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INVITED LECTURES

GENETIC VACCINES AGAINST EMERGING OR RE-EMERGING INFECTIOUS DISEASES

GENETIC VACCINES AGAINST EMERGING AND RE-EMERGING INFECTIOUS DISEASES

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It is becoming increasingly clear that vaccines are the main defense against aggressive infectious diseases. An ideal vaccine should be not only safe and efficacious but also easy to make, within a short timeframe, and it should be cheap enough for distribution in developing countries.
We have developed a platform technology based on viral vectors that has proved efficacious in relevant animal models and also in humans. It is safe in haelthy subjects of a wide range of age and geography and it can be manufactured rapidly and cheaply.

ALL ABOUT VACCINES

HPV VACCINATION: ACHIEVED GOALS AND OPEN ISSUES

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Immunization programmes against Human Papillomavirus (HPV) have been implemented since 2006 in several countries worldwide, pre-adolescent females representing the primary target. Both bivalent and quadrivalent vaccines protect against HPV 16 and 18, responsible for 70-80% of all cervical cancers (CC). The quadrivalent vaccine also protects against HPV 6 and 11, associated with more than 90% of anogenital warts. A 9-valent vaccine, protecting against HPV 6, 11, 16, 18, 31, 33, 45, 52, 58, was approved by the European Medical Agency in 2015.
Immunization programmes goal is CC prevention. However HPV 16 and 18 are responsible for 40% of vulvar cancers, 70% of vaginal cancers, 50% of penile cancers and 80-85% of anal and 20-60% of head and neck cancers in both genders.
Large clinical trials showed a vaccine efficacy against cervical intraepithelial neoplasia (CIN) 2+ of 93-100% in women that were negative for HPV vaccine types at the enrollment. A certain degree of cross-protection against HPV types 31, 33, 45 was reported.
The first effectiveness data are currently available. Population impact against earlier outcomes has been demonstrated. A recent metanalysis reported that HPV 16 and 18 anogenital warts decreased significantly between the pre-vaccination and post-vaccination periods in 13–19 year-old girls. Additionally, in countries with female vaccination coverage ≥ 50%, significant reductions in anogenital warts were reported also in boys < 20 year-old and in women 20–39 years of age, suggesting herd effects.
Ecological studies and studies conducted in Australia, Denmark, and Scotland through linkage of disease, screening and immunization data from population-based registries have recently demonstrated vaccine effectiveness against cervical abnormalities too, including CIN2+.
Prevalence studies are needed to monitor the potential occurrence of type replacement due to the reduction of selective pressure from vaccine types. Some studies recently revealed slight increases of certain HPV non vaccine types. However some authors hypotesized that a reduction in the rate of detection of vaccine types in genital specimens may lead to an apparent increase in some HPV types that were previously masked.
Vaccination of males is also a matter of debate. The advantages could be several: i) reduction of HPV circulation and consequent protection of unvaccinated people, ii) prevention of HPV related cancers in males, iii) protection of men who have sex with men (MSM) who do not benefit from heterosexual herd immunity. There are some concerns on cost-effectiveness. Some models, when considering only the health outcomes HPV vaccines are licensed for, showed that investing resources to improve coverage among females is more cost-effective than extending vaccination to males. Adding a targeted vaccination of MSM seems to be a cost-effective option too. According to more recent models the cost-effectiveness profile of male vaccination im-

proves if all HPV related lesions, a reduced vaccine price and the suboptimal coverage among females are included.

EPITOPE-BASED VACCINES: WHEN AND HOW

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Introduction: The development of effective vaccines is based on the identification of antigens capable of eliciting a protective antibody response. Vaccination with these immunogens induces the production of a great variety of epitope-specific antibodies, only a minority of which possesses the ability to protect against target infections. Many pathogens have adapted to repeatedly or chronically infect their hosts by varying the sequence of protective epitopes and/or by "hiding" them from the immune system. For example, pathogens sometimes adopt the strategy of incorporating, in the context of their virulence factors, immunodominant regions that function as "decoys" by preventing the immune system from targeting the neutralizing epitopes. These cryptic epitopes, however, are potentially capable of inducing protective antibodies once they are made more immunogenic. We propose here a method to map neutralizing epitopes recognized by polyclonal sera using libraries of antigen fragments, paying special attention to cryptic neutralizing epitopes.
Methods: Antigen-specific phage displayed libraries are prepared using DNAse-digested fragments of the gene of interest and expressing them as fusions to coat protein D in a lambda phage vector. Phage libraries are subjected to affinity selection using polyclonal sera from immunized individuals mixed with protein G-coated magnetic beads. Affinity-selected phage populations are subjected to next-generation sequencing using an Illumina system to identify immunoreactive antigenic fragments. Results are interpreted and displayed using an ad hoc developed software.
Results: Phage displayed libraries of the antigens contained in a commercially available vaccine were selected with sera from vaccinated volunteers. The profiles obtained allowed the rapid and reliable identification of immunodominant, as well as non-immunodominant epitopes, and showed characteristic age-related patterns. A comparison of these profiles with those obtained using monoclonal antibodies allowed the identification of one potentially protective cryptic epitope.
Conclusion: Affinity selection of phage-displayed libraries allows a rapid, detailed and reliable identification of the antigen regions preferentially targeted by polyclonal antibody responses. These epitope maps can be used to identify cryptic protective epitopes and to develop empiric interventions, such as the use of different adjuvants or antigen fragments, to make such epitopes more immunogenic.

ANIMAL MICROORGANISMS AND HUMAN DISEASES

FROM PRION DISEASES TO ANIMAL MODELS OF NEURODEGENERATION

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Prion diseases are fatal neurodegenerative diseases of humans and animals.
The most known prion diseases are Creutzfeldt–Jakob disease (CJD) in humans and bovine spongiform encephalopathy (BSE) in cattle. They are caused by prions, a group of peculiar proteinaceous agents devoid of pathogen-coding nucleic acid, which are experimentally transmissible to susceptible hosts and, in some cases, infectious under natural conditions. Prion diseases emerged as a serious concern following the BSE epidemics. More than 190.000 cattle have been found affected worldwide and 222 cases of variant CJD have been diagnosed in humans as a consequence of the consumption of BSE-contaminated meat. Thank to severe control measures, BSE has been almost eradicated in Europe. As a consequence, the interest for prion diseases has declined but they have actually left a formidable legacy to research in neurodegenerative diseases.
Growing evidences show that Alzheimer disease (AD), Parkinson disease (PD) and prion diseases share similar basic pathogenetic mechanisms. These diseases are all characterised by the misfolding and

aggregation of endogenous proteins in the central nervous system of affected subjects. A molecular mechanism referred to as "nucleation-dependent aggregation" is thought to underlie this phenomenon. According to this concept, disease-associated protein particles act as nuclei, or seeds, that recruit cellular proteins and incorporate them, in a misfolded form, into their growing aggregate structure.
Experimental studies have shown that the aggregation of the AD-associated proteins amyloid-β (Aβ) and tau, and of the PD-associated protein α-synuclein, can be accelerated, triggered or caused in laboratory animal models by intracerebral injection of aggregated species of the respective proteins. However, differently from prions that are able to transmit fatal diseases between affected and healthy subjects and represent therefore genuine infectious agents, none of the transmission studies with AD or PD provided evidence for cerebral neurodegeneration or development of fatal disease in challenged rodent models. Nevertheless, the prion-like properties of Aβ-, tau- or α-synuclein aggregates has raised concerns about a potential unrecognized risk of accidental transmission, e.g., during surgery or transfusion interventions.

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EMERGING AND RE-EMERGING INFECTION AGENTS: HUMAN LEISHMANIASIS IN THE EMILIA-ROMAGNA REGION (ITALY)

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Introduction: Leishmania are protozoa transmitted by phlebotomine sandflies and are agents of neglected tropical diseases. However, these parasites are also endemic in southern Europe. Mediterranean leishmaniasis caused by L.infantum is considered a zoonotic infection; human infection may vary from asymptomatic to clinically evident disease, which can remain localized to the skin (cutaneous leishmaniasis, CL) or extend throughout the reticulo-endothelial system (visceral leishmaniasis, VL). The first documented VL outbreak in Italy occurred within the 1970 to 1972 period, near Bologna, north-eastern Italy. In the last 20 years, Italy experienced a generalised increase of VL cases, including an outbreak in Campania region with 789 cumulative cases. Very recently, a re-emergence of VL has been reported in the Bologna Province. In this context, the Microbiology Unit at St. Orsola-Malpighi University Hospital of Bologna has implemented a diagnostic work-flow for identification and microbiological surveillance of human leishmaniasis.
Materials and Methods: An improved algorithm was implemented for the diagnosis of VL by (a) serological methods; (b) molecular methods, employing two real-time PCR assays in peripheral blood samples and bone marrow aspirates. CL cases were identified by histological and molecular tools on cutaneous biopsies.
Results: During the period January 2013-December 2014, we identified 21 cases of VL as well as 19 cases of CL in the Bologna Province, with an incidence of 2.1/100,000 in 2013 and 1.99/100,000 in 2014, respectively. We also observed one case of isolated lymphadenitis. All but three cases clustered in a localized area, which parallels Via Aemilia. Demographic and clinical characteristic of these cases as well as advances in diagnostic methods will be discussed.
Discussion and Conclusions: A human leishmaniasis focus has emerged over the last 2 years in the Bologna Province, with an over 5-fold increase of cases as compared with 2008-2012. Cases concentrated in a very localized area of the province, in line with reports indicating that continental northern Italy is now focally endemic for leishmaniasis. Our findings corroborate the need for an effective microbiological surveillance of human leishmaniasis in this area and for increased awareness on this parasitic infection among general practitioners and clinicians.

HIGHLY DANGEROUS BACTERIAL ZOONOSES

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Microbiology laboratories normally focus on the detection of the pathogens most frequently associated with human disease. This may lead to missed identification of rare or highly dangerous agents, whose identification requires skills and experience that are often lacking. Many of these agents have a zoonotic or environmental origin and in some cases have been listed as potential bioterrorism agents; diagnostic samples are often sent to specialized laboratories, sometimes with veterinary expertise as is the case in Italy for tularaemia and anthrax, or to the National Institute of Health (Istituto Superiore di Sanità). In this presentation, I will describe some of these infections (plague, melioidosis, anthrax, tularemia) and discuss the possibility of employing the diagnostic algorithms normally used in clinical microbiology, integrated if needed with more specific methods. Such diagnostic algorithms have been validated through quality control exercises by the European Network for Highly Pathogenic Bacteria (ENHPB), under the EU-funded Quandhip project (http://www.quandhip.info/Quandhip/EN/Home/Homepage_node.html), of which the L. Spallanzani Institute is a partner. The possibility of using Maldi-TOF analysis to identify these pathogens will be discussed.

DIAGNOSTIC MONITORING IN VETERINARY MEDICINE IN ZOONOTIC AGENTS

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Introduction: Zoonoses are infections and diseases that are naturally transmissible directly, or indirectly via contaminated foodstuffs, between animals and humans. Zoonoses monitoring programmes and antimicrobial resistance surveillance are regulated by DL 191/2006 (2003/99/CE). *Campylobacter spp., Salmonella spp.*, verocytotoxigenic *Escherichia coli* (VTEC), *Mycobacterium bovis*, Brucella control programmes and the diagnostic monitoring in Veterinary Medicine are reported
Materials and Methods: European Food Safety Authority (EFSA) and the European Centre for Disease Prevention and Control (ECDC) reported in 2014 data on the occurrence of zoonoses and foodborne outbreaks in 2012. Diagnostic and control are related to the official methodology used by Official Veterinary Services (OVS) and Istituti Zooprofilattici Sperimentali (IIZZSS).
Results: Successful *Salmonella spp.* control programmes in poultry, through serology, microbiology tests and PCR in breeders in meat and eggs, has been leading to a decreasing trend in confirmed salmonellosis cases in humans with a total of 91,034 cases reported in 2012. As well as for *Salmonella spp. C. jejuni* and *C. coli* are monitored in specific control programme and samples are analyzed with microbiological specific medium and Polymerase Chain Reaction (PCR). Nevertheless the occurrence of Campylobacter continued to be high in broiler meat and Campylobacteriosis is still the most commonly reported zoonosis, with 214,268 confirmed human cases. VTEC infections affected humans as well as domestic and wild ruminants with a total of 5,671 cases is monitired in animal samples and food by microbiology, PCR and other methods (e.g. microarray, sequencing) and positivity are investigated to identify VTX genes. The serogroups considered important regarding pathogenicity in humans and cattle are O26, O91, O103, O111, O145 and O157. Tuberculosis and Brucellosis are monitored at farms level for their eradication. The programmes are based on intra vitam diagnosis; isolation and identification of *M. bovis* by PCR and serological tests in blood and milk (Complement fixation, agglutination test, ELISA) for Brucella antibody detection. The number of human tuberculosis cases due to Mycobacterium bovis was 125, and 328 of brucellosis in humans. The prevalence of tuberculosis in cattle increased while brucellosis prevalence in ruminants is decreased.
Discussion and Conclusions: *Campylobacter spp.* and *Salmonella spp.* control programmes in poultry, Brucellosis and tuberculosis eradication programmes, VTEC monitoring in ruminants diagnostic and antimicrobial resistence monitoring by OVS are the focal point for the contaiment of the trasmission of the main zoonotic agents from animals to human in Italy.

MICROBIOTA IN COMPLEX SYSTEMS

BIFIDOBACTERIA AND THE HUMAN GUT MICROBIOTA: AN EXAMPLE OF SOCIAL BEHAVIOR

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Introduction: Bifidobacteria represent one of the dominant microbial groups that occur in the gut of various animals, being particularly prevalent during the suckling stage of life of humans and other mammals. Their ability to compete with other gut bacteria is largely attributed to their saccharolytic features.
Materials and Methods: Based on genomics and transcriptomics analyses we have dissected the molecular background of the saccharolyitic phenotype for each of 47 bifidobacterial taxa representing the genus *Bifidobacterium.*
Results: *In silico* analyses as well as transcriptomics analyses generated insightful information regarding carbohydrate resource sharing and crosss-feeding among bifidobacteria. The extensive occurrence of bifidobacterial saccharolytic features was confirmed by analysing metagenomic datasets, thereby supporting the notion that metabolic accessibility to dietary and/or host derived glycans is a common and potent evolutionary force that has shaped the genomes of bifidobacteria. Furthermore, we have explored the role-played by gut glycans (derived from the diet or synthesized by the host) in shaping the overall composition of bifidobacterial communities residing in the gut. We have investigated the role of glycans present in the gut in driving the trophic relationships (mutualistic, commensal and antagonistic) between members of bifidobacterial communities.
Discussion and Conclusions: The obtained data has generated novel insights regarding carbohydrate resource sharing among bifidobacteria in the human gut, revealing heretofore unknown cross-feeding phenotypes.

EATING DISORDERS: EFFECT OF STARVING OR OVERFEEDING OUR MICROBIOTA

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Introduction: The composition and activity of the gut microbiota co-develop with the host from birth and is subject to a complex interplay that depends on the host genome, nutrition, and life-style. Particularly, diet can have a marked impact on the gut environment, including gut transit time and pH, and changing the intakes of the three main macronutrients (carbohydrates, proteins and fats) can significantly affect the composition of the microbiota. On the other hand, the impact of gut microbiota composition on body weight gain or host adiposity has been supported.
Materials and Methods: We collected stool samples from obese subjects, without (O) or with type 2 diabetes (O-T2D), patients with anorexia nervosa (AN) and normal-weight (NW) subjects. Investigation of microbial communities has been performed by DGGE analysis and, for AN subjects, by Next Generation Sequencing using 16S rRNA (V3-V4) genomic region on Illumina MiSeq platform with a 250PE protocol. Real time PCR on specific bacterial species was performed on all samples.
Results: A total of 40 subjects were studied. The anthropometric characteristics were: for AN, BMI = 14 ± 2.01 (mean ± SD), age-matched controls, BMI = 21 ± 0.47; for O, BMI = 33.2 ± 2.3; for O-T2D, BMI = 38.3 ± 5.1, age-matched controls, BMI = 23.1 ± 1.1.
We found, despite the low number of analyzed subjects and inter-individual differences, a clusterization in distinct groups. All the AN patients but the one (AN2, BMI > 16) grouped together. Similarly, all NW subjects but the one (NW2, with a very low calories intake) clustered in the second group. Indeed, AN2 was more similar to the NW group, and NW2 more similar to the AN group.
All patients with T2D clustered apart from all other obese subjects.
No significant or minor differences were seen at phylum level. However, we found some species to be present only in AN group compared to NW age-matched controls, such as *Meganonas* spp. and *Prevotella copri*, or in a higher percentage, such as *Desulfovibrio* spp. and *Bacteroides caccae*.

Akkermansia muciniphyla and *Roseburia* were more present in the NW subjects, whereas *Bacteroidetes* group was more abundant in obese patients.
Discussion and Conclusions: We found common dysbiotic traits in very opposite, AN and O, populations. Particularly, *A. muciniphila* and R*oseburia*, both involved in maintaining a healthy mucosa, were found to be decreased compared to NW subjects. An alteration of the mucosa can promote inflammation that in turn can worsen several AN traits (weight regulation, anxiety, depression) or favor obesity comorbidities.

RESCUE OF FRUCTOSE-INDUCED METABOLIC SYNDROME BY ANTIBIOTICS OR FAECAL TRANSPLANTATION IN A RAT MODEL OF OBESITY

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Introduction: A fructose-rich diet is one of the factors inducing the metabolic syndrome, a combination of health disorders significantly increasing the risk of diabetes and cardiovascular disesase. The diet is also known to alter the microbial composition of the gut, although it is not clear whether such alteration is a consequence or one of the causes of the diet-induced health disorders. Aim of this work is to assess whether the gut microbiota is linked to the development of the diet-induced metabolic syndrome in a rat model of obesity.
Materials and Methods: Rats were fed either a standard or high-fructose diet. Groups of fructose-fed rats were treated with either antibiotics or faecal samples from control rats by oral gavage. Body composition, plasma metabolic parameters and tissue oxidative stress were measured in all the groups. A metagenomic approach was used to evaluate the bacterial composition of the gut of animals under different diets.
Results: The fructose-rich diet induced markers of metabolic syndrome such as inflamation, oxidative stress and insuline resistance. These markers resulted significantly reduced in rats treated with antibiotic or faecal samples. Also the gut microbial composition was altered by the fructose rich diet. In particular, the number of members of two genera and one family of bacteria increased with respect to rat under standard diet. This number resulted significantly reduced at control level when the animals were treated with antibiotic or faecal samples.
Discussion and Conclusions: Our data indicate that in rats fed with a fructose-rich diet the development of the metabolic syndrome is directly correlated with the alteration of the gut microbial population. Our data suggest that the manipulation of gut microbiota, here performed with antibiotic or fecal samples treatments, is able to reduce the effects induced by the fructose rich diet and that the manipulation of the gut microbiota can be considered as a potential therapeutic strategy in the treatment of the metabolic syndrome.

DIETARY CHOICES AND HUMAN MICROBIOME: POSSIBLE ROLE OF THE MEDITERRANEAN DIET

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Introduction: The typical diet in Western countries is that of an omnivore (O); however, vegan (V) and vegetarian (VG) dietary patterns are increasing their popularity. 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 cohort of 153 apparently healthy volunteers was assembled comprising

51 VG, 51 V, and 51 O. 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.
Results: The majority of V and VG, as well as 30% of O, had a high adherence to the Mediterranean diet (MD). Specific *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, *Ruminococcus* species belonging to *Lachnospiraceae* and *Streptococcus* were linked to O diet. Moreover, 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 and in individuals with low adherence to the MD.
Discussion and Conclusions: These results suggest that the consumption of vegetable-based diets promotes the presence in the gut of beneficial microbes and provides the right substrate for their activity, leading to the development of health-promoting metabolites. Western omnivore diets are not necessarily detrimental when a certain consumption of plant foodstuffs is included like in the MD, which is associated with beneficial microbiome-related metabolome and decreased level of TMAO, compound associated to the development of cardiovascular diseases.

THE ROOT MICROBIOME: A RESOURCE TO PROMOTE PLANT GROWTH IN ARID ECOSYSTEMS

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Introduction: In many regions of the world food production is primarily limited by the availability of freshwater. As a consequence of the effects of climate change, drought represents a major challenge for crops, negatively affecting growth and development and dramatically reducing product yields. Due to changes in the seasonal distribution of the precipitations, drought is affecting agriculture not only in regions with chronic soil water deficit, but also where a positive annual precipitation to evaporation/water consumption balance occurs.
Materials and Methods: Research is now moving towards the rationalization of the freshwater usage and the recycle of disposed waters. One frontier for the improvement of water usage by plant is the exploitation of soil and rhizosphere microbes as key factors for counteracting drought stress. The understanding of the ecology and the role of microorganisms associated to the rhizosphere and the root endosphere in retaining soil moisture and promoting plant growth under drought is a key for improving the water use efficiency.
Results: Here, the importance and potential of bacteria and bacterial diversity associated to the root system for alleviating drought stress is discussed in the light of the microbial ecology of the root system and the potential effects on the plant root physiology. The bacterial distribution in the plant root system is analyzed, as well as the bacterial colonization patterns of the rhizosphere and the endosphere and the mechanisms bacteria activate to promote the plant growth under drought.
Discussion and Conclusions: The potential use of root system bacterial conditioners is discussed under the strategy of "Desert Farming", the general crop management practice that is frequently used in arid regions. The concepts are reviewed under a perspective of integration with the current and future technologies for water management in agriculture.

HIGHLIGHTS IN PARASITOLOGY

TOXOPLASMOSIS IS FOREVER: BETTER TO PREVENT IT?

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Toxoplasmosis is a global health hazard as it affects 30-50% of the world human population, however in western industrialized Countries and also in Italy

seroprevalence is declining.
Clinically, the life-long presence of the parasite in tissues of a majority of infected individuals is usually considered asymptomatic. However, a number of studies show that this 'asymptomatic infection' may also be associated to the increased risk of occurrence of some human diseases.
Data on relations between atopy and toxoplasmosis are contradictory, depending on the different geographical regions.
On the contrary, it is quite clear from many experimental studies that *Toxoplasma gondii* (*Tg*) can change the behaviour of infected rodents which become less fearful of cats, losing aversion to cat urine. This effect gives an advantage to the parasite, since the transmission is facilitated to the definitive host.
The permanent loss of aversion to predator urine may not necessarily depend on persistent brain infection (and inflammation) caused by the presence of cysts in the brain as hypothesized by some Authors. In fact, the aversion is maintained even when *Tg* has been cleared by the host, suggesting that *Tg* causes a permanent change in the brain during acute infection, thereby not requiring persistence of the parasite cysts or continuing brain inflammation and an ongoing immune response.
Observational studies suggest that latent toxoplasmosis may also cause behavioral changes in humans. The observed differences between infected and non-infected people include: decreased novelty seeking behavior, slower reactions, increased risk of traffic accidents, lower rule-consciousness and greater jealousy (in men), greater warmth, conscientiousness and moralistic behavior (in women).
Evidence is accumulating for an association between psychiatric disorders such as schizophrenia and *Tg* infection; probably related to a dysregulation of the neurotransmitter pathway. *Tg* is also suspected to have a role in the pathogenesis of depressive disorders, obsessive–compulsive disorder, Alzheimer's diseases and Parkinson's disease, epilepsy, headache and or migraine and autism disorder as well.
Conclusion: Toxoplasmosis increases the predisposition to several diseases, for this reason, attempts to prevent infection in immunocompetent individual, and not only in the pregnant woman should be greatly encouraged.

ANIMALS AND *TOXOPLASMA*: IMPLICATION FOR PUBLIC HEALTH

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Still today, *Toxoplasma gondii* is one of the most common parasite in human being and other warm-blooded animals. Monitoring, prevention and control of this parasite is very complex and require a One Health approach. Public health organizations repeatedly encourage the collection of accurate data about *T. gondii* in humans, animals and food in order to plan adequate control strategies. Indeed, toxoplasmosis should be regarded a veterinary public health issue and veterinary practitioners can contribute in controlling the infection in humans.
Besides its vertical transmission, raw or undercooked meat from infected animals can be the major source of infection and other contaminated foods (e.g. milk, vegetables, water) can contribute to the horizontal transmission. Infection by *T. gondii* has important veterinary implications because it causes disease, miscarriage or congenital malformations in the definitive and intermediate hosts. Pigs, cats, sheep and goats are the domestic animal species most seriously affected by the protozoan. Noteworthy, prevalence in small ruminants is generally very high due to the continuous contamination of pastures by *T. gondii* oocysts. In the present talk we review epidemiology of *T. gondii* in animals in Italy and the role of cats in the trasmission of this parasite to humans. This knowledge should be useful to public health workers, veterinarians and physicians. Furthermore, a coordinated national-scale survey on toxoplasmosis in animals is strongly advocated, in order to better assess the actual epidemiological situation of this under-estimated and under-reported zoonosis and to clarify factors that influence its presence and distribution.

ECHINOCOCCUS GRANULOSUS: EPIDEMIOLOGY AND CONTROL

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Echinococcus granulosus is the smallest dog's tapeworm. The larval stages of this parasite causes cystic echinococcosis (CE), one of the most widespread parasitic zoonoses. The life cycle of *E. granulosus* includes canids as definitive hosts and a wide range of domestic and wild mammals and humans as intermediate hosts. Disability Adjusted Life Years resulting from human CE have been calculated as high as 1 million, similar to Dengue, Chagas Disease and Trypanosomiasis.
In the last years, we have studied the distribution and the epidemiology of CE in water buffaloes, cattle and sheep bred in the Campania region (southern Italy). During these studies we used Geographical Information Systems (GIS), in order to better understand the chain of transmission of this parasite.
In cattle, buffaloes and sheep farms the CE prevalence was 12.4%, 22.8% and 63.5%, respectively. No fertile hydatid cysts were found in any of the CE-positive cattle, whilst 20.6% and 25.0% of fertile cysts were found in CE-positive water buffaloes and sheep, respectively. Molecular studies showed the presence of *E.granulosus sensu stricto* (G1, G2 and G3 genotypes) in both large and small ruminants investigated.
Since 2013 we have started a regional project on control and reduction of echinococcosis/hydatidosis in animals and humans. The principal tools of this project are: 1) information, dissemination and health education for dogs' owners, farmers and school-age children; 2) diagnosis and treatment of dogs in sheep farms; 3) diagnosis of CE in humans.
Preliminary data on the prevalence of CE in dogs and human confirm the importance of control of CE in the Campania Region.

PLASMODIUM FALCIPARUM: EXPORT MECHANISMS UNDERLYING PROTEIN ERYTHROCYTE INVASION

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Introduction: Malaria remains a major burden in developing countries (WHO 2013). In infected people, an asymptomatic initial replication of *Plasmodium* parasites in liver cells is followed by continuous asexual multiplication within red blood cells (RBCs) that leads to the clinical symptoms of malaria. The highly differentiated RBC requires extensive modifications by the parasite to support its proliferation. For this remodeling, many parasite proteins are exported into the host cell, where they reside in the cytosol, in the RBC membrane, or in parasite-induced vesicular cisternae in the host cell.
Materials and Methods: 3D7 parasites were cultured in RPMI: GFP-expressing parasites were viewed directly as described (Grüring and Spielmann, 2012); PK protection assays; GBP/SERA was purified from infected RBC saponin supernatants using GFP-Trap-A beads and analyzed by mass spectrometry (MS); Constructs were under the control of the SP6 promoter.
Results: To gain access into erythrocytes, the parasite exports proteins containing a *Plasmodium* export element (PEXEL) into the host cell. Phosphatidylinositol-3-phosphate binding and cleavage of the PEXEL are thought to mediate protein export. We reported that these requirements can be bypassed, taking advantage of a second level of export control in the N terminus generated after PEXEL cleavage that is sufficient to distinguish exported from non-exported proteins. Furthermore, this region also corresponds to the export domain of a second group of exported proteins lacking PEXELs (PNEPs), indicating shared export properties among different exported parasite proteins. Concordantly, export of both PNEPs and PEXEL proteins depends on unfolding, revealing translocation as a common step in export. However, translocation of transmembrane proteins occurs at the parasite plasma membrane, one step before translocation of soluble proteins, indicating unexpectedly complex translocation events at the parasite periphery. Discussion we have shown that PNEPs, and thus TM proteins, need to be unfolded to reach the host cell, indicative of a translocation step at the parasite periphery.
In conclusion, PNEPs and PEXEL proteins appear to share a core export domain in an export path-

way that depends on the (mature) N terminal region. This raises the question of why the PEXEL is required at all. Our finding that a PNEP TM can substitute for it could indicate a general need for membrane association in the initial steps of export.

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CRITICAL ISSUES IN THE EPIDEMIOLOGY AND DIAGNOSIS OF TRICHINELLOSIS

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Introduction: The etiological agents of trichinellosis are nematode worms of the genus *Trichinella*, which can reach the human beings by the ingestion of raw or semi-aw meat and meat derived products from swine, horses, carnivore mammals (e.g. fox, bear), and carnivore reptiles. The global yearly incidence of trichinellosis has been estimated to be of 10,000 cases with a death rate of 0.2%. In the last decades, most of trichinellosis infections have been documented in Europe. The main source of infection is pork from backyard and free-ranging pigs, hunted wild boars, and horses. The aim of the present work was to summarize the main critical issues encountered in the course of epidemiological investigation and diagnosis.
Materials and Methods: Critical evaluation of epidemiological and diagnostic data collected in the course of dozens of trichinellosis outbreaks, which occurred in Italy, Europe and outside Europe in the last thirty years.
Results: In Italy, 1,509 trichinellosis cases have been documented in the course of 32 outbreaks from 1948 to 2015, with an average incidence of 0.04 cases/10^5 inhabitants. The source of infections has been horse meat (1038 cases, 68.8%), wild boar meat (280 cases, 18.5%), pork (180 cases, 11.9%), and fox meat (11 cases, 0.7%). The occurrence of single cases is extremely rare. Due to the long incubation period, the lack of pathognomonic signs and symptoms, and the sporadic occurrence of trichinellosis cases, it is difficult to identify the source of infection in a short period of time to avoid the consumption of the parasitized meat from other persons. On average, the first clinical signs and symptoms occur 10-15 days after the ingestion of the infected meat and additional days elapse before the patient contacts the family physician or the hospital. It is of great support for the physician the appearance of eosinophilia in almost all the patients. As a role, seroconversion can occur between 20 and 60 days post infection, sometimes when the patient is completely recovered. In Italy, the most important aetiological agent of autochthonous cases was *T. britovi*. Recently, *T. pseudospiralis* has been suspected. Most of infections caused by imported meat was due to *T. spiralis*.
Discussion and Conclusions: The joint work between the public health and veterinary services is of great importance to identify the source of infection and to educate consumers, farmers and hunters. Serological kits on the EU market should be used with cautions due to the low quality of the antigens and the high number of cross-reactions. An early diagnosis is important for the efficacy of the anthelminthic treatment.

SYSTEMATICS AND EPIDEMIOLOGY OF SOIL-TRANSMITTED HELMINTHIASES BY *ASCARIS* AND *TRICHURIS*

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Introduction: *Ascaris* and *Trichuris* are soil transmitted helminthes (STHs) requiring a period of development in the soil to reach infectivity (third larval stage) to the definitive hosts.
Among *Ascaris* species, *A. lumbricoides* and *A. suum* are considered to be two separate species, morphologically conservative, with little or no variation in morphological traits. *A. suum* is prevalent worldwide in both intensive and extensive pig production systems, while *A. lumbricoides* infects 1.2 billion people globally. Nematodes of the genus *Trichuris*, known as whipworms, are recognized to infect several mammalian species, including humans. The present work addresses two major issues in the taxonomic status of spe-

cies belonging to these two genera, having implications in their phylogeography, evolution and epidemiology, by elucidating the zoonotic potential of *Ascaris* infections and the taxonomic status of *Trichuris* species.
Materials and Methods: Genetic relationships, molecular epidemiology and phylogeography of *Ascaris* and *Trichuris* species from different hosts including humans were studied by analysing RFLP profiles and sequences of nuclear ribosomal spacers and mitochondrial genes.
Results and Discussion: The main results on the taxonomic distinctiveness of *A. lumbricoides* and *A. suum*, described two different scenarios in transmission patterns: (1) separated host-specific transmission cycles in highly endemic regions, (2) a single pool of infection shared by humans and pigs in non-endemic regions. No fixed differences between human and pig Ascaris were evident, with the exception of a Slovak population. The RFLP analysis confirmed pig as a source of human infection in non-endemic regions and as a corridor for the promulgation of hybrid genotypes.
As for whipworms, the phylogenetic trees showed that *Trichuris* sp. from captive non-human primates collected in the Rome Bioparco represented new, undescribed species, one related but distinct to human *T. trichiura*, another related to *T. suis* from pigs. The molecular epidemiology approach has provided evidences to solve questions regarding: i) the route of parasites introduction in isolated hosts; ii) the management measures to prevent eggs transport within the zoological garden; iii) the zoonosic risk.

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VIRUS-HOST INTERACTIONS IN THE PATHOGENESIS OF CHRONIC DAMAGE

HERPES SIMPLEX VIRUS AND ALZHEIMER'S DISEASE

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Introduction: A growing body of evidence suggests herpes simplex virus type 1 (HSV-1) as one of the potential risk factors for Alzheimer disease (AD), linking recurrent HSV-1 infections to the appearance of the biochemical hallmarks of this devastating disease. These include intra- and extra-neuronal accumulation of β-amyloid peptides (Aβs) and neurofibrillary tangles, mainly composed by hyperphosforilated tau. We previously demonstrated that HSV-1 productive infections cause marked changes in neuronal excitability and intracellular Ca^{2+} signaling modulating the phosphorylation of amyloid precursor protein (APP) and promoting its processing in various neurotoxic fragments, including Abs. Herein we designed *in vivo* studies to verify whether repeated reactivations of the virus, like those occurring in humans during life, lead to the accumulation of these toxic bricks in the brain and in turn to the appearance of an AD-like phenotype.
Materials and Methods: BALB/c mice were inoculated via snout abrasion with a sublethal doses of HSV-1. Viral reactivation was periodically induced by thermal stress. HSV-1 spreading to the brain and reactivations were analyzed in mice through PCR analysis of viral TK gene and RT-PCR analysis of ICP4 mRNA. AD-like phenotype was analyzed in mice through: a) immunofluorescence and western blotting analysis of APP processing and tau phosphorylation; b) Y-MAZE and novel object recognition (NOR) behavioral tests.
Results: HSV-1-infected mice, following virus re-

activations, showed: 1) viral TK and ICP4 genes (markers of viral infection and active replication, respectively) in cortex and hippocampal tissues, indicating that HSV-1 is able to reach and replicate in those brain regions mostly affected during AD; 2) accumulation of Aβs and other APP proteolytic fragments, together with altered tau phosphorylation and signs of neuroinflammation in hippocampus and cortex of aged animals; 3) significant cognitive impairments.
Discussion and Conclusion: Overall, these results strongly support the hypothesis that recurrent HSV-1 infections may contribute to the neurodegeneration typical of AD.

HERPESVIRUS 8 INFECTION AND CELLULAR METABOLISM ALTERATIONS

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Introduction: Kaposi's Sarcoma Herpesvirus, also known as Human Herpesvirus 8 (HHV8), demonstrates a typical tropism for peripheral lymphocytes and endothelial cells. After infection it remains in a latent state as an episome linked to cell-DNA by specific tethering proteins. Like most oncogenic herpesviruses, it induces some relevant modifications in the behavior of infected cells and causes the typical spindle shape and neo-angiogenesis. Moreover, during viral infection, the cells undergo a dramatic modification of their metabolism, showing an increase in glucose uptake and consumption, as well as an enhanced metabolism of fatty acids and triglycerides (TGs).
Experimental Design and Results: We found that HHV8-infection induces an enhancement of neutral lipid synthesis and their accumulation in lipid droplets in HUVEC cells. The TG increase in the lytic phase is likely related to infected cell metabolic requirements. Conversely, cholesteryl esters (CEs) seem to be closely related to the angiogenic properties of the infected cells, mainly in the latent phase, suggesting that these neutral lipids may be functionally involved in regulating the malignant process. Moreover, insulin receptor over-expression, insulin binding, and glucose uptake increase can be metabolically profitable to HHV8-infected cells for proliferation and pathological neo-angiogenesis, as found in clinical Kaposi's sarcoma. In agreement with this observation, a recent hypothesis has suggested that the pathological changes in cell metabolism could indicate an oncogenic driver rather than an adaptation to the tumor environment. All these observations and our epidemiological studies led us to consider the possibility that HHV8 could be involved in the metabolic syndrome and, probably, in type 2 diabetes (DMT2). In fact, HHV8 is the only herpesvirus with a significant prevalence in DMT2 patients, and more than 50% of DMT2 examined subjects were positive for HHV8-DNA and antibodies against viral proteins.
Conclusions: It is therefore reasonable to consider HHV8 as a possible Factor X as defined by Barbara Corkey (Banting Lecture Award, 2011), who stated that "e*levated background levels of insulin, superimposed on a susceptible genetic background, or basal hyper-insulinemia are the root cause of insulin resistance, obesity, and diabetes; in addition exposure to free fatty acid affects basal insulin secretion*". In fact, cell metabolism alterations due to HHV8 could represent the root cause that, through insulin-resistance, leads to DMT2.

ROLE OF NATURAL KILLER CELLS IN THE ANTIVIRAL RESPONSE OF MULTIPLE SCLEROSIS PATIENTS

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Introduction: Natural killer (NK) cells control viral infections killing cells in which virus down-modulates human leukocyte antigen (HLA) class I surface expression, while sparing cells with HLA-I via recognition by killer-cell inhibitory receptors (KIR). We have recently demonstrated that Multiple Sclerosis (MS) patients with KIR2DL2 expression are more susceptible to herpes simplex virus 1 (HSV-1) infection. We explored the molecular mechanisms that differentiate NK cell behavior to HSV-1 infection in association with KIR2DL2 expression.
Materials and Methods: We used an *in vitro* system with NK92 cell line and we confirmed the data in NK cells from controls and MS patients. We evaluated NK cell activation status by flow cytometry and cytokine expression by ELISA.
Results: KIR2DL2^{+} NK92 cells slightly activated during HSV-1 infection and had no ability to con-

trol the infection. The blockade of KIR2DL2 receptor and/or of its HLA-C1 ligand are able to re-establish the NK cell activation towards HSV-1 infected cells. We confirmed the effect of KIR2DL2 expression on NK cell activation towards HSV-1 in MS patients, where KIR2DL2$^+$ NK cells secreted high levels of Th17 cytokines during HSV-1 infection. On the contrary, KIR2DL2$^-$ NK cells from MS patients and control NK cells released Th1 cytokines, mainly IFN-gamma.
Conclusions: Our data suggest that KIR2DL2 makes NK cells less efficient in HSV-1 infection control. Moreover, our data showed, for the first time, a peculiar cytokine secretion in KIR2DL2$^+$ MS patients, with a Th17 prevalence, that could be implicated in MS pathogenesis.

HUMAN ENDOGENOUS RETROVIRUSES AND NEURODEGENERATION

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Introduction: About eight percent of human DNA is constituted by human endogenous retroviruses (HERVs). The HERVs are scarcely, but variably expressed, and both beneficial and detrimental effects were described. Since 1999, we study the MSRV and Syncytin-1 elements of the HERV-W family, in relationship to neurodegeneration in two human diseases: multiple sclerosis (MS) and neuroAIDS, in view of the neuro-immune-pathogenic properties of the MSRV*env* and Syncytin-1 envelope proteins.
Materials and Methods: *in vivo* studies of patients and controls, for molecular epidemiology and follow up studies of disease progression and therapy outcomes; *in vitro* studies of molecular mechanisms involved in pathogenesis, and of the effects of treatments, by MSRV*env*-specific and Syncytin-1-specific RT-PCR assays, Western Blotting and flow cytometry, on peripheral blood mononuclear cells (PBMC) from healthy volunteers and patients, and on astrocytes.
Results: A) Multiple Sclerosis: we found MS-specific HERV-W/MSRV expression in patients (blood, spinal fluid, brain); MRSV presence/load strikingly paralleled MS stages, active/remission phases, and therapy outcome. The DNA of MS patients has increased MSRV*env* copies, while Syncytin-1 copies are unchanged. Presence of MSRV in the spinal fluid predicted worst MS progression, ten years in advance. The Epstein Barr virus (EBV) activates HERV-W/MSRV both *in vitro* and *in vivo*. B) neuroAIDS: HIV and HIV-Tat protein activate HERV-W/MSRV in monocyte-macrophages and astrocytes indirectly, by interaction with TLR4 and induction of TNF-α, without internalization.
Discussion and Conclusions: A) MS: HERV-W/MSRV can be considered a biomarker for MS behavior and therapy outcome. As for MS pathogenesis, we found that the two main links between EBV and MS (late onset of infectious mononucleosis and high anti-EBNA1 IgG titers) are paralleled by activation of HERV-W/MSRV. Thus, we postulate the possibility for EBV of an initial trigger of future MS, years later, and for MSRV of a direct role of effector of neuropathogenicity during MS, and that HERV-W/MSRV activation is the missing link between EBV and MS, and may open new avenues of intervention. B) neuroAIDS: the HERV-W/MSRV/Syncytin-1 activation by HIV-Tat could contribute to HIV-related neurodegeneration, since Tat promotes neuroinvasion by HIV-infected monocytes/macrophages, carrying also the activated, neuropathogenic HERV-Ws. Within CNS, Tat-induced TNFα could induce high levels of the HERV-Ws, in both macrophages and astrocytes, also without HIV replication.

HERVS IN NEURODEVELOPMENTAL DISORDERS

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Introduction
Always more evidences suggest that neurodevelopmental disorders, like Autism spectrum disorders (ASD) and Attention deficit hyperactivity Disorder (ADHD) may result by a complex interaction of environmental, biological and genetic factors. It's established that stressful experiences during pregnancy have long-term consequences on the neurologic

development of new-born and that different types of insults (chemicals, infections, stress) during early life may increase the risk of neurological and psychiatric disorders, through epigenetic mechanism. Human Endogenous Retroviruses (HERVs) could represent the interface between genetic predisposition to neurodevelopmental disorders and environment, as element able on one side to respond to environmental changes and on other side to transfer the information to the individual.
Materials and Methods
ASD and ADHD patients and their parents were enrolled to analyse the transcriptional activity of three HERV families (HERV-H, HERV-K, HERV-W) in peripheral blood mononuclear cells (PBMCs), stimulated or not in culture and in presence of non-nucleoside reverse transcriptase inhibitors (NNRTIs). A questionnaire was administered to parents to identify familiar and related to maternal-fetus system risk factors.
Results
A different expression profile of HERVs was observed among young patients and control individuals, matched for age and sex. Interestingly, families' study highlighted a similar expression profile of HERVs among mothers and patients, significantly different from fathers. Moreover, NNRTIs affected the HERVs transcriptional activity of PBMCs *in vitro*. By the analysis of questionnaires, several risk factors were identified in agreement with those reported in the literature.
Discussion and Conclusions
Our findings support the hypothesis that dysregulation of HERVs expression in response to different stimuli, could represent a new parameter of susceptibility to ASD and ADHD. HERVs may be thought of as components of the genome that interact with environmental factors, capable to interplay with different molecular pathways in determining neurodevelopmental disorders. Our recent findings on a mouse model of ASD will be also discussed.

IN TIMES OF RESISTANCES… NEW ANTIMICROBIAL STRATEGIES

COMBINED STRATEGIES TO COUNTERACT THE VIRULENCE AND ANTIFUNGAL RESISTANCE OF PATHOGENIC FUNGI

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As relatively new human pathogens, many fungal species are well-recognized causes of life-threatening infections so as to represent major medical problems in the real-life setting. Medical practices such as usage of broad-spectrum antibiotics that affect the host microbiome, and of immunosuppressive therapy in the form of corticosteroids and effective chemotherapy for some cancers allow for the treatment of certain diseases but, in the same time, increase the host susceptibility to fungal infections. Other advances, such as complex surgeries, implantable devices and organ transplantation, are contributing to impair host defenses and increase susceptibility to fungi. As a result, invasive fungal infections (IFIs) are associated with substantial morbidity and mortality, and are increasingly caused by fungal species or subspecies with diminished susceptibility or resistance to many standard antifungal agents. Despite the availability of newer antifungal drugs, outcomes for patients with IFIs continue to be poor, thereby stimulating outstanding research in the field of relationships between fungal pathogenesis and antifungal resistance. First, the study of *Candida albicans* molecular chaperone Hsp90 in pathogenesis and drug resistance has led to the notion that this protein and its related molecular network are a hub of circuits associated with responses to stress, morphogenesis, and virulence. Thus, these circuits provide a vulnerable point in the fungal metabolism that could be exploited in the development of new therapies. Secondly, biofilm formation is essential to the pathogenesis of *C. albicans* during device infections, and biofilms provide shelters for the yeast cells against host defense and antifungal agents. Finally, the dynamic

nature of the fungal cell wall in the infection process influences its interplays with host receptors that often determine the outcome of the host-fungus interaction. In this talk, it will present and discuss current research data concerning the discovery of novel targeted strategies against pathogenic fungi, including not only *C. albicans* but also other important *Candida* pathogens such as C. glabrata and C. parapsilosis species complex.

MEASLES FUSION MACHINERY IS DYSREGULATED IN NEUROPATHOGENIC VARIANTS

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Introduction: Measles virus (MV) is a leading cause of child mortality in developing countries despite the availability of a live attenuated vaccine for over 40 years. Severely immune-compromised people are particularly at risk for MV. MV causes periodic outbreaks all over the world. While natural infection with MV elicits long-lasting immunity, protection wanes in vaccinated persons. The most serious manifestations of MV infection, including encephalitis, occur in people with impaired cellular immunity. MV affects the central nervous system (CNS) in up to half of routine cases; with adequate cellular immunity the infection is eradicated, but individuals with impaired cellular immunity are at a disadvantage. Even the vaccine strain can lead to fatal encephalitis in such persons.
Results: In a recent MV outbreak in South Africa several people died of MV CNS infection. We analyzed the viruses from these patients and found that specific intra-host evolution of the MV fusion machinery -- receptor binding protein (H) + fusion protein (F) -- had occurred. A mutation in F of the "CNS-adapted" viruses allows it to promote fusion with less dependence on interaction of H with the two known MV cellular receptors; this F is activated independently of H or receptor. We hypothesize that in the absence of effective cellular immunity, MV variants bearing fusion machinery that enabled efficient spread in the CNS underwent positive selection. We propose that prevention of MV infection immune-compromised individuals must halt infection at an early stage to avoid fatal consequences, and we evaluated fusion inhibitor peptides against MV infection *in vivo* in immune compromised animal models.
Discussion and Conclusions: We found that strains isolated from patients with measles virus infection of the CNS have fusion properties different from those of strains previously isolated from patients without CNS involvement. Specifically, the viral entry machinery is more active and the virus can spread, even in the absence of H. Our findings are consistent with an intrahost evolution of the fusion machinery that leads to neuropathogenic MV variants. We also found that our fusion inhibitors are effective at preventing fatal encephalitis in animal models, and therefore could protect the immune compromised individuals from MV infection and its CNS manifestations.

THE UNIVERSE OF ANTIMICROBIAL AND ANTIVIRAL PEPTIDES FROM THE INSIDE OF THE ANTIBODIES

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The threat of infections has became impellent due to the extensive misuse of therapeutic agents and the large increase of microbial and viral resistance. This trend will become dramatic in the future so that academia and pharmaceutical industry should become more involved in the search of conceptually new drugs. The mechanism of action of conventional compounds produced by soil bacteria, fungi and sponges are based on traditional targeting schemes which can be easily exceeded via mutation or genetic recombination by the infectious agents. More promising anti-infective drugs may refer to molecules

with retained activity in spite of their co-evolution with infectious agents for million of years such as host cationic antimicrobial peptides.
Antibodies (Abs), like other organic macromolecules characterized by specific physiological functions, may be the result of the assembly of evolutionary products of genes that, ancestrally, were codifying for peptides with innate biological activity. Peptide fragments either of the complementarity determining or constant region of Abs have proven to exert *in vitro*, ex vivo and/or *in vivo* a differential antimicrobial and antiviral activity (inclusive of multidrug resistant strains) as well as an immunomodulatory and antitumor effect, mediated by different mechanisms of action, regardless of the specificity and isotype of the belonging Ab, without showing toxic or genotoxic effects on mammalian cells. Targeted amino acid substitutions, adopted as surrogates of point mutations, were shown to implement the magnitude of the biological activity of the native Ab peptide.
Antimicrobial and antiviral activities were displayed *in vitro*, ex vivo and/or *in vivo* by a human serum phosphorylated peptide derived from the C region of IgMs as well as by the synthetic products of J lambda, J kappa, D and J heavy immunoglobulin genes.
These data support the hypothesis that Abs, as putative evolutionary result of the adaptive combination of gene products that ancestrally were devoted to functions of innate immunity, may exert, beyond their half-life, an anti-infective activity through their physiological fragments.
The universe of bioactive internal peptides, foreshadow Abs as an unlimited source of sequences potentially active against pathogenic agents. The easy and cheap production of small sized synthetic peptides and the possibility of their design and chemical optimization, associated to new delivery mechanisms, are expected to give rise to a new generation of anti-infective drugs.

