by a greater penetration of the antibiotic due to changes of membrane structures as evidenced by propidium iodide staining and electron microscopy studies. Regard Hep-2 cell lines, SGG was able to inhibit cell proliferation more efficiently compared to CDO.

Conclusions: The preliminary results, showing a better biological activity of SGG compared with CDO, encourage further studies to highlight the main active components and their action mechanism on prokaryotic and eukaryotic cells.

P 047

ANTIMICROBIAL ACTIVITY OF NATURAL MOLECULES EXTRACTED FROM *HELICHRYSUM ITALICUM* AGAINST *STAPHYLOCOCCUS EPIDERMIDIS*

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Introduction: *Staphylococcus epidermidis* is able to form biofilm and is involved in infectious diseases related to indwelling medical devices, such as peripheral or central intravenous catheters. Bacterial biofilms are highly organized surface-associated communities of bacteria encased within a self produced extracellular matrix. Bacteria within a biofilm exhibit distinct phenotypes from planktonic cells, particularly with respect to growth and gene expression. Biofilm infections are rarely resolved by host defence mechanism and antibiotic therapy generally fails to kill bacteria residing within biofilm. In addition, the increasing emergence of drug-resistant bacteria highlight the need of a continuous screening of new antimicrobials. We reported the antimicrobial activity of new compounds extract of *Helichrysum italicum*, a medicinal plant used in folk medicine as an anti-inflammatory and anti-infective plant.

Materials and Methods: The *H. italicum* compounds dissolved in dimethylsulfoxide and diluted in Luria-Bertani, have been tested for their anti-microbial effect against *S. epidermidis*. The strain has been cultured in LB broth at 37°C. The compounds were added to bacterial cell suspension and the bacterial growth was monitored, measuring the OD in a microtiter plate reader at 600 nm of absorbance. For the rate of killing bacteria the compounds were added to bacterial suspension and incubated at 37 °C for 0, 4, 8, 16 and 24h, after incubations at 37 °C colonies were counted on LB agar by dilution titration method. To assess the ability of molecules to inhibit biofilm formation, compounds were added to microtiter plate at the time of inoculation and the cells were allowed to form biofilms. The biofilm was measured at 570 nm in an microplates reader after Cristal violet (1%) dyes.

Results: *S. epidermidis* growth was differently

affected by treatment with the compounds tested. The 2(FEA/5/4) induced the highest percentage of growth reduction and the best bactericidal activity, 1(FEA/6/4) shows only a bacteriostatic activity, while the other compounds were less efficacious in terms of CFU reduction. In addition compounds 2, 9(FEAC14/88/80) and 20 (FEAT/7/1) were able to inhibit biofilm formation. Compound 2 displayed the best result.
Discussions: Although antibiotic therapy generally reverses the symptoms caused by planktonic cells, bacteria within a biofilm are resistant to antibiotic or biocide treatment. In this context, identification of natural compounds which can limit the formation of bacterial biofilms represent an important task for the researchers. Even though this is a preliminary screening, the force of our study is the ability of natural products to inhibit biofilm formation and to exhibit also a microbicidal activity.

P 048

DIFFERENTIAL FEATURES OF THE TWO CLADES OF ST258 KPC-PRODUCING *KLEBSIELLA PNEUMONIAE* CAUSED BY DIFFERENT EXPRESSION OF THE MRK FIMBRIAL SYSTEM

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Introduction: Dissemination of KPC-producing *Klebsiella pneumoniae* is attributed to the expansion of a dominant Clonal Group, CG258 (including all single locus variants of ST258). Over the past decade, CG258 clonal stability has remained high. However a certain degree of diversity in the cps locus was observed and is at the basis of the differentiation in two distinct sublineages, named clade 1 and clade 2. Strains belonging to clade 2 are more prevalent, geographically disseminated and less virulent in the animal model. Genome comparison of two strains, representative of the two clades of ST258, KK207-1 and KKBO-1 (clade 1 and clade 2, respectively), enabled us to identify an adjunctive difference in the mrk, mannose-resistant, fimbrial gene cluster. The presence of a functional Mrk system has been implicated in biofilm formation and ability to colonize biotic and abiotic surfaces. In this work we investigated the MrkA structural subunit expression in relationship with biofilm formation ability of KK207-1 and KKBO-1 strains.
Materials and Methods: cDNA isolated from *K. pneumoniae* strains was used for quantitative real-time PCR (qRT-PCR) of mrkA gene as previously described. Reactions were performed using the LightCycler instrument. Results normalization was performed against the rpoD housekeeping gene. Biofilm assay was performed using the Calgary Biofilm Device in Cation Adjusted Mueller Hinton Broth (3 days of incubation at 35°C and 100 r.p.m.). Data were obtained in two independent experiments (eight replicates per experiment). Statistical differences were determined by the unpaired t-tests (Graph-Pad Prism 6).
Results: Sequences analysis showed that in KKBO-1 (clade 2) the mrkABCDFHIJ genes cluster is intact, while in the KK207-1 genome (clade 1), the mrkH positive transcriptional regulator is disrupted by an ISEc21-like element. From a screening of the NCBI database we found that this alteration is typical of ST258 clade 1 genomes. qRT-PCR results demonstrated that mrkA expression was 6.2 ± 0.7 fold lower in KK207-1 than in KKBO-1, in agreement with the disruption of the positive regulator. These differences translated into differences of biofilm formation, with KK207-1 showing a significantly decreased ability to form biofilm *in vitro* (3.3 log ± 0.3 CFU/peg) compared to KKBO-1 (5.4 log ± 0.4 CFU/peg) ($p < 0.05$).
Conclusions: The KKBO-1 strain, representative of ST258 clade 2, has an enhanced biofilm formation ability compared to KK207-1 (clade 1). The difference, likely caused by an alteration in the Mrk fimbrial system, could be responsible for diverse adhesion ability and relationship with the host immune response, and could be one of the reasons of the observed predominance of ST258 clade 2 isolates in the clinical setting.

P 049

INDIVIDUATION AND EVALUATION OF NEW BACTERIOPHAGES FOR THE TREATMENT OF CYSTIC FIBROSIS BIOFILM-RELATED LUNG INFECTIONS CAUSED BY *PSEUDOMONAS AERUGINOSA*

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Introduction: *Pseudomonas aeruginosa* represents a serious problem in the management of cystic fibrosis (CF) patients, especially because of its biofilm lifestyle. *Lytic bacteriophages* (phages) might provide an alternative and innovative option for the treatment of *P. aeruginosa* infections. The aim of this study is the isolation and characterization of new phages with lytic activity against *P. aeruginosa* to define their potential for the treatment of CF lung infections.
Materials and Methods: Phages were isolated and purified from sewage by the double agar layer method. The following *P. aeruginosa* strains were considered: 6 (5 from CF patients, and PAO1) for phages detection, 33 CF strains for assessing anti-biofilm activity. Five phages were selected based on their lytic spectra to evaluate the anti-biofilm effect by exposing 24 h-old biofilm to each phage at a MOI (Multiplicity-Of-Infection) of 1 and 100 for 24 h. The total biofilm biomass was measured by crystal violet stain. Morphological characterization of phages was performed by scanning electron microscopy (SEM).
Results: Overall, 22 phages from 5 different water sources were active against PAO1 and one CF strains, and 5 phages (4_ZP1, 9_ZP2, 14_OBG, 17_OBG, 19_OBG) were identified as the most promising to go into further characterization. SEM analysis showed that all phages were tailed. Exposure to phages caused significant increase in biofilm biomass by 18 (MOI: 1) and 12 (MOI: 100) *P. aeruginosa* strains, whereas a decrease was observed in 10 (MOI 1) and 24 (MOI 100) strains. The biomass reduction in biofilm treated with MOI 100 after 4 h-exposure was higher than that obtained after 24 h-exposure. Therefore, the effect of phage exposure on growth of planktonic cells of selected strains (PaPh4, PaPh6, PaPh22, and PaPh28) was evaluated. The highest lytic activity was observed by the first 6 h of treatment, followed by a bacterial regrowth, while phages showed 2-Log increased concentration, thus suggesting that bacterial regrowth was not caused by phage death.
Conclusions: Overall, our results showed that the phage activity on *P. aeruginosa* biofilm is phage-strain specific and MOI-dependent. Further, we confirmed that bacteria could acquire resistance to phage already after the first hours of exposure. Further studies are ongoing on the safety of phage preparation for therapeutic use, and on phage training that, developing more effective virus, could offer the possibility to adapt our library of phages to a wide range of CF clinical strains of *P. aeruginosa*.

P 050

WHOLE GENOME SEQUENCE OF *CLOSTRIDIUM DIFFICILE* BELONGING TO RT018

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Introduction: *Clostridium difficile* infection (CDI) is one of the most common hospital-acquired infections and the leading cause of antibiotic-associated diarrhoea and pseudomembranous colitis. Increasing CDI incidence rates have been mainly attributed to successful clones belonging to few predominant ribotypes (RTs). RT018 has been reported as one of the most prevalent genotype circulating in in Italy and represents the dominant genotype circulating in Florence hospitals settings, with a prevalence rate higher than 70%. This ribotype is highly transmissible and it is associated with com-

plicated outcomes. Here we report on results from whole-genome-sequencing of *C. difficile* CD8-15, belonging to RT018.
Materials and Methods: *C. difficile* CD8-15 was collected from a patient with fatal CDI. The isolate was subjected to ribotyping following the method described by Bidet et al. MICs of rifampicin, levofloxacin, erythromycin, and clindamycin were determined by E-test strips placed on Brucella Blood Agar supplemented with haemin and vitamin K. Genomic DNA was subjected to whole-genome sequencing (WGS) using the MiSeq platform (Illumina Inc., San Diego, CA). The draft genome was then subjected to the following in silico analyses: i) Multi Locus Sequence Typing (MLST) (http://pubmlst.org/cdifficile/); ii) toxinotyping (http://www.mf.uni-mb.si/mikro/tox/); iii) detection of antibiotic resistance mechanisms, using the *C. difficile* 630 strain as reference.
Results: Ribotyping analysis assigned *C. difficile* CD8-15 to RT018. Antibiotic susceptibility tests showed that the strain was resistant to rifampicin, fluoroquinolones, and macrolides, leading to a multi-drug resistant phenotype. According to WGS results, the extimated genome size of CD8-15 was 4,249,791 bp. *C. difficile* CD8-15 was assigned to ST17 by MLST analysis and was classified as non-variant strain (toxinotype 0) producing toxin A and B, and not the binary toxin CDT by toxinotyping. Sequence analysis revealed the presence of point mutations accounting for resistance to rifampicin and fluoroquinolones, while resistance to macrolides was likely attributable to the presence of a multidrug efflux system encoded by the cme gene.
Conclusions: *C. difficile* RT018 has been regarded as one of the most prevalent genotype circulating in Italy. Here we examined the major genetic features of RT018 *C. difficile* CD8-15 in order to gain better insights into the spreading abilities and antibiotic resistance mechanisms of such epidemic clone.

P 051

WIDESPREAD AND PROLONGED DISSEMINATION OF A SUCCESSFUL VIM-1-ENCODING PLASMID AMONG CLINICAL ISOLATES OF DIFFERENT *PSEUDOMONAS SPECIES*

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Introduction: Among P*seudomonas spp.*, carbapenem resistance can result from the acquisition of metallo-β-lactamases (MBLs), with VIM-type enzymes being the most common acquired MBLs in *P. aeruginosa*, and to a lesser extent, in other Pseudomonas species. The diffusion of VIM-type MBLs is mediated by mobile gene cassettes inserted into integrons that, in *P. aeruginosa*, are usually chromosomally located. Descriptions reporting a plasmid-mediated acquisition are limited, and the nature of plasmids carrying blaVIM in *Pseudomonas spp.* remains largely unexplored. Here we report on the characterization of VIM-1-encoding plasmids obtained from different *Pseudomonas spp.* of clinical origin, isolated over a 20-years period.
Materials and Methods: *P. mosselii* AM/94 was isolated in 1994 from the lower respiratory tract of an inpatient in Genoa, *P. aeruginosa* C/57 was isolated in 2014 from a bloodstream infection from an intensive care patient from Milan, and *P. mendocina* AOUC-01/15 was isolated in 2015 from the bronchial aspirate of an inpatient from Florence. Bacterial identification was carried out by MALDI-TOF and confirmed by 16S and rpoD gene sequencing. Antimicrobial susceptibility testing was carried out by reference broth-microdilution method. Plasmid sequencing was performed using the Illumina MiSeq and data were analyzed using SPAdes and RAST. Sequence alignments were performed using Mauve and BLAST.
Results: Sequencing revealed nontypeable plasmids of variable size, ranging from 52-55kb for pMOS94 and pMEN15 to 105kb for pAER57. The three plasmids shared almost identical essential backbone regions and were highly related to

previously sequenced VIM-1-encoding plasmids (pPC9 and pAMBL2) from *P. aeruginosa* clinical strains isolated during 2000s in France and in Spain, suggesting a possible evolution from a common ancestor. Plasmids pMOS94 and pMEN15 showed minimal rearrangements in their structures, and mainly differed in the independent acquisition of distinct mobile genetic elements. Conversely, pAER57 showed a more complex architecture, carrying also a large region sharing high sequence identity with a portion of the pWW0 catabolic plasmid described in an environmental isolate of P. putida. In all plasmids, the blaVIM-1 gene was embedded in a class I integron resembling the structure of In70 and differing from it for the acquisition of additional aminoglycosides and sulphonamides resistance genes or for the shuffling of gene cassettes.
Conclusions: Our findings support the evidence for a key role of plasmids in acquisition and spread of MBLs in *Pseudomonas spp.*, expanding the knowledge about resistance plasmids circulating in this genus and revealing the existence of a plasmid lineage able to promote a wide dissemination of blaVIM-1 over a prolonged period.

P 052

LONG-LASTING PERSISTENCE OF NDM-1-ENCODING PLASMIDS IN MULTIPLE ENTEROBACTERIAL SPECIES IN A PEDIATRIC PATIENT

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Introduction: The emergence of the New Delhi Metallo (NDM) β-lactamase constitutes a critical and growingly important medical issue. This enzyme, frequently detected in *Escherichia coli* and other *Enterobacteriaceae*, compromises the efficacy of almost all β-lactams, including the last resort carbapenems. Genes encoding NDM-type enzymes are usually carried by conjugative plasmids, often harbouring additional resistance determinants against several antibiotic classes. In this work we describe a long-lasting persistence, of at least 1 year, of NDM-1-encoding plasmids in several members of Enterobacteriaceae in a pediatric patient.
Methods: A total of 6 blaNDM-1 positive isolates (*Klebsiella pneumoniae*, n = 3; *E. coli*, n = 1; *Serratia marcescens*, n = 1 and *Citrobacter freundii*, n = 1) were collected from different sites from a pediatric patient of Balkan origin admitted to the Meyer Children Hospital, Florence. Clonality of *K. pneumoniae* isolates was investigated by PFGE. MLST of the *E. coli* and *K. pneumoniae* isolates was performed as previously described. Plasmids transfer was attempted by electroporation and conjugation in *E. coli* laboratory hosts. Antimicrobial susceptibility testing was performed on donor and recipient strains. Plasmid sizes were estimated with S1 nuclease digestion, followed by Southern blotting and detection with a specific $bla_{\text{NDM-1}}$ probe; transferred plasmids were subjected to next generation sequencing (NGS) using a 2x250 paired end approach. Raw data from NGS were analyzed by standard bioinformatic pipelines.
Results: Isolates carrying NDM-1-encoding plasmids were recovered during one year of observation from different sites. *K. pneumoniae* isolates were identical according to PFGE, leading to the selection of the first and the latter isolates for further analysis. Plasmids were transferred both by electroporation and conjugation into *E. coli* hosts; S1 digestion indicated that the estimated plasmid sizes were ~100-120 kb. Recipient cells carrying NDM-1 positive plasmids were not susceptible to all tested β-lactams, except aztreonam, but retained susceptibility to ciprofloxacin, fosfomycin, colistin and trimethoprim/sulfamethoxazole. Variable phenotypes have been observed for aminoglycosides. NGS results demonstrated that plasmids belonged to the IncL/M incompatibility group, with the exception of the plasmid from *E. col*i that was typed as IncA/C group. The immediate genetic context of blaNDM-1 was conserved in all plasmids, and the overall genetic structures of IncL/M-type plasmids were highly related.
Conclusions: To the best of our knowledge, this is the first report of carriage of NDM-1-encoding plasmids in four different species from a single patient. Genetic differences detected from the plasmids deriving from the first and the last isolates

give precious information on the *in vivo* evolution of bla_{NDM-1} carrying plasmids.

P 053

ALTERATION OF THE PMRB SENSOR KINASE RESPONSIBLE FOR COLISTIN RESISTANCE IN AN *ESCHERICHIA COLI* CLINICAL ISOLATE

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Introduction: In the family of *Enterobacteriaceae,* colistin resistance (Colr) was mainly associated to chromosomal mutations, involving gene of the *pmrHFIJKLM* operon and leading to the modification of the colistin target (lipid A). By contrast, recently, a plasmid mediated mechanism of Colr (*mcr-1* gene) in *Escherichia coli* has been described. In the first paper reporting the detection of *mcr-1* positive *E. coli* in Italy, nine Colr strains were investigated. Eight of these strains were positive for *mcr-1* gene while the ninth showed an unknown Colr mechanism. In this work we studied the mechanism responsible for the acquisition of colistin resistant trait in this *mcr-1* negative *E. coli* isolate.
Materials and Methods: The *E. coli* LC711/14 was isolated in an hospital located in Northern Italy, from an urine sample of an outpatient. *E. coli* BW25113 derivative, belonged to the Keio collection, was used as deleted mutant of the *pmrB* gene (*E. coli*_Δ*pmrB*). For complementation experiments, a copy of a functional wild-type *pmrB* gene and some flanking regions were cloned into the pACYC-184 (pACYC184-*pmrB*). The plasmid pACYC184-$pmrB^{T29C}$ was a derivative of pACYC184-*pmrB* containing the $pmrB^{T29C}$ gene generated by direct mutagenesis. pACYC184-$pmrB^{T29C}$ plasmid and, as control, pACYC184 were used to transform *E. coli*_Δ*pmrB*. Quantitative real-time PCR (qRT-PCR) by ΔΔCT (delta delta threshold cycle) method (relative), was used to measure the expression of the pmrK gene.
Results: The *E. coli* LC711/14 showed a multi-susceptible phenotype and was resistant to colistin only (MIC = 8 μg/ml). PCR sequence analysis revealed a single nucleotide polymorphism (t29c) in the *pmrB* gene encoding for the sensor kinase of the two component system PmrAB responsible for positive regulation of pmrHFIJKLM operon. The mutation t29c resulted in a non-synonymous leucine->proline aminoacidic substitution at position 10 of the protein sequence. Complementation of LC711/14 with pACYC184_*pmrB* restored susceptibility to colistin (MIC = 1 μg/ml). The *E. coli*_Δ*pmrB* (MIC = 0.125 μg/ml) after transformation with pACYC184-$pmrB^{T29C}$ acquired resistance to colistin (MIC = 8 μg/ml). On other hand transformation with pACYC184-*pmrB* did not change the susceptibility level. Analysis of the pmrK expression revealed increased levels in all the Colr strains compared with BW25113.
Conclusions: This finding underlines the ability of *E. coli* isolates to evolve towards colistin resistance phenotype through a number of different mechanisms. This work represents the first characterization of an *E. coli* clinical isolate whose resistance to colistin was associated with a point mutation occurred in the *pmrB* gene.

P 054

BACTERIAL KERATITIS: A RETROSPECTIVE STUDY IN OPHTHALMOLOGIC HOSPITAL OF TURIN FROM 2010 TO 2015

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Introduction: Bacterial keratitis is a serious ocular infection that can cause severe visual loss if treatment is not initiated at an early stage. Common risk factors for bacterial keratitis include contact lens wear, ocular trauma, ocular surface disease, ocular surgery, lid deformity, chronic use of topical steroids, contaminated ocular medications or solutions, and systemic immunosuppression.
Material and Methods: This is a monocentric observational retrospective study from 2010 to 2015

in all suspected infective keratitis.
Cultures were grown using both liquid (heart-brain broth) and solid (chocolate agar, blood agar) media. All samples have been identified with standard laboratory procedure. Susceptibility test was conducted using Kirby Bauer method. In case of severe prognosis infections (corneal abscess, deep corneal ulcer) we employed Bactec Peds Plus ™/F culture vials.
Results: In this study, we isolated 823 positive samples with corneal infections. By sample culture, Gram-positive cocci were the predominant causes of infections 536 = 65%. In particular, most frequently isolated organisms were: *Pseudomonas aeruginosa* 194 = 24%, CONS 177 = 22%, *Staphilococcus aureus* 167 = 21%, followed by *Streptococci* 141 = 17% and *Serratiae* 47 = 6%. Interessantly *Pseudomonas aeruginosa* showed a higher susceptibility to Ciprofloxacine, Norfloxacine and Lomefloxacina, while *Staphylococcus aureus* was more susceptible to Netilmicine, Cloramphenicol and Tetramicine and Staphylococcus Coagulase Negative showed susceptibility to Netilmicine, Cloramphenichol, Tetramicine.
Conclusion: Identification of causative pathogens is an important step in the management of infectious diseases. Although some Authors recommend liberal empirical use of antimicrobial agents, pathogen identification and its susceptibility patterns is important to avoid treatment failure and the development of drug resistance.

P 055

DIRECT IDENTIFICATION OF MICRORGANISMS FROM POSITIVE BLOOD CULTURE BY MALDI-TOF

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Introduction: Blood stream infections represent a relevant cause of morbidity and mortality, the impact of which can be reduced by timely initiating of appropriate antibiotic administration. In recent years, MALDI-TOF (Matrix Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry) technology has been used for rapid identification from colony. A relevant application of this technology could consist in the direct identification of microrganisms from positive blood culture, although there is no consensus on operative protocols and no standardized methods are available. In this study, a methodological approach to this application has been investigated by evaluating the potential for identification of different microrganisms.
Materials and Methods: Over a period of 6 months, at the Blood Culture Laboratory, University Hospital Città della Salute e della Scienza di Torino (Turin, Italy), 158 blood culture positive specimens (incubated on the BacT/ALERT bioMérieux instrument using BacT/ALERTPlus bottles) were studied. Following Gram staining, specimens were seeded directly onto Blood Agar plates and incubated at 36+/-1°C for 3, 5 and 24 hours. Subsequently, MALDI-TOF assay was performed from the growth patina using the Vitek MS (bioMérieux) isntrument. Results were compared with those obtained by automated biochemical identification assay (MicroScan Walkaway plus, Beckman Coulter).
Results: Gram staining evidenced the presence of Gram positive cocci in 115/158 (72.8%) specimens, Gram negative bacilli in 32/158 (20.2%), Gram positive bacilli in 3 (1.9%) and yeasts in 8 (5.1%). Considering Gram positive cocci, at 3 hours, specie and genus identification were obtained in 66% and 70.4%, respectively; at 5 in 91.3% and 92.2%; considering Gram negative bacilli, at 3 hours in 78.1% and 78.1%; at 5 hours in ‘87.5% and 90.6%; for Gram positive bacilli, no specie identification was obtained at 3 and 5 hours, whereas 1/3 genus identification was achieved at 5 hours. No yeast specie and genus identification was obtained. No difference was observed at 24 hours.
Conclusions: MALDI-TOF technology has proved to be suitable for identifying most of etiological agents of bacteremia and sepsis, particularly Gram positive cocci and Gram negative bacilli, already at 3 hours of incubation with further 10-20% increase at 5 hours. The limited number of specimens positive for Gram positive bacilli and yeasts did not allow for an adequate evaluation. The use of MALDI-TOF for direct identification of microrganisms from positive blood culture represents an interesing diagnostic application, being able to provide informations on genus and specie identification for a timely clinical and therapeutic management.

P 056

ANTIBIOTIC RESISTANCE PATTERNS AND GENETIC DIVERSITY OF *CAMPYLOBACTER COLI* AND *CAMPYLOBACTER JEJUNI* ISOLATED FROM BROILER CHICKENS

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Introduction: *Campylobacter jejuni* and *Campilobacter coli* are food-borne pathogens that cause gastroenteritis in humans, representing the most commonly reported zoonosis in the European Union. *Campylobacter* has developed resistance to several antimicrobial agents over the years, and this fact represents a significant public health problem. The present study was carried out to assess the genetic diversity of *C. coli* and *C. jejuni* isolated from 8 commercial broiler farms in the Center of Italy, using s-AFLP typing. The susceptibility of *C. coli* and *C. jejuni* isolates to various antibiotics, and the possible relationship between specific genotypes and antibiotic resistance were also evaluated.
Materials and Methods: 99 *C. coli* and 41 *C. jejuni* were recovered from cloacal swabs and carcasses of chicken broiler from eight farms. The samples were processed according to ISO 10272 standard procedure. Strains phenotypically classified as *Campylobacter* were identified by multiplex PCR and typed using the s-AFLP technique. The susceptibility to antibiotics (gentamicin, streptomycin, ciprofloxacin, tetracycline, erythromycin, nalidixic acid, and chloramphenicol) was evaluated with the microdilution method. Statistical analysis was performed.
Results: The AFLP types were strongly associated with farm ($P < 0.0001$) with the presence of a characteristic *Campylobacter* coli s-AFLP genotypes within the same farm. The s-AFLP typing of C. jejuni has revealed, instead, the presence of different patterns and clusters within the same farm, indicating the genetic diversity between strains of *C. jejuni*. We found higher rates of resistance in C. coli than in C. jejuni, to ciprofloxacin (70% vs. 39.0%), to nalidixic acid (70% vs. 39.0%), to tetracycline (70% vs. 10%), and to erythromycin (30% vs. 0%) Conversely, rate of streptomycin resistance was higher in C. jejuni than in C. coli (29.% vs. 0)
Conclusions: The polymorphisms resulting from AFLP analysis were higher within C. jejuni strains than in C. coli. The investigation on antimicrobial resistance showed differences in antimicrobial resistances between C. jejuni and *C. coli* with the latter generally more resistant. We observed high prevalence of fluoroquinolone- and tetracycline-resistant *Campylobacter*. Moreover 30% of *C. coli* isolates were resistant to erythromycin, the drug of choice in human *Campylobacter*iosis treatment. Even thoug the incidence of resistance in human *Campylobacter* isolates is still relatively low, it could become higher, considering the fact that the resistance to erytromycin has been increasing at the production level. With respect to the AFLP type in each farm there was no correlation between genotype and antibiotic resistance patterns.

P 057

GUT MICROBIOTA PROFILE IN PATIENTS WITH INFLAMMATORY BOWEL DISEASE

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Introduction: Several lines of research have provided compelling evidence that the human microbiota plays an important control on various metabolic pathways of the host; also, it may be involved in the pathogenesis of bowel disorders, through the metabolic capacity of the luminal and mucosa-associated microbes to influence the host via immune-microbial interactions. Inflammatory bowel disease (IBD) refers, mainly, to ulcerative colitis (UC) and Crohn's disease (CD), which are diseases of the digestive tract with similar clinical, pathological and epidemiological features. Recent evidence indicates that the intestinal microbiota may play a role in initiating, maintaining, and determining the phenotype of IBD. The aim of the study was to analyze the gut microbial community in faecal samples of IBD in comparison with healthy controls.

Materials and Methods: Overall, 179 stool samples from patients with CD (n = 50), patients with UC (n = 77), and health controls (n = 52) were collected. Total DNA was extracted, purified and quantified from each faecal sample. After amplification with qRT-PCR to estimate the total bacterial load, barcoded amplicon libraries for the bacterial community analysis were generated using primers targeting the V3 and V4 hypervariable region of the bacterial 16S rRNA gene and Nextera XT index kit (Illumina, Inc.). Samples were pooled and sequenced with Ilumina MiSeq platform and data were analysed using the 16S Metagenomic App and the MiSeq Reporter software.
Results: Regardless of the type and the state of disease, *Actinobacteria* and *Proteobacteria* phyla were more abundant in IBD patients than in controls ($p < 0.01$ and 0.05, respectively), as well as sequences representative of the *Firmicutes* ($p < 0.01$). On the other hand, *Bacteriodetes* ($p < 0.0001$) were substantially depleted and the overall microbial diversity reduced ($p < 0.0002$) in samples from IBD patients. With regard to the genera detected, *Bifidobacterium, Escherichia, Lactobacillus*, and *Veillonella* predominated in IBD ($p < 0.0002$-0.04), whereas *Bacteroides, Flavobacterium* e *Prevotella* resulted significantly reduced ($p < 0.0004$-0.05).
Conclusions: While we partially confirmed the previously published differences in phylum-level taxonomic composition between IBD patients and control individuals in several cohorts, we found interesting associations between disease and taxonomic composition of stool microbiomes at phylum (Firmicutes), genus and species level. Further studies exploring the mucosa-associated microbiota in both UC and CD are currently in progress, and preliminary results suggest that a distinctive mucosal microbial profile is detectable in IBD.

P 058

BACTERICIDAL ACTIVITY OF LOW INTENSITY, HIGH FREQUENCY ULTRASOUNDS AGAINST *E. COLI* AND *S. AUREUS*

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Introduction: In addition to the disruption of biofilm, sonication has an effect on bacterial killing, suggesting that ultrasounds might act by degrading the bacterial cell wall and thus serve as a potential adjunctive tool in the treatment of IAIs. The objective of the present study was to evaluate the anti-bacterial effect of low frequency ultrasounds against *E. coli* and *S. aureus*.
Materials and Methods: Bacterial suspensions containing ≈1x10*6 CFU/mL of E. coli (ATCC 25922) and *S. aureus* (ATCC 29213) underwent treatment with a sonicator (Sonics Vibracell VCX500) with a 13 mm probe, which is able to generate low frequency and high intensity ultrasounds. During the treatment, the temperature was monitored throughout an oscilloscope equipped with a thermocouple. The samples were placed in glass test tubes surrounded by water and ice in order to facilitate the heat exchange and were subjected to the sonication treatment at different values of oscillation amplitude, duration of 'on' and 'off' cycle (s) and total treatment time (s). Treatments were performed by applying increasing power density on the same sample (39.46 ± 0.08, 40.97 ± 0.11, 48.15 ± 0.16 and 51.24 ± 0.4 W/cm^2 for *E. coli* and 40.64 ± 0.15, 45.51 ± 0.38, 51.65 ± 0.15 and 57.23 ± 0.4 W/cm^2 for *S. aureus*, respectively). After treatment, bacterial samples were plated and incubated at 37°C for 24h. Colonies were counted and expressed as log10CFU/mL. Experiments were performed in triplicate.
Results: Compared to the initial inoculum, we observed a mean reduction of bacterial load of 4.10 ± 0.42 log10 CFU/mL for *E. coli* and 5.03 ± 0.48 log10 CFU/mL for *S. aureus,* respectively. For *E. coli*, starting from an initial inoculum of 5.24 ± 0.4 log10 CFU/mL, we obtained the following bacterial load: 3.57 ± 0.55 log10CFU/mL at 39.46 ± 0.08 W/cm^2, 2.6 ± 0.83 log10 CFU/mL at 40.97 ± 0.11 W/cm^2, 1.62 ± 0.75 log10 CFU/mL at 48.15 ± 0.16 W/cm^2 and 1.14 ± 0.71 log10 CFU/mL at 51.24 ±

0.4 W/cm^2. Similarly, for *S. aureus* we obtained the following bacterial load: 5.88 ± 0.16 log10 CFU/mL at 40.64 ± 0.15 W/cm^2, 4.51 ± 0.64 log10 CFU/mL at 45.51 ± 0.38 W/cm^2, 2.26 ± 0.95 log10 CFU/mL at 51.65 ± 0.15 W/cm^2 and 1.37 ± 0.56 log10 CFU/mL at 57.23 ± 0.4 W/cm^2 after an initial inoculum of 6.4 ± 0.12 log10 CFU/mL. During the sonication treatment, initial and final temperature were 14.53 ± 0.28°C and 28.19 ± 0.6°C for *E. coli*, 9.79 ± 0.23°C and 45.94 ± 0.76°C for *S. aureus*, respectively.

Conclusions: Low frequency ultrasounds showed a bactericidal effect against both *E. coli* and *S. aureus*. This effect was dependent on the increasing power density. Temperatures were maintained within non-pathologic values and did not have an effect on bacterial killing. These preliminary results might have important clinical implications in the treatment of IAIs by combining both ultrasounds and antimicrobials.

P 059

MULTIDRUG-RESISTANT *SERRATIA MARCESCENS* ASSOCIATED WITH PNEUMONIA DISEASE IN A *CARETTA CARETTA* TURTLE

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Introduction: *Serratia marcescens* is Gram-negative bacterium, naturally found in plants and animals often as a pathogen. In humans it has emerged as the 7th cause of pneumonia and bloodstream infections in newborns, immune-compromised and intensive care patients among America and Europe. Since the alarming increase of antimicrobial resistance in *S. marcescens*, the treatment of diseases caused by this bacterium represents a challenge. In this study we describe an outbreak of respiratory disease in a sea turtle (*Caretta caretta*) with swim problems associated with a multidrug resistant strain of *S. marcescens*.

Materials and Methods: An adult male loggerhead turtle, found from the CRAS (Centro Recupero Animali Selvatici) of the WWF unit of Policoro oasis (MT), was visited at the Sea Turtle Clinic of the Veterinary Medicine Department -University of Bari. During the routine examination, a total body radiography revealed findings likely to be attributed to pneumonia, thus samples from bronchoalveolar lavage (BAL) and pulmonary fibrinous exudate were collected and screened for infectious agents using classical cultural assays. Grown bacteria were identified using the Analytical Profile Index (API)20E (bio-Merieux) and the high-throughput 16S rRNA gene sequencing. Antibiotic resistance profile was obtained testing isolated bacteria to a pattern of antibiotics with a Kirby-Bauer disc diffusion method.

Results: Bacteriological assays showed the presence of non-lactose fermenting Gram-negative rods in pure culture (≥ 107x g of tissue) in all samples. Isolates from all sources were identified using both API20E system and sequencing of the 16 rRNA gene as *Serratia marcescens*. Upon evaluation of antibiotic susceptibility with disc diffusion method, resistances to ampicillin, cephalotin, cephalexin, cefuroxime, erythromycin and tetracycline were observed.

Conclusion: Results achieved in this study revealed for the first time an association between *S. marcescens* and pneumonia findings in a sea turtle according to previous studies in humans. The presence of a multi-drug-resistance bacterium in a marine animal raises concern of public health due to the zoonotic risk mainly for people working at rescue centers. Additionally, as sea turtles are wild animals never treated with antibiotics, the presence of antimicrobial-resistance genes in the marine environment could be linked to their massive consumption both in livestock and human fields supporting the need of prudent antimicrobial use.

P 060

SYNERGISTIC ACTIVITY OF THE N-TERMINUS OF HUMAN LACTOFERRIN IN COMBINATION WITH VARIOUS ANTIBIOTICS AGAINST CARBAPENEM-RESISTANT *KLEBSIELLA PNEUMONIAE* STRAINS

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Introduction: The spread of multi-drug resistant (MDR) *Klebsiella pneumoniae* strains points to a pressing need for new antibacterial agents. To this aim, the *in vitro* antibacterial activity of a synthetic N-terminal peptide of human lactoferrin, further referred to as hLF1-11, was evaluated against *K. pneumoniae* strains with different susceptibility to various antibiotics. The antibacterial activity of hLF1-11 in combination with conventional (gentamicin and tigecycline) or hydrophobic (rifampicin, clindamycin and clarithromycin) antibiotics was evaluated in synergy studies against MDR *K. pneumoniae* strains harboring different resistance genes (i.e. OXA-48, KPC-2, KPC-3, VIM-1, kindly provided by Prof. Gian Maria Rossolini, University of Florence).
Materials and Methods: An antimicrobial peptide susceptibility assay was used to assess the bactericidal activity of hLF1-11 against the different *K. pneumoniae* strains tested. The synergistic activity was evaluated by a checkerboard titration method, and the fractional inhibitory concentration (FIC) index was calculated for the various combinations. A hemolysis assay was performed to evaluate possible toxic effects of hLF1-11 and antibiotics on human red blood cells.
Results: The results revealed that hLF1-11 was more efficient in killing a non-carbapenemase producing than carbapenemase producing *K. pneumoniae* strains. In addition, hLF1-11 exhibited a synergistic effect with the tested antibiotics against MDR *K. pneumoniae* strains.
Conclusions: The results of this study suggest a hLF1-11-induced sensitizing effect of *K. pneumoniae* to antibiotics, especially to hydrophobic antibiotics, which are normally not effective on Gram-negative bacteria. Altogether, these data indicate that hLF1-11 in combination with antibiotics is a promising candidate to treat infections caused by MDR-*K. pneumoniae* strains.

P 061

SANITIZATION PROCEDURES FOR THE CONTROL IN HOSPITAL SETTINGS OF *ENTEROCOCCUS HIRAE* BIOFILMS

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Introduction: The bacterial contamination in the hospital environment is of particular concern because the hospital-acquired infections (HAIs), also due to the biofilms formed on hand-touch surfaces, are responsible for significant morbidity and mortality. This work supply new knowledge both on capability of biofilm production of *Enterococcus hirae* on different surfaces and the action of two biocides on the produced biomasses.
Materials and Methods: The reference strain *E. hirae* ATCC 10541 was used for this study. The biofilm formation was carry out on polystyrene and stainless steel surfaces at 20 and 37°C; the biomass quantification and the cell viability were performed thought the crystal violet and live/dead staining. Moreover, using the O.D. measurements of biofilms formed, *E. hirae* ATCC 10541 was classified as strong, moderate or weak biofilm producer. The effect of two biocides LH IDROXI FAST and LH ENZYCLEAN SPRAY, in the removal biofilms formed on polystyrene and stainless steel was performed after 60 min of contact and their antibiofilm action was expressed as percentage of biofilm biomass reduction.
Results: *Enterococcus hirae* both at 20 and 37°C, produced more biofilm on stainless steel (OD600 = 0.18 ± 0.02 and 0.22 ± 0.03, respectively) in respect to polystyrene (OD600 = 0.17 ± 0.03 and 0.14 ± 0.02, respectively). The bacterium was defined a weak biofilm producer in all conditions tested, except on stainless steel at 37°C, in which the biofilm production was moderate. The amount of

viable cells on polystyrene was greater at 20 than 37°C whereas, on stainless steel this difference was less remarkable. The two biocides expressed the most effect on stainless steel at 37°C, with a biofilm reduction of 81.82% and 86.37% in presence of LH IDROXI FAST and LH ENZYCLEAN SPRAY, respectively.
Conclusions: *Enterococcus hirae* is capable to form biofilm and this skill is dependent both by the surface material and the temperature and can be associated to its increasing incidence in HAIs. It is generally a weak biofilm producer, except on stainless steel at 37°C confirming the correct practice of both the materials analyzed in the hospital setting. Moreover, the tested biocides can be considered suitable disinfectants to guarantee the success of sanitization procedures against *E. hirae* biofilms.