GLOBAL EMERGENCY OF MDR MICROORGANISMS: DRUG-DISCOVERY AND NEW ANTIBIOTICS

ROUTES TO THE DISCOVERY OF NOVEL ANTIBIOTICS

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There is an increased need for novel and more effective antibiotics to combat microbial pathogens which are becoming increasingly resistant to available treatments. The pipeline of compounds under development, however, is scarce and mainly consists of improved derivatives of marketed compounds, which are at best only partially effective against prevailing resistance mechanisms. Therefore antibiotics endowed with new mechanisms of action must be developed in order to effectively fight multi-drug resistant pathogens.
Natural products represent a major source of approved drugs and, despite a decreased interest by large pharmaceutical companies, still play an important role in supplying chemical diversity. As tens of thousands of bioactive natural products are known, novel approaches must be implemented to decrease the chances of re-discovering them. The talk will give an overview of natural product screening, focusing particularly on microbial-derived antibiotics, and of the different approaches that can be implemented to increase the probability of finding new bioactive molecules. Finally, attention will be focused on the technology platform currently used at Naicons, providing some examples of novel antibacterial molecules we have characterized.

NEW ANTIBIOTICS AGAINST GRAM-POSITIVE BACTERIA

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Drug-resistant bacteria present a serious and worsening threat to human health. To ensure that the

supply of new antibiotics keeps pace with these evolving pathogens, it is necessary to have a robust pipeline of new drugs and innovative pathways to get this medicine to the patients who need it most. Developing new drugs involves a great deal of time, effort, scientific research, and expense. At best, only 1 out of 5 drugs that reach the initial phase of testing in humans will receive approval from FDA and EMA.

Development of antibiotics to treat highly resistant bacterial infections is especially challenging, because only a small number of patients contract such infections and meet the requirements to participate in traditional clinical trials. he current assessment of the pipeline shows 36 new antibiotics in development. These drugs would potentially address many, but not all, resistant bacteria. As of March 2015, of the 36 antibiotics in development, eight were in phase 1 clinical trials, 20 in phase 2, and eight in phase 3. Historically, about 60 percent of drugs that enter phase 3 will be approved.

At least 25 of the antibiotics in development have the potential to treat infections caused by Gram-positive pathogens.

Although a few new antibiotics will be availbale in the next 5 years, these new agents are not based on new mechanisms of action and are not necessarily active against MDR pathogens for which there is the highest medical need. Three new drugs against Gram positive pathogens (dalbavancin tedizolid and oritavancin) received EMA authorization and will be market soon. Among six antibiotics currenty in phase III, two of them will also be useful to treat Gram positive infections. At least two antibiotics in early development attack bacteria in an entirely new way by sidestepping the resistance of some bacteria to available antibiotics. Other drugs in the pipeline attack the same targets in bacteria as available drugs but seek to thwart resistance by using new chemical compounds. Since 2010, awareness around MDR issue has dramatically increased and actions have started to be called for implementation. As MDR is a global issue, coordinated action will noe become key in order to optimize resources to develop new antibiotics, as well as to allow their appropriate distribution and use.

DRUG DISCOVERY IN *MYCOBACTERIUM TUBERCULOSIS*

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Introduction: As it is well known, tuberculosis (TB) is an old disease and a re-emergent killer. So, like in the past, there is an urgent need for new antitubercular drugs and novel targets in order to fight the spread of MDR (Multi-Drug-Resistant), XDR (Extensively-Drug-Resistant) and perhaps TDR (Totally-Drug-Resistant) strains.

There are well-defined criteria for developing new TB drug candidates such as:

- A safety profile
- Be more potent than the existing ones in order to reduce the duration of therapy
- Be effective against MDR- and XDR-TB strains- -
- Be compatible with anti-retroviral therapy
- No antagonism to other TB drugs
- Be effective against *Mycobacterium tuberculosis* in its different physiological states including latent TB.

Materials and Methods: In this perspectives several strategies can be pursued:

1. *From target to drug*: With this approach, an enzyme, essential for the pathogen's life, is used in high throughput screening to find inhibitors present in chemical or natural libraries of compounds. Medicinal chemistry is then utilized to progress towards leads and finally to candidate drug (CD).
2. *From drug to targ*et*:* This involves screening of synthetic or natural libraries for whole cell activity against *M. tuberculosis.*
3. Searching for natural products
4. Repurposed drugs and targets

Results: The past 5 years has seen the emergence of a promising TB drug pipeline and most of them were discovered through a phenotypic screening of compound libraries against *in vitro M. tuberculosis* growth. A few candidates are in Phase II and Phase III clinical trials, respectively. It is noteworthy that many of the candidates presently in clinical trials are drugs that were developed for other infectious diseases (like fluoroquinolones, rifamycins, oxazolidinones) and were repurposed.

Evolved from the FP6 project, New Medicines for TB (NM4TB) - which successfully released a can-

didate drug for clinical development - the MM4TB team applied integrated approaches to successfully discover new TB compounds.

Discussion and Conclusion: A strong TB drug discovery portfolio is fundamental to eradicate tuberculosis. However, there are only 10 drugs for TB treatment in clinical development which is inadequate to address the problem of multi-drug resistance. Therefore it is widely supported that there needs to be more early stage drug discovery to feed the clinical pipeline to fight this disease.

HIGHLIGHTS IN VIROLOGY

HIV INFECTION: ERADICATION STRATEGIES TO DEPLETE THE HIV RESERVOIR

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Three decades of research and development have produced more than 30 different antiretroviral drugs that when combined in Highly Active Antiretroviral Therapy (HAART) can drive the viral load down to undetectable levels. But even with chronic HAART treatment, an integrated copy of proviral HIV DNA remains in latent cells, which can re-establish viral production and cause a rebound, producing plasma viremia. The persistent latent HIV reservoir remains the major obstacle in HIV-1 eradication. HIV latency is multifactorial and thus the eradication of HIV may require multiple approaches.

In order to eradicate HIV-1 infection, the cells with integrated HIV-1 proviral DNA must be removed or the integrated proviral DNA damaged. The most prominent current strategy to address HIV latency is, while under HAART therapy, to reactivate latently infected cells so that they can be targeted by the immune system. A major problem with this approach is that specific reactivation of only latent cells has not been achieved and nonspecific reactivation of T-cells can lead to a cytokine storm. Pharmacological reactivation of virus it has been proposed as a cure strategy. Once integrated the HIV genome is subject to the control of the chromatin environment and a variety of different host transcription factors. Histone deacetylases (HDACs) maintain HIV in a transcriptionally silent state. To date, HDAC inhibition by small pharmacologically molecules to induce transcription at the HIV LTR is the most well characterized strategy to purge latent HIV.

Recent approaches in modifying stem cells and enabling them to give rise to potent/resistant T-cells against HIV holds immense hope for eradication of the virus from the host. Specifically, current cell therapy approaches are based on the following: (1) replacement of HIV replication-competent cells with cells naturally resistant or edited to become resistant to infection; (2) transplantation of autologous -lymphocytes spontaneously targeting or redirected against HIV; and (3) autologous cells engineered to secrete anti-HIV proteins.

Because of the efficacy of HAART, future studies must determine whether genetically engineered cell products can restore normal immune function, and can decrease the viral reservoir to such an extent that the mission of curing HIV infection can be accomplished.

NOVEL INSIGHTS INTO THE PATHOGENETIC MECHANISMS OF PARVOVIRUS B19

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Introduction: Parvovirus B19 (B19V) is a human ssDNA virus responsible for an ample range of clinical manifestations. The diversity in pathologies associated with B19V infection suggests an underlying diversity in pathogenetic mechanisms, closely linked to the interaction of virus, cell environment and the response of immune system. The development of model systems to investigate these interactions is now offering novel opportunities to understand virus-cell interactions and to develop effective antiviral strategies.

Materials and Methods: Investigation of B19V lifecycle and pathogenetic mechanisms, other than in the widely used UT7/EpoS1 megakaryoblastoid cell line, have recently been carried out in ex vi-

vo-expanded CD36+ erythroid progenitor cells (EPCs), that demonstrated to be a highly permissive system for B19V replication and expression, and in endothelial cell lines. In the clinical setting, accurate quantitative molecular diagnostic assays and immunoassays are essential tools for a correct definition of the course of infection.

Results: The selective tropism of B19V toward EPCs is the consequence of specific attachment and penetration pathways, genome replication mechanisms and expression profile dynamics. It emerges a close dependence of viral replication and generation of infectious virus on the differentiation stage within the erythroid lineage, and on the cell physiology status, such as Epo stimulation and hypoxic conditions. In turn, B19V infection impacts on cell physiology, mainly by deregulation of cell cycle and induction of apoptosis, that is a major determinant of transient erythroid aplasia. ADE phenomena are relevant in the interaction of B19V with endothelial or monocytic cells, contributing to the necrotic or inflammatory aspects of B19V infection. Finally, the observed persistence of virus in tissues is possibly linked to silencing epigenetic mechanisms. The resulting diversity of pathologies and clinical manifestations imply a critical assessment of laboratory markers of infection.

Discussion and Conclusion: The advances in our knowledge and understanding of B19V-cell interactions, pathogenetic mechanisms and derived clinical manifestations bear implications for future developments. First, these should lead to an increased awareness of the clinical relevance of the virus and consequent implementation of diagnostics algorithms. Then, they offer a framework to develop and test compounds for an efficient antiviral activity, in view of an etiological therapeutic approach replacing the actual supportive or symptomatic treatments.

GENUS BETA PAPILLOMAVIRUS AND POLYOMAVIRUS: UBIQUITOUS VIRUS WITH ONCOGENIC POTENTIAL IN THE IMMUNOCOMPROMISED HOST

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The induction and maintenance of impaired immunological surveillance in transplantation medicine is paralleled by significant increases in the incidence of specific cancers. Most of these cancers are caused by reactivated viruses whose oncogenic potential is suppressed by immunological reactions in healthy individuals. The most prominent tumours arising are EBV-associated B-cell lymphomas, Kaposi's sarcoma, caused by the reactivation of human HHV8, and Merkel cell carcinomas (MCC) of the skin, associated with a novel human polyomavirus (HPyV), namely MCPyV. Of the cancers presenting in this setting that have no established infectious aetiology, skin cancer is the most frequent form of malignant cancer, 95% of which are non-melanoma skin cancer (NMSC). Numerous studies have pointed to a possible causal role of small DNA tumour viruses in the pathogenesis of skin cancer, including b-papillomavirus (b-HPV) and HPyV. Urologic malignancies are reported to be the second or third most common malignancies in transplant recipients.

HPVs and PyVs share a common morphology and structural organization. Over 180 different types of HPV have been identified to date and classified into several phylogenetic groups. Of these, mucosal HPVs belonging to the a-genus and associated with infections of mucosal epithelia can be grouped into 'low-risk' and 'high-risk' types, depending on the relative propensity of the resulting neoplasms to undergo malignant progression. Cutaneous b-HPVs are evolutionarily distinct from the a-genus and appear to cause widespread unapparent or asymptomatic infections in the general population. However, in immunosuppressed patients, and in individuals suffering from the rare inherited disease Epidermodysplasia Verruciformis (EV), these viruses can spread unchecked and have been implicated in the development of skin cancer.

A similar scenario can be envisaged for HPyVs whose causal association with human tumours is also difficult to be established because they may also be constituents of the human skin microbiome and present as DNA in many normal human tissues during the lifelong latent infection which follows HPyVs primary infection. Humans can be infected with as many as 13 different HPyV although MCPyV is the only one causally linked to cancer.
Our group has studied HPV and HPyV infection/reactivation in skin and urinary tract tumours from a cohort of kidney transplant recipients and primary immunodeficient patients. Viral proteins and DNA have been visualized in infected tissues by combined costaining procedures. The results of several recent studies will be presented.

FOCUS ON HUMAN VIROME: THE ROLE OF TTV

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Introduction: With the ever-extending use of next-generation sequencing on a variety of clinical samples, rapid progress on the composition of the human virome (the viral component of the microbiome) and its relationship with immune system are to be expected in the coming years. To date, composition and inter/intra-individual variability of the human virome are not precisely known, however viruses able to establish long-term persistence within their host represent a subpopulation of the virome that is particularly fascinating. Torquetenovirus (TTV) is the prototype of these viruses and recent studies have revealed that it represents the prevalent component of the human virome.
Materials and Methods: TTV kinetics were studied in patients receiving various organ transplantations (bone marrow, liver, kidney, and pancreas) and different regimens of maintenance immunosuppression. Temporal variability of TTV load was measured by a single step real-time TaqMan PCR assay based on an highly conserved segment of the viral untranslated region. The assay has the potential for sensitive and specific detection of all the isolates of TTV present in GenBank at the time of writing, and has a lower limit of sensitivity of 100 viral genomes per ml of plasma.
Results: During the two year of post-transplant follow-up, TTV viremia markedly fluctuated similarly in the different types of transplantation (significantly increased at month 1 post-transplantation, showed the maximum average increase at month 3 and then slowly declined up to month 24 when viral levels returned similar to those in the pre-transplant blood), but highly dependent on the immunosuppressant administered (significantly higher since month 6 in patients administered a cyclosporine-based maintenance regimen versus those receiving a tacrolimus-based regimen).
Discussion and Conclusions: In conclusion, our evidences suggest that the measure of TTV loads in the blood of infected individual could be a solid, handy marker of immune system competence, with potential to tailor-made immune suppression.

LATEST UPDATE IN DIAGNOSTICS

INDUSTRY FOR INNOVATION: THE NEW TECHNOLOGIES TO SUPPORT THE MICROBIOLOGIST AND THE CLINICIANS

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Past President Assodiagnostici

Within Laboratory Medicine Industry Sector, high potential technologies and devices were developed during last years, in terms of automation and new high efficiency in Diagnostic appliances, like genomics, proteomics, pharma genomics and bio molecular. Over 70% of clinical decisions are supported by laboratory diagnostic tests.
Although Budget National Health Service (NHS) expenditure in 2014 increased to 109,9 Bill euro (+2,0% vs. 2013), mainly due to the decision not to introduce a copayment amount to citizen for near

about 2 Bill euro, the various measures that the government has introduced to respond to the situation in the NHS affected medical devices sector and IVD. During 2014 it was also introduced a ceiling on medical devices expenditure (4,4%) based on the national funding of the NHS. This is influencing the acquisition also of IVDs and revenues of Companies in the Sector. This approach may introduce for this Sector, in term of Research and Development investments in the next years and Innovation of new technologies.

MONITORING OF CYTOMEGALOVIRUS-SPECIFIC CD8+ T-CELL RESPONSES USING THE QUANTIFERON®-CMV ASSAY IN HEART TRANSPLANT RECIPIENTS

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Introduction: Reconstitution of cytomegalovirus (CMV)-specific cell-mediated immunity (CMI) after transplant has been associated with control of viral replication. This study evaluates the clinical utility of QuantiFERON®-CMV (QF) (Cellestis-Qiagen) to measure CMI in heart transplant (HT) recipients.
Materials and Methods: Forty-four CMV seropositive HT recipients were enrolled: 19 received valganciclovir prophylaxisis (mean 57 days, range 7-94) and 25 were followed with pre-emptive approach. CMV-DNAemia monitoring was performed by real-time PCR (ELITechGroup). Immunological monitoring was performed during the first biopsy as well as 1, 3, 6 and 12 months post-transplant.
Results: *Prophylaxisis group*. Six out of 19 (31.6%) patients developed an active CMV infection (median 80 days, range 53-199). Following the termination of prophylaxisis treatment, QF-CMV indeterminate results were equal to 83.3% in infected and 20% in not actively CMV-infected patients. Furthermore QF-CMV positive results were equal to 20% in not actively infected patients. No positive results were obtained in the infected patients. Following onset of active CMV infection, only 2/6 (33.3%) infected patients reconstituted CMV-CMI. These two patients showed mean CMV-DNAemia peak lower than patients with QF-CMV indeterminate/negative results. Among patients with an indeterminate result, one developed a CMV disease.
Pre-emptive group. Eighteen out of 25 patients (72%) developed an active CMV infection (median 24 days, range, 2-44). Prior to the onset of active CMV infection, QF-CMV indeterminate results were equal to 66.7% in infected and 71.4% in not actively infected patients. QF-CMV positive results were equal to 27.7% in infected and 28.6% in not actively infected patients. Following onset of active CMV infection, QF-CMV positive results were 27.8% (5/18). One out of 5 patients (20%) with QF-CMV positive results developed a second episode of viraemia as compared to 6 out of 13 patients (46%) with indeterminate/negative results ($p < 0.0001$).
Discussion and Conclusions: QF-CMV assay may not be a support tool within the first month post heart transplantation whereas can provide information regarding the immunosuppression level of the patient (CMI+/CMI-) following the suspension of prophylaxis therapy and the prevention strategies after the first episode of viraemia in both groups. Finally, assays for CMV-specific CMI response may help to customize the anti CMV strategy in the single patient.

AN UPDATE ON DIAGNOSING AND MONITORING OF HEPATITIS B VIRUS INFECTION

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Hepatitis B virus (HBV) infection represents an important health threat considering that it affects more than 400 million people worldwide. The spectrum of disease and natural history of chronic HBV infection are diverse and variable, ranging from an inactive carrier state to progressive chronic hepatitis B (CHB), which may evolve to cirrhosis and hepatocellular carcinoma (HCC).
Historically, the diagnosis and treatment of CHB was based on detection of the hepatitis B surface antigen (HBsAg) and more recently on plasma viral load detected by molecular assays. Our understanding of the natural history of HBV infection and the

potential for management of the resultant disease is however continuously improving.
Here we discuss the recent developments in the molecular and serological diagnosis which allow today, more than some years ago, to classify patients during the natural history of HBV and to monitor therapy and management. Indeed, through the use of rapid and reliable new assays, it is now possible to predict the likelihood of patient response to treatment as well as the clinical course of disease.
Among these "new" assays, probably, the most consolidated is the quantitative HBsAg detection immunoassays which allows the quantification of HBsAg by using automated technology and which, in association with HBV DNA, has been suggested to be helpful in the management of CHB patients. More recently other assays have become available. Among them, there is, for instance, a commercially available assay which allows the quantitative detection of HBcrAg which combines the antigenic reactivity resulting from denatured HBeAg, HBcAg and a HBV core-related protein (p22cr). The HBcrAg level has been suggested as an additional marker of HBV infection. Specifically, it has been suggested that high and low levels of HBcrAg may be an independent risk factor for hepatocellular carcinoma and a positive prognostic factor for the response to nucleos(t)ide analogues, respectively.
It is our opinion that in the near future the introduction in routine diagnosis of the above novel molecular and serological assay and technologies, allowing to carefully identify and stage the different phases of HBV infection, may lead to a significant change of patient monitoring and management.

AUTOMATION AND CLINICAL IMPACT OF DIAGNOSTIC MICROBIOLOGY

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When a specimen is collected in a medical setting, it goes to a lab for analysis. Specimens for suspected infectious conditions (bacterial, viral, parasitological, mycological) go to Clinical Microbiology Labs (CMLs). CMLs provide services critical to the population analyzing specimens from sick patients and gathering data that enable correct diagnoses. In addition, they are sentinels for outbreaks as well as for possible bioterrorism events. CMLs are the first to recognize emerging pathogens, novel drug resistance traits and provide critical information for antimicrobial therapy. They also gather local and global epidemiology. Highly trained professionals work in CMLs and make important decisions that save lives and benefit the sick and at-risk members of society.
CMLs, however, are victims of difficult times due to expense limitations in the rapidly-expanding field of lab medicine. Obstacles in recruiting/retaining qualified workers are also evident. Among scientific issues, it is difficult to quickly translate research achievements into effective means for diagnosing and managing infectious diseases (e.g., microbiome, metagenomics). Difficulties include:
- Limited numbers of labs with expertise and size to perform increasingly complicated tests;
- Pressure to obtain more rapid identification of an increasing variety of pathogens, to better differentiate colonization from infection, to detect key virulence factors and antimicrobial resistances;
- Need to implement effective IT solutions for improving data transmission within Hospitals, in their territories, and to health authorities;
- Challenge of obtaining adequate reimbursement from the healthcare system, especially for cutting-edge technology;
- Current shortage of qualified clinical microbiologists that may worsen in coming years due to the aging of workforce and the shortfall of replacements, since incentives for young people are largely absent.

Thus, implementation of automated methods and harmonization of interpretation criteria have become a necessity. This is due to the continuous progress in medicine, the request for shorter hospital stays, the need of Labs consolidation.
CMLs have automated several tasks (blood cultures, biochemical identification of bacteria and yeasts, AST, Gram staining, NA extraction, gene amplification). In critical conditions, early detection of positive blood cultures, rapid ID of pathogens and their susceptibility profiles do significantly reduce mortality. It is likely that MS will progressively replace phenotypic ID. One recent advance in automation is the development of plate streakers that couple a bi-directional interface with the LIS and allow

to inoculate different plates with a unique sample. When streakers are coupled to plate readers and rapid ID instruments, the time to results is reduced by at least 30%. If IT systems may convey results in real time to Clinical Wards over 24hrs, clinical benefits become evident. Reduction of hospital stays and costs has been documented, together with quicker diagnosis, improvement of clinical outcomes, control of nosocomial infections.

MULTIDIMENSIONAL HTA APPROACH FOR THE EVALUATION OF FILMARRAY TECHNOLOGY APPLIED TO THE DIAGNOSTIC ALGORITHM OF SEPSIS

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Introduction: Rapid and broad-spectrum identification of microorganisms, with the rapid identification of the major antibiotic resistance genes, through FilmArray technology can play a relevant role in the sepsis management, guiding the choice to the right antibiotic therapy.The "MICROTAT" project at the ASO of Alessandria measures the clinical and economic impact of new technologies on the patient management/healthcare system.
Materials and Methods: The MICROTAT data allow the electronic collection from clinical records and from hospital databases. The "alert" alarm of bacteremia activates the Infections Control System collecting data linked to specific parameters. The analysis of those parameters allows to score the following indicators to determine clinical appropriateness:
- Compliance the hospital algorithms on empiric antibiotic therapy (0 = no, 1 = yes).
- Time Gap between gram stain results and initiation of the target therapeutic strategy. Score: 0 points if > 4h; score 1 if ≤ 4 h.
- Appropriateness of target therapy after communication of preliminary/presumptive identification (score range from 1 to 3).
- Appropriateness of the adjusted therapy (score range from 1 to 3).

Minimum score 6.
Maximum score 8.
Infectious diseases Specialists regularly evaluate the records. Records with a low performing score (< 6) are sent back to the *Antimicrobial Stewardship Commission* for corrective actions.
MICROTAT data form has been customized and used to evaluate the health economic impact of the FilmArray technology on the diagnosis of sepsis.
Standard costs of sepsis are calculated using the *hospital patient costing* method.
Results: Analisys of 119 records in 2015 shows an appropriateness value of 78% with score ≥ 6 DDD *(defined daily dose)*/100 bed-days of used antibiotics in sepsis is decreasing after implementation of the MICROTAT tool.
The MICROTAT data form allows a continuous monitoring of the therapeutic appropriateness and an evaluation of the clinical and economic impact of the FilmArray technology.
Discussion and Conclusion: Considering the impelling economic crisis, every investment in new technologies need to be evaluated according to the specific organization model based on resources, availability and sustainability of healthcare services. MICROTAT is an health technology assessment approach which evaluates the impact of new technologies under different dimensions to make an appropriate use of any resource.

NOVEL STRATEGIES FOR THE TREATMENT OF OCULAR INFECTIONS

RECENT ADVANCES IN EYE BACTERIOLOGY: A TURIN OPHTHALMOLOGICAL HOSPITAL EXPERIENCE

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Introduction: Ophthalmological microbiology is very important in the diagnosis of ocular infections. It has specific identity and characteristics: amount

of material for examination, bacterial microflora, lack of commercial classical methods for diagnosis. It is very important for microbiologist a closely collaboration with ophthalmologist.
Materials and Methods: In our laboratory we have introduced a method for severe infections – keratitis, ophthalmitis, abscesses- of rapid culture using emoculture bottles. Culture with broth enriched is currently the subject of depth examination and analysis. In all cases of ocular toxoplasmosis we have associated with polymerase chain reaction the blotting, which allowed to increase the sensibility and to reduce the time required for the reliable diagnosis. I will also present emerging pathogens in our experience
Results: Method using emoculture bottles allows to reach 80% of positive cases in 18-20 hours. The use of two or plus methods for few material's amount allows to increase the diagnostic sensitivity and specificity. What bacteria and virus can we regard as emerging pathogens? For us Coagulase Negative Stafilococci, Bacillaceae and EBV
Discussion: For microbiological ocular diagnosis we can use non conventional methods. Use of two or plus methods in little specimens must carefully assess on results. We must always compare ours results with ophthalmologists for evaluation of new pathogens.

ANTIBIOTIC THERAPY IN OPHTALMOLOGICAL PRACTICE

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Introduction: In ophthalmology there are many diseases against which we must establish a prompt and targeted antibiotic therapy, and many times and depending on the mode of appearance and the rapidity of the clinical course, we are often induced to prescribe antibiotic eye drops without the need for an antibiogram.
Matherial and methods: The author reviews in different clinical focusing particularly on bacterial keratitis, which have resulted in a potential permanent opacity of the cornea, but especially on postsurgical endophthalmitis, infectious sequelae dreaded by the devastating consequences on the functionality and sometimes also on the anatomy of the eyeball subjected to a routinely operation as that for the extraction of cataracts.
Discussion: The author then offers some of the most current standards for proper praparazione patient preoperative antibiotics and proper antisepsis in the operating room.
Results: Data in the literature, starting from large-scale clinical studies show a clear drop in the incidence of postoperative endophthalmitis from quanldo in Italy are applied protocols for preventing endophthalmitis, anda reduction in unfavorable outcomes by applying proper treatment in case of appearance.

ACANTHAMOEBA KERATITIS IN ITALY

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Acanthamoeba keratitis (AK) is a potentially blinding disease, occurring mainly among users of soft contact lenses (Panjwani, 2010). The causative agents are species of the genus Acanthamoeba, opportunistic free-living protozoans found in the environment worldwide. Acanthamoeba species have been traditionally identified on the basis of their morphology (Pussard and Pons, 1977). However, since it is not always possible to unequivocally establish differences by microscopy, molecular genotyping mainly based on the analysis of 18S rRNA, allowed to establish 17 different genotypes (T1-T17) among the genus Acanthamoeba (Nuprasert et al. 2010). It is widely accepted that the current cut-off limit of similarities between all sequences of each distinct genotype from all other 18S rRNA gene sequences should be at least 5% (Stothard et al. 1998). To date genetic studies have shown that the genotype T4 represent the most commonly type found in human AK as well as in environmental isolates (Khan, 2006; Ledee et al. 2009; Magnet et al. 2012). The preponderance of genotype T4 in human AK infections could be most likely due to their potential virulence as well as to its relative presence in the environment (Khan, 2006). Recently, a high genetic diversity has been observed amongst T4 isolates and a classification of the T4 cluster in sub-genotypes is currently applied on the basis of differences in the sequence (Booton et al. 2002; Maciver et al. 2013). Although epidemiological and molecular studies from patients with amoebic keratitis have frequently been reported in several countries worldwide including Europe (Magnet et al. 2012; Nagyová et al. 2010; Yera et al. 2008),

data about the epidemiology of Acanthamoeba in Italy are still scarce and the pattern of genetic variation in Acanthamoeba isolated from the environment and from patients infected by this pathogen is poorly known (Corsaro and Venditti, 2010; Di Cave et al. 2009; Gatti et al. 2010). In the present study, the genotypes among clinical Acanthamoeba were determined.

NANOCARRIERS AS PLATFORM FOR OCULAR DRUG DELIVERY

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Drugs applied onto the eye need to bypass different biological barriers in order to reach the targeted ocular structures. Depending on the target sites of the different ocular pathologies, drugs either need to be retained at the cornea and/or conjunctiva or cross these barriers and reach the internal structures of the eye. These barriers consist of the (i) muco-aqueous layer of the tear film, (ii) corneal epithelium, and (iii), in case of pathologies associated with the back of the eye, vitreous humor. In addition, the rapid renewal rate of the outer layers of the lachrymal fluid together with the blinking reflex, severely limits the residence time of drugs in the precorneal space and, thus, the ocular bioavailability of the instilled drugs. Nanotechnology offers the possibility to develop delivery systems (nanocarriers) particularly adapted to overcome the eye-associated barriers. Nanocarriers have shown the capacity to (i) associate a wide variety of drugs, (ii) reduce the degradation of labile drugs, (iii) increase the residence time of the associated drugs onto the ocular surface, and (iv) improve their interaction with the corneal and conjunctival epithelia and consequently their bioavailability. To date, nanocarriers loading various active molecules are currently in pre-clinical studies for the treatment of several ocular diseases. For example, ocular infections can affect different structures in the eye surface, such as the conjunctiva or the inner part of the eye, as in the case of endophthalmitis or uveitis. However, the efficacy of conventional topical treatments is limited by the short residence time of the drugs in the ocular surface, leading to frequent instillations and a significant systemic absorption. The inclusion of anti-infectious drugs within nanocarriers has been reported as an efficient strategy to overcome the limitations of conventional therapies. *In vivo* studies indicated that the drug was retained on the precorneal area when administered by nanocarriers, contrary to the marketed formulation, which was quickly cleared and absorbed into the systemic circulation. In addition, drug-loaded nanocarriers can be a good alternative to the frequent intraocular injections used in the treatment of pathologies of posterior segment of the eye. As a biological drug reservoir, nanocarriers can be injected once in the eye taking advantage of the ability to achieve long-term drug delivery. In summary, nanotechnology represents a promising strategy for the treatment of ocular diseases, a fact that is supported by the increasing number of commercialized drugs-loaded nanocarriers for ocular delivery.

EFFICACY OF GEMIFLOXACIN IN *EX VIVO* EXPERIMENTAL KERATITIS DUE TO METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS*

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Introduction: Bacterial ulcerative keratitis is a serious condition that requires an early diagnosis as well as an immediate and adequate treatment. *Staphylococcus aureus* is the predominant pathogen isolated from the majority of cases of keratitis. In recent years, the emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) strains has been observed in ocular infections. The resistance of MRSA also to second-and third-generation fluoroquinolones has increased the interest in the fourth-generation fluoroquinolones. The aim of this study was: (a) to monitor *in vitro* the susceptibility of ocular isolates versus gemifloxacin and others antibiotics; (b) to test the effectiveness of gemifloxacin 0.3% solution in an ex vivo model of MRSA-induced keratitis.
Materials and Methods: In vitro susceptibility studies were performed on various strains of S.

aureus ocular isolates (including MRSA) to determine the minimum inhibitory concentration (MIC) of gemifloxacin, ofloxacin, levofloxacin, ciprofloxacin, moxifloxacin and gentamicin using E-test method. MRSA-keratitis were performed using a modified ex vivo rabbit model. Corneal buttons with sclera rims, placed in colture, were intrastromally injected with 50 µl of the bacterial suspension (5 x 10^5 colony forming units/ml-CFU/ml) of each strain. Twenty hours after the injection (late treatment), 1 topical drop of gemifloxacin ophthalmic solution (0.3%) (treated group) and balanced salt solution (BSS) (untreated group) was applied to each cornea every 30 min for 14 administration. The corneas were examined both before and after treatment. The tissues from treated and untreated samples were homogenized and serially plated to determine the number of recovered CFU/g.

Results: *In vitro* susceptibility study findings indicated that the MIC of gemifloxacin was lower than the MIC of other fluoroquinolones and gentamicin. Experimental keratitis showed a statistically significant decrease ($p < 0.05$) of MRSA (1 to 2 $\log_{10}$ CFU/g) in gemifloxacin-treated corneas.

Discussion and conclusions: Topical gemifloxacin 0.3% may be effective for the treatment of MRSA-induced keratitis. Additionally, this reproducible, ethical and economic *ex vivo* model can be used as a mechanistically-based alternative to *in vivo* animal testing bridging the gap between *in vitro* and *in vivo* results.

MICROBIOLOGY FOR ARTS AND ARCHEOLOGY

MUMMIES AND INFECTIOUS DISEASE

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Paleopathological surveys, in past generations, have done useful groundwork in detecting, describing and quantifying diseases, and setting them in their temporal context, through macroscopic, radiological and microscopic analyses of mummified soft tissues, bones and teeth. However, the key to further significant advances lies with the integration of skeletal with the biomolecular evidence. Improved techniques for aDNA detection and sequencing are now providing interesting results with regard to ancient pathogens infections like Treponema pallidum, Brucella sp., Mycobacterium tuberculosis. Interest in pathogens is also focussed on their evolutionary trajectories in the interaction with the human host. Besides DNA, other biomolecules (i.e. haemoglobines, human leukocyte antigens, hemozoin) are also providing further means of diagnosis. The search for ancient molecules extends beyond mummies and mineralized tissues, even resorting to dental calculus and coprolites as sources of data. Furthermore, the field has been moving out of descriptive research on individual cases, into multidisciplinary approaches applied to whole populations, marshalling evidence from histology, anatomy, microbiology, physiology, biochemistry, medicine, archaeology, history, ecology. At the same time, much effort has been dedicated to validating new research methods and addressing limitations in data assessment and interpretations. This contribution will report the state of the art of advanced biomolecular, morphological and histomorphological techniques in order to assess the biocultural adaptation of past population to infectious diseases and epidemics, with special reference to the richest sources of data: the human mummified bodies.

BIOARCHEOLOGY: FROM THE ARCHEOLOGICAL SITES TO THE MICROBIOLOGY LABORATORY

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Bioarcheology is the study of human biological remains inside their archeological context and it is the convergence of several methods and disciplines. The leading cause of death in preindustrial cultures, historic or modern, was and is infectious disease. Urbanization and the creation of densely populated environments contribute to transmission, zoonotic or person to person, of infectious diseases. In the last decades particular exciting for scientists was the study of humans, animals and bacterial ancient DNA (aDNA) using a range of advanced molecular tech-

niques including next-generation sequencing (NGS). Since the first identification of Mycobacteria in 1993 from ancient skeletons several paleomicrobiological studies using the traditional or Real Time PCR, advance sequencing techniques or metagenomic analysis have led the identification of ancient microbial pathogens including bacteria, protozoa, viruses and other microorganisms from bones, coprolites, teeth, mummies tissues and paraffin embedded histological preparations. A draft of the complete sequencing was achieved in *Yersinia pestis* and *Mycobacterium leprae* The use of metagenomic analysis in mummies identified tuberculosis patients and some case of co-infections. Particularly paleoparasitology, is one of the most studied branch in the constellation of disciplines that deals with the diseases of the past commonly called paleopathology. Although there has been much excitement surrounding the publication of these studies, the field of aDNA is accompanied by extensive criticism due to the ease of contamination and DNA degradation. In order to minimize contamination and reduced DNA degradation a procedure will be discussed to collect samples directly from archeological site. Several applications of bioacheology will be also presented that permitted the identification of *Y. pestis* in teeth of individual buried in a crypt of the Cathedral of Castelsardo (Sassari, Italy) in XVII century; of parasites in the contents of a latrines sediment obtained during the excavation of a Palace in Italy utilized in XIX century and of several pathogens in the teeth of austro-hungarian prisoners of first world war died in the Island of Asinara in 1915-1916. A metagenomic approach for studying the soils collected in archeological context and the content of ancient vases will be showed.

RECENT ADVANCES IN OUR UNDERSTANDING OF MICROBIAL DETERIORATION OF CULTURAL HERITAGE

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This presentation highlights recent advances in our understanding of microbial deterioration of cultural heritage (CH), i.e. any undesirable change in the properties of a CH material caused by microorganisms. As culture-based methods provide limited information regarding CH microbial diversity, during the last two decades, methods based on the analysis of ribosomal RNA genes have been exploited that allowed a more exhaustive analysis of microbial communities. In the last decade the idea that our understanding of the role of microorganisms in CH deterioration was incomplete without information on metabolically active bacteria using 16S rRNA was explored by some researchers. Lately, it has been recognized a significant gap between the detection of a metabolically active microorganism and the understanding of its real role in surface deterioration. To this end, some progress was made with the study of functional genes. Additionally, it has become evident that microbial deterioration should be increasingly understood in an ecological context. The concept of biofilm implies to take into consideration the interactions established among microorganisms, microorganisms with the substratum and the immediate environment, a view that overcomes the mere identification of microorganisms and their activities as a single taxon. Overall, taking into account interactions among taxa, between microorganisms and the substratum and microorganisms and air, there are some promising avenues of research that are likely to yield a deeper understanding of microbial communities on CH that shift from the observation-based question of 'Who is there?' to the more relevant question 'What are they doing?'. This contribution shows a set of case studies that exemplifies a scenario where the sessile microbial community interact with the substratum and air and how this is relevant to CH conservation. Only a few reports have shown that the drastic changes in biological diversity observed on surfaces are directly connected to differences in air quality, especially regarding anthropogenic input, and reported the use of RNA-based molecular analyses to investigate the key active members of biofilm communities inhabiting artistic surfaces. In the case study presented, the biofilm microflora of historic limestone tombstones located in a highly polluted urban environment (Cambridge, MA) and in a less polluted location (Lexington, MA) were compared using comprehensive RNA-based molecular analyses of 16S rRNA gene sequences as well as sequences of genes for different pathways of sulphur metabolism. This was the first case-study available regarding the sulphur-oxidizing bacteria and sulphate-reducing bacteria community structure inhabiting stone monuments based on functional genes (soxB, apsA, dsrA). Although it is now well known that the interactions between biofilm, stone and atmosphere are of outstanding importance, these interactions have often proven difficult to explore with field experiments. To overcome these limitations, we developed

a unifying methodology to obtain a laboratory model of a dual-species biofilm that is relevant to CH studies. Our experiments underscore the ability of the dual-species biofilm model to capture functional traits characteristic of biofilms inhabiting lithic substrate such as: i) microcolonies of aggregated bacteria; ii) network like structure following surface topography; iii) autotroph-heterotroph interactions; iv) ability to change the chemical parameters that characterize the microhabitats; v) survival under desiccation and vi) biocide tolerance. Ultimately, recent advances in the study of CH biofilm allow us to provide in-depth information on its actual role, i.e. if either it is deteriorative or protective to the CH substratum, keeping into account the surface conservation state and different environmental conditions.

BIO-CLEANING AND BIO-RESTORATION FOR CULTURAL HERITAGE: NEW FRONTIER OF ENVIRONMENTAL MICROBIOLOGY

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Introduction: The presentation focuses on the biocleaning and bioremediation of stone cultural heritage surfaces. Surfaces are continuously subjected to physical, chemical and biological harm. Among the biological agents that cause deterioration, microorganisms are of paramount importance. For decades, abatement of microbial growth has commonly been achieved by using biocides. We propose some environmentally friendly biocleaning strategies. More, growing experimental evidence has shown that some microorganisms can be used to remedy chemical alterations on historical objects of artistic importance.
Materials and Methods: The use of viable bacteria for the cleaning of cultural heritage is an alternative strategy to both the use of organic solvents and mechanical treatments. Sulphates are transformed into H_2S, nitrates into N_2, and organic matter into CO_2 respectively by sulfate-reducing bacteria, nitrate-reducing bacteria and organic degrader bacteria. H_2S, N_2 and CO_2 are gases that are liberated into the air.
Results: The bioremediation case-studies related to the Monumental Cemetery in Pisa, the Cathedral of Milan (biocleaning + bioconsolidation), the base of Michelangelo's Pietà Rondanini, and, the Cathedral of Matera, are presented. In this presentation I will be looking in particular at the themes dedicated to *bio-restoration*, in which *the development of biological agents is actively encouraged because*, rather than playing a negative role, *it assumes a positive function*; some microbial species, thanks to their metabolic characteristics, are utilised in order to make good the chemical and physical damage on certain materials.
Discussion and Conclusions: Our works demonstrated that multiple short-term applications of aerobic degrader, aerotolerant sulphate (and nitrate) reducing bacteria within an appropriate delivery system can be very successful in removing organic residues, black crusts (sulphate or nitrate) from frescoes and marble, both in the laboratory and *in situ* artworks. The use of microorganisms to help bio-preserve, bio-protect and bio-restore C.H. (frescoes, building stone) is a new biotechnology that offers a different approach for conservators.

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OPPORTUNISTIC INFECTIONS IN IMMUNOCOMPROMISED PATIENTS

INFECTIONS BY MULTIRESISTANT BACTERIA IN IMMUNOCOMPROMISED PATIENTS

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Introduction: Bacterial infections represent the most frequent infectious complication after solid

organ transplantation. Determinant factors, clinical characteristics, antibiotic resistance and clinical outcomes vary according to time of onset after transplantation. In particular, most bacterial infections occurring in the first month posttransplantation are hospital-acquired and usually caused by multidrug-resistant agents with high mortality. The main etiological agents include *S. aureus*, enterococci, Gram-negative enteric and non-fermentative bacilli. Subsequently, other opportunistic infections from community-acquired pathogens, play a role. In this context, bloodstream infection is the most severe complications, being associated with mortality rates as high as 50%. Intrinsic virulence factors, antibiotic resistance and the use of inappropriate therapy are also associated with higher mortality. Surgical site, lower respiratory tract and urinary tract infections may also cause significant morbidity and/or mortality.

Materials and Methods: A correct diagnosis of multidrug-resistant bacterial infections is essential in order to optimize clinical management. The diagnostic pathway include traditional methods based on the phenotypic characteristics, that also allow for study of sensitivity to antimicrobials and epidemiological molecular markers. Molecular and genotypic methods have been progressively developed as complementary or alternative techniques, as well as proteomics-based methods (MALDI-TOF). Data on antimicrobial susceptibility for multidrug-resistant can be obtained using highly standardized methods, usually by automated processes. The most importante break points used in the interpretation of antimicrobial susceptibility are indicated by the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST).

Results: Multidrug-resistant bacteria with relevant clinical impact include methicillin-resistant *S.aureus*, *Enterococcus* spp., *Enterobacteriaceae*, *P.aeruginosa* and *A. baumanii*. Among these, in recent years, a growing importance has acquired carbapenemase-producing *K.pneumoniae* (KPC) and other enterobacteria.

Discussion and Conclusion: Data about epidemiology, diagnostic protocols and clinical management of multidrug-resistant bacteria (in particular KPC) will be presented and discussed, with insights in possible future impact on microbiology lab organization and work flow.

CULTURE-BASED AND NONCULTURE-BASED DIAGNOSTICS FOR IFIS: ARE THEY COMPLEMENTARY APPROACHES?

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Invasive fungal infections (IFIs) are associated with high morbidity and mortality, particularly in patients admitted to intensive care units or in patients with severely impaired immune system. Diagnosis of IFIs is a challenge, because current (culture-based) diagnostics are not sufficiently sensitive or specific, and results are often available too late to be clinically helpful. From a diagnostic standpoint, it is essential that clinicians not only assess the risk of IFI in a particular patient, but also are familiar with newer laboratory diagnostic markers and methods as they become available and continue to evolve. While blood culture remains an essential for bloodstream infection (BSI) diagnosis, it is noticeable that the turnaround time to species identification can now be speeded through the use of new technologies directly on positive blood cultures. Among them, matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) has recently proved to improve the clinical management of bloodstream yeast infection, allowing patients to early receive appropriate antifungal therapy. As an alternative to MALDI-TOF MS, the PCR-based FilmArray BCID panel allows the simultaneous detection of 24 microbial pathogens, including 5 *Candida* species. Newer nonculture-based assays of surrogate markers are being incorporated or investigated as tools to improve the early diagnosis of IFIs caused by *Candida*, Aspergillus, or other fungal pathogens. From an antifungal stewardship standpoint, the use of nonculture-based assays, such as detection of fungal antigens, antibodies, or fungus-specific nucleic acids could aid to establish whether an early approach (i.e., 'initiation of antifungal therapy in at risk patients followed by close follow-up and discontinuation of antifungal therapy when IC is excluded') can impact the outcomes of patients with IFI. The comparison of beta-D-glucan (BDG), mannan, anti-mannan antibodies, and Cand-Tec *Candida* antigen showed that BDG and mannan are the best biomarkers for invasive *Candida* infection.

Interestingly, BDG assay, used in combination with the *Candida* albicans germ-tube antibody detection, provides negative predictive values potentially usable for the therapy decision-making process and discontinuing of empirical antifungal therapy. In this talk, it will present and discuss the current data regarding the potential use of integrated workflows for the laboratory diagnosis of IFIs, particularly those caused by *Candida* species.

KILLER AGENTS IN IMMUNOCOMPROMISED HOSTS FOR HEMATOLOGICAL MALIGNANT DISEASES: FOCUS ON BACTERIA, FUNGUS AND VIRUS

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Introduction: To investigate the clinical characteristics and management of breakthrough bacterial, fungal, and viral infections in patients undergoing cytotoxic agent treatments for hematological malignancies.
Materials and Methods: Particular attention was focused on factors affecting outcome of neutropenic enterocolitis (NEC), on impact of conventional diagnostic criteria for invasive fungal infection (IFI) in the era of posaconazole prophylaxis, and on hepatitis B virus (HBV) reactivation.
Results: NEC has emerged as common and alarming infectious abdominal complication in patients with acute myeloid leukemia with the wide-spread use of chemotherapeutic agents which cause severe gastro-intestinal mucositis. Management of this complication requires prompt administration of potent antibiotic treatments. Prophylaxis with posaconazole is currently a well-defined form of therapeutic strategy, characterized by a reduction in overall IFIs and specifically of infections due to *Aspergillus spp.* However, this drug may affect the diagnostic accuracy of radiological and laboratory tests. HBV reactivation has been widely reported in patients undergoing immunosuppressive therapy in onco-hematological settings, with a high frequency of hepatic failure. Routine antiviral prophylaxis with lamivudine or other drugs is recommended for HBsAg+ subjects and specific guidelines have been proposed by scientific societies.
Discussion and Conclusions: New approaches for the management of NEC, IFIs and HBV reactivation are now available.

THE PATHOGEN SPREAD IN THE ERA OF MIGRATION

THE SPREAD OF PATHOGENS IN THE AGE OF MIGRATION

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Migration flows, legal and illegal, are a constant phenomenon, even though with cyclical fluctuations, and will last over time, and with which our country is confronted, as long as the socio-economic and political life of the countries of the near East and in sub-Saharan Africa will not be changed. As to the health issue rather illegal migrants, the Ministry of Health (MoH) has undertaken numerous initiatives for surveillance to verify the absence of conditions that pose a risk to public health, like "Mare Nostrum" initiative, which has allowed us to anticipate health checks during navigation, routinely made on the ground also by the staff of the Office of Maritime Health Air and Frontier (USMAF) of the MoH, and to identify health conditions, in migrants rescued at sea, which could actually be a warning to public health, and to implement all appropriate measures of public health. Of course, the checks continue to be carried out on the ground on landing, despite the great difficulties in carrying out the tasks of international prophylaxis. Through this activity, and the operation of the system as routine surveillance of infectious diseases and syndromic surveillance system activated since 2011, it can be said that in Italy, despite massive irregular migration, revealed no increase in the incidence and the prevalence of infectious diseases requiring public health interventions. In addition, surveillance of

infectious diseases does not end upon arrival but must continue, under the responsibility of the structures of the National Health System, for the duration of residence in the country. In fact, the system of infectious disease surveillance and inspections carried out routinely on landing, did not reveal any situations that could constitute a health emergency and have, however, allowed to operate immediately, and as appropriate, suspected cases of infectious diseases interest of the International Health Regulations (IHR), as well as other health situations requiring immediate attention, both in the case of infectious diseases and in the case of pathological conditions such as burns, trauma, heart disease, diabetes, sequelae of poliomyelitis or other neurological diseases or physiological conditions (pregnancy), of undeniable interest in the health of the individual but not for the community, with start-up of cases to appropriate places of care. Among more than a hundred thousand irregular migrants, evaluated when entering the country in the manner mentioned above, from October 2013 to date, the signs or symptoms of infectious diseases in place were found in a small percentage of cases, concerning mostly dermatological (skin parasites) and no confirmed case of diseases that pose a real threat to public health. However, it is essential not only to carefully monitor the evolution of the epidemiological diseases endemic or currently epidemic in the countries from migrants depart and pass through or where they stop before arriving in our country, but also provide, where possible, the impact on health migrants arising from ethnic-religious wars, insurrections and/or environmental disasters in their countries, which inevitably lead to displacement, feeding problems, diseases, violence of various kinds, disruption and collapse of social and health services, environmental degradation.

EMERGING AND RE-EMERGING PARASITIC INFECTIONS

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Introduction: The exponential growth of international travel and the relatively recent immigrant wave have caused a change in the local epidemiology with the emergence (or re-emergence) of a number of infections, most of which responding to the definition of "Neglected Tropical Diseases" (NTD). A few examples Malaria was formerly endemic in Italy, declared malaria-free by the WHO in 1970. Thereafter and until 1990 cases were rare. In the last 25 years the number of imported cases has increased to reach over 1000 notified cases per year around the year 2000, predominantly caused by *Plasmodium falciparum*, with several, perfectly avoidable deaths. Chagas disease, caused by *Trypanosoma cruzi*, exclusive of Latin America, is a typical example of "emerging" parasitic infection. Immigrants from Bolivia in particular present a high prevalence of this infection, causing potentially fatal consequences if not recognized and treated when still clinically silent. We estimate that thousands of infected subjects are currently present in Italy, with implications, besides the individual clinical burden, for transplant and transfusion policy, not to mention the problem of vertical transmission. Schistosomiasis (from the trematodes *S. mansoni* and *S. haematobium* and occasionally other species), causing life-threatening consequences to the liver, the genito-urinary system and potentially many other organs, has a striking prevalence in some nationalities, especially from Sub-Saharan Africa. Again, the diagnosed cases are just the tip of the iceberg, as most cases remain undiagnosed, with a potential, heavy burden for the national health service. Other helminths, such as *Ascaris lumbricoides*, hookworm and *Trichuris trichiura* (Soil Transmitted Helminths or STH) were highly endemic in our country, but have virtually disappeared as a consequence of an improved hygiene and sanitation. Another STH, *Strongyloides stercoralis*, has remained endemic in Italy because of its peculiar life cycle, and for different reasons is also likely to become the only significant helminth infection for decades also in the countries in transition, unless specific control programmes are undertaken, which has not yet been the case.

Conclusion: Immigrants are often regarded as a problem and a potential source of infectious diseases for the local population. Nevertheless, most tropical parasitic infections are not directly transmissible from person to person, but are a significant health problem for the affected individual. They represent a challenge but also a great opportunity for medical and laboratory professional to broaden their knowledge and experience.

ORAL COMMUNICATIONS

Latest on Microorganism / Host Interactions

INTRODUCTION *MYCOBACTERIUM AVIUM* SUBSPECIES PARATUBERCULOSIS AND AUTOIMMUNE DISEASES

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Mycobacterium avium subspecies paratuberculosis (MAP) is the cause of Johne's disease, a chronic intestinal inflammation, in ruminants. It's role in Crohn's disease has been debated for more than a century due to the similarities between the two diseases. However, MAP can be readily detected in infected ruminants but it is much more difficult to detect in humans due to the extreme slow growth. Except the uncultivable Mycobacterium leprae, MAP has the slowest growth rate among harmful mycobacteria. After inoculum of infected samples from infected animals and incubated under optimal conditions, MAP colonies usually appear not before 3 months or more. MAP can be found in pasteurized milk, milk powder for children, surface water, soil, piles of cow manure that contaminate the soil and supply of drinking water, all contributing to human exposure. Perhaps more incriminating for MAP as a zoonotic agent is the increasing number of human diseases with which MAP has been related: Type 1 Diabetes, Hashimoto Thyroiditis and Multiple Sclerosis, just to mention some. In this overview we'll focus on discussing several arguments which might support a causal role for MAP as one of the microorganisms involved in the trigger of different autoimmune diseases suggesting a possible course of action attempting to explain how the bacteria could ignite autoimmunity. Indeed, recently an independent association has been observed between MAP, Epstein Barr Virus and Multiple Sclerosis focusing on the same human targets. In conclusion, the role of MAP in different autoimmune diseases has progressed from controversial to conspicuous to compelling, there should likely be a major shift in the public health approach to MAP and human diseases. Early indications of such a shift are two clinical trials employing anti-mycobaterial drugs in Crohn's disease and Multiple Sclerosis.

C 001

BTBR MICE, A POTENTIAL MODEL TO INVESTIGATE MICROBIOTA-GUT-BRAIN CONNECTION IN AUTISM SPECTRUM DISORDERS (ASD)

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Introduction: Alterations of gut microbiota composition have been described in children affected by ASD and a few mouse models, showing ASD related behavioural phenotype, has been used to elucidate the role of gut microbiota composition in this disorder.
We analysed the gut microbiota of female and male BTBR T+tf/J (BTBR) mice, a genetic spontaneous model of autism, with respect to non-autistic C57/B6 (C57) mice. Variations of gut microbiota profiles and behaviour were also investigated upon treatment of BTBR and control mice with PEA (palmitylethanolamide), a neuroprotective compound.
Materials and Methods: High resolution metagenomic analysis was performed by next generation sequencing approach (MiSeq, Illumina platform) from: 1) faecal material of 3 female and 3 male of inbred BTBR and C57 mice (age: 10-13 months); 2) caecal material of 3 BTBR, BTBR treated with 30mg/kg PEA and C57 control female mice (age: 4 months).
Operational taxonomic units (OTUs) definition and tassonomic classification were performed using QIIME. Key OTUs, that discriminates between C57 and BTBR groups were identified with Metastats and Galaxy platform-based LDA Effect Size analysis.
Results: Alpha- and beta-diversity analyses showed that female BTBR mice displayed a different assortment of gut bacteria with respect to control mice namely in terms of species richness and diversity. Interestingly, male BTBR mice showed an intermediate gut microbiota profile with respect to control mice and BTBR female mice and PEA treated BTBR female revealed microbiota reassortment.
Discussion and Conclusions: We have identified

108 OTUs that discriminate between BTBR female and control mice. The preponderance of specific OTUs in BTBR female microbiota and in control mice gives rise to the hypothesis of their possible promoting or counteracting role in the ASD pathogenesis. Treatment with PEA significantly reduced the discordances between BTBR and control mice. *Sutterella* and *Ruminococcus gnavus* appreciably increased in BTBR mice. These taxa were also reported as key features in ASD patients. The obtained data indicate that BTBR mouse model could represent a useful tool to futher investigate the possible gut-brain connection in autism.

C 002

ROLE OF LACTOFERRIN IN MODULATING INFLAMMATORY RESPONSE BY EPITHELIAL AND MACROPHAGIC CELLS

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Introduction: Lactoferrin (Lf), an iron-binding glycoprotein secreted by exocrine glands and by neutrophils in infection and inflammation sites, is a key component of innate immunity and possesses several biological effects dependent and independent on its iron binding ability. In particular, Lf modulates infection and inflammatory processes. Conflicting data on pro- or anti-inflammatory function of Lf may be due to the different cell models as epithelial cells or macrophages infected by bacteria or stimulated by LPS.
Here we evaluate the effect of Lf on different in vitro models.
Materials and Methods: In this research we used: i) a primary bronchial epithelium from a cystic fibrosis (CF) patient stimulated by LPS or infected by *Pseudomonas aeruginosa* LESB58, an epidemic strain isolated from a CF patient; ii) differentiated intestinal CaCo-2 cells infected by Adhesive/Invasive *Escherichia coli* LF82, isolated from Crohn diseases (CD) patient; iii) human THP-1 macrophagic cell line stimulated by *E. coli* LPS.
Highly purified bovine milk Lf (bLf) lactoferrin was kindly provided by Morinaga Milk Industries Co., Ltd. (Tokyo, Japan). The bLf iron saturation was about 20%.
Results: Results showed that bLf significantly reduced the bacterial survival in both the CF epithelium and intestinal cell line modulating the inflammatory response and the synthesis of ferroportin (Fpn), a key component of the cellular iron homeostasis and, consequently, of the intracellular multiplication of bacteria.
It has been recently shown that the macrophagic M1 (inflammatory) and M2 (tollerogenic) phenotypes are characterized by a different synthesis of Fpn. Our results show that bLf modulates inflammatory response as well as Fpn synthesis in THP-1 cells stimulated by LPS.
Discussion and Conclusion: These data demonstrate that bLf affect both inflammation and iron export ability in differently stimulated cells with possible beneficial effects contrasting infection/inflammation related damage.

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C 003

THE SECOND LIPID MESSENGERS PHOSPHATIDIC ACID AND PHOSPHATIDYLINOSITOL 5-PHOSPHATE PROMOTE INNATE ANTIBACTERIAL IMMUNITY

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Introduction: We have recently shown that Apop-

totic-Body like Liposomes (ABL) carrying phosphatidic acid (PA) strongly enhance anti-mycobacterial innate immunity. In the present study, ABL carrying second lipid messengers involved in different phases of phagocytosis, from internalization to phagosome maturation, were tested as a possible tool to improve innate antimicrobial response.
Materials and Methods: ABL were generated as described (PNAS, 2012; 109: E1360-8) in order to carry the following second lipid messengers: PA, Phosphatidylinositol 3-phosphate (PI3P), PI5P, Arachidonic acid (AA), Sphingosine 1-phosphate (S1P), Lysobisphosphatidic acid (LBPA). Human macrophages infected or not with P. aeruginosa PAO1 and Bronchalveolar Lavage (BAL) cells, from patients with pulmonary infections, were stimulated with the different liposome formulations. Intracellular bacterial viability was monitored by CFU whereas reactive oxygen species (ROS) and phagosome acidification was assessed by fluorometry.
Results: In vitro analysis showed that only ABL carrying PA, PI5P or PI3P stimulate phagosome acidification and ROS production in human macrophages. Thus, they were ex vivo tested for the possible capacity to promote antimicrobial response in BAL cells from patients with pulmonary infections caused by different Gram+ or Gram- bacteria. ABL carrying PA or PI5P induced a significant increase in intracellular killing of the pathogens. As cystic fibrosis (CF) transmembrane conductance regulator (CFTR) is involved in phagosome acidification, we also tested the same liposome formulations for the possible capacity to improve the antimicrobial response of macrophages from patients with CF. Interestingly, PA or PI5P also significantly increased the intracellular killing of PAO1 in CF macrophages.
Discussion and Conclusions: Results show a novel role for the second lipid messengers PA and PI5P in the activation of antibacterial response against a wide range of Gram+ and Gram- pathogens and suggest ABL as a possible immunotherapeutic tool to improve the treatment of drug resistant or recurrent infections, such as those occurring in CF.

C 004

A HYPER-GLYCOSYLATION OF HBV SURFACE ANTIGEN CORRELATES WITH IMMUNOSUPPRESSION-DRIVEN HBV REACTIVATION AND STRONGLY AFFECTS HBsAg QUANTIFICATION *IN VITRO*

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Introduction: HBV surface antigen (HBsAg) is a glycosylated protein. Here we investigated the HBsAg N-linked glycosylation profiles in immunosuppression-driven HBV-reactivation and evaluated their impact on HBsAg-antigenicity.
Methods: Mutations associated with acquisition of N-linked glycosylation (NLG) site were investigated in 127 HBsAg genotype-D sequences from 47 patients with immunosuppression-driven HBV-reactivation (HBV-R) (defined as Hwang,2014), and 80 chronically HBV-infected drug-naïve patients as control.
The impact of NLG sites on HBsAg-antigenicity was analyzed by transfecting HepG2-cells with a plasmid encoding wild-type and mutated HBsAg linked to a streptavidin-tag (strep-tag). The strep-tagged HBsAg amount in supernatants was quantified by a specifically-designed ELISA targeting the Strep-tag (thus, not affected by HBsAg-mutations), and by ELISAs targeting the HBsAg (Architect-Abbott, Monolisa-Biorad).
Results: Additional NLG sites are found in 19.1% of HBV-R patients versus 0/80 controls ($p < 0.001$). They localize in the major hydrophilic HBsAg-region (MHR), target of antibodies. In 7 patients, a single additional NLG site results from the mutations T115N (n = 2), T123N (n = 2), T131N (n = 2), and from the insertion of an N between 114 and 115 position (ins114-115N) (n = 1). In the remain-

ing 2 patients, 2 additional NLG sites result from S113N+T131N and ins114-115N+T117N.
Notably, 5/9 patients with ≥ 1 additional NLG sites remain HBsAg-negative by diagnostic-test at HBV-R ($p = 0.002$).
In-vitro, all additional NLG sites decrease the strep-tagged HBsAg quantification by the 2 ELISAs targeting the HBsAg. Among them T115N, T123N, ins114-115N determine a > 90% decrease in HBsAg-quantification by both ELISAs. No decrease of strep-tagged HBsAg is revealed by ELISA targeting the Strep-tag, suggesting that additional NLG sites hamper HBsAg-recognition by antibodies without affecting HBsAg-release.
Conclusions: Additional NLG sites in MHR correlate with false HBsAg-negativity at ELISA despite HBV-R, and profoundly affect HBsAg-antigenicity *in-vitro*. This supports the role of immune-escape mutations in HBV-R during immune-suppression and the importance of HBV-DNA (more than HBsAg) in HBV-reactivation diagnosis.

C 005

PRIME-BOOST VACCINATION STRATEGIES BASED ON THE COMBINATION OF ADJUVANTS AND IMMUNIZATION ROUTES INDUCE DIFFERENT ANTIGEN-SPECIFIC CD4+ T CELL RESPONSES

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Introduction: The design of heterologous prime-boost vaccine combinations, including different vaccine formulations and routes of delivery that optimally shape the immune response, is of primary importance for the development of next generation vaccines. Here we assessed the antigen-specific local and systemic CD4+ T cell responses elicited by different prime-boost combinations using the *Mycobacterium tuberculosis* vaccine antigen H56 mixed with different adjuvants administered by the parenteral and nasal routes.
Materials and Methods: Different prime-boost combinations were tested in C57BL/6 mice using the H56 antigen mixed with CAF01 and CpG ODN adjuvants. Both adjuvants were tested by the nasal route, while for parenteral injection CAF01 was used. Prime-boost strategies were obtained crossing different vaccine formulations and routes of immunization in homologous and heterologous combinations. Antigen-specific CD4+ T cells were tracked following primary and booster immunizations by using peptide-MHC class II tetramers.
Results: Both parenteral priming with H56 plus CAF01 and nasal priming with H56 plus CpG elicited significant expansion of CD4+ tetramer-positive T cells in the spleen, however only parenterally primed cells responded to booster immunization. Subcutaneous priming with H56 and CAF01 followed by nasal boosting with H56 and CpG showed the greater expansion of CD4+ tetramer-positive T cells in the spleen and lungs compared to all the other homologous and heterologous prime-boost combinations. Nasal boosting exerted a recruitment of primed CD4+ T cells into lungs that was stronger in subcutaneously than nasally primed mice, in accordance with different chemokine receptors expression induced by primary immunization.
Discussion and Conclusions: Combination of different vaccine formulations and routes of delivery is critical for rationale design of prime-boost strategies. The optimal prime-boost combination for eliciting activated CD4+ T cells, not only in the spleen but also in the lungs, appeared to be parenteral priming with CAF01 followed by nasal boosting with CpG. These data demonstrate that subcutaneous priming is fundamental for eliciting CD4+ T cells that can be efficiently boosted by the nasal route with recruitment of antigen-specific cells into the lungs.

C 006

THE IFI16 RESTRICTION FACTOR COOPERATES WITH HCMV pUL83 TO DOWN-REGULATE UL54 GENE EXPRESSION AND VIRAL DNA SYNTHESIS

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Introduction: During the early phase of human Cytomegalovirus (HCMV) infection, the Interferon-γ-Inducible factor 16 (IFI16) behaves as a pattern recognition receptor (PRR) sensing viral DNA and triggering antiviral cytokine release. Later on, it restricts virus replication by down-regulating expression of viral genes committed to DNA synthesis including UL54 and UL44. These activities are modulated by viral proteins including pUL83, a tegument protein involved in viral evasion. 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: Here, we demonstrate that pUL83 interacts with IFI16 relieving its inhibitory activity on UL54 gene transcription. We also establish that, starting from 48 hours post-infection, IFI16 is stabilized and protected from degradation by pUL83 as observed infecting HFF with v65Rev or the v65Stop lacking pUL83 expression. Upon infection with the HCMV mutant virus RV-VM1 IFI16 is retained in the nucleus and does not migrate into the cytoplasm. Interestingly, accumulation of nuclear pUL83 prevents the formation of discrete puncta and dissipates aggregation of IFI16 filaments. We observe that IFI16 shows a half-life of less than 1h in the absence of pUL83 compared with 2h in the presence of pUL83 demonstrating that IFI16 is less stable in the absence of pUL83. Consistent with this, we observe restoration of IFI16 protein in v65Stop-infected cells compared to v65Rev-infected cells in presence of the proteasome inhibitor MG132.
Discussion and Conclusions: Our results demonstrate a novel role for the pUL83 protein that stabilizes and protects IFI16 from proteasome degradation during HCMV infection and modulates suppression of UL54 gene activity.

ORAL COMMUNICATIONS

Latest on Virology

C 007

DETECTION OF HUMAN POLYOMAVIRUS 6 DNA IN THE CEREBROSPINAL FLUID OF HIV NEGATIVE PATIENTS WITH NEUROLOGICAL DISEASES

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Introduction: The family *Polyomaviridae* comprises thirteen human viruses that have been detected in various specimen types, mainly urine, skin and blood. Among all of them, only JC Polyomavirus (JCPyV) genome has been detected in Cerebrospinal Fluid (CSF). Recently, the unexpected finding of Human Polyomavirus 6 (HPyV6) DNA in the CSF from an HIV positive patient with leukoencephalopathy was reported. JCPyV being a recognized cause of Progressive Multifocal Leukoencephalopathy (PML) in immunodeficient subjects provides a precedent for polyomavirus causing neurological symptoms, but alternative explanations should be considered. The aims of the study was to explore the prevalence of the newly discovered HPyV6, Human Polyomavirus 7 (HPyV7) and Human Polyomavirus 9 (HPyV9), Merkel Cell Polyomavirus (MCPyV) in neurological diseases, in comparison with those of JCPyV.

Material and methods: CSF have been collected from 51 HIV-positive patients affected with HIV-related leukoencephalopathies and 191 HIV-negative patients affected with other neurological diseases (OND), such as meningitis, encephalitis, and encephalomyelitis. DNA was isolated and real-time PCR assays for JCPyV, HPyV6, HPyV7, HPyV9 and MCPyV were conducted.

RESULTS: JCPyV genome was detected in the CSF of 16/51 (31.4%) HIV-related leukoencephalopathies patients, HPyV6 genome in the CSF of 2/51 (3.9%) HIV-related leukoencephalopathies patients and in 37/191 (19.4%) HIV-negative patients with OND. MCPyV DNA was present in 9/191 (4.71%) CSF from HIV-negative OND patients, while HPyV7 and HPyV9 genomes were not amplified in any clinical specimens.

Discussion and Conclusions: HPyV6 genome was found in a high percentage of the CSF of the OND patients, followed by MCPyV, whereas HPyV7 and HPyV9 genomes were not detected. HPyV6 has not been previously associated with any disease, and it is nonetheless possible that the HPyV6 sequences originated from the skin during CSF collection. Whether HPyV6 detection in CSF is associated with patients's neurological disease, is indicative of contamination with skin microbiota, or reflects increased blood-brain permeability is unknown. Demonstrating a causative role will require further studies.

C 008

SELECTIVE miRNA DOWN-REGULATION IN THE INHIBITION OF HIV-1 REPLICATION BY A HUMAN SERUM ANTIBODY-DERIVED PEPTIDE

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Introduction: K40H, a phosphorylated peptide derived from the constant region of IgMs found in human serum, proved to reduce *in vitro* HIV-1 replication besides being endowed with microbicidal activity. MiRNAs are short non-coding sequences of RNA, known to have a key role in the modulation of many cellular processes, including humoral and cell-mediated immunity in response to viral infections. The present study was aimed at analyzing miRNA profile in HIV-1 infected cells after K40H treatment.

Material and Methods: CD4+ cells purified by negative magnetic separation from healthy donors were seeded and exposed to HIV-1 R5 (BaL) and CXCR4 (IIIB) strains at 0.5 multiplicity of infection for 2 hours. K40H treatment (1 μg/ml) began after viral adsorption step. Viral replication

was monitored by p24 antigen detection at 8 and 12 days post-infection. In addition, cells were harvested after 4 days of infection in order to purify total RNA and perform miRNAs analysis by GeneChip® miRNA 3.0 Array. As negative controls, infected and untreated and uninfected cells were used. Validated targets and their functional activity were investigated by bioinformatics tools.
Results: After quantification, 213 miRNAs whose expression showed at least 1 Log_2 fold change were identified, 94 were down-regulated and 119 up-regulated. Three miRNAs (miR-548aa, miR-548aj, miR-3663-3p) discriminated treated vs untreated samples, being up-regulated by HIV-1 infection and down-regulated after K40H treatment. Enrichment analysis of their validated targets revealed that the most abundant Gene Ontology terms were post-translational protein folding (Biological Process), chaperonin containing T-complex (Cellular Component), and unfolded protein binding (Molecular Function). Querying pathway libraries, the most significant protein-protein interaction resulted the cooperation of prefoldin and TriC/CCT in actin and tubulin folding.
Discussion and Conclusions: K40H might be involved through miRNAs modulation in the control of protein folding process. As altered interactions between protein-folding machinery or chaperonins and substrates (i.e. transcription factors) led to the gain or loss of function of misfolded proteins with a consequent deregulation of many cellular processes, these findings suggest that K40H may play a key role in inactivating proteins involved in HIV-1 replication.

C 009

OXYSTEROLS POTENTLY INHIBIT HUMAN ROTAVIRUS INFECTION BY SEQUESTERING VIRUS PARTICLES INTO ENDOCYTIC VESCICLES

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Introduction: The oxysterols are oxidized derivatives of cholesterol playing critical roles in regulating lipid metabolism, bile acid synthesis, sterol transport, and gene expression. Recently, one hydroxylated derivatives of cholesterol, 25-hydroxycholesterol (25HC), has been indicated as an important modulator of immune responses against viral pathogens. 25HC is induced in macrophages by type I interferon (IFN) signaling and displays broad antiviral properties against several enveloped viruses (e.g. human immunodeficiency virus, hepatitis C virus and ebola virus). Despite the growing literature investigating the efficacy and mechanism of action of 25HC against enveloped viruses, the antiviral potential of 25HC and other oxysterols against non-enveloped viruses has yet to be explored. To this end, we tested the antiviral activity of oxysterols against human rotavirus (HRoV), a non-enveloped virus causing severe gastroenteritis in infants.
Materials and Methods: Focus reduction assays were performed in order to test 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). 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: We showed that 25HC and 27HC can block the infectivity of different HRoV strains at 50% inhibitory concentrations (EC_{50}) in the low micromolar range, with no impairment of cell viability (SI > 100). These oxysterols can prevent 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.
Discussion and Conclusions: These findings suggest that appropriate modulation of endogenous production of oxysterols might be a primary host strategy to counteract HRoV infection. Moreover, 25HC and 27HC could be considered for new therapeutic strategies against HRoV.

C 010

EFFECT OF HA-TARGETING THIAZOLIDES AGAINST AVIAN INFLUENZA VIRUS INFECTION

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Introduction: The recent emergence of new avian strains of influenza A viruses (IAVs) able to infect humans represents a serious threat to global human health. The thiazolide nitazoxanide (NTZ) has recently proven to possess antiviral activity against several RNA viruses, and is in late-stage clinical trial for treating acute uncomplicated influenza. While the molecular mechanism of thiazolide antiviral activity has not been fully elucidated, NTZ and its active metabolite tizoxanide (TIZ) were shown to inhibit A/PR/8/34(H1N1) IAV replication by a novel mechanism, impairing hemagglutinin (HA) maturation and virus morphogenesis. In the present study we investigated the activity of NTZ, TIZ and second-generation thiazolides (SGT) against avian influenza virus infection in vitro, and explored the mechanism of the antiviral action.
Materials and Methods: Madin-Darby canine kidney (MDCK) cells and human A549 alveolar type II-like epithelial cells were infected with the following low-pathogenicity avian IAV strains: H1N1 A/Goose/Italy/296246/03, H5N9 A/Chicken/Italy/9097/97 and H7N1 A/Turkey/Italy/RA5563/99. NTZ, TIZ and SGT, dissolved in DMSO, were diluted in culture medium before treatment. Virus yield was determined by hemagglutinin titration and infectivity assay, and cell viability was determined by MTT assay. Viral protein synthesis and HA maturation were evaluated by SDS/PAGE-autoradiography after [^{35}S]methionine/cysteine-labeling, Endo H-digestion, Western-blot and immunofluorescence analysis.
Results: Nitazoxanide was found to cause a dose-dependent inhibition of avian IAV replication, with IC_{50} ranging from 0.3 to 1.0 mg/mL, and SI ranging from > 50 to > 100, depending on the m.o.i. Similar results were obtained with the NTZ metabolite tizoxanide, whereas SGT RM4848 and RM5038 were found to be more effective with IC_{50} ranging from 0.03 to 0.8 mg/mL, and SI ranging from > 70 to > 500. As previously shown for the A/PR/8/34(H1N1) IAV, thiazolides did not affect virus entry into target cells, whereas they acted at post-translational level by inhibiting the maturation of the HA glycoprotein.
Discussion and Conclusions: The results indicate that thiazolides possess potent activity against avian influenza virus infection. These drugs target HA glycoprotein maturation independently of the subtype. The fact that NTZ was also able to inhibit HA maturation in cells constitutively expressing IAV hemagglutinin (H2 subtype) in the absence of viral infection, suggests a cell-mediated mechanism.

C 011

FULL VIRAL SUPPRESSION, RESIDUAL VIREMIA AND LOW LEVEL VIREMIA IN HIV-1 ART TREATED PATIENTS: RISK OF VIROLOGICAL REBOUND/ FAILURE AND ASSOCIATION WITH INFLAMMATION MARKERS AND HIV-1 DNA

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Introduction: Clinical significance of persistent residual viremia (RV) in HIV-1 treated patients is still unknown. The aim of the study was to clarify the role of RV on virological rebound (VR) and failure (VF), and its influence on immune activation status. Moreover, the relationship between RV and size of the latent reservoir was investigated.
Methods: 961HIV-1 patients from Policlinico Umberto I Hospital were retrospectively examined for 45 months. HIV-1 RNA was measured by Versant kPCR (Siemens). sCD14, TNFα and IL-6 were measured by ELISA kit. HIV-1 DNA was performed by commercial kit (Biocentric).
Results: According to basal viremia levels, full sup-

pression (TND; n = 596), residual viremia (TD; n = 251), and LLV (37-200 copies/ml; n = 114) groups were defined. VR (2 HIV-1 RNA values > 200 copies/mL) rates were 0.1% for TND 0.1%, for TD, and 6.1% for LLV. VR risk was significantly higher in LLV than in TND or TD ($p < 0.0001$). VF (HIV-1 RNA value > 400 copies/mL) rates were 3.4% for TND 6%, for TD, and 24.6% for LLV. VF risk was significantly higher in LLV than in TND or TD ($p = 0.001$) and in TD vs TND ($p = 0.045$). The levels of inflammatory markers were analyzed in 113 patients with viremia TND (I), 113 patients with RV (II) and 95 patients with at least 2 value of VL > 37 copies/ml (III) during follow up. Median (range) values for sCD14 were 7.25 μg/ml ($2.7 \leq 10$) in I, 8.8 μg/ml ($3.6 \leq 10$) in II and 10 μg/ml ($4 \geq 10$) in III (I vs II: $p < 0.0001$; I vs III: $p < 0.0001$; II vs III: $p = 0.001$). No difference between groups about TNF-alpha was found. A significantly higher percentage of patients with IL-6 value > 15.6 pg/ml was detected in patients TND vs LLV (I vs III $p = 0.003$). When HIV-1 DNA levels were stratified on basis of RV, a significant difference between TND versus TD[12.23 (IQR: 9.4-14.3)log10/10^6 PBMC ($p = 0.001$) and 14,3 (IQR: 12.4-15.9) log10/10^6 PBMC] and LLV [(15.4 (IQR: 12.3-17.2) log10/10^6 PBMC ($p < 0.0001$)] were found.

Conclusions: Higher risk of VR and VF in RV and LLV than TND were found. Patients with RV and LLV showed higher levels sCD14 markers than individuals with a persistent TND viremia. In addition, a relationship between RV and HIV-1 DNA was found.

C 012

IDENTIFICATION OF A NOVEL VARIANT OF THE TR2 PROTEIN OF HUMAN HERPESVIRUS TYPE 8 ISOLATED IN A SARDINIAN POPULATION

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Introduction: Kaposi's sarcoma (KS)-associated herpesvirus (KSHV), also known as human herpesvirus 8, is a human DNA tumor virus and the etiological agent of KS and other lymphoproliferative disorders. Its prevalence varies geographically, and some studies have reported an increasing presence of infection in the general population and, in particular, in Sicily, Sardinia, and the Po river valley. However, only a small fraction of individuals infected with KSHV develops neoplastic diseases, suggesting that other risk factors are involved in tumor development.

Some papers support the hypothesis that KSHV could play a role in the development of Diabetes Mellitus type II (DM2).

KSHV possesses a typical herpesvirus icosahedral capsid composed of four structural proteins: the hexameric and pentameric capsomers are composed of the major capsid protein (MCP) encoded by orf25; the heterotrimeric complexes, forming the capsid floor between the hexons and pentons, are each composed of one molecule of orf62 and two molecules of orf26 (which encode respectively TR1 and TR2 proteins). K. Nealon et al. identified three distinct capsid species that arise during lytic KSHV replication.

Over the last 6 years, we have investigated KSHV prevalence in the South Sardinian population and performed molecular characterization of the isolates. This study aims to identify the role of orf26 polymorphisms in the development of the TR-2 protein and accordingly of the capsids.

Materials and method: In the study we examined 646 samples from diabetic subjects and 363 blood donors. KSHV genotyping was performed by a nested PCR for k1 and for orf26. The expected bands were purified and cloned with TOPO® TA Cloning® Kit for Sequencing (Life Technologies).

Results: Orf26 and k1 genotyping showed that all samples were attributable to genotype C1 and C2 and B3 (one sample); the orf26 polymorphisms were characteristic of 3 subgroups, 2 of which are already present in the literature (Endo et al. 2003), and one new subgroup, called "NEW TYPE", only detected in DM2 patients. These polymorphisms cause an amino acidic substitution and, therefore, a change in the TR2 structure.

Conclusion: The different orf26 subgroups could be suggestive of a different step in infection or could be due to the production of defective viral particles that cannot cause tumors and which can be linked to diabetes onset.

ORAL COMMUNICATIONS

Latest on Bacteriology / Mycology / Parasitology

INTRODUCTION SYMBIOSIS AMONG PATHOGENS; THE STRANGE STORY OF *TRICHOMONAS VAGINALIS* AND *MYCOPLASMA*

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Symbiotic relationships between unicellular eukaryotic microorganisms and bacteria are widely represented in nature. In most cases, these associations involve free-living organisms, while only very limited examples involving human pathogens are described.
The symbiosis between the eukaryotic parasite *Trichomonas vaginalis* and the bacterium *Mycoplasma hominis* is the first one described involving two obligated human parasites producing independent diseases in the same anatomical site: the lower urogenital tract. The mucosal parasite *T. vaginalis* is the causative agent of trichomoniasis, the most common nonviral sexually transmitted disease, worldwide, while *M. hominis* has been frequently associated with various pathological conditions in female genito-urinary tract and with adverse pregnancy. Studies *in vitro* have demonstrated the ability of *M. hominis* to invade, survive and multiply intracellularly in *T. vaginalis* cytoplasm, suggesting a role for protozoan parasite as Trojan horse in mycoplasma infections. Despite several aspects of this unique relationship have been investigated many questions on the influence of the symbiosis on pathobiology of *T. vaginalis* still remain unanswered I'll discuss about the influence of *M. hominis* parasitism on protozoan replication rate, virulence, and on production of ATP.

C 013

EXPOSURE TO DNA-METHYLATING AGENTS IMPAIRS BIOFILM FORMATION AND INVASION OF EUKARYOTIC CELLS VIA DOWN REGULATION OF THE SIALIDASE NanA

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Introduction: DNA methylation damage can be induced by both endogenous and exogenous chemical agents. All living organism have developed strategies to counteract the effects of DNA modifications.
We investigated *E.coli* response to methylation stress by differential proteomics approaches carried out on alkylating agent methyl-methane sulfonate (MMS) treated and untreated E.coli cells to obtain a global view of the protein expression and the cellular pathway(s) affected by the alkylating agents.
The biologica role of the sialidase NanA in cell-cell interactions was then investigated.
Materials and Methods: DIGE experiments were performed as described in Caterino M. et al (2009) *J. Proteome Res* 8, 1515-26. The spots of interest were analyzed by LC-MSMS.
Results: Among the downregulated proteins, our data showed a severe decreasing in the sialidase N-acetylneuraminate lyase (NanA), a protein involved in cell-cell interactions.
Experiments of biofilm formation and bacterial adhesion and invasion on eukaryotic cells carried out using both pathogenic and non pathogenic *E. coli* strains showed that methylation stress affected bacterial adhesive properties. The pivotal role of NanA in this mechanism was demonstrated by using a null *nanA* mutant, indicating that the MMS-dependent reduction in *E.coli* adhesion properties is mediated by down-regulation of NanA.
Discussion and Conclusions: Following methylation stress, *E.coli* shows a general down regulation of proteins involved both in cell wall structure and in metabolic pathways.

Since NanA was heavily downregulated upon MMS treatment, we investigated the biological role of the sialidase in biofilm formation and adhesion/invasion capabilities of both pathogenic and non pathogenic *E.coli* strains. Biological assays using a null *nanA* mutant and a specific sialidase inhibitor demonstrated that the effect of methylation stress on biofilm formation is mediated by NanA and the absence of sialidase activity strongly decreases the bacterial adhesive properties. These data point to a pivotal role of NanA in biofilm formation and invasive properties of pathogenic *E.coli* indicating that the sialidase might represent a new target to counteract bacterial infections.

C 014

CLINICAL *BACILLUS* ISOLATES: IDENTIFICATION AND VIRULENCE DETERMINANTS

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Introduction: Despite often considered as contaminants in clinical cultures, *Bacillus* spp. can cause serious human diseases that frequently present in primary care settings. This study aimed to i) evaluate the use of matrix-assisted laser desorption–ionization time of flight mass spectrometry (MALDI-TOF MS) for the identification of clinical *Bacillus* isolates, and ii) decipher the pathogenic potential of these strains by evaluating specific virulence determinants and motility factors.
Materials and Methods: Clinical *Bacillus* isolates (n = 76) were collected from deep or superficial body sites over a 2-year period. All the isolates were subjected to biochemical and MALDI-TOF MS identification. In the case of identification discrepancy or failure, 16S rRNA gene sequencing was performed. The pathogenic potential of each strain was determined by evaluating production of hemolysins, hemolysin-BL (HBL), phosphatidylcholine-specific phospholipase C (PC-PLC), and proteases, as well as by searching for toxin encoding genes (phosphatidylinositole phospholipase C, *plcA*; sphingomyelinase, *sph;* cytotoxin K, *cytK;* non-hemolytic enterotoxin, *nheA, nheB, nheC*). Other virulence traits such as motility, swarming, biofilm formation and antibiotic resistance were also assessed.
Results: MALDI-TOF MS was significantly better than the biochemical system for correct species identification (81.6% vs 59.8%). All *B. cereus* and 93% of *B. pumilus* isolates were hemolytic. Production of HBL and PC-PLC was a feature of *B. cereus* strains. Extracellular proteases were detected in almost all species. *B. cereus* and *B. pumilus* were the only species possessing all three genes encoding the NHE complex. Most of the isolates were able to swim (81%), swarm (66%), or produce biofilms (89%). *B. cereus, B. licheniformis, B. simplex,* and *B. mycoides* strains were resistant to penicillin, as well as three out of the four *Paenibacillus* spp. isolates. Almost all the isolates were susceptible to tetracycline.
Discussion and Conclusions: Herein, we demonstrate that soil-related *Bacillus* species are frequently isolated from clinical samples. MALDI-TOF MS proved useful for rapid and accurate *Bacillus* spp. identification. In addition to their ability to produce spores, the variety of virulence factors expressed by *Bacillus* isolates and their resistance to penicillin can explain the growing relevance of these bacteria in human diseases.

C 015

ROLE OF THE GLUTAMATE DECARBOXYLASE (GAD)-DEPENDENT SYSTEM OF *BRUCELLA SPP* IN THE RESISTANCE TO EXTREME ACID STRESS

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Introduction: *Brucella* is the causative agent of

brucellosis, the major bacterial zoonosis worldwide. This debilitating infection is transmitted to humans through direct contact with infected tissues, inhalation of airborne bacteria and, mainly, by ingestion of contaminated and unpasteurised dairy products. In the last years, new species and atypical strains of *Brucella* (as *B. microti, B. inopinata* and isolates from bullfrog) have been isolated from unusual hosts. These strains are more acid-resistant (AR) than the classical *Brucella* species (as *B. abortus, B. melitensis,* and *B. suis*). In several food-borne pathogens such as *Escherichia coli*, the glutamate decarboxylase (GAD)-dependent system and the glutaminase are most efficient for survival under extreme acid stress. Recently, our team has demonstrated that the GAD system of *B. microti* allows survival of the bacterium at pH 2.5 and contributes to murine infection by oral route [1].

Materials and Methods: The existence of potentially functional GAD systems in the new *Brucella* species was investigated by an *in silico* analysis of their genome sequences. The role of the GAD system in the resistance of these strains to acid stress was studied by bacteriological, biochemical and genetic approaches.

Results: In contrast to the classical *Brucella* species studied, new and atypical strains were found GAD-positive (established by a simple qualitative colorimetric test), able to export GABA, and AR in the presence of glutamate or glutamine. Functional complementation of a *gad* mutant of *E. coli* with the *gad* loci of these new strains demonstrates strong homologies between the 2 systems.

Discussion and Conclusions: A functional GAD system may contribute to improving the adaptability of new species of *Brucella* in certain natural habitats and/or in the gastrointestinal apparatus of their hosts. Furthermore, the GAD phenotype may be a useful diagnostic tool to distinguish the new and atypical Brucella strains from classical terrestrial pathogenic species and closely related *Ochrobactrum* species [2].

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C 016

A COMBINATION OF TWO MENINGOCOCCAL ANTIGENS GLTT AND HrpA AS POTENTIAL VACCINE TARGET

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Introduction: Although vaccines have been developed against major *Neisseria meningitidis* serogroups, an universal vaccine remains a challenge due to successful immune evasion strategies of the meningococcus. The recent vaccine formulations are composed by a combination of surface antigens, but their efficacy is not yet determined, because they may not protect all recipients against meningococcal strains. From these considerations comes the need to identify new protective antigens to develop effective immunoprophylaxis. We propose a combination of two proteins essential for bacterial survival and virulence: the periplasmatic component of ABC type GltT transporter for L-Glutamate and hemagglutinin/hemolysin related protein, HrpA. The pivotal role of GltT transporter for the establishment of systemic meningococcal infection and the functional homology of HrpA protein with the main constituent of the pertussis vaccine, render these two proteins interesting as vaccinal candidates.

Materials and Methods: The above mentioned proteins were expressed in pGEX2TK vector in fusion with the glutathione S-transferase using *E. coli* BL21 (DE3) cells and successfully purified. GltT protein has been used in active immunization assay in murine host to produce specific antibodies. To verify the ability of obtained anti-sera to induce *N. meningitidis* killing, we performed an *in vitro* serum bactericidal assay.

Results: The GltT anti-serum showed the ability to kill bacteria both alone and with complement indi-

cating that GltT represent a good candidate as vaccine target. Currently, we are proceeding with massive purification of HrpA antigen in order to perform active immunization assays.
Discussion and Conclusions: The obtained results so far indicate that the GltT protein shows a high immunogenicity in the murine host and antibodies against-GltT mediate a significant bactericidal effect on meningococcal growth. This protein component in combination with the HrpA protein could represent a cocktail antigenic effective against the main serogroups responsible of meningococcal disease.

C 017

THE RAPIDLY EVOLVING EPIDEMIOLOGICAL LANDSCAPE OF TUBERCULOSIS IN SICILY: INSIGHTS FROM MOLECULAR TYPING

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Introduction: Epidemiology of tuberculosis (TB) in Sicily is evolving with the most striking feature being the rapidly increasing proportion of cases in the foreign-born population.

Materials and Methods: An observational study of the TB notifications in the five-year period 2010-2014 has been performed by analyzing the *Sistema Informativo delle Malattie Infettive* (SIMI) database. A molecular epidemiological analysis of 151 isolates of *Mycobacterium tuberculosis* complex (MTBC), of which 81 from foreign-born patients, identified in Palermo in the years 2012-2013, was also performed by spoligotyping and 24-loci mycobacterial interspersed repetitive units - variable number of tandem repeats (MIRU-VNTR) typing. Susceptibility testing to streptomycin, isoniazid, rifampin and ethambutol was also carried out.
Results: ln the period 2010-2014, 1124 new TB cases have been notified in Sicily with a mean annual notification rate of 4.50 cases per 100.000 inhabitants, ranging between 3.96 in 2011 and 5.00 in 2014. The proportion of TB cases in the foreign-born individuals was increasing from 36.4 in 2010 to 58.8 in 2014. The median age of foreign-born patients was significantly lower than the Italian-born (30.75 vs. 48.92 years, $p < 0.001$). Moreover, the distribution by age class was also significantly different, with the largest proportion of TB cases among the foreign-born sub-population in the 15-44 age class, whereas the Italian-born cases were equally distributed in the 15-44, 45-64 and > 64 age classes.
Fourteen lineages and 33 sublineages showing a different distribution among the two patients sub-populations were detected. Of interest, five M. bovis isolates, of which two BCG, were identified from Italian patients. Only two multidrug resistant (MDR) MTBC isolates were identified from an Italian born elderly patient and an Eritrean young patient (Beijing lineage), respectively.
Discussion and Conclusions: TB epidemiology in Sicily is a complex mix of reactivation in the autochthonous population and in sub-populations of immigrants from high endemic countries and recent transmission among vulnerable subgroups of both foreign and Italian origin. An integrated approach using both conventional and molecular tools is necessary to accurately assess and monitor TB epidemiology in this geographical area.

C 018

INDOLICIDIN AND SILVER NANOPARTICLES: ANTIMICROBIAL AND ANTI-BIOFILM ACTIVITIES

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Introduction: Antimicrobial peptides (AMPs) and silver nanoparticles (AgNPs) have attracted increasing attention due to their potent antibacterial activity. The combined use of AMPs and AgNPs seems to be very versatile and they can be involved in multiple interactions and are becoming of huge interest as an alternative to antibiotics. Among AMPs, indolicidin is a peptide with extremely high tryptophan content, exhibits broad-spectrum antimicrobial activity which is, nevertheless, accompanied by a high hemolytic activity.
The aim of our study is to combine AgNPs with the indolicidin peptide (IndAgNP) and to evaluate the possible antimicrobial activity of IndAgNP and its capacity to inhibit biofilm formation and to disaggregate mature biofilms.

Materials and methods: Indolicidin was synthesized using the standard solid-phase-9-fluorenylmethoxycarbonyl (Fmoc) method. Silver colloids were prepared in the presence of Indolicidin using Electron Microscope. Antibacterial activity of IndAgNP was analyzed by minimal inhibitory concentration (MIC). The morphology of silver nanoparticles was analyzed using a transmission microscope on three model bacteria strains: *Escherichia coli* ATCC 11219, *Pseudomonas aeruginosa* ATCC 13388 and *Staphylococcus aureus* ATCC 6538. The model bacteria strains utilized to evaluate the effect of compound on microbial biofilm were *Proteus mirabilis* and *Pseudomonas aeruginosa*, both biofilm-producing bacteria. Inhibition of biofilm formation and established biofilms was measured.
Results: The compound IndAgNP is provided with a stronger antibacterial effect with similar MIC value for both Gram-positive and Gram-negative bacteria. Quantitative tests on the biofilm of *P. aeruginosa* and *P. mirabilis* showed a reduction of its formation. Finally, through observation by confocal microscopy, it is observed a bactericidal effect and a marked disorganization of the biofilm.
Discussion and Conclusions: According to the study conducted, IndAgNP shows a marked bactericidal effect both on bacteria "in suspension" and on forms organized in biofilms. This can be a favorable prerequisite for the use of such a compound in vivo, as additional therapeutic agent and/or alternative to conventional antibiotics

POSTERS

Virology

P 001

DETERMINATION OF LOW HCV VIRAL LOAD BY ABBOTT REALTIME HCV ULTRASENSITIVE PROTOCOL AFTER DIRECT ACTING ANTIVIRAL AGENTS THERAPY

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Introduction: New drugs called Direct Acting Antiviral Agents (DAAs) have been developed to clear chronic HCV infection. Highly sensitive quantitative real-time PCR assays are recommended to monitor patients on antiviral therapy. The aim of the study is to assess whether patients with detectable but not quantifiable viremia have more frequently a return of viral replication compared to patients with undetectable viral load.
Material and Methods: An ultrasensitive (US) protocol was designed by introducing a modification of the Abbott RealTi*m*e HCV assay: larger sample input volume (1 ml) was used to detect and quantify HCV RNA below the validated LOD (Limit of detection) of 12 IU/ml of the standard assay. RNA extraction was performed with the Abbott *m*2000sp and amplification/quantification with the Abbott *m*2000rt.
To evaluate the analytical performances of the US protocol a clinical sample with an HCV RNA concentration of 304292 IU/ml pre-determined by the Abbott RealTi*m*e HCV assay was diluted with Basematrix to the target concentrations of 51.4, 25.7, 12.84, 6.42, 3.21 1.60 and 0.80 IU/ml, respectively.
To evaluate intra-run variation and the LOD, ten replicates of each dilution were tested in 1 run and to evaluate inter-run variability, 3 replicates of each dilution were analyzed in 4 runs.
Results: The modified protocol showed high detection rate in the range from 51.4 IU/ml to 0.80 IU/ml: 82.8% (58/70). The LOD calculated by probit analysis following the CLSI Guideline EP17-A2 was 4.381 IU/ml or 0.6416 log IU/ml. The intra-run CV % spanned from 13.6% to 43.3% based on the quantified results. The CV % across 4 runs per target concentration with 3 replicates each, respectively, spanned from 18.9 % to 82.3% based on the quantified results.
Discussion and Conclusions: The US protocol of the Abbott RealTi*m*e HCV showed high precision and adequate LOD and it is thus appropriate to evaluate the predictive value of very low on-treatment HCV RNA concentrations for DAA therapies.

P 001A

SPECIFIC HPV GENOTYPES DISTRIBUTION IN *CHLAMYDIA TRACHOMATIS* CO-INFECTION IN A LARGE COHORT OF WOMEN FROM NORTH-EAST ITALY

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Introduction: Infections caused by *Chlamydia trachomatis* (CT) and human papillomavirus (HPV) are the two main sexually transmitted infections showing potential oncogenic characteristics linked to persistence of infection and cervical dysplasia. CT may damage the mucosal barrier thereby supporting HPV cell entry, and chronic inflammation produces a local immune perturbation decreasing the number of antigen-presenting cells that influence the clearance of the HPV. Epidemiological data on Italian women with CT chronic infection, CT/HPV co-infection and HPV genotypes in the setting of co-infection are only preliminary.
Materials and Methods: CT/HPV co-infection and distribution of HPV genotypes were investigated in 6214 cervical swabs using Luminex technology. A quantitative Real time-PCR was performed to assess CT chronic infection by Hsp60 gene expression. Moreover, HPV genotypes were investigated in a subgroup of 921 women at risk for HPV

infection for data comparison. Results: The overall prevalence of CT/HPV co-infection was of 58%, of which 57% showed a chronic infection. The highest prevalence was found in women ≤ 25y (68%), with chronic infections reaching 72%. In this group, HPV multiple infections were found in 78% of samples. HPV genotypes distribution showed that specific genotypes (HPV6-31-42-44-51-56-66-73) were strongly associated with CT.
Discussion and Conclusions: The study indicated a high frequency of co-detection of multiple HPV genotypes and CT chronic infection in young women suggesting that CT-Hsp60 gene expression may positively select uncommon HPV genotypes as compared to national data. In conclusion, the increased association among CT and HPV multiple genotypes and young age supports the assumption that CT may increase the risk of pre-cancer lesions in elderly, in line with the active role of CT in favoring HPV and cell transformation.

P 002

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) AND BOVINE HERPESVIRUS 1 INFECTION IN CATTLE: AN EPIDEMIOLOGICAL ANALYSIS IN CAMPANIA REGION (ITALY)

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Introduction: 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD), commonly known as dioxin, is a toxic and persistent environmental contaminant which induces immune suppression and increased susceptibility to infectious agents. Bovine herpesvirus 1 (BHV-1), a cattle pathogen, can provoke infectious bovine rhinotracheitis (IBR), genital infections, conjunctivitis, abortions, encephalitis or other fatal diseases in newborns. BHV-1 induced immune suppression which may lead to secondary bacterial infections that can cause pneumonia. Hence, BHV-1 infection might cause substantial economic loss in the cattle industry. Following BHV-1 infection in bovine cells (MDBK), we previously showed that TCDD influences the infection by increasing virus replication. Moreover, TCDD anticipates BHV-1-induced apoptosis, by accelerating the down-regulation of telomerase activity when virus-induced apoptosis occurred, and by anticipating in the cytoplasm the presence of bICP0, the main transcriptional regulatory protein of BHV-1. Furthermore, TCDD enhances the free intracellular iron availability, which might promote the onset of BHV-1 infection and render bovine cells more vulnerable to the virus.
Objectives: Herein, we performed an epidemiological analysis on prevalence of IBR in some areas where high levels of TCDD have been detected in dairy products.
Materials and Methods: We collected serum and plasma samples to detect antibodies for IBR from cattle raised on farms in Campania Region (Italy), by using IBR-gB and IBR-gE E.L.I.S.A. kit, which represents the test procedure of choice in many European IBR programs.
Results: We revealed a significant prevalence of IBR on samples collected from farms in contaminated areas compared to uncontaminated areas.
Discussion and Conclusions: Very low doses of TCDD cause the damage previously showed in bovine cells. In addition, herein we provided evidence that TCDD influences BHV-1 infection, promoting the prevalence of IBR in cattle. Thus, TCDD may act as an additional risk factor for progression of BHV-1 infection in cattle. We suggest that this risk should be given adequate consideration in the care of farm animal health.