P 062

RETT SYNDROME IS ASSOCIATED WITH ALTERED GUT MICROBIOTA COMMUNITY

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Introduction: Rett syndrome (RTT) is an X-linked neurodevelopmental disorder and the second most common cause of mental retardation in females. Changes in microbiota composition, as observed in other neurological disorders such as autism, may account for several typical symptoms associated to RTT. Indeed, it is shown that a dysbiotic microbiota in gastrointestinal tract may affect the function of the nervous system. The main goal of this preliminary study was to characterize gut microbiota in RTT patients, and to compare it with a healthy control group of female of the same age.
Materials and Methods: Eight RTT patients were enrolled at the Child Neuropsychiatry Department of San Paolo Hospital of Milan. Age and sex-matched healthy women (CTR), working at the University of Milan, were recruited. From all subjects we collected stool samples, anthropometric data and dietary habits. Microbiota characterization was achieved by amplicon sequencing using 16S rRNA regions (V3-V4) genomic region with a Next Generation Sequencing approach on Illumina platform. Concentration of short chain fatty acids (SCFAs) was determined by gas-chromatography analysis.
Results: Body mass index (BMI, kg/m^2) was 17.4 ± 3.9 (mean ± SD) in RTT patients, and 20.9 ± 2.2 in control group ($p = 0.0284$). We did not observe differences in the mean value of Kcal/die ($p = 0.43$), but RTT diets were characterized by an increase in protein content ($p = 0.029$) and a lower intake of sugars ($p = 0.0035$). Microbiota analysis showed a significant lower alpha-diversity in RTT samples compared with control group (chao index and species richness $p < 0.0001$; shannon index $p = 0.0015$; and inverse simpson index $p = 0.013$). The predominant bacterial taxa in both groups were *Firmicutes* and *Bacteroidetes*. Although the *Firmicutes/Bacteroidetes* ratio was similar, at family level *Bacteroidaceae* were significantly higher in RTT samples ($p = 0.0009$), whereas C*lostridiaceae, Ruminococcaceae* and *Christensenellaceae* were strongly reduced. Desulfovibrio spp., as seen in autistic patients, was found increased in RTT patients ($p = 0.0216$). We did not find differences in butyrate and acetate concentration ($p = 0.1144$ and $p = 0.6456$, respectively) whereas fecal propionate was increased in RTT patients ($p = 0.0248$).
Conclusions: We demonstrated in a small sample of patients that RTT gut microbiota is significantly different from the control group. Our hypothesis is that a dysbiotic gut in RTT patients could result in alterations of SCFAs that can worsen clinical symptoms by interacting at various levels (gut, brain, liver). Understanding critical changes could offer new tools for a diet intervention or probiotics supplementation to improve RTT associated symptoms and, ultimately, psycho-physical wellness.

P 063

IMPROVING THE CONCORDANCE BETWEEN THE RAPID ANTIMICROBIAL SUSCEPTIBILITY TEST AND THE TRADITIONAL SEMI-AUTOMATED ANTIMICROBIAL SUSCEPTIBILITY TEST

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Introduction: Sepsis is a widespread and life-threatening condition which clinical diagnosis is crucial and time-based. The onset of antimicrobial resistance has made also the results of microbiological examinations an urgent need. Rapid identification of bloodstream pathogens by MALDI-TOF Mass Spectrometry and the recently introduced rapid antimicrobial susceptibility testing (rAST) directly from positive blood cultures allow clinicians to promptly achieve a targeted therapy, thus improving the patient outcome. In this study we propose an upgrading of a rAST, available in 3-5 hours, using Alfred 60AST system (Alifax) directly from positive blood cultures.
Materials and Methods: Bacterial identification from positive blood cultures was performed using a modified method previously described (Barnini et al. BMC Microbiology (2015;15:124). After the identification, a suspension of Gram-negative (G-) bacilli (0.6 McFarland) or Gram-positive (G+) cocci (0.9 McFarland) was prepared in 2 ml of liquid medium (Alifax). Each germ (80 G+ and 100 G-) was assigned to one of the 5 antimicrobial designed panels, namely, *Enterobacteriaceae*, G- non-fermenting bacteria, CoNS, *Staphylococcus aureus, Streptococcus-Enterococcus* and monitored for growth. Antibiotics tested were chosen among cefoxitin, linezolid, teicoplanin, ampicillin, cefotaxime, ceftazidime, gentamicin, levofloxacin, meropenem, amikacin and colistin. Absence of growth was interpreted as sensitivity while growth as resistance to the antibiotic, in terms of clinical category (SIR). Results were compared with a traditional semi-automated system.
Results: Direct bacterial identification was 99% concordant at species level with the identification from colonies. In the first place, 180 rASTs were performed to assess the system: the average concordance with Phoenix (Becton Dickinson) was 87%. The major disagreements were related to gentamicin, teicoplanin and cefoxitin tests. Further analysis showed the possibility to improve the concordance for these tests by purifying the bacterial inoculum from debris. Then, 474 rASTs were performed to improve the concordance, with an agreement of 92% for G+ and 97% for G- bacteria. For G+ bacteria the concordance increased to 96% with a manual correction of growth curves for teicoplanin tests.
Conclusions: During sepsis events a prompt and efficient communication of the results of Gram staining, microorganism identification and mostly ASTs are essential for providing effective care of patients. The main issues observed in this study were exceeded increasing both the amount of pellet and the number of bacterial pellet washings to eliminate interfering erythrocytes, and regulating in turn the bacterial suspension for each germ associated with a specific antimicrobial panel.

P 064

TOXIGENIC *CLOSTRIDIUM DIFFICILE*: DIAGNOSIS AND EPIDEMIOLOGY IN A TERTIARY CARE UNIVERSITY HOSPITAL IN ROME, ITALY

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Introduction: Toxinogenic *Clostridium difficile* infection (CDI) is one of the major causes of infectious diarrhea in patients treated with long lasting antibacterial and antacid therapy, older age and immune-compromisation. The introduction of the PCR in the laboratory diagnosis of *C. difficile* (CD), increased sensitivity and speeded the diagnosis. However, the clinical relevance of PCR results is often unclear. The aim of our study is to investigate the molecular epidemiology of toxinogenic *C. difficile* in a tertiary care Academic hospital (Policlinico Umberto I) in Rome, Italy.
Materials / Methods: In this study, a retrospective analysis was carried out in patients screened for CDI in a 1300-bed University Hospital (Policlinico Umberto I) in Rome, from November 2013

until October 2015. In brief, adequate stool samples (≥ 5 Bristol stool chart) were firstly tested for GDH antigen positivity by *C. difficile* Chek-60-GDH (Techlab) and positive samples were further analysed for the presence of gene-encoding toxins by RT-PCR with Xpert® *C. difficile* (Cepheid). All RT-PCR outputs were recorded and analysed.
Results: Diarrheic stool specimens (n = 2785) were collected from 1846 patients (averaging 61.04 ± 24.97 years, 52% male). GDH antigen detection assay resulted positive in 23% of the patients and thereafter tested by Xpert® *C. difficile* for gene-encoding toxins. In our patients population 73% (314/426) presented toxinogenic CD by RT-PCR, with a cumulative incidence of 2.6 episodes/1000 admission and a total frequency of 17% (314/1846) of diarrhea attributable to CD. Unexpectedly, the higher frequency of diarrhea due to CD was observed in outpatients (28%, n = 57/202), and in particular in those with an hospital admission in the previous 30 days. Moreover, the retrospective analysis of RT-PCR curves of the 426 test performed revealed that 20 samples (4.6%) belonging to 20 patients, reported by the software as negative, had cycle threshold and endpoint for toxin B and binary toxin ranging between 37.1-40 and 26-63. These values were very close to the negative detection limit of the software, but a rumor was noticed. In 14/20 of these patients a previous positivity for toxigenic CD was recorded within 2-23 days, and metronidazole or vancomicin treatments were undertaken. In 5/6 of the remaining patients, antimicrobic treatments that could reduce CD toxins concentration were ongoing at the time of the analysis.
Conclusions: CDI incidence is increasing in our hospital setting. The use of the RT-PCR assay is a powerful tool for quick diagnosis of toxinogenic CDI. However, a careful interpretation of the results is needed to obtain the clinical significance of these results. In fact, the presence of vital CD in stool specimens in addition to the toxin DNA detection need to be addressed.

P 065

ANTIBIOTIC SUSCEPTIBILITY PROFILES AND DIFFERENT CPE ENZYMES: IS THERE A CORRELATION?

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Introduction: Carbapenemase producing *Enterobacteriaceae* (CPE) are involved in numerous outbreaks. Data reported in the literature on the susceptibility profiles of strains carrying different carabapenemase genes, are rare. The genotypic detection assays for carbapenemase genes (NDM, OXA48, IMP, KPC, VIM) are rapid and helpful diagnostic tools. In the present study, RT-PCR cycle threshold and EndPoint values of CPE strains were investigated and correlated to the susceptibility profiles for different antibiotics.
Materials and Methods: In this retrospective observational study, CPE randomly isolated from April 2014 until June 2016 in the Clinical Microbiology Laboratory of Policlinico Umberto I in Rome, were investigated. Strains were isolated from different clinical samples on different culture media (i.e. Trypticase soy agar+5% sheep blood, McConkey agar, Chromo ESBL agar) and analyzed through IVD FDA approved RT-PCR assay (CARBA-R, Cepheid) according to manufacturer instructions. Susceptibility profiles to different antibiotics were obtained through an automated system (Vitek 2, AST-N202 card, Biomerieux).
Results: In the 27 months of observation, 164 tests were performed and 158 CPE were observed. The enzyme most frequently isolated was KPC (n = 113) followed by VIM (n = 27), OXA48 (n = 8), VIM-OXA48 (n = 8) and VIM-KPC (n = 1). The higher number of carbapenemases were harbored by Klebsiella pneumoniae (75.4%), followed by Escherichia coli 14.2%, Enterobacter aerogenes 6.4% and Enterobacter cloacae complex 4%. Antibiotic susceptibility tests were performed on 131 isolates. KPC positive CPE displayed resistance to colistin (MIC90 ≥ 16 ug/ml) in 22% of the isolates. Good residual activity to ceftazidime and 100% of susceptibility to tigecyclin was observed in OXA48 positive isolates. For VIM expressing CPE, fosfomycin displayed better activity than in other strains

harboring other enzymes and all the strains were susceptible to colistin. On the other hand, VIM/OXA48-positive strains were susceptible to amikacin in 89% of the isolates and showed MIC values for tigecyclin of 2 ug/ml. The retrospective analysis of RT-PCR curves of the 164 test performed revealed that 2 strains reported by the software as KPC, had cycle threshold and endpoint for IMP/NDM and OXA48 with some rumors as well. These values were very close to the negative detection limit of the software and, as such, reported negative for these three enzymes.

Conclusions: In our setting, multiplex RT-PCR displayed high diagnostic feasibility and rapidity, although the interpretation of the results requires caution and expertise. Antimicrobial susceptibility profiles exhibited peculiar characteristics according to the resistance gene carried by the strain.

P 066

STENOTROPHOMONAS MALTOPHILIA PHENOTYPIC AND GENOTYPIC DIVERSITY DURING A 10-YEAR INFECTION IN THE LUNGS OF A CYSTIC FIBROSIS PATIENT

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Introduction: *Stenotrophomonas maltophilia* is one of the most common emerging multi-drug resistant pathogens found in the lungs of cystic fibrosis (CF) patient, although it is unclear whether it simply colonizes CF lungs or causes true infection leading to pulmonary inflammation and clinical deterioration. In several studies, we found evidences highly suggestive of the pathogenic role of *S. maltophilia* in CF patients. Particularly, this microorganism can grow as biofilm on CF-derived epithelial monolayer, probably because of a selective adaptation to CF airways. In contrast to *P. aeruginosa* CF lung colonization, where genetic adaptations leading to phenotypic variation are well known, the diversity generated by *S. maltophilia* persistence in the lungs is mostly unknown. Thus, the present study was carried out to understand the adaptive strategies developed by *S. maltophilia* for chronic colonization of the CF lung and to evaluate whether there is a typical phenotypic profile related to chronic infection.

Materials and Methods: Twelve strains isolated from the sputum of a CF patient over 10-year (2005-2014) were comparatively evaluated for growth rate, biofilm formation, motility, mutation frequencies, antibiotic resistance, virulence exhibited in *Galleria mellonella* and pathogenicity on human respiratory A549 epithelial cells. The epidemiological relatedness of the strains was also studied by pulsed-field gel electrophoresis (PFGE).

Results: PFGE showed over time the presence of 2 distinct groups, each consisting of 2 different pulsotypes. The pattern of evolution followed by *S. maltophilia* was dependent on pulsotype considered, with strains belonging to pulsotype 1.1 resulting to be the most adapted, being significantly changed in all traits considered. Pulsotype 2.1 strains showed variations in all traits but mutation frequency and twitching motility, sharing with pulsotype 1.1 the same trend for growth rate, biofilm formation, pathogenicity, and antibiotic resistance. All pulsotypes were affected in A549 pathogenicity and antibiotic susceptibility. Generally, *S. maltophilia* adaptation to CF lung led to increased growth rate and antibiotic resistance, whereas both *in vivo* and *in vitro* pathogenicity as well as biofilm formation were decreased.

Conclusions: Our results showed, for the first time, that *S. maltophilia* can successfully adapt to a highly stressful environment such as CF lung by paying a "biological cost", as suggested by the presence of relevant genotypic and phenotypic heterogeneity within bacterial population. This indicates that *S. maltophilia* populations are significantly more complex and dynamic than can be described by the analysis of any single isolate and can fluctuate rapidly to changing selective pressures.

P 067

DEVELOPMENT OF A NOVEL PARATRANSGENIC SYMBIOTIC-BASED CONTROL STRATEGY FOR VECTOR-BORNE INFECTIOUS DISEASES

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Introduction: Paratransgenesis for the control of vector-borne infectious diseases is based on reintroduction into the host insect of genetically modified symbiotic bacteria expressing effector molecules that interfere with pathogen transmission. Key features for the success of a paratransgenic system are the ability of engineered bacteria to display or excrete the effector molecule and the fitness of the recombinant symbionts in insect physiological conditions. Objective of the present study is the development of a paratransgenic strategy to control vector-borne human diseases stimulating insect immunity against invading pathogens.
Materials and Methods: The stable dominant insect-associated bacterium *Asaia platycodi* wild-type (wt) strain SF2.1, was transformed with a plasmid bearing the gene of the potent insect immunomodulating surface protein of *Wolbachia* (WSP). To prejudicially verify the feasibility of the approach, the expression of the recombinant WSP by *A. platycodi* was evaluated.
In addition, the fitness of the recombinant and wt isolates were compared *in vitro* by maximal growth rate (MGR) analysis in different culture conditions and by pairwise competition assays.
Results: Expression studies showed that recombinant *A. platycodi* displayed WSP on bacterial surface. MGR analysis showed that the transformation might differently affect the fitness of the bacterial host, in relation to the increase of the pH value of growth medium. Competition experiments carried out at *A. platycodi* pH optimal growth showed a slight reduction in vitality of the recombinant strain.
Conclusions: A recombinant insect-associated symbiotic bacterium displaying WSP on its surface was produced. We expect that paratransgenic immune activation of insects induced by WSP could hamper their infection by pathogens. Fitness studies indicated that the suggested symbiotic approach can be assumed in case of compatible pH values of colonized organs of the insect vectors and that possible problems of competitiveness of the transformed strain relatively to the wt should be considered. To increase the fitness of recombinant symbionts, bacteria may be engineered to stably include the WSP gene in the bacterial chromosome.

P 068

FOETAL LIFE: STERILE OR NOT STERILE?

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Introduction: Foetal development is a period of unparalleled cellular proliferation, tissue formation, and organ construction within the confines of the womb-a incubator that surrounds the foetus with amniotic fluid and provides nutrients for growth and protection of vulnerable tissues. Although historically the womb and foetus have been considered sterile until birth or rupture of the amniotic sac, recent evidence shows that womb hosts a diversity of microorganisms that closely resemble the mother's oral microbial community. If this is proven to be correct, it would imply that new-borns have a starter culture of bacteria received from the mother in utero. This starter culture may change during pregnancy, which would explain why neonate's gut microbiota could differ depending on the time of birth. For this reason, evaluation of the antenatal microbiota (microbial composition during pregnancy) is extremely important. Here, we present preliminary results conducted on an animal model.
Materials and Methods: Eight CD albino rats (Sprague-Dawley) were maintained in standard conditions (light 6 a.m.-6 p.m., T= 22 ± 2°C, humidity = 55 ± 5%) with tap water and food ad libitum. Virgin females were caged overnight with males of proven fertility. The day of positive vaginal smear was considered as day 0. Stool samples were collected at different time-points. At 16 days of gestation, oral and vaginal swab were collected. After sacrifice, amniotic fluid, placentae and foetal intestines were collected in sterile conditions. Total bacterial DNA was extracted from collected

samples and analyzed by 16S rRNA amplification followed by denaturing gradient gel electrophoresis (DGGE) analysis. Banding patterns of DGGE profiles were analyzed with Fingerprinting II software using the Pearson correlation coefficient and the unweighted-pair group method with averages (UPGMA) for he generation of dendrograms.
Results: Four rats resulted pregnant and the remaining were considered as control group. The analysis of stool samples pointed out a different microbial composition in pregnant rats. The vaginal swab clustered with stool samples of the first two days of gestation, while the oral swab was in the same similarity group of amniotic fluid, placentae, intestine and stool samples from day 6 to day 11.
Conclusions: These preliminary results indicate a different microbial composition in maternal stool samples that changes through gestation. The presence of microorganisms in placentae, amniotic fluid and foetal intestines suggests an in utero microbial colonization. Next Generation Sequencing analysis is ongoing to identify the foetal microbiota.

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P 069

FUNCTIONAL FOODS, A LESSON FROM AFRICAN TRADITIONAL DIET

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Introduction: Functional foods contain biologically active components able to improve our nutritional and health status. Dietary fibres from cereals and legumes contain phytochemicals and indigestible fermentable fractions able to select beneficial gut microbial communities. In this study, we evaluated how diet, especially foods with nutraceutical properties, and environmental factors are able to shape gut microbiota.
Materials and Methods: Dietary habits of different populations of children living in different areas of Burkina Faso (rural village, little town, capital city) were compared with a westernized population living in Florence. A selection of typical cereals and legumes from Burkina Faso and Tuscany were chemically compared for fibre, poly-and oligosaccharide and phytochemical content. The gut microbiota of these populations were characterized by pyrosequencing of bacterial 16S rDNA gene (V5-V6 region). We associated meta-taxonomic data with metabolomic analyses, in order to evaluate relations among microbial communities, metabolites and different diets.
Results: African children living in the rural village (BR) have a vegetarian diet, eating local cereals (Millet and Sorghum), legumes (Niebè), vegetables (Néré and Baobab leaves), fruits and fermented products (Soumbalà). Children from Nanoro town (BT) eat cereals and legumes and occasionally meat, fish and egg. Children from capital city (BC) supplement African diet with bread, milk, cheese, eggs, fruit juice, similarly to children from industrialized countries. Chemical analysis of cereals from Burkina and Tuscany showed different characteristic of dietary soluble and unsoluble fibre, protein and bioactive components. Metagenomic analysis of gut microbiota showed profound differences between rural African and Italian children (EU). BR microbiota was enriched in Xylanibacter and Prevotella, able to extract energy from the plant polysaccharides and producing Short Chain Fatty Acids (SCFAs), known for antinflammatory properties. Prevotellaceae resulted progressively reduced in urban BT and BC, and absent in EU. Conversely, Bacteroidaceae, Lachnospiraceae, Ruminococcaceae, Porphyromonadaceae and Bifidobacteriaceae were enriched in urban African children (BT and BC) and in EU, reflecting the protein-rich diet and milk derived-food consumption. SCFAs levels were associated to dietary fibre content and progressively reduced from rural African to EU children.
Conclusions: Migration from rural to urban area and different dietary habits influence gut microbiota composition and their metabolites. Our results suggest the need to preserve ancient microbial communities, in association with functional foods derived from traditional dietary habits that are at risk of disappearing due to globalization.

P 070

ROLE OF ERYTHROCYTE MEMBRANE MICRODOMAINS IN P*LASMODIUM FALCIPARUM* INFECTION

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Introduction: Malaria is one of the most deadly diseases worldwide: in 2015, it caused around 214 million cases and 438,000 deaths, mostly in children under 5 years of age. The vast majority of the lethal events are caused by the parasite *Plasmodium falciparum*, which develops and replicates inside erythrocytes. Plasma membrane microdomains (MMs) are involved in various biological processes, such as signalling via receptors, intracellular trafficking, cellular differentiation and, most importantly, infection by pathogens. It was reported that disruption of erythrocyte MMs prevents invasion by merozoites, suggesting an essential role of erythrocyte MMs in susceptibility to invasion by *P. falciparum*. In the present work we aim to characterize erythrocyte MM proteins, in relation to their role in malaria infection.
Materials and Methods: Being enriched in cholesterol and sphingolipids, MMs are resistant to solubilization by certain non-ionic detergents at low temperature. This feature allows to isolate them and analyze their protein content. With the aim of confirming and improving existing information on erythrocyte MM composition, we performed a new proteomic analysis of erythrocyte detergent resistant membranes. Purified erythrocyte membranes were lysed using 1% Triton X-100 on ice, a treatment known to preserve MMs, and lysates were separated on sucrose gradient.
Results: The proteomic analysis led to the identification of 158 proteins, corresponding to around 6% of the whole erythrocyte protein content and around 20% of the membrane-associated proteins. With the aim of characterizing distinct erythrocyte MM types, all the gradient fractions were analysed by quantitative proteomic analysis and profiles reflecting the abundance of each protein along the gradient were generated. The resulting profiles were then used to categorize the proteins into different groups by hierarchical cluster analysis. Only 11 erythrocyte proteins have been described so far to be involved in *P. falciparum* invasion process. Strikingly, 8 of them were identified in our analysis and in particular 6 of them show the same floating profile and fall in the same cluster, comprising a total of 18 proteins.
Conclusions: Our results strongly suggest that a specific MM type may be involved in *P. falciparum* attachment and invasion. The invasion-related cluster includes many proteins involved in the classical signal transduction cascade, thus enforcing the concept that parasites exploit the host signal transduction machinery to invade.

P 071

DETERMINATION OF ANTIFUNGAL ACTIVITY OF A VAGINAL CREAM FORMULATION CONTAINING 1% ECONAZOLE AND 0.12% BENZYDAMINE HCL

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Introduction: Aim of the study was to compare the antifungal activity against *Candida albicans* of a new vaginal cream containing 1% econazole and 0.12% benzydamine HCl (test formulation) vs. a marketed cream containing 1% econazole only.
Materials and Methods: *In vitro* fungicidal activity. Growth inhibition of *C. albicans* ATCC 10231 and of *C. albicans* MYA 574 (fluconazole-resistant) by the two cream formulations was determined by agar dilution assay (Alsterholm M.et al., 2010). Weighed creams were dissolved directly in agar (Diagnostic Sensitivity Test Oxoid) at cream final concentrations of 1% to 0.03% (w/v). The surface of each plate was inoculated with 4 spots of *C. albicans* and plates were incubated at 37°C for 48 h.
Image acquisition and analysis. Selected plates were scanned using ImageQuant LAS4000 densitometer, setting the instruments with the white light table inside and choosing different parameters in order to quantify the spots corresponding to the *C. albicans* colonies by ImageQuant TL7.0 Image Analy-

sis Software (GE Healthcare). Colony density was measured as densitometric arbitrary units (AU) and EC50s were expressed as % cream dilution.
Results: The new test cream formulation showed a reduction of visual growth on *C. albicans* ATCC 10231 up to the 0.06% cream concentration corresponding to an EC50 of 0.08%. Differently, the marketed cream reduced the growth of *C. albicans* ATCC 10231 to a lower extent, as resulted from the EC50 value of 0.251%. A similar effect was observed on fluconazole-resistant *C. albicans* MYA 574 where the test formulation showed a reduction of visual growth up to the 0.250% cream concentration with an EC50 of 0.366%, while the marketed cream reduced the growth of *C. albicans* MYA 574 to a lower extent (EC50 value > 1%). It has been reported (Pina-Vaz C. et al., 2000) that benzydamine can induce membrane alterations in yeast cells modifying their permeability. Hence, it can be speculated that the association of benzydamine to the econazole containing cream facilitates the entry of econazole into the *Candida* cells and increases its fungicidal effect.
Conclusions: We tested the antifungal activity of a vaginal cream formulation containing 1% econazole and 0.12% benzydamine HCl on two *Candida* strains in comparison with a 1% econazole marketed cream. Visual inspection results showed that the addition of benzydamine increases the fungicidal effect of econazole, even in a fluconazole-resistant strain. Image quantification confirmed the observations of the plate visual inspection analysis.

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P 072

ANTIBIOTIC RESISTANCE AND BIOFILM PRODUCTION IN ANIMAL STRAINS OF *PSEUDOMONAS SPP*

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Introduction: *Pseudomonas aeruginosa* is one of the most resistant bacteria to antibiotic therapy. Italy is the fifth country in the consumption of antibiotics. However, a wrong use of these drugs may accelerate the diffusion of multidrug resistant strains. The aim of the study was to collect and phenotypically characterize 28 *Pseudomonas spp.* strains isolated from different clinical samples of animal origin, at the Department of Veterinary Medicine and Animal Production of the University of Naples "Federico II" in the year 2015. The antibiotic-resistance of Pseudomonas strains and the ability to produce biofilm was evaluated. Finally, the correlation between biofilm production and multi-resistance profiles of each strain, was evaluated.
Material and Methods: Each sample was grown on three different selective medium: Columbia Blood Agar, MacConkey II Agar and *Pseudomonas* CFC. Catalase and oxidase test, Gram staining and biochemical test were used to identify bacterial species. Antibiotic susceptibility testing was performed by Kirby-Bauer disc diffusion technique, following Clinical and Laboratory Standard Institute (CLSI) guidelines. The ability of the isolates to form biofilm was examined by Crystal violet assay. Pseudomonas strains were classified by Stepanovic's criteria into strong, moderate, weak and no biofilm producers.
Results: The most frequently isolated species were *P. aeruginosa* (71%) and *P. luteola* (21%). Many strains showed high levels of resistance to aminoglycosides, others to fluoroquinolones and to all III generation of cephalosporins and carbapenems. *P. luteola* presented resistance levels comparable to *P. aeruginosa*. All the strains tested were able to produce biofilm, but only one was found to be a strong biofilm producer. The others strains were moderate or weak biofilm producers. However, no correlation

between the levels of biofilm production and antibiotic-resistance was observed.
Conclusions: In the literature the antibiotic resistance among biofilm producing bacterial strains is generally found significantly higher compared to biofilm non-producing strains. In our study all Pseudomonas strains studied have shown the ability to produce biofilm and high resistance rate (78%) to almost all the tested antimicrobial agents. However, from our data we can assume that the different ability of the strains to form biofilm does not represent a variable factor affecting the antibiotic resistance level. It is likely that additional mechanisms of antimicrobial resistance of *Pseudomonas spp.* might contribute to the observed high percentage of antibiotic resistance.

P 073

SENSITIVITY OF *CANDIDA ALBICANS* TO AZOLE ANTIFUNGAL AGENTS

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Introduction: *Candida spp.* are commensal yeasts found on the skin, mucous membranes, gastrointestinal tract, blood and vagina of animals and humans. In the last decades, the incidence of human fungal infections has increased with *Candida spp.* being the major agents of nosocomial ones. Among them, *Candida albicans* is the most frequently isolated in human candidiasis. The proliferation of *C. albicans* is usually controlled by the immune system of the host; however, in immuno-compromised patients, especially AIDS ones or those undergoing chemotherapy and immunosuppressive therapy, this yeast causes severe opportunistic infections. Fungal infections are commonly treated with antifungal drugs, mainly belonging to the azoles (clotrimazole, fluconazole, itraconazole, ketoconazole) and polyenes (amphotericin B, nystatin) categories. Azoles exert a fungistatic effect, inducing a partial growth inhibition of fungal cells; however, they are frequently associated with drug-resistance. The aim of this work was to assess the sensitivity of 30 different vaginal isolated strains of *C. albicans* to the three main used drugs (clotrimazole, fluconazole, itraconazole); moreover, cytological analyses were performed to better characterize azole response in treated cells.
Materials and Methods: Thirty strains of *C. albicans* were isolated from vaginal swab on CHROMagarTM Candida. The agar disk diffusion method was employed to determine the sensitivity for three antifungal drugs (clotrimazole, itraconazole and fluconazole). The antifungal activity of the drugs was investigated using a microdilution method. Transmission and scanning electron microscopy analyses were also performed.
Results: The first step of our approach was the screening of the antifungal activity by using the agar disk diffusion method. The results obtained for three different drugs, both itraconazole and fluconazole generally induced a regrowth halo. Only clotrimazole completely inhibited *C. albicans* growth. Microdilution test showed a general resistance to Clotrimazole. Transmission and scanning electron microscopy showed, in untreated cells, a cluster of well preserved and dividing cells, characterized by intact cell walls with clear external fibrillar layer. Clotrimazole treated cells showed shrunken and smooth surfaces.
Conclusions: Pharmacologically-active antifungal substances used in medicinal products typically interact with cell metabolism by inhibiting the synthesis of specific enzymes (azoles). Acquired resistance to these antifungal agents has been described and represents an increasing problem both in Europe and in USA and China. This work is a preliminary work to better understand the cellular effect of azole antifungal agents. Future prospective is to perform proteomic analysis.

P 074

BASIC KNOWLEDGE AND MISCONCEPTION ON ANTIBIOTIC USE: A SURVEY AMONG VETERINARY STUDENTS

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Introduction: Microbial resistance to antibiotics is an aspect of bacterial homeostasis. However, the inappropriate use of antibiotics in human and veterinary medicine has led to the spread of this phenomenon. Misconceptions about the nature and effectiveness of antimicrobials among people contribute to persistence of antimicrobial resistance. To this purpose, the European Commission conducted two surveys in 2009 and 2013, to determine knowledge and use of antibiotics by Europeans. Surprisingly, Europeans aged from 15 to 23 years were found to have wrong perceptions regarding this topic. For this reason, students from the Veterinary Faculty were interviewed about personal assumption of antibiotics and perception of their use.
Materials and Methods: From 2013 to 2015, 110 students from the Department of Veterinary Medicine of Bari were recruited for the survey, before the beginning of microbiology course. A questionnaire was designed according to previous surveys, and the 14 questions covered the following issues: i) personal use of antibiotics, ii) knowledge and the source of information about the topic, iii) the presence in the family of health workers.
Results: Ninety-seven out of 110 students fulfilled the questionnaire (response rate 88.1%). The mean age was 21 years (20-23 years) and 58% were females. Seventy-five % of students said they had taken antibiotics in the last 12 months, mostly for flu, cold or dental practices. Sixty-eight% of the interviewed knew that antibiotics are not active against virus, and 40% of them were aware that antibiotics are not effective against cold and flu. Eighty% of the respondents knew that abuse of antibiotics makes them ineffective and 73% that taking antibiotics often produce side-effects. Regarding the correct use of antibiotics, 70% of the respondents said that compliance with the prescriptions is important. Forty-eight% knew that the use of "old" molecules is preferable to "new" ones, and 45% that medical prescription is mandatory for antibiotics. As expected, mass media were major source of information. Thirty-seven % of students lived with one or more heath-workers, and they had a better understanding of the risks of antibiotic misuse.
Conclusions: Veterinary students declared a higher consumption of antibiotics when compared to people, in the same range of ages, enrolled in previous surveys, and only a minority of them (40%) knew that antibiotics are ineffective against cold and flu. Nonetheless, students appeared to be conscious of the importance of side-effects, dosage and the need for prescription.

P 075

EFFECTIVENESS OF THE ANTIBODY-DERIVED KILLER PEPTIDE ON *CANDIDA ALBICANS* BIOFILM

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Introduction: *Candida* spp. colonize human skin and mucosae of healthy individuals, behaving as harmless commensals. Nevertheless, in susceptible patients (subjects with medical devices or immunosuppressed individuals), they behave as opportunistic pathogens also because of their ability to produce biofilms on mucosal surfaces or medical devices. When embedded in a biofilm, *Candida* cells become more resistant to common disinfectants and antibiotic treatments. From here, the demand of novel therapeutic approaches. Recently, several antibody-derived peptides (collectively indicated as "PeptAb") have been shown to have antimicrobial, antiviral, immunomodulatory and antitumor activity *in vitro* and *in vivo*. The aim of the present study was to evaluate the effect of the

parental PeptAb, the killer peptide (KP), on the formation and persistence of *Candida* biofilm.
Materials and Methods: The reference strain *C. albicans* SC5314, two *C. albicans* fluconazole-resistant, two *C. albicans* fluconazole-susceptible isolates and KP (AKVTMTCSAS) were employed. The effects of KP were assessed against *Candida* biofilm at different stages of development, by microscopy, crystal violet and tetrazolium salt reduction assays.
Results: KP affected biofilm both in terms of total mass and metabolic activity, in a time- and dose-dependent manner; maximal impairment was observed with KP treatment for to 6 hours against both 24 and 48 hours biofilm (early and mature biofilm, respectively). Similar inhibitory effects were observed against all the *Candida* strains employed, including the reference strain and irrespective of their fluconazole resistance or susceptibility.
Conclusions: This pilot *in vitro* study provides initial evidence on the KP effectiveness against *Candida* biofilm, thus pointing to the possibility that PeptAbs might be considered as potential novel tools for treatment and prevention of biofilm-related *Candida* infections.
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P 076

ANTIBACTERIAL ACTIVITY OF TETRACYCLINE AGAINST ESCHERICHIA COLI ANIMAL STRAINS: AN ITALIAN RETROSPECTIVE MULTICENTRE STUDY

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Introduction: Tetracyclines exhibit the activity against a broad spectrum of pathogenic microorganisms; they are well absorbed, with a low toxicity and they are relatively inexpensive. In veterinary medicine, the tetracyclines are widely used mainly for the treatment of gastrointestinal, respiratory and skin bacterial infections, osteomyelitis, genito-urinary tract as well as systemic infections and sepsis. A retrospective multicentre surveillance of the tetracycline activity against *Escherichia coli* strains, isolated from domestic and wild animals, was carried out.
Materials and Methods: This study involved hospital microbiology laboratories located in Northern (Turin and Milan), Central (Camerino) and South Italy (Naples and Messina) between January 2014 and December 2015. In all participating laboratories, the collected samples were plated on MacConkey agar plates, which were incubated aerobically at 37°C for 24-48 h. To identify the isolated bacteria, commercial biochemical kits were used. The tetracycline susceptibility pattern was determined by the Kirby-Bauer disk-diffusion method according to EUCAST guidelines.
Results: *E. coli* isolates of domestic animal origin showed a range of tetracycline resistance from 61.5% to 82.0%. In particular, Turin showed a

percentage of 80.0 and 82.0, Milan 61.5 and 72.7, Camerino 70.0 and 73.5, Naples 66.7 and 72.4, Messina 61.7 and 73.0, in the year 2014 and 2015, respectively. Generally, in the 2015 the percentage rate resulted higher than that one observed in the 2014. *E. coli* isolates of wild animal origin showed a lower percentage of tetracycline resistance in samples collected in departments that presented data on wild animals (Turin, Camerino, Naples and Messina).

Conclusions: Resistance in Gram-negative bacteria has been increasing in the last years. This multicentre study gives a clear indication of the present spread of tetracycline-resistant *Escherichia coli*, the most common Gram-negative bacterial pathogen, between domestic and wild animals. Regardless of their geographical origin, all isolates displayed an interesting percentage of resistance. Generally, *E. coli* isolates from domestic species showed a higher resistance to tetracycline than those ones from wild animals. The extension of the monitoring systems in Italian territory is required to evaluate the prevalence of tetracycline-resistant microorganisms. The obtained data could suggest a correct use of antibiotics in Veterinary Medicine, because animals may represent one of the major sources of multidrug-resistant bacteria also for humans. Therefore, this investigation opens up new areas of exploration and cooperation about antibiotic resistance in veterinary and in human medicine.

P 077

DISCRIMINATION OF *ASPERGILLUS* SECTION FLAVI ISOLATES BY MALDI-TOF MASS SPECTROMETRY: A FEASIBLE APPROACH?

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Introduction: Due to the impact of genomic era on the identification of fungi, the taxonomy within the genus *Aspergillus* section Flavi species is ever evolving and new cryptic species have been discovered. The appropriate identification of these cryptic species, indistinguishable basing on their morphological traits, usually requires a polyphasic approach including morphological, biochemical and molecular analysis of specific genomic regions. The aim of this study was to investigate the applicability of a mass spectrometry approach to differentiate *Aspergillus* section flavi isolates.

Material / Methods: Eight clinical isolates, previously identified by molecular methods as A. flavus/A. oryzae, were subcultured on PDA plates and grown at 30°C. The surfaces of the fungal colonies were scraped, and the mycelium and conidia were suspended in distilled water. One µL of the mixture was directly spotted onto a polished target plate and air dried. Each sample was overlaid with 1 µL of absolute ethanol and 1 µL of a solution of a-cyano-4-hydroxycinnamic acid in 50% acetonitrile/2.5% trifluoroacetic acid. Measurements were performed with a microflex LT mass spectrometer (Bruker Daltonics, Bremen, Germany). Each spectrum profile was matched against the in house-Fungi Database and Log(score) values of $\geq$ 2.0 were considered sufficient for species identification, instead Log(score) values between 1.9 and 1.7 were sufficient for genus only. For Main Spectra Profiles (MSPs) creation, ten spectra of each clinical isolates and each reference CBS strains were manually acquired, using the MALDI Biotyper 3.0 software package. MSPs were included in the dendrogram for the cluster analysis.

Results: All clinical isolates were identified as *A. flavus* or A. oryzae with Log(score) values between 1.9 and 1.7 by MALDI-TOF MS, with a not reliable species identification. In order to discriminate the different isolates, a MSP dendrogram was generated by MALDI Biotyper 3.0 software including spectra of clinical isolates and the reference strains belonging to *A. flavus* complex. Clinical isolates clustered in an isolated group with respect to *A. flavus* complex reference spectra, suggesting a possible new species. Additional study would be performed to deeply understand whatever these isolates could belong to new undiscovered species.

Conclusions: Since the correct identification of *Aspergillus* section flavi may affect antifungal treatment and aflatoxins produced by these isolates are proven to be hepatocarcinogenic their accurate identification and discrimination within this section is of detrimental importance for patients management and outbreak control strategies. Mass spectrometry could be a feasible approach to discriminate among cryptic species belonging to *A. flavus*

complex and possibly suggest the existence of novel sibling isolates.