P 003

HIGH FREQUENCY OF JCV DNA DETECTION IN PROSTATE CANCER TISSUES

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Introduction: Prostate cancer (PC) represents the most frequently diagnosed cancer in men. Exposure to infectious agents has been considered to induce prostatic inflammation and cancerous transformation. Controversial data exist concerning the role of the human polyomaviruses BK (BKV) and JC (JCV) in PC etiology. Therefore, a possible association between these polyomaviruses and PC was investigated.
Materials and Methods: Urine, blood and fresh prostatic tissue specimens were collected from 26 patients with PC. The presence of BKV and JCV, the possible non-coding control region (NCCR) variations and the genotyping analysis of the viral protein 1 (VP1) of both viruses were assessed.
Results: Data showed a preferential viral reactivation in the urinary compartment and a statistically significant prevalence of JC viruria and of BKV in PC tissues. A BKV DDP-like NCCR sequence was isolated in two patients, whereas JCV NCCR was consistently of an archetypal structural organization. A prevalence of the European genotypes was observed for both viruses.
Discussion and Conclusions: Our data demonstrated the presence of JCV DNA in approximatively 60% of cancerous prostatic tissue specimens, confirming the results obtained in a previous study, in which JCV has been defined as common inhabitant of the prostate, and opening the discussion about its potential role in PC.

P 003A

DIFFERENT INFECTION PATTERNS IN PATIENTS DISPLAYING ABNORMAL SUSCEPTIBILITY TO HPV ASSOCIATED WITH DEVELOPMENT OF MULTIPLE SKIN AND MUCOSAL LESIONS

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Objectives: A crucial role in determining the clinical outcome of a given HPV infection is played by host-related factors. Here, we present the data obtained from 7 patients with abnormal susceptibility to HPV infection.
Methods and Results: In these patients, HPV infection has been characterized by immunostaining for viral proteins (E4 and L1) and viral genome replication (FISH) in tumour tissue sections, PCR analysis in eyebrow hair bulbs, and deep-sequencing of virion preps from skin swabs. Genotype-specific infection pattern was defined by E4 immunostaining. Mutations in the *EVER* genes were only found in 2 patients (*EVER2* gene), despite the fact that they all displayed EV-like clinical features. General immunophenotyping on whole-blood samples revealed lower frequencies of CD4+ T cells in comparison with healthy individuals in 5 patients; the two EVER2-null patients failed to reveal any abnormalities. Unbiased deep-sequencing analysis on skin swab virion preps from four of them revealed a mixture of genus *Alpha, Beta*, and *Gamma* HPVs, including new types and species but neither any new HPV genera nor any polyomaviruses. Notably only *Beta*-HPVs were detected in EVER2-null background. A variety of new Anelloviruses were found in skin swabs from one patient.
Conclusions: These patients are massively infected by HPVs but not skin-tropic polyomaviruses, and subjects carrying EVER genetic abnormalities display less severe immune defects and a unique HPV signature restricted to beta genotypes. HPV overproduction occurring in these patients may interfere

with or prevent the replication of the other common viral components of the skin microbiota.

P 004

HUMAN POLYOMAVIRUS JC REPLICATION IN MULTIPLE SCLEROSIS PATIENTS TREATED WITH NATALIZUMAB: VIRAL MARKERS AS TOOLS FOR AN EARLY PML DIAGNOSIS?

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Introduction: The human polyomavirus JC (JCV) is the aetiological agent of the demyelinating disease progressive multifocal leukoencephalopathy (PML). PML onset represents a growing concern about the safety of Natalizumab, a biological drug used to treat multiple sclerosis. The precise mechanism by which this medication may have facilitated PML pathogenesis is a matter of debate. On these bases, JCV infection monitoring, the possible non-coding control region (NCCR) variations and the genotyping analysis of the Viral Protein 1 (VP1) were investigated.
Materials and Methods: JCV-specific quantitative PCR was performed on biological samples, collected at the enrollment (t0) and every 4 months in the first year (t1,t2,t3) and at two time points (t4,t5) in the second year of Natalizumab treatment. PCR products corresponding to JCV NCCR and VP1 were sequenced. JCV-specific antibodies were assessed by STRATIFY JCV® in serum at t0 and t3.
Results: A significant correlation between patients with viruria and positive JC-specific antibody response and patients without JCV-specific antibodies after 1 year of natalizumab was found. The prevalence of viremia rather than JC viruria was observed only in the second year of treatment. Regarding NCCR, sequencing revealed the presence of 4 rearranged structures in peripheral blood mononuclear cells of patients with JC-specific antibodies after 12 natalizumab infusions (t3). In particular, two of them were compatible with the neurotropic variant found in a PML patient. Finally, VP1 sequence analysis showed the prevalence of genotypes 1A, 1B and 4.
Discussion and Conclusions: In summary, JC viruria evaluation seems to be useful to identify early those patients who do not already develop a humoral immune response against JCV. It may also be important to study NCCR rearrangements since they could give us new insights on the onset of neuro-invasive viral variants.

P 004A

SULFONAMIDE COMPOUNDS INHIBIT LATENT HUMAN HERPESVIRUS 8 REPLICATION BY INTEFERING WITH MDM2-P53 COMPLEX FORMATION

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Introduction: Human Herpesvirus 8 (HHV8) is the causing agent of Kaposi sarcoma (KS) and also of some other lymphoproliferative pathologies. KS can be present in different forms, namely classical, iatrogenic, and post-transplant, but the necessary condition for its onset is a substantial decrease in immune system efficiency, as usually happens in AIDS and in post-transplant and anti-cancer immunosuppressive therapies.
KS is cured as a normal skin angiosarcoma by the use of chemiotherapic drugs and immunomodulators, such as anthracyclines and interferon; typical anti-AIDS compounds are also usually employed for KS treatment. However, the anti-tumor treatment can even depress the immune system often

leading to therapy failures. Moreover, HHV8, after a brief lytic infection, enters into a state of latency inside the cells, which persists for the cell's lifetime and involves the formation of stable complexes with cell proteins such as p53 and MDM2. In this case, anti-cancer chemotherapy can slow KS evolution, but is not able to definitively cure the disease.
Several drugs capable of inhibiting a lymphotropic herpesvirus, such as latent Epstein-Barr virus, have recently been described [1]. In addition, nimesulide has also been found to block latent HHV8 replication.
Methods and Experimental Design: In this study we examined some sulfonamide derivatives for their ability to suppress the replication of HHV8 in human endothelial HUVEC cells. Clearance of viral DNA and viral proteins was detected by the use of rt-PCR and cytofluorimetry respectively. Furthermore, the ELISA test was used for studying the effect of sulfonamide drugs on the MDM2-p53 cellular complex.
Results: At a concentration of 50 μM some of the sulfonamide compounds tested were able to inhibit latent HHV8 in the infected cells by 60-95% after 6 days of contact. The ELISA test revealed that the sulfonamide drugs were able to disrupt the MDM2-p53 complex needed by the HHV8 to remain bound to the cell genome by a"tethering" condition.
Conclusions: Some sulfonamide drugs are able to clear latent HHV8 from infected human endothelial cells. These drugs can be a convenient alternative or a complementary KS treatment for the complete cure of this threatening disease.

1. Li et al. (2010) PLoS ONE 5(4): e10126

P 005

RELATIONS BETWEEN SERUM REACTIVE OXYGEN METABOLITES AND ALPHAHERPESVIRUS INFECTION IN ITALIAN MEDITERRANEAN BUFFALOES - *BUBALUS BUBALIS*

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Introduction: In recent years, oxidative stress has been postulated to be an important factor in pathogenesis and development of diseases. Reactive Oxygen Species (ROS) are well known for being both beneficial and deleterious and have long been known to be a component of the killing response of immune cells to microbial invasion. Measuring the free radical activity in serum samples is the best way to investigate the association between oxidative stress and infectious diseases. The aim of this study is to determine the association between alphaherpesvirus infection and serum reactive oxygen metabolites (ROM) in Italian Mediterranean buffaloes (*Bubalus bubalis*).
Materials and Methods: The presence of antibodies against bovine herpesvirus type 1 (BoHV-1) or bubaline herpesvirus type 1 (BuHV-1) was investigated by enzyme-linked immunosorbent assay (anti-gB/gE blocking ELISA) (Idexx).
We carried out the derivatives of ROM (d-ROM) and the antioxidant activity (anti-ROM) tests by spectrophotometry in sera of BoHV-1 (A) or BuHV-1 (B) infected animals compared to seronegative animals (C).
Results: The concentrations of d-ROM in the three groups, expressed in Carratelli Units (U-CARR) were, respectively, 64.6 ± 11.6, 85.0 ± 10.7 and 56.2 ± 10.5. Although the serum d-ROM levels in group A and B were higher than that in group C, only the group B compared with the control group C was significant at $p < 0.05$. At anti-ROM test, the three groups of animals showed values respectively of: 287.5 ± 66.1, 318 ± 64.3 and 276 ± 53.0. The

results of group A and B compared to that of control group C were not significant.
The oxidative stress index (OSI), calculated as d-ROM/anti-ROM x 100, showed significant differences between the groups B (26) and C (20) whereas no significant difference was detected between the groups A (22) and C (20).
Discussion and Conclusions: Our data indicate that in alphaherpesvirus infected buffaloes, in particular those in BuHV-1 infected, the d-ROM and the OSI values were significantly increased. These results suggest that the presence of BuHV-1, the virus species-specific, seems to induce a worsening balance in ROM levels, whereas that of BoHV-1 has no substantial effects. Future studies are needed to assess the role of oxidative stress in *Bubalus bubalis* infected by alphaherpesvirus.

P 005A

STUDY OF ARCHIVED HIV-1 RESISTANCE MUTATIONS IN MULTIDRUG-EXPERIENCED HIV-1-INFECTED PATIENTS TREATED WITH A SALVAGE REGIMEN: 4-YEARS FOLLOW-UP

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Introduction: The role and the stability of mutational archive during HIV-1 infection have not been fully addressed. The aim of this study was to examine the changes in intracellular drug resistance mutations (DRMs) and to assess the ability of archived viral variants to re-emerge in 20 multidrug-experienced HIV-1 positive patients during 4 years follow-up of darunavir/ritonavir(DRV/r)-based salvage therapy.
Methods: All individuals were multidrug-experienced, with a mean treatment time of 17.6 years. Plasma HIV-RNA levels were monitored by Versant kPCR (Siemens) and intracellular DRMs were examined in PBMCs at T0 and after 18, 36, 54, 72, 176 and 192 weeks, (Siemens). Phylogenetic analysis was performed to investigate the evolution of archived variants; phylogenetic trees were generated with the generaltime reversible model (GTR+I+G) of nucleotide substitution. Fixed and Random Effects Likelihood were used to identify the sites under positive selection pressure. Hypermut 2.0 program (www.hiv.lanl.gov) was used to identify hypermutations in pol gene sequence.
Results: During follow-up all patients maintained undetectable viremia. At T0 all patients showed high number of DRMs, the mean being 12 (±5.2). In the majority of patients a fluctuation in the number of mutations was detected. Moreover, the analysis of DRV/r DRMs revealed that 18 patients had intracellular mutations associated with resistance at T0, 6 of them with score > 3.5; after 192 weeks, in 4 patients the score changed, owing to loss and/or acquisition of some DRV/r DRMs. Phylogenetic analysis revealed that most of viral variants displayed polymorphisms in pol region, but only in 2 patients significant evolutionary divergence was observed. Analysis of codon and amino acid changes has provided statistical evidence that 3 codon are under positive selection pressure, suggesting an evolution of the virus. Moreover, pol gene sequence, did not show the presence of APOBEC-induced hypermutations, suggesting that that archived viruses could be potentially able to replicate.
Conclusions: In multidrug-resistant patients treated with salvage therapy, the archived drug-resistant viral variants may change during suppressive ART favoring the evolution of the virus; however the presence of DRMs associate to DRV does not affect virological success.

P 006

MOLECULAR CHARACTERIZATION OF THE FIRST EBOLA VIRUS ISOLATED IN ITALY FROM A HEALTH CARE WORKER REPATRIATED FROM SIERRA LEONE

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Introduction: Until recently, the virus Ebola was a rarely encountered human pathogen that caused small size outbreaks with extraordinarily high lethality. At the end of 2013, in Western Africa, Zaire Ebola virus (EBOV) initiated an unprecedented disease outbreak that is still ongoing, causing thousands of deaths.
Materials and Methods: The full genome sequence of a recent EBOV isolate obtained from a health care worker repatriated from Sierra Leone to Italy in late November 2014 was obtained, and was analyzed in respect of possible mutations affecting diagnostic and therapeutic targets as well as of amino acid signatures hypothesized to play a role in enhanced virulence, at least in animal models. The INMI isolate sequence was aligned with routinely used primers and probe sets, to assess the potential impact of SNV on diagnostic PCR efficiency.
Results: The INMI isolate felt in the clade 3 of Western Africa sequences, with most mutations being synonymous. Single Nucleotide Variants (SNV) of the INMI isolate are located both in coding and in noncoding regions. Eleven SNV are unique for the INMI isolate: 6 intergenic, 1 synonymous in VP24 gene, 2 nonsynonymous, 1 synonymous and 1 synonymous back mutation in L gene. Concerning the impact on diagnostic PCR efficiency, two unique variations were identified in the forward primer of a described RT-PCR targeting L gene. As compared to EBOV genomes used for drug design, some SNV were identified in the siRNA target regions, none in the PMOs target regions; several amino acid mutations were identified in the antibody-binding sites, whose significance deserves further evaluation. No mutations potentially associated, at least in animal models, with increased pathogenicity, were identified.
Discussion and Conclusion: The INMI1 isolate sequence shows high similarity with the previously published sequences from the western African outbreak, grouping in the phylogenetic maximum-likelihood tree within the Sierra Leone clade 3.

P 006A

LIVER microRNA hsa-miR-125a-5p MAY EXERT AN ONCOSUPPRESSOR EFFECT ON HEPATOCELLULAR CARCINOMA (HCC)

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Introduction: MicroRNAs are small non-coding RNAs that modulate gene expression at post-transcriptional level, playing a crucial role in cell differentiation and development. To evaluate in patients with HCC the hsa-miR-125a-5p concentration in HCC tissue and in non-HCC liver tissue to ascertain whether this microRNAs may have a clinical relevance.
Materials and methods: 45 consecutive patients with liver cirrhosis and HCC (31 at first diagnosis and 14 with a recidivate HCC) enrolled from June 2013 to April 2014 underwent a diagnostic liver biopsy: The mean age was 69 ± 6.5 years and 51.1% of patients were males. The etiologic agent was

HCV in 35 patients, HBV in 5 and the remaining 5 had NASH-related cirrhosis (Chiild-Pugh class A in 88.9% of cases and class B in 11.1%). Twenty-six (64.4%) showed a unifocal and 19 (35.6%) a multifocal HCC. According to the Barcelona Clinic Liver Cancer (BCLC) class, 39 (86.7%) had class A, 5 (11.1%) class B and 1 (2.2%) class C. Two patients showed also portal thrombosis.
For each patient, real-time PCR was used to quantify miR-125a-5p in HCC and non-HCC liver tissue in relation to RNU6B.
Results: Considering all 45 HCC patients, lower levels of hsa-miR- 125a-5p were observed in HCC tissue than in non-HCC liver tissues (M ± SD 4.84 ± 3.69 vs. 8.27 ± 4.5 A.U., p = 0.0002). This difference was highly significant to statistical analysis in the 35 HCV-patients, 4.21 ± 3.47 vs. 7.79 ± 4.75 AU, p = 0.0009 and lower and not statistically significant significance in the 5 HBV-patients, 7.38 ± 2.08, vs. 10.7 ± 2.4AU, and. in the 5 patients with NASH-related cirrhosis, 6.72 ± 5.24 vs. 9.2 ± 3.99AU. In addition, the level of miR-125a-5p in the HCC tissue of the 35 HCV-related cirrhosis was lower than that observed in the 10 non-HCV patients (4.21 ± 3.47 vs. 7.05 ± 3.77 AU, p = 0.023). Besides, the levels of has-miR-125a-5p in HCC tissue were moderately lower in the 41 patients with Child-Pugh score A (4.67 ± 3.47 AU) than in the 4 with score B (7.05 ± 3.77 AU, p = 0.7), such as in the 40 patients with BCLC class A as compared with the 5 with class B or C (4.66 ± 3.78 vs. 6.31 ± 2.79 AU, p = 0.21).
Discussion and Conclusions: These data suggest an oncosuppressor effect of microRNA hsa-miR-125a-5p on HCV-related HCC.

P 007

BOVINE HERPESVIRUS TYPE-1 MARKER VACCINE INDUCES CROSS-PROTECTION AGAINST BUBALINE HERPESVIRUS TYPE 1 IN WATER BUFFALO

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Introduction: The water buffalo (Bubalus bubalis) is susceptible to various viral and bacterial pathogens causing infectious diseases in cattle. In particular, water buffaloes are susceptible to two herpesviruses: Bovine herpesvirus type 1 (BoHV-1) and a species-specific herpesvirus, Bubaline herpesvirus type 1 (BuHV-1). In this study, the protection from viral replication induced by an inactivated marker BoHV-1 based vaccine against BuHV-1 infections was investigated.
Materials and Methods: One group of water buffalo calves was immunized with an inactivated BoHV-1 marker vaccine. A second group was not vaccinated and used as control. In the post-vaccination period, we monitored the humoral immune response. The efficacy of the vaccine was tested after intranasal challenge of the calves with a BuHV-1 strain. Seroconversion was assayed by both Virus Neutralization as well as by competittive gB and gE Enzyme-linked immunoadsorbent assay (ELISAs). Viral Shedding was montiored by viral isolation and PCR assays.
Results: Our data shows that the vaccine was able to protect animals from BuHV-1 replication. Control animals showed high levels of virus shedding and mild signs associated with BuHV-1 infection, while calves immunized with the inactivated BoHV-1 marker vaccine did not show BuHV-1 clinical signs and significantly reduced virus shedding. Unvaccinated animals did not develop any antibody titres in the post-vaccination period and seroconverted 15 days after challenge. Following BuHV-1 challenge, VNT antibody levels continued to rise, in vaccinated groups reaching maximum titres of 2.6 log10 at 105 D.P.V.
Antibodies levels were also measured by ELISA tests. As for the VNT, during the post vaccination period the mock-infected animals did not develop any detectable serological reaction in the two ELISAs. In contrast, all vaccinated animals reacted positively in gB ELISA but, as expected, not reacted in gE ELISA.
Discussion: We observed that a cross-protection between BoHV-1 and BuHV-1 in buffalo calves exists and this could explain the rare occurrence of BuHV-1 clinical signs especially in regions where infections with BoHV-1 and BuHV-1 coexist.
The analysis of our data on gE ELISA shows that although sero-conversion was observed in the ELISA gB, the gE ELISA tests showed no seroconversion for E-glycoprotein in groups subjected to challenge with BuHV-1, both in controls and in the vaccinated groups. Our findings clearly demonstrated that gE-marker vaccinated and BuHV-1 infected animals share the same serological pattern and this aspect raise the need of a specific test able to clearly discriminate between BuHV-1 infected and gE marker vaccinated animals.

P 007A

HEPATITIS B VIRUS INFECTION IN IRREGULAR AND REFUGEE MIGRANTS IN NAPLES, ITALY

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Introduction: To define the characteristic of HBV infection in a cohort of irregular or refugee immigrants living in Italy.
Materials and Methods: A screening for HBV, HCV and HIV infections was offered free of charge and of bureaucratic procedures to 1,254 illegal or refugee immigrants living from 5 years or more in Naples or in its surroundings (Italy). Of these 1,254, 1,212 (96.6%), accepted to be screened at one of 4 first-level clinical centers operating for years in this setting,. The median age of screened subjects was 32 years, range 12-74, 75.2% were males, 52% came from Sub-Saharan Africa, 18% from Eastern-Europe, 13% from Indo-Pakistan Area, 7% from Northern-Africa and the remaining 10% from others countries.
Results: One-hundred sixteen migrants (9.6%) were HBsAg positive, 490 (40.4%) HBsAg negative/anti-HBc positive and 606 (50%) sero-negative for both. All positive subjects ignored their serological status. A high HBsAg sero-prevalence was found in migrants from sub-saharan Africa (13.8%) and an intermediate one in those from Eastern Europe (6%), Northern Africa (3.4%) and Indo-Pakistan area (2.8%). A logistic regression analysis identifie as factors independently associated with HBV infection (both HBsAg positive and HBsAg negative/anti-HBc positive) the male gender ($p = 0.002$), the sub-Saharan African origin ($p < 0.0001$) and a low level of schooling ($p = 0.04$). Of the 116 HBsAg positive migrants, 78 (67.2%) were HBV-DNA positive, 11(14%) with HBV genotypes A, two (2.5%) with genotype C, 12 (15.4%) with genotype D and 53 (68%) with genotype E. Of the 116 HBsAg positive migrants, 79 (68.1%) concluded the diagnostic program, 58 (73.4%) were considered asymptomatic carriers, 17 (21.5%) with chronic hepatitis, 2 with liver cirrhosis and 2 with liver cirrhosis plus HCC.
Conclusions: The HBsAg sero-prevalence is very high in the immigrants investigated, particularly in those from Sub-Saharan Africa who are introducing HBV-genotype E in Italy.

P 008

CIRCULATION OF G12P[8] ROTAVIRUS STRAINS AMONG CHILDREN IN THE CENTRAL ITALIAN REGION OF UMBRIA DURING TWO CONSECUTIVE SEASONS (2012-13 AND 2013-14)

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Introduction: Group A rotaviruses (RVA) are the major cause of acute gastroenteritis in children worldwide. In 2007, RotaNet-Italy has activated a surveillance program for rotavirus acute gastroenteritis to investigate the diversity of rotavirus genotypes and the possible emergence of uncommon or novel genotypes. In Italy, as in other European countries, rotavirus gastroenteritis is mainly associated with five genotypes (G1P[8], G2P[4], G3P[8], G4P[8], and G9P[8]), but emerging strains are also reported sporadically. The aim of this study was to investigate the diffusion of an uncommon G12P[8] genotype, occurred in the Italian region of Umbria during two consecutive seasons (2012-13 and 2013-14), causing an important epidemic outbreak.
Materials and Methods: A total of 136 rotavirus

positive stool samples were collected from patients with acute gastroenteritis between September 2012 and August 2014. After viral RNA extraction, samples were genotyped for VP7 (G-type) and VP4 (P-type) genes and the aminoacid sequence was assessed, in accordance with the EuroRotaNet methods.
Results: During the first season, the emerging G12P[8] RVA genotype was detected in 69% of samples, followed by G1P[8] (14%), G2P[8] (7%), G3P[4] (5%) and G4P[8] (5%). During the second season, circulation of G12P[8] genotype decreased (18%), whereas G1P[8] was detected in 41% of samples followed by G9P[8], that didn't circulated in the previous season (25%), G4P[8] (13%) and G2P[4] (3%). In both seasons, G12P[8] infections were observed mostly in infants less than 1 year old. Sequence analysis of VP4 and VP7 genes showed close relationship between all Umbria G12P[8] stains and other global G12 strains, but revealed various substitution in the main antigenic regions between Italian G12 and vaccine strains.
Discussion and Conclusions: The rapid emergence in Umbria and the recent circulation in other Italian regions of uncommon G12P[8] genotype confirms that these strains might become a new common genotype in Italy. Uncommon genotypes such as G12P[8] infect mainly younger children, presumably because the weaker protection conferred by maternal antibodies. Since some Italian regions have introduced rotavirus vaccination, the continuous surveillance is important to obtain information about genotype distribution and evolution.

P 008A

INCREASING CIRCULATION OF NOROVIRUS GII.4 NO2009/SY2012 RECOMBINANT STRAIN

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Introduction: Noroviruses (NoVs) are important enteric pathogens of humans in both children and adults. GII.4 genotype is associated with the majority of NoV gastroenteritis outbreaks and sporadic cases worldwide. Starting from 2002 surveillance for NoV has been enacted in Palermo and from 2011 data are being shared in the Italian ISGEV network. In the 2012-13 winter season a new GII.4 variant, Sydney 2012 (Sy2012), progressively replaced worldwide the previous New Orleans 2009 (NO2009).
Materials and Methods: Stool samples from children hospitalized for gastroenteritis at the Di Cristina Hospital in Palermo were screened for NoV by RT-RealTime-PCR. NoV isolates were characterized by sequence analysis of both region A (ORF1) and region C (ORF2). When inconsistencies between region A and region C characterization suggested a recombinant origin, a 3' RACE-PCR protocol was used to generate a 3.2 kb amplicon encompassing the 3' end of ORF1, the full-length ORF2 and ORF3.
Results: During 2011-15 NoV infections progressively increased among children hospitalized for gastroenteritis in Palermo, from 12% in 2011 to 35.7% in early 2015. Characterization of NoV-positive samples showed a substitution in the GII.4 genotype variants. While NO2009 decreased from 65.1% in 2011 to 5.4% in 2013 and then disappeared, Sy2012 increased conversely, from 4.7% in 2011 to 77.4% in 2013. However, thereafter Sy2012 decreased to 59.4% in 2014 and represented only 10% of the isolates in 2015, being overcome by a novel recombinant GII.4 strain (recNO2009/Sy2012), possessing the ORF1 of the former variant NO2009. RecNO2009/Sy2012 already represented 9.7 and 12.5% of the isolates in 2013 and 2014, respectively, but was roughly half of the sampling at the beginning of 2015.
Discussion and Conclusions: NoV is on the way to replace Rotavirus as the first agent of gastroenteritis in children in Palermo, possibly due to the use of rotavirus vaccines. Recombinant GII.4 strains were already detected in 2012-14 but have become the leading NoV in Palermo in the last winter season. This finding is relevant for understanding the evolutionary pathways followed by NoV evolution. Continued surveillance for NoV infections will enable precise assessment of the public health implications of the continuous remodeling, also through recombination, of the pool of circulating strains.

P 009

CHARACTERIZATION OF CIRCULATING WILD-TYPE MEASLES VIRUSES IN SICILY (PALERMO AREA)

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Introduction: Measles virus (MV) based on the variability of 450 bps of C-terminal nucleoprotein (N-450) was classified in 23 genotypes that show a distinct geographic distributions. Genotypes contain multiple distinct lineages.
Although the WHO has planned the eradication of Measles in 2015, in 2011 large outbreaks of measles occurred in Italy and in many European countries. Aim of this study is to analyze the intra-genotype variability and to follow the importation and the spread of new MV strains in Sicily
Patients and Methods: The 450 bps of MV C-terminal nucleoprotein were sequenced from sera of 73 Sicilian patients(mean age 25, range 13–44 years) with symptomatic measles occurred between 2010 and 2011. A sequence obtained from a sporadic measles disease occurred in 2009 and 73 MV sequences from Gene Bank were included for phylogenetic analysis.
Results: Six MV strains were of genotype D4 and 68 of D8.The MV/D4 collected in 2009 was identical to MV/D4-Enfield variant, and 5 sequences collected in 2010-2011 differ ina synonymous mutation G1386T. Sequences with this nucleotide variation were never reported outside Italy.
The sequences of MV/D8 are included in the first and third clusters with strains detected in India. Sixty-one MV/D8 sequences named PA_2010-11, identical each other, was homologous to the well known variant MV/D8-Villupuram including in cluster 1, and 5 isolates that showed single nucleotide mismatches [G1223T (R408I), G1490A (R497K), G1494A, C1533T]. Two strains (PA_1768-10 and PA_1780-10) were related to cluster 3 of genotype D8 and were identical to the version found in Birmingham in 2006 and other than D8-Villupuram to 15 nucleotides C1152T, G1183A, G1225T, G1254A, T1269C, G1323A, T1359C, G1375C, C1383T, G1404A, C1412A, C1454T, C1483T, T1500C and G1525A that caused the amino acid mutations A409S, L473I and P485L in the C-terminal nucleoprotein.
Discussion and Conclusion: The MV/D4 strains identified in Palermo area highlight the wide circulation of MV/D4-Enfield variant and its introduction in Sicily regardless of the spread of Hamburg variant in Roma people, as happened in other European countries. Also,this study showed the co-circulation of two different lineages of MV-D8 and the multiple origins of the outbreaks.This finding highlights the necessity of national strategies based on investments, legislations and campaigns to encourage the adherence to vaccination.

P 009A

ANTIVIRAL ACTIVITY OF AGMA1 POLYMER AGAINST HUMAN PAPILLOMAVIRUSES AND PRECLINICAL STUDY AS A TOPICAL MICROBICIDE

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Introduction: Human papillomaviruses (HPV) are widespread human pathogens and many sexually transmitted types cause anogenital lesions including cancers. Indeed some viral types are highly oncogenic and therefore are named "high-risk HPV" (e.g. types 16, 18, 31, 45) . HPV is the only etiological agent of cervix carcinoma which caused 266 000 deaths in 2012. Currently two vaccines are available: Gardasil and Cervarix, but even in the era of HPV vaccination, effective inhibitors of HPV infection are required particularly in low resource settings where there is the highest burden of HPV infection.
The aim of this work was to assess the antiviral potency and the spectrum of activity of an amphoteric polyamidoamine, AGMA1 against a panel low-risk and high-risk HPV and to elucidate its mechanism

of action.
Materials and Methods: Pseudovirions of different types of HPV were generated and carachterized and then subjected to neutralization assays and to time of addition assays. Biacore technology was used to investigate AGMA1 interaction with heparin. Furthermore EpiVaginal tissues and lactobacilli of the normal flora were used to assess the biocompatibility of AGMA1.
Results: AGMA1 was found to be a potent inhibitor of mucosal HPV types (i.e., types 16, 31, 45, 6). The 50% inhibitory concentration was between 0.34 µg/ml ad 0.73 µg/ml and no evidence of cytotoxicity was observed. AGMA1 interacts with immobilized heparin and with cellular heparan sulfates preventing viral binding, moreover it is active also when added post viral attachment. Furthermore AGMA1 shows a good biocompatibility profile on vaginal lactobacilli and on human cervicovaginal histo-cultures along with a good stability in vaginal fluid.
Discussion and Conclusions: The findings from this study indicate AGMA1 prevents HPV attachment by masking HSPGs on the cell surface and suggest that AGMA1 can be a leading candidate compound for further development as an active ingredient of a topical microbicide against HPV and other sexually transmitted viral infections, since it is active also against HSV-2.

P 010

TORQUE TENO VIRUS IN FECAL SAMPLES FROM PATIENTS WITH INFLAMMATORY BOWEL DISEASE

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Introduction: Torque teno virus (TTV), a single-stranded DNA virus belonging to the *Anelloviridae* family is highly prevalent worldwide, and it is currently considered a component of the host microbiota. However, it is probable that TTV could have a pathological role at a high viral load, usually not reached in healthy individuals, or act as a cofactor during co-infection with other pathogens, and finally as trigger for impairment of immune surveillance. Inflammatory bowel disease (IBD) is a group of inflammatory conditions of the colon and small intestine including Crohn's disease (CD) and ulcerative colitis (UC). It is currently though that a deregulated immune response to the gut microbiota in a genetically susceptible host under certain environmental influences can contribute to the pathogenesis of the gut inflammation. The goal of this study was to determine the TTV prevalence in fecal samples of IBD patients and to investigate whether the TTV load correlates with the course of the disease.
Materials and Methods: TTV prevalence and viral load were evaluated in stool samples from 130 IBD patients and 56 healthy controls. DNA was extracted from 200 mg of stool, and TTV-specific TaqMan®-based qPCR assay was used to detect and quantify viral DNA.
Results: The prevalence of TTV DNA in stool was significantly higher in IBD patients (76.9%; 100/130) than in healthy controls (42.9%; 24/56) ($p < 0.0001$), but no significant difference was observed between CD (77.6%; 38/49) and UC (76.5%; 62/81) ($p > 0.05$). qPCR results showed that the mean TTV load in IBD patients was significantly increased (1.61 ± 0.82 $\log_{10}$ copies per mg of stool) compared to the healthy controls (0.38 ± 0.47 $\log_{10}$ copies per mg of stool) ($p < 0.0001$), but no significant difference was observed between CD and UC ($p > 0.05$).
Discussion and Conclusions: Our study shows that TTV prevalence and viral load in IBD patients stool are significantly higher than in healthy controls, and that dynamics of replication correlates with the different stages of the disease (active or dormant). Because the immunobiology of TTV and the immunopathology of IBD are still poorly understood, we can only speculate on the correlation between TTV load and pathogenesis/modulation of IBD. Further studies exploring the role of TTV as a surrogate marker of immune competence in patients with IBD could yield interesting outcomes.

P 010A

IMPACT OF HIV-1 SUBTYPE ON VIROLOGICAL RESPONSE TO FIRST LINE PI/r REGIMENS

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Introduction: We evaluated the impact on virological response of HIV-1 subtypes (B, CRF02_AG, C, F) at first line ritonavir-boosted protease inhibitor (PI/r) based regimens.
Materials and methods: The impact of HIV-1 subtype on virological-success (VS) and virological rebound (VR) was evaluated by survival analyses. The prevalence of subtype associated polymorphisms at baseline and the resistance at failure were explored.
Results: Among 1057 patients (pts), 83.3%, 8.0%, 4.8% and 3.8% were infected with B, CRF02_AG, F and C subtypes, respectively. Compared to subtype B, non-B infected pts were younger (years [IQR]: B, 40 [34-47] vs. non-B, 38 [31-44], p = 0.002), and showed a lower proportion of men (B: 81.2% vs. non-B: 55.1, p < 0.001) and of Italian native (B: 82.5% vs. non-B: 39.8%, p < 0.001). By 12 months of treatment, CRF02_AG infected pts showed a lower rate of VS compared to others (C: 93.7%; B: 90.1%; F: 88.9%; CRF02_AG: 75.4%; p = 0.076). By 24 months after VS, CRF02_AG infected pts showed also the highest probability of VR compared to others (34.2% vs. 15.0% in F, vs. 19.4% in B, vs. 20.8% in C, p = 0.026). By multivariable Cox regression (adjusting for gender, age, pre-therapy CD4/HIV-RNA and treatment), CRF02_AG infected pts showed a higher hazard of VR (RH [95% CI]: 1.97 [1.20-3.24, p = 0.007) compared to B infected pts. 23 out of 236 mutations detected were significantly associated to CRF02_AG. Among these mutations, by 24 months after VS, K20I, K70R or L89M were associated with a significantly higher rate of VR (mutation: present vs. absent; K20I: 31.7% vs. 19.4%, p = 0.019; K70R: 43.1% vs. 19.0%, p = 0.001; L89M: 26.7% vs. 19.2%, p = 0.026).
In 76 pts with a genotype at PI/r failure, 17.1% showed resistance (2.6% to PI; 13.5% to NRTI). CRF02_AG infected pts treated with lamivudine/emtricitabine showed at failure a significantly higher prevalence of M184V mutation compared to B subtype infected pts (B vs. CRF02_AG: 26.6% vs. 2.0%, p = 0.017).
Discussion and conclusions: Despite the high success rate in PI/r treated pts at first line regimen, CRF02_AG infected pts result more likely to experience VR. K20I, K70R and L89M mutations may correlate with this phenomenon. Further investigations are needed to clarify these findings.

P 011

MOLECULAR AND SEROLOGICAL STUDY OF *EPSTEIN BARR VIRUS* (EBV) IN PATIENTS WITH MULTIPLE MYELOMA

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Introduction: Multiple myeloma (MM) is a chronic lymphoproliferative disorder with a clonal proliferation of plasma cells. *Epstein Barr virus* (EBV) has been recognized as possible etiologic cofactor in the development of a chronic lymphoproliferative disease lymphoma, such as multiple myeloma. The aim of this study was to evaluate whether there is an association between multiple myeloma and EBV in Sardinian patients by serological and molecular methods.
Materials and Methods: This case-control study was performed on 34 blood samples of multiple myeloma patients and 25 Healthy donors controls. The patients and control groups were matched according to gender and age. Presence and levels of IgG antibodies against EBV antigen specific EBNA-1 and EA were evaluated using commercially available kit anti-EBV EBNA and EA IgG ELISA (Bio-Rad). In the next step specific DNA Real-time PCR

was carried out for detection of EBV genome, DNA was extracted from 10^6 cells, by DNAzol as described elsewhere (19). Selective amplification of the qualitative and quantitative EBV DNA BamHI W fragment was obtained by a real-time polymerase chain reaction (PCR) assay, data have been expressed according to the 2^{-DCt} Method.

Results: Sardinian MM patients were found to have increased prevalence of anti-EBNA-1 IgG (88% vs. only 60% of HD, p = 0.002) while anti-EA IgG only 8% in MM compared with 22 % of HD). About EBNA-1 and EA titers, there were differences in anti-EBNA-1 IgG levels between EBV-positive MM patients (69,9%) and HD (57,7%) whereas anti-EA IgG levels were significantly higher in HD (56,4%) than in MM patients (51,2%). We found EBV-DNA positivity was more frequent in MM PBMCs than in HD (73% vs. 42% respectively, p = 0.03). Similarly EBV relative load was increased in MM EBV positive patients than in HD EBV positive PBMCs [3.5 (3,8) vs. 0.9 (1) 2-DCt EBV-DNA, respectively, p = 0.002].

Discussion and Conclusions: The present study suggest an association between EBV infection and Multiple Myeloma. One of the possible explanations of the pathogenetic role of the virus may lie in the NFkB mechanism. In fact, It is closely involved in neoplastic plasma-cell proliferation and also involved in the life cycle of EBV and its oncogenic role. Future studies will be needed to clarify the possible implication of EBV in the development of the myeloma disease.

P 011A

POTENTIATION OF CAF ACTIVITY BY THYMOSIN ALPHA 1: LOOKING FOR NEW ANTIVIRAL PEPTIDES

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Background: Thymosin alpha-1 (Ta1) exploits a specific action on lymphoid cells and is able to induce a strong transcriptional response in peripheral blood mononuclear cells (PBMCs). CD8 antiviral factor (CAF) activity could play a role in the control or prevention of Human Immunodeficiency Virus 1 (HIV-1) infection by a non-cytolytic mechanisms. We aimed to investigate the ability of Ta1 to modulate the release by $CD8^+$ cells of soluble factors endowed with potential antiretroviral activity.

Methods: Supernatants from $CD8^+$ isolated cells, treated with LPS and Ta1, were screened on *in vitro* infection of human monocyte derived macrophages (MDMs) and PBMCs with HIV-1, and of PBMCs with human T lymphotropic virus 1 (HTLV-1). In $CD8^+$ cells, as well as in PBMCs of healthy donors as from HIV^+ individuals, a microarray analysis to assess the transcriptional response after treatment was performed.

Results: Ta1 potentiates in LPS stimulated $CD8^+$ cells the release of soluble factors, able to inhibit the *in vitro* HIV-1 infection of MDMs and PBMCs. Ta1-mediated CAF activity was effective also on the inhibition of the HTLV-1 *in vitro* infection of PBMCs. Distinctive transcriptional profile was induced by Ta1 in PBMCs from HIV+ donors.

Conclusions: These findings suggest a re-evalu-

ated approach of Ta1 in the antiretroviral therapy, to be used in combination with modern innovative treatments and with vaccine administration. Future studies are necessary to identify the players of the potentiated anti-HIV activity, accomplished by Ta1 in CD8+ cells. Proteomic analyses are in progress in our laboratory for the identification of possible novel antiretroviral peptides.

P 012

NITAZOXANIDE POTENTLY INHIBITS PARAMYXOVIRUS REPLICATION *IN VITRO*: EFFECT ON VIRAL GLYCOPROTEINS MATURATION

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Introduction: Nitazoxanide (NTZ), a safe, orally bioavailable drug licensed in the USA for treating *Cryptosporidium parvum* and *Giardia lamblia* infections, has been recently shown to possess antiviral activity against influenza and rotavirus infection. The molecular mechanism of NTZ antiviral activity has not been fully elucidated. In the case of influenza A virus infection, NTZ and its active metabolite tizoxanide (TIZ) were shown to inhibit virus replication by a novel mechanism, impairing hemagglutinin maturation and virus morphogenesis. Herein we investigated the effect of NTZ and TIZ treatment during Paramyxovirus infection *in vitro*, using Sendai virus (SeV) as a model, and explored the mechanism of the antiviral action.
Materials and Methods: Confluent monolayers of 37RC monkey kidney cells and human A549 alveolar type II-like epithelial cells were infected with SeV under single-step and multi-step growth conditions. NTZ and TIZ were dissolved in DMSO and diluted in culture medium before treatment. Virus yield was determined by hemagglutinin titration and infectivity assay, and cell viability was determined by MTT assay. Viral protein synthesis was characterized by SDS/PAGE-autoradiography after [^{35}S]methionine/cysteine-labeling, Endo H-digestion, Western-blot and immunofluorescence analysis.
Results: NTZ and TIZ caused a dose-dependent inhibition of SeV replication, with IC_{50} ranging from 0.26 to 1 mM, and SI ranging from > 50 to > 625, depending on the m.o.i. NTZ does not affect virus entry into target cells and does not cause a general inhibition of viral protein expression. Instead it acts at post-translational level by selectively inhibiting the maturation of the two viral glycoproteins: HN, with hemoagglutinating and neuroaminidase activity, and F, which plays an essential role in cell fusion and infectivity of the virion. Interestingly, NTZ and TIZ were also found to be effective against Respiratory Syncytial virus (RSV) type-A2.
Discussion and Conclusions: The results indicate that NTZ is the lead molecule in a novel class of antivirals possessing potent activity against Paramyxovirus infection. The fact that NTZ was also able to inhibit RSV replication suggests a general effect of the drug on Paramyxoviridae family members.

P 012A

LIPOSOMES AS DELIVERY SYSTEM FOR ACYCLOVIR AND AMINOMETHYL-NAPHTOQUINONES

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Introduction: Herpes viruses are leading causes of human infections which result in severe manifestations in neonates, immunocompromised and/or transplanted individuals. The most common drug used for the treatment of Herpes simplex virus-1 (HSV-1) infections is acyclovir but the wide use of this antiviral has been associated with toxicity and drug-resistant HSV strains. In previous work, our group demonstrated that aminomethylnaphtoquinone (AMNQ) were able to reduce HSV-1 replication being more effective than acyclovir. We analysed the effect of acyclovir and naphtoquinone (NQ) derivatives loaded in liposomes in HSV-1

replication to obtain biocompatible nanostructures using lower quantities of these compounds.
Material and Methods: Vero cells (2 x 10^5 cell/well) were infected with HSV-1 (MOI of 0.1), incubated for 1h and treated with 4 different concentrations of the liposomes for 20 h. The viral titer was determined in 12-multiwell plates (4 x 10^5cells/well) and infected with various dilutions (1:10) of the HSV.
Results: Using acyclovir with liposome as control we demonstrated that liposome could efficiently deliver the compound inside the cell in all concentrations tested. Moreover, all three compounds exhibited high antiviral activity in a dose-dependent manner. The compounds 1 and 2 at concentration of 5uM showed the lowest antiviral effect (74.5% and 76% of inhibition, respectively) comparable to ACV (68.75%). At 1uM all the liposomes were able to inhibit the viral replication, but AMNQ derivatives were more effective (48% and 66%, respectively for compound 1 and 2) than acyclovir (33%).
Discussion and Conclusion: Liposomes have been considered an optimal drug-carrier system because the similarity of cellular membranes and ability to incorporate various substances. We investigated the ability of liposomes as delivery system to acyclovir and AMNQ. Our results demonstrated that new formulations of these antiviral compounds have the same high antiviral profile e therefore must be investigated in pre-clinical and clinical tests to antiviral drugs.

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P 013

EVALUATION OF DNA EXTRACTION METHODS FOR DETECTION OF CYTOMEGALOVIRUS IN DRIED BLOOD SPOTS

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Introduction: Cytomegalovirus (CMV) is a leading cause of viral intra-uterine infection; congenital CMV infection (cCMV) is the most frequent vertically transmitted viral infection. It's responsible of up to 20% sensori-neural hearing loss in infants and children, with approximately 90% of infections asymptomatic at birth. Detection of CMV on dried blood spots (DBS) collected at birth represents a useful tool for retrospective diagnosis of cCMV. Unfortunately, the current CMV molecular assays in DBS exhibit low sensitivity and specificity. The goal of the study was to evaluate different DNA extraction methods on DBS, aiming to increase sensitivity for CMV diagnosis by quantitative Real-Time PCR.
Materials and Methods: Analysis was conducted on 50 CMV-positive whole blood samples (viral loads range: 10^2-10^6 copies/mL); 10 samples per logarithmic category were included. A volume of 50 µL was spotted on DBS, dried at room temperature, scheduling evaluations at 1, 6, 12 months. DNA extraction from 3 punches (3 mm diameter) was performed by automated QIAsymphony (Qiagen) system, according to methods: DNA Investigator kit (protocol Reference 200V5), and DSP Virus/Pathogen midi-kit (protocol Pathogen Complex 400V4). CMV-DNA quantification was with qRT-PCR (CMV Elite™ MGB, ELITechGroup), and results analyzed for single amplification and in triplicate.
Results: Preliminary results on 46 DBS at 1 month after spotting showed sensitivity for CMV detection by qRT-PCR of 45.7% and 60.9% for Investigator and Virus/Pathogen kit, respectively; sensitivity raised to 58.7% and 69.6% when considering amplifications in triplicate. In details, amplifications in triplicate showed sensitivity of 20% and 50% for both the extraction methods for viral load categories 10^2 and 10^3; in 10^4 category, sensitivity raised to 90% with the Virus/Pathogen, versus 40% with Investigator. Considering initial CMV viral loads $\geq 3*10^4$/mL, both the extraction methods showed 100% concordance and sensitivity, either in single or triplicate amplification.
Discussion: Both extraction methods exhibited 100% sensitivity in CMV detection in DBS for starting viral loads $\geq 3*10^4$/mL. Furthermore, using amplifications in triplicate for starting viral loads £10^4 resulted in increased sensitivity. These preliminary results indicate that the use of well-established procedures for sampling, storing and processing of DBS is essential.

P 013A

IDENTIFICATION OF A MULTI-REASSORTANT G12P[9] ROTAVIRUS IN A CHILD WITH ACUTE GASTROENTERITIS

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Introduction: Group A rotaviruses (RVA) of genotype G12 are recognized as globally emerging genotype. G12 strains have been demonstrated to be strongly versatile due to reassortment events, which may have contributed to their capability to spread in the human population. In Sicily, G12 RVAs, were first detected in 2012, more often in association with the P[8] and in one case with P[9]. G12P[9] strains are uncommon strains and probably originated by multiple reassortments events. The aim of the this study is to determine the genetic constellation of the G12P[9] strain by full-length genome analyses, in order to investigate the origin of the Sicilian strain.
Materials and Methods: A RVA (ME848/12) was identified in stool sample of a child hospitalized with severe acute gastroenteritis at the "G. Martino" University Hospital of Messina. The complete genotype constellation was determined and phylogenetic analyses were performed by MEGA 6 software.
Results: Based on the nucleotide sequence identities and according to the novel phylogenetic genotype classification system, the G12-P[9]-I17-R12-C12-M11-A12-N12-T7-E6-H2 genetic constellation was assigned to the ME848/12 strain. This genes combination did not corresponded to any of the established rotavirus genetic backbones and included segments of different animal origin. Phylogenetic analyses revealed that the VP1-3 and NSP2 genes of the Italian strain were distant from all established genotypes. Therefore, the Rotavirus Classification Working Group assigned them new genotypes R12, C12, M11 and N12.
Discussion and Conclusions: Comparison of the ME848/12 genotype constellation with other completely sequenced RVA genomes revealed that this strain shared several genome segments with either mono- or multi- reassortant human and animal RVAs. Whole genome analysis revealed an impressive genetic diversity and allowed to identify novel VP1-3 and NSP2 genotypes. In order to allowed identification of novel alleles, and whether the complex gene constellation observed is unique or it is retained in other RVAs, it will be important to gather sequence data on other human and animal RVAs.

P 014

EPIDEMIOLOGY OF HUMAN HERPESVIRUS TYPE SIX (HHV6) VARIANTS A AND B

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Introduction: Human herpesvirus 6 (HHV6) infection is widespread, with seroprevalence approaching 100%. HHV6 isolates are classified into two variants, termed A and B. HHV6-B has been recognised as the cause of *roseola infantum* in infants and primary infection usually occurs between 6 months and 2 years of age. In addition, HHV6-B has been frequently isolated in the blood of transplant patients. As concerns HHV6-A, its epidemiological role and clinical significance is rather unclear. In particular, HHV6-A has been found in multiple sclerosis patients and has been implicated as a cofactor in several other diseases, including encephalitis, meningoencephalitis, pneumonia and organ failure, especially in individuals with AIDS or lymphoproliferative disorders.
In this study we investigated the epidemiology of HHV6-A and HHV6-B in a cohort of individuals positive for HHV-6 with different levels of viral load. Particular attention has been focused on the analysis of the prevalence of the infection and the onset of the pathogenic role of HHV-6 related diseases.
Materials and Methods: We prospectively studied

40 individuals (18 males/22 females; mean age, 31.4 years; range, 0-83) grouped in solid organ or hematopoietic stem cells transplant patients, and individuals with cancer and neurological disorders. The identification and quantification of HHV6-A and HHV6-B DNA was performed using a real-time PCR specific for the two variants in different clinical samples such as whole blood, bronchoalveolar lavage, cerebrospinal fluid.
Results: Overall, 37/40 (92.5%) patients were positive for: HHV6-B, whereas only 3/40 (7.5%) were positive for HHV6 variant A. In particular, HHV6-A DNA was found in two oncohematological patients with lymphoblastic lymphoma and in one patient with multiple sclerosis. In all three cases, the presence of the variant HHV6-A has been associated with high viral load in blood (3,717,400 copies/mL in the patient with multiple sclerosis and 3,274,000 copies/mL and 2,476,900 copies/mL in the two oncohematological patients). HHV6-B variant was found in the majority of transplant patients and pediatric oncohematological patients.
Discussion and Conclusions: Our results confirm the data of the literature showing a high prevalence of HHV6-B variant compared to HHV6-A. In particular, the presence of HHV6-A was correlated with high viral load and with neurological and oncohematological disorders.

P 014A

MOLECULAR EPIDEMIOLOGY AND GENETIC DIVERSITY OF *BLASTOCYSTIS* INFECTION IN HUMANS, IN ITALY

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Aim: The study aimed to describe the molecular epidemiology and genetic diversity of *Blastocystis* infection in Italy.
Patients and Methods: 189 fecal isolates, collected during the years 2012-2014 from mildly symptomatic patients, or those affected by IBD, IBS or chronic diarrhoea, or otherwise immunosuppressed, were subtyped by sequencing analysis of the SSU rRNA gene (536bp). Sequences obtained were aligned with those previously deposited in GenBank. Phylogenetic and genetic diversity analyses were performed. Epidemiological data were treated statistically by Wilcoxon test and multivariate analysis (MCA).
Results: Six STs were detected: ST1 (15.3%), ST2 (13.8%), ST3 (46.0%), ST4 (21.7%), ST6 (3.2%) and ST8 (0.5%). They clustered in distinct clades, as inferred from BI phylogenetic and Median Joining Network analyses. A high genetic differentiation was found at the inter-subtype level. At the intra-ST level, a high genetic homogeneity was found in ST4.While, high values of haplotype and nucleotide diversity were observed in ST1, ST2 and ST3. No association was found between patient gender and subtype. A significant occurrence of *Blastocystis* ST4 in patients suffering of IBS, IBD or chronic diarrhoea was observed; in addition, a slight significant association between ST1 and ST3 and IBS patients was found. MCA showed some significant contribution of different variables (STs, haplotypes, age) in the observed pattern of ordination of the 189 isolates in the symptom categories.
Conclusions: The low level of genetic variability found in the ST4 could be related to the fact this ST has extended its range to humans during its more recent evolutionary history; according to this hypothesis, its pathogenic role could be greater than that observed for other STs. In support of this premise, the percentage of patients here found infected by ST4 and characterised by IBS, IBD or chronic diarrhoea was very high (87.2%). On the contrary, ST3, showing higher genetic variation, was suggested as the only subtype of human origin having a human-to-human transmission; it may have coevolved with human hosts over a longer period. ST3 was found here associated with patients complaining of IBS belonging to age groups between 23-59 years, but it was not correlated to a pathological state in older patients (aged 60 ≥ 75), suggesting its role as 'Old Friend' in human microbiota.

P 015

DISTRIBUTION OF HCV GENOTYPES IN CATANIA

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Introduction: The epidemiological profile of HCV infection is evolving in Europe such as in Italy. In Europe approximately 8 million people (1.3%) are infected by HCV, but a heterogeneous pattern of prevalence has been reported; the prevalence is intermediate-to-high in Eastern and Southern Europe, while it is low in Western and Northern Europe. The most common subtype are 1b followed by 2a/2c and genotype 3. Among European countries, the highest prevalence of HCV infection has been found in Italy, with a percentage between 3 and 26%.

Materials and Methods: In our study we considered rates of HCV genotypes according to gender and age and the HCV genotype distribution in the period between June 2005 and May 2015. Six hundred forty four patients, referred to the Clinical Virology Unit, were studied: 379 males (58,9%) and 265 females (41,1%). The mean age was 60,2 years (range 20-82). They were divided in three age groups: 20-40 years, 41-60 years and > 60 years.
The genotyping was performed by the VERSANT HCV AMPLIFICATION 2.0 and VERSANT HCV GENOTYPE 2.0 ASSAY (LiPA) (Siemens, Germany).

Results: Subtype 1b was the most prevalent (57,9%) followed by subtype 2a/2c (13,7%). Genotype 1a was the third most frequent (9,8%) in association with genotype 3a (8,9%). Genotype 1b was common in males (52,5%) and in females (65,7%), while the genotype 2a/2c was more frequent in females (19,6%). Genotype 1b e 1a were prevalent in all age groups, while genotype 3a was almost absent in > 60 years age group.

Discussion and Conclusions: Our data suggest that there has been no substantial changes in the overall epidemiology of HCV genotypes in the area of Catania over the last 10 years. Genotype 1b remains the most prevalent (57,9%), followed by subtype 2a/2c (13,7%). Genotypes 1a and 3a were as common in our geographical area, as found in other Italian regions.
The gender distribution shows a higher percentage prevalence of 1b among females (65,7%), as well as genotype 2a/2c (19,6%).
Considering the age group distribution, genotypes 1b e 1a were equally distributed among patients over and below 60 years, while genotype 3a was almost absent in > 60 years age group. These data confirmed the reduced risk of transfusion-related transmission. Few cases of genotype 4 were detected only in 2010.
In the light of migration flows in Sicily, future evaluation of new emerging subtypes will be necessary.

P 016

INFLUENZA SEASON 2014-2015 IN TUSCANY: VIRAL CHARACTERIZATION

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Introduction: Historically, influenza surveillance has been conducted through sentinel surveillance for influenza-like illness (ILI), with respiratory specimens being collected for virological monitoring and vaccine strain selection. The 2009 influenza pandemic highlighted the need for improved surveillance for severe influenza-associated disease. The World Health Organization (WHO) now recommends countries to perform surveillance for both ILI and influenza-associated severe acute respiratory infection (SARI)
Following these recommendations, also in Tuscany the virological survey of the last influenza season, 2014-2015, has been based on the analysis of respiratory samples obtained by sentinel physicians and hospitalized patients. Relying on the indications emerged from the last seasons and the composition of the seasonal vaccines, a deeper molecular characterization of the influenza virus circulating in Tuscany has been carried out. The aim of this study was to obtain information useful to better evaluate the efficacy of the seasonal vaccine and the severity of influenza infection caused by different subtypes.

Materials and Methods: Altogether, 660 clinical respiratory samples (throat swabs, TS, or broncho-

alveolar lavages, BAL) since November to April, have been analyzed. A commercial real time RT PCR (BioMerieux) was used to detect influenza A and influenza B viruses. The influenza A positive samples were then subtyped by an already described real time RT PCR in house assay. The H3N2 strains were further characterized by sequencing of a small fragment of the HA gene. Influenza B lineage was established by the use of a lineage specific RT-PCR.
Results: Altogether, 160 influenza A and only 19 influenza B viruses were detected. Fifty one % of the influenza A viruses were of the H1N1 2009 subtype and 49% were of subtype H3N2. All the influenza B characterized were of the B/Yamagata lineage. Among the influenza A viruses, subtype H3N2, the variant A/Texas/50/2012-like included in the vaccine was prevalent in circulation in comparison with the variant A/Switzerland/9715293/2013 (H3N2)-like viruses.
Conclusion: In the influenza season 2014-2015 both subtypes of influenza A viruses co-circulated with similar prevalence. The subtype H1N1 was the most prevalent among patients hospitalized in Intensive Care Units. The influenza A (H3N2) among ICU patients were similar to the variant A/Texas /50/2012-like.

P 017

PRESENCE OF HUMAN PAPILLOMAVIRUS (HPV) GENOTYPES IN BIOPSIES FROM WOMEN OF SICILIAN INTERLAND

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Introduction: The HPV is the main cause of cervical carcinoma. This is one of the most frequent causes of malignant disease in women of childbearing age, and it is worldwide the second malignant tumor of women. The aim of the study was to evaluate genotypes circulating in the Sicilian Interland and HPV-related lesions.
Materials and Methods: We analyzed the HPV genotypes present in 124 cervical biopsies by the LINEAR ARRAY HPV Genotyping test. The samples were collected by women aged between 20 and 85 years. Histological studies were carried out sections of tissue.
Results: We found 50% (62/124) of single infections and 50% (62/124) were multiple infections. HPV-16 had the highest prevalence in 29,8% (37/124) followed by HPV-42 in 16,9% (21/124), HPV-53 in 10,4% (13/124), HPV-66, 73 and 89 in 9,6% (12/124), HPV-52 and 31 in 8,8% (11/124), HPV-6, 39, and 84 in 7,2% (9/124), HPV-51 and 58 in 6,4% (8/124), other genotypes were present with an percentage inferior to 5%. 79% (98/124) of the cases contained high risk genotype (HR-HPV). About single infection the HR-HPV was present in 74,2% (46/62) of the cases. With respect to multiple infection in 83,9% (52/62) of the cases was present HR-HPV. In 31,2% (34/109) of CIN1 was present HPV-16 genotype alone or as multiple infection always in association to HR-HPV. All cases of CIN3 presented only HR-HPV.
Discussion and Conclusions: Epidemiologic and molecular studies have shown that infection with a HR-HPV genotype is the most important etiologic risk factor for the development of cervical carcinoma. As is the case in most regions worldwide, HPV-16, is the most prevalent HPV genotype in Sicilian women from Interland. The data show, however, that the only case of squamous cell carcinoma are responsible for HPV-58 and 73.

P 018

IN VITRO INFECTIVITY REGULATION OF HSV-1 BY GLYCOPROTEIN DERIVED SYNTHETIC PEPTIDES

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Introduction: Herpes simplex virus (HSV) is a human pathogen that infects epithelial cells. The mild skin lesions, caused by the virus, spread to the peripheral nervous system creating several complications. Fusion of host membranes with the viral envelope is necessary for the HSV-1 infection mechanism. Penetration is mediated by a group of glycoproteins conserved in all Herpesviridae subfamilies, such as the glycoproteins B (gB), H (gH), L (gL) and D (gD). The entry process starts with the virus binding to a cell receptor and is followed by fusion between the viral envelope and a cellular membrane mediated by gB and gH/gL via conformational changes.
Materials and Methods: Two library of overlapping peptides homologous to the ectodomains of gH and gL were prepared and screened for the infection modulation. Solid phase-9-fluorenyl-methoxycarbonyl (Fmoc) on an automatic synthesizer SYRO multisyntech standard method for peptide synthesis was used. RP-HPLC-MS was apply for peptides purification. We obtained 80% yields of synthesis and over than 95% of purification. Several assays of viral entry inhibition have been performed.
Results: We investigated the inhibitory activity mediated by synthetic peptides overlapping both gH and gL glycoproteins,. We have performed an analysis of the whole ectodomains of gH/gL in order to explore the inhibitory activity of peptides modelled on these glycoproteins against HSV-1 infection. 24 of the gH peptides at 150 μM as final concentration reached the cut-off of 50% of inhibition. Interestingly, them are mainly located in the gH ectodomain carboxy-terminal region. Out of these 24 peptides, 8 were selected for further studies. In the same time none of the gL peptides had a clear inhibiting effect.
Discussion and Conclusions: Actually the golden standard therapy for HSV-1 patient is acyclovir therapy. This drug act on viral DNA polymerase, but some resistant strains are emerging. In this scenario, innovative approachs for HSV-1 treatment are necessary. Our data support the direct involvement of the described domains in membrane fusion, therefore, these results are of relevance to the potential development of novel therapeutic compounds to prevent HSV-1 infections.

P 019

DISTRIBUTION OF HPV GENOTYPES AMONG ITALIAN WOMEN WITH AND WITHOUT CERVICAL PATHOLOGY

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Introduction: The distribution of human papillomaviruses (HPV) varies greatly across populations. To assess the characteristics of HPV infection women in Italy, we examined 4376 cervical samples of women (mean age: 21.7 ys) who had come to the Virology laboratory at the Department of Hygiene and Microbiology (Polyclinic University Hospital of Palermo, Italy) with a request for HPV testing.
Materials and methods: HPV detection was performed by the Linear Array HPV Genotyping Test (Roche Diagnostics).
Results: A total of 1794 (41%) samples were HPV positive. The highest HPV detection rate (54.2%) was found in the 15-24 year age group, followed by the 25-34 group (47.8%). Multiple HPV type infections were shown in 550 (30.7%); oncogenic types were found in 1466 (81.7%) women, alone (52.9%) or with non-oncogenic types (28.8%).
Thirty-seven different HPV types were identified: the mostly frequent were HPV-16 (22.2% of HPV positive patients), -66 (10.1%), -51 (9.3%), -31 (9%), -52 (8,6%), -53 (8,2%), -6, (6.8%), -59 (5,5%), -61 (5.3%), -62 (5.1%), -18 (4.8%), -58 (4.4%), -54 (4.3%); other viral types occurred at a frequency of less than 4.0%.
The association between HPV infection and presence of cervical lesions (SIL) was examined in a subset of 644 patients with known histological diagnosis, of which 332 (51.6%) had no SIL, 248 (38.5%) had low-grade (L)SIL and 64 (9.9%) high-grade (H)SIL. HPV infection was evident in 82 (24.7%) women without SIL, 109 (43.9%) LSIL and 58 (90.65) HSIL *($p < 0.001$)*. Infection with oncogenic HPV genotypes was present in 69 (20.8%) women without SIL, 82 (33.1%) with LSIL and 55 (85.9%) with HSIL *($p < 0.001$)*. The most frequently detected type was HPV-16 (4.5% cases of no SIL, 7.7% LSIL and 42.2% HSIL). Common types oth-

er than HPV-16 were: in no SIL, HPV-31 (3.6%), HPV-39 and HPV-51 (2.4% each), HPV-66, -52 and -42 (2.1% each), HPV-18, -58, and -6 (1.2% each); in LSIL, HPV-31 and -66 (5.6% each), HPV-51, -6 (5.2% each), HPV-52 and-53 (3.6% each), HPV-18 (3.2%), HPV-42 (2.8%), HPV-45, -58, -73, and -89, (2% each); in HSIL, HPV-31 (12.5%), HPV-33 (7.8%), HPV-52 (6.2%), HPV-45, -51 and -42, (4.7% each).

Discussion and Conclusions: This study showed a high prevalence of genital HPV infection in Italian women. This information will contribute to elucidating the epidemiology of HPV infection, and it will also be helpful in the implementation of future prevention strategies.

P 020

SERUM TOTAL ANTIOXIDANT CAPACITY IN PATIENTS WITH HEPATITIS C

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Introduction: Hepatitis C virus (HCV) infection affects chronically more than 150 million human beings worldwide, and is one of the major causes of chronic hepatitis that can lead to liver failure or hepatocellular carcinoma. During infection, HCV induces oxidative stress that affects virus replication as well as progression and severity of HCV infection. Nowadays, few data have been reported on the general redox state in patients infected with different HCV genotypes.

Materials and Methods: The aim of this study was to estimate the level of serum Total Antioxidant Capacity (TAC) in patients with chronic HCV infection. One hundred eighty-three HCV patients with different underlying clinical conditions, different genotypes, and twenty-five healthy donors were enrolled in the study. Serum TCA was measured by TCA Colorimetric Assay (BioVision USA) and serum Hydrogen Peroxide concentration was measured using Hydrogen Peroxide Colorimetric Assay Kit (BioVision USA).

Results: In HCV infected patients, mean serum TAC was 5.74 mM Trolox equivalents and was significantly lower ($p < 0.001$) compared to the control group (7.34 mM Trolox equivalents). This difference was particularly evident in two groups of patients affected by different HCV genotypes. In fact, the mean serum TAC was significantly lower in patients infected by genotypes 1 and 2 with respect to healthy donors and to those with genotypes 3 and 4. Consequently, high levels of Hydrogen Peroxide were found in the serum of infected patients.

Conclusions: The results indicate an imbalance of redox state in HCV infected patients versus an oxidative state. This alteration is significantly present in serum from patients infected by genotypes 1 and 2 that are mostly diffuse in Italy. The evaluation of redox state in these patients could represent a biomarker of severity of infection and contribute to the choice of therapy.

P 021

AUTOPHAGIC PATHWAY IS BLOCKED BY INFLUENZA A VIRUS THROUGH INHIBITION OF HDAC6-MEDIATED ACETYLATION OF CORTACTIN

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Introduction: Autophagy is a cellular catabolic pathway in which portions of cytoplasm are sequestered into autophagosomes and delivered to the lysosomes for degradation. It can be considered a pathway of defense against invading pathogens. Therefore strategies to evade autophagy or to subvert it for their own advantage have been developed by pathogens, including influenza virus. It has been

shown that influenza virus blocks autophagosome fusion with lysosomes via its matrix protein.
It is also known that acetylation plays a crucial role in the regulation of autophagic pathway. Histone Deacetylase 6 (HDAC6), a class II HDAC member, is required for autophagosome-lysosome fusion, inducing cortactin deacetylation. This phenomenon promotes an actin remodeling that provides a platform for vesicles fusion. This study was aimed at verifying whether influenza virus blocks autophagy through the modulation of cytoskeleton proteins.
Materials and Methods: Influenza Virus Infection In A549 Epithelial Cell Line, western blot analysis, immunoprecipitation and immunofluorescence studies of expression level and intracellular localization of cortactin, LC3, Lamp1 and F-actin, real time-PCR analysis of HDAC6 mRNA level, plasmid transfection experiment to over express HDAC6.
Results: We found by immunofluorescence analysis of influenza A virus-infected epithelial cells that the virus did not allow the physiological colocalization of cortactin and F-actin. Immunoprecipitation of cortactin followed by a western blot analysis with acetyl-lysine antibody revealed that the protein is hyperacetylated in the late phases of infection. Moreover we found that the virus induced HDAC6 downregulation, both at mRNA and protein levels. The overexpression of the enzyme reverted the effect allowing cortactin and actin to colocalize.
Conclusions: Our data suggest that influenza virus blocks autophagy by modulating the acetylation state of cortactin. Identification of cellular proteins involved in the host-virus interactions may provide new potential targets for the control of influenza virus replication.

P 022

TEMPORIN B, AN AMPHIBIAN ANTIMICROBIAL PEPTIDE, IS ABLE TO INHIBIT *IN VITRO* HERPES SIMPLEX TYPE I VIRUS INFECTION

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Introduction: Several viruses, including herpes simplex virus type 1 (HSV-1), develop quickly resistance to conventional drugs; therefore, the search for new natural or *de-novo* designed molecules becomes indispensable. Thus, our studies have been focused on the evaluation of the potential antiviral activity of amphibian antimicrobial peptides (named *temporins*) obtained from frog-skin secretion. Temporins are mildly cationic short peptides, synthesized by frog dermal glands and stored within granules and are released by a holocrine-type mechanism upon stress or physical injury. While bactericidal activity of temporins has been described, very little is known about their antiviral action. This study was aimed at investigating their potential antiviral activity.
Methods: HSV-1 infection in monkey kidney epithelial (Vero) cells; temporin A (TA) and B (TB) treatments at the following steps of infection:
a) during adsorption phase
b) after adsorption phase and for the subsequent 24 h
c) during adsorption phase and for the subsequent 24 h
d) 3 h before infection.
Plaque assay evaluation of viral titer in cell supernatants; western blotting analysis of viral protein expression in cellular lysates.
Results: We demonstrated that TA was not able to reduce virus titer, while TB significantly inhibited viral replication (> 2 log) when the cells were treated during and after viral adsorption.
To verify whether TA and TB exerted a direct effect on the viral particles (virucidal effect) or on the attack and/or penetration of virus to the host cell (virustatic effect), HSV-1 was pre-incubated with each peptide for 1 h at 37°C and the mixtures were used to infect VERO cells for 24 h. We found a total inhibition of HSV-1 replication when the virus was pre-incubated with TB. Then we performed attachment and entry assays to characterize TB antiviral activity. Vero cells were incubated with HSV-1 for 1h at 4°C (synchronization phase) in the presence (attachment assay) or absence (entry assay) of TB. After adsorption phase at 37°C and following 24 h of infection, the viral titer was estimated: HSV-1 replication was inhibited only during the attachment assay (2 log).
Conclusions: These results indicate that TB exerted its antiviral activity mainly affecting HSV-1 envelope. Further studies are in progress to deepen the mechanisms underlying the antiviral activity.

POSTERS

Bacteriology / Mycology / Parasitology

P 023

LUCIFERASE *IN VIVO* ASSAY VALIDATION FOR OROPHARYNGEAL CANDIDIASIS IN MICE

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Introduction: Bioluminescence *in vivo* imaging technique, allow the real-time progression of oropharyngeal candidiasis, hence potentially useful to evaluate the efficacy of antifungal therapies. In the present study, the *in vivo* imaging technique was compared with CFU measurement of target organs for monitoring and quantifying oropharyngeal candidiasis.
Materials and Methods: An engineered luminescent *Candida albicans* (*C. albicans*) strain was used to infect mice rendered susceptible to oral candidiasis by injection of cortisone- acetate. At different times post-infection (day +3, +6, +8) scatter plots, Pearson correlation and Student's t test were used to compare the methods.
Results: Our results show that CFU method failed to detect fungal burden in the tongue after 3 and 6 days post-infection, but not on day 8 after challenge. In contrast, the bioluminescence technique revealed fungal infection in the oral cavity of all mice at each time point tested. This was also evident following the introduction of a variable such as treatment with fluconazole.
Discussion and Conclusions: The results described in this study could validate the bioluminescence *in vivo* imaging technique as a method to monitor and quantify oropharyngeal candidiasis and to assess early discovery of active compounds *in vivo*.

P 023A

MOLECULAR DETECTION OF *CHLAMYDIA TRACHOMATIS* IN CERVICAL SAMPLES TESTED FOR *HUMAN PAPILLOMAVIRUS*

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Chlamydia trachomatis (*Ct*), a bacteria transmitted through sexual contact, in women exhibits tropism for squamous epithelial cells of the cervical mucosa with risk of cervicitis. Persistent chlamydial infection may be related to cervical hypertrophy and squamous metaplasia, cytological cervical lesions, often, associated to high-risk *Human Papillomavirus* (HPV) genotypes.
Different authors suggest a possible Ct/HPV synergic effect in lesion progression such as low-grade squamous intraepithelial lesion (L-SIL) to high-grade squamous (H-SIL) and finally to cervical squamous cancer (CSC).
This study investigate the prevalence of *Ct* in 100 cervical samples of women with abnormal cytology assayed for HPV and in 50 sample with normal cytology and HPV negative.
Materials and Methods: One hundred cervical samples with abnormal cytology (88 L-SIL, 11 H-SIL, 1CSC) were earlier collected for HPV detection and tested for the INNOLiPA HPV Genotyping assay and HPV-DNA by nested PCR/ sequencing, resulting positive in 50 cases. The samples were, also, assayed for *Ct* detection by a Real Time PCR according to "Chlamydia tr. ELITe MGB" kit (ELITechGroup). As control group, 50 cervical samples with normal cytology and negative to HPV, were chosen and analyzed for *Ct* DNA.
Results: The prevalence of *Ct* in cervical samples with abnormal cytology was 2% without difference between HPV-positive and HPV-negative samples. Only one cervical HPV-positive sample and only one HPV-negative sample revealed presence of *Ct* DNA, both associated to L-SIL. In the *Ct*/HPV-positive sample multiple genotipes (58, 42, 73, 51, 59) were identified.
Discussion and Conclusions: Our preliminary results suggested a casual association between

Ct and HPV infection in women with cervical lesions. No significant difference we have revealed between HPV-positive and HPV-negative samples for the presence of *Ct*. Furthermore the same percentage of *Ct* positivity was observed in the control group. We can assert that in women, cervical lesions are mainly associated to HPV which represented the major risk factor to develop cervical cancer. Because previous studies have demonstrated an association between HPV and *Ct* infection within cervical carcinoma, suggesting that infection with this bacterium could increase the risk of lesion progression to invasive cervical cancer, more analysis are necessary to confirm this consideration.

P 024

ANTIMICROBIAL ACTIVITY, CO-AGGREGATIVE AND INTERFERENCE ABILITY OF SEVERAL LACTIC ACID BACTERIA TOWARD HUMAN INTESTINAL PATHOGENS

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Introduction: The effectiveness of probiotic strains depends on their ability to survive during passage through the stomach, as well as on their ability to persist and compete with pathogens through competitive exclusion of binding sites, modulation of the host's immune system and production of inhibitory compounds. In this study, several Lactic Acid Bacteria (LAB) strains were tested for antimicrobial activities, co-aggregative abilities, interferences studies against human intestinal pathogens to define their potential as probiotics.
Materials and Methods: The potential probiotic activity of 7 LAB strains, provided by Winclove Probiotics, was evaluated against *Salmonella enteritidis* ATCC 13076, *Listeria monocytogenes* ATCC 7644, *E. sakazakii* ATCC 51329 and *E. coli* O157:H7 ATCC 35150. In view of this, interferences studies between LAB strain and pathogens were carried out to evaluate: i) antimicrobial activities by killing studies in co-culture; ii) co-aggregative abilities by spectrophotometry; iii) invasion inhibition by competition, displacement and exclusion tests on Caco-2 cells monolayers.
Results: Regarding the antimicrobial activities, a decrease of viable cells was observed for *S. enteritidis* ATCC 13076 in co-culture with *Lactobacillus salivarius* W24 and *Lactobacillus casei* W56 and for *E. coli* O157:H7 ATCC 35150 in co-culture with *L. salivarius* W24, *L. lactis* W58 and *L. plantarum* W 62. Co-aggregation abilities were observed in most LAB strains with percentages variable between the different intestinal pathogens. As regards interference studies, percentages of invasion inhibition of pathogens by LAB strains were observed in exclusion and competition infections on Caco-2 cells, particularly remarkable for *L. monocytogenes.*
Discussion and Conclusion: The LAB examined in this study may protect the intestinal epithelium through a series of barriers (antimicrobial activity, co-aggregation with pathogens, adherence) and interference mechanisms. In addition, co-aggregation may be useful for preliminary screening in order to identify potentially probiotic strains. Consequently, some of these LAB strains may be considered candidates for different uses, such as prophylactic agents, and studies to further evaluate their feasibility are underway.

P 024A

SELECTION OF BACTERIOPHAGES LYTIC FOR *SALMONELLA* AND *E. COLI* FOR ANIMAL FEED BIOCONTROL

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Introduction: *Salmonella* and *Escherichia coli* cause gastroenteric syndromes both in hu-

mans and animals. The main route of transmission is oro-feacal and is associated with pathogen-spreaders subjects. The diseases are generally treated with antibiotics, even though a great number of antibiotic-resistant bacteria is arising in the recent years. In this context the present study aims at isolate and characterize lytic bacteriophages as potential biocontrol agents alternative to antibiotics.

Materials and Methods: The isolation of bacteriophages lytic for *E. coli* and *Salmonella* spp. was performed on 30 fecal samples collected from water buffalo farms in the Campania region. Feces were incubated overnight at 37°C in TSB inoculated with a mixture of five strains of either *E. coli* or *Salmonella* spp. After incubation the suspensions were centrifuged and the supernatant was filtered and chloroform treated. Phage titer and host ranges were determined by the spot assay. Phages were characterized by electron microscopy. Feed biocontrol assays were performed on hay samples (100g) inoculated with 9 ml of S. Typhimurium (2x10^7 UFC/ml) and phage treated (MOI 10) 24 h after contamination. Bacterial counts were performed 4, 8 and 24 h after phage treatment.

Results: Two *E. coli* and fourteen *Salmonella* spp. bacteriophages were isolated from water buffalo calves. All phages were characterized for plaque morphology and host range on 20 *Salmonella* strains and 15 toxinogenic *E. coli*. The phages exhibited lytic activity against different strains including S. Typhimurium, S. Enteritidis, S. monophasic Typhimurium, S. Napoli, and Shiga-toxin producing *E. Coli*. Six phages were further characterized by electron microscopy, showing different morphologies. Preliminary experiments carried out on hay indicated a 2log reduction in *Salmonella* counts already at 4h after phage treatment *in vitro*.

Discussion and Conclusions: These results demonstrate the efficacy of this experimental approach for isolation of multiple phage types and suggest the possibility to use them either as a single agent or a therapeutic cocktail for the biocontrol of gastroenteric pathogens in farms and animal feed.

Key words: Bacteriophages, *Salmonella* spp., *E. coli*

P 025

ANTIBIOTIC RESISTANCE IN *HELICOBACTER PYLORI* STRAINS ISOLATED IN CENTRAL ITALY

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Introduction: *Helicobacter pylori* expresses in time an increased resistance in respect to antimicrobial agents currently used in therapy. The aim of this study was to evaluate the antimicrobial profiles of H. pylori isolates to nine conventional antibiotics used in the Abruzzo Region (Italy).

Materials and Methods: Biopsy specimens were taken from antrum and fundus of 115 Urea Breath Test positive patients with gastroduodenal symptoms and analyzed for *H. pylori* culture and antibacterial activity. Antimicrobial susceptibility tests were performed for clarithromycin, metronidazole, levofloxacin, moxifloxacin, ciprofloxacin, tetracycline, amoxicillin, ampicillin and rifabutin, by a modified agar dilution susceptibility test.

Results: Bacterial culture was successful in 100 (86.95%) out of 115 patients. *H. pylori* strains were isolated from 98 antrum and 83 fundus samples. *H. pylori* strains were recovered in 90.50% (181/200). The percentages of resistance were as follows: clarithromycin 72.44 antrum, 72.28 fundus; metronidazole 34.69 antrum, 42.16 fundus; levofloxacin 42.85 antrum, 53.01 fundus; moxifloxacin 37.35 antrum, 46.57 fundus; ciprofloxacin 39.47 antrum, 44.28 fundus; tetracycline 2.63 antrum, 2.85 fundus; amoxicillin 1.02 antrum, 1.20 fundus; ampicillin 0 antrum, 0 fundus and rifabutin 0 antrum, 1.20 fundus. Moreover, a total of 35 subjects (35/100) harbored multiresistant strains.

Discussion and Conclusions: The high levels of clarithromycin, quinolones and metronidazole resistance together with multiple resistance, recommend to avoid empiric therapy. Even if, the isolation of *H. pylori* from bioptic samples requires experience and costs for detection of the microorganism and antimicrobial analysis, culture and susceptibility test (antrum and fundus), should be performed to prevent the emergence of multiresistance and to assess the most efficacious regimen.

P 025A

NEXT-GENERATION 16S rRNA GENE SEQUENCING FOR BACTERIAL VAGINOSIS INVESTIGATION BY ION TORRENT PGM PLATFORM

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Introduction: Bacterial Vaginosis (BV) is a common, complex polymicrobial disorder resulting in a reduction of *Lactobacillus species (spp.)* and a concomitant overgrowth of strict or facultative anaerobic bacteria. Studies on BV are preliminary in explaining the pathobiology of the disease, maybe to the detection limit of the current laboratory techniques. Recently, the presence of a dense polymicrobial biofilm on the vaginal epithelium seems to characterize BV where *G. vaginalis* seems to play a central role among resident bacteria. The next-generation sequencing platform could deeply describe the vaginal microbiome in colonization and causing BV infection. An optimized 16S gene protocol based on the Ion PGM sequencing technique has been used on a selected series of vaginal samples from women symptomatic for BV and compared to a matched healthy group.
Materials and Methods: Cervical swabs were collected from women clinically symptomatic (S) for BV (n 30) and from asymptomatic women (control) (n 27). S women were grouped according to Nugent score (NS): 15 with a score of 4-6 and 15 with a score of 7-10. The vaginal microbial communities were qualitatively analysed using the V3 region of 16S gene by Ion PGM 200bp chemistry. The output files were aligned on MG-RAST pipeline and grouped according to OTUs.
Results: A different microbial profile was observed between the groups. Comparing to control, *Streptococci, Staphylococci, Enterococci, Megasphaera, Proteus, Pediococcus, Kluvyera, Finegoldia, Dialister* and *Gardnerella spp.* were identified only in S women. Specifically, *Prevotella, Bifidobacterium* and *Ureaplasma spp.* were predominant in women with NS 4-6 while high abundance of uncultured bacterium was present in women with NS 7-10.
Interestingly, in S women the presence of *Lactobacilli iners* and *crispatus* was low (10%; 8%) comparing to controls where the same *spp.* were around 28% and 33%. Conversely, a specific cluster including *Lactobacilli acidophilus, helveticus, delbrueckii, johnsonii, gasseri* and *reuterii* was found only in S group.
Discussion and Conclusions: Our results identify in BV symptomatic women a specific bacteria cluster associated with the severity of the clinical manifestations and NS. Moreover, we confirm the protective role of *L. crispatus* in healthy women while different underrepresented *Lactobacillus spp.* seem involved in BV.

P 026

BIOFILM-ASSOCIATED PROTEINS: NEWS FROM *ACINETOBACTER*

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Introduction: A 8621 aminoacid repetitive protein called BAP (biofilm-associated protein), because involved in biofilm formation, was identified in the *A. baumannii* strain AB307-0294. The organization of BAP and two similar proteins, called BLP1 and BLP2 (for BAP-like proteins 1 and 2), had been been thoroughly analyzed in hundreds of sequenced strains of the *Acinetobacter* genus.
Materials and Methods: The genes of the *A. baumannii* AYE strain encoding BLP1 and BLP2 were mutagenized by allelic replacement.
Results: The AB307-0294 BAP features seven repeat units (A-G) ranging in size from 70 to 104 aminoacids. Repeats A-D exhibit Big_3_4 (Bacterial Ig-like domain, group 3) motifs, fitting the consensus TDnAGN. Repeats E-G lack Big_3_4 motifs, and are reiterated in tandem in the large 1002 aa COOH region. B, C and D repeats are over-represented, and account for 2/3 of the protein. The survey of 541 *A. baumannii* genomes belonging to 108 ST (Sequence Type) revealed that BAP is highly polymorphic, and is distinguishable for changes both in the repetitive and the COOH region in three main types. The type-3 variant is restricted to *A. baumannii*, proteins homologous to type-1 and type-2 variants occurr also in other *Acinetobacter*

species. Two smaller proteins, BLP1 (3369 aa) and BLP2 (728 aa) also featuring Ig-like motifs have been identified in *A. baumannii*. The pattern of distribution of BAP, BLP1 and BLP2 genes in the population is highly suggestive of a functional hierarchy linking the three proteins. The hypothesis is directly supported by gene disruption analyses, since the knock-out of both BLP1 or BLP2 genes of the A. baumannii AYE strain severely affected both biofilm formation and adherence to epithelial cells, as revealed by confocal scanner analyses.

Discussion and Conclusions: *A. baumannii* BAP, BLP1 and BLP2 are proteins featuring similar repeated modules, that plausibly functionally interact to make up structures involved both in biofilm formation and host cells contacting. Future experiments should clarify how these protein interact, and also whether additional proteins are involved in the process.

P 026A

STUDIES OF IMMUNE RESPONSES IN *CANDIDA* VAGINITIS AND VACCINE DEVELOPMENT

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Introduction: Vaginal candidiasis is one of the most frequent infections of the female genital tract of women of reproductive age. At least 75% women suffer once in their life from one episode of a *Candida* infection. 8%-15% of women have recurrent forms of the disease (RVVC), that devastates their quality of life, causes high cost of medical visits and large use of drugs with possible subsequent upsurge of drug resistance. Moreover, in contrast to systemic candidiasis, relatively little is known about the role of mucosal immunity in protection against *Candida.* The widespread occurrence of mucosal candidiasis and the development of resistance against anti-fungal agents has stimulated interest in understanding the pathogenesis of this disease. The aim of our work was to characterize the immune response to mucosal candidiasis.

Materials and Methods: To this purpose, in an animal model of vaginal candidiasis, we have tried to evidence the mechanisms that play a role in the induction of mucosal immunity against *C. albicans* and that characterize the interaction between innate and adaptative immunity.

Results: Our studies evidenced the elicitation of CMIs and Ab mediated immunity with a Th1 protective immunity. To further investigate the host defence mechanisms at vaginal level we have evaluated the role of the Toll like receptors (TLRs) and C-type lectin like receptors (CLRs) in vaginal candidiasis. The results suggest that TLRs and CLRs have a role in the innate and adaptive immunity to *Candida* at vaginal level. An immune response of this magnitude in the vaginal compartment was very encouraging to identify the proper targets for new strategies for vaccination or immunotherapy of vaginal candidiasis. We were working with two recombinant proteins: an aspartyl-proteinase (Sap2) and a protein of 65 kDa (Mp65) which are important immunodominant antigens and virulence factors of *C. albicans* acting in mucosal infections.

Discussion and conclusions: Overall, our data provide a clear evidence that is possible to prevent *C. albicans* vaginal infection by active intravaginal immunization with Sap2. This opens the way to a modality for anti-*Candida* protection at mucosal level. The recombinant protein Sap2 was assembled with virosomes and a vaccine PEV 7 was obtained. The results have given evidence that the vaccine constitute by virosomal and Sap2 (PEV7) has an encouraging therapeutic potential for the treatment of recurrent vulvovaginal candidiasis.

P 027

EFFECT OF DIFFERENT TITANIUM IMPLANT SURFACES AND COMPOSITIONS ON *PORPHYROMONAS GINGIVALIS* BIOFILM FORMATION

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Introduction: Surface properties and composition of titanium (Ti) play a crucial role for osseointegration as well as for microbial proliferation and implant failure. The aim of this work was to evaluate the biofilm formation of *Porphyromonas gingivalis* on disks of Ti grade 4 (G4), and Ti-6Al-4V alloy grade 5 (G5) with different surface topographies.
Materials and Methods: *P. gingivalis* ATCC 33277 was used to develop an *in vitro* mature biofilm on disk-shaped specimens of laser-treated (L), sandblasted (S) and machined (M) surfaces of Ti G4 and Ti G5. Surface roughness (Ra) and the wettability contact angle (WCA) were measured to characterize the surface of the specimens. The bacterial biofilm was evaluated by biomass quantification, bacterial viability, visualization of the biofilm extracellular matrix and bacterial cell count. Data were analyzed using One-Way ANOVA and Holm-Sidak tests.
Results: The Ra for the L group was 0.10 (± 0.07) µm inside the craters and 0.40 (± 0.08) µm in the area surrounding the craters; for the S group 1.30 (± 0.61) µm and for the M group 0.75 (± 0.23) µm. The L group showed a higher WCA than S and M groups both for G4 (109.9°± 6.6) and G5 (104.2°± 5.9) materials. The L groups displayed the less P. gingivalis bacterial biomass, polysaccharides production and total cell number than S and M groups both for G4 and G5 materials. In particular, G4-L showed the best performances in hampering the sessile bacterial proliferation.
Discussion and Conclusions: Within the limits of the present study, the results showed that G4-L appears to be significantly efficient in the reduction of the *P. gingivalis* biofilm formation. The S surface treatment overcomes the differences in material composition without any significant difference in the biofilm formation between G4 and G5. The M surface treatment showed a remarkable increase of the *P. gingivalis* biofilm formation. The present findings could have a clinical impact. Since, lesser implant surfaces colonization by *P. gingivalis* it may decrease the risk of dental implant surgery morbidity.

P 027A

MARKED DYSBIOSIS CHARACTERIZE DUODENAL MICROBIOME IN ADULT ACTIVE CELIAC DISEASE PATIENTS

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Introduction: Celiac disease (CD) is an autoimmune condition triggered by gluten in HLA-DQ2/DQ8 genetically susceptible subjects; however, increasing evidence implicates the intestinal ecosystem in the pathogenesis of CD. To address more properly this issue, we studied the gut microbiome directly on the duodenal mucosa of CD patients.
Materials and Methods: The microbiome was evaluated by 454-based DNA sequencing (NGS) of 16S rRNA libraries in duodenal biopsies from 2 groups of adult subjects, 10 active celiac patients and 10 controls. In addition we performed microbiological analysis by differential culture media and mass spectrometry analysis of the biopsy specimens.
Results: *Proteobacteria* were the most abundant and *Firmicutes* the less abundant *phyla* in the NGS microbiome profiles of active CD patients. Within the *Proteobacteria phylum* members of the *β-proteobacteria* and *γ-proteobacteria* classes were more and less abundant, respectively, in active CD patients than in controls. Microbiological differential analysis revealed a cultivable biodiversity in duodenal microflora of the tested samples, in particular confirmed within *Firmicutes* a decreased levels of

Lactobacillus species and within *Actinobacteria* an increased levels of *Rothia* and within *Proteobacteria* of *Klebsiella oxytoca, Enterobacter aerogene* and *Neisseria flavescens* species. Preliminary data on penetration into intestinal cells and *in vitro* effects on inflammatory reactions will be presented for some bacterial species.

Discussion and Conclusions: Marked dysbiosis (increased *Proteobacteria* and decreased *Firmicutes*) characterize duodenal microbiome in active CD patients. Our preliminary finding support that the CD-associated microbiota could represent a further "trigger" in CD development in addition to genetics and gluten.

P 028

IN VITRO ANTIFUNGAL ACTIVITY OF TOTAL EXTRACTS AND ISOLATED COMPOUNDS FROM *MORUS NIGRA L.* AGAINST PLANKTONIC FORM AND BIOFILM OF *BOTRYTIS CINEREA*

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Introduction: *Botrytis cinerea* is a fungal pathogen that attacks over 200 different plant species. Fungicides against *Botrytis* represent 10% of the world fungicide market. The use of plant extracts could be a valid alternative to use of synthetic compounds. Numerous plant species have been studied for their ability to synthesize antifungal secondary metabolites. In the present study, an *in vitro* assay was established to screen different extracts and isolated compounds obtained from *Morus nigra L.* (Moraceae), for their ability to inhibit *B. cinerea* growth.

Materials and Methods: Methanolic and acetonic extracts rich in diels-alder adducts were obtained from root bark of *M. nigra*. Leaves of *M. nigra* exposed to UV-B radiation were subjected to extraction in chloroform and acetone. Kuwanon G, morusin were purified from the root bark and kuwanon R, chalcomoracin from cell cultures. For the antifungal evaluation, *B. cinerea* DSM 877 and isolated strain were tested. Antifungal susceptibilities were determined using the broth microdilution method in accordance with CLSI guidelines with some modification. Inhibition of *B. cinerea* biofilm formation was performed using conidia suspension with serial dilution of the extracts into polystyrene microtiter plates and the biofilm was quantified by crystal violet staining. The measure of absorbance was proportional to the quantity of biofilm biomass.

Results: The root acetonic extract was the most active against *B. cinerea* planktonic cells with a MIC_{100} values ranged from 64 to 128 µg/ml. Kuwanon G, kuwanon R, morusin and chalcomoracin were tested. Chalcomoracin showed antifungal activity with MIC_{100} (100% reduction in growth) values ranged from 8-32 µg/ml. Regarding *B. cinerea biofilm*, acetonic extract showed a MIC_{80} (80% reduction in growth) value of 64 µg/ml.

Discussion and Conclusions: *Morus* root bark has been used for centuries in folk medicine. The acetonic extract of the root bark of *M. nigra* showed the best antifungal activity. *M. nigra* root is a rich source of isoprenylated phenolics with significant bioactivities. The chalcomoracin, a diels-alder type adducts purified from cell cultures, showed the best anti-*Botrytis* activity. Further studies are required on a broader panel of pathogens.

P 028A

CANDIDA ALBICANS HYPHAL DEVELOPMENT AND BIOFILM FORMATION/ PERSISTENCE ARE DIFFERENTIALLY AFFECTED BY MOUTHWASHES DEPENDING UPON THEIR COMPOSITION

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Introduction: Oral candidiasis is a frequent opportunistic fungal infection, occurring especially in susceptible individuals. This pathology, mainly associated with *Candida albicans* species, may be prevented by a good oral hygiene, the daily use of toothbrush and mouthwashes. Among several virulence factors, *C. albicans* has the ability to switch from yeast-to-hyphal forms and to produce biofilm, thus contrasting antimicrobial agents and host immune defences as well [1-3].
The aim of this study is to investigate the susceptibility of *C. albicans,* in terms of growth, hyphal formation and biofilm production/persistence, to mouthwashes with different composition.
Materials and Methods: *Candida albicans* SC5314 and 7 commercial mouthwashes have been employed: 3 with 0.2% chlorhexidine digluconate; 1 with 0.06% chlorhexidine digluconate and 250 ppm F^- sodium fluoride; 3 with 125-250ppm F^- sodium, amino and/or stannous fluoride. The effects of the mouthwashes on *C. albicans* were assessed by crystal violet, tetrazolium salt reduction assays and morphological analysis by microscopy. By using four different protocols, combining different concentrations and different contact times, the mouthwashes were tested against: 1) *C. albicans* growth on Muller-Hinton agar, as assessed by disk diffusion assay; 2) hyphal formation and biofilm production by yeast cells, cultured in RPMI + 10% FBS; 3) early pre-formed (24 h-old) *Candida* biofilm and 4) mature (48 h-old) *Candida* biofilm.
Results: The highest anti-*Candida* activity was consistently exhibited by the chlorhexidine digluconate- containing mouthwashes, irrespective of the protocols employed. Morphological and functional impairments occurred and fungal survival/growth were impaired as well; the effects strictly depended on both the dilution employed and the time of contact.
Discussion and Conclusions: Both *C. albicans* hyphal development and biofilm formation/persistence are affected by mouthwashes, provided that they contain chlorhexidine digluconate. Thus, special attention should be used when choosing mouthwashes for prevention and/or treatment of *Candida*-associated oral pathologies.

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P 029

IN VITRO AND *IN VIVO* ANTICANDIDAL ACTIVITY OF KILLER PEPTIDE DERIVATIVES

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Introduction: We have previously demonstrated that the synthetic decapeptide AKVTMTCSAS (killer peptide, KP), derived from the variable region of a recombinant yeast killer toxin-like anti-idiotypic antibody, exerts a significant activity *in vitro, ex vivo* and/or *in vivo* against fungi, bacteria, viruses and protozoa, as well as an immunomodulatory effect on dendritic cells [1]. Based on the wide possibilities of manipulation of the amino acid sequence, the present study was aimed at analyzing the candidacidal properties of peptides derived from KP by substitution of the first amino acid, maintaining unchanged the remaining residues.
Materials and Methods: KP-derived peptides were obtained by solid-phase synthesis chemistry and evaluated for their *in vitro* and/or *in vivo* anticandidal activity by consolidated experimental models. Structural characteristics of selected peptides were analyzed by circular dichroism spectroscopy. Furthermore, their effect on *Candida albicans* cells was studied by transmission electron and confocal microscopy, while potential apoptotic effects were evaluated by flow cytometry.
Results: The selected peptides proved to exert differential *in vitro* and/or *in vivo* anticandidal activities without showing toxic effects on mammalian cells. The change of the first amino acid in the sequence led to different structural organization in solution. Microscopy studies allowed to establish that selected peptides penetrate within *C. albicans* cells and cause gross morphological alterations. Most substituted peptides, likewise KP itself, were able to induce apoptosis in *C. albicans* cells.
Discussion and Conclusions: The different physical and chemical properties of the first substituted residue were found to be important for structural properties, candidacidal activity and possible mechanism of action of the selected peptides. The easy production and low cost of small sized peptides, and the

possibility of their manipulation, associated to new delivery mechanisms, could be exploited for the production of a new generation of antimicrobial drugs.

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P 029A

HIGH-LEVEL OF COLISTIN RESISTANCE IN *IN-VITRO* SELECTED MUTANTS OF KPC CARBAPENEMASE-PRODUCING *KLEBSIELLA PNEUMONIAE*

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Introduction: Colistin is a key component for treatment of KPC carbapenemase-producing *Klebsiella pneumoniae* (KPC-KP) infections. However, the emergence of colistin-resistant KPC-KP has been reported. Inactivation of *mgrB* and alteration of pmrB genes can be responsible for acquired colistin resistance in KPC-KP clinical isolates, by upregulating the Pmr LPS modification system. In this work we investigated the stepwise evolution of KPC-KP to colistin resistance.
Materials and methods: A previously characterized colistin-resistant KPC-KP (KPB-2) (colistin MIC = 4 μg/ml) was used as representative of resistance phenotype associated to PmrB (L82R) alteration. A total of 1 x 10^9 CFU of early-stationary phase cells of KPB-2 was plated onto MHA containing 32 μg/ml of colistin sulphate for *in-vitro* selection of highly resistant colistin mutants. Colistin MICs were measured by Etest. *mgrB* and *pmrB* status was checked by PCR and sequencing. *mgrB* wild type genes was cloned in a pACYC184 derivative for complementation experiments. Alteration in the expression of *pmrHFIJKLM* operon was measured by qRT-PCR on *pmrK* gene.
Results: High-level resistant (selected on colistin, 32 mg/L) KPB-2 mutants were selected at the frequency of 6.03 x 10^{-7}. One mutant (MKPB-2) was selected for further characterization. The MKPB-2 colistin MIC was 48 μg/ml, 12-fold higher than the parental strain MIC. Additionally to the *pmrB* parental alteration, MKPB-2 showed an insertional inactivation of *mgrB* (IS3-like at nt. 75). The pmrK expression was 5-fold higher in MKPB-2 than in KPB-2. MKPB-2 complementation with *mgrB* wild-type decrease colistin MIC and *pmrK* expression to KPB-2 levels.
Discussion and Conclusions: Stepwise evolution to higher levels of colistin resistance by *mgrB* inactivation was documented following in-vitro selection with a colistin-resistant parental strain with *pmrB* alteration. Stepwise increase of colistin MIC can be caused by accumulation of different resistance mechanisms in KPC-KP. It will be interesting to further address the potential clinical relevance of this phenomenon.