P 078

THE VARIABLE ABILITY OF FORMING BIOFILM IN *C. PARAPSILOSIS* BLOODSTREAM ISOLATES IS ASSOCIATED TO DIFFERENT PATHOGENICITY

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Introduction: *Candida* is the fourth most common cause of bloodstream infections in hospitalized patients. In several studies, *C. parapsilosis* has now become the second most common isolated species from blood cultures in Europe, Canada and Latin America, and the third one in United States. *C. parapsilosis* is able to produce biofilm. The aim of this study is to investigate the variable ability of clinical isolates of *C. parapsilosis* to form biofilms and how this impacts on clinical outcome.
Materials and Methods: We studied 195 clinical strains of *C. parapsilosis* isolated from 2000 to 2015 at Catholic University Hospital in Rome. The biofilm production was classified as having low, moderate, or high metabolic activity, according to XTT reduction assay (LMA, MMA and HMA respectively), or having low, moderate, or high biomass according to crystal violet (CV) assay (LBA, MBA and HBA respectively).In addition, we have used an insect model, *Galleria mellonella*, to demonstrate a correlation between biofilm formation and pathogenicity of different clinical strains of *C. parapsilosis*.
Results: Biomass production and metabolic activity of the sessile cells for each *C. parapsilosis* are measured by CV and XTT procedures. The analysis of biofilm structure by optical microscope revealed different biofilm frameworks that were consistent to the classification of isolates by the CV and XTT procedures. Most isolates were LBF (85%) or LMA (83%), followed by HBF (66%) or HMA (68%) and MBF (40%) or MMA (38%). There is a correlation between variable ability of isolates of *C. parapsilosis* to form biofilms and clinical data of infected patients. In fact, among 152 (80%) intravascular device there were 91 (47.9%) CVC-related candidemia cases with 62 (58.5%) patients infected with MBA/HBA biofilm-forming group and 29 (34.5%) with LBA biofilm-forming group ($p = 0.001$). Before they were affected by candidemia, 15 (7.9%) patients had been staying in the ICU; the majority of them were infected with MBA/HBA 13 (12.3%). The hospital mortality was 55.6% (59 of 106 patients) in the MBA/HBA, compared to 35.7% (30 of 84 patients) in the LBA ($p = 0.001$). Furthermore we have demonstrated a correlation between biofilm formation and pathogenicity using an insect model *G. mellonella*.
Conclusions: We studied the mass and the metabolic activity of biofilm produced by the clinical strains of *C. parapsilosis* using a cut-off to classify isolates as having low, moderate, or high biomass and metabolic activity respectively. Furthermore our data show that biofilm growth has an adverse impact on clinical outcomes of patients and the better outcomes were found for those patients are infected by a low producer of biofilms. Thanks to the insect model *G. mellonella* we have demonstrated that the greater is the ability to form biofilms, the greater is pathogenicity of the strain.

P 079

MOLECULAR MECHANISMS OF AZOLE RESISTANCE IN CLINICAL ISOLATES OF *CANDIDA ORTHOPSILOSIS*

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Introduction: *Candida albicans* is the main cause of invasive candidiasis worldwide; nevertheless a large proportion of bloodstream infections are due to non-*albicans Candida* species. The *C. parapsilosis* complex, the second most common isolated species includes three cryptic species: *C. parapsilosis* sensu strictu, *C. orthopsilosis* and C. metapsilosis. Recent clinical data indicate that *C. orthopsilosis* isolates are significant pathogens responsible for a broad variety of clinical manifestations and that a decrease of susceptibility to azole might become a cause for clinical concern. The alteration or up-regulation of the enzyme target lanosterol

14-α-demethylase, encoded by the ERG11 gene, or enhanced drug efflux due to overexpression of efflux pump such as ABC transporters have been recognized as the most common mechanisms of azole resistance in Candida. Nowadays relatively few reports still describe the azole resistance in C. orthopsilosis. The aim of this study is to evaluate the molecular mechanisms in azole-resistant *C. orthopsilosis* clinical strains.
Materials and Methods: Twenty-nine isolates of *C. orthopsilosis* identified by MALDI-TOF MS were selected from a collection of clinical samples (ROCANET: Rome *Candida* Network). Antifungal susceptibility test was performed by Sensititre YeastOne and voriconazole (VOR) and fluconazole (FLC) MIC values were in parallel evaluated by broth microdilution method in accordance with CLSI document M27-A3. The entire open reading frame (ORF) of the ERG11 gene was amplified and sequenced with specific primers and compared with *C. orthopsilosis* isolate ATCC 96139 wild-type ERG11 sequence. A flow-cytometry based high-throughput screen (HTS) assay was performed using Rhodamine 6G (R6G) as fluorescent substrate, in order to understand the involvement of efflux pump in the resistance.
Results: All *C. orthopsilosis* isolates were categorized as VOR and FLC susceptible, susceptible dose dependent or resistant according to *C. parapsilosis* CLSI species-specific clinical breakpoints, in particular 8 out of 29 turned to be VOR and FLC resistant. Interestingly none of these resistant isolates showed enhanced efflux of R6G in flow-cytometric HTS assay, whilst 7 isolates harbored the aminoacidic substitution p.Y132F in the ERG11 gene.
Conclusions: Basing on our results it is inferable that azole resistance in *C. orthopsilosis* should not involve ABC transporters efflux pump. Conversely, substitution p.Y132F in ERG11 gene is probably a mutation able to prevent the binding of azoles to the active site of the target. In this context it would be worthy to go deeper in the involvement of such mutation in *C. orthopsilosis* resistance.

P 080

INHIBITION OF *CANDIDA GLABRATA* AND *CANDIDA TROPICALIS* ADHESION ON MEDICAL-GRADE SILICONE BY RHAMNOLIPIDS FROM *PSEUDOMONAS AERUGINOSA*

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Introduction: Biofilm-associated infections on medical devices are a serious problem for the public health system with high morbidity and mortality rates. In biofilm, microbial cells become resistant to antibiotics and less susceptible to the immunological defense mechanisms, thus leading to increasing difficulties in the clinical treatment. In the last years, a significant increase in the incidence of *Candida* spp. medical-device associated infections has been observed, representing a significant problem in terms of patient management and healthcare costs. New strategies in preventing biofilm development have drawn attention to bacterial antagonistic products. Among these, biosurfactants have shown the ability to inhibit biofilm formation, reducing surface hydrophobicity of biomaterials, thus interfering with microbial adhesion.
Materials and Methods: The ability to form biofilm on medical-grade silicone elastomer disks (SEDs) of six *Candida* spp. clinical isolates (*C. krusei* 1, *C. glabrata* 1, *C. glabrata* 2, *C. glabrata* 3, *C. glabrata* 4, *C. tropicalis* 1) was carried out by means of the XTT assay. Subsequently, the anti-adhesive activity of rhamnolipid biosurfactants from *Pseudomonas aeruginosa* 89 (R89BS) was tested against the two most efficient biofilm producer strains on SEDs by means of the crystal violet staining method. SEDs were coated by three different concentrations of R89BS (0.5 mg/mL, 1 mg/mL and 2 mg/mL) and its anti-adhesive activity was assessed in the presence of 10% fetal bovine serum. Furthermore, the optimal carbon source and medium for *P. aeruginosa* 89 growth and rhamnolipid production were selected and the Critical Micellar Concentration (CMC) of R89BS was evaluated.
Results: For the optimal production of rhamnolipids, *P. aeruginosa* was grown in mineral Siegmund

medium supplemented with 3% glycerol for 72 h. The CMC value of R89BS crude extract was 46.1 µg/mL. Among the six *Candida* spp. isolates, *C. glabrata* and *C. tropicalis* strains were classified as high biofilm formers. In particular, the strains *C. tropicalis* 1 and *C. glabrata* 3 produced the highest amount of biofilm on silicone after 48 h of incubation and were selected for the anti-adhesion assay. On the contrary, *C. krusei* 1 was a low biofilm former. R89BS treated SEDs were able to reduce significantly the adhesion of both *Candida* spp. isolates at all the tested concentrations. In particular, the highest activity was observed at 0.5 mg/mL for *C. tropicalis* 1, with a reduction of adhesion to SEDs of 51%, whereas for *C. glabrata* 3 the highest inhibition was detected at 2 mg/mL with a reduction of adhesion of 44%.
Conclusions: In conclusion, R89BS deserves a promising role as anti-adhesive agent to prevent or slow *Candida* spp. biofilm formation on medical devices.

P 081

RAPID GROWTH OF FUNGI IN LIQUID CULTURE: A PROMISING DIAGNOSTIC TOOL FOR FUNGAL INFECTIONS

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Introduction: One of the major problems in diagnosing of fungal infections is the time for microbiological results, nevertheless these opportunistic pathogens are very frequent in a getting older population: speeding the cultural examinations could be helpful for a timely overview of patients. Dermatophytes, although don't cause life-concerning infections, are among the slower fungi to be identified, by colony and microscopic morphology observation. In this study, we propose a rapid method for fungi detection using light-scattering technology to monitor the growth into a new liquid medium, thus reducing considerably the time of culturing.
Materials and Methods: Two hundred respiratory samples and 8 cutaneous samples, collected in the tertiary-care University Hospital at Pisa, Italy, were tested using HB&L Sabouraud kit (Alifax Srl, Italy). For respiratory samples, an aliquot of diluted samples were inoculated into culture broth vials and incubated into the HB&L System (Alifax) at 37°C. The fungal growth was monitored for 48 hours. The traditional semi-quantitative culture method was considered the reference method. The plates were observed at 24 and 48 hours, microbial load(s) was calculated and colonies were identified by MALDI-TOF Mass Spectrometry (Bruker Daltonics). The medium suitability was tested for 19 moulds species belonging to genera *Alternaria*, *Aspergillus*, *Fusarium*, *Penicillium* and *Trichophyton*. Skin swabs were double inoculated into culture broth vials and incubated into the HB&L System. Results were compared with traditional culture followed by microscopic identification. Samples with positive results by the HB&L system were identified directly using MALDI-TOF MS.
Results: Positive respiratory samples were 76 out of 200: 95% of samples were positive within 24 h and 93,5% of bacteria were inhibited in this medium. Filamentous fungi in the liquid medium were positive in few hours (3-29) compared to the traditional cultures, even at low inoculum (0.1 Mc Farland). Positive skin swabs were 3 out of 8: 2 positive for dermatophytes (*Microsporum canis* and *Trichophyton rubrum*; with identification scores of 2.085 and 2.291, respectively) and 1 for *Candida parapsilosis*.
Conclusion: Results for respiratory samples show a rapid growth of yeasts compared to the traditional method; even more important, 33% of yeasts were detected only with the new method. Preliminary results for dermatophytes show a remarkable and unexpected reduction of the turnaround time for the mould growth. Dermatophytes identified from clinical samples were positive within 20h compared with 12 days using the traditional method (*Microsporum canis*), and within 90h vs 15 days using the traditional method *(Trichophyton rubrum).*

P 082

MICROSATELLITE GENOTYPING OF *CANDIDA PARAPSILOSIS* STRAINS ISOLATED FROM BLOOD CULTURES BY TWO MULTIPLEX PCR

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Introduction: *C. parapsilosis* is a human opportunistic pathogen that is frequently implicated in superficial and invasive disease. Most of the infections are due to exogenous acquisition of the fungus, which shows a marked tendency to colonize hospital environment, including central venous catheters and several other medical devices. To identify similarities among the isolates and the cross-transmission of the microorganism the molecular typing methods are frequently used. However methods as random amplified polymorphic DNA (RAPD) or pulsed-field gel electrophoresis (PFGE) not discriminate enough to distinguish closely related isolates. Today it is preferred microsatellite analysis as a highly accurate typization method.
About that, we examined eighteen strains of *C. parapsilosis* isolated from blood culture with two molecular typing methods to evaluate their possible clonality. In particular microsatellite analysis was performed, setting up two multiplex PCR, and eight loci were chosen for evaluations .
Materials and Methods: To confirm phenotipical identification, the 18 strains of *C. parapsilosis* were analyzed by sequence analysis of the ITS region. As typing methods we used RAPD and microsatellite analysis of 8 loci. For microsatellite analysis two different multiplex PCR were setted and products were size-separated by capillary electrophoresis performed on a genetic automatic sequencer (ABI Prism 3130). Electropherograms were verified using Gene Mapper v.4.1 software. The R package 'poppr' was employed to identify unique multi-locus genotypes (MLGs) based on microsatellite allele sizes by isolate and to calculate microsatellite loci statistics. Pairwise genetic distance between isolates was calculated and plotted in a UPGMA dendrogram based on Nei's genetic distance.
Results: The unique RAPD profiles showed a homogeneity among *C. parapsilosis* isolates. The 18 samples analyzed at 8 microsatellite loci showed 11 different MLGs. The remaining 7 isolates probably originated from clonal duplication.
A total of 22 alleles were detected with an average of 2.75 alleles for locus. The cluster analysis of the isolates match with the provenience of samples. According to the genetic distance, 3 clusters can be clearly distinguished.
Conclusions: The recent clinical relevance of *C. parapsilosis* in intensive care units has highlighted the need for the proper identification and epidemiological study of this yeast. Molecular techniques as PFGE, RAPD, RFLP are excellent tools for typing of the microorganism but the high discriminatory power of simple sequence repeat (SSR) analysis is essential for epidemiological investigations.
In this study only the microsatellite analysis of eight loci has permitted to observe three different clusters in 18 *C. parapsilosis* strains isolated from blood colture.

P 083

INHIBITION OF *CANDIDA ALBICANS* BIOFILM FORMATION BY A HUMAN LACTOFERRICIN DERIVED PEPTIDE (HLF1-11)

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Introduction: Biofilm formation represents one of the major virulence factors contributing to the pathogenesis of *Candida albicans* infections, due its high levels of resistance to conventional antifungal treatments. Hence, the development of alternative approaches to prevent and/or eradicate biofilm is urgently needed. A synthetic N-terminal peptide of human lactoferricin (hLF1-11) exerts potent antifungal activity against planktonic *C. albicans* cells. The present study aims at investigating the *in vitro* anti-biofilm activity of hLF1-11 against clinical isolates of *C. albicans* with different fluconazole susceptibility.

Materials and Methods: Three different *C. albicans* clinical isolates and the SC5314 reference strain were selected for their ability to produce biofilm. *C. albicans* cells were co-incubated with various concentrations of hLF1-11 into flat bottomed 96 well plates at 37°C for different time points. The inhibitory effect of hLF1-11 was also evaluated at 24 h following fungal adhesion for 90 min and 6 h. The anti-biofilm activity of the peptide was assessed in terms of biofilm biomass reduction, metabolic activity, viable cell (CFU/ml) reduction. Quantitative RT-PCR was used to evaluate the expression of 12 genes related to cell adhesion, hyphal development and extracellular matrix production in hLF1-11 treated and untreated biofilms. Exogenous dibutyryl-cAMP (dbc-AMP) was used to rescue the morphogenesis in cells treated with hLF1-11 for 4 h.
Results: The hLF1-11 peptide exhibited an inhibitory effect on biofilm formation by all the *C. albicans* strains tested dose-dependently, regardless of fluconazole susceptibility. The results obtained from co-incubation experiments revealed a significant reduction ($p < 0.05$) in biofilm biomass, metabolic activity (100%) and CFU/ml (~2 log) of sessile cells treated with hLF1-11, as compared to the untreated control. Microscopic observation of biofilm cells with an inverted microscope showed that hyphal formation was significantly reduced in hLF1-11 treated cells. This anti-biofilm effect was evident as early as 3 h. Moreover, hLF1-11 showed a reduced activity on pre-adherent cells. The transcription level of genes associated with biofilm formation was downregulated by hLF1-11. Interestingly, most of these genes were regulated by Ras1-cAMP-Efg1 pathway. The exogenous dbc-AMP rescued the hyphal growth in hLF1-11 treated cells.
Conclusions: The hLF1-11 peptide significantly inhibited biofilm formation by *C. albicans* mainly at early stages, interfering with biofilm biomass and metabolic activity, and significantly affected morphogenesis through the Ras1-cAMP-Efg1 pathway. Our findings suggest that hLF1-11 could represent a potential agent to prevent biofilm formation by *C. albicans*.

P 084

EFFECTS OF CHOLINERGIC AGONISTS AND ANTAGONISTS ON THE PATHOGENESIS OF SYSTEMIC *CANDIDA ALBICANS* INFECTIONS

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Introduction: Systemic candidiasis continues to carry a high mortality, despite available antifungal treatment. *Candida albicans* biofilms have been observed on the majority of commonly used indwelling devices, and represent an important virulence factor contributing to drug resistance and pathogen persistence. Recently, non-neuronally derived acetylcholine (ACh) has been demonstrated to play a role in regulating favorable innate immune responses to *C. albicans* infection resulting in effective clearance with limited bystander organ damage. Furthermore, ACh was also found to directly inhibit yeast-to-hyphae transition and biofilm formation both *in vitro* and *in vivo*. In this study, we investigated, in a *Galleria mellonella* infection model, the effect of cholinergic agonists and antagonists (pilocarpine, scopolamine, tubocurarine) on the pathogenesis of systemic *C. albicans* infection. We aimed at determine whether the promotion of larval survival was attributable to an enhanced immune response, inhibition of fungal growth, or both.
Materials and Methods: The effect of cholinergic drugs on *C. albicans* biofilm formation *in vitro* was assessed using crystal violet assay and confocal laser scanning microscopy. Drugs modulation of host immunity to *C. albicans* infection was determined using haemocyte counts, cytospin analysis, and larval histology, and real time PCR on antimicrobial peptides.
Results: Cholinergic antagonists failed to affect larva survival during *C. albicans* infection. However, the non-specific muscarinic receptor agonist pilocarpine was found to inhibit biofilm formation and yeast to hypha transition to a greater extent than ACh. Nevertheless, ACh promotes a more effective cellular immune response compared to pilocarpine,

enhancing neutrophil-like immunity function, with a strong impact on infection outcome. In terms of larvae survival there was no difference in protection from *C. albicans* infection between larvae inoculated with ACh and larvae inoculated with pilocarpine ($p = 0.99$).

Conclusions: This data suggests that *C. albicans* possesses a putative muscarinic receptor which may mediate cholinergic modulation of *C. albicans* pathogenicity. The fact that ACh and not pilocarpine induced hemocyte recruitment and aggregation suggests that nicotinic receptors may play a more important role in immune regulation in the host.

P 085

CELLULAR IMMUNOLOGY TECHNIQUES APPLIED TO THE SPECIATION OF TRICHINELLA GENUS

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Introduction: Trichinellosis is a zoonotic parasitic infection caused by a nematode of the genus *Trichinella*, worldwide distributed. There are nine recognized species and two clades: encapsulated and not encapsulated. The cellular immune response to *Trichinella* is well known in the rodent, in which after a short Th1 response a Th2 polarization, follows and maintained during the whole period of the infection. However, such studies in humans are few. We studied the cellular immune response in the muscle phase of a patient infected along with other 51 people in a trichinellosis outbreak in which the aetiological agent was not possible to identify. The blood sample has been collected after 10 months of the infection.

Materials and Methods: We studied a patient infected during a dinner attended by 52 persons of which 36 resulted serologically positive for trichinellosis. We isolated peripheral blood mononuclear cells (PBMCs) that were incubated with Excretory / Secretory (E / S) antigens, of two *Trichinella* species: *T. spiralis* (T1) and *T. pseudospiralis* (T4) to determine the proliferative index . Other PBMCs were was used to produce antigen specific T cell clones from which the phenotype (T helper or cytotoxic cells) and the cytokine profile (IFN-ɤ, IL-17, IL-4) were analysed by flow cytometry and Elisa respectively.

Results: The specific test performed using antigen E/S T1 or T4 on PBMCs has helped to identify the *Trichinella* species responsible for the infection. In fact, the Proliferative Index (M.I.) of PBMCs was 2.5 in response to T1 and 12.7 to T4. The cell lines produced increased levels of in IFN-ɤ, IL-4 and IL-17 after treatment with phorbol 12-myristate 13 - acetate and ionomycin. The lymphocytes that produce IL-4 and IFN-ɤ were considered Th0, those producing IFN-ɤ but not IL-4, Th1, those producing IL-4 but not IFN-ɤ, Th2 and those producing only IL-17, Th17. 28 clones CD4+ from T1 line were obtained distributed as follows: 18.6% were Th2, 22.8% Th1, 6.6% Th17, 6 % Th0, 2.2% Th1/Th17 and 0.7% Th2/Th17. 11 clones CD4+ from T4 line were obtained: 23.7% were Th2, 27.2% Th1, 3% Th17, 10.3 %Th0, 1.9% Th1/Th17 and 1% Th2/Th17.

Conclusions: Through the application of cellular immunology techniques it was possible to identify the species of *Trichinella* responsible of the infection, confirming the results previously obtained by serological analysis. Based on the present results, it is possible to conclude that the species responsible for the outbreak where the patient was involved was *T. pseudospiralis*. The over all results show in human trichinellosis a mixed response of Th1 and Th2. For the first time it has been revealed in a human chronic infection the presence of Th17 cells.

P 086

SALINOMYCIN AND OTHER MONOVALENT IONOPHORES: POTENTIAL MALARIA TRANSMISSION BLOCKING AND ANTI-LEISHMANIAL AGENTS

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Introduction: Malaria and Leishmaniasis are major tropical protozoal diseases affecting millions of people, especially in developing countries. In the absence of a vaccine, chemotherapy remains the only strategy available, but the increased drug resistance urgently requires the identification of new molecules. Monovalent sodium and potassium ionophores, largely used in veterinary medicine and proposed as human anticancer agents, have antimalarial activity against asexual intraerythrocitic stages of *Plasmodium falciparum (Pf)*. We recently demonstrated that salinomycin and monensin possess transmission blocking activity as well. They are able to kill *Pf* gametocytes, the intraerythrocitic sexual forms, and to inhibit the formation of ookinetes and oocysts, the stages which develop in the mosquito vector, at nanomolar doses (D'Alessandro et al. AAC 2015, 59(9):5135-44). Up to now, only salinomycin has been tested for anti-leishmanial activity, with micromolar IC50 on promastigotes of *Leishmania donovani*. Here, we extended these observations to different *Leishmania spp* and investigated combination treatment to reduce the toxicity of these molecules, one of their major liabilities.
Materials and Methods: *Leishmania spp* viability was assessed by MTT assay, the same method used to evaluate cytotoxicity on different human and mouse cell lines (THP-1 Human monocytes; Human dermal fibroblasts; Human Microvascular Endothelian Cells; Immortalized Murine Bone Marrow Derived Macrophages). The pLDH assay was used as viability measure for asexual Pf parasites and the luminescence assay for gametocytes, using the transgenic 3D7elo1-pfs16-CBG99 strain.
Results: The activity of salinomycin was confirmed on different *Leishmania spp* (*L. tropica* and *L. brasiliensis*) and the analysis extended to additional ionophores. Of those, monensin and nigericin showed an inhibitory activity against promastigotes higher than that of salinomycin (IC_{50} lower than 1μM compared to salinomycin IC_{50} ~5μM). Experiments on Leishmania amastigotes are on-going. The potential toxicity of this class of molecules is an alarming issue. All the ionophores displayed different cytotoxicity depending on the cell type tested. To reduce the dose of treatment and thus ameliorate the selectivity index, association experiments were performed on Pf asexual parasites. Additive effects were observed when different doses of monensin were tested in association with dihydroartemisinin, the active metabolite of most artemisinins and the mainstay of antimalarial therapy.
Conclusions: Although further studies are needed, the monovalent ionophores are interesting anti protozoal agents and could be considered potential partner drugs for antimalarial or anti-leishmanial combination therapy.

P 087

NOVEL POTENTIAL ANTIMALARIALS WITH TRANSMISSION BLOCKING ACTIVITY FROM A SET OF NEW DERIVATIVES OF THE OXYBISBENZOIC ACID

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Introduction: The malaria drug treatment recommended by WHO is the artemisinin-based combination therapy (ACT). However, over the last 5 years, the rate at which the artemisinin derivatives clear malaria parasites has declined significantly in Southeast Asia. To substitute the artemisinins and to achieve the goals of the malaria elimination/eradication agenda, new antimalarials able to cure the disease and to kill the transmissible sexual stages of the parasite, the gametocytes, are strongly needed. A new promising chemotype, constituted by a 4,4'-oxybisbenzoic acid linker bound to two amino acids, has been investigated by our group. One derivative, named DC18, has been selected as

novel hit, because of its good water-solubility; fast and low cost synthesis; fulfilment of the Lipinski's rules of 5, except for the molecular weight (MW = 579). This work presents additional data, including the assays of DC18 against gametocytes as transmission blocking agent, either alone or in association with other drugs. In addition, the liability for patients with G6PD deficiency was also evaluated.
Materials and Methods: The tests were done *in vitro* against the asexual stages of different *Plasmodium falciparum (Pf)* strains using the pLDH method, whereas the transgenic 3D7elo1-pfs16-CBG99 strain expressing luciferase under a gametocyte specific promoter was used for the gametocytes assays. Cellular cytotoxicity was assessed by the MTT assay against normal cells. Haemolysis and formation of methemoglobin were evaluated spectrophotometrically.
Results: DC18 possesses excellent activity *in vitro* (IC50 0.5-5 nM) against all the *Pf* strains tested, equivalent or better than artemisinin derivatives. DC18 reacts very fast, mostly killing ring stage parasites, but also trophozoites. DC18 is active on both young (IC50 130nM on stage II-III) and mature *Pf* gametocyte (IC50 370 nM on stage IV-V). DC 18 activity is selective, since is not active against Leishmania spp, not toxic against different cell lines (IC50 > 25 μM) and it is not haemolytic (up to 200 μM). Differently from primaquine, DC18 does not induce methemoglobin thus is should be safe to patients with G6PD deficiency. Moreover, DC18 shows additive properties with chloroquine and dihydroartemisinin (DHA) on asexual blood stage, additive with methylene blue and synergistic with DHA on gametocytes.
Conclusions: These results suggest that molecules with oxybisbenzoic scaffold are potential partner with other antimalarial drugs and could be explored for further development. The similarity with artemisinins (high activity, fast time to kill, effects on rings stages) in addition to the activity against gametocytes, justifies the necessity to improve the pharmacology and oral bioavailability of these molecules.

P 088

ANTIFUNGAL ACTIVITY OF THE C-TERMINAL PEPTIDE FROM HUMAN SERUM ALBUMIN

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Introduction: In recent years, a number of studies showed the biological activities of peptides derived by proteolytic cleavage of physiological proteins. These functional units, called cryptides, can be characterized by activities similar or different to those of the precursor protein. The present study was aimed to evaluate the fungicidal properties of a synthetic peptide (K13L, KKLVAASQAALGL), representing the C-terminal fragment of human albumin found in serum.
Materials and Methods: The synthetic peptide was obtained by solid-phase synthesis chemistry and evaluated for its biological properties by consolidated experimental methods. The *in vitro* fungicidal activity was investigated against different yeast species, inclusive of multidrug resistant strains. The ex vivo and *in vivo* anticandidal efficacy was evaluated in a model of *C. albicans* infection in porcine oral mucosa and in experimentally infected larvae of *Galleria mellonella*, respectively. Furthermore, haemolytic, cytotoxic and genotoxic properties were verified. The effect of peptide treatment on *C. albicans* cells was studied by confocal microscopy and transmission and scanning electron microscopy.
Results: K13L peptide proved to be fungicidal *in vitro* against the investigated yeasts at micromolar concentrations, to reduce fungal infiltration in porcine oral mucosa ex vivo, and to exert a therapeutic effect *in vivo* in the invertebrate animal model, without showing toxic effects on mammalian cells. Microscopic studies demonstrated that the peptide penetrates and accumulates in fungal cells causing gross morphological alterations in cellular struc*ture.*
Conclusions: Overall, our data prove that fragments from physiological serum proteins, as K13L peptide, may exert an antifungal activity, as previously shown for antibody-derived peptides [1], sugge-

sting a potential role of these molecules in antifungal homeostasis and establishing serum proteins as a source of new antimicrobial agents.

1. Ciociola T, Giovati L, Sperinde M, Magliani W, Santinoli C, Conti G, Conti S and Polonelli L. Peptides from the inside of the antibodies are active against infectious agents and tumours. J Pept Sci 2015); 21: 370-378.

POSTERS

VIROLOGY

P 089

DETECTION OF MALAWI POLYOMAVIRUS SEQUENCES IN SECONDARY LYMPHOID TISSUES FROM ITALIAN HEALTHY CHILDREN: A TRANSIENT SITE OF INFECTION

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Background: In the last years, in Italy, particular attention has been dedicated to the new Polyomavirus, called Malawi polyomavirus (MWPyV), since a sero-epidemiological survey on a large sample of subjects, has documented the circulation of this virus with a trend of frequency similar to that reported for the human polyomaviruses JC and BK. MWPyV was initially detected in stool specimens from healthy children and children with gastrointestinal symptoms, mostly diarrhea, indicating that this virus play a role in human gastroenteric diseases. Recently, MWPyV sequences were additionally identified in respiratory secretions from both healthy and acutely ill children suggesting that MWPyV may have a tropism for different human tissues. This study was designed to investigate the possible sites of latency/persistence for MWPyV in a cohort of healthy Italian children.
Materials and Methods: Specimens (n° 500) of tonsils, adenoids, blood, urines and feces, from 200 healthy and immunocompetent children (age range: 1-15 years) were tested for the amplification of the MWPyV LT antigen sequence by quantitative real-time PCR. Samples (n° 80) of blood and urines from 40 age-matched children with autoimmune diseases, were screened for comparison. Polyomaviruses JC/BK and Epstein-Barr Virus (EBV) were also tested as markers of infection in all samples using the molecular technique.
Results: In our series of healthy children, MWPyV was detected only in the lymphoid tissues showing a prevalence of 6% in tonsils and 1% in adenoids, although with a low viral load. No JCPyV or BKPyV co-infection was found in MWPyV positive samples, while EBV showed a similar percentage of both in tonsils and adenoids (38% and 37%). Conversely, no MWPyV DNA was detected in stool from babies with gastroenteric syndrome. With regards to autoimmune children, neither MWPyV nor BKPyV were detected in blood, while JCPyV viremia was observed in 15% (6/40) of children treated with Infliximab. Urinary BKPyV shedding was observed in 12.5% (5/40) while JCPyV in 100% of the samples.
Conclusions: The detection of MWPyV sequences in tonsils and adenoids of healthy children suggests that secondary lymphoid tissues can harbour MWPyV probably as transient sites of persistence rather than actual sites of latency. However, only very few cells proved to be infected suggesting that these tissues are unlikely to be the preferred site of latency, at least in this series of children. Nevertheless, the high MWPyV seroprevalence found in young children from this geographic area, and the *in vitro* ability of MWPyV to bind to cellular tumor suppressor factors, seem to support the hypothesis that infection/reactivation and transmission of MWPyV may have clinical consequences only in severely immunocompromised hosts.

P 090

HERPES SIMPLEX VIRUS TYPE 1: NEW ANTIVIRAL STRATEGIES

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Introduction: Herpes simplex virus type 1 (HSV-1) infects mucocutaneous tissues in a latent way to cause cold sores and, in severe cases, may cause encephalitis. It enters cells through fusion of the viral envelope with a cellular membrane involving multiple interactions between viral glycoproteins and cellular receptors. The envelope glycoprotein B, D, H and L are all essential for the entry process. In particular, gB and gH/gL interact with each other concomitantly with fusion and this interaction is triggered by binding of gD to its cellular receptor. The glycoprotein B is the key fusion protein. The now known crystal structure of the ectodomain,

composed by a trimer, shows that each protomer is divided into five distinct domains; the domain III contains a trimeric coiled coil and a long nonhelical C-terminal arm packing against the coiled coil in an antiparallel mode. Previous results from our laboratory have shown that a peptide comprising the N-terminus of the helical sequence (residues from 500 to 523, named gBhN) is an active inhibitor of HSV-1 infection prior to virus penetration into cells.
Materials and Methods: The aim of this work is to improve the antiviral activity of the peptide gBhN through binding it to a cholesterol tail. Solid phase-9-fluorenyl-methoxycarbonyl (Fmoc) on an automatic synthetizer SYRO multisyntech standard method for peptide synthesis has been used. Then the peptides has been purified with HPLC, characterized with HPLC/MS and bound to cholesterol. A cytotoxicity assay has been realized, while a co-treatment and a cell pre-treatment assays have been performed to test antiviral activity of the complex.
Results: The compounds have not shown any toxicity on eukaryotic cells, even at concentrations higher than the ones used in antiviral testing. Moreover, we have obtained an improvement in the antiviral activity of the complex gBhN/cholesterol compared to the peptide alone.
Conclusions: Our results provide some new spotlights in HSV-1 entry mechanism but further studies are in progress to deepen the mechanisms underlying the interaction between the peptide and cellular membrane.

P 091

LEVELS OF NON-DIOXIN-LIKE POLYCHLORINATED BIPHENYLS (NDL-PCBS) IN MUSSELS INFECTED BY ENTERIC VIRUSES

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Introduction: Marine bivalves, like mussels, are often used as sentinel organisms of aquatic pollution that can provoke disorders in their growth, reproduction or survival. In particular, mussels filter large volumes of seawater and their immune capacities can be adversely affected by exposure to contaminants, potentially increasing their susceptibility to infectious diseases. Investigating immunity toxicity can provide relevant information both on the quality of the marine environment and on infectious diseases affecting bivalves in coastal areas.
Objectives: The main purpose of this paper is the evaluation the mussel Mytilus galloprovincialis as a sentinel organism regarding both environmental contaminants and enteric viruses, which are recognized as a source of food-borne diseases.
Materials and Methods: We collected 21 samples of mussels from Italian aquaculture farming. They were examined to evaluate, by real-time reverse transcription PCR, the presence of three enteric viruses: norovirus genogroup I (NoVGI), norovirus genogroup II (NoVGII) and astrovirus (AsV). Subsequently, the samples were analyzed to measure the concentration of six NDL-PCBs (28, 52, 101, 138, 153, 180), through a clean-up on acid diatomaceous columns and florisil cartridges (SPE) and quantified by high-resolution gas chromatography/high-resolution mass spectrometry (HRGC-HRMS).
Results: We revealed a significant prevalence of AsV (62%), NoVGII (52%), NoVGI (24%) on examined samples. About 33% of the mussels resulted co-infected by at least two of the three viruses investigated. Additionally, mussels contained $\sum 6$ NDL-PCBs ranged between 0.93 and 6.22 ng/g wet weight. In particular, a correlation between NDL-PCBs levels and the presence of viruses was detected in 89% of examined mussels.

Discussion and Conclusions: In this study, the biological response of mussels (*Mytilus galloprovincialis*) to contamination with anthropogenic pollutants was investigated. Original data on the relationship between the concentrations of NDL-PCBs and the occurrence of enteric viruses (AsV and NoVs), bio-accumulated by mussels samples from Italian farming, are reported. Although a deep discussion is no possible because no literature data are available. Further work is in progress for a broaden characterization of POPs burdens in mussels from different Italian coasts.

P 092

HYDROXYUREA CAN INHIBIT PARVOVIRUS B19 REPLICATION IN ERYTHROID PROGENITOR CELLS

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Introduction: Parvovirus B19 (B19V) is a human ssDNA virus with a strict tropism towards erythroid progenitor cells (EPCs) in bone marrow. In subjects with underlying hematological disorders or immunological deficits, the viral-induced arrest of erythropoiesis can lead to severe clinical manifestations requiring hospitalization and supportive treatments. No specific antiviral drugs are available for B19V, although recent research showed activity for the acyclic nucleotide analogue cidofovir. In this work, hydroxyurea (HU), an S-phase specific inhibitor of DNA synthesis, was evaluated for its possible inhibitory effect on B19V replication.
Materials and Methods: UT7/EpoS1 cell line and EPCs were infected with B19V, in the presence of 0.1 μM-50 mM HU. The inhibition of B19V replication was assessed by qPCR evaluation of viral nucleic acids and expressed as EC50 values. Cellular growth, viability and proliferation in the presence of HU was determined in both systems and expressed as CC50 values. EPCs were analysed by flow cytometry to evaluate the expression of CD36, CD71 and CD235a erythroid markers following B19V infection and HU treatment.
Results: B19V replication was inhibited by HU in a dose-dependent manner yielding EC50 values of 139 μM and 175 μM for UT7/EpoS1 and EPCs. Assessment of the inhibitory effects on cellular replication yielded CC50 values of 457 μM and 491 μM, respectively. Flow cytometry analysis indicated that both HU and B19V blocked EPCs differentiation along the erythroid lineage, without additive effects.
Conclusion: Our study provides evidence of an antiviral activity of HU against B19V. HU is an approved drug for the therapy of sickle cell disease (SCD), and recent data indicate that HU therapy reduces symptoms of anemia during B19V-induced transient aplastic crisis (TAC) in SCD. Our findings lend experimental support for the observed attenuation of clinical symptoms during TAC episodes caused by B19V infection in the SCD population in the context of HU therapy.

P 093

PLASMA AND CEREBROSPINAL FLUID BIOMARKERS OF INFLAMMATION AND NEURONAL DAMAGE DO NOT CHANGE IN HIV/HCV CO-INFECTED INDIVIDUALS ACCORDING TO HCV GENOTYPE

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Introduction: Approximately 25%-40% of individuals infected with human immunodeficiency virus (HIV) are co-infected with hepatitis C virus (HCV). Six major HCV genotypes have been associated with differences in geographical distribution, natural history and response efficacy of current hepatitis C therapy. During chronic HCV infection a number of pro-inflammatory cytokines are activated. The aim of this study was to evaluate the relation between HCV genotype, inflammatory response and neuronal damage in HIV/HCV co-infected patients (pts).
Material and Methods: Retrospective analysis of

58 CSF/plasma pairs from HIV/HCV co-infected pts followed at the INMI Spallanzani, Rome, between 2002-2014 has been performed. In all pts, CSF and plasma HIV/HCV-RNA determination and CD4 measurement were contextually available. HCV-RNA levels were assayed by Roche COBAS Ampliprep-Taqman HCV v2.0 while HCV genotype by Abbott RealTime HCV genotype II. By ELISA assay the concentrations of the following biomarkers were obtained: neopterin and sCD14 (intrathecal and monocytes activation), Neurofilament light-protein (NF-L) (neuronal injury). The results were expressed as median (interquartile range, IQR), the differences between groups were analyzed by Kruskal-Wallis test.

Results: 58 HIV/HCV co-infected pts with HCV RNA+ were included. Overall, 82% were male, median (IQR) age was 48 y (42-52), and HIV acquisition IDU in 74.5%, heterosexual in 12.7% and other in 11%. CD4 cells/mm^3 median (IQR) was 208 (108-451). The median (IQR) plasma and CSF HIV-RNA was 1.9 (1.6-4.1) and 1.7 (1.6-2.7) cps/ml, respectively, while the median (IQR) plasma and CSF HCV-RNA was 5.8 (5.4-6.2) and 1.04 (1.04-1.08) IU/ml. 14 (24%) pts had HCV-RNA detectable in CSF. The prevalence of HCV genotype among the population was the following: 23/58 (39.7%) genotype 1a (G1a), 4/58 (6.9%) (G1b), 1/58 (1.7%) (G2), 19/58 (32.8%) (G3/3a), 8/58 (13.8%) (G4) and 3/58 (5.2%) (nd). The same prevalence was also observed in pts with HCV-RNA detectable in CSF. According to HCV genotype stratification, data showed no significant difference in the plasma and CSF NFL ($p = 0.136$; $p = 0.559$), neopterin ($p = 0.144$; $p = 0.378$) and sCD14 ($p = 0.443$; $p = 0.192$) concentrations. For each HCV genotype group was further evaluated the median of difference between plasma and CSF log10 HCV-RNA; comparing these medians no difference were found ($p = 0.128$).