P 030

IN VITRO ACTIVITY OF KILLER PEPTIDE AGAINST *TOXOPLASMA GONDII* TACHYZOITES

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Introduction: *Toxoplasma gondii* is one of the most important protozoan parasite globally widespread. Pregnant women and immunocompromised individuals are at higher risk of disease. Killer peptide (KP) is a synthetic decapeptide derived from the sequence of the variable region of a single-chain anti-idiotypic antibody acting as a functional internal image of a *Wyckerhamomyces anomalus* killer toxin characterized by the wide spectrum of microbicidal activity. KP proved to be active against bacteria, fungi, viruses and protozoa, including multidrug resistant strains, through different mechanisms of action. The present study was aimed at evaluating the *in vitro* activity of KP against *T. gondii* tachyzoites.

Materials and Methods: Vero cells seeded onto 12 wells plates were infected with *T. gondii* (RH strain, Type I) tachyzoites and treated with KP at 50, 100 and 200 μg/ml. After 4 hours of incubation, cells were washed and new medium was added. Eight-well chamber slides were prepared, infected and treated as described above. RNA was extracted from wells at 24, 48 and 72 h post-infection and analyzed in real-time PCR for parasite quantification (*T. gondii* SAG1 gene). Chamber slides were fixed and stained at 72 h post-infection for calculation of infection and intracellular proliferation indices.
Results: Real-time PCR showed a dose-dependent reduction of parasite RNA, with the strongest effect by treatment with 200 μg/ml of KP at 48 and 72 h. Evaluation of infection index showed a range of inhibition from 33% to 73% at 50 μg/ml and 200 μg/ml KP concentration, respectively. Parasite proliferation was inhibited in a similar manner, with a reduction of 65% at 50 μg/ml and 92% at 200 μg/ml.
Discussion and Conclusions: This is the first description of KP's activity against an apicomplexan protozoan. KP has a direct effect on *T. gondii* extracellular tachyzoites. KP treatment at the time of seeding reduced the number of infected cells and parasite intracellular proliferation. It has been reported that the microbicidal activity of KP is mediated by receptors, including β-1,3-glucan and α-1,3-linked mannose residues. Although these have not been described in *T. gondii* tachyzoites, β-1,3-glucan is present on the membrane of *T. gondii* oocysts. Further studies will be needed to elucidate the mechanism of KP action against different forms of *T. gondii*.

P 030A

HIGHLIGHT ON FITNESS COST OF RIFAMPICIN-RESISTANCE IN *NEISSERIA MENINGITIDIS*

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Introduction: *N. meningitidis* is a leading cause of bacterial meningitis and sepsis worldwide. Chemoprophylaxis with rifampicin is one of the major measures to avoid further spread and possible epidemics. Nevertheless, although rifampicin has been used routinely in the management of contacts and mutator strains with an elevated mutation frequency to rifampicin-resistance (Rif^R) are rather common in hypervirulent lineages, only few cases of meningococcal disease caused by Rif^R strains have been reported so far reflecting a decreased biological fitness. The mutations conferring Rif^R change aminoacids directly involved in antibiotic binding to RNA polymerase in the central segment of the β-chain.
Materials and methods: We selected *in vitro* spontaneuos Rif^R rpoB mutants from the hypervirulent serogroup C strain 93/4286 and analyzed a number of phenotypic features. The whole sequencing of *rpoB* gene in resistance and susceptible strains was performed. To evaluate the fitness of Rif^R mutants we tested the ability to grow/survive in different culture media and in differentiated human monocytes and to compete with the wild type strain. Finally, we performed RNA-seq experiments in selected strains.
Results: Our results demonstrate that *rpoB* mutations may have different effects on bacterial fitness ranging from low-cost (H553Y) to high-cost (H553R, S549F). As a consequence of metabolic perturbation the H553Y mutant exhibits a reduced fitness in macrophages and resistance to oxidative damage, confirmed also by RNA-seq experiments. In the H553Y strain, many genes involved in energetic (carbon) and aminoacids metabolism were significantly up-regulated, while the master genes of host-pathogen interaction were down-regulated.
Discussion and Conclusions: Altogether these findings seem to be consistent with the hypothesis that the H553Y mutation in the RNA polymerase determines a global metabolic perturbation with effects on transcription dynamics, bacterial fitness, physiology, metabolism and virulence of meningococcus.

P 031

RE-MERGING INFECTIONS IN TRANSFUSION THERAPY FROM IMMIGRANT POTENTIAL DONORS: A MONITORING PROJECT IN CAMPANIA REGION

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Introduction: Migration flows have greatly increased in recent years and have contributed to the rise of new or re-emerging pathogens, potentially dangerous for human and animals. Re-merging infections, such as malaria and tuberculosis, and emerging infectious diseases such as West Nile fever and Chikungunya fever transmitted through blood transfusion are an important public health problem in endemic and not endemic areas. The increasing number of immigrants that request to become regular donors requires an appropriate assessment of the problems related to endemic diseases of the native regions.
Materials and Methods: We have collected 4059 foreign donors coming from Asia, Africa, Est Europe, Centre and South America and resident in the Campania Region. They have been subjected to routine laboratories tests and to confirm the positivity of pathogen detection, we also used real-time PCR assays that discriminate between microorganisms based on a signal from specific nucleic acid sequences.
Results: The project has been designed to implement a plan that monitors: P. falciparum, M. tuberculosis, West Nile virus, Chikungunya virus, responsible of infectious diseases and to develop an appropriate prevention and surveillance program. A total of 4059 foreign donors were collected: 62.6% of samples became from Asia, 24.61% were from Africa, 12.52% from East Europe and the remaining donors were from Centre and South America (0.21%). Noteworthy we found 1128/4059 (27.79%) foreign donors to be positive for one or more routine serological markers (HbsAg, Anti-HCV, Anti-HIV and TPHA), 16.9% of samples to be positive for malaria antibodies, and 2.5% to be positive for M. tuberculosis DNA.
Discussion and Conclusions: These findings suggest that integrated human and entomological surveillance is crucial to monitor the spread of emerging vector-borne diseases and to implement public health measures in order to avoid transmission and risks associated with transfusion therapy.

P 031A

EVALUATION OF THE ANTIBIOFILM PROPERTIES OF THE ANTIMICROBIAL PEPTIDE TEMPORIN B AND ITS POTENTIAL IN THE LOCK THERAPY OF CENTRAL VENOUS CATHETERS COLONIZED BY BIOFILMS OF *STAPHYLOCOCCUS EPIDERMIDIS*

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Introduction: With the increased use of medical devices in health care procedures, biofilm associated infections have emerged as one of the major threats of modern medicine. Biofilm formation on central venous catheters (CVC) is particularly relevant as it may represent the starting point of catheter-related blood stream infections. This work evaluates the potential of the frog-skin derived antimicrobial peptide temporin B (TB), used alone or in combination with conventional or un-conventional

antimicrobial agents, to prevent the formation of and/or to eradicate mature biofilms of *S. epidermidis*.
Materials and methods: *S. epidermidis* clinical isolates were obtained from blood cultures or CVC at the Microbiology Unit of Pisa University Hospital. Antibiofilm activity of TB or its combinations was evaluated in terms of biofilm biomass and/or number of biofilm-associated viable cells.
Results: A striking ability of TB to kill both forming and mature *S. epidermidis* biofilms was observed, especially in combination with cysteine or EDTA. Kinetics studies demonstrated that the combination TB/EDTA was active against mature biofilms already after 2-4h exposure. A double 4h exposure of biofilms to TB/EDTA further increased the therapeutic potential of the same combination, causing 3 $\log_{10}$ reduction in CFU number as compared to the control. Of note, TB/EDTA was able to eradicate *S. epidermidis* biofilms formed *in vitro* on silicon catheters. Possibility to further optimize the TB therapeutic potential was investigated by encapsulating the peptide in chitosan nanoparticles and evaluating the microbicidal properties of the nanosystem against *S. epidermidis*.
Discussion and conclusions: The lock therapy of contaminated catheter (i.e. instillation of high concentration of antibiotics into the catheter lumen to salvage the colonized catheter) is a widely used procedure, but still far to be optimal. The possible use of TB in combination with EDTA in the lock solution may reduce the risk of development of resistance as compared to conventional antibiotics, while taking advantage of the intrinsic antibiofilm and anticoagulant properties of EDTA. TB encapsulation in a proper delivery system may further contribute to the long-term prevention of catheter-related blood stream infections.

P 032

EVALUATION OF THE POSSIBLE INVOLVEMENT OF BACTERIAL BIOFILMS IN THE PATHOGENESIS OF CHRONIC RHINOSINUSITIS: A PILOT STUDY

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Introduction: Chronic rhinosinusitis (CRS) is a frequent disorder that remains poorly understood from a pathogenic standpoint and has a significant impact on patient quality of life, as well as healthcare costs. Recently, CRS is thought to have an underlying biofilm etiology. To evaluate the role of biofilm in the pathogenesis of CRS, in this study we assessed the presence of biofilm at the infection site and the biofilm-forming ability of bacteria isolated from CRS patients.
Materials and Methods: Patients with CRS (n = 7) and 2 controls who underwent nasal surgery for other pathologies were included in the study. Samples collected were: i) nasal swabs before the surgical intervention as an indicator of the "normal" bacterial flora, ii) swabs from the specific infection site, iii) biopsies at the time of functional endoscopic sinus surgery. In addition, samples by sinus puncture and aspiration were obtained when possible. The identification and drug sensitivity tests of the isolated bacteria were performed by routine microbiology techniques. In addition, confocal scanning laser microscopy (CM) was used to detect the presence of biofilms on biopsies. Microtiter plate assays were employed for *in vitro* assessment of i) biofilm formation, evaluated as crystal violet staining after 24h culture, and ii) biofilm drug-susceptibility.
Results: Staphylococci represented the majority of the isolates obtained from the infection site, with *S. epidermidis* being the most frequently isolated species. Other isolates were represented by *Enterobacteriaceae* or by species present in the oral flora. CM of the mucosal biopsies taken from patients with

CRS revealed biofilm formation in almost all specimens. In concordance, the vast majority of bacteria isolated from the specific infection site of the CRS patients were able to form biofilm *in vitro*. Drug-susceptibility tests demonstrated that the antibiogram profile of planktonic bacteria differs from that of sessile bacteria in biofilms.
Conclusion: The consistent demonstration of biofilms on mucosa biopsies and the presence of biofilm-forming bacteria in CRS patients suggests that they may play a significant role in the pathogenesis/persistence of the disease.

P 032A

ANTIBACTERIAL ACTIVITY OF TEMPORIN B ANALOGUES, ALONE OR IN COMBINATION WITH OTHER ANTIMICROBIAL AGENTS, AGAINST BIOFILMS OF MEDICALLY RELEVANT BACTERIAL SPECIES

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Introduction: Treatment of biofilm-associated infections is notoriously difficult and requires high doses of antibiotics. Biofilm-forming bacteria acquired properties that render them extremely tolerant to conventional drugs, making the discovery of new antibiofilm compounds particularly pressing. Antimicrobial peptides (AMPs) have been repetitively proposed as a future class of antibiotics. Recently, their potential use in preventing biofilm formation or in treating mature biofilms has also been highlighted. The aim of the present study was to optimize the antibiofilm properties of the frog skin-derived AMP temporin B (TB) against biofilms of medically relevant bacterial species, by designing peptide's analogues and testing their activity alone or in combination with other antimicrobial agents.
Methods: A computational approach was used to design a series of TB analogues. Sequences of other TB analogues were obtained from the literature. All the peptides were tested for their antimicrobial and cytotoxic activity in bactericidal or hemolysis assays. Biofilms were formed in 96-well plates and biofilm-biomass was evaluated by crystal violet staining.
Results: A number of TB analogues showed an improved antibacterial activity against planktonic Gram-pos. and Gram-neg. bacterial strains. Of note, the analogues resulted particularly improved against Gram-neg. bacteria (including *P. aeruginosa)* as compared to the parental peptide. The most active analogues (KK-15 and FL-13) were tested for their antibiofilm activity. Both peptides strongly inhibited the formation of *S. aureus* biofilms, while only KK-15 was able to prevent biofilm formation by *P. aeruginosa.* Interestingly, a marked synergistic antibiofilm effect against biofilms of the latter species was observed when both peptides were used in combination with EDTA, levofloxacin or amikacin.
Conclusions: Optimization of peptide sequences and identification of combinatorial strategies with conventional and un-conventional drugs may highly improve the therapeutic potential of AMPs against biofilms of medically relevant bacterial species allowing to reduce the active antibiotic concentrations and the possible side-effects.

P 033

PHENOTYPIC AND GENOTYPIC CHARACTERIZATION OF METHICILLIN-, TETRACYCLINE- AND ERYTHROMYCIN-RESISTANT STRAINS OF *STAPHYLOCOCCUS INTERMEDIUS* OF CANINE ORIGIN

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Introduction: *Staphylococcus intermedius (S. intermedius)* is a coagulase positive zoonotic staphylococcus found in several domestic animals. It is a common commensal of oral, nasal and skin flora in healthy dogs, where it can also cause invasive diseases. Studies on genotypic characterization of antibiotic resistance have shown that the distribution of the antibiotic resistance genes seems to be vary among staphylococci of different animal origin.
Materials and Methods: The specimens were collected from diseased dogs which attended the Microbiology Laboratory of the Department of Veterinary Medicine and Animal Production of the University of Naples Federico II.
The swabs from different origins were inoculated onto Mannitol Salt Agar (MSA) and identified by automated VITEK 2 (bioMérieux®). The isolated strains were tested for their susceptibility to 14 antibiotics by using Kirby-Bauer disk diffusion test. Multiplex PCR were performed to determine genetic profiles of antibiotic resistance.
Results: We isolated 25 strains of *S. intermedius*. Phenotypically penicillin resistance was present in 18 bacterial strains; however, only 4 of these isolated strains carried the *mecA* gene.
The resistance rates for tetracycline and erythromycin were 60 and 56 per cent, respectively.
Tetracycline resistance genes *tet*(K) and *tet*(M) were found positive in 15 isolates which were phenotypically tetracycline-resistant. Erythromycin resistance gene *erm*(B) was found positive in 14 isolates which were phenotypically erythromycin-resistant. In particular, nine of the tetracycline-resistant strains carried only *tet*(K) gene, while six carried both *tet*(K) and *tet*(M) genes. All the erythromycin-resistant isolates had *erm*(B) gene.
Discussion and Conclusions: We noticed 56 per cent multidrug resistant *S. intermedius* strains. Moreover, our results show that the phenotypic and genotypic characterization of methicillin resistance are not correlated and so further studies are needed, whereas the phenotypic and genotypic characterization of tetracycline and erythromycin resistance are well correlated. The distribution and the prevalence of the antibiotic resistance genes among Staphylococcus spp. suggest that effective measures should be taken to control antibiotic use in pet animals.

P 033A

A COMPARISON BETWEEN INTERFERON GAMMA RELEASE ASSAYS AND SEROLOGY OF *MYCOBACTERIUM TUBERCULOSIS*

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Introduction: Tuberculosis (TB) caused by *Mycobacterium tuberculosis* remains a major world disease with approximately nine million new cases each year. Serology may offer increased detection of active disease in patients with Interferon Gamma Release Assay (IGRA). We evaluated the presence of antibodies against *M. tuberculosis* in patients with suspect of active disease.
Materials and Methods: The detection of anti-*Mycobacterium tuberculosis* IgG antibodies was carried out by an Enzyme-Linked Immunosorbent As-

say (Tb Eurospital) and the results were compared with the most commonly-used Interferon Gamma Release Assay (Quantiferon). We studied 189 samples sent to our laboratory from January 2014 to March 2015 for testing *M. tuberculosis* with IGRA in according to the manufacturer's protocol.
Results: Clinical information was obtained for 189 patients and they were divided in terms of disease. Patients who were positive by the IGRA with "suspected" tuberculosis disease (clinical symptomatology present) had about 39% (73/189) anti-*M. tuberculosis* IgG antibody positivity, while patients who were positive by the IGRA with confirmed active tuberculosis disease had an anti-*M. tuberculosis* IgG antibody positivity rate of 76% (143/189).
Discussion and Conclusions: Currently, neither serology nor IGRA should be used for the diagnosis of active tuberculosis disease, as recommended by WHO, but the combined use of IGRA and the anti-*Mycobacterium tuberculosis* IgG antibodies would improve the diagnosis of active tuberculosis. For the moment, in this study, we did not consider patients with indeterminate or negative IGRA results.

P 034

DIAGNOSIS OF ENDOCARDITIS DUE TO *NEISSERIA ELONGATA* SUBSPECIES *NITROREDUCENS*: CASE REPORT

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Introduction: *Neisseria elongata* subsp. *nitroreducens* is a nasopharyngeal commensal organism and an uncommon pathogen able to reduce nitrates and nitrites to amines without the formation of gas. Other biochemical characteristics of the organism include negative catalase, urease, and indole reactions; a positive oxidase reaction; and lack of acid production from carbohydrates. *N. elongata* subsp. *nitroreducens* causes a variety of infections but is most often associated with bacteremia and infective endocarditis. We describe a patient with prosthetic-valve endocarditis due to this organism.
Materials and Methods: A 40-year-old male affected by Marfan syndrome with a prosthetic aortic valve and graft was admitted to the Operative Unit of Cardiology for evaluation of fever (temperature, to 39°C). A transesophageal echocardiogram (TEE) showed an echolucent zone around the graft and a vegetation on the prosthetic valve. Blood cultures were performed, and the patient was treated with iv amoxicillin-clavulanic acid and gentamicin.
Gram-negative, aerobic, nonmotile coccobacilli were detected in 3 blood culture bottles after 1 day of incubation. Subcultures were performed, and growth was observed after 24 hours of incubation with 5% CO_2 on plates containing chocolate, blood agar. The colonies were small and nonpigmented. No hemolysis on sheep or horse blood was observed. The organism was strictly aerobic, catalase negative and oxidase positive. To identify this gram-negative short rod with good agreement, we used the technique of mass spectrometry (MALDI-TOF) confirmed by a molecular approach based on 16S ribosomal DNA (rDNA) sequencing.
Results: The bacterium was identified as *N. elongata* subsp. *nitroreducens* by MALDI-TOF with a score of 2.07. This identification was confirmed by 16S ribosomal DNA (rDNA) sequencing. Our sequence had an homologies with sequence of *N. elongata* subsp. *nitroreducens* GenBank accession number AJ 247254.
Discussion and Conclusion: The isolation of the organism from three blood cultures from this patient indicates the clinical significance of this subspecies. Since unequivocal identification of the isolate could not be established by phenotypic tests, the use of 16S rDNA sequencing in combination with the technique of mass spectrometry (MALDI-TOF), allowed us to correctly identify *N. elongata* subsp. *nitroreducens.*

P 034A

INDUCTION AND CHARACTERIZATION OF PROPHAGE Φ1207.3 IN *STREPTOCOCCUS PNEUMONIAE*

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Introduction: Φ1207.3 is a prophage of *Streptococcus pyogenes* which carries the macrolide efflux resistance genes *mef(A)/msr(D)*. Complete nucleotide sequence showed that Φ1207.3 is 52,491 bp in length and contained 58 ORFs. Φ1207.3 codes for two different C-methylation systems, several phage structural genes, a lysis cassette, and three site-specific resolvases of the serine recombinase family. The aim of this study was to characterize Φ1207.3 in a prophage-free *Streptococcus pneumoniae* model strain.

Materials and Methods: *S. pneumoniae* FP10 and FP11 were used as host strains, while *S. pyogenes* 2812A served as Φ1207.3 donor. Φ1207.3 lytic cycle was induced in early exponential phase with 100 ng/ml of mitomycin C for 2 hours. Induction was monitored by Real Time-PCR using primers targeting *mef(A)* and phage particles were prepared from culture supernatants. Phage particles were processed by standard negative stain and imaged by a Tecnai G^2 transmission electron microscope (TEM). The virion morphology was also predicted using the Virfam webserver (http://biodev.cea.fr/virfam/) which allows for an automatic classification of phages.
Results: *S. pneumoniae* strain FR1 carrying the prophage Φ1207.3 was obtained by conjugation. Culture supernatants, prepared after mitomycin C treatment, were analysed. Induction assays demonstrated that the *mef(A)* gene was increased in culture supernatants after ultracentrifugation (7.7×10^8 mef(A) copies/ml). Phage particles were obtained from strain FR1 and used for electron microscopy. TEM showed phage particles with an icosahedral head and a noncontractile tail, placing them in the *Siphoviridae* family. An average of $3,6 \times 10^3$ particles/mesh were obtained. The bioinformatic analysis using Virfam assigned Φ1207.3 to the family *Siphoviridae*, Neck Type 1 - Cluster 2, order *Caudovirales*, which is composed by phages infecting *Firmicutes.*
Discussion and conclusions: In this work we showed that Φ1207.3 is a phage belonging to the *Siphoviridae* family. The phage can be transferred from *S. pyogenes* to *S. pneumoniae* where it is still functional. This is the first demonstration of a functional phage carrying an antibiotic resistance determinant which is transferable among differents species.

P 035

EFFECTS OF CAPSAICIN ON *STREPTOCOCCUS PYOGENES*

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Introduction: Capsaicin (8-methyl-N-vanillyl-6-nonenamide) is the active component responsible for the fruit pungency of *Capsicum* plants, cultivated for food and also for medicinal uses since ancient times. Besides its multiple pharmacological and physiological properties (pain relief, cancer prevention, weight reduction, cardiovascular, and gastrointestinal benefits), capsaicin has recently received attention because of its antimicrobial activity and anti-virulence properties. The aim of the present study was to investigate the effects of capsaicin on *Streptococcus pyogenes*, the most common cause of acute bacterial pharyngotonsillitis.
Materials and Methods: The erythromycin-resistant [*erm*(B)/cMLS], high cell-invasive, and strong biofilm producer *S. pyogenes* pharyngeal isolate SP1070 was used throughout the study. Capsaicin was purchased from Sigma-Aldrich and stored (10 mg/mL stock solution) in absolute ethanol at -20°C. The MIC and MBC were determined according to the CLSI guidelines. Survival in presence of capsaicin was studied by the live/dead assay. Biofilm formation was tested by a microtiter assay and quantified by measuring the absorbance at 690 nm. Cell experiments were performed using the human alveolar carcinoma A549 cell line.
Results: The MIC and the MBC of capsaicin were both 128 μg/mL. In the live/dead assay, several red cells were detected as early as 15 min after incubation with capsaicin at MIC; all cells were red after 60 min of incubation. At capsaicin sub-MICs (1/2

– 1/16 × MIC), a significant increase in biofilm production and in the number of streptococci adherent to A549 cells was observed; whereas a strong reduction in the number of intracellular bacteria was detected.

Discussion and Conclusions: Our findings reveal that capsaicin has a dual effect on *S. pyogenes*. High-level capsaicin exerts a bactericidal effect, probably due to the disruption of the cell membrane, this result being in agreement with previous studies on Gram-positive and Gram-negative pathogens; while sub-lethal capsaicin modifies virulence properties *in vitro*, such as the ability to form biofilm and to adhere/invade epithelial cells. Capsaicin-induced effects on biofilm formation seem to be similar to those observed for a variety of antibiotics that at sub-lethal concentrations can act as agonists of bacterial biofilm production *in vitro*. Overall, capsaicin-induced effects on *S. pyogenes* deserve further studies.

P 035A

OXA-23-PRODUCING ST25 *ACINETOBACTER BAUMANNII*: FIRST REPORT IN BOLIVIA

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Background: Carbapenem-hydrolysing class D beta-lactamases (CHDLs) are the main mechanism of carbapenem resistance in *A. baumannii* and five groups of acquired CHDLs have been identified in this pathogen, with OXA-23 enzyme being the most widespread worldwide. In South America, *A. baumannii* producing OXA-type carbapenemases have been described since the late 1990s and sequence type (ST) 25 has recently been identified as an emerging clone, associated either to OXA-23 or to the NDM-1 metallo-beta-lactamase. Here we describe the first OXA-23-producing ST25 *A. baumannii* from Bolivia.

Methods: *A. baumannii* 9495/13 was isolated in 2013 from an inpatient in Villa Montes (Bolivia). Identification was confirmed by Matrix-assisted laser desorption/ionization mass spectrometry. Minimum Inhibitory Concentrations were determined by broth microdilution according to Clinical and Laboratory Standards Institute. Multilocus sequence typing (MLST) was performed according to the Pasteur Institute scheme and a PCR-sequencing approach was used for detection of CHDLs. The genetic environment of $bla_{\text{OXA-23}}$ was characterized by inverse PCR.

Results: *A. baumannii* 9495/13 showed a multidrug resistance phenotype including carbapenems, expanded-spectrum cephalosporins, fluoroquinolones, aminoglycosides, tetracycline, trimethoprim-sulfamethoxazole, while remaining susceptible to colistin and tigecycline. MLST assigned it to the emerging clone ST25. $bla_{\text{OXA-23}}$ gene was found to be associated to a Tn*2008* transposon, inserted in an original chromosomal location and surrounded by a 9 bp duplication of the target sequence.

Discussion and Conclusion: To the best of our knowledge, this is the first report of OXA-23-producing *A. baumannii* in Bolivia, and the first description of transposon Tn*2008* in Latin America. The finding of an OXA-23-producing *A. baumannii* belonging to ST25 in Bolivia is consistent with recent studies indicating ST25 as a potential emerging clone in Latin America. Finally, sequence analysis of the chromosomal location of Tn*2008* in *A. baumannii* 9495/13 supports the recent hypothesis that Tn*2008* might be able to move as a discrete transposable element.

P 036

ANTIMICROBIAL-RESISTANCE AND PHYLOGENETIC GROUPING OF *ESCHERICHIA COLI* ISOLATES FROM NATURAL BEDS OF VENUS CLAM

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Introduction: Faecal contamination of coastal areas is a major concern especially in shellfish harvesting sites. *Escherichia coli* and enterococci used as faecal indicator bacteria of water pollution have been proved to survive in sand and sediment that could represent potential reservoirs of pathogenic strains. The bivalve *Chamelea gallina* living buried into sandy sediment, can concentrate microrganisms from the surrounding water by filter-feeding activity. In this work *E. coli* strains, from *Chamelea gallina* collected at seven natural beds along Marche region coast, have been screened for: (i) phylogenetic group and clade assignment, (ii) antimicrobial susceptibility and (iii) genetic correlation of isolates.
Material and Methods: Phylogenetic grouping and clade assignment of *E. coli* isolates were performed by specific multiplex-PCR assays. Susceptibility of 141 *E. coli* isolates to eight antibiotics was evaluated by the disk diffusion method. Enterobacterial Repetitive Intergenic Consensus PCR was used for the typing and the phylogenetic analysis of isolates.
Results: The majority of isolates (60%) belonged to phylogroup A or B1, 31% to the other groups (B2, C, D, E, F), 8% to *Escherichia* cryptic clades and only 1% was untypable. A third of isolates (47) was resistant to at least one drug and 16 strains (11%) were multidrug resistants (MDR). Resistance to tetracycline, ampicillin and streptomycin was the most frequently found. Typing analysis disclosed high genetic heterogeneity among strains, including those from the same sampling site. Clonality was not observed.
Discussion and Conclusions: This findings show survival of *E. coli* in bivalve mollusc from all the seven sampling sites. The high genetic diversity of strains reveal multiple sources of microbiological contamination of the analysed coastal areas. The presence of MDR isolates belonging to phylogroups encompassing human extraintestinal pathogens, suggest a clinical origin of some isolates. Therefore a potential risk to public health as a result of raw or undercooked bivalve consumption may occur if purification is not performed or it is not effective.

P 036A

EFFICACY OF THE PROBIOTIC *L. BREVIS* CD2 IN THE INHIBITION OF BIOFILM-GROWING ORAL PATHOGENS

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Introduction: The use of Lactobacilli as probiotics has been demonstrated to be health-promoting. The potential application of probiotics to displace pathogenic microorganisms is gaining considerable interest. In addition to the already demonstrated activity in a variety of gastrointestinal disorders, new evidences are accumulating to support the effect of probiotics in the prevention of urinary tract, wound and oral infections. These infections are more and more often caused by microbial pathogens able to form biofilm, thus generating biofilm-related infections. Taking this into consideration, the aim of this study was to evaluate the efficacy of a probiotic lactobacillus strain in preventing pathogenic biofilm development in the oral cavity.
Materials and Methods: The strictly anaerobes *Fusobacterium nucleatum* subsp. *polymorphum* DSM 20482, *Actinobacillus actinomycetemcomitans* HG569 and *Porphyromonas gingivalis* W83 were selected to evaluate the probiotic L. brevis CD2 ability to counteract their biofilm formation. The classical quantitative assay was applied to determine the influence of CD2 supernatant on biofilms, while Field Emission Scanning Electron Microscopy and Confocal Microscopy were used test the CD2 adhesive ability in inhibiting pathogenic oral biofilm.

Results: Supernatant was tested as it is, neutralized to a pH 6.5-7.0, and incubated at 100 ◦C for 15 min. Supernatant and heat shocked supernatant are both of them able to strongly reduce biofilm of the selected strains while neutralized supernatant did not affect biofilm formation. No differences have been detected when supernatant was added to pre-formed biofilms. When we used cells of *L. brevis* CD2, the *A. actinomycetemcomitans* HG569 and *P. gingivalis* W83 growths were inhibited, while a less compact biofilm was observed in *F. nucleatum* DSM 20482-*L. brevis* CD2 dual species biofilm.
Discussion and Conclusions: It seems that L. brevis CD2 is able to counteract other potentially pathogenic bacteria growing as biofilm. In particular, organic acids seem to inhibit *F. nucleatum* and *P. gingivalis* biofilm development. Organic acids, in addition to their antimicrobial activity, also could function as permeabilizers of the outer membrane and act as potentiators of the effects of other antimicrobial substances possibly present into the supernatant.

P 037

DETECTION OF ESBL-PRODUCING GRAM-NEGATIVE BACTERIA FROM SURFACE AND GROUND-WATERS IN THE PO VALLEY

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Introduction: The aim of the study is to inspect the occurrence of Extended Spectrum β-Lactamases (ESBLs)-producing Gram-negative bacteria in the Po Valley wells, streams and treatment plants.
Materials and Methods: A total of 159 water samples from 11 wells, 5 streams and 3 treatment plants were collected once a month, during the period from December 2014 till June 2015. The water samples were filtered using filter membranes methodology, utilizing 100ml and 1ml of water from wells and streams/treatment plants, respectively. The filters were placed on Plate Count Agar (PCA), McConkey Agar (MCA) and selective MCA containing cefotaxime 8mg/L (MCA+CTX). Identification/antimicrobial susceptibility profiles of bacteria grown on MCA+CTX were obtained using the MicroScan/A4 System (Beckman Coulter) and interpreted according to EUCAST 2015 guidelines. PCR for detecting $bla_{CTX\text{-}M/TEM}$ genes and Pulsed Field Gel Electrophoresis (PFGE) were performed.
Results: Using MCA+CTX, 48.7% *Enterobacteriaceae*, 17.1% *Vibrio fluvialis* and *Acinetobacter* spp., 10.5% *Pseudomonas* spp., and 6.6% *Aeromonas hydrophila* were identified. All treatment plants, 80% of streams and 15.4% of wells sampled resulted positive for the presence of CTX resistant strains. Out of 37 suspected ESBL-producing *Enterobacteriaceae*, 17 were *Yersinia enterocolitica,* 11 *Escherichia coli*, 6 *Klebsiella* spp., 2 *Enterobacter* spp. and 1 *Leminorella* spp. All the above *Y. enterocolitica* strains showed resistance to tetracycline amoxicillin/clavulanate (80%), followed by ciprofloxacin (33.3%), chloramphenicol (20%), amikacin and gentamicin (13.3% each). All the 11 *E coli* and 6 *Klebsiella* spp. isolates resulted MDR, showing resistance to β-lactams and tetracycline (100%), ciprofloxacin (81.8% and 50%, respectively) and amikacin (18.2%; 16.6% each).
Eight out of nine *E. coli* and 3 out of 4 *Klebsiella* spp. chosen as representative, resulted CTX-M positive by PCR. PFGE on 13/17 representative ESβL *E. coli* and *K. pneumoniae* producers yielded different pulsotypes and clonal diversity also in the case of isolates from the same sample source.
Discussion and Conclusions: There appears to be a high occurrence of ESBL-enterobacteria, almost exclusively from surface waters. The persistence and spread of these species in the environment poses a threat to exposed human populations.

P 037A

ONYCHOMYCOSIS IN DIABETIC PATIENTS: EPIDEMIOLOGY AND DIAGNOSTIC METHODS

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Introduction: Patients with diabetes represent a unique group of individuals who appear more prone to develop infections than others. The diabetic foot complication represents one of the most complex and serious complications in these patients. Fungal infections can also contribute to the severity of the diabetic foot. The aim of the present study was to evaluate the prevalence of toenail mycoses in a group of 47 subjects with diabetic foot complication and in a matched control group.

Materials and Methods: Over a period of 1 year, 715 patients (47 diabetic and 668 nondiabetics) were observed. Nail material was taken through scraping from clinically abnormal nails. The nail scrapings were incubated in 15% potassium hydroxide for 90 minutes and examined under microscope for the presence of fungal elements. Cultures were performed using Sabouraud glucose agar + chloramphenicol +/– cycloheximid and incubation at 25 ºC for up to 2 weeks. Moreover for some patients an alternative PCR-based method for the detection of dermatophyte nail infections was performed. Data were analyzed using MedCalc program. Chi-square test was used to compare differences between diabetic and nondiabetic patients.

Results: Diabetic subjects presented onychomycosis in 55.3% showing a prevalence of fungal infection significantly higher ($p < 0.0001$) than that observed in the control group (25.2%). In diabetic group, dermatophytes were the most common isolate (50%), followed by yeasts and moulds in 30.8% and 19.2%, respectively. In control group, the distribution of dermatophytes, yeasts and non-dermatophyte moulds was 67.4%, 5.3% and 27.8%, respectively. In both diabetic and control groups, dermatophytes were the most common fungal pathogens isolated from toenails.

Discussion and Conclusions: In our study, the presence of onychomycosis was found to correlate significantly with increasing age. In the diagnosis of onychomycosis laboratory studies are important to confirm the clinical diagnosis, and also to identify the etiologic agents. The efficiency of direct microscopic examination emphasizes the importance of the method, when performed by experienced professionals, favoring the speed of diagnosis and treatment of patients. On the patients with diabetes the PCR-based methods may be considered a good alternative for the cases of onychomycosis for which the etiological agents are dermatophytes.

P 038

TUBERCULOSIS VERSUS UVEITIS

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Introduction: Uveitis is an umbrella term used to describe a wide variety of inflammatory conditions occurring inside the eye. Tuberculosis-related uveitis (TRU) is an extra pulmonary form of the disease with multiple clinical presentations. Difficulties in the microbiological confirmation of TRU cases makes indirect testing with Mantoux and QuantiFERON TB Gold (QFT) the main support for diagnosis. The correct diagnosis of TRU has important therapeutic consequences, since tuberculous uveitis should be treated with anti-tuberculosis drugs to prevent visual loss. We present a preliminary study concerning with the usefulness of QFT and of anti-tuberculosis treatment (ATT) in patients that fulfilled the definition of TRU.

Materials and Methods: Four patients with presumed TRU were examined at the Institute of Ophthalmology, University of Sassari, Italy, between September 2014 and January 2015. Blood samples were obtained from all patients and QFT was performed according to the recommended guidelines at the Laboratory of Mycobacteriology, University of Sassari. The patients were given standard anti-tubercular treatment (ATT) and the QFT was carried out again in all of them after three months and the completion of ATT.

Results: Bilateral panuveitis was identified in 3 patients and unilateral posterior uveitis was observed in the remaining one. The clinical manifestations

observed included cyclitis + vitreous inflammation, cyclitis + vitreous inflammation + vasculitis, vasculitis + vitreous inflammation and papillitis. The most common ocular complication was macular edema. Before the beginning of ATT, QFT was positive in all subjects. After three months, a patient showed a reversion of QFT result, which went from positive to negative; in two patients the QFT was still positive, but with a concentration of Interferon-gamma (IFN-γ) that decreased in one cases and increased in the other. At present 3 out 4 patients completed ATT; all showed decreased IFN-γ concentration in comparison with pre-treatment values. There was clinical improvement in all of them.

Discussion and Conclusions: Our results suggest that QFT is useful in the diagnosing of TRU. An interesting finding of our study is the fact that after specific treatment all the patients with presumed TRU had a reduction of IFN-γ concentration and clinical improvement. However further research should focus on the relation between decrease in IFN-γ concentration and ocular improvement in patients with TRU receiving ATT.

P 038A

NEW ARTEMISININ DERIVATIVES WITH ANTILEISHMANIAL AND ANTIPLASMODIAL ACTIVITY

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Introduction: Artemisinin derivatives are the most effective antimalarials available to-date. They possess anti-leishmanial activity, as well, at micromolar concentrations. Malaria and leishmaniasis are two main tropical parasitic diseases responsible for million cases every year. Co-infections exist in those populations living in geographical areas where the diseases overlap. In the absence of any vaccine, chemotherapy remains the only weapon to fight these infections. However, cost, toxicity and resistance to conventional drugs, make very urgent the need for new drugs. Here we describe the antiplasmodial, antileishmanial and cytotoxic activity of new artemisinin derivatives. Artemisinin, dihydroartemisinin and artesunate were used as control drugs.

Materials and methods: The antiplasmodial activity on different *Plasmodium falciparum* strains was tested using the pLDH assay. The activity on promastigote stage of *Leishmania infantum*, *L. tropica* and *L. braziliensis* was evaluated using the MTT assay and the IC_{50} was calculated for each compound. Intracellular amastigotes were obtained infecting PMA differentiated THP-1 cells with metacyclic promastigotes. The percentage of infected macrophages in control and in drug treated cells was determined by Giemsa staining and light microscopy. Cytotoxicity on human cells was tested by the MTT assay.

Results: Artemisinin derivatives were toxic against promastigotes of *Leishamnia* spp with IC_{50} values in the micromolar range. The most active compound was the new derivative GC12 whereas artemisone was the less active compounds on Leishamnia, but also the less cytotoxic on human cells and the best on *Plasmodium*. Mild activity was exhibited against intracellular amastigotes. As expected, all derivatives explained high antiplasmodial activity with IC_{50} values in the nanomolar range.

Discussion and conclusion: These data indicate that artemisinin derivatives have promising anti-leishmanial activity. Further work is needed to develop and select new drug candidates against both diseases

P 039

INHIBITION OF *CANDIDA ALBICANS* BIOFILM FORMATION BY A HUMAN LACTOFERRICIN-PEPTIDE DERIVED FROM THE N-TERMINUS

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Introduction: *Candida albicans* is characterized by a high propensity to produce biofilm on medical implants and is often associated with catheter-related infections in hospitalized patients. *C. albicans* biofilms are highly resistant to most conventional antifungal agents, limiting the choices for appropriate therapeutic intervention. Hence, there is a growing need to develop alternative approaches to eradicate biofilm-associated infections. A synthetic N-terminal peptide of human lactoferricin (hLF1-11) has previously shown to exert antifungal activity against planktonic *C. albicans* cells. In this study, the *in vitro* anti-biofilm activity of hLF1-11 was evaluated against clinical isolates of *C. albicans* with different fluconazole susceptibility.
Materials and Methods: Ten *C. albicans* clinical isolates were investigated for biofilm production. Among these, four different strains, SC5314, CA22, CA37, and a fluconazole-resistant isolate (CA688), were selected based on different RAPD profiles. To evaluate the effect of the hLF1-11 peptide on biofilm formation, *C. albicans* cells were co-incubated into flat bottom 96 well plates at 37°C for 24 h with various concentrations of hLF1-11. The anti-biofilm activity of the peptide was assessed by measuring (i) biofilm biomass at ODλ490 nm; (ii) the metabolic activity, using the XTT (2,3-bis-2- methoxy-4-nitro-5-sulfophenyl-2H-tetrazolium-5-carboxanilide) reduction assay and (iii) viable cell (CFU/ml) reduction by scraping biofilm from wells.
Results: hLF1-11 exhibited an inhibitory effect on biofilm formation by all the *C. albicans* strains tested, in a dose-dependent manner. Indeed, a significant reduction ($p < 0.05$) of biofilm biomass was observed following co-incubation with 64 and 128 μM hLF1-11, in comparison with the untreated control. These peptide concentrations also reduced metabolic activity (100%) and CFU/ml (~2 log) of sessile cells. A visual inspection of treated or untreated biofilm cells was also performed with an inverted microscope, indicating a significant reduction in hyphal formation by hLF1-11 treated cells.
Discussion and Conclusion: Co-incubation of hLF1-11 and *C. albicans* cells significantly altered biofilm formation, mainly interfering with biofilm biomass and metabolic activity and affected morphogenesis. Taken together, our findings suggest that hLF1-11 could represent a promising agent against *C. albicans* biofilm.

P 039A

ADAPTIVE RESPONSE OF *STAPHYLOCOCCUS AUREUS* TO LONG-TERM EXPOSURE TO CARVACROL

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Introduction: Carvacrol (2-methyl-5-(1-methylethyl)-phenol) is an aromatic essential oil constituent with high potential to control clinical and foodborne pathogens. Previously, we studied the antimicrobial activity of carvacrol against drug-resistant and biofilm forming *Staphylococcus aureus* strains, also when it was delivered from polymeric films for a prolonged contact time. The aim of this study was to provide additional data in this field and to evaluate the effects of long-term exposure to carvacrol on the adaptive response of *S. aureus* strains.
Materials and methods: The adaptive response was evaluated on American Type Culture Collection (ATCC 6538 and 43300) and clinical isolates of *S. aureus*. Parent strains were serially exposed at sublethal concentrations of carvacrol (0.5 x minimum inhibitory concentration, MIC) for 3 and 10 days to generate adapted A3 and A10 cells that were then passaged on carvacrol-free medium to generate X3 and X10 cells. Subsequently, the cells of all stages were assayed for: a) planktonic and biofilm susceptibility to essential oil constituents (carvacrol, cinnamaldehyde and eugenol) and seven antibiotics; b) growth rate and biofilm formation c) coagulase activity; d) colony morphology. Parent strains were also grown at 1, 2, 4 x MIC of carvacrol for

single-step resistant-selection.
Results: Although not regarded as significant, A3 and A10 cells decreased the susceptibility to carvacrol (MIC values 0.015-0.031%, v/v), compared with non-adapted cells (MIC values 0.007-0.015%, v/v) either in planktonic and biofilm phase. On the contrary, no modification in susceptibility to cinnamaldehyde, eugenol and antibiotics was displayed except for *S. aureus* 6538 A10 that changed susceptibility category from gentamycin-susceptible to gentamycin-resistant. This strain showed low growth rate and increase of biofilm production, reduced colony size, pigmentation, hemolysis and coagulase activity that were partially restored in X10 cells. No carvacrol-resistant mutants were obtained in single-step selection.
Discussion and Conclusions: The results of the current study showed that the adaptive response of *S. aureus* to the presence of carvacrol can be strain-specific. Repeated exposure to sublethal concentrations led to minor changes in susceptibility to antimicrobial agents, only one of which may be relevant.

P 040

RAPID DETECTION OF STAPHYLOCOCCI, STREPTOCOCCI AND ANTIBIOTIC-RESISTANCE, MAJOR VIRULENCE GENE BY REAL TIME qPCR AND HRMA

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Introduction: Our goal was to develop a diagnostic tool based on a multiplex real time qPCR and High Resolution Melting (HRM) platform to detect *Staphylococcus* and *Streptococcus spp.* genes allowing an early determination of virulence and/or antibiotic-resistance profiles vs drugs used in clinical practice having a significant clinical impact in the patient outcome and treatment costs.
Materials and Methods: We set up a new Taqman real time qPCR and HRM platform on Rotor-GeneQ. On control staphylococcal DNA we identified: i) *S. aureus* and coagulase-negative Staphylococci (CoNS) (*gap* and *nuc* genes); ii) HA-CA *S. aureus* (pvl); iii) *mec*A, *van*A, *cfr* (linezolid), *erm*A-C resistant genes; iv) hVISA-VISA and DAP reduced-susceptibility (*hld* transcription).
HRMA was performed to detect SNPs related to resistance: i) LIN (G2576T in 23SrRNA); ii) RIF (C1441A - C1586T in *rpo*B); iii) FQs (C251T in *gyr*A - C239T in *grl*A); IV) VAN/DAP (C1862A in *rpo*B).
S. pyogenes and *S. pneumoniae* were identified by *spy*1258 and *lyt*A. On *S. pyogenes*, we detect *cov*-R/S and *spe*B genes. *lytA* and *Spy1258* probe specificity was tested in biological samples: *lytA* in blood including 3 positive for *S. pneumoniae* 19A; and *lytA/Spy1258* in throat swabs including *S. pneumoniae/S.pyogenes*+; *S. pneumoniae*+/*S. pyogenes*-; *S. pneumoniae*-/*S. pyogenes*+; *S. pneumoniae* -/*S. pyogenes* -.
Results: We evaluated the target copy number with a high sensitivity (10^3-10^2 gCopies) and specificity (No cross-reaction with the DNA of other species). No inhibition was found with increasing concentrations (up to 10^3 copies) of other species DNA. All tests were repeated more than 10 times to optimize assays.
All probes, both in Singleplex and Duplex assays, showed high-quality standard curve parameters in terms of R^2 (coefficient of correlation), M (slope) and E (efficiency) of ≥ 0.99, -3.1/-3.6, 90-100%, respectively.
All 10-fold DNA dilutions, (10^8-10^2), were amplified with C_T of 13- 37 included in the theoretical C_T range. Streptococcal identification probes show no interference with biological matrix.
HRM peaks correctly distinguished LIN, RIF and FQs resistant *S. aureus* vs the susceptible ones with a confidence threshold of 90%.
Discussion and Conclusion: We describe a new combined real time qPCR and HRM platform for the identification of Staphylococci, Streptococci, their antibiotic resistance and major virulence genes, meeting the need to have rapid diagnostic methods for early patient treatment and ongoing pathogen-oriented therapy management.

P 040A

PHENOTYPIC AND GENETIC RELATIONSHIP BETWEEN AIEC LF82 STRAIN AND THE UPEC PATHOVAR

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Introduction: Adherent/invasive *Escherichia coli* (AIEC) strains are a new potentially pathogenic group of *E. coli*, involved in Crohn's disease (CD) pathogenesis. AIEC strains are able to adhere and invade epithelial cells, to survive within macrophages and carry many virulence-associated genes characteristic of extraintestinal pathogenic *E. coli* (ExPEC) strains. In addition to their suspected role in CD, AIEC strains could be involved in extraintestinal diseases, including urinary infections. Uropathogenic *E. coli* (UPEC) is the most frequent cause of urinary tract infections (UTI) and an important etiological agent of bacterial prostatitis. Genetically distinct from commensal *E. coli* found in the intestine, these strains possess virulence factors contributing to their ability to cause disease, to facilitate growth and persistence within the host urinary tract. UPEC are able to bind, invade and replicate within host cells. Furthermore, UPEC are generally believed to originate from the gut, where they represent a small fraction of the *E. coli* flora. The aim of the study is to compare the behavior of AIEC to that of UPEC pathovar regard to the ability of AIEC strains to invade, survive and induce inflammation in human prostate cells line.
Materials and Methods: The reference AIEC strain LF82 and UPEC EC73 strain isolated from a patient with recurrent UTI were characterized and compared for their adhesiveness, invasiveness and persistence ability in human prostate cells line RWPE-1. Furthermore, phylogenetic groups, virulence-gene carriage, intracellular localization, and cellular response such as signal transduction pathways, cytokines expression and production were also evaluated.
Results: LF82 and EC73 strains are able to adhere, invade and survive in RWPE-1 cells within vacuoles as small clusters. Both strains belong to B2 phylogroup, are strong biofilm producers and share some virulence associated genes. Moreover, both *E. coli* strains induce IL-8 and IL-6 expression and production during infection, although LF82 strain shows to be a stronger inducer than EC73 strain. Western blotting analyses revealed different ability of two strains in modulating NF-κB and MAPK phosphorylation in RWPE-1 cells.
Discussion and Conclusion: The present study shows that AIEC LF82 and UPEC EC73 strains are able to infect and survive in RWPE-1 cells. However, the two strains show a dissimilar behavior in the induction of inflammatory response that may be due to a different lifestyle.