Conclusions: Our results show that 24% of pts has detectable HCV RNA also in CSF and that the inflammatory profile (sCD14 and neopterin), neuronal damage (NFL) and HCV viral load in both compartments were completely independent of the infecting HCV genotype. Moreover, the prevalence of HCV genotype in pts with detectable CSF HCV-RNA showed that no specific genotype was more prone to CNS invasion.

P 094

THIAZOLIDES INHIBIT HENDRA VIRUS F PROTEIN MATURATION AND INTRACELLULAR TRAFFICKING IN HUMAN CELLS

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Introduction: Hendra virus (HeV) is an emerging deadly virus belonging to the Henipavirus (HNV) genus of the *Paramyxoviridae* family. HeV causes a zoonotic disease: fruit bats are the natural host, whereas horses represent HeV intermediate hosts, transmitting infection to humans through close contact. In humans HeV can lead to respiratory illness with severe flu-like symptoms, that may progress to fatal respiratory or neurological disease; although HeV infection is rare, the case/fatality rate is high (60-75%). The lack of vaccine or antiviral therapy underscores the need for novel drugs effective against HNV. We have previously shown that the thiazolide nitazoxanide (NTZ) has antiviral activity against several RNA viruses and inhibits influenza virus replication by a novel mechanism, impairing hemagglutinin maturation and virus morphogenesis. Herein we investigated the effect of NTZ and second-generation thiazolides (SGT) on the maturation and intracellular trafficking of HeV fusion glycoprotein (HeV-F) in an in-vitro HeV-F expression model.

Materials and Methods: Confluent monolayers of HeLa cells were transiently transfected with a pCMV-driven construct containing the gene expressing the HeV-F protein linked to a FLAG-tag or the pcDNA3 vector as control. Thiazolides were dissolved in DMSO and diluted in culture medium before treatment. HeV-F stability, oligomerization, maturation and intracellular trafficking were evaluated by SDS/PAGE-autoradiography after [35S]-methionine-labeling, sensitivity to glycosidase digestion, Western-blot, cell-surface protein biotin labeling and confocal immunomicroscopy.

Results: Thiazolides caused a decrease in intracellular HeV-F protein levels. This effect was not due to protein degradation via the ubiquitin-proteasome system or autophagy; instead HeV-F protein was found to be present in an insoluble state in NTZ- or

SGT-treated cells, revealing a drug-induced defect in maturation leading to HeV-F protein aggregation. Thiazolides were also found to prevent HeV-F protein translocation to the host-cell membrane.
Conclusions: The HeV-F protein plays a critical role in cell fusion and infectivity of the virion and therefore represents an interesting target for novel antiviral drugs. The fact that NTZ and SGT were found to interfere with HeV-F maturation and translocation to the cell membrane, an effect that would prevent progeny virus morphogenesis, cell-to-cell fusion and syncytium formation, suggest that thiazolides my represent a novel therapeutic strategy against HeV infections.

P 95

QUANTITATIVE HCV RNA TEST FOR MONITORING PATIENTS ON TREATMENT WITH DIRECT ANTIVIRAL AGENTS

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Introduction: Hepatitis C virus (HCV) infection has reached epidemic proportions. Chronic infection induces continuous inflammation in the liver, progression of hepatic fibrosis, eventual cirrhosis and possible hepatocellular carcinoma. A number of promising new direct-acting anti-virals (DAAs), which interfere with specific steps in the HCV replication cycle through a direct interaction with the HCV genome, polyprotein, or its polyprotein cleavage products, have been developed. Thanks to their increased efficacy, safety and tolerability DAAs are approved for patients with HCV infection, including those with cirrhosis. Viral load is recommended for monitoring antiviral therapy.
Materials and Methods: 13 patients treated with DAAs were selected: 11 patients with cirrhosis, 1 with liver transplant and 1 with chronic hepatitis. Their viral load ranged from 1*105 to 5*106 UI/mL (VERSANT HCV RNA 1.0 SIEMENS) and HCV genotypes (GEN-C 2.0 NUCLEAR LASER MEDICINE) are: 1a (2/13), 1b (7/13), 3a (2/13), 3a/3c (1/13) and 2a/2c (1/13). Two patients are in therapy for 24 weeks with PARITAPREVIR/OMBITASVIR/RITONAVIR+DASABUVIR+RIBAVIRIN and 2 with the same therapy for 12 weeks, 2 with PARITAPREVIR/OMBITASVIR/RITONAVIR+DASABUVIR for 12 weeks, 3 with DACLASTAVIR+SOFOSBUVIR+RIBAVIRIN for 12 weeks and 3 for 24 weeks and 1 patient SOFOSBUVIR+RIBAVIRIN for 16-20 weeks. Viral load has been tested after 2 and 4 weeks of treatment and at the end of therapy, as AISF (Associazione Italiana Studio Fegato) guidelines. Haemochrome, alkaline phosphatase, urea, creatinine, albumin, prothrombin time, transaminases are also tested for monitoring any adverse effects.
Results: At the first testing during the therapy 6/13 patients had no more HCV-RNA detectable into blood, 5/13 had a decrease of viral load of 4.0-5.0 log, 2/13 patients have just started treatment and they will be tested in the next weeks. At the second and third testing, 2/5 patients further decreased viral load of 0.5-2.5 log, while in the others 9/13 (70%) viral genome was undetectable (0 UI/mL). No significant biochemical parameters variations are observed.
Conclusions: The ideal outcome of the new anti-HCV therapy is the achieving sustained virologic response (SVR), defined as undetectable viral RNA into the plasma of patients after completion of treatment, in fact the SVR corresponds to virological healing which gives patients health benefit. Our preliminary results show the efficacy of DAAs treatments in selected patients, without significant adverse effects. Because of the high cost of these treatments AIFA (Associazione Italiana Farmaco) has restricted the use to selected patients; the hope is decreasing of price of the drug in order to enlarge the treatment to all patients with chronic hepatitis.

P 096

ANTIVIRAL ACTIVITIES OF ANTIBODY-DERIVED PEPTIDES

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Introduction: Human antibody fragments, indicated as PeptAbs, have been shown to have a wide spectrum antimicrobial and antiviral activity *in vitro*. Initial data argued on the efficacy of such molecules, and in particular of parental killer peptide KP (AKVTMTCSAS), against HIV and Influenza viruses. The aim of the present study was to evaluate the antiviral effect of 12 different PeptAbs (including KP) against enveloped and naked viruses.
Materials and Methods: Herpes Simplex-1 Virus (HSV-1), Coxsackievirus B5, Adenovirus and VERO cells were used. Cytotoxicity was evaluated by MTT assay, while the PeptAb-mediated antiviral activity was assessed by a microtiter viral yield reduction assay. Different protocols were applied in order to investigate whether PeptAbs were effective directly on virus particles (by a 1 h pre-incubation of the viral inocula) or on cells (either pre-infected or concomitantly exposed to the virus and PeptAbs).
Results: Several PeptAbs (including KP) affected viral yield to a different extent, depending upon the virus investigated. PeptAbs were effective in the range between 50 and 100 μg/ml. Enhanced anti-HSV-1 activity was achieved when the viral inoculum had been pre-incubated with KP.
Conclusions: This preliminary *in vitro* study provides evidence on the effectiveness of several PeptAbs against both enveloped and non-enveloped viruses. KP appeared to exert also a direct activity against HSV-1 particles. These results open to the possibility that such molecules may offer a wide-spectrum and low-cost therapeutic approach against viral infections.

P 097

EFFECTS OF D(–) LENTIGINOSINE ON *IN VITRO* HTLV-1 INFECTION

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Introduction: We have recently demonstrated that D(-) lentiginosine [-LENT], a non-natural iminosugar with glycosidase inhibitor properties, can cause a preferential, mitochondrial-dependent caspase 3 activation in tumour cells in comparison with non-tumour cells. Moreover, preliminary results suggest that the latter activity could be related to a down-regulation of GLUT-1 receptor expression in tumour cells. Considering the possible role of GLUT-1 in HTLV-1 entry into target cells, we then asked whether [-LENT] could act as a potential drug candidate towards infection caused by HTLV-1.
Materials and Methods: The effects of [-LENT] on PBMC from healthy donors infected in vitro with HTLV-1 by co-culture with irradiated, chronically infected MT-2 or C91/PL cells, were evaluated through real time PCR and flow cytometry analysis. Expression of GLUT-1 was evaluated by confocal microscopy.
Results: The [-LENT] inhibited HTLV-1 expression in a dose-dependent fashion, at concentrations in HTLV-1 infected cells. Proliferation of MT-2, C91/PL and HTLV-1-immortalized IL-2-dependent CD4+ cells (CD4/HTLV-1) were inhibited, in comparison with chemotherapeutic agent, after 24 hours of treatment, with an IC50 of 327, 113 and 131 μM, respectively, while stimulated PBMC were inhibited with an IC50 of 170 μM. In addition cell growth of HTLV-1 infected cell lines was more efficiently inhibited after long-term treatment with [-LENT] at the concentration of 5 μM in comparison with stimulated PBMC and 5 μM AZT treated HTLV-1 infected cells. Confocal microscopy studies and flow cytometry analysis showed that [-LENT] inhibited by 50% GLUT-1 receptor expression. In addition in [-LENT] treated cells GLUT-1 was differently dis-

tributed in the cytosol in comparison with untreated cells.
Conclusions: These data suggest that [-LENT] could protect from both HTLV-1 infection and HTLV-1 immortalization processes by interfering with GLUT-1 and/or GLUT-1 related glucose metabolism of HTLV-1. The use of metabolic inhibitors, in combination or not with other agents, seems an interesting, potential novel strategy against HTLV-1 infection and HTLV-1 associated diseases, owed to presumably low chances for outcome of resistance. Further studies are necessary to verify this hypothesis.

P 098

ANTIMICROBIAL SULFONAMIDES INHIBIT HERPESVIRUS 8 LATENCY BY IMPAIRING THE MDM2-P53 COMPLEX FORMATION

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Introduction: Human Herpesvirus 8 (HHV8) is the causative agent of the Kaposi sarcoma (KS), a rare malignant angiosarcoma, that becomes very frequent in immunosuppressed subjects, AIDS patients and transplant recipients [1, 2]. At present this disease is treated with conventional anti-tumor chemotherapeutic agents and interferon alfa. Such therapy is able to control the disease evolution, but does not cure the HHV8 infection, since the virus can remain in a latent state as an episome bound to the cell DNA. Moreover, classical anti-herpes drugs are able to suppress the virus replication in the lytic phase, but not in the latent state [3]. Recently some studies demonstrated that latent herpesviruses, such as Epstein-Barr and HHV8, can be inhibited by some synthetic chemical compounds, namely sulfonamide derivatives and Nimesulide, respectively. In this work we tested some antibiotic sulfonamide drugs for the ability to suppress the expression of both HHV8 DNA and latency factor LANA in order to clear the virus in the PEL BC3 cells.
Materials and Methods: Several sulfonamide compounds, widely used for treating bacterial infections, namely sulfaguanidine, sulfanilamide, sulfathiazole and sulfamethoxazole, were used to suppress latent HHV8 DNA and protein expression in permanently infected lymphoblastic BC3 cells by RT-PCR and cytofluorometry, respectively [4]. The interaction of the drugs with the MDM2-p53 complex formation was studied by an ELISA test by the use of specific anti-MDM2 monoclonal antibodies.
Results: Here we show that some sulfonamide antibiotics, worldwide used for antibacterial therapy, are able to suppress the HHV8 latent state in permanently infected BC3 lymphoma cells after a short contact at μM concentration. Studies on the mechanism of action revealed that these compounds could interfere with the formation of the MDM2-p53 complex, that is necessary to HHV8 to remain bound to cell DNA in an episomal state and triggers the tumor transformation of the cells.
Conclusions: These findings give a new perspective for treating and curing HHV8-induced lymphoproliferative diseases, either as a complementary or even alternative therapy to the conventional anti-tumor chemotherapy.

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CIRCULATION OF TYPE-1 HUMAN ASTROVIRUS IN A SICILIAN PEDIATRIC POPULATION

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Introduction: Human astroviruses (HAstVs) are relevant enteric pathogens that belong to the family Astroviridae. HAstV RNA genome contain three open reading frames (ORFs): ORF1a and ORF1b, at the 5' end of the genome, encode the non-structural viral proteins, including the RNA-dependent RNA polymerase, while ORF2, at the 3' end, encodes the capsid protein. HAstVs can be classified into eight sero/genotypes (HAstV-1 to -8) and different lineages within each HAstV type. Genotyping surveys have shown that HAstV-1 is the most common type identified in children, followed by HAstV-2, -3, -4 and -5, whereas HAstV-6, -7 and -8 have been detected more rarely. This study describes the genetic diversity of HAstVs circulating in Palermo over 14 years.
Materials and Methods: A total of 2966 stool samples were collected from children under 5 years of age hospitalised with acute gastroenteritis at the "G. Di Cristina" Children's Hospital of Palermo from 1999 to 2014. The samples were screened for the presence of HAstVs by RT-PCR with specific primers, amplifying the 5' end of ORF 2. The ORF2 amplicons of positive samples were sequenced and submitted to phylogenetic analysis using MEGA 6.0 software with the Kimura 2-parameter model and the maximum-likelihood method.
Results: Seventy-eight samples were positive for HAstV (2.63%). The yearly detection rates ranged from 0.53% in 2005 to 10.14% in 2006. No HAstV was detected in 2001. Sequence analysis of the 5' portion of ORF 2 revealed the presence of five genotypes (HAstV-1 to -5) circulating during the study period. HAstV-1 represented the predominant genotype, accounting for 84.6% of all cases positive for HAstV. The other four genotypes (HAstV-2, -3 -4 and -5) were detected at low frequency, indicating a minor epidemiological role. Sequence and phylogenetic analysis of the HAstV strains detected in Sicily revealed high heterogeneity within the same genotype. Molecular analysis of the HAstV-1 strains showed significant sequence variation, with three different HAstV-1 lineages (1a, 1b and 1d) being observed over the study period. HAstV-1d and HAstV-1a circulated sequentially, persisting for four and six consecutive years, respectively in 2003-2006 and in 2007-2012. No HAstV-1c strains were identified. Other HAstV genotypes, HAstV-2c, HAstV-3, HAstV-4b and HAstV-5, were detected sporadically in this survey.
Conclusions: The study of the circulation of HAstV genotypes and lineages in a settled population over 14 years allowed to evaluate the patterns of variation of HAstV infections over the years. The genetic variability of HAstVs correlated to their epidemiology, since the circulation of HAstV-1 genotype was sustained by the continuous introduction and re-introduction of the three main lineages.

P 100

ROLE OF MOUSE MAMMARY TUMOR VIRUS-LIKE IN HUMAN BREAST CANCER: DETERMINATION OF VIRAL PRESENCE IN A PRIMARY CELL LINE

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Introduction: Mouse mammary tumor virus is an oncogenic betaretrovirus that induces mammary tumors in mice. It is trasmitted to the offspring by lactation. Proviral integration in proximity of regulatory gene promoters, such as WNT-1, modify their expression and causes oncogenesis. Epidemiological and laboratory findings suggest that the presence of MMTV-like env sequence in human breast tumor cells but not in the normal breast may be responsible for breast cancer. For this reason, the existence of a human mammary tumor virus (HMTV) is being investigated. HMTV nucleotide sequence

has a homology of 98% with MMTV and only 57% with Human endogenous retrovirus (HERV K-10). The aim of this project is 1) to confirm the presence of HMTV in human mammary tumor cells; 2) set up reagents to search for antibodies against HMTV in patient sera.
Material and Method: A breast tumor cell line that was positive for HMTV by PCR was cloned with limiting dilution in order to have positive and negative clones. To identify the presence of viral protein in these clones, was used immunofluorescence assay (IFA) and SYBR Green I-based product-enhanced reverse transcriptase assay (SG-PERT). To verify the presence of HMTV, Seminested PCR was used. To test patient sera for anti HMTV antibodies, MMTV gp52 and p27 were expressed as bacterial proteins.
Results: The clones obtained from primary cell line was used in IFA assay and two of these turned out to be positive for the presence of Env protein. SG-PERT has given ambiguous results, while Semi-Nested PCR was positive. We have obtained the purified protein p27 and gp52-env mature peptide. Patients sera are tested in order to search antibody against both protein with Western Blot method. Earliest results suggest that this approach may bring very intresting data.
Conclusion: Detection of env viral sequence and viral protein in this line was confirmed, whereas the search for the whole genome/virions in such a cell line gave inconclusive results. Patient sera will be collected and tested to obtain a clinical records proceed with statistical survey. This approach may will be used as a predictive tool to test the population. In order to strengthen our results, we will produce monoclonal antibody to research p27 and gp52 proteins not only in this cell line in question, but also in breast cancer cells from patients.

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NOROVIRUS GASTROENTERITIS OUTBREAK AT A SEASIDE RESORT NEAR TAORMINA

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Introduction: Noroviruses (NoVs) are considered as the major agent of outbreaks of gastroenteritis (GE) in various epidemiological settings, including restaurants, schools, day-care centers, hospitals, nursing homes, and cruise ships. NoVs are classified into six distinct genogroups (GI-GVI) on the basis of the major capsid protein VP1 sequence. GI, GII and GIV NoVs infect humans and they have been divided in more than 30 genotypes based on the sequences of polymerase (ORF1) and capsid protein (ORF2). GII.4 genotype has been associated with the vast majority of NoV-related cases of GE worldwide although many genotypes, belonging to both GI and GII genogroups are involved in food-borne and waterborne outbreaks. We report a NoV outbreak, occurred in May 2016, involving an high school class visiting a seaside resort near Taormina (Mascali, Sicily).
Materials and Methods: Twenty-nine stool samples, from 26 symptomatic and 3 asymptomatic travellers, were collected soon after the onset of symptoms and tested for NoV and bacterial enteric pathogens. An immunochromatographic rapid test (IC), RIDA QUICK Norovirus r-Biopharm, and a RT-PCR assay that allows to differentiate between GI and GII NoVs were used. All NoV-positive samples were sequenced in ORF1 and ORF2 to identify the NoV genotype. Phylogenetic analyses were conducted to track the source of infection. A questionnaire was sent to all participants to obtain data on their symptoms and on possible correlations with food consumption.
Results: The survey indicated that most of the students involved in the outbreak (18) showed symptoms on the second day of journey, while 6 more students and a teacher fell ill the following day and a last student was sick two days after the first cases. Symptoms included vomit, diarrhea and fever, and 12 students required hospitalisation. Five

stool samples from symptomatic subjects were IC positive for NoV, but NoV genome was detected by RT-PCR in 25 stool samples from symptomatic subjects, although 2 showed low viral load values. Samples from 3 students without symptoms were negative for NoV both with IC and RT-PCR tests. The GII.P2_GII.2 NoV genotype was linked to the outbreak by ORF1/ORF2 sequence analysis.
Conclusions: The GE outbreak described in this study was due to NoV and possibly linked to tap water consumption. NoV epidemics are not unusual and should always be suspected when symptom are dominated by vomiting and duration of disease is short (< 48h). Waterborne transmission is suggested by the rapid spread of disease and the high attack rate. NoV detection is not usually included in the diagnostic laboratory procedures for GE but in case of outbreaks NoV testing would be imperative, possibly preferring genome screening to antigen detection.

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INTRODUCTION OF THE NEW NOROVIRUS GENOTYPE GII. P17_GII.17 IN SICILY

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Introduction: Noroviruses (NoVs) have been recognized as an important cause of sporadic cases and outbreaks of gastroenteritis in children and adults. NoV genome possesses three open-reading frames (ORFs) that encode for RNA-dependent RNA polymerase (ORF1), the major capsid protein VP1 (ORF2) and minor structural protein VP2 (ORF3). NoVs can be classified into six genogroups and three of these, genogroup I, II and IV (GI, GII and GIV), occur in human infections. Although more than 30 genotypes can infect humans, since the mid-1990s a single genotype, GII.4 has been related with most outbreaks and sporadic gastroenteritis events. Point mutations and intra-genotype recombination events seem to account for the emergence of novel NoV variants that contribute significantly to the global morbidity from NoV infections. During the winter season 2014/15 a novel GII.P17_GII.17 NoV strain, Kawasaki 2014, emerged in Asian countries, China and Japan, replacing the previously dominant GII.4 genotype. The Italian Study Group for Enteric Viruses (ISGEV;http://isgev.net) monitors the epidemiology of enteric viruses in distinct areas of Italy. In Sicily, NoV surveillance was based on the collection of stool samples from children hospitalized at the ARNAS Civic Hospital of Palermo.
Materials and Methods: A total of 590 faecal specimens were collected from January 2015 to February 2016. NoVs were detected by a RealTime-PCR assay that allows to differentiate between GI and GII. All positive samples were sequenced in ORF1 and ORF2 to identify NoV pol/cap genotype and phylogenetic analyses were conducted using Mega 6 software.
Results: From January 2015 to February 2016, in Palermo NoV prevalence was 21.4% (126/590). Six GII.P17_GII.17 NoV strains (Kawasaki 2014) were detected and represented the third most prevalent clone (10.3%) over the whole study period, following the recombinant strain GII.P4 New Orleans 2009/GII.4 Sydney 2012 (56.9%) and the former pandemic GII.Pe/GII.4 Sydney 2012 strain (17.2%). However, Kawasaki strains were mostly detected in January-February 2016, accounting for 23.8% of typed strains. Sequence analysis revealed that all Palermo strains fitted to the prototype of GII.P17_GII.17 (PR668/2015/ITA) circulating at very low prevalence during February 2015 in Italy and displayed close relationship to NoVs detected in China, Hong Kong and USA.
Conclusions: The finding of GII.P17_GII.17 among the major NoV genotypes circulating in Sicily demonstrates that no restriction to its circulation due to diversity between European and Asian populations is under way. Therefore, more detections are expected in the near future in Western Countries and the global expansion of GII.17 clones could represent a challenge for the efficacy of the candidate NoV vaccines.

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CONCORDANCE AND DISTRIBUTION OF HPV GENOTYPES IN HPV INFECTED SEXUAL COUPLES

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Introduction: The characteristics of HPV infection in women have been extensively investigates, however, only a few studies have analyzed the characteristics of HPV infection in men and in sexual couples.
Materials and Methods: 195 sexual couples positive for HPV-DNA were examined, at the Virology laboratory of the Department of Sciences for Health Promotion and Mother and Child Care (Policlinico, University of Palermo, Italy). HPV-DNA detection was performed by the INNOLiPA HPV Genotyping Extra II Test (Fujirebio) and nested PCR/sequencing method. All women (range: 20-60, mean age: 31.5 ys) had performed a pap smear and knew the cytological diagnosis: 73 (37.4%) negative at Pap smear, 21 (10.8%) with atypical squamous cell of undetermined significance (ASCUS), 82 (42.1%) with low grade squamous intraepithelial lesion (LSIL), 18 with high grade squamous intraepithelial lesion (HSIL) and 1 carcinoma, For ease of computation, these two latter categories were grouped together, and thus represented a total of 19 cases of ≥ HSIL (9.7%). As for the partners (range 20-70; mean age: 36.7 ys) only two had genital warts .
Results: 36 types of HPV were identified: 27 were present in both men and women, two (HPV-67, -90) only in women and seven types (HPV-43, -81, -82, -83, -87, -91, -107) only in men. Infection with or containing high risk HPV types (HR-HPV) was in 112/195 (57.4%) women and in 57/195 (29.2%) men; low risk HPV types (LR-HPV) was in 17/195 (8.7%) women and in 32/195 (16.4%) men. Multiple infections was in 66/195 (33.8%) women and in 106/195 (54.4%) men. Mostly frequent types, the same in men and women, were: HPV-16 (27.7% and 21% respectively), HPV-51 (13.8% and 19%), and -66 (13.8% and 18.5%). HPV-group specific (HR or LR) concordance between sexual partners was found in 163/195 (83.6%; 95%CI = [78.4-88.8]) couples. HPV-type specific concordance was found in 99/195 (50.8%; 95%CI = [43.8-57.8]) couples, of which 82 (82.8%) shared one types, 12 (12.1%) two types and 4 (4.1%) three types, 1 (1%) four types. Cytological diagnosis was not statistically significantly associated neither with HPV-group specific concordance (p = 0.206) nor with HPV-type specific concordance (p = 0.312).
Conclusions: partners of positive women represent a population at high risk of infection and in turn can be a source of (re)infection to the partner. Studied are needed to improve knowledge of the natural history of HPV infection in sexual couples, to control viral transmission and provide adequate counseling to HPV infected sexual partners.

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DISTRIBUTION OF GENITAL HUMAN PAPILLOMAVIRUS IN SICILIAN MEN WITH AND WITHOUT CLINICAL MANIFESTATIONS

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Introduction: Infection Human Papillomavirus (HPV) is the cause of several disease in men and in women: genital warts, penile and cervical intraepithelial neoplasia, invasive penile carcinoma and cervical cancer. However, less is known about HPV infection, prevalence and distribution of HPV types in men.
Materials and Methods: 820 genital samples of men (age 19-77; mean age: 36.7 ys) who had come to the Virology laboratory of the Department of Sciences for Health Promotion and Mother and Child Care (Policlinico, University of Palermo, Italy) were examined for HPV infection. The study included men with genital warts, men with atypical genital lesion, partners of HPV-positive women and asymptomatic men for Sexually Transmitted Diseases (STD) diagnostic evaluation. HPV-DNA genotyping was performed by the INNOLiPA HPV

Genotyping Extra II Test (Fujirebio) and nested PCR/sequencing method.
Results: 461/820 (56.2%) genital samples were HPV positive. The highest HPV detection rate was found in the 25-34 year age group (41.4%), followed by the 35-44 group (31.7%). Oncogenic types were found in 360/461 (78.1%) positive samples. Multiple HPV type infections were shown in 225/461 (48.8%) samples of whom 109 (23.6%) had two genotypes, 58 (12.6%) three genotypes, 38 (8.2%) four genotypes, 15 (3.2%) five genotypes, 3 (0.6%) six genotypes and then only 2 (0.4%) eight genotypes. Thirty-eight different HPV types were identified: the mostly frequent were HPV-16 (19.9% of HPV positive patients), -51 and -6 (18.2%), -31 (13.9%), -66 (13.7%), -53 (11%), -18 (7.6%), -44 (7.1%), -56 (7%), other viral types occurred at a frequency of less than 7%. Men who have made the HPV test: 138 (16.8%) were diagnosed with genital warts, 3 (0.4%) carcinomas, 413 (50.3%) were HPV-positive women partners, 30 (3.6%) presence of an atypical genital lesion, 236 (28.7%) men who wanted a full assessment of sexual transmitted diseases. HPV infection was evident in 100% of men with carcinomas, in 103/138 (74.6%) men with genital warts, in 254/413 men (61.5%) partners of HPV-positive women, in 11/30 (36.7%) men with presence of an atypical genital lesion and in 90/236 (38.1%) in asymptomatic men. HPV-16 was prevalent in 2 (66.7%) men with carcinoma, in 55 (21.6%) men HPV-positive women partners and in 3 (27.3%) men with atypical genital lesion; HPV-6 in 36 (35%) men with genital warts and in 19 (21.1%) asymptomatic men.
Discussion and Conclusions: Different prevalence and distribution of HPV types in different categories including men in study will contribute to elucidating the epidemiology of HPV infection in men, and it will also be helpful in the implementation of future prevention strategies.

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RESISTANT GENETIC PROFILE OF HEPATITIS C VIRUS GENOTYPE 4 IN DIRECT-ACTING ANTIVIRAL AGENTS NAÏVE PATIENTS

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Introduction: Direct-acting antiviral agents (DAAs) become the new standard of anti-HCV therapy and show a very high sustained virological response (SVR) rate. Due to HCV genetic variability, drug resistance associated substitutions (RAS) are detected in patients prior the start of DAAs therapy according to genotype/subtype. More studies have focused on genotype 1 and little has known about the impact of other HCV intra-genotype variability on DAAs resistance. HCV-4 is highly heterogeneous, with 19 recognized subtypes which relative prevalence changes geographically. In Italy, the prevalence of the HCV-4 increase in recent years, with 2-6% of chronic hepatitis reported in southern regions, and due to migration flows, new subtypes can be introduced. We study the presence of RAS and the genetic barrier of HCV-4d in isolates from Sicilian chronically infected patients naive for DAAs therapy.
Materials and Methods: Serum samples from 50 Sicilian HCV-4 chronically infected patients naive for DAAs were collected. Two fragments of NS3/4A and NS5A genes were amplified with specific primers and sequenced by Sanger method. Thirty-six HCV-4 sequences of all subtypes, obtained from data base, were included. A phylogenetic analysis, using MEGA 6 software, was conducted to identify the subtypes and the presence of RAVs and polymorphisms was evaluated by visual inspection. The genetic barrier was studied using the minimal score (*m.s.*) calculation which assigned a score of 1 to each transition and of 2.5 to transversion.
Results: Phylogenetic analysis of NS3 and NS5A sequences show that forty-nine HCV strains from Sicilian patients were classified as subtype 4d and only one as subtype 4a. The nucleotide distance of HCV-4d strains was 5.8% for NS3/4A and 6.4% for NS5A genes. Most of HCV-4d strains showed RAS

in NS3 and NS5A gene as genetic signatures. V36L, S122T and M175L were detected in 96%, 94% and 100% in NS3 gene and Q30R, L31M and D62E in 100%, 94% and 94% in NS5A gene, respectively. HCV-4d strains showed in NS3 a low genetic barrier for Q41R, F43L, V55I, T122A, A156T/V, D168N, V170A selection (*m.s.* 1). A strain showed a nucleotide polymorphisms that reduced the genetic barrier for R155K (*m.s*. 1vs 3.5). In NS5A single isolates . showed a low genetic barrier for M31V and P58S (*m.s.* 1), while all isolates have *m.s.* 1 for Y93H. The most of isolates showed the association of many RAS in NS3 and NS5A genes, especially in 10% of isolates in which V36L+T122A+M175L+Q30R+P58T, V36L+T122P+M175L+Q30R and V36L +D168N+M175L+Q30R were detected.
Conclusion: Because of the low genetic barrier and the high presence of RAS in HCV-4d isolates, the definition of genetic profile prior DAAs treatment seems to be necessary for choose the optimal therapy.

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HERPES SIMPLEX VIRUS TYPE 1 (HSV-1) INFECTION, NEUROINFLAMMATION AND NEURODEGENERATION: A POSSIBLE ROLE FOR OXIDATIVE STRESS?

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Introduction: Several evidence suggest HSV-1 as a potential risk factors for Alzheimer disease (AD), a neurodegenerative disorder characterized by the accumulation in the brain of β-amyloid peptides (Aβs) and neurofibrillary tangles (mainly composed by hyperphosphorylated tau), high levels of oxidative stress markers and neuroinflammation (De Chiara et al, 2012). Herein we design in vitro and in vivo study to clarify the role of oxidative stress in virus-induced neurodegeneration.
Methods: Levels of oxidative stress marker, i.e. reactive oxygen (ROS) and 4-hydroxynonenal (HNE), were measured in HSV-1-infected neuronal cells by fluorimetry and western blotting (wb). BALB/c mice were inoculated via snout abrasion with HSV-1, virus reactivation was periodically induced by thermal stress, and virus replication in the brain was verified through PCR and RT-PCR analysis of viral TK gene and ICP4 mRNA. AD-like hallmarks were analyzed in brain tissues by Bio-plex technology, immunofluorescence and wb.
Results: We found that HSV-1 induces in neuronal cells a significant increase in ROS and HNE levels 4 hours after virus infection. This timing is consistent with our previous data showing the activation of redox-regulated pathway inducing Aβ production. Similar effects were also found in primary culture of murine cortical neurons following HSV-1 infection. These results prompted us to investigate whether virus-induced oxidative damages may accumulate following recurrent HSV-1 infections. We found that repeated HSV-1 reactivations induce in mouse brains: 1) virus spreading and replication in those brain region most affected in AD (i.e. cortex and hippocampus), as assessed by amplification of viral TK and ICP4 genes; 2) a significant increase in levels of oxidative stress markers (i.e. HNE and nitrosylated proteins) in cortical tissues; 3) enhanced levels of IL-6 and IL-1β cytokines and gliosis.
Discussion and Conclusions: These data, together with those showing accumulation of Aβs and altered tau phosphorylation in HSV-1-infected mouse brains (De Chiara et al, manuscript in preparation), strongly support the hypothesis that repeated HSV-1 infections may contribute to the neurodegeneration typical of AD and suggest the involvement of the oxidative stress elicited by the virus.

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ENHANCING PATHOGEN IDENTIFICATION IN PATIENTS WITH ACUTE MENINGITIS AND ENCEPHALITIS USING THE BIOFIRE FILMARRAY MENINGITIS/ENCEPHALITIS PANEL

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Introduction: Acute infection of the central nervous system is a severe disease that can result in high morbidity and mortality and despite recent advantages in diagnostic tests the etiology of acute meningoencephalitis remains unknown in up to 60% of the cases. Prompt, appropriate therapy is crucial to minimize neurological permanent damages and death: hence diagnostic tests which allow a rapid and multiple identifications of microbial and viral pathogens are necessary. Recently, a novel molecular technology for simultaneous detection of 15 neurotropic pathogens has been developed by BioFire, FilmArray Meningitis/Encephalitis (FA-ME). In the present work we evaluated the performance of the FA-ME test in patients presenting acute neurological symptoms, comparing it to routine laboratory diagnostic methods.
Materials and Methods: Cerebrospinal fluid (CSF) of 34 patients with acute meningitis or encephalitis admitted to Emergency Unit, between March and June 2016, were analyzed by FA-ME; the panel requires 200µl of CSF and simultaneously tests for 6 bacteria (*S. pneuminiae, N. meningitidis, S. agalactiae, E.coli* K1, *L. monocytogenes, H. influenzae*), 7 viruses (HSV1, HSV2, VZV, Enterovirus (EV), Parechovirus (PeV), HHV6, CMV) and 2 fungi (Cryptococcus *gatii/neoformans*). 28/34 CSF specimens underwent additional testing by routine microbiological test and virological methods (qPCR and RT-PCR): 19 were analyzed to viral infection and 13 by routine microbiological cultural techniques
Results: Pathogens were identified in 21 of 34 of CSF (61.76%) analyzed by FA-ME [EV (6), PeV (3), HSV1 (2), HSV2 (1), VZV (1), HHV6 (1), *S. pneumoniae* (3), N. meningitidis (3), H. influenzae (1). Results showed that viruses were the pathogens most frequently detected (66,7%), followed by bacteria (33.3%), no fungi were detected. Routine evaluations performed on 28 CSF confirmed all FA-ME results with the exception of two discordant results. One of the thirteen FA-ME negative specimens resulted positive for HHV6 (2145 cp/ml) by qPCR; by contrast, one specimen positive for N. *meningitidis* with FA-ME resulted false negative using routine cultural methods.
Conclusions: Our data show that FilmArray ME Panel is able to detect rapidly a broad range of pathogens directly in CSF with good performance compared to conventional diagnostic test. It is more sensitive than classical CSF cultural test in N. *meningitidis* detection, a fastidious organism which does not grow easily in conventional culture. The panel detects seven viral pathogens, the highest cause of infective meningo-encephalitis, and *L. monocytogenes* which are not usually included in the emergency diagnostic protocol. In conclusion, the routinely use of this comprehensive and rapid test in the diagnosis of CNS infections might improve patient outcomes and antimicrobial management.

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"*IN VITRO*" MODEL OF THE HUMAN JC POLYOMAVIRUS REPLICATION

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Introduction: JC virus (JCV) is an human polyomavirus that causes the fatal demyelinating disorder progressive multifocal leukoencephalopathy (PML) as a result of its productive replication in oligodendrocytes within the brain. JCV infect approximately 80% of the human population in early childhood and seems to establish latency in the kidney. Upon reactivation it causes PML most frequently in AIDS patients and in patients treated with monoclonal antibodies.

The JCV genome is a double-stranded circular DNA encoding the viral early and late proteins. The non-coding control region (NCCR) regulates both JCV replication and gene expression. The NCCR is an high variable region able to promote differences in the rate of viral replication and in gene expression. Moreover this genetic variability accounts for viral tropism, indeed it can infect several human cell types, including hematopoietic progenitor cells, B lymphocytes, fetal Schwann cells, stromal cells and glial cells which is the key step for PML development. There are few data describing the molecular mechanism by which JCV exerts the different cell tropisms. Moreover there is no animal model for PML, and the evidence for the tissue origins of viral latency, reactivation, and trafficking to the brain depends on studies of patients' tissues and disease progression. Hence, the aim of the work is to evaluate the replication rate of JCV archetype (CY strain) in "in vitro" model of infection, using the renal immortalized Cos-7 and the glial immortalized SVGp12 cell lines.

Materials And Methods: Cos-7 and SVGp12 cell lines and CY archetype JC genome (pCY/cl1) were purchased by ATCC. The cells were maintained in medium supplemented with 10% fetal bovine serum (FBS), and incubated at 37° C in the presence of 5% CO2. Both cell lines were transfected with the CY DNA following manufacture instruction (Xfect™ Transfection Reagent kit). At selected time point, the quantification of JC viral-genome copy number in JCV-infected SVGp12 and Cos-7 cell lines was evaluated using a quantitative real-time PCR (qPCR) with JCV Mad-1-specific primers.

Results: In our study, the ability of the archetype JCV replication in the renal Cos-7 and in the glial SVGp12 cell lines was evaluated. The level of JCV replication was assayed in a time course infection starting from 2 to 35 days using qPCR. An increase in JCV replication, in both the supernatant and within the Cos7 and the SVGp12 cell lines, during the time course infection was observed, indicating an efficiently propagation of JCV in cell culture.

Conclusions: A successful JCV replication was observed in the "in vitro" model of JCV infection in both Cos-7 and SVGp12 cell lines. Both cell lines represent a possible latency sites for JCV, hence, this model will be helpful to study the molecular pathways of viral infection and to test drugs that can inhibit its replication.

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ANTIVIRAL ACTIVITY OF HUMAN COLOSTRUM AND MILK AGAINST CYTOMEGALOVIRUS IN SAMPLES FROM MOTHERS OF PRETERM INFANTS AND EFFECTS OF PASTEURIZATION

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Introduction: Colostrum contains large quantities of natural bioactive substances and plays important roles in the health maintenance of newborns. Although breast-feeding represents a main maternal–infant transmission of several infections, there are increasing evidences about the protective role of colostrum for infectious diseases. In this contest, the aim of our study was, firstly, to assess the spectrum of antiviral activity of human colostrum against a panel of viral pathogens selected as representative of different routes of infections, secondly, to compare the spectrum of antiviral activity of human and bovine colostrums, thirdly, to investigate the anti-HCMV activity of milk fractions from mothers of preterm infants, and to study the effect of pasteurization on antiviral activity.

Materials and Methods: Colostrum (days 1-7), transitional milk (days 8-15) and mature milk (beyond day 15) were collected from a cohort of 18 healthy mothers of preterm infants with gestational age below 32 weeks and immediately frozen at -20°C. Aqueous fractions were obtained after centrifugation of whole milk samples. Antiviral assays were performed in vitro by plaque reduction against Rhinovirus, Respiratory Syncytial Virus, Herpes Simplex Virus, Human Rotavirus (HRoV), Bovine Rotavirus, HCMV, Vesicular Stomatitis Virus and against Human Papillomavirus type 16 using a pseudovirion-based assay.

Results: Human colostrum did not exert a broad spectrum antiviral activity but was endowed with specific inhibitory activities against several human viruses, as HRoV and HCMV. By contrast bovine colostrum exerted a remarkable antiviral activity against several genotypes of HRoV. Being the anti-HCMV activity of human colostrum the most po-

tent, we investigated the antiviral profile of aqueous fractions of milk samples from mothers of preterm infants. All fractions exerted strong anti-HCMV activity with statistically significant variation between colostrum and transitional milk from HCMV IgG-positive mothers. Interestingly, colostrums from IgG-positive mothers exhibited lower ID50s (dilutions of sample that reduce of 50% the infectivity) than samples from receptive mothers. Finally, we demonstrated that Holder pasteurization did not affect the antiviral activity of colostrum.
Conclusions: Despite breast milk can serve as a vector for mother-to-child HCMV transmission, we demonstrated that colostrum and milk exert a potent anti-HCMV effect. The absence of variation of antiviral activity of milk following Holder Pasteurization has important implications for preterm infants fed with banked human milk. Studies are in progress to investigate which factors (presence of IgA, biochemical composition of milk, etc.) can be responsible for the antiviral activity.

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DIFFERENT TRENDS IN HIV-1 DRUG RESISTANCE TRANSMISSION ACCORDING TO SUBTYPE IN ITALY OVER THE YEARS 2000-2014

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Introduction: The dynamics and phylogenetic relationships of transmitted drug-resistance (TDR) and subtype in HIV-1 infected patients diagnosed in Counselling and Testing Sites of North/Central Italy from 2000 to 2014 were evaluated.
Material and Methods: 4323 HIV-1 PR/RT sequences from drug-naïve patients (1 per patient) were analysed. TDR was evaluated over time by considering WHO-2009 list with the additional RT mutations 138GKQR/179L/215N/221Y/227C/230I listed in IAS/Stanford 2015. Phylogeny was generated by GTR model and 1000 bootstrap with maximum-likelihood method by RAxML. Transmission clusters (TCs) were recognized by Cluster Picker with bootstrap > 90% and genetic distance ≤ 0.02; TCs included large TCs (≥ 3 sequences, LTCs) and pairs (2 sequences). Factors associated with TDR were evaluated by logistic regression.
Results: Most patients were males (80.2%) and Italian (72.1%), with a median (IQR) age of 37 (30-45)

years. Men who have sex with men accounted for 44.2% of cases, heterosexuals for 36.4%, injection drug users for 4.1%. Non-B infected patients accounted for 30.8% (N = 1331) of the overall population (CRF02_AG = 329; F = 248; C=219; other = 535). Non-B subtypes increased over time (< 2005-2014: 19.5%-38.5%, p < 0.001), particularly in Italians (< 2005-2014: 6.5%-28.8%, p < 0.001). TDR prevalence was 8.8% (B subtype: 9.7%; non-B subtypes: 6.9%, p = 0.003). TDR to any class increased over time in non-B subtypes (< 2005-2014: 2%-7.1%, p = 0.018), while decreased with a slightly trend toward significance in B subtype (< 2005-2014: 13.4%-8.1%, p = 0.156). Overall, 467 TCs (86 pairs and 40 LTCs in non-B subtypes; 259 pairs and 82 LTCs in B subtype) involving 1207 (28%) patients were identified. A higher proportion of recently diagnosed patients (2011[2009-2013] vs 2010[2008-2012], p < 0.001), women (22.4% vs 12.1%, p < 0.001), and heterosexuals (42.1% vs 25.4%, p < 0.001) was found in non-B vs. B subtype TCs. TCs involving subjects with TDR accounted for 13.3% (non-B TCs: 18; B TCs: 44), mainly represented by pairs both in non-B (72.2%) and B subtypes (79.5%). TDR TCs were mainly related to NNRTI and NRTI mutations for non-B and B subtypes, respectively. At multivariable analysis, CFR02_AG was the only factor significantly associated with a lower probability of TDR detection.

Conclusions: In the period 2000-2014, 28% of patients newly diagnosed with HIV-1 in the study population took part in TCs, non-B diagnoses increased, and TCs were involved in TDR spread both in B and non-B strains. Even if TDR was mainly represented by B subtype infection, a TDR increase over time was observed in non-B subtypes. These results highlight epidemiological changes in HIV-1 infection occurring in Italy, which require an improvement in HIV-1 prevention strategies and screening activities.

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HCV RESISTANCE TESTING AFTER PROTEASE INHIBITORS FAILURE GUIDES OPTIMAL RE-TREATMENT STRATEGIES

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Introduction: In clinical studies, retreatment of

patients who have failed first-generation protease inhibitors (PIs) involve drugs from other classes, but real-life practice can be much different. We analyzed the efficacy of re-treatment strategies after PI-failure with various DAA regimens, and the role of natural and acquired resistance associated substitutions (RASs) on viral failure to a 2nd line regimen in a real-life setting.

Material and Methods: This is a multi-center, real-life, observational study of patients undergoing HCV re-treatment after PI plus peg-IFN and ribavirin (RBV) failure. Sustained viral response (SVR) was evaluated at week-12 of follow-up. HCV resistance testing was performed after PI-failure and/or before the 2nd regimen by Sanger sequencing.

Results: 61 patients (60 GT1 [GT1b = 54.1%] and 1 GT4; cirrhosis = 86%), we re-treated after a median(IQR) of 92 (35-123) weeks from PI failure. Overall SVR rate was 88%. SVR rate was 96% in the 27 patients receiving a 2nd line regimen according to EASL and AASLD indications of 2015 (sofosbuvir[SOF]+daclatasvir/ledipasvir +RBV). The only 1 GT1b pt who relapsed had a natural L28M NS5A-RAS, while 2 other GT1b patients with natural L31M/Y93H NS5A-RASs reached SVR after 24 weeks of treatment. SVR rate was 100% in the 9/61 patients who started a 2nd line PI-regimen (SOF+simeprevir[SIM]+/-RBV) chosen according to baseline NS3-NS5A testing, reporting absence of NS3 RASs and/or presence of major NS5A RASs. On the contrary, SVR rate was strongly reduced in the 25/61 patients, who received a not recommended, not RASs-guided regimen (including 12 SOF+SIM+/-RBV. Only 76% of them reached SVR (p-trend = 0.04), including 3/7(43%) treated with SOF+RBV+/-pegIFN. 1 GT-4d was wrongly treated with BOC. He was a non-responder but he didn't show any RAS at failure. He reached SVR after SOF+SIM+RBV treatment. Overall, 27/61 patients were re-treated with a PI (SIM or paritaprevir) and 25/27 (92.6%) achieved an SVR. 2 GT1a patients relapsed after SOF+SIM+RBV, though PI-RASs were absent at baseline. While 4 patients with baseline PI RASs (Q80K, R155K) achieved SVR. SVR rates were not affected by prior failure for virological or non-virological reasons (89% vs 83%, p = 0.62).

Conclusions: DAA's re-treatment after first-generation PI failure can lead to very high SVR rates if guided by resistance testing. According guidelines, the use of SOF+NS5A inhibitors+RBV for 24 weeks has excellent success rate, even when natural NS5A RASs are present. Empirical re-treatment with only one active DAA has limited efficacy and should be avoided.

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HBV GENETIC EVOLUTION IN HBSAG CAN CONSTRAIN HDV REPLICATIVE POTENTIAL

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Introduction: HDV genome encodes only HDV antigen (HDAg) that is present in viral capsid. This antigen interacts with the envelope glycoprotein (HBsAg) encoded by HBV genome, thus allowing HDV assembly and release. Although the interaction between HBsAg and HDAg is known to be critical for HDV morphogenesis, mechanisms underlying this process are not well defined. Here, we characterize the extent of genetic variability in HBsAg and HDAg in the setting of HBV+HDV infection.

Methods: Among 78 patients with chronic HBV+HDV infection, HBsAg gen-D sequences (aa:1-226) are obtained for 31 patients and HDAg gen-1

sequences (aa:1-214) for 47 patients. The 31 patients with HBsAg sequences are matched (1:2) with 62 HBV mono-infected patients according to sex, nationality, age, HBV-DNA, and anti-HBV drug received. Shannon Entropy (SE) is used to measure the amino-acid variability at each HBsAg/ HDAg position. For HBsAg, SE is calculated among HBV+HDV infected and HBV mono-infected patients, and stratifying HBV+HDV infected patients according to serum HDV-RNA (14 patients with HDV-RNA< 3.5 log IU/ml and 17 with HDV-RNA > 3.5 log IU/ml). Positions with SE = 0 are defined conserved.

Results: In HBV+HDV infected patients, median(IQR) HBV-DNA is 3.0(1.8-3.4) log IU/ml and median(IQR) HDV-RNA is 3.7(2.0-6.1) log IU/ml. By SE, the number of conserved positions in HBsAg is significantly higher in HBV+HDV infected patients than in HBV mono-infected patients (69.6% vs 52.6%, p = 0.001). In particular, 19 positions in the major hydrophilic region and 12 positions in C-terminus are conserved only in HBV+HDV infected patients. Some of them (171/196/197/219), residing in C-terminus, are known to be critical for HDV assembly. By stratifying patients according to serum HDV-RNA, mutations at positions 204 and 206 in HBsAg C-terminus are detected only in patients with log HDV-RNA < 3.5 IU/ml and never in patients with log HDV-RNA > 3.5 IU/ml (position 204: 5/14 vs 0/17, p = 0.007; position 206: 3/14 vs 0/17, p = 0.045). This suggests that mutations at these positions may affect HDV replicative potential. In HDAg, a higher number of conserved positions is detected in the viral-assembly signal (VAS), known to interact with HBsAg and critical for HDV morphogenesis, than in other HDAg domains including nuclear localization signal (NLS), RNA-binding domains (RBD) and multimerization domain (MD) (70% [VAS] vs 52.4% [NLS], 31.3% [RBD] and 27.3% [MD], p = 0.004).

Conclusions: HBV+HDV coinfection is characterized by a higher degree of HBsAg conservation than HBV mono-infection. This suggests that the extent of genetic variability in HBsAg, mainly clustered in HBsAg C-teminus, can hamper HDV replicative potential. Conserved HBsAg/ HDAg regions may pose the basis for design of innovative antiviral targets.

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HHV-6A IS PRESENT IN ENDOMETRIAL EPITHELIAL CELLS FROM UNEXPLAINED INFERTILE WOMEN

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Introduction: Human herpesvirus 6 (HHV-6) comprises 2 viral species: HHV-6B, associated to roseola infantum and ubiquitous in the adult population in a latent state, and HHV-6A, rarely found in the healthy population and with still undetermined firm ethiologic associations. Literature data describe the detection of HHV-6 in genital female secretions, without identification of the viral species. Currently, it is not clear if genital HHV-6 infection could influence female tract functions. We evaluated the presence of HHV-6A/B infection in endometrial biopsies from unexplained infertile and fertile women.

Materials and Methods: A prospective randomized study was conducted on a cohort of primary unexplained infertile women (N = 30) and a cohort of control fertile women (N = 36). HHV-6 DNA was analyzed and the percentage and immune-phenotype of resident endometrial Natural Killer (NK) cells, as the first line of defense towards viral infections, were evaluated in endometrial biopsies. Cytokine levels in uterine flushing samples were analyzed.

Results: HHV-6A DNA was found in 43% of endometrial biopsies from primary unexplained infertile women, with high viral loads, transcription of lytic genes and expression of viral antigens. Control women were negative for HHV-6A. On the contrary, HHV-6B DNA was absent in endometrial biopsies, but present in PBMCs of both cohorts. Infertile women with HHV6-A infection had a lower percentage of endometrial specific CD56brightCD16- NK cells compared to infertile women without HHV6-A infection and to controls. HHV-6A positive infertile women showed also an enhanced HHV-6A-specific endometrial NK cell response. The analysis of uterine flushing samples

showed an increase in IL-10 levels and a decrease of IFN-gamma concentrations in infertile women with HHV6-A infection.
Conclusions: Our study indicates, for the first time, that HHV-6A infection might be an important factor in female unexplained infertility development, with a possible role in modifying endometrial NK cells immune profile and ability to sustain a successful pregnancy.

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HCV-RNA QUANTIFICATION IN LIVER BIOPTIC SAMPLES OF TRANSPLANTED PATIENTS BY USING THE ABBOTT REALTIME HCV-ASSAY: A REAL TIME QUANTITATIVE ASSAY FOR HCV-RNA TISSUE TESTING

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Introduction: During post-transplant follow up, recurrence of hepatitis C is highly frequent in the 95% graft cases. The quantification of HCV-RNA in both serum and liver might provide helpful and more accurate information regarding the HCV burden. We developed a rapid method to quantify the HCV-RNA in tissue, by using the Abbott Real*Time* HCV-assay.
Materials and Methods: 18 HCV-infected patients (pts) (4 GT1a, 8 GT1b, 5 GT3a, 1 GT2) undergoing orthotopic liver transplantation (OLT) (N = 16) or liver resection (LR) (N = 2) were analyzed. 4/18 pts were treated with DAA (Direct Acting Antivirals) before OLT. HCV-RNA was quantified in 3-4 sections of the same liver-sample and the mean value was considered for the analysis. 13mg liver-bioptic sample was homogenized in BufferRLT+β-Mercaptoethanol by TissueRuptor (QIAGEN) homogenizer; the sample was then used for HCV-RNA quantification by Abbott Real*Time* HCV-assay. Total RNA and DNA extractions from liver biopsies were performed to allow normalization of HCV-RNA concentrations expressed in IU/µg of total RNA and IU/106 liver-cells.
Results: Among non-treated pts, the median (IQR) HCV-RNA resulted always higher in NT respecting to TT: logIU/µgRNA = 4.3 (3.1-4.9) in NT vs 4.0 (1.2-4.3) in TT (Mann-Whitney, p = 0.193); and IU/106 cells = 6.4 (5.6-7.1) in NT vs 5.4 (3.8-6.2) in TT (Mann-Whitney, p = 0.209). A positive and significant HCV-RNA correlation between serum and NT liver samples was observed among the 14 untreated pts (Pearson: rho = 0.609, p = 0.021), but not between serum and TT (rho = 0.207, p = 0.541). Moreover, the same correlation between serum and NT resulted significant in cirrhotic pts (N = 9/14) (rho = 0.702, p = 0.035) but not in non-cirrhotic (N = 5/14) (rho = 0.775, p = 0.124). About the 4 DAA-treated pts, at OLT time, 3 were still in treatment, while 1 had completed therapy and showed a sustained-virological-response (SVR) at week-12. Notably, at the moment of OLT, all treated pts had undetectable serum HCV-RNA. However, the 3 pts who were still in treatment had still detectable HCV-RNA in liver tissues; the only patient who had undetectable HCV-RNA in both serum and all liver samples was the SVR patient.
Conclusions: This study shows the Abbott Real*Time* HCV-assay is fast and accurate for the HCV-RNA quantification also in liver tissues. This may be of help to further attest HCV clearance in pts under DAA-treatment while waiting for OLT, even when HCV-RNA is "undetectable" in serum. Moreover, HCV-RNA quantification by a commercial assay in liver tissue can allow easier diagnosis of HCV-infection in pts with "occult hepatitis C infection", defined by negative HCV-RNA in serum and anti-HCV positivity. Finally, a valid liver-tissue HCV-RNA quantification may help in assessing residual HCV burden in HCV infected pts.

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HTLV-1 REVERSE TRANSCRIPTASE ACTIVITY AS BIOMARKER OF CLINICAL RESPONSE TO AZT/IFN THERAPY IN ATL PATIENTS

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Introduction: Adult T cell leukemia lymphoma (ATL) is a chemoresistant malignancy associated with human T lymphotropic virus type 1 (HTLV-1) infection. The combination of the antiviral agents zidovudine (AZT) with interferon-alpha (IFN) represents a breakthrough in ATL therapy, through induction of high response rate and prolongation of survival in the leukemic subtypes of ATL. However, the biological basis for the therapeutic efficacy of this combination is still unclear.
Materials and Methods: In a retrospective study, we assessed the HTLV-1 reverse transcriptase (RT) activity, tax/rex expression, and P19 levels in samples from short-term cultures of peripheral blood mononuclear cells (PBMC) from six ATL patients, before and after AZT/IFN treatment, and from three untreated ATL patients. RT activity and tax/rex expression were assessed by PCR-based assays developed in our laboratory, while P19 levels were evaluated by a commercial ELISA.
Results: HTLV-1-RT activity was completely inhibited after therapy in all samples from patients who clinically responded to AZT/IFN, despite presence of viral core protein p19. No inhibition of HTLV-1-RT activity was found in the only one patient classified as a clinically non-responder.
Conclusions: These results suggest that HTLV-I-RT activity from short-term cultured PBMC may represent a good biomarker of clinical response to AZT/IFN therapy in ATL patients and suggest that the therapeutic efficacy of this combination presumably reflects the inhibition of RT-related functions.

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ROLE OF G6PD ACTIVITY IN REGULATING INFLUENZA VIRUS REPLICATION

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Introduction: It has been demonstrated that viral replication cycle is favored by pro-oxidant conditions mainly determined by an increase of reactive oxygen species (ROS) production and decrease of intracellular glutathione (GSH) content. GSH redox homeostasis depends on glucose- 6-phosphate dehydrogenase (G6PD) activity, the first and the rate-limiting enzyme of pentose phosphate pathway, responsible for the production of reducing equivalents in the form of NADPH, which is used for regenerating the reduced form of GSH. It has been reported that G6PD-deficiency could increase the susceptibility to different viral infections even if the mechanisms promoting viral replication are not well elucidated yet. The aim of the study is the evaluation of the role of G6PD in regulating specific steps of influenza virus life-cycle.
Materials and Methods: Infection was performed with different strains of influenza virus. The expression of G6PD and NOX4 was evaluated by western blot analysis. The enzymatic activity of G6PD was evaluated through a colorimetric Assay kit that detects the formation of reaction product NADPH.
Results: A549 human carcinoma cells were infected with different strains of influenza virus and 24 and 48 hours after the infection, cells were detached and lysed for western blot analysis. We found that 48 hours post infection the expression level of G6PD is decreased in infected cells compared to uninfected ones. Interestingly, at the same time the activity of the enzyme is strongly reduced in influenza virus-infected cells compared to that of uninfected ones. In this condition the expression of NOX4 enzyme, a member of NADPH family, is up-regulated.
Conclusions: Our data indicate that G6PD activity may contribute to virus-induced redox imbalance and play a role in viral replication. Further studies are in progress to clarify the mechanisms by which G6PD deficiency could influence the spread of viral infections.

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LONGITUDINAL EVALUATION OF RESERVOIR SIZE DECAY IN NAÏVE HIV PATIENTS STARTING ANTIRETROVIRAL TREATMENT

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Background: Despite the success of antiretroviral therapy (ART) in suppressing viral replication, HIV can persist in patients in presence of effective treatment. During long-term viral suppression, integrated forms of HIV persist in a silent, but replication-competent state in resting CD4 T cells, which constitute the major viral reservoir. A small latent reservoir is associated to a higher likelihood of controlling viral replication after ART withdrawal and thus reduction of reservoir size is one of the major goals of the new strategies aimed at curing HIV. In this project we will determine the effect on reservoir decay of two different therapeutic strategies (backbone plus either an integrase inhibitor or a protease inhibitor). TILDA assay, a new assay specifically developed to measure the frequency of replication-competent provirus will be used to assess the size of the reservoir.
Methods: Clinical study: HIV patients receiving a regimen including either an integrase inhibitor or a protease inhibitor will be enrolled in the study. Blood samples will be collected at time 0 and at 3, 6, and 12 months after starting therapy. TILDA assay. After 16h stimulation with a potent mitogen, CD4 T cells are serially diluted in culture medium. Presence of cells harbouring active HIV is evaluated using a nested PCR assay targeting the tat/rev mRNA. The frequency of latently infected cells is calculated with the maximum likelihood method. Sensitivity of the PCR method will be estimated using serial dilutions of RNA standards. Performances of the method will be further evaluated on cell suspensions containing the latently infected J-Lat11.1 cells (harboring a latent copy of the HIV genome) and Jurkat cells at know ratios.
Results: The first part of the project was aimed at evaluating the performances of the assay using RNA standards, and cell lines models of latency. The PCR method showed good sensitivity, showing a limit of detection of 20 RNA copies/reaction. Furthermore, TILDA assay proved to be able to correctly estimate the frequency of latently infected cells in mixtures containing 10, 50 and 100 J-Lat cells/106 Jurkat cells. Finally, using TILDA, we were able to quantify the size of the reservoir in a long-term treated HIV patient. Despite the low frequency of infected cells in this sample (3 infected cells/106 CD4 T cells), the assay showed a good inter-assay reproducibility (95% CI, 1-6). The second part of the project is ongoing and will include the enrollment of patients and the longitudinal evaluation of the status of their reservoir during therapy.
Conclusion: A rapid achievement of complete virological suppression is necessary in order to prevent the seeding of a large HIV reservoir. Here we evaluate the correlation between the frequency of latently infected cells, as estimated by the TILDA assay, and the therapeutic regimen. We expect to see a faster decay of reservoir in patients treated with integrase inhibitors, as these drugs prevent viral integration. This study will provide insights about the optimal regimen for HIV patients starting therapy.

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FIRST CASES OF FECAL MICROBIOTA TRANSPLANTATION (FMT) VIA ENEMA FOR CLOSTRIDIUM DIFFICILE RECURRENT INFECTION (RCDI) AT THE POLICLINIC UMBERTO I OF ROME

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Fecal microbiota transplantation has been described to restore resistance to C.difficile (CD) colonization by re-establishing a healthy microbial ecosystem in the guts of patients with a cure rates of disease quoted from 84% to 93% .

Aim: To determine the efficacy of FMT via enema in RCDI and evaluate the FMT impact on gut microbiota composition.

Methods: Fecal transplant protocol. The study was conducted at the Policlinico Umberto I Hospital, in Rome. We included patients with RCDI. Patients received an abbreviated regimen of vancomycin (500 mg orally four times per day for 4 or 5 days); polyethylene glycol-based colonoscopy preparation on the day prior to FMT; loperamide, 3 hours prior the infusion, to aid the retention of transplanted material. Donors and fecal samples preparation. Healthy volunteers donors (< 30 years of age) were subjected to microbiological and parasitological screening. In addition we introduce, for the first time, a donor fecal ecosystem balance evaluation by qPCR. Popolation study. The average patient age was 69 years (range, 42-83 years). Patients were randomly allocated to receive frozen or fresh FMT via enema. They received a 250-ml suspension on 75% (range 250-500 mL). Height-seven percent of patients experienced resolution (7/8), 85.7% after a single treatment. No major adverse events correlated to FMT was registered. Microbiota characterization. Fecal microbiota composition was evaluated before and after the FMT in all patients and in healthy donor too. To this purpose 16S rDNA sequencing (MiSeq) was employed. The real Time PCR was employed for a quick evaluation of the fecal ecosystem, by analyzing the relative abundance of the bacterial phylum Bacteroitedes and Firmicutes.

Results: In all patients before FMT, was possible to observe the prevalence of the Proteobacteria phylum, and a significant decrease of beneficial phylum such as Firmicutes and Bacteroides (a dysbiosis status). After the FMT a rapid lowering of the Proteobacteria is observed with a parallel increase of potentially beneficial phylum such as Firmicutes and Bacteroitedes. Interesting the case were an additional treatment was necessary because CD was still present in the fecal sample after the first infusion. In this patient was possible to observe a clear failure in restoring the microbiota balance after the one week from the first infusion.

Conclusion: Those are the result of the first cases of FMT at the Policlynic Umberto I of Rome. FMT via enema is efficacy and safety. FMT showed a rapid recovery of the microbiota balance. The incredible success of this therapy approach indicate the curative potential of the FMT, that could be used as strategy to restore gut to indicate that we could bypass the bacterial antibiotic resistance problems, using the FMT as the gold standard therapy in the treatment of CDD. Furthermore the FMT could be approached in other intestinal disorder, and pathology liked to an intestinal dysbiosis status.

POSTERS

MICROORGANISM / HOST INTERACTIONS

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CELL LYSATES OF TWO DIFFERENT CAMPYLOBACTER JEJUNI STRAINS INDUCE LETHAL AND SUBLETHAL FEATURES IN DIFFERENT CELL LINES

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Introduction: *Campylobacter jejuni* is the most common cause of bacterial gastroenteritis in humans, probably due to the production of a cytolethal distending toxin (CDT), delivered by outer membrane vesicles (OMVs) into host cells. The aim of this study is to investigate cellular effects of *C. jejuni* ATCC 33291 and *C. jejuni* ISS1 strains, positives for the cdtA, cdtB, cdtC genes, compared to *C. jejuni* 11168H cdtA mutant.
Methods: *C. jejuni* ATCC 33291 and *C. jejuni* ISS1 suspensions were centrifuged at 3,500 rpm for 10 min, and the supernatants were collected as the "Campylobacter cell supernatants" (CCSs). The bacterial cell pellets were resuspended in Dulbecco's Modified Eagle Medium (D-MEM), lyzed by sonication and centrifuged. The resultant supernatants were collected as "Campylobacter cell lysates" (CCLys). Both CCSs and CCLys were 0.2 µm sterilized and tested on HeLa cells to verify microscopically the morphological changes after 24, 48 and 72h of incubation. At this point, the fractions showing the typical CDT effect were added to cells from different lineages (HeLa, U937 and donor monocytes). Cytometric and confocal microscopy analyses were conducted for evaluating mitochondrial and lysosomal cell death pathways. Particularly, cells were analysed to investigate mitochondrial and lysosomal features, p53 and Bcl-2 status, CD54, CD14 and CD59 alterations.
Results: We observed the CDT effect with typical progressive cellular distension, and ultimately death on HeLa cells in the samples treated with the CCLys and not in those with the CCSs.
After CCLys treatment, all cell lines showed significant mitochondrial alterations (by TMRE and NAO), the apoptotic behaviour (by Bcl-2, PI and AnxV), particularly for *C. jejuni* ATCC 33291 and the increment of their endolysosomal and ER compartments, specifically for *C. jejuni* ISS1. Myeloid cells highlighted deep modulations of CD14, endoplasmic reticulum stress and lysosomal exocytosis, whereas CD54 and CD59 alterations were detected in all cell lines. After CCLys treatments (at 48h-72h), all cell lines showed a certain apoptotic behaviour (particularly for ATCC 33291) and modulation of surface receptors. In each experiment CCLys of *C. jejuni* 11168H cdtA mutant was used as negative control.
Conclusion: In conclusion, we observed cell morphological changes CDT-related induced by CCLys and, following treatment with the different CCLys, lethal and sublethal (and reservoir-transforming) effects of both lysates on the different cell lines investigated, depending on the specific *C. jejuni* strains.

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METAGENOMICS REVEALS MARKED DYSBIOSIS AND A POTENTIALLY PATHOGENIC *NEISSERIA FLAVESCENS* STRAIN IN DUODENAL MICROBIOME OF ADULT ACTIVE CELIAC DISEASE PATIENTS

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Introduction: Celiac disease (CD)-associated duodenal dysbiosis has not yet been clearly defined, and the mechanisms by which dysbiosis could concur to CD development or exacerbation are unknown. In this study, we analyzed the duodenal microbiome of CD patients by 16S ribosomal RNA (rRNA) metagenomics.
Materials and Methods: The microbiome was evaluated by 454-based DNA sequencing (NGS) of 16S rRNA libraries in duodenal biopsy samples of 20 adult patients with active CD, 6 CD patients on a gluten-free diet, and 15 controls. Bacterial species were cultured, isolated and identified by MALDI mass spectrometry. Characterized bacterial species were used to infect CaCo-2 cells, and to stimulate normal duodenal explants and cultured dendritic cells (DCs). Inflammatory markers and cytokines were evaluated by ELISA.
Results: In the microbiome profiles of active CD patients the Proteobacteria was the most abundant and Firmicutes and Actinobacteria the least abundant phyla. Members of the *Neisseria* genus (Betaproteobacteria class) were significantly more abundant in active CD patients than in the other groups. In particular *Neisseria flavescens* (CD-Nf) was the most abundant Neisseria species in active CD duodenum. Interestingly, the whole-genome sequencing of CD-Nf and control-Nf showed genetic diversity of the iron acquisition systems. CD-Nf was able to escape the lysosomal compartment in CaCo-2 cells and to induce an inflammatory response in DCs and in ex-vivo mucosal explants.
Conclusions: Marked dysbiosis and an abundance of a peculiar *N. flavescens* strain characterize the duodenal microbiome in active CD patients, suggesting that the CD-associated microbiota could contribute to the many inflammatory signals in this autoimmune disorder.

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PRODUCTION OF IL-1BETA FROM HUMAN NK-CELLS STIMULATED WITH *MYCOBACTERIUM TUBERCULOSIS* AND *M. BOVIS* BACILLUS CALMETTE-GUÉRIN

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Introduction: The cellular components of the host innate immune system, resident in the lung or attracted to the infection site by the inflammatory process, play a crucial role in determining the outcome of the host-parasite interaction in tuberculosis (TB) infection and the development of an efficient antigen-specific immune response against *Mycobacterium tuberculosis* (Mtb). In fact, these cells perform several effector functions aimed at controlling the infection that include the activation of potent microbicidal mechanisms or the production of pro-inflammatory cytokines such as IL-1β, TNFα, and IFNγ. Recently, there is increasing evidence that NK cells are important mediators of the innate resistance to a variety of microorganisms, including Mtb. We have previously demonstrated that the direct interaction of mycobacteria with human NK cells activates the effector functions of these cells and natural cytotoxicity receptor NKp44 and Toll-like receptor 2 (TLR-2) expressed on human NK cells are involved in recognition of mycobacteria cell-wall components (arabinogalactan and mycolic acids for NKp44; peptidoglycan for TLR-2). In particular, TLR-2 has been demonstrated to be essential for the activation of NK cells by mycobacteria.
Methods: Herein we studied the stimulating/immunomodulating capacities of Mtb and *M. bovis* BCG on human NK cells with a focus on the production of IL-1β, a cytokine that represents a key molecule in the triggering and maintenance of the inflammatory response, contributing to the host defense through the increase of the microbicidal capacity of the macrophages and the induction of the specific immune response by Th1 type lymphocytes. Human NK cells were stimulated *in vitro* with Mtb or *M. bovis* BCG.
Results: The results obtained demonstrated that both Mtb and *M. bovis* BCG are able to induce NK cells to produce IFN-γ, TNF-α, and interestingly IL-1β. The production of IL-1β was correlated with the level of

NK-cell activation as determinated by the expression levels of the activation marker CD69. Moreover, the secretion of IL-1β from NK cells was also observed when these cells were stimulated with polystyrene beads coated with mycolyl-arabinogalactan-peptidoglycan, a fundamental component of Mtb cell-wall. The production of IL-1β from NK cells stimulated with mycobacteria was confirmed at a single cell-level with an assay (PrimeFlow) that measures IL-1β mRNA expression by using flow cytometer.
Conclusion: Such results demonstrate the ability of NK cells to produce IL-1β and suggest that the production of IL-1β during the early phases of Mtb-host interaction may represent another important effector function of NK cells with a protective and/or immunopathologic role.

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SERRATIOPEPTIDASE MODULATES CELL INVASION AND IMMUNE RESPONSE IN OSTEOBLASTS DURING STAPHYLOCOCCAL INFECTION

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Objectives: *Staphylococcus aureus* (*S. aureus*) is a flexible microbial pathogen frequently isolated from community-acquired and nosocomial infections, that is responsible for a wide variety of human diseases. In particular *S. aureus* is the prevalent etiological agent of osteomyelitis (bone infection), which is characterized by progressive inflammation and bone tissue damage. *Staphylococcus epidermidis* (*S. epidermidis*), belonging to coagulase-negative staphylococci, is considered a commensal of human skin and mucous membranes but is now recognized as an important opportunistic pathogen responsible for several nosocomial infections. It was demonstrated that *S. aureus* and *S. epidermidis* can invade bone cells and this ability can therefore explain some difficulties encountered in the treatment of bone infections. Adhesion and invasion of host cells are two important components of the infectivity of staphylococci that contribute to human disease progression by protecting bacteria from the host immune system and traditional antibiotics. The aim of this research was to investigate the ability of serratiopeptidase (SPEP), a non-cytotoxic anti-inflammatory natural compound, to reduce *S. aureus* and *S. epidermidis* adhesion and invasion in osteoblasts and to modulate their inflammatory response.
Materials and Methods: Osteoblasts were infected with *S. epidermidis* O47 and *S. aureus* 6538P at a multiplicity of infection (MOI) of 300:1 and 30:1. To quantify internalized bacteria, suspension diluitions of cell lysates were plated in TSA plates followed by an overnight incubation at 37oC. For cell proliferation, osteoblasts were incubated with bacterial strains viable or heat-inactivated. The number of living cells was determined by colometric MTT assay after 24h incubation. The concentration of MCP-1 was determined in the culture supernatants after 24 and 48 h incubation. All experiments were performed with or without SPEP.
Results: In this work we describe the action of SPEP on the ability of different strains of *S. aureus* and *S. epidermidis* to invade a human osteoblastic cell line. Our results show that SPEP modulates the invasiveness of staphylococci without affecting viability and proliferation of bone cells. Furthermore we evaluated SPEP action on osteoblasts proliferation in the presence and in the absence of bacteria, and on the secretion of cytokines IL-6 and Il-17 and chemokine MCP-1 by infected osteoblasts. Our data show that SPEP modulates the production of molecules that contribute to bone destruction.
Conclusions: Therefore the synergistic use of traditional antibiotics with SPEP may be a strategy to control inflammation in bone infection by the control of invasion and the regulation of immune response.

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ENDOMETRITIS ASSOCIATED WITH *GLOBICATELLA SANGUINIS* AND *STAPHYLOCOCCUS LENTUS* IN A MARE: A CASE REPORT

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Introduction: Bacterial endometritis represents one of the most important reproductive diseases in mares causing considerable economic loss. Bacterial pathogens are a potential cause when a mare fails to conceive to a fertile stallion on a well-managed breeding farm on one or more cycles in the same season. *Streptococcus* group C and *Escherichia coli* are the most frequently bacterial strains associated with fertility problems. Furthermore, emerging bacterial resistance to commonly used antibiotics has been demonstrated. Here, we present a case of endometritis in a mare which was attributed to a co-infection by a strain of *Globicatella sanguini*s, an unusual gram-positive coccus, which is only very rarely isolated in animal clinical specimens, and a strain of *Staphylococcus lentus*.
Materials and Methods: Cytological examination of the sample stained and examined under microscopy was performed to evaluate the presence of polymorphonuclear (PMN) cells. For the bacteriological examination the uterine swab sample was inoculated in Brain Heart infusion (BHI) medium and then, after 24 h of incubation, on different agar plates (blood agar, MacConkey agar, Mannitol Salt Agar, Sabouraud Dextrose Agar) and incubated for further 24 h at 37°C. The isolated bacteria were identified by a miniaturized panel of biochemical tests, API systems. Antimicrobial resistance testing was performed by disk diffusion method on Mueller Hinton agar plates.
Results: Cytological examination showed presence of PMN and absence of bacteria. The uterine swab incubated in BHI and then streaked onto different agar plates provided microbial culture. Based on their growth capacity on the different plates and on biochemical identification, *Globicatella sanguini*s and *Staphylococcus lentus* were identified. The both isolates showed a multi-drug resistant profile, precisely they were resistant to 13/16 and 9/16 of the tested antibiotics, respectively. Ceftriaxone (2 g in 100 ml physiological solution) was administered in intrauterine infusion for 3 days after the insemination, and gentamicin (7 mg/kg) for seven day intravenously. The mare was pregnant 25 days after the ovulation.
Conclusions: To our knowledge, this is the first case of *Globicatella sanguini*s associated with endometritis in mares. Furthermore, this strain showed an alarming antimicrobial resistance profile. However, we conclude that bacteriological results of uterine swabs obtained through enrichment culture step may substantially increase the culture sensitivity, give more trustworthy results and provide advantage to define the correct therapy.

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RAT INTESTINAL DISBIOSIS INDUCED BY DICLOFENAC CAN BE COUNTERACTED BY RIFAXIMIN TREATMENT

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Introduction: Nonsteroidal anti-inflammatory drugs (NSAIDs), besides exerting detrimental effects on the upper digestive tract, can also damage the small and large intestine. Although the underlying mechanisms remain unclear, there is evidence that enteric bacteria could play a prominent role. In particular, NSAIDs increase mucosal permeability, thus facilitating the entrance and action of bacteria, which trigger the inflammatory cascade via activation of Toll-like receptors (TLRs). The present study examined the changes in enteric bacterial load and composition in a rat model of diclofenac-induced enteropathy and the effects of rifaximin, a poorly absorbed antibiotic, on this model.
Materials and Methods: Enteropathy was induced

in male rats (40-weeks old) by intragastric diclofenac administration (4 mg/kg bis in die, BID) for 14 days. Control animals received drug vehicle (0.3 ml of 1% methylcellulose). A group of rats received Rifaximin-EIR (coated microgranules of rifaximin) (50 mg/kg BID), 1 hour before diclofenac (n = 6-7 per group). At the end of treatments, ileum was excised and processed for the evaluation of total bacterial load and quantitative analysis of phyla and genera, via 16S real-time PCR. The microbiota composition was also analyzed through 16s rRNA MiSeq sequencing analysis.
Results: In rats treated with diclofenac, an overall increase of bacterial load with respect to control animals was observed. In particular, Proteobacteria (*Enterobacteriaceae*) and Firmicutes (*Lactobacillus*, *Clostridiaceae*, *Peptostreptococcaceae*) increased while Bacteroidetes decreased in diclofenac treated animals. In rats treated with diclofenac plus Rifaximin-EIR, microbiota composition was similar to the control group.
Discussion and Conclusions: In the small bowel, treatment with diclofenac leads to quantitative and qualitative alterations of enteric bacteria. Under these conditions, treatment with Rifaximin-EIR seems to counteract the bacterial changes. These peculiar pharmacological actions of rifaximin may represent the underlying mechanism(s) of its preventive activity against NSAID-induced intestinal damage, recently shown in rats [1] and humans [2].