P 041

COLISTIN-RESISTANT *ACINETOBACTER BAUMANNII*: *IN VITRO* SYNERGISTIC ACTIVITY OF ANTIBIOTIC COMBINATIONS BY TIME-KILL CURVES

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Introduction: Multiresistant (MDR) *Acinetobacter baumannii* is undoubtedly one of the major problems for healthcare systems, especially in ICUs. This microorganism has a very versatile genome, and this characteristic allows it to rapidly involve resistance mechanisms to almost all antibiotic classes including colistin, a previously abandoned polymyxin antibiotic that has re-emerged as a last-resort therapeutic option. Recently, a few strains of colistin-resistant *A. baumannii* have been isolated, requiring assessment of use of colistin in combination with other antibiotics for the treatment of these high risk infections.
Materials and Methods: Eight colistin-resistant (MIC range 16-256 mg/L) *A. baumannii*, belonging

to ST2, were isolated from severe infections in an ICU in Catania (Italy) and Amasya (Turkey). The antibiotic combinations were evaluated by time-kill curves, at MIC concentration, following standard methods. The antibiotic combinations were: Colistin + Meropenem; Colistin + Rifampicin; Colistin + Tigecycline; Meropenem + Rifampicin; and Tigecycline + Rifampicin.

Results: All MDR isolates harboured the bla_{OXA-23} gene and were resistant to all antibiotics including colistin.

Time-kill assays of colistin plus rifampicin or meropenem exhibited synergistic activity against all clinical isolates with full bactericidal activity from 4 h until 24 h.

Indifference was observed for tigecycline/colistin, rifampicin/meropenem and tigecycline/rifampicin for 4, 5 and 2 strains respectively. No antagonism was observed in any of the combinations for the all isolates tested.

Discussion and Conclusions: Combination therapy represents the only option for treatment of infections caused by MDR *A. baumannii* due to the lack of drugs against anti-gram negative microorganisms; therefore, the use of colistin with other antibiotics is an option in the treatment of serious infections sustained by MDR *A. baumannii*. In fact, our results suggest that colistin plus rifampicin or meropenem are the most consistently synergistic combinations against all colistin-resistant *A. baumannii.*

P 041A

EFFECT OF TEMPORINS AGAINST CLINICAL BACTERIAL ISOLATES AND HERPES SIMPLEX VIRUS TYPE

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Introduction: Antimicrobial peptides (AMPs) are evolutionarily conserved molecules of the innate immune system of a wide range of organisms and protect the host from the constant interactions with invading microbes before the adaptive immunity is activated. Temporins (first isolated from the skin secretions of specimens of the European red frog Rana temporaria) are the largest family of AMPs, with more than 100 isoforms. Temporins are among the smallest AMPs (10-14 residues long) found in nature. Temporin-1 (TL, sequence: Phe-Val-Gln-Trp-Phe-Ser-Lys-Phe-Leu-Gly-Arg-Ile-Leu) has a broad spectrum of antimicrobial activity and is cytotoxic to three different human tumor cell lines (Hut-78, K-562, and U-937) but it also kills human erythrocytes at microbicidal concentrations.

Materials and Methods: A TL analogue ([Pro3] TL), bearing a substitution at position 3 (Gln → Pro), has proved to posses similar antimicrobial activity compared to TL but with much lower toxicity. In the present work we have analysed [Pro3] TL focusing on its antibacterial activity against Gram-positive bacteria isolated from clinical patients. We tested both high resistant isolates to common antibiotic and susceptible strains. Moreover, we investigated the antiviral activity of [Pro3]TL against HSV-1. Studies on the cytotoxicity and hemolytic activity were also performed and related to the biochemical characteristics of the peptide.

Results: Results showed that [Pro3]TL has a strong antibacterial activity against Gram-positive with minimal inhibitory concentrations in the micromolar range. The activity was retained in the case of antibiotic resistant strains. Cytotoxicity was greatly reduced in the [Pro3]TL analogue and more importantly we found that the percentage of helicity of TL and its analogue directly correlated to their hemolytic and cytotoxicity activity but not to their antimicrobial activity. The peptide showed also antiviral effect against HSV-1.

Discussion and Conclusions: We identified and analysed a TL analogue with properties that make it an attractive topic for future research. The results of these experiments will assist in engineering analogues of TL with a better therapeutic index.

P 042

IN VITRO ACTIVITY OF A NOVEL *ALOE VERA* FORMULATION APPLIED FOR THE HYGIENE OF THE PERIOCULAR AREA

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Introduction: Periocular surfaces are normally colonized by a variety of commensal and pathogenic organisms that are often responsible for ocular infections when ocular defence mechanisms are altered. A variety of products specifically formulated for eyelid hygiene are available and effective in reducing the microbial burden of this area. However, most of them contain chemical compounds that can be toxic or exert a sensitizing effect on the eye.
Herein, we evaluated sterility and antimicrobial activity of a non-toxic novel gauze formulation containing 0.2% *Aloe vera* and hyaluronic acid that is commercialized for the hygiene of the periocular area.
Materials and Methods: Gauze sterility was assessed by incubation in broth for up to 30 days. Bacterial or fungal growth was evaluated seeding broth aliquots at different time intervals.
The *in vitro* susceptibility test of the gauze solution against bacteria and fungi commonly colonizing the periocular area was assessed following the CLSI M07-A9 (2009) and M27-A3 (2008) broth methods, respectively. The solution was diluted volumetrically two-fold in broth from 50 µl (25% v/v) to 0.025 µl (0.0125% v/v) in microtitre plates. Plates were incubated at 37°C for 24 hours and MICs were read visually.
The antimicrobial effectiveness test was performed by inoculating the gauze solution with microbial suspensions at the initial concentration of 10^5-10^6 CFU/ml, as recommended by the international Pharmacopoeias. At different time intervals, test samples were tested for microbe enumeration.
Results: The results obtained indicate sterility of the formulation. The MIC value of the solution ranged from 25% to 12.5% for bacteria and 6.25% for *C. albicans*. Furthermore, by assessing antimicrobial effectiveness, we found that the solution meets the criteria reported by the European and US Pharmacopoeias.
Discussion and Conclusions: This study suggests that the novel gauze formulation herein tested possesses antimicrobial activity against bacteria and yeasts commonly found in the periocular area. The microbial death curves obtained following deliberate contamination of the gauze solution revealed a strong bactericidal and fungicidal activity of the formulation.

P 042A

THE ROCANET STUDY: RESULTS FROM A PROSPECTIVE OBSERVATIONAL SURVEY OF CANDIDAEMIA IN THE ROME CITY

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Introduction: *Candida* species are leading causes of nosocomial bloodstream infections and are associated with substantial morbidity, mortality and costs. While the antifungal susceptibility pattern is closely linked to the species, it is yet greatly important to understand and monitor the local species epidemiology as well as the antifungal resistance rate. The ROCANET (Rome Candida Network) was established in December 2012 in order to perform prospective surveillance of all candidaemias among patients hospitalized at selected medical centres in the Rome city area. Its aim is to improve the knowledge of the burden of candidaemias in different groups of patients, better define patients at risk and understand the epidemiology, species distribution, antifungal susceptibility and outcomes of candidaemias from the study sites.
Materials and Methods: All the patients admitted to 10 large hospital medical centres of Rome (Italy) from January 2013 to December 2014 and diagnosed with candidaemia will be studied. All the *Candida* isolates from the study patients will be

collected and maintained at the Clinical Microbiology Laboratory of the Università Cattolica del Sacro Cuore of Rome. The isolates will be re-identified by the MALDI-TOF MS method and sequence identified using the ITS gene region, whereas susceptibility testing will be performed against 7 antifungal agents (anidulafungin, caspofungin, micafungin, fluconazole, itraconazole, posaconazole, voriconazole) using CLSI and EUCAST methods. In the case of antifungal drug-resistant isolates, underlying molecular resistance mechanisms will be assessed, as well all the isolates will be genotyped using multi-locus sequence typing, as appropriate.
Results and Conclusions: From January 2013 to October 2013, a total of 668 isolates of *Candida* species were studied, including 319 *C. albicans*, 197 *C. parapsilosis* complex (including 4 *C. orthopsilosis* and 2 C. *metapsilosis*), 65 *C. glabrata* complex (including 1 *C. nivariensis*), 41 *C. tropicalis,* 2 *C. guilliermondii*, 12 *C. krusei,* 7 *C. lusitaniae,* 5 *C. guilliermondii*, 5 *Rhodotorula mucilaginosa*, and other 17 belonging to other 9 species. Overall, resistance to the echinocandins was very low, with *C. albicans* (1 isolate) and *C. glabrata* (1 isolate) being resistant to anidulafungin, caspofungin or micafungin and shown to have *fks* mutations. Resistance to fluconazole was low among isolates of *C. albicans* (1 isolate) and *C. tropicalis* (1 isolate), whereas 12 isolates of *C. parapsilosis* complex (11 *C. parapsilosis* and 1 *C. metapsilosis*) were found to be resistant to fluconazole. Surprisingly only 4 isolates were resistant to azoles. Voriconazole and posaconazole were active against all *Candida* species except *C. glabrata* and 2 isolates of *C. parapsilosis.*
Conclusion: Overall, echinocandin and triazole resistance were uncommon. Although no fluconazole and echinocandin co-resistance among *C. glabrata* isolates was observed, continued close surveillance is locally warranted.

P 043

DIRECT IDENTIFICATION AND ANTIMICROBIAL SUSCEPTIBILITY TESTING OF BACTERIA FROM POSITIVE BLOOD CULTURES BY COMBINING MALDI-TOF AND ALFRED 60AST ARE RAPID AND RELIABLE

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Introduction: Rapid identification (ID) and antimicrobial susceptibility testing (AST) of the pathogens causing bloodstream infections can lead to prompt and appropriate antimicrobial therapy. Direct ID by MALDI-TOF of bacteria spotted onto the target plate and direct AST by the ALFRED 60AST automated system (Alifax) allow to speed up the microbiological diagnosis. ID and AST results were compared with those by current methods.
Materials and Methods: MALDI-TOF analysis was performed using a Microflex LT system mass spectrometer (Bruker Daltonics) following the manufacturer's instructions. Two different protocols (PR1 and PR2) for rapid AST were evaluated. Direct AST by ALFRED 60AST by PR1 used bacteria recovered by serum separator tubes from positive blood cultures; by PR2 used an aliquot of the positive blood culture incubated in broth culture vials for 3-5 hours at 37°C. Routine ID was performed by MALDI-TOF and AST by Vitek 2 (bioMérieux, France) from isolated colonies. Cefotaxime, ceftazidime, gentamicin, levofloxacin, meropenem, amikacin, and colistin were tested for Gram-negative bacteria; linezolid, teicoplanin, cefoxitin, and ampicillin for Gram-positive cocci.
Results: The direct MALDI-TOF method correctly identified 96% of 103 Gram-positive cocci and 98% of 80 Gram-negative bacteria contained in monomicrobial blood cultures. For Gram-positive cocci, concordant/correct AST was observed for 93% and 93.8% of antimicrobial/isolate combinations by PR1 and PR2, respectively. Total AST agreement was seen for streptococci/enterococci with linezolid by PR1 and PR2, and with ampicillin by PR2; to-

tal AST agreement was observed for staphylococci with linezolid by PR2. For Gram-negative bacteria, concordant/correct AST was found for 88.2% and 90.8% of antimicrobial/isolate combinations by PR1 and PR2, respectively. Complete AST agreement was found for Enterobacteriaceae with levofloxacin by PR1 and PR2, and for nonfermenters with amikacin and cefotaxime by PR1 and PR2, and with colistin by PR2.
Discussion and Conclusions: Rapid identification by MALDI-TOF and direct AST of bacteria from positive blood cultures by ALFRED 60AST allow to report reliable ID and AST results of Gram-positive cocci and Gram-negative bacteria one day earlier.

P 043A

FIRST DESCRIPTION OF THE bla_{GES-5} CARBAPENEMASE IN AN ST235 *PSEUDOMONAS AERUGINOSA* FROM ITALY

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Introduction: Carbapenem resistance in *P. aeruginosa* is a matter of major concern and can be due to several mechanisms including reduced permeability and production of carbapenemases. Class B carbapenemases are the most frequent acquired enzymes in *P. aeruginosa,* while class A enzymes are overall less common. GES-type enzymes are extended-spectrum β-lactamases, with few variants only showing carbapenemase activity, such as GES-5. To date, GES-5 has been described in few isolates of *P. aeruginosa* and it has never been reported from Italy. Here we report on the first description of a GES-5-producing *P. aeruginosa* from Italy and on the mobile genetic element (MGE) carrying the bla_{GES-5} gene.
Materials and Methods: *P. aeruginosa* LC-030 was isolated from an inpatient at Lecco Hospital in 2013, within an Italian nationwide survey. MICs were determined by broth microdilution according to the CLSI guideline and interpreted according to the EUCAST breakpoints. Carbapenemase activity was assayed spectrophotometrically. PCR and sequencing were carried out using probes on carbapenemase genes and known MGE. MLST was performed according to pubmlst.org/paeruginosa guidelines.
Results: LC-030 isolate exhibited a multidrug resistant phenotype, including carbapenems; carbapenemase activity, not inhibited by EDTA, was observed in crude extracts. PCR screening and sequencing revealed the presence of a bla_{GES-5} gene, embedded in a ΔTn402/class 1 integron, named DTn402:In717, also carrying aminoglycoside resistance determinants (aacA4 and aphA15). DTn402:In717 was part of a complex Tn3-like transposon located on a chromosomal genomic island. LC-030 belongs in ST235, a high risk clone (HRC) responsible of the dissemination of major resistance traits worldwide. This complex GES-5 encoding transposon, previously described in a *P. aeruginosa* strain from Australia, represents to date the only fully characterized bla_{GES-5}-harbouring transposon.
Discussion and Conclusions: This report represents the first description of the GES-5 carbapenemase in *P. aeruginosa* from Italy. Although GES-type enzymes have been rarely reported, their association with complex MGE and successful HRC could represent a major treat for dissemination of relevant resistance traits.

P 044

DEVELOPMENT OF A NEW TECHNOLOGY FOR HIGH EFFICIENCY BIOAEROSOL REMOVAL

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Introduction: Bioaerosol of pathogenic microorganisms, as bacteria and viruses, causes a variety of healthy problems, and should be controlled in public and private indoor ambient. Filtration is largely adopted but bacterial growth on the filter is a significant limitation of this process. We are developing a new technique aimed to capture microorganisms, characterized by low operating costs and very high efficiency. This technique is based on the integration of a wet electrostatic scrubber (WES), with a heterogeneous condensation system (HC). The process aims to achieve an improved washing of the air with a disinfectant liquid. This contribution reports the first experiments concerning the design and optimization of the lab-scale prototype and deals with the methods to generate bioaerosol of specific microorganisms.

Materials and Methods: It was developed a lab-scale set up able to generate a bioareosol containing both of Gram-negative (*E. coli*) and Gram-positive (*S. epidermidis*) bacteria. Pilot experiments were carried out at 1 atm and 300 l/h flow rate using 10^9-10^{10} CFU/ml bacterial suspensions in 50 ml deionized H_2O. The system was set up by an aerosol generator equipped with a Laskin nozzle. In order to remove the water droplet, the bioaerosol was subjected to a diffusion dryer, composed by a silica-gel tube. Mixed cellulose esters filters were used to evaluate bacterial viable counts at different masses of silica gel.

Results: The experiments showed that the maximum viable count was reached without silica gel, with about 10^6 CFU/m^3 and 10^5 CFU/m^3 for Gram-positive and -negative bacteria respectively. Increasing the silica-gel mass, the Gram-positive concentration was constant, instead the Gram-negative one decreased by two order magnitude, this could be explained by the higher sensitivity to mechanical stresses in Gram-negative bacteria.

Discussion and Conclusions: We have generated a controlled flow of air containing *S. epidermidis* cells, which can be used as a stable source of Gram-positive bioaerosol required to proceed with our experimental work. Our next goal will be the development of a HC-WES system on a lab scale to remove microorganisms.

P 044A

LIVE RECOMBINANT *STREPTOCOCCUS GORDONII* AS A VACCINE VECTOR FOR DELIVERY OF THE CHIMERIC ANTIGEN H56 OF *MYCOBACTERIUM TUBERCULOSIS* TO THE MURINE UPPER RESPIRATORY TRACT

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Introduction: Combination of priming and boosting represents a vaccination strategy able to modulate magnitude, quality and localization of the immune response. In this work we constructed a recombinant *Streptococcus gordonii* expressing the chimeric antigen H56 of *Mycobacterium tuberculosis* and used it to colonize the murine upper respiratory to induce a H56-specific immune response.

Materials and Methods: *M. tuberculosis* chimeric antigen H56 was expressed on the surface of S. gordonii as a fusion with streptococcal protein M6 as previously described. An inoculum of $2x10^9$ CFU of live recombinant *S. gordonii* was given intranasally to C57BL/6 twice at a 3-week interval. Animals were boosted by the subcutaneous route 3 weeks after the last inoculum using killed recombinant *S. gordonii* or the soluble protein H56. Antigen-specific antibodies were determined in serum by ELISA and cytokines were studied by ELISPOT and Multiplex Assay in splenocytes and lymph nodes.

Results: Live recombinant *S. gordonii* colonized efficiently the mouse upper respiratory tract and this colonization primed mice to produce a secondary antibody response to recombinant antigen H56. Animals immunized with live recombinant *S. gordonii* proved to produce a Th17 type of cellular response which, upon subcutaneous boosting, showed to be markedly inhibited in its capacity of producing IFN-γ and IL-2.

Discussion and Conclusions: The vaccination strategy investigated in this work, by achieving

suppression of IFN-γ and IL-2 production by $CD4^+$ T cells, represents a promising result in the perspective of developing tuberculosis vaccines with low potential of causing lung tissue damage due to T cell-mediated immunopathology.

P 045

CHARACTERISATION OF NOVEL CONJUGATIVE MULTIRESISTANCE PLASMIDS CARRYING CFR FROM LINEZOLID-RESISTANT STAPHYLOCOCCUS EPIDERMIDIS CLINICAL ISOLATES

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Introduction: Linezolid inhibits protein synthesis by binding to the peptidyl transferase centre of the bacterial ribosome. Acquired resistance to linezolid emerged shortly after introduction of the drug in clinical practice, due to ribosomal mutations at the 23S rRNA or in ribosomal proteins L3 and L4. Transferable linezolid resistance appeared later, due to the *cfr* gene. *cfr*-mediated resistance leads to co-resistance to phenicols, lincosamides, oxazolidinones, pleuromutilins, and streptogramins A ($PhLOPS_A$ phenotype). The potential for dissemination is underscored by the frequent location of cfr on mobile genetic elements, typically plasmids, which are important vehicles for its spread. In Italy, *cfr*-mediated linezolid resistance, though less common than mutation-mediated forms, has been reported in coagulase-negative staphylococci in the last few years. In this study we analysed two linezolid-resistant, *cfr*-positive clinical isolates of *Staphylococcus epidermidis* from Italy and investigated the genetic context and transferability of the *cfr* gene.
Methods: The two strains (SP1 and SP2) were phenotypically and genotypically characterised. Transferability of *cfr* was assessed by conjugation and transformation. The *cfr* genetic contexts were investigated by PCR mapping, sequencing and comparative sequence analyses.
Results: SP1 and SP2 belonged to sequence types ST23 and ST83, respectively. In both strains, *cfr* was located on a plasmid, which could be transferred to *Staphylococcus aureus* by conjugation and transformation. pSP01, the cfr-carrying plasmid from SP1, had a larger number of additional resistance genes and was sequenced (76,991 bp). It disclosed a distinctive mosaic structure, with four cargo regions interpolated into a backbone 95% identical to that of *S. aureus* plasmid pPR9. Besides cfr, other resistance genes were distributed in the cargo regions. A closely related cfr plasmid (pSP01.1, ~49 kb), differing from pSP01 for the lack of a cargo region, was detected in SP2.
Conclusions: Conjugative multiresistance plasmid pSP01 is the first *cfr*-carrying plasmid to be sequenced in Italy. This is the first time *cfr* is found in association with particular resistance genes, and in a strain (SP2) belonging to ST83.

P 045A

CLINICAL CASE OF CONGENITAL TOXOPLASMOSIS: QUANTIFICATION OF PARASITES OVER TIME AND STRAIN TYPING

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Introduction: *Toxoplasma gondii* is an obligate intracellular parasite that infects all warm-blooded vertebrates. Up to one-third of human population in the world is chronically infected. Human infections are caused by ingesting undercooked meat containing viable tissue cysts. While toxoplasmosis is a mild disease in immunocompetent individuals, the illness is severe in immunocompromised patients. Congenital toxoplasmosis (CT) is the most serious manifestation of the disease resulting from transplacental contamination of the fetus with *T. gondi* during pregnancy.
Materials and methods: The DNA was extracted from liquor and blood of infant. The nested-PCR-RFLP of *sag-2* gene was performed on liquor extracted and the nested-PCR product was also sequenced. Quantitation of *T. gondii* load over time from blood and liquor was performed by Quantitative Real-time PCR kit specific for *T. gondii* (Genesig).

Results: Here we report a case of severe CT in an infant. The strain responsible of the infection was characterized and the parasite load was monitored over time from different samples. The RFLP analysis of *sag-2* gene reveals that the isolate has the most similarity with type II of *T. gondii.* The sequence analysis demonstrated that the isolate has 98% identity with those of available sequences for type II *T. gondii* ME49 in GenBank. According to therapy administered the parasite load decreases over time in liquor samples from $5x10^3$ to 5 parasites.

Discussion and conclusions: *T. gondii* infection during pregnancy may result in fetus infection in about 30% cases. Severity of the disease mainly depends on the gestational age of transmission. Unfortunately although the values of Toxoplasma serological tests clearly showed a seroconversion between the first and second trimester the mother refused to undergo prenatal diagnosis and to begin appropriate treatment. The untreated fetus has developed hydrocephalus and the newborn was then monitoring for the presence of parasite over time during the therapy. The report case highlights the crucial role of prenatal diagnosis in suspected toxoplasmosis acquired during pregnancy.

P 046

EMERGENT PATHOGENS IN OCULAR INFECTIONS

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Introduction: Ocular infections are potentially blinding diseases. In this study we present our 15-years' experience dealing with bacterial pathogens isolated from ocular infections and we focus our attention on new emergent species.
Materials and Methods: This is a monocentric observational retrospective study. The microbiological records of about 40,000 ocular swabs performed in our microbiology laboratory between 2000 and 2014 were retrospectively reviewed.
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.
Ocular cultures media were, for adult population, heart-brain broth and chocolate agar both incubated at 37° C in aerobiosis. For child population we used also blood agar and haemophilus agar both incubated at 37° C, with the latter used in capnophilia.
Results: In this study, we collected about 40,000 ocular samples and analyzed to identify pathogens. During study, we isolated 11225 positive samples. We separated study in three periods: from 2000 to 2004, from 2005 to 2009 and from 2010 to 2014. By sample culture, Gram-positive cocci were the predominant causes of infections. In particular, most frequently isolated organisms were Streptococci and Staphylococci, followed by Enterobacteriaceae, Moraxella, Pseudomonas, Haemophilus, which showed quite similar values in all three considered periods.
Interestingly, Coagulase Negative Staphylococci (CoNS) were not present in the first period from 2000-2004 but they appeared in the second period and increased in the last one. Otherwise, Bacillaceae, absent from 2000 to 2009, appeared in the last five years.
We also observed that Pasteurella spp. and Enterococci were more frequent in 2000-2004 whereas they decreased in the last two periods.
Discussion and Conclusions: Identification of causative pathogens is an important step in the

management of infectious diseases.
We observed that in 15 years many bacteria maintained almost the same frequency whereas some microorganisms as CoNS and Bacillaceae appeared in 2005-2009 and increased in 2010-2014.
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 047

RAPID DETECTION OF CARBAPENEM RESISTANCE IN *ENTEROBACTERIACEAE* DERIVED FROM BLOOD CULTURES BY MATRIX-ASSISTED LASER DESORPION IONIZATION-TIME OF FLIGHT MASS SPECTROMETRY (MALDI-TOF MS)

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Introduction: β-Lactam antibiotics, in particular carbapenems, are the most potent compounds for the treatment of sepsis caused by Gram- negative (Gram-) bacteria. The emergence of multiresistant phenotypes described in *Enterobacteriaceae* requires the need of having an early marker of resistance against these drugs. Recently, MALDI-TOF MS has been used to detect resistance against β-lactam antibiotics. This assay is based on a short co-incubation of the Gram- bacteria with the antibiotic in question. If the bacteria produce the β-lactamase hydrolysis of the β-lactam ring occurs. Hydrolyzed and non-hydrolyzed substances differ in their molecular weights, and these differences can be detected by MALDI-TOF MS.
Methods: In the present study, we evaluated this technique in patients with Gram-negative bacteremia. 170 consecutive blood cultures (BCs) containing *Enterobacteriacea* were tested. After Gram staining, extraction of bacterial pellets from BCs flasks was performed in order to identify and detect resistance against ertapenem. The identification and the carbapenemase-resistance assays were performed also from subcultures of these isolated strains. The samples were measured using an autoflex speed mass spectrometer. For all isolates, a complete antibiogram was generated using the Vitek 2 automated system for the phenotypic characterization while a genotypic characterization was performed by PCR and sequencing.
Results: Of the 170 BCs tested, 25 yielded Klebsiella *pneumonia* carbapenemase producer (KPC). The results of the carbapenemase assay by MALDI-TOF MS were compared with those of conventional methods and the data obtained revealed identical classification of the bacteria according to resistance. The assay detected and discriminated KPC from other carbapenemases in *K. pneumonia* after only 1hour incubation with ertapenem. Moreover, the comparison between the carbapenemase assays performed on bacteria grown on solid media and that on bacteria extracted from BCs fluid gave the same results.
Conclusions: This assay allows a very rapid detection of KPC within 1hour after the BCs were flagged as positive. It is easy to perform and the cost is low.

P 048

PHARMACOKINETIC ASSESSMENT OF PRULIFLOXACIN IN PATIENTS WITH RENAL IMPAIRMENT USING POPULATION PHARMACOKINETICS MODELLING AND SIMULATIONS

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Introduction: Prulifloxacin (Unidrox® 600 mg tablets) is the pro-drug of ulifloxacin, a fluoroquinolone antibacterial agent.
A 2-centre, open label, parallel group study has been performed to investigate the influence of renal impairment on the pharmacokinetics (PK) of ulifloxacin following single and repeated administration of prulifloxacin.
A population-PK (POP-PK) model was developed to assess the dosing regimen of prulifloxacin for renal impaired subjects.
Materials and Methods: The POP-PK model was developed using data available from 11 prulifloxacin studies. The stochastic component and covariates were introduced into the model to optimize the final PK model.
The POP-PK analysis was performed using NONMEM.
Steady-state simulations were performed according to the validated model by reducing the dose/prolonging the frequency.
Results: The best POP-PK model was 2-compartment linear model with 1^ order absorption and elimination. Creatinine Clearance (CRCL), body weight and health status significantly influenced the clearance of ulifloxacin. The final model was validated by non-parametric bootstrap and Visual Predictive Check (VPC).
Based on the model, PK profiles of ulifloxacin were simulated at steady-state in renal (mild, moderate, severe) and healthy subjects.
The results indicated that dose adjustment may be not required in mild and moderate renal subjects. In severe renal impairment a 2-fold dose reduction was suggested.
Preliminary PK results of the multiple dosing phase of the clinical study seem to confirm the previously simulated PK profiles.
Discussion and Conclusions: Following oral administration of prulifloxacin, ulifloxacin PK is described by a 2-compartment linear model with 1^ order absorption and elimination. The covariate CRCL described the differences in the observed ulifloxacin PK profiles. Simulations to design the dosing regimen for the renal patients suggested that no dose [600 mg Once A Day (OAD)] adjustment may be required for mild and moderate renal subjects as compared to healthy subjects. A dose reduction to 300 mg OAD was instead recommended for the severe renal subjects. These dosing regimens were applied during the multiple dose phase of the renal study. The preliminary mean PK results seem to confirm the simulation predictions

P 049

ELECTROSPUN NANOFIBERS EMBEDDED WITH SILVER NANOPARTICLES: PREPARATION, CHARACTERIZATION AND ANTIBACTERIAL ACTIVITY

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Introduction: The electrospinning of biodegradable polymers and its potential applications have recently been the subject of a large number of studies in the field of biomaterials. The high surface area and porosity of the obtained nanofibers make them particularly attractive to a large number of medical applications, i.e.: filtration, tissue engineering, implants, wound dressing and drug delivery. In parallel, a considerable interest on the use of silver nanoparticles use, due to their known antibacterial activity, has developed. The purpose of this work was to evaluate the antibacterial activity of both nano polymeric fibers of poly lactic acid (PLA) and PLA

containing nanoparticles of silver (Ag) produced by an alternative, one-step, electrospinning method.
Materials and methods: The nanofibers (PLA and PLA + Ag) obtained by an electrospinning technique have been characterized morphologically by SEM and TEM. Both nanofibers were subsequently tested to evaluate their antibacterial activity against two reference bacterial strains: *Staphylococcus epidermidis* (ATCC 35984) and *Escherichia coli* (ATCC 25922). The nanofibers PLA and PLA+Ag were sterilized by UV, placed in the presence of a bacterial inoculum of 10^7 CFU/ml and incubated for 15, 24 and 48 hours at 37°C. Controls without nanofibers were also performed. After the incubation time, for each sample the number of CFU/ml was quantified by TSA plate count. All the experiments were performed in triplicate. The results were analyzed by descriptive statistics and tested by unpaired T-test ($p < 0.05$).
Results: The resultant fibers exhibited uniform morphology with silver nanoparticles distributed throughout the fiber. Our results demonstrated that no antibacterial activity was detected for the PLA nanofibers. Further on, a significant ($p < 0.01$) activity against both *S. epidermidis* and *E. coli* was observed to be more pronounced for the PLA embedded with Ag, during the course of the experiments.
Discussion and Conclusions: Although there has been only a partial Ag release from nanofibers during the experiment times, a significant antimicrobial activity, exerted by PLA+Ag against two common types of pathogens, was detected and attributed to the presence of Ag nanoparticles. Further studies on the biocompatibility, cytocompatibility, and antimicrobial properties of the obtained nanofibers will pave the way for potential applications in medical areas such as in the field of wound dressing.

P 050

COMPARISON OF PHENOTYPIC AND GENOTYPIC METHODS FOR DETECTION OF CARBAPENEMASES IN *KLEBSIELLA PNEUMONIAE*

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Introduction: *Klebsiella pneumoniae* is one of the most important cause of nosocomial infections expecially in ICUs. The strains are multidrug- resistant in particular also to carbapenems; therefore the mortality related to these infections is very high. The aim of this preliminary study was to compare the results obtained by phenotypic and molecular methods for detecting the carbapenemases production in *K. pneumoniae* and to identify the KPC variant.
Material and Methods: Seventy five strains of *K. pneumoniae*, isolated from seven hospital of Apulia, Southern Italy, were analyzed by phenotypic methods (Modified Hodge test, Meropenem ± Dipicolinic Acid Combination test, Meropenem ± boronic acid Combination test, Temocillin Disk Diffusion test, ESβL test, AmpC screening) and genotypic methods: PCR for detecting carbapenemases resistance genes, *bla*-KPC and sequencing were used to identify the KPC variant.
Results: The 75 *K. pneumoniae* strains (32 isolated from blood, 19 from urine, 7 from sputum, 1 from throat swab, 6 from wound swab, 1 from CSF, 1 from faecal sample, 5 from Centre Venous Catheter, 1 from urinary catheter) resulted positive for carbapenemases production and all were KPC. No *K. pneumoniae* MBL, OXA, AmpC and ESβL producing with porin loss were found. All the strains tested carbapenemases positive were confirmed also by genotypic methods. In addition KPC-3 was the only variant identified.
Discussion and Conclusions: Even if referring to a small number of *K. pneumoniae* strains, phenotypic methods for detection of carbapenemases in *K. pneumoniae* are simple, sensitive, specific, low expensive for a rapid diagnosis of carbapenemases producing strains and of considerable utility for routine clinical

laboratories without access to more specialized diagnostic procedures such as the molecular methods requiring highly skilled staff and more expensive in comparison to phenotypic methods.

P 051

HRM-TYPING OF MRSA: THE NEW FRONTIER TO REPLACE MLST FOR EPIDEMIOLOGICAL SURVEILLANCE STUDIES

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Introduction: Molecular epidemiology of methicillin-resistant *Staphylococcus aureus* (MRSA) is important to survey the spread of the main HA- and CA-MRSA clones and monitor local and national epidemiology. Several molecular typing methods have been used, leading to a current agreement in considering multi-locus sequence typing (MLST) (together with SCCmec- and spa-typing) the most useful and comprehensive technique to perform molecular epidemiology. Although these methods are extremely effective, they are also laborious, expensive and time-consuming in the context of epidemiological surveillance studies.
We report an implemented typing method, based on high-resolution melting analysis (HRMA), to discriminate SNPs within internal fragments of the MLST loci, giving genotypic profiles - defined as "Melting Types" (MelT) - correctly translating their corresponding STs and reducing the time of analysis.
Materials and Methods: Real-time PCR and HRM were performed on a Rotor-Gene Q (Qiagen) instrument, in a single-closed tube run. Discrimination of ST5 from 105 and ST239 from 241, was obtained by considering flanking region identified SNPs inside the yqi gene.
100 MRSA clinical isolates, previously phenotypically and molecularly characterized, were selected among a large Italian collection, and were re-evaluated by HRMT. 11 control strains, belonging to known clones and representative of the major international MRSA lineages circulating in Italy, were further included in the study.
Results: Our results show an overall agreement between HRM-typing and MLST (94/100, 94%). All strains belonged to ST1 (5/5 MelT1), ST5 (11/11 MelT4a), ST105 (4/4 MelT4b), ST8 (26/28 MelT7), ST22 (21/22 MelT16), ST228 (21/23 MelT44), ST239 (2/3 MelT52a), ST241 (1/1 MelT52b), ST58 (1/1 MelT27), ST63 (1/1 MelT30) and ST398 (1/1 MelT24). Compared to sequence analysis, HRM-typing was approximately 9 times cheaper and 12 times faster (single strain sequencing = € 120, 120h; HRM reaction = €15, 11h).
Discussion and Conclusions: This study provided a solid evidence that HRMT could be the new frontier, alternative to MLST, to predict STs by MelTs, reducing time and cost of analysis, in the context of large epidemiological surveillance studies.
We applied HRMT to re-evaluate the genotypes of a large Italian MRSA collection, demonstrating the high reproducibility of this method.
This new attractive typing technique will allow a manageable approach to trace quickly and cheaply, the molecular epidemiology of MRSA.

P 052

ANTIFUNGAL ACTIVITY OF ESSENTIAL OILS AGAINST *CRYPTOCOCCUS NEOFORMANS*

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Introduction: *Cryptococcus neoformans* is the etiological agent of cryptococcosis, a cosmopolitan infectious disease that affects humans, domestic and wild animals. It usually causes infection and disease in immunosuppressed patients. For treatment of cryptococcal infections in these patients, intravenous amphotericin B combined with flucytosine, followed by azole such as fluconazole (FLC) and itraconazole (ITZ) are recommended. Since the current therapies have certain limitations due to side effects such as toxicity, nephrotoxicity and

emerging of resistant strains, effective treatments and the development of novel antifungal drugs will be necessary for the future. The essential oils (EO) had a wide application in folk medicine, fragrance industries, food flavoring and preservation, and in recent years, they have started to be recognized for their antimicrobial properties. The object of this study was to evaluate the antifungal activity of different EO and their major components alone and in combination with ITZ against some clinical isolates of *C. neoformans*, with different susceptibility or resistance patterns to FLC and voriconazole (VRZ).
Materials and Methods: Antifungal activity (minimum inhibitory/fungicidal concentration, MIC/MFC) of thyme red, clove, pine, lemon balm, sage, lavender, fennel EO and some components (carvacrol, thymol, α-pinene) was evaluated *in vitro* against *C. neoformans* FLC/VRZ-susceptible (FLC/VRZ-S) and FLC/VRZ-resistant (FLC/VRZ-R) strains by a broth microdilution method, according to the CLSI. Moreover, the potential synergistic action of different OE and ITZ was evaluated by the checkerboard assay and calculation of the Fractional Inhibitory Concentration (FIC) Index.
Results: The results showed that most of EO and their major components displayed low MIC (% v/v) and possessed a significant fungicidal activity against *C. neoformans* FLC/VRZ-S and FLC/VRZ-R strains. The results also showed a significant synergistic relationship between some EO (e.g.thyme) and ITZ, with a consequent reduction of the MIC values of both compounds used in combination.
Discussion and Conclusions: These results help to broaden the knowledge on antifungal action of EO, in view of the development in the future of new therapeutic strategies that favor the resolution of fungal infections and containment of toxicities and drug resistance.

P 053

A NOVEL APPROACH AGAINST BIOFILM-GROWING MICROBIAL STRAINS: FUNCTIONALIZED VIRUS-LIKE PARTICLES WITH ANTIMICROBIAL PEPTIDES

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Introduction: The search for new antimicrobial drugs minimizing antibiotic use and bacterial resistance is becoming imperative. One of the strategies is to explore the efficacy of antimicrobial peptides (AMP), even if the success of antimicrobial therapeutics is not only merely due to the mechanisms of molecular interaction between peptide and its target, but also to the effectiveness of drug delivery. This is particularly true when we consider that the most threatening form of microbial life is represented by the biofilm, a polymeric complex matrix that confers a strong resistance towards antibiotic drugs. Virus like particles (VLPs), that are obtained by the self-assembly of viral selected proteins (in our case the VP6 from human rotavirus), are in this sense a new and potent technology to be pursued, due to the possibility, by means of protein engineering, to spatially co-localize active peptide epitopes with different molecular architecture. We selected and inserted at the C-terminus of VP6 two natural AMPs: anoplin (A) and eumenitin (E) both from wasp venom, and verified the efficacy *in vitro* of the VLPs obtained in comparison with the antimicrobial activity of the selected AMPs alone.
Methods: A biotechnological platform in *Escherichia coli* was setup to produce recombinant VP6 coupled with small ubiquitin-like modifier (SUMO) fusion system. Three different constructs were expressed and purified: VP6 wild-type, VP6-A and VP6-E and the corresponding VLPs were assem-

bled both in form of functionalized nanoparticles and nanotubes.
Results: Our results clearly show that functionalized VLPs are efficacious against different strains of human pathogenic yeasts such as *Candida albicans, Candida glabrata and Candida tropicalis.* Furthermore, preliminary results also demonstrated efficacy against *in vitro* biofilm form of *C. parapsilosis.*
Conclusions: Novel antimicrobial drugs obtained by the combination of selected AMPs and VLPs may be used to further improve the battery of bullets against threatening bacterial and fungal organisms.

P 054

ESCHERICHIA COLI SEQUENCE TYPE 131 (ST131) SUBCLONE H30, THE MAIN CAUSE OF MULTIDRUG-RESISTANT URINARY TRACT INFECTIONS AMONG PATIENTS IN PALERMO, ITALY

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Introduction: Extraintestinal pathogenic *Escherichia coli* ST131 (ExPEC ST131) has been reported to cause a wide range of extraintestinal infections, including urinary tract infection (UTI) worldwide. The H30 subclones of ExPEC ST131 have expanded more extensively than other ST131 variants. The H30 subclone, so named because it contains allele 30 of *fimH* (type 1 fimbrial adhesin gene), comprises almost all current fluoroquinolone- resistant ST131 isolates. In current study we determined the prevalence of ST131 and its H30 subclone among isolates from urinary tract infections caused by extended spectrum β-lactamase (ESBL)-producing *E. coli* strains at the main hospital of Palermo, Italy.
Materials and Methods: During 2013-2015, two hundreds de-identified ESBL producing *E. coli* clinical isolates were collected from patients with urinary tract infections. Phylogenetic group typing and detection of virulence genes were performed using multiplex PCRs. Classification of isolates as ExPEC was based on the presence of two or more of the following virulence genes: *pap, sfa* or *foc, afa* or *dra, iutA*, and *kpsM II*. ST131 and its H30 subclone were detected by two different single nucleotide polymorphism PCR (SNP PCR).
Results: Out of 200 clinical *E.coli* isolates from urinary tract infection, 142 isolates (71%) were belonged to phylogenetic group B2. Based on the molecular definition of ExPEC, 137 /142 of these B2 isolates (96.5%) were attributed with the status of ExPEC. The most prevalent virulence factors were *kpsMT II* and *iutA*. SNP-PCRs results confirmed that 131 out of 142 (92.2%) isolates of B2 were ST131 and among these ST131 isolates 119 (90.8%) were positive for H30 subclone.
Discussion and Conclusions: Among clinical isolates, ExPEC ST131 and primarily its H30 subclone, accounts for most antimicrobial-resistant *E. coli* and is the dominant *E. coli* strain worldwide. Here we determined the prevalence of ST131 and its H30 subclone and explored their associations with resistance phenotypes, ESBL types, and virulence profiles, among prospectively collected *E. coli* clinical isolates from the Palermo area (2013 to 2015). We confirmed the well-established association between ST131 and ESBL production , and found that the recently identified H30 ST131 subclone has expanded in the study region more than non-H30 ST131 subclones.

P 055

THE MACROLIDE RESISTANCE GENE *erm*(TR) AND *erm*(TR)-CARRYING GENETIC ELEMENTS IN *STREPTOCOCCUS AGALACTIAE*: CHARACTERIZATION OF ICE*sag*TR7, A NEW COMPOSITE ELEMENT CONTAINING IME*SP*2907

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Introduction: Four *erm*(TR)-carrying integrative and conjugative elements (ICEs) have been described in streptococci: two (ICE10750-RD.2 and Tn*1806*) in *Streptococcus pyogenes* and *Streptococcus pneumoniae*, respectively; and two (ICE*Sp*1108 and ICE*Sp*2905) in *S. pyogenes*. While ICE10750-RD.2, Tn*1806*, and ICE*Sp*1108 are closely related, ICE*Sp*2905 features a completely different organization, being formed by two independent mobile elements, one [the *erm*(TR)-carrying integrative and mobilizable element IME*Sp*2907] integrated into the other (ICE*Sp*2906). In this study, 17 macrolide-resistant *Streptococcus agalactiae* isolates harboring *erm*(TR) were characterized and investigated to evaluate their *erm*(TR)-carrying genetic elements, a topic never dealt with in this species.
Materials and Methods: The 17 *S. agalactiae* isolates were phenotypically and genotypically characterized. Their *erm*(TR)-carrying elements were explored by analyzing the distinctive recombination genes of known *erm*(TR)-carrying ICEs and by PCR mapping. The new genetic context and organization of IME*Sp*2907 in *S. agalactiae* were explored using several experimental procedures and *in silico* analyses.
Results: 5 isolates harbored ICE10750-RD.2/Tn*1806* and 5 ICE*Sp*1108, and 5 bore unknown *erm*(TR)-carrying elements. The remaining 2 isolates, with identical serotype and pulsotype, harbored IME*Sp*2907 in a new genetic environment, which was investigated in depth in one of the 2 isolates, SagTR7. IME*Sp*2907 was circularizable in *S. agalactiae*, as described in *S. pyogenes*. The new junctions of IME*Sp*2907 were identified: the att sites were almost identical to those in *S. pyogenes*. In strain SagTR7, *erm*(TR)-carrying IME*Sp*2907 was embedded in an *erm*(TR)-less variant of ICE10750-RD.2/Tn*1806* which, in turn, was embedded in an ICESde3396-like element. The resulting whole ICE, ICE*Sag*TR7 (~129 kb), was integrated downstream of the chromosomal *rplL* gene, and was excisable in circular form and transferable by conjugation.
Discussion and Conclusions: This study provided the first picture of the nature and distribution of *erm*(TR)-carrying elements in *S. agalactiae*. Of special interest was the characterization of a new, large, matryoshka-like ICE*Sag*TR7, that represents the first ICE*Sa*2603 family element carrying and capable of transferring resistance determinants for both antibiotics and heavy metals.

P 056

ANTIBACTERIAL EFFICACY OF ANTIBIOTIC COMBINATIONS FOR ENDODONTIC REGENERATIVE PROCEDURES EVALUATED BY CONFOCAL LASER SCANNING MICROSCOPY

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Introduction: The high complexity of the transversal anatomy of the root canal system protects bacteria from chemo-mechanical disinfection frequently resulting in root canal treatment failures. Because

of its ability to form biofilms and survive for long time in filled root canals, *Enterococcus faecalis* is usually isolated from failed root canals that undergo retreatment. The aim of this study was to assess and compare the efficacy, even in depth, of endodontic antimicrobial mixtures in an ex vivo tubule infection model treated with a nucleic acid-binding fluorescent stain and examined by confocal laser scanning microscopy (CLSM).
Material and Methods: 72 human single-root teeth with fully formed apex were used. Cylindrical root dentine blocks were longitudinally sectioned. Root canals were infected with E.faecalis for 3 weeks to allow penetration into dentinal tubules. Samples were divided into 3 groups: group A was exposed to TRIMIX-M (ciprofloxacin, metronidazole, minocycline), group B to BIMIX (ciprofloxacin, metronidazole) and group C to TRIMIX-C (ciprofloxacin, metronidazole, clarithromycin) for 3 weeks; after that, all specimens were treated with Live/Dead BacLight Viability Stain and examined by CLSM, and the ratio of dead/live bacteria into dentinal tubules was quantitatively analyzed. Differences among groups were analyzed with Kruskall-Wallis and post-hoc Dunn's test ($p < 0.05$). Mean penetration depth of action was recorded and differences were analyzed with one-way ANOVA and post-hoc Bonferroni's test ($p < 0.05$).
Results: Ratio of red fluorescence over total green/red signal in TRIMIX-C, TRIMIX-M and BIMIX groups was 87.1, 84.2 and 76.4%, respectively. The mean depth of action was: TRIMIX-C 520 ± 30 μm, TRIMIX-M 500 ± 60 μm and BIMIX 320 ± 60 μm.
Discussion and Conclusions: Our previous studies demonstrated the higher TRIMIX-C capacity to kill endodontic pathogens *in vitro* compared with TRIMIX-M and its ability to avoid tooth discolouration after 3 weeks of incubation. The results of this study confirm that TRIMIX-C even in an *ex vivo* model shows a higher bactericidal activity, accompanied by excellent penetration in dentinal tubules.

P 057

AN EXPERIMENTALLY-BASED RATIONALE TO TEST THE MICROBIAL CHARACTERISTICS OF PROBIOTIC STRAINS USED IN COMBINATION

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Introduction: Few studies have been performed about the effects on the microbiological characteristics of a probiotic strain when it is used in combination with other probiotic strains.
Object of this study was to investigate *in vitro* the antagonistic activity between the probiotic strains *Bifidobacterium longum* BB536 and *Lactobacillus rhamnosus* HN001 and to evaluate for these strains tested alone and in combination the resistance to simulated gastrointestinal conditions, the ability to adhere to epithelial intestinal cells and their competition for adhesion.
Materials and Methods: The antagonism between *B. longum* BB536 and *L. rhamnosus* HN001 was tested modifying the method described by Abdel Daim et al. (2013). The *in vitro* resistance of the two strains used alone or in combination to low pH and bile salts was carried out modifying the method described by Muñoz-Quezada et al. (2013). The adhesion ability and the competition between the tested strains for adhesivity on human colon cancer HT-29 cells were evaluated modifying the methods described by Ruas-Madiedo et al. (2011) and by Serafini et al. (2013).
Results: The results demonstrated that *B. longum* BB536 and *L. rhamnosus* HN001 did not show *in vitro* inhibition effect each other and had a good resistance to low pH and to different concentrations of bile salts, that was enhanced when they were tested in combination. Moreover, the two strains tested alone and in combination showed a good adhesion on HT-29 cells and no mechanism of competition.
Discussion and Conclusions: Several studies demonstrated that probiotic bacteria can have antagonistic effects against enteropathogens due to bacteriocin production and competition for the adhesion to intestinal cells. These mechanisms could not be selective against enteropathogens, therefore probiotic strains used in combination could show

antagonistic effects each other, generally not investigated. In our study *B. longum* BB536 and *L. rhamnosus* HN001 showed an enhanced resistance to gastrointestinal conditions when they were used in combination. Moreover, the results showed that there was not antagonism between *B. longum* BB536 and *L. rhamnosus* HN001 due to bacteriocin production or competition for adhesion to HT-29 cells. In conclusion, the results suggest that *B. longum* BB536 and *L. rhamnosus* HN001 in combination show no antagonism and could have functional endosymbiotic effects in intestinal host-microbiota.

Keywords: Bifidobacterium longum BB536, Lactobacillus rhamnosus HN001, combination, antagonism, adhesion.