1. 2. Gastroenterology 2015; 148 (Suppl 1): A-398, A-307.

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EFFECTS OF PROTON PUMP INHIBITORS ON THE GASTRIC MUCOSA-ASSOCIATED MICROBIOTA IN DYSPEPTIC PATIENTS

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Introduction: Besides being part of anti-*Helicobacter pylori* treatment regimens, proton pump inhibitors (PPIs) are increasingly used to treat dyspepsia. However, little is known about PPI effects on the human gastric microbiota, especially those related to H. pylori infection. The goal of this study was to characterize the stomach microbial communities in patients with dyspepsia and to investigate their relationships with PPI use and H. pylori status.
Materials and Methods: Using 16S rRNA gene pyrosequencing, we analyzed the mucosal biopsies microbial populations of 24 patients, of whom 12 were treated with the PPI omeprazole and 9 (5 treated and 4 untreated) were positive to *H. pylori* infection. Multitag ('multiplex') pyrosequencing was performed using the GS Titanium technology targeting the V1-V3 variable regions corresponding to position 28 to 519 of the *Escherichia coli* 16S rRNA gene. Sequence data were processed with the QIIME (Quantitative Insights Into Microbiology Ecology) 1.8.0 pipeline.
Results: The Proteobacteria, *Firmicutes, Bacteroidetes, Fusobacteria*, and *Actinobacteria* phyla accounted for 98% of all the sequences, with *Helicobacter, Streptococcus*, and *Prevotella* ranking among the 10 most abundant genera. Either *H. pylori* infection or PPI treatment did not influence the number of microbial species composing the gastric community under dyspepsia. Principal coordinate analysis of beta diversity in these communities revealed clear but significant separation only accor-

ding to *H. pylori* status. However, in PPI-treated patients, *Firmicutes*, particularly *Streptococcaceae*, were significantly increased in relative abundance compared to untreated patients. Consistently, *Streptococcus* was also found to significantly increase in relation to PPI treatment, and this increase seemed to occur independently on H. pylori infection.
Discussion: Our results suggest that *Streptococcus* may be a key indicator of PPI-induced gastric microbial composition changes in dyspeptic patients. If these changes may be linked with altered gastric H. pylori abundance, it needs to be elucidated in the future.

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TRANSGLUTAMINASE TYPE 2 PLAYS A KEY ROLE IN THE *MYCOBACTERIUM TUBERCULOSIS* INFECTION PATHOGENESIS

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Introduction: *Mycobacterium tuberculosis* (*Mtb*), the etiologic agent of tuberculosis (TB), is a highly successful human pathogen causing more deaths than any other single infectious agent. Failure to eradicate *Mtb* infection is a consequence of complex mechanisms employed by *Mtb* to evade innate and adaptive host immune responses, which warrant *Mtb* persistence and replication inside macrophages.Autophagy is an ancient evolutionary conserved cellular process which plays a protective role through its action in different aspects of immunity, including the elimination of invasive microbes and the participation in antigen presentation. The molecular underpinnings of these mechanisms remain to be elucidated and we wondered the role of autophagy during *Mtb* infection.For this purpose it has been raising our interest the protein Transglutaminase type 2 (TG2) a pleiotropic enzyme belonging to the family of transglutaminase which catalyzes post-translational modifications of proteins through Ca2+-dependent. The aim of the present study was to investigate whether TG2 is involved in the *Mtb* pathogenesis
Materials and Methods: To this purpose we have used genetically engineered mice lacking TG2, and examined the course of infections with BCG and with the virulent *Mtb*, both *in vitro* and *in vivo*. Finally, we investigated whether pharmacological modulation of TG2 function could be beneficial to inhibit *Mtb* infection
Results: In this study we show that both the genetic and the pharmacological inhibition of TG2 leads to significative reduction of the *Mtb* replicative capacity both *in vivo* and *in vitro*. We demonstrate that TG2 is dispensable for the ability of *Mycobacterium bovis* (BCG) to persist intracellularly, but it is required for *Mtb* persistence in macrophages and host tissues. Interestingly, the same inhibitory effect can be pharmacologically reproduced by using the highly specific inhibitor of TG2's transamidating activity (Z-DON). The inhibition by Z-DON of the TG2 cross-linking activity does not affect the ability of *Mtb* to enter macrophages but rather the ability of *Mtb* to persist intracellularly in macrophages. Furthermore, we show that the massive cell death observed in TG2 proficient macrophages is prevented by the absence of the enzyme and can be largely reduced by the treatment of wild type macrophages with the TG2 inhibitor. Finally our data suggest that the reduced *Mtb* replication in cells lacking TG2 is due to the impairment of LC3/autophagy homeostasis.
Conclusion: These results indicate for the first time in the inhibition of TG2 transamidating activity a potential novel approach in the possible treatment of TB.

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SECRETORY ASPARTYL PROTEINASES OF *CANDIDA ALBICANS* INDUCE NEUTROPHILS CHEMOTAXIS

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Introduction: *Candida albicans* utilizes secretory aspartyl proteinases (Sap), a family of ten proteins that are known to be its key virulence traits, as active enzymes to induce damage of epithelial cells. Neutrophils are actively recruited in the vaginal cavity during Sap-induced inflammatory response, suggesting that Sap have direct neutrophil chemotactic activity.
Materials and Methods: Neutrophil migration in response to Sap2 and Sap6, which are well-studied members of the Sap1-3 and Sap4-6 sub-families, was measured by a chemotaxis chamber (in vitro) and was assessed in an ex vivo and in vivo mouse model. Chemotactic cytokines such as MIP-2 and IL-8, were tested in vaginal fluid in a mouse model of Sap-induced vaginitis without infection.
Results: Our results show that Sap2 and Sap6 induce migration of neutrophils in vitro, ex vivo and in vivo models. MIP-2 and IL-8 are produced during Sap-induced vaginal inflammation. The vaginal fluid of Sap-treated mice did not affect neutrophil candidacidal activity; in contrast, the vaginal fluid harvested from C. albicans-treated mice drastically inhibited anti-Candida properties of neutrophils.
Conclusions: Overall, Sap-mediated neutrophil chemotaxis can contribute to immune-pathogenesis of vaginal candidiasis and be a potential therapeutic target.

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RELATIONSHIP BETWEEN RIFAMPIN- AND METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) GENETIC BACKGROUND, RPOB NUCLEOTIDE POLYMORPHISMS AND HOST-PATHOGEN INTERACTION

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Introduction: Rifampin is one the major anti-MRSA drugs normally used alone or in combination with vancomycin, for difficult-to-treat bone and joint infections. Due to the observation that in Italy, rifampin resistance is an increasing trait of MDR-MRSA isolates (16.4%), with respect to the European trend (5.7%), we aimed to evaluate the relationship between *rpo*B mutations, genetic backgrounds and hVISA and daptomycin-non-susceptible (DNS) phenotypes, leading to antibiotic resistance and altered host-pathogen interactions.
Material and Methods: We analyzed *rpo*B mutations and the susceptibility to the main anti-Gram-positive drugs of 50 RIF-R MRSA strains isolated from hospitalized patients. Antimicrobial susceptibility testing was performed by broth-microdilution-method; VISA/hVISA phenotype was evaluated by population analysis. All isolates were typed by MLST/SCC*mec/spa*-typing. The internalization frequency of 7 RIF-R MRSA strains was evaluated in an *ex-vivo* model of infection within human MG-63 osteoblasts at a multiplicity of infection (MOI) of 100:1. *S. aureus* ATCC53657 and ATCC12598 reference invasive strains were included.
Results: hVISA and DNS were 60% and 22% of all RIF-R. The most common clone was ST228-SCC-*mec*I, of those 86.3% were hVISA and 18.2% DNS. 13 diverse amino acid substitutions were identified. 50% of RIF-R MRSA harbored the substitution H481N, conferring low-level resistance. Single mutation at the equivalent locus or in other loci, and multiple mutations, including H481N, conferred high-level resistance. H509R and D471G novel variants were identified in hVISA. Different invasion rates were observed using an initial *inoculum*of 5x106 CFU/50.000 osteoblasts. ST239/241-SCC-

*mec*III-t037 showed the best ability to internalize (0.37-0.74 CFU/cell); internalization rates of the other clones were ST5-SCC*mec*II-t002/t2154, 0.23-0.27 CFU/cell; ST8-SCC*mec*IV-t008/t121, 0.57-0.089 CFU/cell; ST228-SCC*mec*I-t041, 0.09 CFU/cell. ATCC53657 and ATCC12598 internalization rates were 0.448 and 0.20 CFU/cell, respectively.
Conclusion: The RIF-R isolates analyzed in this study represent the major MDR-MRSA clones circulating in Italy, with a high percentage of hVISA and DNS phenotypes. All the amino acid substitutions were present in the RNA polymerase conserved "rifampicin resistance-determining region" (RRDR). High-level resistance was ascribed to multiple mutations, including H481N, indicating a step-by-step *mec*hanism in resistance development, probably due to rifampicin-therapy exposure. The *ex-vivo* assays suggest that the ability to invade osteoblasts is correlated to the genetic background: ST239/241 and ST5 showed higher internalization efficiency; conversely, ST228, mainly involved in severe infections, did not exhibit significant rates of internalization.

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HERV-K ENV SURFACE ANTIGEN SPECIFIC PEPTIDES ELICIT AN HUMORAL IMMUNITY RESPONSE IN SERUM AND CSF OF AMYOTROPHIC LATERAL SCLEROSIS PATIENTS. CAUSE OR CONSEQUENCE?

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Introduction: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by the progressive loss of spinal and cortical motor neurons, the cause of which is unknown. Previous studies showed that HERV-K is activated in a subpopulation of patients with sporadic amyotrophic lateral sclerosis (ALS) and that its envelope (env) protein may contribute to neurodegeneration. In this study for the first time we investigated the HERV-K env antigen-specific humoral immunity response in serum and cerebral spinal fluid (CSF) of amyotrophic lateral sclerosis patients, and also in serum and CSF of Multiple Sclerosis and Other Neurological Disease patients.
Materials and Methods: The study included peripheral blood and CSF samples of 21 ALS patients, 26 Multiple Sclerosis patients and 13 Other Neurological Disease (OND) patients. In silico analysis performed by IETB Analysis Resource software allowed us to identify 4 antigenic peptides derived from HERV-K env surface protein. An indirect enzyme-linked immunosorbent assay (ELISA) was set up to detect Abs specific for the 4 HERV-K env surface peptides. Antibody Index (AI) for all ALS, MS and OND patients was calculated with the following formula AI = QIgG[spec]/Qlim.
Results: Abs against HERV-Kenv 1 were found in 90% of serum and in 81% of CSF from ALS patients; only in 23% of serum and in 50% of CSF from MS patients and in 23% and 47% of both serum and CSF of OND patients ($p < 0.0001$); Abs against HERV-Kenv2 peptide were found in 91% of serum and CSF of ALS patients, only in 23% of MS sera and 47% of MS CSF whereas OND patients were positive in 7% of serum and 23% of CSF ($p < 0.0001$). Concerning the other HERV-K surface peptides we didn't found any significant reactivity in ALS patients compared to MS and OND patients in both serum and CSF samples. A high number of positive samples in the ALS group had an AI > 1.5 that demonstrate an intrathecal IgG-specific antibody production against HERV-K surface peptides.
Conclusion: HERV-K env1 and HERV-K env2 peptides were strongly recognized in serum and CSF of ALS patients when compared to MS and OND patients. It is to establish if it is a cause or a consequence of the disease.

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VITAMIN D REDUCES THE INFLAMMATORY RESPONSE BY *PORPHYROMONAS GINGIVALIS* INFECTION BY MODULATING HUMAN B-DEFENSIN-3 IN HUMAN GINGIVAL EPITHELIUM AND PERIODONTAL LIGAMENT CELLS

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Introduction: Periodontitis is a multifactorial polymicrobial infection characterized by a destructive inflammatory process. *Porphyromonas gingivalis*, a Gram-negative black-pigmented anaerobe, is a major pathogen in the initiation and progression of periodontitis; it produces several virulence factors that stimulate human gingival epithelium (HGE) cells and human periodontal ligament (HPL) cells to produce various inflammatory mediators. A variety of substances, such as vitamin D, have growth-inhibitory effects on some bacterial pathogens and have shown chemo-preventive and anti-inflammatory activity.
Materials and Methods: We used a model with HGE and HPL cells infected with *P. gingivalis* to determine the influence of vitamin D on *P. gingivalis* growth and adhesion and the immunomodulatory effect on TNF-α, IL-8, IL-12 and human-β-defensin 3 production.
Results: Our results demonstrated, firstly, the lack of any cytotoxic effect on the HGE and HPL cells when treated with vitamin D; in addition, vitamin D inhibited *P. gingivalis* adhesion and infectivity in HGE and HPL cells. Our study then showed that vitamin D reduced TNF-α, IL-8, IL-12 production in *P. gingivalis*-infected HGE and HPL cells. In contrast, a significant upregulation of the human-β-defensin 3 expression in HGE and HPL cells induced by *P. gingivalis* was demonstrated.
Conclusions: Our results indicate that vitamin D specifically enhances the production of the human-β-defensin 3 antimicrobial peptide and exerts an inhibitory effect on the pro-inflammatory cytokines, thus suggesting that vitamin D may offer possible therapeutic applications for periodontitis.

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NEW INSIGHTS INTO THE ROLE OF MULTIPLE SCLEROSIS TREATMENT ON POLYMORPHONUCLEAR LEUKOCYTE FUNCTIONS AGAINST PATHOGENS

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Introduction: Multiple sclerosis (MS) patients run an increased risk of microbial infections. Although immunotherapy may increase infection risk in some cases, data as to the relationship among microbial factors, disease-modifying treatments (DMTs) and alterations in the innate immunity of these patients are still scanty. Our previous study indicated that MS polymorphonuclear cells (PMNs) have a significant reduction in intracellular killing activity compared to healthy subject (HS) PMNs, related to the therapeutic management of patients. On this ground, the aim of this study was to evaluate the direct role of most common immunosuppressive or immunomodulatory MS therapeutic agents on the functions of HS neutrophils subjected to an in vitro drug pretreatment.
Materials and Methods: To achieve this purpose HS PMNs were pre-treated with natalizumab (NAT), fingolimod (FTY), interferon β 1b (INFβ) or glatiramer acetate (GA), and then their intracellular killing activity towards *Klebsiella pneumoniae*, cytokine release profile, apoptosis, reactive oxygen species (ROS) production and surface molecule expression were investigated.
Results: In vitro assays with drug pre-treated HS PMNs showed a reduction in bacterial killing with significantly lower values ($p < 0.001$) than those registered for the un-pre-treated controls for all the DMTs assayed. In the same experimental conditions variable results, depending on the DMT used,

for the other examined neutrophil functions were achieved. In fact, this defective neutrophil killing was also associated with a significantly lower ROS production, a slight increase of survival and cytokine production for FTY, and a lower cytokine release pattern for both INFβ and GA.
Conclusions: In this regard, a deeper understanding of the priming condition exerted by treatments in neutrophils from MS patients could be a clue for the complete comprehension of MS pathogenesis and may be later utilized for therapeutic purposes that may prevent neutrophil-mediated damage to host tissues. In the end, the validation of these results could help in identifying a subset of patients at high risk of infection who could benefit from a closer follow-up and/or antibiotic prophylaxis.

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IN VITRO ANTAGONISM OF *BIFIDOBACTERIUM LONGUM* BB536 AND *LACTOBACILLUS RHAMNOSUS* HN001 AGAINST PATHOGEN ADHESION TO HT-29 CELLS

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Introduction: Little data exist in literature about the molecular players that sustain the ability of probiotic strains to colonize the human gut and to antagonize the enteric pathogens. The aim of this study was to test the in vitro antagonistic activity of *Bifidobacterium longum* BB536 and *Lactobacillus rhamnosus* HN001 against the adhesion of gram-negative strains to human epithelial intestinal HT-29 cells.
Materials and Methods: The in vitro antagonism of *B. longum* BB536 and *L. rhamnosus* HN001, both individually and in combination, against the adhesion of *Escherichia coli* EC3960, *Escherichia coli* EC4219, *Salmonella enteritidis* SEN6 and *Salmonella thypi* STN12 to HT-29 cells was performed using standardised culture techniques (Serafini et al. Anaerobe 21: 9-17, 2013; Inturri et al. Minerva Gastroenterol Dietol. 61(4):191-7, 2015).
Results: The results showed that both probiotic strains have a good capacity to inhibit pathogenic adhesion to HT-29 cells. Moreover, the study showed that *B. longum* BB536 and *L. rhamnosus* HN001, when they are used in combination, have an improved ability to inhibit the adhesion of enteric pathogens and also to compete with them.
Conclusion: The simultaneous presence of the two probiotics could promote competitive mechanisms that reduce the adhesion properties of pathogen strains. The good adhesive behaviour displayed by *B. longum* BB536 and *L. rhamnosus* HN001 cells may thus reflect their ecological potential within the highly competitive environment of the human gut.

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CHLAMYDIA TRACHOMATIS INDUCES UP REGULATION OF MOLECULAR BIOMARKERS PODOPLANIN WILMS' TUMOUR GENE 1 OSTEOPONTIN AND INFLAMMATORY CYTOKINES IN HUMAN MESOTHELIAL CELLS

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Introduction: *Chlamydia trachomatis* is the most prevalent infection of the genital tract in women worldwide. *C. trachomatis* has a tendency to cause persistent infection, inducing a state of chronic inflammation. Epithelial cells of the human genital tract can secrete different cytokines and chemokines in response to abnormal conditions, such as infections.
Materials and Methods: Primary cultures of mesothelial cells (Mes1) and an established cell line NCI-H2452 (NCI) human mesothelioma cells (ATCC CRL-5946), were infected with C. trachomatis at a multiplicity of infection of 4 inclusion-forming units/cell for 3, 7 and 14 days. Here we reported that persistent C. trachomatis infection

increases cell invasion, the expression of inflammatory tumorigenic cytokines, and up-regulates podoplanin, Wilms' tumour gene 1 and osteopontin of a primary cultures of mesothelial cells (Mes1).
Results: Mes1 cells infected with C. trachomatis showed many intracellular inclusion bodies. After 7 day infection an increased proliferative activity was also observed. ELISA analysis revealed an increase of VEGF, IL-6, IL-8, and TNF-α release in Mes1 cell infected for a longer period (14 days). Finally, real-time PCR analysis revealed a strong induction of podoplanin, Wilms' tumour gene 1 and osteopontin gene expression in infected Mes1 cells.
Conclusions: Given the association between infection, inflammation and cancer, the present study investigated the inflammatory response elicited by *C. trachomatis* infection and the role played by inflammation on perturbation of cell proliferation and increased molecular biomarkers of cancer. The results of this study suggest that increased molecular biomarkers of cancer by persistent inflammation of *C. trachomatis* infection might support cellular transformation, thus increasing cancer risk.

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INNOVATIVE TITANIUM ALLOY SURFACES ABLE TO PROMOTE BONE INTEGRATION AND AVOID BACTERIAL CONTAMINATION

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Introduction: An ideal surface for orthopedic implants should improve cellular adhesion and reduce bacterial one. A proper stimulation of the cell activity is the last request to the new biomaterials, intended for bone substitution and osseointegration. In this regard, the scientific literature suggests that the surface modification on a nanoscale is a major source of innovation. Furthermore, numerous solutions have been proposed to face the problem of the bacterial contamination and to prevent the development of prosthetic infections. The considered solutions are based on inorganic antibacterial agents, such as silver (Ag) copper or zinc, which have been introduced to overcome the growing problem of bacterial resistance to antibiotics. The aim of the present research work is the development of innovative antibacterial and bioactive titanium alloy (Ti6Al4V) surfaces, able to promote fast and physiological bone integration and avoid bacterial contamination.
Materials and Methods: Ti6Al4V disks were surface modified by means of a patented process that foresees a first etching in diluted hydrofluoric acid and a subsequent controlled oxidation in hydrogen peroxide, added with Ag.The surface topography and chemical composition of modified surfaces were characterized by means of Field Emission Scanning Electron Microscopy, X-ray Photoelectron Spectroscopy (XPS) and Fourier Transformed Infrared Spectroscopy (FT-IR). *In vitro* bioactivity was investigated by soaking samples in Simulated Body Fluid (SBF) and Ag release was quantified in double distilled water. The modified surface antibacterial activity was tested against *Staphylococcus aureus* ATCC 29213 by means of inhibition halo on agar medium and quantitative bacterial adhesion assays by using a sonication protocol to dislodge adherent microorganisms.
Results: Modified Ti6Al4V samples present a titanium oxide layer with a peculiar nanotexture that can be described as a nanometric sponge. Ag nanoparticles are embedded in this surface layer by the addition of an Ag precursor in the hydrogen peroxide bath. XPS analyses indicate that Ag is in the metallic form. Ag nanoparticles are responsible of Ag ion release in water: the released Ag amount is higher than what required to have an antibacterial action and lower that the cytotoxic limit reported in the literature. The results of antibacterial tests confirm these data and reveal an effective antibacterial behaviour of modified surfaces against S. aureus. Moreover a reduced bacterial adhesion has been observed on nanotextured surfaces compared to the polished ones. The modified surfaces are rich in hydroxyls groups (FT-IR and XPS evidence) and they are able to induce hydroxyapatite precipitation after immersion in SBF.
Conclusions: In this study, a nanotextured titanium oxide layer rich in hydroxyl groups and embedded with Ag nanoparticles has been obtained. Modified surfaces are bioactive by inducing hydroxyapatite precipitation in SBF, release Ag ions and present an antibacterial action against *S. aureus*. Considering the reported results, the obtained innovative Ti6Al4V surfaces are promising for orthopaedic

applications to induce fast and physiological bone integration and to reduce the incidence of prosthetic infections.

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LUNG LEUKOCYTES AS BIOMARKERS OF PNEUMOCOCCAL INFECTION OF THE MURINE LOWER RESPIRATORY TRACT

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Introduction: *Streptococcus pneumoniae* is a major human pathogen causing life-threatening diseases. In order to develop alternative vaccination strategies it is essential to have a reliable animal model of the infection. Since mice are poorly susceptible to pneumococcal pneumonia, highly virulent strains or high doses are used to obtain a clear lung pathology and mortality. We developed a murine model to detect biomarkers of infection with a sublethal inoculum.
Materials and Methods: C57BL/6 mice were infected intranasally with 107 CFU of *S. pneumoniae* strain TIGR4 and monitored for clinical signs and body weight loss. A group of mice was previously immunized subcutaneously with the pneumococcal conjugate vaccine (Prevnar13). Mice were sacrificed at different time points (1, 2, 4 or 7 days after infection); after perfusion, lungs were excised and homogenized to enumerate neutrophils, lymphocytes, monocytes, macrophages and natural killer cells by flow cytometry. The lungs of 2 animals/group were also graded histologically. Splenocytes were cultured for 72 hours and culture supernatants were assayed for cytokine concentrations.
Results: Mice did not show any clinical sign at any time point but started to lose weight after infection until 48 h post-infection. After that point animals gradually regained their initial weight. Mice immunized with Prevnar13 did not show body weight loss. Lung cell populations reached their maximum counts at 7 days, except for neutrophils that were highest 4 days post-infection. Seven days after infection, mice immunized with Prevnar13 had very low counts for all cell populations, resembling uninfected animals. All infected mice presented a lymphocyte infiltrate in the lungs, with some granulocytes present at 4 and 7 days post-infection. Mice sacrificed 4 days post-infection had the highest lung histological damage. Cytokine production was studied in the splenocytes harvested 7 days after the infection, following *in vitro* stimulation. Infected mice showed significantly higher levels of IFN-γ IL-17 and IL-10 compared to uninfected mice. Splenocytes from vaccinated and infected mice produced low levels of cytokines, except for IL-2 which was comparable to infected mice.
Conclusions: In this work we showed that by inducing a sublethal respiratory infection with *S. pneumoniae* we can find, still at 7 days, cellular and humoral factors that can be used as biomarkers of the infection. In fact mice immunized with Prevnar were protected and did not show increase of lung cellular population nor cytokine production. Therefore, this model can be used to evaluate new vaccination strategies without relying just on the mortality rate.

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TARGETED GENE DELETION IN CANDIDA ORTHOPSILOSIS DEMONSTRATES A ROLE FOR CORT0C04210 IN ADHESION TO HUMAN BUCCAL CELLS

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Introduction: Agglutinin-like sequence (*ALS*) proteins are cell wall molecules directly involved in the adhesion of *Candida albicans* to host surfaces. The present project was aimed at dissecting the contribution of *ALS* proteins to the adhesion ability of human pathogen *Candida orthopsilosis*, by generating null mutants for each *CoALS* genes. In a previous study, we successfully achieved the deletion of one of the 3 *ALS* genes, *CORT0B00800* (*Co800*). However, null mutant and the wild type strains adhered to a similar extent to human epithelial cells. A second *C. orthopsilosis ALS* gene, *CORT0C04210*

(*Co4210*), was then selected for site specific mutagenesis.
Material and Methods: The deletion of *Co4210* was performed using the SAT1-flipper cassette system. Two independent lineages of heterozygous and null mutant strains were obtained in *C. orthopsilosis* 90125 genetic background. Southern blot analysis was used to check for correct genotypes. Heterozygous and null mutant strains were characterized for phenotypic traits such as growth rate in liquid medium, ability to undergo morphogenesis under inducing conditions, growth in presence of cell perturbing agents (congo red, caffeine, fluconazole and calcoflour white) and adhesion to human buccal epithelial cells (HBECs).
Results: *Co4210* mutants were not impaired in their ability to grow in liquid medium and to form pseudohyphae. Moreover, similar results between the wild type and the mutant strains were observed when the ability to grow in presence of cell wall-perturbing compounds was evaluated. Interestingly, the deletion of both copies of *Co4210* resulted in a decreased ability to adhere on HBECs, compared to the wild type strain, as demonstrated by a $\geq$ 50% reduction in the adhesion index (0.67 and 0.53 for lineage a and b, respectively).
Conclusion: The deletion of *Co4210* did not alter the growth ability of *C. orthopsilosis* in any of the conditions examined. However, the results obtained with the adhesion assay provide the first evidence for a direct role of the encoded protein in *C. orthopsilosis* adhesion to host surfaces. Further characterization on other human cell types will help in our understanding of the binding properties of *Co4210* and *Co800* proteins.

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NEUTROPHILS PRODUCE HIGH LEVELS OF CHEMOKINES (CXCL1/2) AFTER SENSING GROUP B STREPTOCOCCUS THROUGH ENDOSOMAL TLRS

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Introduction: Neutrophils have a crucial role in host defenses against *Streptococcus agalactiae* (Group B *Streptococcus*, GBS), a leading cause of serious infections in neonates, post-partum women and elderly people [1]. Since little is known about anti-GBS responses in neutrophils, here we studied the mechanism underlying chemokine production after innate immune recognition of these bacteria by this cell type.
Materials and Methods: Bone-marrow-derived neutrophil preparations were obtained from wild type mice or mice deficient in Toll-like receptors (TLRs) or TLR adaptors/chaperones. Cells were exposed to live GBS and supernatants were collected for cytokine measurements by ELISA.
Results: Chemokine (CXCL1 and CXCL2, Chemokine receptor ligand 1 and 2), but not TNF-alpha, production by neutrophils in response to live bacteria was considerably higher than that observed after stimulation with LPS or killed bacteria. This response required the TLR adaptor MyD88 (Myeloid Differentiation factor 88) and UNC93B1, a chaperone protein required for the localization of TLRs to endosomal compartments [2, 3].
Conclusions: Neutrophils play a crucial role in host defenses against GBS infection [1]. Neutrophils have been assigned the role of short-lived effector cells with limited ability to influence the function of other cell types through cytokine production. Our results show that neutrophils produce high levels of CXCL1 and CXCL2 directly in response to GBS through a mechanism that required endosomal TLRs, thereby amplifying their own recruitment

1. Biondo C et al. Infect Immun 2014; 82: 4508-17.
2. Tabeta K et al. NatImmunol 2006; 7: 156-64.
3. Biondo C et al. mBio 2014; 5: 01428-01414.

P 137

COINFECTION OF *HELICOBACTER PYLORI* AND EPSTEIN-BARR VIRUS IN PATIENTS WITH GASTRIC DISEASE IN SOUTHERN ITALY

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Introduction: *Helicobacter pylori* and Epstein-Barr virus (EBV) infections are the most common worldwide infections and recently it was reported that co-infection is involved in development of gastric diseases.
Objective: To study the association of *H. pylori* and EBV infection in patients with gastric diseases in Sicily.
Methods: Biopsy samples were collected from 23 adult patients with chronic gastritis active (CGA) undergoing upper gastrointestinal endoscopy. *H. pylori* infection was diagnosed by PCR for *ureaseA* gene while EBV-DNA was detected by Real time PCR for region Bam HI-W.
Results: We founded that the *H. pylori* and EBV infection was present in 43% and 39% of patients respectively while the dual prevalence of *H. pylori* infection and EBV-DNA was present in 26% of the patients. In all positive patients for EBV the peripheral blood was drawn and antibodies against EBV (IgG anti-VCA, EBNA and EA) were analyzed. Only 1 patient presented reactivation of infection.
The *cagA* gene and *vacA* (*s, i* and *m* region) genotypes were assessed in all positive biopsies. *cagA* gene was detected in 20% of the cases with EPYA ABC motif. Regarding the *vacA* gene, the s1 allele was found in 2 samples, *m*1 allele in 1 case and *i*1 was found in 2 cases.
Conclusion: In our study there was a strong association between the simultaneous presence of *H. pylori* and EBV infection suggesting a possible cooperation in development of gastric diseases.

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NEUTROPHILS ARE MAINLY RESPONSIBLE FOR IL-1B PRODUCTION DURING GROUP B *STREPTOCOCCUS* INFECTION

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Introduction: IL-1β has a central role in host defenses against group B streptococcus (GBS or *Streptococcus agalactiae*) [1, 2], a frequent cause of serious infections in neonates and elderly people. Since IL-1β has a crucial role in attracting neutrophils to infection sites in response to GBS infection, we investigated here the cell types and mechanisms involved in the production of this cytokine.
Methods: Cell influx and cytokine release were analyzed using an *in vivo* model of GBS-induced inflammation. ELISA and Western Blot analysis were performed to detect cytokine production in neutrophil cultures stimulated with live GBS.
Results: We first found that neutrophils are the predominant IL-1β-producing cell type in GBS-induced peritoneal exudates, as indicated by a selective reduction of IL-1β, but not TNF-α or IL-6, after neutrophil depletion with anti-Ly6G antibodies. In neutrophil cultures, pro-IL-1β production followed bacterial recognition by mechanisms requiring the toll-like receptor (TLR) adaptor MyD88 and the chaperone protein UNC93B11,3. Moreover, pro-IL-1β processing and IL-1β release required caspase-1, ASC and NLRP3, but not serine proteases or caspase-83.
Conclusions: Neutrophils are largely responsible for *in vivo* IL-1β production during GBS infection, thereby amplifying their own recruitment. Moreover, GBS recognition in neutrophils requires endosomal TLRs and caspase 1 inflammasome components.

1. Biondo et al. mBio 2014; 5:e01428-01414
2. Gupta et al. J biolchem 2014; 289: 13701-5.
3.Tabeta et al. Nat Immunol 2006; 7: 156-64.

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AN IN VIVO RNA-SEQ APPROACHES IN *ENTEROCOCCUS FAECIUM* REVEALS THE OVER-EXPRESSION OF LYSM DOMAIN CONTAINING PROTEINS: *IN VITRO* E *IN VIVO* CHARACTERIZATION

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Introduction: *Enterococcus faecium* is considered as an harmless commensal of the gastro-intestinal tract of animals since long time. In the last two decades, however, it has been recognized as an important cause of health-care associated infections. *Enterococcus faecium* infections are a major threat due to the high level of antibiotic resistance. Our previously RNA-sequencing in vivo analysis conducted on bacteria isolated from mice peritoneum after 24 hours revealed the over expression of three highly homologues LysM domain containing proteins whose functions are unknown. In bacteria, LysM module binds the peptidoglycan and most of LysM proteins are peptidoglycan hydrolases.
Material and Methods: For our study, we used the vancomycin-resistant strain AUS0004. Deletion mutants of EFAU004_0494, EFAU004_01059, EFAU004_01150 were constructed using PWS3 vector. The effective deletion was confirmed by sequencing of the regions of interest. The virulence of AUS004 and the deletion mutants was assessed in systemic infection models. Biofilm and Z- potential were evaluated by atomic force microscopy (AFM) microscopy and light scattering.
Results: Mice were infected via intravenous injection of each enterococcal strain. The log10CFUs/g of bacteria recovered from the kidneys infected by AUS0004 were significantly higher than that recovered from the kidneys infected by the ΔEFAU004_0494($p < 0.05$) whereas ΔEFAU004_01059, ΔEFAU004_01150 did not show differences. Due to the high homology, double ΔEFAU004_01150-01059 and triple ΔEFAU004_01150-01059-00494 mutants were constructed to asses a possible redundant effect. These mutant strains show a lower colonization capacity compared to the wild type and ΔEFAU004_0494. Again, a comparison of the mean log10 CFUs of bacteria recovered from the liver showed that the differences between AUS004 and the mutants were statistically significant, but to a lower extent. These results are consistent with an increased tropism of *E. faecium* for the kidney relative to the liver. The analysis at the light scattering reveals a decreased Z-potential in the double and the triple mutant.
The biofilm analysis at the AFM displays different morphology and an increased adhesion force of the deletion mutants.
Discussion: LysM domain containing proteins are known to be involved in the cell wall turn-over as hydrolases or muramidases. To date, nothing is known about their functions in *Enterococcus faecium* In order to address their roles with respect to the high up regulation during in vivo transition, we decide to investigate the specific phenotypes of the deletion mutants. Mouse model shows a significant impact in the virulence. As future prospective we will further investigate the capacity of the deletion mutants in the mouse urinary tract infections model (UTI) to confirm the role in the phatogenicity. Further, recombinant proteins will be used in in vitro assay to fully understand their function.

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TUNING OF A WHARTON'S JELLY-DERIVED MESENCHYMAL STEM CELL ENGINEERING SYSTEM WITH EPSTEIN-BARR DERIVED INTERLEUKIN-10

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Introduction: Lentiviral vectors (LVs) are quite effective delivery systems. Upon appropriate modifications, they target a huge variety of active and quiescent cells. LVs are versatile and can deliver up to 9 Kbp heterologous DNA organized in one or more genes and guarantee constitutive expression of heterologous proteins thanks to the fact that they integrate in the host genome. LVs expressing viral inteleukin-10 (encoded by Epstein Barr virus) and/or herpes simplex virus type-1 thymidine kinase (TK) were developed to engineered Wharton's Jelly-derived mesenchymal stem cell (WJ-hMSCs). TK can be tracked in vivo by positron electron tomography (PET).
Materials and Methods: A monocistronic and a bicistronic LV have been generated by cloning the genes for vIL10 and/or TK into LAW34, a feline immunodeficiency virus-derived vector and with a cytomegalovirus promoter to control transgene expression. LAW34-vIL10, and LAW34-vIL10/TK vectors were generated by transfection of HEK-293T cells with packaging plasmid pΔenv1 and pVSV-G, in the presence of polyethyleneimine. LAW34-GFP, delivering enhanced-green fluorescent protein (GFP) has been used as a control of efficiency of transfection and of transduction which were assessed by FACS. WJ-hMSCs were transduced with increasing amounts of LAW34-GFP and various concentrations of polybrene or protamine sulfate (PS).
Results: 1) LAW34-vIL10 and LAW34-vIL10/TK produce both vIL-10 and TK as showed by western blot and were able to release high level of vIL-10 in cell culture medium as showed by ELISA. 2) The treatment with aciclovir (an anti-herpetic guanosine analogue) and propidium iodide demonstrated TK bioactivity, in transfected HEK-293T cells. 3) WJ-hMSCs were efficiently transduced by LAW34-vIL10 and LAW34-vIL10/TK in the presence of PS even at low MOI. 4) Engineered WJ-hMSCs keep the phenotype and functional features of wild type cells.
Conclusions: We developed an efficient method to engineer WJ-hMSCs by vIL10/TK and to track cell *in vivo* by PET.

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GUT MICROBIOTA COMPOSITION IN MECP2-MUTANT MURINE MODELS OF RETT SYNDROME

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Introduction: Rett Syndrome (RTT) is a pervasive developmental disorder due, in more than 90% of cases, to a mutation in gene encoding for Methyl-CpG-Bindig protein 2 (MeCP2). Often RTT patients have gastrointestinal problems (like esophagitis and diarrhea). Different studies have shown an altered gut microbiota in autistic patients with respect to normal children. Moreover, animal studies suggest that changes in the abundances of gut bacteria may affect the function of nervous system of the brain and/or the gut. To understand if the gut microbiota alterations may underlie intestinal dysfunction of RTT patients we analysed the gut microbiota of two different RTT mouse models.
Material and Methods: The analysis of gut microbiota of female MeCP2-null (+/-), MeCP2-308 (+/-) and wt controls was done by Next Generation Sequencing (NGS) approach. The DNA was extracted and sequenced using Illumina amplicon

sequencing method on Illumina MiSeq sequencing machine. The data generated were filtered, assembled, trimmed and the remain reads were analysed with QIIME to pick the Operational Taxonomic Units (OTUs). The taxonomic classification was performed against Greengenes database. The alpha and beta diversity indices were calculated to analyse the diversity intra- and inter-communities. To identify the differences in gut microbiota between MeCP2-mutant mice and wt we used LEfSe algorithm and Metastats comparison. The script of QIIME compute_core_microbiome.py was used to identify the core-OTUs that characterize the gut microbiota of MeCP2 mice and the wt.
Results: The comparison of MeCP2-null and wt using LEfSe and Metastats showed that the MeCP2-null mice differed from wt in the abundance of different OTUs. We found that MeCP2-308 mice also had differences in OTUs abundances from wt studied. We observed that there was a variability in gut microbiota in the same group of MeCP2-mutant mice. Through compute_core_microbiome.py of QIIME we found that MeCP2-null and MeCP2-308 shared a core gut microbiota with specific bacterial taxa which was different from its wt counterpart.
Conclusion: In this study we identified some features characterising the gut microbiota of MeCP2-mutant mice which may have a role in the development of certain symptoms (like gastrointestinal problems) of autistic-like phenotype in these mice.

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ST258 K. PNEUMONIAE STRAINS BELONGING TO THE CLADE 1 ESCAPE INFLAMMASOME ACTIVATION BY INFLAMMATORY MONOCYTES THROUGH THE MODULATION OF NF-KB AND P38MAPK-ACTIVATED PATHWAYS

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Introduction: Strains of *Klebsiella pneumoniae* (KP) of sequence type 258 (ST258) are mainly responsible for the global spread of KPC carbapenemases. Two distinct clades mainly differing in capsular composition, were reported in ST258 KP clone. Strains from the two clades are equally uptaken and killed by neutrophils. We have shown however relevant differences in the ability to activate functional properties in myeloid dendritic cells and consequently in activating adaptive response. Indeed KK207-1/clade 1 was revealed less efficient than KKBO-1/clade 2 either in activating IL-1β, IL23 production by MDC either in inducing TH17 differentiation. Here we studied the effects of KKBO-1/clade 2 and KK207/clade 1 on CD14+ monocytes. the phenotype,the inflammatory/anti-inflammatory properties of CD14+ cells induced by bacterial cells from the two clades were studied.
Materials and Methods: KK207-1 and KKBO-1 cells were used live or UV-inactivated. Peripheral Blood CD14+ cells were isolated through immune-adsorption. Cytokines were measured by immunoplex array and gene expression evaluated by RT-PCR. Caspase-1 and pro-IL-1β cleavage were studied by Western Blot.
Results: Phenotype: CD14+ cells cultured with ST258 KP-KPC strains increased expression of CD14 and decreased those of CD16 resembling an intermediate "pro-inflammatory" phenotype. Furthermore the expression of CD86 and HLA-DR were also induced by ST258-KP. Cytokine production: CD14+ cells cultured with ST258 KP

produced high amounts of IL-6, TNF-α, IL-1β. We noted that KK207-1/clade 1 was much less active in inducing the production of IL-1β compared to KKBO-1/clade 2. Accordingly KK207-1/clade 1 was less effective to induce IL-1β gene expression compared to KKBO-1/clade 2. NLRP3 inflammasome activation: ST258-KP strains activate the NLRP3 inflammasome pathway, leading to caspase 1 activation and pro-IL-1beta cleavage. KK207-1/clade 1 was a less efficient stimulus compared to KKBO-1/clade 2 in all stages of inflammasome pathway: NLRP3 gene expression, caspase 1 and pro-IL-1beta cleavage. Purified capsular polysaccharides (PCP) from ST258-KP strains behave as PAMPs, since they activate molecular pathways involved in the regulation of NLRP3 inflammasome genes, namely p38MAPK and NF-kB. Again PCP from KK207-1/clade 1 were significantly less efficient than PCP from KKBO-1/clade 2 in these activities suggesting that the capsular composition help bacterial cells to escape NLRP3 recognition.
Conclusion: Our data suggest that ST258-KP/clade1 may escape NLRP3-inflammasome activation by pro-inflammatory monocytes recruited at the infection site being helped by the peculiar composition of capsular polysaccharydes.

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A RARE CASE OF SEPTIC ARTHRITIS WITH RICE BODY FORMATION CAUSED BY *CANDIDA ALBICANS* IN PREMATURE INFANT

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Introduction: The incidence of septic arthritis in children ranges from 5 to 12 cases per 100,000 occurring more frequently in children younger than three years. This condition is usually caused by microrganisms that enter the bloodstream and infect the joint, causing inflammation and pain. Children with immune deficiency disorders have a higher risk of septic arthritis. *Staphylococcus* and *Enterobacteriaceae spp*. are commonly implicated in septic arthritis. This condition is less frequenting by *Candida* species. In this report we describe an unusual case of *Candida albicans* septic arthritis in an infant with rice body formation.
Materials and Methods: Our patient was a born prematurely at 30 weeks of gestational age, with a birth weight of about 1000g. When she was two week old showed fever and laboratory examination revealed an increased leukocyte count (12 × 109/l, normal range 3.5-8.8 × 109/l) with eosinophilia (15.2% , normal range 2-4%), an elevated erythrocyte sedimentation rate (25 mm/h, normal range 0-2 mm/h) and increased serum procalcitonin (6 ng/ml, normal range 0-0.1 ng/ml). *Candida albicans* was isolated from a blood culture. Antifungal drug susceptibility testing were performed using a Vitek 2 system (AST-YS07 cards). The patient received a therapy with fluconazole (16 mg/ml e/v). At 50 days of age the infant showed swelling, warmth, pain and limited range of motion of the left knee and was brought by her parents to the Department of Pediatric Surgery of the University of Messina Polyclinic "G. Martino". Knee's ultrasound examination revealed copious effusion with abundant corpuscular bodies. Culture of aspiration fluid on Sabouraud agar revealed *Candida albicans* while the blood culture was negative. Antifungal drug susceptibility testing was performed using Sensititre YeastOne system and it showed that the yeast was susceptible to all antifungal tested. Intra articular injections with amphotericin B was performed.
Discussion: *Candida* septic arthritis infections are most often due to hematogenous seeding of the joint or bone in patients who have been candidemic and although *Candida albicans* is the most common pathogen, also other species, such as *C. glabrata*, *C. parapsilosis*, and *C. tropicalis*, can be involved.
Conclusion: *Candida* arthritis is infrequent in infants younger than three months and to our knowledge, this is the first reported case of rice body formation in an infant. Septic arthritis is a serious cause of morbidity and for proper evaluation and treatment, fungal septic arthritis should be included in the differential diagnosis.

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ADAPTIVE IMMUNE RESPONSE INDUCED BY A HYPERVIRULENT K2 *K. PNEUMONIAE* STRAIN

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Introduction: *Klebsiella pneumoniae* (*KP*) is a common Gram-negative pathogen causing community and nosocomial infections, including pneumonia, urinary tract, septicaemia and wound infections. Most *KP* strains are encapsulated and resistant to neutrophil phagocytosis. Hyper virulent (HV) *KP* strains produce high amounts of mucoviscous exopolysaccharides and mainly belong to K1 and K2 serotypes. The spread of HV *KP* strains, as well as the acquisition of antibiotic resistance among both classical and HV strains, may contribute to the rise in morbidity and mortality of *KP* infections in both immunocompromised and healthy populations. In this work we evaluated the interaction of a *KP*-HV strain, CIP 52.145, with the immune system of immune-competent hosts.
Materials and Methods: CIP 52.145, ST258 *KP*C-producing *KP* strains, XL1Blue *Escherichia coli* strain were grown in Luria Broth (LB) or LB-agar. Live, UV- or heat-inactivated bacteria were used as stimulant. Myeloid dendritic cells (MDC) were obtained from circulating CD14+ cells. HEK-293-HuTLR4 with NFkB-reporter gene were used for TLR assay. Cytokine production was determined by immunoplex array. Tbet and RORγ mRNA was quantified by RT-qPCR. The percentage of TH1, TH17, and regulatory T cells was assessed by cytofluorimetry. Capsular polysaccharides were measured evaluating the amount of glucuronic acid after sulfuric acid treatment, using VICTOR microplate reader (Perkin Elmer).
Results: CIP 52.145 *K. pneumoniae* strain activated the TLR4 pathway significantly less than ST258 *KP*C-*KP* or *E. coli* strains. The production of IL23 and IL12 by MDC cultured with CIP 52.145 was significantly lower compared to that induced by ST258 *KP*C-*KP* or *E. coli* strains. In contrast no differences were found in the production of inflammatory cytokines, as IL6, TNF-α. The expression of the transcription factors RORγ (TH17) e Tbet (TH1) by CD4+ T cells cultured with MDC pulsed with CIP52.145 was significantly lower compared to that induced by MDC pulsed with ST258 *KP*C-*KP* isolates and *E. coli*. When cultured with PBMC from normal donors CIP52.145, either UV-inactivated or heat-killed induced a weak TH17 response which was significantly lower compared to that induced by ST258 *KP*C-*KP* or *E. coli*. In contrast no differences were recorded in the percentage of TH1 and Treg cells.
Conclusions: Our data indicate that CIP52.145 *KP* strain does not induce significant TH17 response. The low activation of TLR4-mediated pathway likely represent the mechanisms leading to limited availability of cytokines inducing TH17 differentiation. We suggest that, in addition to capsule-mediated resistance to phagocytosis, HV *KP* strains escape the adaptive TH17 response being facilitated in the process of mucosal colonization even in immune competent donors.

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SETTING UP A RAT MODEL OF INVASIVE *PULMONARY ASPERGILLOSIS*

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Introduction: Invasive pulmonary aspergillosis (IPA), caused by *Aspergillus fumigatus*, affects immunocompromised patients and it frequently leads them to death. In this study we aimed to set up a useful and reproducible model of IPA in the rat.
Materials and Methods: Sprague-Dawley rats were immunosuppressed by subcutaneous injection (s.c.) of 150mg/kg of Cortisone Acetate (CA) on days -6, -4, -2, 0, followed every other day by repeated doses of CA at 80mg/kg. A total of 22 animals were infected at day 0 by a single intratracheal inoculation of *A. fumigatus* conidia (5x107 in 0.2mL). Rats were divided in 3 experimental groups: control group (i.e. immunosuppressed and untreated), positive control group, immunosuppressed, infected with *A. fumigatus* and Voriconazole-treated (oral), experimental group, immuno-

suppressed, infected with *A. fumigatus* and vehicle-treated. We evaluated immunosuppression by FACS analysis and its impact on rat weight. Fungal burden was assessed by analyzing explanted lungs and by measuring galactomannan index (GMI) in lungs by ELISA. Mortality rate was recorded every day till the end of the study.
Results: FACS analysis on rat PBMCs revealed that immunosuppression was successful, since the lymphocyte population mostly disappeared following treatment with CA. Rats' weight decreased over the duration of the study in a comparable manner among groups, as consequence of the immunosuppression and the low-protein diet. Following animal death or upon euthanasia (if the animal was experiencing severe breath insufficiency), lungs were explanted. Lungs of rats infected with *A. fumigatus* and treated with Vehicle showed large lesions involving one or more lung lobes, while most of the animals treated with Voriconazole did not present detectable signs of aspergillosis. Lung weight was also determined as a measure of Aspergillus growth, since it increased about two fold after infection and was significantly suppressed by the treatment with Voriconazole. GMI evaluation revealed an increased galactomannan concentration in the lungs of rats infected with Aspergillus and a reduced concentration in the infected and Voriconazole-treated group. Furthermore, no deaths were recorded in the Voriconazole-treated group.
Conclusions: Taken together, these results show that we have developed a promising rat model of IPA, wherein suitable parameters of immunosuppression, infection with *A. fumigatus* conidia and appropriate drug treatment have been set up. This model holds promise for the evaluation of novel agents and drug combination for the treatment of IPA, as well as the identification of new biomarkers of disease evolution and control.

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ACTIVATION OF NF-KB PROTECTS U937 MONOCYTIC CELLS AGAINST BOTH INFECTION AND APOPTOSIS INDUCED BY HSV-1

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Introduction: The transcription factor nuclear factor-kappa B (NF-κB) is a crucial player of the antiviral innate response. Intriguingly, however, NF-κB activation is assumed to favour herpes simplex virus (HSV) infection rather than restrict it. Apoptosis, a form of innate response to viruses, is completely inhibited by HSV in fully permissive cells, but not in cells incapable to fully sustain HSV replication, such as immunocompetent cells. NF-κB activation is assumed to contrast apoptosis.
Materials and Methods: To resolve the intricate interplay among NF-κB signalling, apoptosis and permissiveness to HSV-1 in monocytic cells, we utilized U937 monocytic cells in which NF-κB activation was inhibited by expressing a dominant-negative IκBα. HSV-1 'F' strain, was used in all experiments. Virus replication was assayed by different methods including immunofluorescence or western blot analysis of viral proteins, analysis of viral gene expression by PCR, virus titration. Apoptosis was detected by microscopy analysis of nuclear morphology following Hoechst 33342 staining or flow cytometry analysis following propidium iodide staining. Merged images were used to simultaneously evaluate gD and apoptosis double-positive cells. NF-κB activation was assessed by electro mobility shift assay (EMSA).
Results: Surprisingly, viral production was increased in monocytic cells in which NF-κB was inhibited. Moreover, inhibition of NF-κB led to increased apoptosis following HSV-1 infection, associated with lysosomal membrane permeabilization. High expression of late viral proteins and induction of apoptosis occurred in distinct cells. Transcrip-

tional analysis of known innate response genes by real-time quantitative reverse transcription-PCR excluded a contribution of the assayed genes to the observed phenomena.
Conclusions: Thus, in monocytic cells NF-κB activation simultaneously serves as an innate process to restrict viral replication as well as a mechanism to limit the damage of an excessive apoptotic response to HSV-1 infection. This finding may clarify mechanisms controlling HSV-1 infection in monocytic cells.

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MODULATION OF IMMUNITY IN CACO-2 CELLS BY FAECAL LACTOBACILLI AND BIFIDOBACTERIA FROM INDIVIDUALS FOLLOWING OMNIVOROUS, VEGETARIAN AND VEGAN DIETS

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Introduction: The intestinal epithelium is located between the bacterial flora and the associated lymphoid tissue (GALT). Enterocytes are involved in the defense against pathogens and prevent the penetration of commensal microbes in the tissues. They also have an important role in intestinal mucosal immunity giving the specialization of dendritic cells. The microbiota is directly involved in the development and modulation of the intestinal immune system. In particular, lactobacilli and bifidobacteria play a primary role in the modulation of the immune response. The aim of this study was to evaluate the immunomodulatory ability of isolates lactobacilli and bifidobacteria, derived by faecal microbiota, on the inducible immune response following incubation with CACO-2 cells.
Materials and Methods: Fecal samples were recovered from 155 healthy adult volunteers who followed an omnivorous, ovo-lacto-vegetarian or vegan diet recruited in four Italian cities (Turin, Parma, Bologna and Bari). Differentiated Caco-2 cells were pre-incubated for 0-72 h with irradiated bacteria resuspended in complete DMEM medium at a 30:1 bacteria : Caco-2 cell ratio. Following bacteria exposure, cells were stimulated with PMA/Ionomycin (PMA/I) for 24-72 h in complete medium. IL-8 and Thymic stromal lymphopoietin (TSLP) levels, collected from Caco-2 supernatants, were analyzed by sandwich ELISA. We used as positive control cells treated only with PMA/I.
Results: The significant increase in IL-8 release was observed in media of Caco-2 cells exposed to PMA/I, while release of TSLP was not modulated by PMA/I. IL-8 release was significantly down-regulated, in comparison with positive control, only by lactobacilli isolated from omnivore group. On the contrary, a significantly down-regulation of this cytokine, was observed in cells treated with bifidobacteria independently from the diet, compared to positive control. Significantly down-regulation of TSLP release was found in lactobacilli and bifidobacteria isolated in stimulated Caco-2 cells supernatant, regardless of the diet regimen, in comparison to positive control.
Conclusions: In this study, compared faecal populations of lactobacilli and bifidobacteria isolated from individuals followings three different diets , we found that both genera down-regulated cytokines release in Caco-2 cells after stimulus with PMA/I; however, only lactobacilli activity was influenced both by the dietary habit and by the geographical origin.

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INFLUENCE OF *MENTHA X PIPERITA* ESSENTIAL OIL OF PANCALIERI (TO) ON INTRACELLULAR KILLING ACTIVITY BY PMNS AGAINST *CANDIDA KRUSEI*

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Introduction: The growing threat of antimicrobial drug resistance has stimulated the search for new therapeutic alternatives, including essential oils (EO) that are now recognized for their potential antimicrobial role against microorganisms. *Mentha x piperita* L. Huds has been used in folk medicine since antiquity and recent studies documented the antimicrobial efficacy of its EO. In Piedmont (Pancalieri, TO) it has spread the OE production of a cultivar of *M. x piperita*. Since clinical experience showed that the efficacy of antimicrobial drugs depends both on their direct effect on microorganisms and on the activity of the host immune system, we evaluated the influence of this oil on intracellular killing by PMNs against *Candida krusei* in comparison with anidulafungin (AND), as reference drug.
Materials and Methods: A clinical *C. krusei* strain was used. The EO was purchased from Erbe Aromatiche Pancalieri,Turin and analysed by GC (Drug Science and Technology Dept.). AND (Pfizer) was provided by Prof. Milici (University of Palermo). Susceptibility testing was based on the CLSI M27-A3 method, with some modifications. Intracellular killing was investigated by incubating yeasts (106 cfu/mL) and PMNs (106cells/mL) at 37°C for 30, 60, 90 min in presence of 1/4xMIC or 1/8×MIC of EO and 1/2MIC of AND. Killing values were expressed as Survival Index. To differentiate between any separated effect of EO/AND on yeasts/PMNs, the experiments were conducted after exposure of each of them to EO or AND for 1h. EO/AND-free controls were included. Statistical evaluation of the differences between test and control results was performed by Tukey's test.
Results: EO at 1/4 and 1/8xMIC produced a significantly further decrease in the survival of yeasts, with killing values ranging from 56 to 61% and 53 to 67%, respectively during the 90 min period, in comparison with controls (22-33%) ($p < 0.01$). In the presence of ½×MIC AND, intracellular yeasts were killed in similar values to the control (27 to 33%). The data showed that pre-treatment with EO acts both on the yeasts and on phagocytic cells, suggesting a positive interaction between EO and PMNs. In fact, pre-treatment of PMNs with 1/8xMIC EO resulted in a significant enhancement of intracellular killing, as compared with 1/2xMIC AND and controls. Pre-treated yeasts with ¼ - 1/8xMIC EO were killed more efficiently by PMNs than AND pre-treated yeasts: 46-44-57% and 50-64-60% vs 17-18-13% (1/2xMIC AND) and 22-29-33% (yeasts not treated) ($p < 0.01$).
Conclusions: We observed that EO was more efficacy at 1/8xMIC than at 1/4xMIC (eagle effect), indicating that decreasing concentrations did not cause lower candidacidal activity. These data show a promising potential application of this EO against *C. krusei*, often resistant to conventional drugs.

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POTENTIAL IMPACT OF A NONAVALENT VACCINE ON HPV RELATED LOW-AND HIGH-GRADE CERVICAL INTRAEPITHELIAL LESIONS

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Introduction: Demonstration of the role of persistent infection, with high-risk (HR) human papillomaviruses (HPV) as the causal agent of cervical cancer made the development of first and second generation prophylactic vaccines. Bivalent and quadrivalent HPV vaccines are at the moment available in Europe. In 2014 is licensing a nonavalent HPV vaccine against HPV types: 6/11/16/18/31/33/45/52/58. The aim of our study was to evaluate the potential impact on HPV infection and related low- and high-grade cervical lesions (LSIL, HSIL) of the candidate nonavalent

HPV vaccine, compared to the impact of the quadrivalent, in a female population living in Sicily.
Materials and Methods: HPV genotypes was identified by Linear Array HPV Genotyping Test (Roche Diagnostics) and with the INNO-LiPA HPV assay (Innogenetics) for ambiguous HPV 52 status. Low estimates of HPV vaccine impact was calculated as prevalence of HPV 6/11/16/18/31/33/45/52/58 genotypes alone or in association but excluding presence of another HPV type; high estimate as prevalence of HPV 6/11/16/18/31/ 33/45/52/58 genotypes alone or in association, in presence of another HPV type.
Results: 1794 samples had a HPV positive finding. HR HPV types, alone or with LR types, were present in 1466 (81.7%) samples. 584/1794 (32.5%) samples harboured at least one of the four HPV types covered by the quadrivalent vaccine (HPV 6/11/16 and 18), while 984 (54.8%) samples harboured at least one of the genotypes included in nonavalent vaccine, implying a significantly higher estimated coverage of HPV infection from the developing vaccine than the current quadrivalent (54.8% vs 32.5%; $p < 0.001$). Of the samples with a known histological diagnosis a total of 362 cases (72.2%) of LSIL and of 58 cases (90.6%) of ≥ HSIL were HPV positive. The nonavalent HPV vaccine showed increased impact on both categories of lesions, compared to the quadrivalent vaccine. Estimates of potential impact varied from 30.9% (low estimate) to 53.3% (high estimate) for LSIL, and from 56.9% to 81% for HSIL. Compared to the quadrivalent vaccine, the proportion of additional cases potentially prevented by the nonavalent vaccine was 14.4%-23.8% for LSIL, and 19%-32.8% for HSIL. The benefit of the nonavalent vaccine compared to the quadrivalent vaccine was more than 80% for both low and high impact estimates for LSIL and more than 50% for both low and high impact estimates for HSIL.
Discussion and Conclusions: The present study confirms that the switch to a nonavalent HPV vaccine would increase the prevention of high grade cervical lesions in up to 90% of cases. Implementation of nonavalent vaccination programs could become thus a cost effective public health prevention approach, based on the potential to produce substantial incremental benefits.

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RESVERATROL-LOADED NANOEMULSIONS: IN VITRO ACTIVITY ON CANCER CELL LINES OF DIFFERENT ORIGIN

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Introduction: Polyphenols like resveratrol (RV), curcumin, quercitin, catechin constitute a common groups of anti-oxidant substances present in plants and reported to have antimicrobial and anti-proliferative activities. Although natural bioactive substances hold promises for supporting more conventional therapies, inconsistent results due to the low level of aqueous solubility, stability, bioavailability are often found in the literature. Nanoemulsions (NEs) are colloidal dispersion in which main components are oil, emulsifying agents, and aqueous phases: nanometric oil droplets are dispersed in an external aqueous phase and the system can be stabilized by a suitable surfactant or surfactant mixture. The aim of our research is to study the efficiency of newly designed NEs as carriers of natural bioactive compounds, such as resveratrol, in cancer cell lines of different origin.
Materials and Methods: Water based NEs from neem seed oil and Tween 20 or 80 as a surfactants were loaded with RV and characterized in terms of size and stability. The anti-proliferative activity of the synthesized polyphenol-loaded NEs on the tumoral and macrophage cell lines was tested by MTT and trypan blue assays. Cell lines used in the study were: Hep-2 (larynx derived epithelial cells), Caco-2 (colon epithelial cells), PC-3 (prostate; derived from metastatic site: bone),T-24 (from urinary bladder,carcinoma), Raji (B Lymphocytes from Burkitt's Lymphoma), RAW 264.7 (murine macrophages).
Results: Among the different preparations, RV-loaded NEs of different size with Tween 20 or with Tween 80, were chosen to evaluate anti-proliferative activity. In general, all RV-loaded NEs more

efficiently reduced cell proliferation as compared to the empty NE controls as well as to RV alone.
Conclusions: NEs are widely used in pharmaceutical systems for targeted drug delivery of various therapeutic agents. Oil-in-water NEs delivery of hydrophobic phytochemicals such as resveratrol, could represent a good strategy to improve intracellular availability of these compounds and boost their potential effects on tumors and cancer cells.

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GENOTOXIC MUCOSA-ASSOCIATED *ESCHERICHIA COLI* IN COLON DISEASES: BAD BUGS IN OUR GUT

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Introduction: The complex communities of microorganisms that live in the human digestive tracts play a central role in human health. It has demonstrated that *Escherichia coli* strains belonging to phylogenetic group B2 are found with increasing frequency in the feces of healthy individuals and are responsible for a range of extraintestinal diseases. These extraintestinal pathogenic *E. coli* (ExPEC) strains carry different virulence determinants that promote host colonization damaging. Among these, it was shown that the *pks* genomic island is present in 30% to 40% of *E. coli* B2 strains. *pks* codes for the production of a polyketide-peptide genotoxin that causes double-strand (ds) breaks in DNA and chromosome instability, that might have superlative influence on development of colorectal cancer. Moreover, it has been shown that there is a higher prevalence of psk positive *E. coli* in colorectal cancer patients compared to the healthy people, supporting the idea that genotoxic activity by *pks* positive *E. coli* exerts specific carcinogenic effects. Hence, the aim of this work is the evaluation of *pks* positive *E. coli* frequency in polipoid patients, as the precancerous group, compared to the healthy controls and the distribution of different phylogenetic groups within the *pks* positive *E. coli*.
Materials and Methods: 4 colon biopsies from healthy controls and 14 colon polyp biopsies were collected from colonoscopy center of Policlinic hospital "Umberto I" of Rome. For bacterial recovery, lysates of each biopsy sample were plated on MacConkey agar. *pks* island and phylogenetic groups of thirty randomly selected *E. coli* isolates were determined by PCR, using "*pks*" specific primers and the method recently described by Clermont et al., respectively. *E. coli* strain IHE3034 (gifted by Prof. Jean-Philippe Nougayrede) was used as *pks* positive control.
Results: In our study, we found *pks* positive *E. coli* colonizing two patients with polypoid lesion and none *pks* positive *E. coli* were found in the control biopsies. PCR analysis showed that the frequency of *pks* positive *E. coli* in the polyp lesion and in the normal tissue of both patients was high [patient A: (26/30; 86.6%), (22/30; 73.33%) and patient B: (22/30; 73.33%), (18/30; 60%)], respectively. The predominant phylogenetic group was B2 (105/120; 87.5%), followed by D (9/120; 7.5%), B1 (3/120; 2.5%), A (2/120; 1.7%), and F (1/120; 0.8%).
Conclusions: Overall, a low presence of *pks* positive *E. coli* among the patients was observed. However, a high abundance of *pks* positive *E. coli*, in two cases both in normal tissue and in polyp lesion, was obtained, indicating that they can abnormally colonize the tissue. It might be speculated that the high abundance colonization of *pks* positive *E. coli* strains belonging to the B2 phylogroup could represent development of sporadic colorectal cancer.

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TH2 RESPONSE OF MONOCYTES INDUCED BY LTA AND PA OF *S. AUREUS* MODIFIES EPIDERMAL BARRIER FUNCTION

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Introduction: Atopic dermatitis (AD) is a common, fluctuating skin disease that is often associated with atopic conditions such as asthma and IgE-mediated food allergy and whose skin lesions are characterized by a Th-2 cell-mediated response to environ-

mental antigens. The increasing prevalence and severity of atopic diseases including AD over the last three decades has been attributed to decreased exposure to microorganisms during early life, which may result in an altered Th-1/Th-2-balance and/or reduced T cell regulation of the immune response. Patients with AD exhibit defects in innate and acquired immune responses resulting in a heightened susceptibility to bacterial, fungal and viral infections, most notably colonization by *S. aureus*. Toxins produced by *S. aureus* exacerbate disease activity by both the induction of toxin-specific IgE and the activation of various cell types including Th-2 cells, eosinophils and immune cells recruited. However, half of *S. aureus* isolated from AD patients have been found to be enable to produce superantigens. In the present work The implicance of S.aureus, lipoteichoic acid (LTA) and protein A (PA) in the immunostimulation of monocytes U937 was analyzed.

Methods: U937 have been stimulated for 4 and 16h with S . aureus LTA and PA. TH2 cytokines; IL10, and IL25 have been analyzed by qPCR. The supernatants of treated cells for 24h have been used to stimulate HaCat to evaluate the epidermal barrier protein through the expression of filaggrin and acquaporin 3.

Results: *S. aureus*, LTA and PA are able to modulate gene expression of IL-25 and IL-10 in U937 cells HaCat cells treated with supernatants of U937 stimulated or not, showed an alteration in the epidermal barrier targets.

Conclusion: The result obtained showed a clear involvement of TH2 cytokines during *S. aureus* skin infection and that the released product from monocytes on the site of infection can alter the epidermal barrier. It can be speculated that in patients with AD in which there is already an altered epidermal barrier, the Th2 response, especially IL-25, may provide for the establishment of a chronic disease. Microorganisms play an influential role in AD pathogenesis, interacting with disease susceptibility genes to cause initiation and/or exacerbation of disease activity.

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"INDIRECT PATHOGENICITY" IN CYSTIC FIBROSIS DURING PULMONARY EXACERBATIONS: *STENOTROPHOMONAS MALTOPHILIA* MODULATES *PSEUDOMONAS AERUGINOSA* VIRULENCE IN A GALLERIA MELLONELLA MODEL

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Introduction: During cystic fibrosis (CF) airway disease, periods of stability are punctuated by acute pulmonary exacerbations (PEs), whose physiology is not yet completely understood. Although *Pseudomonas aeruginosa* is considered the primary cause of PEs, it is often co-isolated with *Stenotrophomonas maltophilia*, whose pathogenic role is not yet clear. It is, therefore, plausible to hypothesize the occurrence of interspecies interactions able to affect *P. aeruginosa* virulence and persistence. In this study, we evaluated the interactions between *P. aeruginosa* and *S. maltophilia* in a *Galleria mellonella* systemic infection model, using strains co-isolated from the same lung of five different CF patients.

Materials and Methods: Eleven *P. aeruginosa* (RR8, BR1, DIN1, DIN2, DAT8, DAT9, DAT10, AC12a, AC12b, AC13a, AC13b), and six *S. maltophilia* (RR7, BR2, BR3, DIN3, DAT7, AC8) strains were co-isolated, during PE episode, from the sputa of five CF patients (RR, BR, DIN, DAT, AC). The in vivo effects of *S. maltophilia* on *P. aeruginosa* host response were evaluated in a wax moth model. Overnight cultures of *S. maltophilia* and *P. aeruginosa* grew in Trypticase Soy broth were washed and resuspended in PBS. Each larva (n = 20/group) was inoculated, directly into the hemocoel via the right proleg, with 103 CFU of each strain, alone or in combination, or PBS only (control). Larvae were incubated in the dark at 37°C until 96 h, and checked daily for survival.

Results: Survival curves of "RR" and "BR" strains showed no differences between groups of larvae infected with *P. aeruginosa* alone (RR8 and BR1) and in combination with the respective strains of *S. maltophilia* (RR7, BR2 and BR3, respectively). With regard to "DIN" strains, all S.maltophilia-P. aeruginosa combinations exhibited a comparable mortality rate compared to *P. aeruginosa* tested alone, but significantly higher ($p < 0.0001$) than *S. maltophilia*. The same trend was observed for the combination AC8+AC12a with respect to *S. maltophilia* tested alone ($p < 0.0001$). In "DAT" strains, no differences were observed between groups except for DAT7+DAT10 combination where mortality was significantly reduced ($p < 0.05$) when compared to that observed in larvae infected with *P. aeruginosa* DAT10 alone (mortality at 24h: 5 vs 30%, respectively). The same trend was observed in larvae infected with AC8+AC13b when compared to larvae infected with the respective strains tested alone ($p < 0.05$).

Conclusions: Overall, our results showed that *S. maltophilia* could indirectly contribute to lung disease, modulating *P. aeruginosa* host response. Particularly, the presence of *S. maltophilia* seems to mitigate *P. aeruginosa* virulence in G. mellonella model in a strain-dependent way. Further in vivo studies are needed to gain new insights in the pathogenic significance of microbial cooperation.

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IN VIVO BACTERIAL COOPERATION IN CYSTIC FIBROSIS: EXPOSURE TO *STENOTROPHOMONAS MALTOPHILIA* AFFECTS *PSEUDOMONAS AERUGINOSA* VIRULENCE IN MURINE MODEL OF ACUTE LUNG INFECTION

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Introduction: Several studies suggested that a complex microbiota resides in the lungs of cystic fibrosis (CF) patients, thus indicating that pathogenic processes in CF airways might be due to polymicrobial interactions. Stenotrophomonas maltophilia, whose pathogenetic role in CF is not yet clear, is often co-isolated from CF lungs with Pseudomonas aeruginosa. Our hypothesis is that S. maltophilia can modulate, directly or indirectly, P. aeruginosa virulence, thus contributing to the respiratory function decline in CF patients. In this study, we evaluated the in vivo effects exerted by S. maltophilia on P. aeruginosa virulence.

Materials and Methods

P. aeruginosa DIN2 and S. maltophilia DIN3 strains, co-isolated from the lung of a chronically colonized CF patient, were tested. The in vivo effects of S. maltophilia on P. aeruginosa virulence were assessed in C57BL/6N mice following intratracheal exposure to each strain alone and in combination. Changes in mice weight, quantitative lung bacteriology, lung histology, and pulmonary interleukines were evaluated over 24h.

Results

Infection caused by P. aeruginosa and S. maltophilia, alone or in combination, provoked a reduction of mice body weight compared to controls, although not statistically significant. Macroscopic score index revealed that DIN2+DIN3 co-infection caused a higher damage than DIN3 alone, but comparable to DIN2 alone, as confirmed by mortality rate. When tested alone or in co-culture, P. aeruginosa

exhibited longer pulmonary persistence than S. maltophilia alone did. Histological analysis revealed that alveolar involvement and intraluminar infiltrate were higher in the lungs of co-infected mice, although these differences were not statistically significant. Infection caused by DIN2 alone induced higher pulmonary levels of interleukines, compared to mixed DIN3+DIN2 infection.
Conclusions
Overall, our results showed that in vivo S. maltophilia is able to modulate P. aeruginosa virulence, thus suggesting that studying bacterial interactions should be considered in evaluating CF disease progression. Further studies are warranted to confirm our findings, to identify the underlying mechanisms, and to evaluate the impact of these interactions on the management of CF patients.

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EVALUATION OF CELLULAR IMMUNE RESPONSE TO HSV-1 BY ELISPOT ASSAY IN LUNG TRANSPLANT PATIENTS

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Introduction: Cellular immunity plays a relevant role in control of HSV-1 infection/reactivation with a potential important impact on clinical-therapeutic management of immunocompromised patients, such as transplant recipients.
Materials and Methods: In this study, we quantitatively evaluated T-cell response directed at HSV-1 by a newly developed IFN-γ EliSPOT assay in 53 patients (including 45 lung transplant recipients and eight subjects in waiting list).
Results: Overall, 62.2% of transplant patients and 62.5% of subjects in waiting list evidenced a response to HSV-1 with no significant difference in the level of virus-specific cellular immunity. Response tended to be lower in the first 3 months posttransplantation with progressive recovery of pretransplantation status by the second year and in patients with occurrence of HSV-1 DNA positivity in bronchoalveolar lavage. As expected, no response was found in seronegative patients, whereas no significant difference in the level of response according to IgM and IgG status was found.
Conclusions: Quantitative evaluation of HSV-1 specific cellular immunity in lung transplant patients could allow for a patient's tailored clinical-therapeutic management in terms of immunosuppression and antiviral therapy. Further studies are required to define the role and level of HSV-1 specific cellular immune response in this and other clinical settings and to define cut-off levels discriminating between absence/low and strong response to be related to the risk of viral infection/reactivation.

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PEPTIDES DERIVED FROM THE PROTEOLYSIS OF SERUM PROTEINS AND IMMUNOGLOBULINS INDUCE IL-1BETA PRODUCTION INDEPENDENTLY OF INFLAMMASOME ACTIVATION

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Introduction: It has been reported that, independently from the specificity of the native antibody (Ab) for a given antigen (Ag), complementarity determining regions (CDR)-related peptides and peptides deriving from the enzymatic cleavage of the constant region (Fc), may display differential antimicrobial, antiviral, antitumor and immunomodulatory activities.
Materials and Methods: The capacity of peptides derived from the Fc of human IgM and from the alpha-chain of the complement factor C4 to induce production of inflammatory cytokines such as: IL-1beta, TNF-alpha and IL-6 by human monocytes was tested by using immunoenzymatic assay. Western blot was used to test inflammasome activation and caspase-1 expression.
Results: Our results show that the endocytosis of the selected peptides by human monocytes induced

a significant increase of pro-inflammatory cytokines production. In particular, peptides-induced IL-1beta was independent of NLRP3-inflammasome activation but occurred via cleavage of the precursor pro-IL-1beta in the bioactive form IL-1beta.
Conclusions: These results open a new scenario about the possibility that selected peptides derived from the proteolysis of different serum proteins and immunoglobulins may induce an unexpected regulation of inflammatory response.

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IMMUNOSUPPRESSION-DRIVEN HBV REACTIVATION IN PATIENTS WITH RESOLVED HBV INFECTION CORRELATES WITH A RELEVANT RISK OF DEATH AND EVOLUTION TO CHRONICITY OF HBV INFECTION

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Introduction: The issue of immune-suppression driven HBV-reactivation (HBV-R), in patients (pts)

with previously resolved HBsAg-negative infection is often underestimated. This study provides a snapshot of virological and clinical features of pts with resolved HBV-infection, undergoing HBV-R driven by immunosuppressive-therapy (IS-T).

Materials and Methods: This study includes 51 pts with HBV-R (Hwang, 2013), all HBsAg/HBV-DNA negative before IS-T. For 29 pts, virological/biochemical follow-up is available after HBV-R. Mean genetic distance (GD) is used to estimate the extent of HBsAg genetic variability. Prevalence of HBsAg mutations associated with HBV-R (Salpini, 2015) is investigated in 36/51 genotype D infected pts.

Results: Before IS-T, serological profiles are: 58.8% isolated anti-HBc, 27.5% anti-HBc/anti-HBs pos, 7.8% isolated anti-HBs, and 5.9% negative to all HBV-markers. At HBV-R, median (IQR) HBV-DNA and ALT are 6.7 (4.5-7.7) logIU/ml and 195 (39-762) IU/L. Notably, 17.7% remains HBsAg-negative despite HBV-R (HBV-DNA:3.0-7.5 logIU/ml). In 9/9 HBsAg negative pts at HBV-R, > 1 new N-linked glycosylation in HBsAg is detected. 52.1% of HBV-reactivated pts is treated with rituximab (RTX), 12.5% with corticosteroids alone, and 35.4% with other chemotherapeutics. LAM-prophylaxis is used in 15.7% of pts with HBV-R (median[IQR]duration: 21 [10-33] months). In 59.1%, HBV-R occurs after completing IS-T (range:1-48 months). RTX-use correlates with HBV-R after completing IS-T (72% after vs 28% during, $p = 0.05$), while corticosteroids alone use correlates with HBV-R during IS-T (0% after vs 100% during, $p = 0.01$). After HBV-R, death for hepatic failure occurs in 9.8%. Among 35 pts starting anti-HBV treatment after HBV-R, ALT-normalization is observed in 74.3%, virological-suppression in 42.9%, and HBsAg-loss in only 31.4%. Pts with RTX-related immunosuppression are characterized by a higher HBsAg genetic variability than pts with immunosuppression related to other chemotherapeutics/corticosteroids (median GD±SE: 0.022± 0.008 vs 0.008 ± 0.004, $p < 0.001$). > 1 HBsAg-mutation associated with HBV-R occurs in 90% of RTX-related pts. In particular, Q129R (known to hamper HBsAg recognition) correlates with RTX use (6/20 on RTX vs 0/16 not on RTX, $p = 0.01$).

Conclusions: In pts with resolved HBV infection, immunosuppression-driven HBV-R, can occur in a large variety of anti-HBV serological profiles and immunosuppressive settings. A higher degree of genetic variability and specific mutations in HBsAg, such as Q129R, are correlated with RTX use and may favor HBV reactivation in the setting of drug-induced B-cell depletion. Overall, these data support the need of an optimized management of HBV-R in terms of adequate monitoring pre- and during IS-T, and improved prophylaxis.

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RESIDUAL VIREMIA AND INFLAMMATION MARKERS: GENDER DIFFERENCE IN ARV TREATED HIV-1 INFECTED PATIENTS

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Introduction: Chronic immune activation and inflammation represent a key component of HIV pathogenesis. The process of chronic inflammation could derive from a multifactorial process in which host and virus actively participate, then factors related to gender differences could influence this process. The aim of the study was to evaluate gender difference in HIV-1 infected patients about immune activation status and its relationship with residual viremia.

Methods: Two hundred and twenty-one patients [113 female (F) and 108 male (M)] from Policlinico Umberto I Hospital were retrospectively examined for 45 months. The median age of patients was 52 (IQR 48-57) for F and 53 (IQR 47,5-57,7) for M. According to basal viremia the patients were divided in three groups. Specifically, 68 F and 59 M had full suppression viremia (gI), 25 F and 33 M had a viral load detectable but below the threshold value (gII) and 20 F and 16 M show viremia between 37-200 copies/ml (gIII). HIV-1 RNA was measured by Versant kPCR (Siemens). D-dimer, sCD14 and C-reactive protein (CRP) were measured by ELISA tests.

Results: The median levels of sCD14 and CRP were similar in F and in M (sCD14: 2.51 ug/ml (IQR 1.78-3.62) vs 2.79 ug/ml [(IQR 2.04-3.97 $p = 0.065$; CRP: 0.85 ug/ml (IQR 0.44-3.35) vs 1.59 ug/ml (IQR 0.44-2.83) $p = 0.68$]. However when we compared patients with undetectable viremia, lev-

els of sCD14 was higher in M than in F [(2.84 ug/ml (IQR 2.33-3.76) vs 2.21 ug/ml (IQR 1.64-3.58) p = 0.017]. At the contrary, comparing gII and gIII, no differences of activation marker levels between F and M were observed. The median levels of D-dimer in F was higher than in M [(214.53 ug/ml (IQR 134.97-363.09) vs 157.09 ug/ml (IQR 82.9-285.9) p = 0.039]. When we stratified the levels of D-dimer according to basal viremia a statistically significant differences between gIII was found. Specifically, F showed higher levels of D-dimer than M [(210.9 ug/ml (IQR 145.5-373.2) vs 124.5 (IQR 49.1-214.1) p = 0.028]. Furthermore, the levels of D-dimer correlate positively with age of patients (r = 0.15 p = 0.024), at the contrary, no correlation between levels of others two markers with age were detected.

Conclusions: The higher levels of sCD14 detected in male patients than in female patients with undetectable viral load and the higher levels of D-dimer found in female than in male suggest that factors gender related could contribute to chronic inflammation status.

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MOLECULAR MECHANISMS DRIVING *STREPTOCOCCUS MITIS* ENTRY INTO HUMAN GINGIVAL FIBROBLASTS IN PRESENCE OF CHITLAC-NAG AND SALIVA

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Introduction: In dental biofilm a wide range of oral bacteria might invade buccal cells and among them streptococci appear to be the most represented. The aim of this work was to investigate the molecular mechanisms governing *Streptococcus mitis* internalization into human gingival fibroblasts (HGF) in presence of Chitlac-nAg and/or saliva, to evaluate how HGFs react to this entry and to study the effects of the Chitlac-nAg colloidal system on both eukaryotic and prokaryotic cells.

Materials and Methods: The invasion of HGFs by S. mitis in presence of Chitlac-nAg and/or saliva after 3 h and 48 h was examined using antibiotic protection assay and visualized by fluorescence and transmission electron microscopy. In addition, the fibroblast F-actin, vinculin, integrina β1, Focal Adhesion Kinases (FAK) and Metalloproteinase 2 (MMP2) were also evaluated. Furthermore, additional experiments were performed to evaluate the ability of *S. mitis* to survive or come out HGFs after 72 h and 96 h.

Results: After 3 h, S. mitis entry occurs and HGFs show adhesive contacts and cell surface rearrangements with formation of filopodial structures which engulf bacteria and determine F-actin cytoskeleton rearrangement. Early bacterial entry, mediated by intracellular signaling involving FAK, integrin β1, vinculin and F-actin cytoskeleton, is modulated by the presence of saliva. After longer time intervals (48 h), Chitlac-nAg treatment increases significantly the number of internalized bacteria, and it seems to be related to the increased expression of vinculin, integrin β1 and FAK, further supporting their involvement in bacterial entry, while when saliva is added to the culture bacterial entry as well as protein expression are reduced. After 24 h and 48 h of re-incubation upon antibiotic treatment (72 h and 96 h samples), our results show a reduction in the *S. mitis* internalized bacterial number into HGFs suggesting that streptococci did not grow in the cytoplasm and evidencing bacterial release outside HGF cells. Probably, the intracellular environment is unfavorable for bacterial growth in terms of nutritional intake and space, so bacteria prefer escape the cells.

Conclusions: All in all, these results seem to suggest that HGFs tolerate *S. mitis* internalization also in the presence of Chitlac-nAg and, bacteria inside HGFs, partly die for nutrients lack and in part are released outside to restore the microbial balance of the mouth. Thus, the antimicrobial action of Chitlac-nAg on the bacterial planktonic phase makes it a promising device for daily oral care and, on the other hand, induces bacterial entry in HGFs but this effect is skillfully balanced by the key role of saliva.

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TWENTY-SEVEN YEARS EXPERIENCE WITH IMMUNOGENICITY OF INACTIVATED INFLUENZA VACCINES IN THE ELDERLY

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Introduction: Influenza vaccination of elderly people is efficacious and cost effective for the prevention of influenza and its complications, but generally elderly respond suboptimally to influenza vaccination because of the presence of comorbidities and immunosenescence. The aim of our study was to examine the immunogenicity of influenza vaccines in subjects aged over 60, focusing on the weight that some factors, i.e. age (60-84 and ≥ 85), sex, or type of vaccines, may have on the antibody response. We analysed the data obtained by our research group in 27 winter seasons (from 1988-1989 to 2014-2015), in 4,461 elderly people, most of them with underlying medical conditions, vaccinated with different commercially available seasonal trivalent inactivated influenza vaccines.
Materials and Methods: The antibody response was examined comparing haemagglutination inhibition antibody titers in sera collected before and 30 days after vaccination.and post-vaccination results were evaluated according to the criteria of the Committee for Medicinal Products for Human Use (CHMP) for approval of influenza vaccines. All statistical analyses to compare the different subgroups were carried out using Matlab® of MathWorks Inc. release 2014b.
Results: Responses in most instances satisfied at least one of the 3 CHMP criteria. Higher responses were found against A/H3N2 vaccine components in female as compared with male subjects. The response in elderly (60-84 y) and very elderly (≥ 85y) was in most instances similar, in both age groups the difference between males and females was still observed. The two enhanced vaccines used, MF59-adjuvanted and intradermal, induced similar and higher responses compared with conventional vaccines against A/H3N2 antigen. Against A/H1N1, the response induced by MF59-adjuvanted vaccine was in most instances higher than conventional and intradermal vaccines, whereas against B antigen, intradermal vaccine induced higher HI response than that induced by conventional and MF59-adjuvanted vaccines.
Conclusions: Our data evidenced that the use of influenza vaccination appear to be an appropriate strategy to address the challenge of influenza infections of the elderly. Moreover they underline the necessity to expand researches and approaches to understand immunosenescence and its relationship to vaccine-induced immunity in order to have more valid vaccines. The vaccine induced stimulation of HI antibody response following vaccination was found to be influenced by different factors as age, sex and type of vaccine. It is therefore important to understand the mechanisms that result in these differences and to use such information to devise more immunogenic influenza vaccine candidates.

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MODULATION OF THE IMMUNE RESPONSE BY DIFFERENT VACCINE ADJUVANTS

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Introduction: Vaccine-induced immune response is enhanced and shaped by adjuvants. Here, we investigate, in mice, early biomarkers of adjuvanticity after primary immunization, employing four different adjuvants combined with the chimeric tuberculosis vaccine antigen H56.
Materials and Methods: C57BL/6 mice were primed by the subcutaneous route with the tuberculosis vaccine antigen H56 combined with four different adjuvants: the liposome system CAF01, a squalene-based oil- in- water emulsion (o/w Squalene), Alum and CpG ODN 1826. T and B cell primary responses were analyzed within the local draining lymph nodes, blood and spleen 7 and 12 days after

subcutaneous priming.
Results: The presence of the adjuvant in the vaccine formulation shaped the antigen-specific immune response, eliciting different effector mechanisms of the adaptive immunity. Adjuvantation with CAF01, o/w Squalene and Alum elicited a significant primary antigen-specific CD4+ T cell response compared to antigen alone. The effector function of activated cells was skewed towards Th1/Th17 response by CAF01, while o/w Squalene elicited a mixed Th1/Th2 response. On the contrary Alum and CpG did not stimulate significant cytokine secretion compared to H56 antigen alone. Nevertheless, these two compounds, together with o/w Squalene, elicited the highest frequency of short-lived plasma cells that correlated with a high serum H56-specific IgG response, while immunization with antigen alone or combined with CAF01 stimulated a weaker humoral response. The induction of the germinal centre reaction was observed only in lymph nodes of mice immunized with Alum, o/w Squalene and CAF01.
Conclusions: These data indicate that both CD4+ T and B-cell priming are early biomarkers of adjuvanticity and characterize the different priming properties of CAF01, Squalene-based emulsion, Alum and CpG ODN 1826 adjuvants. This is particularly relevant for the rational design of prime-boost vaccination strategies to elicit optimal immune responses.

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HUMAN ENDOGENOUS RETROVIRUSES EXPRESSION IS A MOLECULAR SIGNATURE IN THE FAMILY OF AUTISTIC PATIENTS

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Introduction: Autism spectrum disorders (ASD) are a heterogeneous group of neurodevelopmental disorders characterized by impairment in social communication and interaction, and repetitive or restricted behaviours, resulting by a complex interplay of genetic susceptibility, environmental factors and immune alterations. Human Endogenous Retroviruses (HERVs), originated by the infections of germ cells by exogenous retroviruses during primate evolution, are proposed as possible cofactors in the aetiology of complex diseases. Based on their ability to be modulated under specific stimuli, HERV can be seen as the spanning bridge between genetic predisposition and environmental factors. In a previous study, we demonstrated high HERV-H expression in ASD young patients, higher levels in patients with severe impairment in communication and motor development, and negative correlation with the age of patients.
On the basis of obtained data, we extended the study to parents of ASD patients, in order to evaluate the transcriptional activity of other HERV families, their modulation in response to the in vitro stimulation and to the treatment with valproic acid or non-nucleoside reverse transcriptase inhibitors, plus the expression profile of cytokines.
Materials and Methods: The transcriptional activity of the env sequence of HERV-H, HERV-W and HERV-K families and the expression levels of IL-1β, IL-6, IL-8, IL-10, TNFα, IFNγ in PBMCs of ASD patients and their parents respect to healthy controls age and sex matched, at the time of sampling and after stimulation in culture were analysed by quantitative Real-time PCR.
Results: HERV families are differentially expressed in ASD patients and their parents in comparison to healthy individuals. In particular, PBMCs from mothers of the ASD patients showed a similar expression profile to that of their children, and also showed an increased potential to up-regulate HERV expression upon stimulation in culture. Conversely, no differences in HERV expression level were found in fathers of both groups. Pharmacological treatments modified HERV expression in PBMCs of ASD patients and mothers and, although to a minimal extent, in the fathers. Finally, high transcriptional levels of IL-10 and TNF-α were found in ASD patients and mothers compared to controls.
Conclusions: The results, although preliminary, support the use of HERV as biomarkers for the disease and as possible molecular signature inside the ASD families. In addition data obtained encourage us to further investigate the maternal features, supported by the idea that the intrauterine development and the alteration of the delicate balance between mother and fetus has a key role in the development of the autistic phenotype.

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EVALUATION OF HIV-DNA, SOLUBLE CD14 AND INFLAMMATORY MARKERS IN HIV-1 POSITIVE PATIENTS RECEIVING ANTIRETROVIRAL THERAPY

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Introduction: Antiretroviral therapy suppress viral load but, HIV infected individuals may have persistent residual viremia (RV) and, low grade inflammation and immune activation that have been associated with non-AIDS defining events. The impact of persistent RV and HIV DNA load on immune activation/ inflammation remain unclear.
The purpose of this study was to gain new insights into the relationship between residual viremia, markers of inflammation and the levels of HIV DNA.
Methods: Three-hundreds-twenty-one HIV-1 infected patients, from Policlinico Umberto I Sapienza University Hospital, were analyzed retrospectively for 48 months. Patients were grouped according to viral load (VL): 113 patients with a sustained undetectable viremia (gI);113 patients who had at least 2 values of VL detectable but below the threshold value (gII); 95 patients with at least 2 values of VL between 37-200 copies/ml (gIII). TNFα, IL-6 and sCD14 were evaluated by ELISA assay. Limit of detection were 15.63 pg/ml, for TNFα and 7.81pg/ml for IL-6. Quantification of total HIV-1 DNA was performed by commercial Kit (Biocentric).
Results: There was no difference about proportion of patients with TNFα > 15.63 pg/ml and levels of TNFα among groups. The proportion of patients with IL-6 > 7.81 pg/ml were higher in gI than in gIII (35% vs 17%; p = 0.005) while IL-6 levels > 7.81 pg/ml were significantly higher in gIII than in gI [28 pg/ml (IQR 13-45) vs 15.5 pg/ml (IQR 10-30); p = 0.0047]. Significantly lower levels of sCD14 were detected in gI and gII [7.25 μg/ml (IQR 2.7 ≤ 10) and 8.8 μg/ml (IQR 3.6 ≥ 10)] compared to the median sCD14 level in gIII [10 μg/ml (IQR 4 ≥ 10)], as well as significant difference was detected between gI and gII. An higher percentage of patients, with sCD14 levels greater than high limit of quantification (10 μg/ml), was detected in gIII compared to gI and gII (58% vs 15%, p < 0.0001 and 58% vs 35%, p = 0.001). Again, a significant difference was observed between gI and gII (15% vs 35%, p < 0.001). The quantification of HIV DNA revealed that in patients with detectable viremia the HIV DNA levels were significantly higher than those detected in individuals with undetectable plasma viremia [gIII: 15.3 copies HIV DNA /106 PBMC (IQR 12.0-17.1) vs gI: 12.1 copies HIV DNA /106 PBMC (IQR 9.4-14.2), p < 0.0001; gII: 14.2 copies HIV DNA /106 PBMC (IQR 12.4-15.8) vs gI, p = 0.001].
Conclusions: This study indicated that low/minimal levels of viremia are associated with more elevated levels of sCD14 and IL-6. In addition a higher intracellular viral load was detected in individuals with low/minimal viremia than in patients with undetectable viremia. Further studies are needed to clarify whether the above markers may represent prognostic indicators of progression of the inflammatory disease.

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THE INTESTINAL INVOLVEMENT IN SYSTEMIC SCLEROSIS IS CHARACTERIZED BY A PECULIAR GUT MICROBIOTA

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Introduction: Gastrointestinal involvement is recognized as a major cause of morbidity and mortality in systemic sclerosis (SSc). The pathophysiology is still unclear and includes impairment of motility, digestion, absorption and excretion. Few data on composition and function of gut micro-environment in SSc are reported in literature but there is a growing body of evidences supporting the hypothesis of a relation between gut microbiota, the host immune

system and metabolic/nutritional status. The goal of this study was to characterize the gut microbiota in SSc patients compared to healthy subjects to investigate whether specific microbial species may be responsible of dysbiosis in SSc patients with gastrointestinal involvement. Furthermore, we investigated the composition of microbiota in different subsets of SSc according to patient's nutritional status to verify if microbiota characteristics may be used as a biomarker for malnutrition and poor prognosis in patients with SSc.
Methods: A total of 66 SSc patients were enrolled: 66.7% presented a normal BMI, 15.1% was under-weight, while 18.2% was overweight. Gastrointestinal involvement was evaluated through UCLA-GIT 2.0 questionnaire while nutritional status was assessed through the MUST and selected blood biomarkers (albumin, Vitamin D, Vitamin B12, folate, Ferritin, cholesterol). Faecal samples were obtained from SSc patients and healthy controls. The composition of microbiota was determined through 16SrRNA pirosequencing performed using the GS Titanium technology (Roche 454). Dedicated statistic (LEfSe) was used to identify taxa that showed differential expression between the groups; α- and β-diversity and Firmicutes/Bacteroidetes ratio were determined.
Results: In our cohort of patients the mean total UCLA GIT 2.0 score was 0.4 ± 0.3. The values representing species richness were significantly different in the SSc group compared to controls ($p = 0.009$ for Shannon index mean values). Noticeably, this difference was mostly accountable to BMI > 25 subgroup. Firmicutes/Bacteroidetes ratio was inverted (> 1) in the SSc group compared to controls, as already reported in literature for obese patients. At genus level SSc patients showed a differential expression in 21 taxa compared to controls with higher levels of genera such as Ruminococcus, Roseburia, Lactobacillus and Faecalibacterium and a decrease of genera such as Clostridium, Odoribacter, Veillonella and Prevotella. The differences in microbiota composition between SSc patients and controls were supported also by principal coordinate analysis (PCoA) of the values representing phylogenetic distance of microbial communities between specimens. Conversely, there were no substantial differences among subgroups of SSc patients according to BMI and according with the any specific gastrointestinal tract symptoms reported in the questionnaires.
Conclusion: Our analysis demonstrated an altered and distinct composition of gut microbiota in SSc patients compared to healthy controls. This may be the result of the complex pathophysiology of the disease and, at the same time, may perpetuate immunologic aberrations and contribute to its clinical features.
Though in the overweight SSc patients seemed to exist an overlap with the distinctive microbiota of base disorder, no definitive data are available to explain the relation between the nutritional status and gut microbiota in SSc patients.

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ESTABLISHMENT AND CHARACTERIZATION OF AN IN VITRO MODEL OF PROSTATE NORMAL CELLS INFECTED WITH THE POLYOMAVIRUS BK

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Introduction: Prostate cancer (PCa) is one of the most common male tumor in the Western word, the most commonly diagnosed non-skin cancer and the second leading cause of cancer death. Various potential factors are considered potential onset triggering events, including exposure to infectious agents, such as the human Polyomavirus BK (BKV). BKV is a good candidate as risk factor of PCa because it naturally infects the human reno-urinary tract, is able to establish latency, and encodes oncoproteins that interfere with tumor suppressors pathways, thus altering the normal progression of cell cycle. The aim of the study is to establish an in vitro model of infection and to investigate the possible co-factorial role of BKV in PCa onset and progression.
Materials and Methods: To investigate the potential relationship between BKV infection and PCa, an in vitro model was established using the normal epithelial prostate cell line RWPE-1. The titration of the viral load was performed by means of BKV specific-quantitative real time PCR (qPCR) and droplet digital PCR (ddPCR). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was performed at different time points to evaluate the effect of BKV infection on the growth of RWPE-1 cells. The expression profiles of a panel of 48 cytokines/chemokines were analyzed to identify differences in their kinetics in infected and uninfected cells by multiplex assay. To assess whether BKV infection was able to modify the cells morphology, ultrastructu-

ral analysis and analysis of epithelial-mesenchymal transition (EMT) markers in BKV infected, uninfected and cleared cells were conducted.
Results: RWPE-1 cell line was found to be both susceptible and permissive to BKV infection, reaching a peak of infection after 3 days (3.9*10^6 copies/mL) and the infection lasted for 14 days. The infected cells showed an higher rate of proliferation than the uninfected cells ranging from +37% to +18%. The expression of IL-6, -9 -18 and TNF-α was higher in the infected cells than in the uninfected. Regarding the EMT markers, E-cadherin was expressed in some uninfected RWPE-1 at cell boundaries and upon BKV infection, E-cadherin expression was mainly located in the cytoplasm and in the perinuclear region. Moreover, after infection some big multinucleated cells could be detected. This pattern was maintained also after 30 days post infection.
Conclusions: The RWPE-1 cell line could be used as a model of BKV infection. The viral infection induces molecular and morphological changes in the cells, but the possible tumor progression due to the virus infection or needs to be still elucidated.

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HUMAN CYTOMEGALOVIRUS PP65 INHIBITS INTERFERON TYPE I PRODUCTION THROUGH ITS INTERACTION WITH THE CGAS/STING AXIS

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Introduction: The innate immune response against Human Cytomegalovirus (HCMV) plays a pivotal role during primary infection. Indeed, HCMV infection of primary fibroblasts rapidly triggers a strong induction of interferon-type I (IFN-type I), accompanied by proinflammatory cytokines release. This tightly regulated defense system serves to establish an antiviral state in infected and neighboring cells and protect them against virus replication.
Materials and Methods: To assess the interplay between IFI16 and pUL83 we employed human foreskin fibroblasts (HFF) infected with the wild type HCMV strain (v65Rev), and the HCMV v65Stop lacking pUL83 expression.
Results: Here, we show that HFFs produce IFN-type I when infected with HCMV strain TB40/E, v65Rev. Interestingly, significantly higher IFN-type I levels are observed when HFFs are infected with HCMV unable to express UL83-encoded pp65 (v65Stop), suggesting that the tegument pp65 protein might downregulate IFN-type I production. To clarify the mechanisms pp65 relies on to inhibit IFN-type I production, we analysed the activation of the cGMP-AMP synthase (cGAS)/STING axis in HFFs infected with the v65Rev or v65Stop.
Discussion and Conclusions: The results obtained revealed that pp65 binds to cGAS and prevents its interaction with STING thus interfering with the cGAS/STING axis. These data identify a previously unknown role for pp65 that leads to inhibition of IFN-type I production and evasion from its antiviral activity.

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CONTRIBUTION OF THE HUMAN ENDOGENOUS RETROVIRUS TYPE K IN THE EXPANSION AND MAINTENANCE OF PUTATIVE CANCER STEM CELLS IN HUMAN MELANOMA

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Introduction: The Human Endogenous Retrovirus type K (HERV-K) has been found aberrantly activated during melanoma progression and has been suggested to be also implicated in the etiopathogenesis of the disease. Melanoma is a heterogeneous tumor in which cellular plasticity and expression of the stem cell marker CD133+ has been associated with metastasis promotion and chemotherapy resistance. CD133 positive cells have also been suggested as the putative cancer stem cell (CSC) subpopulation of melanoma tumor. We already demonstrated that

HERV-K is associated to aggressiveness and immune evasion of metastatic melanoma under stress culture conditions. Thus, we investigated the potential role of HERV-K in cellular plasticity and stemness features of melanoma cells upon the modification of the microenvironment.
Materials and Methods: Different melanoma cell lines were cultivated in standard, differentiation and stem cell media. Flow cytometry, sphere-forming and migration/invasion assays were used to assess cell phenotypic modifications and stem and metastatic features. To analyze HERV-K involvement, RT-PCR analysis and RNA interference were used.
Results: The exposure of melanoma cells to a stem cell medium promoted pro-invasive phenotype-switching and expansion and maintenance of a CD133+ subpopulation, accompanied by HERV-K activation. Notably, the sorted CD133+ subpopulations, that experienced HERV-K activation, presented features of putative CSC, showing self-renewal, elevated migration and invasion ability. Interference experiments demonstrated HERV-K has a decisive role in the expansion and maintenance of the putative CD133+ melanoma CSC. Interestingly, antiretroviral drugs, such as the non-nucleoside reverse transcriptase inhibitors (NNRTIs) efavirenz and nevirapine, were effective to restrain the activation of HERV-K in melanoma cells and to inhibit the expansion of CD133+ subpopulation of melanoma cells.
Conclusions: HERV-K activation promotes melanoma cells phenotype-switching and is mandatory to expand and maintain the putative CD133+ melanoma CSC under microenvironment modifications. These results highlight the role of HERV-K on the etiopathogenesis of melanoma, suggesting new diagnostic biomarkers and therapeutic targets.

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PRIME-BOOST VACCINATION STRATEGIES BASED ON DIFFERENT ADJUVANTS

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Introduction: The design of prime-boost vaccine combinations employing adjuvants with different mode of action is of critical importance for the development of next generation vaccines. Here, we dissect the antigen-specific local and systemic T- and B-cell immune responses upon different prime-boost schedules based on the use of the Mycobacterium tuberculosis fusion protein H56 combined with CAF01, a liposomal adjuvant system, or a squalene-based oil-in-water (o/w Squalene) adjuvant.
Materials and Methods: Mice were primed with H56 alone or mixed with CAF01 and boosted with the antigen alone or combined with CAF01 or o/w Squalene. The T- and B-cell immune responses induced by different prime-boost regimens were analysed in draining iliac lymph nodes, spleen and serum 3 and 10 days after the booster immunization. The modulation of the immune response was characterized analysing the induction of H56-specific CD4+ T cells and their effector function, germinal center formation, plasma cell generation and serum antigen-specific antibody production.
Results: Priming with H56 and CAF01 and boosting with H56 and o/w Squalene induced the highest recall response of antigen-specific T helper cells and an increase of short-lived plasma cells that correlated with the highest serum H56-specific IgG response compared to the other vaccine formulations. Upon boosting, a strong cytokine response was observed in mice primed with H56 and CAF01 and not in mice primed with antigen alone. The adjuvant used for boosting shaped the T cell effector function with a clear shift to a Th1/Th17 response with CAF01 or a mixed Th1/ Th2 response with o/w Squalene.
Conclusions: These data demonstrate that priming and booster immunizations are strategic events in the induction of the vaccine immune response and the choice of the adjuvant shapes both the cellular and humoral response to the vaccine antigen playing a

fundamental role in the rationale design of prime-boost strategies.

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EVIDENCE OF HUMAN PAPILLOMAVIRUS AND POLYOMAVIRUS INFECTION IN TUMOURS FROM A SINGLE-CENTRE COHORT OF KIDNEY TRANSPLANT RECIPIENTS

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Defective immune surveillance in transplant recipients is specifically linked to an increased risk for virus-associated tumours, including non-melanoma skin cancer (NMSC) and cancers of the kidney and urinary tract; reactivation of β-papillomavirus (βHPV) and BK polyomavirus (BKPyV) has been reported in these cancers, respectively, as detected by immunohistochemistry in sections of pathological skin and urinary tract. In this single-center long-term retrospective study of 1028 kidney transplant patients (KTR) attending the University-Hospital in Novara, we have analysed the cumulative incidence of cancer, focusing on cancers associated or potentially associated with infection/reactivation of small DNA tumour viruses belonging to either the papillomavirus or polyomavirus family. In this cohort, the 10-year cumulative incidence of NMSC was 11.8% and that of urological malignancy was 1.6%. This study has allowed us to extend our previously reported investigation addressed at identifying the presence of active βHPV infection in skin tumours from KTRs through the detection of viral protein expression by immunohistochemistry, including the E4 and L1 proteins. In this new round of screening, we found positive staining in 3 tumours that were all removed from the same patient. This female patient in question was exceptional in that she had a very long history of immunosuppression (> 30 years), had developed many skin cancers (>15), and displayed a clinical picture that highly resembled that of epidermodysplasia verruciformis patients. BKPyV reactivation was detected by immunohistochemistry in the ureter of just 1 of the 10 kidneys analysed, but not in the tumour cells. Homogeneous large T antigen staining was also found in one bladder carcinoma out of three, although FISH signalling for the BKPyV genome was limited to some cells, indicating that this virus can enter its non-lytic life cycle accompanied by deregulated expression of the oncogenic large T antigen in transformed cells.

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M48U1 AND TENOFOVIR COMBINATION SYNERGISTICALLY INHIBITS HIV-1 INFECTION IN ACTIVATED PBMCS AND HUMAN CERVICOVAGINAL HISTOCULTURES

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Introduction: Microbicides are currently considered a promising strategy for preventing human immunodeficiency virus (HIV-1) transmission and disease. Recently, it has been proposed that combined treatment with two or more drugs targeting different phases of the HIV-1 replication cycle could increase the antiviral effects of microbicides during HIV-1 transmission. MiniCD4 M48U1 is a peptide that inhibits HIV-1 infection34 through the binding with

gp120, showing a higher affinity (100 fold titer binding) than soluble CD4, due to a higher rate of association and its slow dissociation rate. This drug may represent a good candidate as microbicide, because previous observations in simian model demonstrated that M48U1 formulated in hydroxyethylcellulose (HEC) and pluronic-based hydrogels inhibited HIV-1 infection.

Materials and Methods: IN this work, we first analysed the antiviral activity of miniCD4 M48U1 peptide formulated in hydroxyethylcellulose hydrogel (HEC) on activated peripheral blood mononuclear cells (PBMCs) infected by R5 and X4–tropic HIV-1 strains. In the next series of experiments, we tested M48U1 alone or in combination with tenofovir in HEC hydrogel with an organ-like structure mimicking the human cervicovaginal tissue.

Results: The results demonstrated that M48U1 prevented infection by several HIV-1 virus strains including laboratory strains, and HIV-1 different subtype strains isolated from patients in activated peripheral blood mononuclear cells. M48U1 has also inhibited two HIV-1 transmitted/founder infectious molecular clones (pREJO.c/2864 and pTHRO.c/2626). In addition, M48U1 was tested in association with tenofovir, and these two antiretroviral drugs synergistically inhibited HIV-1 without any induction of significant tissue toxicity.

Conclusions: Together, these results indicate that co-treatment with M48U1 plus tenofovir can exert an effective antiviral activity, and this association may represent a new topical microbicide to prevent HIV-1 transmission.

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ANTIBODY RESPONSES TO HELMINTH PARASITE ANTIGENS IN MALARIA ENDEMIC POPULATIONS

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Introduction: Co-infection with *Plasmodium falciparum* and helminths in Sub-Saharan Africa could modulate the immune response towards the parasites as well as susceptibility to disease (Salgame et al. Nat Rev Immunol 2013). Our aim is to investigate the impact of helminth infections on the different immunity to malaria shown by ethnic groups from Burkina Faso (Modiano et al. PNAS 1996). Specific objectives are: i) to measure immunoglobulins against helminth parasite antigens in plasma samples collected among Mossi, Fulani and Rimaibe populations; ii) to assess differences in prevalences and levels of specific IgG in relation to age, sex, village, ethnic group, and infection with *P. falciparum*.

Material and Methods: Plasma samples were collected during a cross-sectional survey conducted in August 2007 in rural villages of Burkina Faso. A subset of samples (N = 288) was assayed by ELISA to measure IgG against: i) *Strongyloides stercoralis* antigens (Bordier Affinity); ii) filarial nematodes antigens (Bordier Affinity) iii) *Schistosoma haematobium* Soluble Egg Antigen (SEA, Mutapi et al. Paras Immunol 1997).

Results: The prevalence of IgG against antigens of *S. stercoralis*, filarial nematodes and *S. hematobium* is 5%, 16%, 63% respectively. These measures lye within the infection prevalence ranges as obtained by direct diagnosis, suggesting ELISA may be suitable for population screening and evaluation of control programmes. The prevalence of anti-SEA IgG is zero in infants, increases during childhood to reach its peak in teens, and decreases from 20 years onwards. Females show a lower prevalence than males ($p = 0.003$). Differences in prevalence are not observed among villages or ethnic groups, but the Fulani show

lower levels of anti-SEA IgG (p = 0.0001) indicating that lighter S. haematobium infections may occur in the ethnic group known for a marked lower susceptibility to P. falciparum. Individuals infected with P. falciparum show higher levels of anti-SEA IgG (p = 0.0002) suggesting that common host factors may affect susceptibility to *P. falciparum* and *S. haematobium* (e.g. age, ethnicity).
Conclusions: Experimental models indicate that helminths Excretory/Secretory molecules induce Dendritic Cells to produce immunoregulatory cytokines (TGFβ, IL10) promoting the expansion of Tregulatory cells (Tregs) and the suppression of effector responses against intracellular pathogens such as malaria (Salgame et al. Nat Rev Immunol 2013). The malaria resistant Fulani population have been previously described to show lower levels of TGFβ and lower number of Tregs (Torcia et al. PNAS 2008). The observation of lower anti-SEA IgG levels in the Fulani warrants further investigation into the immunological cross-talk between *S. haematobium* and *P. falciparum* in this population.

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ANTI-INFLAMMATORY EFFECTS OF GSH-C4 IN LPS-INDUCED RAW 264.7 CELLS

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Introduction: Glutathione (GSH) is the main endogenous thiol antioxidant, which plays a fundamental role in cell protection from reactive oxygen/nitrogen species (ROS/RNS) [1].It is known in the literature that the levels of GSH are lower in sepsis [2]. Despite these evidences, it has been difficult to ascribe causal relationships between changes in GSH levels or redox state and development of inflammation. In fact, in the present study, the anti-inflammatory role of the cell-permeable N-butanoyl GSH derivative (GSH-C4) [3] was investigated in lipopolysaccharide (LPS)-induced responses in RAW 264.7 cells.
Materials and Methods: RAW 264.7 mouse macrophages were cultured in DMEM medium (Lonza, Basel) with 10% FBS (Lonza, Basel). LPS from E.Coli 055B5 was from Sigma Aldrich. Cells were plated in 6 well plates, one day later, was added N-acetyl cysteine (NAC) and GSH-C4 to a final concentration of 10 mM for 2 hours and removed by washing with PBS before performing LPS stimulation (100 ng/ml LSP for 24 hours). After 24 hours NAC and GSH-C4 was again added to the cells at the same concentration.
Results: LPS stimulation determines the nuclear translocation of the phosphorylated form of NF-kB (p65), as well as expression and production of pro-inflammatory cytokines such as interleukin-1β (IL-1β) and tumor necrosis factor α (TNF-α) in RAW 264.7 mouse macrophages. Moreover, LPS stimulation induces a decrease of intracellular GSH levels. NAC and GSH-C4 treatment added 2 hours before LPS stimulation did not affect TNF-α and IL-1β production. However, the release of these pro-inflammatory cytokines was completely inhibited when the cells were co-treated after and before LPS stimulation with GSH-C4 treatment. The same result was obtained for the nuclear translocation of p65-Nf-kB and GSH levels.
Conclusions: These results indicate that GSH-C4 exerts anti-inflammatory effects in LPS-stimulated mouse macrophages, functioning as inhibitor of nuclear translocation of p65-NF-kB activation. Consequently, the pro-inflammatory cytokines did not produce, suggesting that GSH-C4 may be useful in adjunctive therapies that complement conventional anti-inflammatory treatments.

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THE VIRAL DNA SENSOR IFI16: A BRIDGE BETWEEN PATHOGENS AND AUTOIMMUNITY

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Introduction: The PYHIN200 family encodes evolutionary-related human and murine proteins. Among these, the human IFI16 have been implicated in the recognition of pathogen DNA and classified into the ALR (AIM2-like receptor) group. Upon activation by pathogen DNA, IFI16 translocates into the cytoplasm, triggers type I IFN and inflammatory cytokines production, inflammasome activation, and eventually cell death. IFI16 is then released into the extracellular milieu where it behaves as an alarmin, contributing to the progression of inflammation and autoimmune diseases. Among these, inflammatory bowel disease (IBD) is characterized by a chronic inflammation of the gut, partly driven by defects in the expression and function of pattern recognition receptors (PRR), including the IFI16 protein. Since this protein is a target for autoantibodies, we studied its expression and specific seroresponse in IBD patients before and after infliximab (IFX) therapy.
Materials and Methods: IFI16 expression was evaluated in immunohistochemistry in IBD tissue samples. Moreover, anti-IFI16 antibodies (IgG and IgA subtypes) were measured in the sera of 48 Crohn's disease (CD) and 26 ulcerative colitis (UC) patients, prospectively harvested before and after IFX therapy. Anti-GP2 antibodies (both IgG and IgA subtypes) were also tested for comparison. The patient antibody statuses were qualitatively and quantitatively associated with disease phenotype and response to IFX therapy.
Results: An aberrant epithelial IFI16 expression in inflamed colon mucosa from both CD and UC patients by immunohistochemistry was demonstrated. Moreover, significantly higher titres of anti-IFI16 IgG were found in both CD and UC patients compared with healthy controls (HC). Anti-IFI16 IgA titres were also present in CD patients. Anti-GP2 IgG subtype titres were significantly increased in CD patients, as were IgA subtype titres. Significant changes in anti-IFI16 IgG subtype titres were observed after IFX in CD patients that correlated with clinical remission or response.
Conclusions: Our results highlight the importance of IFI16 in IBD pathogenesis showing that its de novo overexpression in the gut epithelial cells leads to a breakdown in immune tolerance and the subsequent development of specific autoantibodies. Considering that: i) gut epithelial cells do not express IFI16 in the normal setting; ii) IFI16 is a nuclear DNA sensor for pathogen exogenous DNA; and iii) IBD likely results from an aberrant immune response against bacteria or viruses that are able to invade cells of the gut mucosa and release or produce exogenous dsDNA, it is not surprising that PRRs, such as IFI16, are aberrantly upregulated in these patients and may trigger the production of specific autoantibodies.

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MYCOPLASMA HOMINIS MHOM_0730 IS A CALCIUM DEPENDENT, SURFACE-EXPOSED NUCLEASE EXPRESSED DURING NATURAL INFECTION

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Introduction: *M. hominis* is commonly found in the urogenital tract of humans and its presence is associated with a number of pathological conditions. A genomic survey lead us to detect a homolog of a *Staphylococcus aureus* Nuclease (SNase), a virulence factor involved in the evasion of innate immune defenses. SNase is able to degrade Neutrophil Extracellular Traps (NETs) via a specific nuclease activity, which in turn promotes the apoptotic death of macrophages at the site of infection. Aim of this work is the functional characterization of the *M. hominis* homolog of SNase, MHOM_730.

Materials and Methods: MHOM_730 was cloned and overexpressed in *E. coli*, and the recombinant protein used for nuclease activity assays over several nucleic acid substrates. rMHOM_730 was used to produce a rabbit polyclonal antibody. Biotinylation and subsequent affinity purification of *M. hominis* surface proteins was performed to assess the subcellular localisation of MHOM_730. Sera from patients which were previously tested for the presence of anti-*M. hominis* antibodies were used to evaluate the immunogenicity of MHOM_730 during human infection.

Results: Affinity purified rMHOM730 showed both exo- and endonuclease activity over DNA substrates. The production of a a specific rabbit polyclonal antibody allowed us to detect the presence of MHOM_730 in the surface subcellular fraction, showing the surface exposure of this putative nuclease. We also indirectly demonstrated that MHOM_730 is expressed during natural infection. A group of *M. hominis*-positive sera were shown to be reactive against rMHOM_730, as compared to negative controls.

Conclusions: Membrane nucleases play a key role for the survival of mycoplasmas into their hosts. Being unable to perform de novo sinthesys of nucleotides, mycoplasmas rely on salvage pathways to acquire nucleic acid precursors. In this work we show that *M. hominis* possesses a putative membrane nuclease, which is surface-exposed, expressed and immunogenic in vivo, and is able to cleave DNA *in vitro*. Similarly to other bacterial nucleases, MHOM_730 may play a role as a virulence factor involved in host cell death. Quite importantly, MHOM_730 might play a role in the evasion from innate immune defenses, similarly to SNase. Indeed, the nuclease activity of MHOM_730 could potentially interfere with the antimicrobial activities of NETs, allowing *M. hominis* to avoid clearance by neutrophils. In recent years we showed that *M. hominis* is able to establish a symbiosis with a sexually transmitted eukaryotic pathogen, *Trichomonas vaginalis*. While the relative advantages for one or both microorganisms resulting from this relationship are still under debate, we could hypotesize for MHOM_730 a role as a virulence co-factor in *M. hominis*-associated *T. vaginalis*.

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