P 058

MONOSODIUM URATE CRYSTALS PROMOTE INNATE ANTI-MYCOBACTERIAL IMMUNITY AND IMPROVE BCG EFFICACY AS A VACCINE AGAINST TUBERCULOSIS

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Introduction: A safer and more effective anti-Tuberculosis (TB) vaccine is still an urgent need. We probed the effects of monosodium urate crystals (MSU) on innate immunity to improve the Bacille Calmette-Guerin (BCG) vaccination.
Material and Methods: Human macrophage were infected or not with BCG at the MOI of 1 and i) mycobacterial viability was analysed by CFU assay, ii) ROS production by fluorometry and iii) phagolysosome maturation by confocal microscopy and fluorometry. Finally, the efficacy of MSU in enhancing BCG efficacy as an anti-TB vaccine has been tested in mice following aerosolic infection with *Mycobacterium tuberculosis* (MTB).
Results: Results showed that *in vitro* MSU cause an enduring macrophage stimulation of the anti-mycobacterial response, measured as intracellular killing, ROS production and phagolysosome maturation. The contribution of MSU to anti-mycobacterial activity was also shown *in vivo*. Mice vaccinated in the presence of MSU showed a lower number of BCG in lymph nodes draining the vaccine inoculation site, in comparison to mice vaccinated without MSU. Lastly, we showed that MSU improved the efficacy of BCG vaccination in mice infected with MTB, measured in terms of lung and spleen MTB burden.
Conclusions: These results demonstrate that the use of MSU as adjuvant may represent a novel strategy to enhance the efficacy of BCG vaccination.

P 059

LACTOBACILLUS PLANTARUM SUPERNATANT AFFECTS *PROTEUS. MIRABILIS* AND *CANDIDA ALBICANS* BIOFILM FORMATION

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Introduction: Many pathogenic and nosocomial bacteria with the ability to form biofilms are responsible for acute and chronic infections because of the decreased susceptibility of bacteria, within the biofilm, to host defenses and antibiotic treatments. *Proteus mirabilis* and *Candida albicans* biofilm play an important role in several urinary tract infection diseases. Lactobacilli play a major role in maintaining the urogenital health by preventing the overgrowth and invasion of pathogenic bacteria by a combination of competitive exclusion, competition for nutrients and antimicrobial substances

production such as, hydrogen peroxide, organic acids, bacteriocins and biosurfactants The aim of this study was to evaluate the ability of different culture supernatants derived from *Lactobacillus spp.* to contrast *P. mirabilis* and *C. albicans* biofilm formation.
Materials and Methods: Bacterial isolation and culture conditions: the six *Lactobacillus* strains used in this study were originally isolated from a vaginal swab of healthy post-pubertal and pre-menopausal woman, after informed consent. Lactobacilli analyzed in this study were first grouped by a multiplex PCR and then identified to the species level by four multiplex PCR assays. *P. mirabilis* was isolated from the urine of an adult woman with urinary-catheter-associated bacteriuria, after informed consent. Identification of pure colonies were identified by API 20E test kit and Vitek2 GN ID card. *C. albicans* was obtained from clinical sample of an healthy patient, after informed consent. Two methods were used to identify the strain: API ID 32 C™ and Vitek2 YST card. Biofilm formation: Biofilm formation was assayed by measuring the ability of cells to adhere to sterile 96-well polystyrene flat-bottom microtiter plate.
Results: We demonstrated that supernatants derived from *L. plantarum* markedly contrast *P. mirabilis* and *C. albicans* biofilm formation. The supernatants did not lost their activity if collected at 24, 48 or 72 hours. In addition, *L. plantarum* supernatant reduced the vitality of *P. mirabilis* and *C. albicans* sessile cells.
Conclusions: The results here presented might suggest the ability of molecules released by *L. plantarum* to act both on preformed biofilm and on microorganisms released by dispersed biofilm, avoiding distant colonization.

P 060

SOL-GEL SYNTHESIS OF SILICA GLASSES WITH DIFFERENT CONCENTRATIONS OF SILVER OXIDE AND GOLD NANOPARTICLES: CHARACTERIZATION OF ANTI-BACTERIAL PROPERTIES

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Introduction: The prosthetic infections are a serious health problem. They are difficult to treat, lead to extended hospitalizations, additional surgical procedures, hospital readmissions, and time off from work. The ideal solution would be to coat the prosthesis with a material capable of releasing antimicrobial agents in situ. In the last decades, bioactive sol-gel glasses have been investigated for drug delivery and their characteristic of being osteoconductive makes these materials particularly suitable in the prosthetics field. Silver and gold have been exploited as antimicrobials from ancient period. With the evolution of nanomedicine as a study for treating infections, metallic silver and gold in the form of NPs have regained their significance. In this work, silicate glasses with increasing percentages of silver oxide (Ag2O) and gold nanoparticles (AuNP) have been synthesized by the sol–gel method. Antimicrobial properties of the obtained materials have been evaluated by means of *in vitro* tests and as function of the Ag_2O and AuNP content.
Materials and Methods: Sol-gel glasses were synthesized starting from tetraethyl orthosilicate (98%, TEOS), silver nitrate (≥ 99%, $AgNO_3$) and gold nanoparticles (AuNP, 5 nm diameter, OD1, stabilized in citrate buffer) as sources of SiO_2, Ag_2O and AuNP respectively. This solution was prepared using the following molar ratio: TEOS:EtOH:H_2O:HNO_3= 1:5:1:1. The materials, after complete gelation, were treated at 500°C for 2 h to remove the residual nitrate content of the synthesis. The antimicrobial activity of prepared nanobioactive glass

particles was tested against clinical pathogens such as *S. epidermidis* and *P. aeruginosa*. Bacterial suspensions were incubated with different percentages of Ag2O and AuNP for 24 h at 37°C. After incubation the colonies were counted to calculate the CFU.

Results: Gel glasses with different concentrations AuNP or AgNP showed antimicrobial activity against S. epidermidis, while only gel glasses with AuNP was effective against *P. aeruginosa*

Conclusions: The sol-gel technique was successfully used to synthesize silver/gold-containing silicate glasses with antibacterial properties. This study shows that sol-gel glasses with AuNP or AgNP are potential candidates for medical applications where antimicrobial activity is essential.

P 061

IN VITRO SYNERGIC EFFECT OF POLYETHYLENIMINE ON THE ANTIMICROBIAL ACTIVITY OF TOBRAMYCIN AND AZTREONAM AGAINST *PSEUDOMONAS AERUGINOSA*

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Introduction: Over the past two decades, *P. aeruginosa* has attracted attention as an opportunistic pathogen responsible for chronic infections. Antimicrobial treatment is often difficult because of development of resistant strains and for the difficulties encountered by drugs in overcoming intra and extracellular barriers imposed by the lungs. To improve the activity of antimicrobials, a particular attention was recently focused on cationic polymers, such as polyethylenimine (PEI), which could increase the outer membrane permeability of several Gram-negative bacteria, promoting drug interaction with intracellular targets. Nevertheless, the ability of PEI to interact with the cell membrane is not selective towards bacteria and toxicity against lung cells can be exerted. The entrapment of PEI inside advanced sustained-release carriers for inhalation may be of help to control the amount of drug administered *in situ,* while allowing co-delivery of different drugs. In this work, we have evaluated the antimicrobial activity of two conventional drugs, aztreonam (AZT) and tobramycin(TB) in association with PEI free and PEI encapsulated into large porous particles (LPP) made of poly(lactic co-glycolic acid) (PLGA) (LPP-PEI).

Materials and Methods: *in vitro*against a clinical isolate of The synergic effect of PEI free at different concentrations (from 40 to 5 mg/ml) orPEI released by LPP-PEI (5mg/ml) on the antimicrobial activity of TB (from 3.9 to 0.49 mg/ml) and AZT (from 32 to 0.25 mg/ml) was evaluated *P. aeruginosa.* The cytotoxicity of PEI free and PEI released from LPP-PEI was carried outon a pulmonary cellular model of A 549 cells, by Tripan blue exclusion Test and LDH release.

Results: The synergic effect of PEI on AZT was demonstrated, while no significant differences where observed in the association with TB. Furthermore, PEI released after 72h from LPP-PEI in PBS is able to reduce the MIC of AZTfrom 8 mg/ml to 4 mg/ml. PEI free showed a very high toxicity which is significantly reduced when PEI is released in a slow and continuous manner from LPP-PEI.

Conclusion: Our data encourage the use of AZT as emerging antibiotic against *P. aeruginosa* in FC patients when in association withLPP-PEI. The encapsulation of PEI into LPP can provide the reduction in AZT MIC while reducing the cytotoxicity effect of free PEI.

P 062

IFN-γ RELASE ASSAY VERSUS TUBERCOLIN SKIN TEST FOR MONITORING TB INFECTION IN HEALTHCARE WORKERS: OUR EXPERIENCE FROM 2012 TO 2014

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Introduction: Healthcare workers (HCW) are a risk group for TB. IFN-γ release assays (IGRA) facilitate the screening of HCW for latent TB infection (ITL). In comparison with the tuberculin skin test (TST), the IGRA reduces the number of x-rays and the amount of chemoprevention needed.
Materials and Methods: From April 2012 to December 2014 we evaluated the performance of the QuantiFERON ®TBGoldInTube (QFT;Cellestis,-CA,USA), one of the IGRAs tests commercially available, compared to the TST. Until recently, TB screening was performed using a MantouxTST, as with the IGRA, TST measures the cell-mediated immune response to antigens specific for *M. tuberculosis*. The QFT test detects the level of IFN-γ produced in response to M.tubercolosis antigens ESAT-6, CFP-10 and TB 7.7, using an enzyme-linked immunosorbent assay (ELISA). Out of 717 samples tested with IGRA, 598 belonged to healthcare workers and students of the Second University of Naples, and out of these 202 reported were vaccinated with BCG, 151 were not vaccinated and 244 did not remember.
Results: Out of 394 samples tested with IGRA, for whom the outcome of the TST was available, 370 were TST positive and 24 TST negative. All negative TST samples confirmed a negative result even with QFT, but for those with positive TST correlation with QFT positive results occurred in only 37% of patients (137) while in the remaining 63% of patients (233), we had a negative QTF. In addition on 139 HCW BCG, vaccinated with BCG and TST+, we had a negative QFT result.
Discussion and Conclusion: Given that IGRA and TST cannot as yet distinguish between latent infection and TB disease , in our experience QFT showed a sensitivity overlapped to TST, but higher specificity. Moreover our data confirm the ability of this assay to well discriminate a real ITL state among vaccinated patients false positive after TST. This two-step approach based on the concept of sequential testing choosing as the first step a test with high sensitivity and cheaper, followed by a second step using a test more expensive but with higher specificity it could be a good choice. The drawback of sequential testing are the costs for two tests, problem that could be overcome when the costs of QFT will decrease enough to be used as single screening test.

P 063

EXPRESSION OF IL10 IN PATIENTS AFFECTED BY *CHLAMYDIA TRACHOMATIS*

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Introduction: *Chlamydia* species are obligate intracellular parasites within the host cell. In women, this bacterium causes several diseases including cervicitis, endometritis, urethritis, acute and chronic pelvic inflammatory disease, infertility, and ectopic pregnancy. *C. trachomatis* usually causes asymptomatic genital tract infections in both men and women, and the high prevalence of undiagnosed infected individuals provides a reservoir for spreading the infection to men and women through sexual contact. Previous studies demonstrated that *Chlamydia* species are able to regulate immune responses via modulated expression of some immune system molecules including cytokines. In particular IL-10 plays important roles in the regulation of immune responses in the pathogenesis of *Chlamydia* infection. Interestingly, investigations demonstrated that IL-10 expression was increased during *Chlamydia* infections. For this aim we evaluated IL10 release in samples of patients positive for *C. trachomatis.*
Materials and Methods: We assessed 84 samples (77.4% cervical swabs, 7.1% urine and 15.5% semen): 44 positive for *C. trachomatis* and 40 negative used as control. The presence of IL10 was eval-

uated with IL-10 ELISA KIT (Sigma Aldrich). For statistical analysis we used the Mann-Whitney test and receiver operating characteristic (ROC).
Results: All samples positive for *C. trachomatis* have released the cytokine analyzed. Cut-off value was 0.093 UI/Ml. Statistical analysis revealed that the differences between evaluated groups regarding levels of IL-10 was significant ($p < 0.0001$).
Discussion: In the present study, the relationship between levels of IL-10 and *C. trachomatis* infection was investigated. The results demonstrated that levels of IL-10 was significantly increased when compared to healthy controls. Several studies showed that seminal levels of IL-10 increased in infertile patients, have been found in prostate secretion fluids of chronic prostatitis patients and may possibly play a key role in the process of chronic prostatitis; it was also demonstrated that endocervical concentrations of IL-10 significantly increased after genital *Chlamydia* infection. So IL-10 can be used in future as a diagnostic marker for the presence of this microorganism.

P 064

SERRATIOPEPTIDASE: OLD ENZYME WITH NEW NON ENZYMATIC ACTIVITY AGAINST *S. AUREUS* BIOFILM?

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Introduction: The use of indwelling medical devices is associated with a significant risk of infections by *Staphylococcus aureus* which possesses a variety of virulence factors including many toxins and the ability to invade eukaryotic cells or form biofilm on biotic and abiotic surfaces.
Previously reports evaluated the anti-infective properties of serratiopeptidase (spep), an extracellular metalloprotease produced by *Serratia marcescens*, in impairing virulence-related staphylococcal properties, such as attachment to inert surfaces and adhesion/invasion on eukaryotic cells. However, to date its mechanism of action is unknown.
Spep has a zinc binding consensus HEXXHXXGXXH, where the three histidine are zinc ligands, and the glutamic acid is the catalytic base.
Materials and Methods: Spep gene was PCR amplified and cloned into expression vector pET28b(+). The resulting construct was indicated as pET28b-Spep. The mutant EspepA was constructed from plasmid pET28b-Spep applying the one-step overlap extension PCR strategy. The resulting plasmids were costransformed in EcBL21(DE3) cells with the plasmid pRuW4inh1 harboring the *Erwinia chrysanthemi* secretion system.
Bacterial pellets and supernatants were collected and analyzed by SDS-PAGE and zymography.
The unambiguous identification and a detailed structure characterization of both the wild type and the mutant Spep were obtained by mass spectrometric analyses.
The resultant supernatants sterilized by filtration were separately used to condition biofilm formation of *S. aureus*. Quantification was based on crystal violet method.
Results: In this work we constructed Spep mutant by substituting the glutamic acid in the catalytic site with a residue of alanine. In this manner we were able to evaluate the anti-biofilm activity of Spep mutant in absence of proteolytic activity. This mutant did not display protease activity but it retained its anti-biofilm properties, suggesting that this action is independent by enzymatic activity.
Discussion and Conclusions: New knowledge obtained from data reported in this paper calls attention to a novel mechanism of action of Spep. This protein could be developed as a potential ‘‘antipathogenic agent’’ capable to impair the ability of *S. aureus* to form biofilm on prostheses, catheters and medical devices, exploiting a mechanism different from the proteolytic activity.

P 065

MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS ISOLATION FROM BLOOD OF T1D PATIENTS

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Introduction: Recently our group hypothesized a role of *Mycobacterium avium* subspecies *paratuberculosis* (MAP) as an environmental agent in the onset of Type 1 diabetes (T1D). MAP is the causative agent of Johne's disease in livestock ruminant species and has been associated to Crohn's disease in humans.
Material and Methods: In order to strengthen this finding, we investigated the presence of MAP within Sardinian T1D population. The blood of T1D Sardinia patients was collected; after blood separation, the buffy coat was cultured in conventional media, including Middlebrook 7H9 broth base with ADC (growth supplement) and Middlebrook 710 broth base with OADC (growth supplement); all media were prepared with mycobactin J (final concentration 2μg ml^{-1}) and in the VersaTREK Automated Cultere System. 500μl of the broth was collected after 12 weeks incubation and processed to detect MAP using IS900 Real Time PCR.
Results: We have isolated 5 strains of MAP. The DNA of MAP was sent out to be sequenced to CRIBI Genomics.
Discussion and Conclusion: These preliminary results confirm the association of MAP to T1D patients and suggest that MAP may plays an important role in T1D etiology. The work is still in progress.

P 066

MATRIX METALLOPROTEINASE (MMP)-9 AS MARKER OF INFLAMMATION IN HUMAN TRICHINELLOSIS

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Introduction: MMPs are involved in many physiological and pathological processes. In parasitic infections, these proteins have been particularly studied in malaria, neurocysticercosis and angiostrongyloidosis.
Recently, we analysed serum levels of MMP-9 and -2 (gelatinases) in mice experimentally infected with *Trichinella spiralis* or *Trichinella pseudospiralis*, (characterised by different levels of myositis) and we found they significantly increased in the former and at a lesser extent in the latter, thus suggesting the possibility that these gelatinases might represent a marker of inflammation.
Our aim was to evaluate the levels of MMP-9 and 2 in trichinellosis patients, to verify their possible clinical significance.
Materials and Methods: Serum samples from *Trichinella britovi*-infected individuals (n = 31) were analysed for MMP-9 and MMP-2 activities. Patients were infected after the consumption of raw or undercooked wild boar meat. Sera were collected before, and after anti-inflammatory therapy, aliquoted and stored at -20°C until use. Sera from healthy subjects were taken as a control group.
The gelatinolytic activity of MMPs was analysed by gelatin zymography on 8% polyacrylamide-SDS gels containing 0.1% porcine gelatin, under non-reducing conditions. Clear bands corresponding to the digested areas were evaluated with an appropriate software. MMP-9 levels were additionally determined using a commercial ELISA kit for human MMP-9.
Results: Differences in the gelatinase activity between the two groups were assessed using a two-tailed Student's t-test assuming equal variances. The significance level was set at $p < 0.01$.
The zymographic analysis of the gels showed the presence in serum samples of gelatinase bands at approximately 92-kDa and 72-kDA, corresponding

to the pro-enzyme form of MMP-9 and MMP-2, respectively. Areas of lysis of *T. britovi*-infected patients were compared to controls. A significant ($p < 0.01$) increase in gelatinolytic activity in patients compared to the control group was observed for pro-MMP-9 in 25 out of 31. The mean increase in activity was 39.25% ± 16.67%. No significant differences were observed for pro-MMP-2 activity. The MMP-9 level detected by ELISA test showed a significant concordance with zymographic data ($r^2 = 0.62$, $p < 0.003$).

Discussion and Conclusions: MMP-9 showed to be a good marker of inflammation in patients infected with *T. britovi*. On the contrary, MMP-2 did not show any significant difference in patients compared to controls.

P 067

STAPHYLOCOCCUS AUREUS EXOPROTEIN ACTIVE AGAINST *STAPHYLOCOCCUS EPIDERMIDIS* BIOFILM

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Introduction: Staphylococci are recognized as the most frequent causes of biofilm-associated infections, thanks to their ability to adhere on both eukaryotic cells and abiotic surfaces. Staphylococcal biofilms display extraordinary resistance to antimicrobial killing and limit the efficacy of antibiotic therapy. *Staphylococcus aureus* and *Staphylococcus epidermidis* are prevalent species on skin and mucosae of animals and humans.

The interest in the development of alternative anti-infective approaches for the prevention and treatment of infections increased in recent years. Our rationale is to look for new antimicrobials inhibiting virulence, in particular bacterial adhesion and biofilm formation, rather than bacterial growth. This latter should exert a weaker selective pressure for the development of drug resistance.

Materials and Methods: We examined the anti-biofilm activity of cell-free supernatants deriving from *S. aureus*. Its effect on *S. epidermidis* biofilm formation was evaluated both in static and dynamic condition. The dynamic condition was assessed in BioFlux System that precisely controls the flow of growth medium, in order to acquire sequential bright-field images of any growing biofilm. Physico-chemical characterization of active compounds was also performed.

Protein purification was performed firstly using a selective precipitation by salting out technique. The active precipitate was further fractionated using a hydrophobic interaction chromatography. Protein profiles of fractions derived from chromatography were analyzed by SDS-PAGE and tested for their anti-biofilm activity.

Results: Data obtained have shown the anti-biofilm activity of *S. aureus* supernatant able to inhibit the biofilm formation, and to disrupt mature biofilms of *S. epidermidis* without affecting the bacterial viability. Treatment with protease eliminated anti-biofilm activity in supernatant. No surfactant activity was detected. An active fraction after salting out and chromatography was identified.

Discussion and Conclusions: The active fraction derived from chromatography with a discrete number of proteins having anti-biofilm activity was identified. The isolation of active molecule necessarily requires additional steps of purification.

In conclusion, in order to understand the mode of action of active molecule, its identification is a fundamental prerequisite.

P 068

IDENTIFICATION OF NEW ANTI-INFECTIVE MOLECULES FROM MARINE BACTERIA ACTIVE AGAINST THE BIOFILM OF *S. AUREUS* AND *P. AERUGINOSA*

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Introduction: *Pseudomonas aeruginosa* and *Staphylococcus aureus* are the most common bacte-

rial pathogens isolated from the cystic fibrosis (CF) respiratory tract. CF infections treatment is hampered by the ability of bacteria to form biofilms intrinsically resistant to antibiotics. Impeding biofilm formation is an important step towards infection control and they might be considered major target for the development of novel therapeutic agents. Many bacteria secrete anti-biofilm molecules that function regulating biofilm architecture or mediating the release of cells from it during the dispersal stage of life cycle.
Cold-adapted marine bacteria represent an untapped reservoir of biodiversity able to synthesize a broad range of bioactive compounds, including anti-biofilm molecules.
Materials and Methods: The anti-biofilm activity of 20 cell-free supernatants derived from sessile and planktonic cultures of Polar bacteria belonging to *Pseudoalteromonas* and *Psychrobacter* species were tested against the biofilm formation of *S. aureus* and *P. aeruginosa* strains. Biofilm formation was quantified using crystal violet assay. A preliminary physico-chemical characterization was also performed. Their activity was evaluated also in dynamic condition by BioFlux system.
Results: Reported results demonstrate that we have selected supernatants containing non-biocidal agents able to destabilize *S. aureus* and *P. aeruginosa* biofilm matrix without killing cells. In particular, Polar supernatants are active to disaggregate the biofilm produced by all *P. aeruginosa* and by almost all *S. aureus* tested strains. In such cases, the production of anti-biofilm activity is related to the growth condition (sessile or planktonic). The physico-chemical characterization of the supernatants highlighted the presence of molecules of different nature that act inhibiting biofilm formation (proteins, surfactants or polysaccharides).
Discussion and Conclusions: The present study reports about the marine bacterial culture supernatants that inhibit the biofilm formation of *S. aureus* and *P. aeruginosa*. Some of them are also able to impair the initial attachment to the surface, acting as surfactant molecules. Most promising supernatants will be further analysed in order to characterize the active components. Thus they could be combined with a conventional antibiotic to eradicate biofilm infection.

P 069

ACUTE OTITIS EXTERNA IN PATIENTS WEARING HEARING AIDS: A MICROBIOLOGICAL STUDY

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Introduction: Hearing aids are essential devices to keep the social, emotional, psychological, and physical well-being of people with hearing loss. However, the use of hearing aids is a predisposing factor to the development of acute otitis externa (AOE). AOE is defined as diffuse inflammation of the external ear canal, associated with severe pain, fever and otorrhea. In addition, this infection has a high incidence and recurrence, representing a major concern for hearing aid users. In the current literature, microorganisms responsible of AOE in patients wearing hearing aids remain poorly identified. Therefore, the objective of this study was to determine qualitatively and quantitatively the causative microorganisms responsible for AOE in patients wearing hearing aids.
Materials and Methods: A total of 115 samples, 56 ear swabs and 59 hearing aids, fit open and closed, were collected. Microorganisms were identified by conventional phenotypic systems (API® systems; bioMerieux) and by 16S rDNA gene sequence analysis.
Results: A total of 83 samples (89%) were infected and, of these, 34% (19/56) of ear swabs and 22% (13/59) of hearing aids, had a bacterial counts of more than 10^4 CFU/ml. Interestingly, almost all samples with a high bacterial load had a polymicrobial profile, suggestive of a possible synergy among different bacterial species. Coagulase negative staphylococci were the most frequent microbial isolates. However, pathogens, such as *Staphylococcus aureus, Streptococcus agalactiae,* and potentially infectious microorganisms, such as *Enterobacter cloacae, Klebsiella pneumoniae, Pseudomonas aeruginosa, Corynebacterium pseudodiftericum, Corynebacterium afermentans, Bacillus* spp., and

Enterococcus faecalis were also recovered. Experiments are in progress to analyze the biofilm-forming ability of isolated strains in mono and polymicrobial biofilms.
Discussion and Conclusions: This study provides insights into the types and amounts of microbial growth detectable on hearing aid and hearing aid-related surfaces. Taken together our results show that the efficacy of cerumen in inhibiting microbial growth may be uniquely challenged in people wearing hearing aids and cross-contamination is a concerning issue especially in elderly and immunocompromised. Therefore, it is important for hearing aid users to manage these devises properly using effective cleaning solutions to prevent AOE.

P 070

A NOVEL qPCR ASSAY BASED ON THE GbpA ADHESIN FOR THE DETECTION OF *VIBRIO CHOLERAE* IN STOOL AND ENVIRONMENTAL SAMPLES

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Introduction: Colonization of environmental chitin surface and human intestinal cells by *Vibrio cholerae* involves a number of bacterial adhesins including the N-acetyl glucosamine-binding protein A (GbpA). We previously investigated the distribution and genetic variations of gbpA in a large collection of *V. cholerae* strains and found that the gene is consistently present and highly conserved in this species. As a follow up to our previous observations, this study developed a new, rapid, sensitive and quantitative species-specific Taq-Man based Real-Time PCR protocol targeting the *gbpA* gene of *V. cholerae*.
Materials and Methods: A total of 129 bacterial strains belonging to different *Vibrio* and other related species were used. The entire *gbpA* ORF in 36 representative *V. cholerae* strains was amplified, purified and sequenced by using the Sanger dye-terminator method. The *gbpA* gene sequences were then aligned using the BIOEDIT software, and primers and a probe were designed to target a species-specific region of the gene showing the lowest sequence variability (> 99%) within *V. cholerae*. The chosen primer and probe sequences were: Vc gbpA F (5-ccg cag ctt cct tct aca ac-3), V.c gbpA R (5-ggc ttt ggt tag cgt ctc ag-3) and V.c gbpA pr (5-FAM-aac cca gca ggt caa atc att cca agt a-BBQ) probe (amplified fragment: 206 bp).
Results: We found that the GbpA-based qPCR assay is sensitive (50 gene copies), highly specific for *V. cholerae* and failed to amplify strains of the closely-related species *Vibrio mimicus*. The sensitivity of the assay applied to environmental and stool samples spiked with *V. cholerae* ATCC 39315 was comparable to that of pure cultures and was of 10^2 genomic units/l for drinking and seawater samples, 10^1 genomic units/g for sediment and 10^2 genomic units/g for bivalve and stool samples. The method also performs well when tested on artificially formalin-fixed and degraded genomic samples and was able to amplify *V. cholerae* DNA in historical "Continous Plankton Recorder" samples, the earliest of which date back to August 1966.
Discussion: The developed protocol provides a new tool for the robust and sensitive detection and quantification of *V. cholerae* in problematic matrices such as stool and environmental samples. The assay has potential application for studies investigating the ecology and epidemiology of *V. cholerae* and in the field of public health.

P 071

START-UP COMPANY FOR PRODUCTION OF SPIRULINA BIOMASS IN SOUTHERN ITALY. FIRST EXPERIENCE IN CALABRIA

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Introduction: *Arthrospira platensis* (Spirulina) is an economically important filamentous cyanobacterium that is commercially produced as a source of human health food, animal feed and cosmetic colorants. Nowadays there are no literature data, which report experiment in Calabria. Therefore, to our knowledge this paper is the first report for

Calabria. We did a preliminary evaluation of the growth characteristics of *Arthrospira platensis'* strain in open-raceway in order to evaluate the potential economic impact.
Material and Methods: *A. platensis* used in this study was collected from "Provence Spirulina" farm in the south of France and maintained in Zarrouk's medium (pH 9) under rotatory conditions and illuminated continuously at a light intensity of 3200 LUX. When the culture was at the stationary phase of growth, a 20 l bottle was used to start to scale-up process. We started with experiment and evaluated the growth in open raceway from April 2014 to March 2015. Every 10 days the biomass in culture was harvested and renewed for 50 percent of volume medium. We calculated correlation between weight and volume as g/L and improved microscopic examination of sample using light microscope.
Results: The growth curve of *A. platensis* represents a typical microbe growth curve. It shows a lag, log, and stationary phase, where the log phase is faster in summer than in winter months. The total yield of *A. platensis* biomass was 0.98g/L harvested after about 240 h. From literature data, the optimum temperature for the growth of *A. platensis* is around 36 °C.
Conclusion: The production in open raceway is limited by temperature. Therefore, the temperature is the most important environmental parameter that limit the growth of *A. platensis* in open raceway. However, in south Calabria the average temperature allow the growth in open raceway as our data shown. The national investments in green economy improved in last years. Favorable environmental conditions and strategic location of south Calabria might draw more funding in the green enterprise.

P 072

MICROBIOLOGICAL RISKS AND VIRULENCE ROLE OF *LISTERIA MONOCYTOGENES* IN RTE SALADS

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Introduction: Vegetables are major components of a healthy and balanced diet. However, 25% of foodborne diseases are linked to the consumption of vegetables, especially minimally processed ready to eat (RTE) vegetables. The main foodborne pathogens associated with RTE vegetables are *Enterobacteriaceae (E. coli, Salmonella* spp. and *Yersinia* spp.) and psychrophilic microorganisms such as *Listeria monocytogenes*. The aim of this work was to assess the microbiological risks associated with consumption of RTE salads. Microbiological challenge tests were carried out for the evaluation of the growing potential of *L. monocytogenes* in RTE salads stored at different temperatures. In order to evaluate the virulence role of *L. monocytogenes*, the temperature-dependent transcription of major virulence genes (inlA, inlB, hlyA, actA, plcA, plcB, iap) was also investigated.
Materials and Methods: A total of 60 pre-packaged mixed raw vegetables were used for the preparation of the microbiological challenge test. A suspension (10-100 CFU/ml) of *L. monocytogenes* ATCC 35152 and a wild type were used for contamination. The samples, stored at 4°, 8° and 25°C, were examined at fixed times. Evaluation of the gene expression of *L. monocytogenes* was carried out by RT-PCR.
Results: Data obtained show the ability of *L. monocytogenes* to survive and grow in RTE mixed raw vegetables at the different storage temperatures considered. Furthermore, the virulence gene expression of *L. monocytogenes* shows different intensities in relation to different temperatures: nevertheless, no stop in transcription is highlighted at refrigeration temperatures (4°C).
Discussion and Conclusions: The results obtained in this study highlight that *L. monocytogenes* is able to grow in RTE mixed raw vegetables. The risk associated with eating RTE mixed raw vegetables depends on the correct application of good manufacturing practices (GMP) in each stage of the production

process, during distribution and consumption and the long period of shelf life (more than10 days).

P 073

IDENTIFICATION OF CRITICAL RESIDUES FOR OMPA-PHON2 BINDING

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Introduction: *S. flexneri* is the agent of bacillary dysentery. Apyrase (PhoN2) is a virulence periplasmic protein involved in the *S. flexneri* intra and inter-cellular motility. PhoN2 interacts with the outer membrane protein A (OmpA). OmpA is a β-barrel protein embedded in the bacterial OM, highly conserved among Gram- bacteria. OmpA plays a role in the structural integrity of the OM. OmpA presents a β-barrel structure and a globular C-terminal domain exposed into the periplasm where it can bind peptidoglican. The aim of this work is to identify the OmpA amino acid residues involved in the interaction with PhoN2.
Materials and Methods: The structure of OmpA of *S. flexneri* M90T strain was predicted using SWISS-MODEL workspace. The model was built by selecting OmpA templates presenting at least 80% of sequence identity. For two-hybrid assay, the *phoN2* gene was cloned in the pGBKT7 vector. Selected regions of *ompA* gene were amplified with primer pairs containing sites for homology recombination with pGADT7 vector. The assay was performed following the manufacture's protocol (Clontech). The interaction of PhoN2 and the different regions of OmpA was carried out by plating the transformed cells on minimal medium lacking leucine, tryptophan, histidine and adenine. The obtained colonies were screened using pGADT7 specific primers and the DNAs from PCR positive colonies were sequenced.
Results: The architecture of OmpA from S. flexneri M90T in the built model is a double-domain protein. The β-barrel consists of N-terminal 8-β strands (172 residues). A short poly proline rich linker region (residue 173-190), that could have high flexibility, connects the β-barrel to the C-terminal globular domain (136 residues). The model showed structural similarity to a transmembrane protein with the β-barrel embedded in the OM and the globular structure located at the periplasm. Based on these structural data we used specific regions of the C-terminal domain of OmpA to test their interaction with PhoN2. The screening of positive colonies showed the presence of a specific region of OmpA starting from amino acids stretch $_{188}$EVQ$_{190}$.
Discussion and Conclusions: Through computational analysis of the structure of *S. flexneri* OmpA protein and two-hybrid assay we identified the amino acid motif $_{188}$EVQ$_{190}$, located in the linker region, as responsible of the binding to PhoN2. This flexible region could be a scaffold for protein interactions revealing an important characteristic of this conserved protein.

P 074

CHARACTERIZATION OF LIPID PROFILES IN *STAPHYLOCOCCUS SP.*: IMPLICATION WITH ANTIBIOTIC RESISTANCE

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Introduction: *Staphylococcus aureus* and Methicillin-resistant *Staphylococcus aureus* (MRSA) strains are responsible for nosocomial infections. The search for novel and more efficient antibiotics has considerably grown over the last decades, mostly to identify low toxicity molecules. Very limited information is available on the lipid composition of ATCC and clinical isolates of *Staphylococcus aureus* and the correlation with antibiotic resistance. In this work we try shed some light on this possible correlation.
Materials and Methods: Antibiotic resistance of 2 ATCC strains of *S. aureus* (ATCC 43300 and ATCC 6538P) and thirty clinical isolates has been performed by the disc diffusion method. Bacterial profiles of the tested strains have been identified by GC-MS.
Results: The results obtained demonstrated ampi-

cillin and penicillin G resistance in all the analyzed strains. However, several strains showed specific antibiotic resistance such us, for instance, *Staphylococcus aureus* clinical isolates 581, 530 and 531. GC-MS analyses of fatty acids separation showed the presence of common cluster of compounds in some strains and more specific for other strains. In *Staphylococcus aureus* clinical isolates 581, 530 and 531 for instance, there is a richness in compounds with eighteen carbon atoms rather than in *Staphylococcus aureus* clinical isolates 526 and 550. Curiously we noticed a correlation with the pattern of antibiotic resistance and the similarity in fatty acid composition. Very little or no correlation has been found with cellular hydrophobicity.

Discussion and Conclusions: The interaction and the ability of bacterial strains to establish covalent or weak interactions with macromolecular components and antibiotic substances are crucial for the pathogenicity of each strain and for our potentiality to avoid infection. In this work we show a direct correlation between the similarity in fatty acid composition of the tested strains and their antibiotic resistance.

P 075

IN VITRO ANTIMICROBIAL ACTIVITY OF A WHITE GRAPE JUICE (*VITIS VINIFERA*) EXTRACT

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Introduction: Consumption of grapes (*Vitis vinifera*) and grape products has been associated to beneficial effects related to the presence of polyphenols, mainly flavonoids and phenolic acids, with antioxidant, anti-inflammatory, antimicrobial, antiviral and cancer preventive properties. Due to the increased antibiotic resistance as well as the difficulty to inhibit and eradicate the Gram-positive and Gram-negative biofilm formation, the antimicrobial properties of natural compounds has been gaining attention. In the present study we evaluated the antimicrobial effect of white grape juice extract (WGJe) against a range of Gram-positive and Gram-negative bacteria.

Materials and Methods: The antimicrobial effect of a polyphenols-rich white grape juice extract, was evaluated against clinical isolates of *S. aureus*, both methicillin-resistant and methicillin-sensitive, and *S. epidermidis* according to CLSI. The same extract was also tested on the production of bacterial biofilms *in vitro*. Chemical composition were evaluated by UPLC/QqQ-MSMS.

Results: WGJe was effective against all Gram-positive bacteria tested, *Staphylococcus aureus* and *Listeria monocitogenes* being the most sensitive strain (MIC values between 3.9 and 62.5 µg/ml). The effect was bactericidal at the concentration of 250 µg/ml. Amongst the Gram-negative bacteria, *Escherichia coli* was the only susceptible strain (MIC and MBC of 2000 µg/ml). Moreover, WGJe inhibit the biofilms formation of *S. aureus, S. epidermidis, E. coli* and *Pseudomonas aeruginosa* in dose- dependent manner.

Discussion and Conclusion: The presented results could be used to develop novel strategies for the treatment of infection. WGJe could also be used in combination with antibiotics to help eradicate resistant bacterial biofilms.

P 076

RAPID DETERMINATION OF THE ANTIBIOTIC SUSCEPTIBILITY IN PATIENTS WITH GRAM-NEGATIVE BACTEREMIA DIRECTLY FROM BLOOD CULTURES FLUID

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Introduction: The identification of pathogens directly from blood cultures (BCs) by Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) is almost a valuable tool for improving the timeliness of infection diagnosis and the treatment of patients with bacteremia and sepsis. However, the increasing incidence of multidrug-resistant Gram-negative bacteria makes

it difficult to predict susceptibility profiles based only on pathogen identification while the delay in appropriate antimicrobial therapy is associated with increased mortality. Thus, there is an urgent need to provide timely and cost-effective information on infection status, which allows the clinicians to set up more informed decisions on appropriate antibiotic therapy at an earlier stage of bloodstream infection (BSI). The aim of this study was to establish a rapid antibiotic susceptibility test directly for positive BCs.

Methods: A preliminary validation study was undertaken by analysis of 50 consecutive BCs from patients with Gram-negative bacteremia. After the results of the Gram staining, bacterial pellets were extracted from the BCs bottles for the identification as well as for the antimicrobial susceptibility testing (AST) of the isolates. About the identification, the samples were measured using an autoflex speed mass spectrometer (Bruker Daltonik) while a preliminary AST was performed using the Vitek 2 system (BioMérieux). A definitive AST was performed according to the conventional procedures from subcultures of the isolates on solid media.

Results: The overall sensitivity and specificity of the results of AST performed on bacteria extracted from positive BCs compared with those of conventional AST performed on bacteria grown on solid media were 100%. The reporting of results was available within 9 hours after the BCs was flagged as positive so the difference between the average Turn Around Time (ΔTAT) in the two different approaches about the determination of the antibiotic susceptibility was about 5 hours.

Conclusions: Considering the highly sensitivity and specificity of this assay as well as the significant time saving in obtaining a definitive AST the introduction of this approach could be adapted to any clinical microbiology laboratory.

P 077

PRESENCE OF *HELICOBACTER PYLORI* INFECTION IN SICILIAN CHILDREN

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Introduction: *H. pylori* infection is usually acquired during childhood but complications develop in adulthood. In children, the prevalence of *H. pylori* ranges from less than 10% to over 80% in developing countries. The risk factors for acquisition of infection include socioeconomic status, house hold crowding, ethnicity, migration from high prevalence regions, and infection status of family members.

Moreover the prevalence of *H. pylori* varies according to the socioeconomic level of the country or region: 7-33% of children in developed countries and Europe, 48-78% of children in South America, and 37.5-66% of children in Asia. In southern Italy we do not know the percentage of infection of *H. pylori* in children. Therefore the aim of this study was to determine the presence of *H. pylori* infection among children in Sicily.

Materials and Methods: Samples were collected from 75 asymptomatic children and from their mothers. The data were collected in two phases: an interview with the mothers or participating children during March 2015, and the screening of the children for *H. pylori* infection during April 2015. All procedures and tests were carefully explained to the parents of patients and informed consent was obtained.

The data regarding age, sex, duration of breastfeeding, family size, previous antibiotic usage, and infection status were recorded for each child. No child was tested within a week of taking antibiotics. The *H. pylori* stool antigen test was used to detect the infection.

Results: Stool samples were positive for *H. pylori* in 3 (4%) children and in 7 (9%) mothers. Two children were brothers, and in all three cases, the parent with the infection was the father but not the mother. There was no correlation between a positive *H. pylori* status, gender, socioeconomic status, duration of breastfeeding and family size.

Discussion and Conclusions: The *H. pylori* infection is primarily acquired in childhood and unless treated, persists throughout the patient's life. Many studies indicate that stool antigen test is a sensitive and specific method to diagnose *H. pylori* infection. This test could be used both safely and cost effectively to screen patients who were positive for *H. pylori* without alarming symptoms. Our results suggest that in Sicily the presence of *H. pylori* in the pediatric population is low but the number of cases should be increased. Moreover the family has an important role in the transmission of infection.

P 078

CORIANDER (*CORIANDRUM SATIVUM L.*) ESSENTIAL OIL: EFFECT ON UROPATHOGENIC *ESCHERICHIA COLI*

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Introduction: Urinary tract infections (UTIs) are one of the most frequent infectious diseases around the world affecting both outpatients and inpatients. Uropathogen *Escherichia coli* (UPEC) is the prevalent pathogen associated with UTIs. Recently, treatment of UTIs has become difficult due to the increase in antimicrobial resistance of urinary pathogens worldwide. Coriander (*Coriandrum sativum* L., Apiaceae), an annual herb native to the Mediterranean region and extensively grown all over the world, is used as culinary ingredient and as a medicinal plant. Chemical composition and biological activities including antioxidant, hypoglycemic, hypolipidemic, anxiolytic, analgesic, anti-inflammatory, anti-convulsant and anti-cancer are reported. Antimicrobial activity of coriander against both Gram positive and negative bacteria as well as *Candida* strains has been reported. The aim of study was to examine the antibacterial potential of commercial essential oil from coriander (CDO) against UPEC clinical strains.

Materials and Methods: Identification and susceptibility tests were performed by automated VITEK-2 System. Chemical composition of CDO was analyzed by gas chromatography mass spectrometry (GC-MS). Antimicrobial activities of essential oil against UPEC strains was determined using micro-dilution method. To determine the activity of CDO with gentamicin and ciprofloxacin, a checkerboard titration method was used. Bacterial biofilm-forming ability was tested by crystal violet assays.

Results: The strains were characterized by high antibiotic resistant profile towards some drugs commonly used in therapy. The chemical composition of CDO, analyzed by GC-MS showed that linalool (45,84%) and bergamol (17,70%) were dominant constituents. The antimicrobial activity of CDO was strain specific: MICs ranging from 3,12 to 6,25 mg/ml against tested bacteria. Depending on strains features and on level of sub inhibitory concentrations, great variability in biofilm production was observed.

Discussion and Conclusions: Preliminary data show that the combination of sub-inhibitory concentration of CDO with gentamicin or ciprofloxacin determined a decrease of MICs. The effect of CDO against multidrug resistant UPEC strains encourages further studies on interactions of this essential oil with bacterial strains.

P 079

VIRULENCE OF UROPATHOGENIC *ESCHERICHIA COLI* STRAINS IN THE HOST MODEL *CAENORHABDITIS ELEGANS*

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Introduction: Urinary tract infections (UTIs) are among the most common of bacterial infections in humans. Although a number of bacteria can cause

UTIs, most cases are due to infection by uropathogenic *Escherichia coli* (UPEC).UPEC isolates are a genetically heterogeneous group that exhibit several virulence factors associated with colonization and persistence of the bacteria in the urinary tract. *Caenorhabditis elegans* is a tiny, free-living nematode found worldwide. Because many biological pathways are conserved between *C. elegans* and humans, the nematode is being increasingly used as a simple model organism to study virulence mechanisms of microbial infections and innate immunity. *C. elegans* has been also used to evaluate the virulence of intestinal and extraintestinal *E. coli* strains. Aim of the study was to assess the pathogenicity and/or colonization ability of clinical UPEC in this host.

Materials and Methods: The intestinal load of nematode grazing on a lawn of urinary E.coli was monitored by determination of worm-associated colony forming units. UPEC and control strains transformed with GFP-encoding plasmids allowed to monitor bacterial colonization by fluorescence microscopy.

Results: The virulence of UPEC, evaluated by measuring the survival of *C. elegans* fed on pure cultures of these strains, showed that urinary strains can kill the nematode faster than the *E. coli* OP50, which is the standard laboratory food. The reduction of the mean lifespan observed for the worms fed on different studied strains varies from 2 to 3 days relative to mean lifespan observed for worms fed on OP50. Interestingly, among UPEC isolates, some were able to efficiently colonize the nematode gut.

Discussion and Conclusions: In order to identify differences in virulence factors that could explain strain dependent pathogenicity in this model, *E. coli* isolates were characterized for antimicrobial resistance, pathogenicity-related genes associated with virulence and phylogenetic group belonging.
Until now, it has not been possible to identify key UTI virulence factors that could discriminate the different pathogenetic behavior. Moreover our finding showed that *C. elegans* could represents a suitable virulence model for urophatogenic *E. coli.*

P 080

ENTAMOEBA HISTOLYTICA AND *ENTAMOEBA DISPAR* DIAGNOSIS IN A NON-ENDEMIC SETTING

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Introduction: Amoebiasis affects about 500 million people, mostly in developing countries. However, only 10% of the infections lead to severe disease, whereas 90% of the carriers remain asymptomatic. Epidemiologic and molecular studies have established that the organism previously known as *Entamoeba histolytica* (EH) now comprises two genetically distinct but morphologically identical species: EH, pathogenic that causes invasive disease, and *Entamoeba dispar* (ED) non-pathogenic, considered commensal organism. Amplification of amoeba DNA fragments by PCR has been proven to be a sensitive and specific method, circumventing the problems of microscopic or culture-based diagnosis. The aim of this study was to determine the relative proportions of infections caused by the pathogenic EH and the non-pathogenic ED, allowing us to obtain a picture of the epidemiological situation in a non-endemic setting.

Materials and Methods: One hundred fifty-two samples (128 stools/formalin-fixed stools, 13 liver abscess samples, 6 intestinal biopsy, 1 peripheral blood, 2 brain tissues, 1 pleural liquid and 1 gluteal abscess) were collected from patients with risk factors for EH/ED complex infection, between January 2010-June 2015 at the National Institute for Infectious Disease Lazzaro Spallanzani. A nested-PCR (Evangelopoulos 2000) was used for detection and differentiation of EH and ED.

Results: Forty-four samples were positive for ED (89.8%; all stools/formalin-fixed stools) and 5 samples were positive for EH (10.2%; 3 liver and 1 gluteal abscess samples and 1 formalin-fixed stools). In 4 EH PCR-positive samples microscopy were found to be negative for the presence of EH/ED cysts. In all the ED PCR-positive samples, microscopy evidenced amoebic cysts, identified as ED by PCR.

Discussion and Conclusions: PCR was crucial for the detection and identification of EH and ED in

clinical samples to routinely diagnose amoebiasis. These methods avoid the limitations of microscopy that is able to detect but not to identify and differentiate the two species. Moreover, our work allowed to estimate the respective proportions of EH and ED in a non-endemic area (Rome, Italy), although the analysed samples were not random but belonged to individuals (travellers and immigrants) who mostly presented with suspected intestinal parasitosis.

P 081

ANTIBACTERIAL AND ANTIVIRAL ACTIVITY OF A CORE DOMAIN OF HUMAN β-DEFENSIN 3

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Introduction: Host defense peptides (HDP) are a critical component of innate immunity and a first line of defense against pathogens. Despite their diversity, HDP share common features like small size, positive charge and amphipathic structure. For the broad family of cysteine-stabilized HDP a common structural signature, named the γ-core motif, has been identified. The presence of the γ-core in antimicrobial peptides, peptide toxins, microbicidal chemokines supports the hypothesis that it represents an archetypal domain present in a common ancestor of this family of HDPs. We tested the hypothesis that each γ-core motif should represent the evolutionary starting point of the molecule. To this end, we analyzed in detail the antibacterial and antiviral activity of the γ-core of human β-Defensin 3 (HBD3).
Materials and Methods: A CFU assay of the antibacterial activity of the synthesized peptides was performed against *Escherichia coli* ATCC-25922, *Pseudomonas aeruginosa* ATCC-27853, and *Staphilococcus aureus* ATCC-6538P. An HSV-1 strain carrying a lacZ gene driven by the cytomegalovirus (CMV) IE-1 promoter to express β-galactosidase was used for antiviral assays. Cellular toxicity was also assessed.
Results: Two peptides corresponding to the HBD3 γ-core with or without the C-terminal charged tail were synthesized and tested against the gram negative bacteria *E. coli* and *P. aeruginosa*, and the gram positive S. aureus, in the presence of increasing concentrations of NaCl. The reduced and oxidized forms of both peptides had comparable activity to full-length HBD3. The antiviral assays showed that the γ-core retains most of the anti-HSV-1 activity showed by its parental molecule. Similar to full-length HBD3, the γ-core peptides were more efficacious in cell pre-treatment and co-exposure experiments than in virus pre-exposure experiments, while no efficacy was observed in post-exposure conditions.
Discussion and Conclusions: We show that a peptide corresponding to the γ-core of human β-defensin 3 shares various antibacterial and antiviral activities of the parent molecule. Gaining insight into the evolutionary construction of γ-core HDP might enable the *de novo* design of antibiotics, using nature's rules.

P 082

CORRELATION BETWEEN CAPSULAR CONTRACTURE AND SUBCLINICAL BACTERIAL INFECTION

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Introduction: Capsular contracture (CC) around breast implants is a very common and unpredictable complication of breast augmentation and reconstruction, and is the main cause leading to reoperation. The etiology of capsular contracture appears to be multifactorial. Currently, different studies suggest that the subclinical infection and the mechanisms that underlie the formation of bacterial biofilms are involved in the formation of the capsular contracture.
Materials and Methods: Between July 2013 and July 2014, a series of 67 female patients in the area

of Naples (Italy) and its province, who presented for breast plastic surgery with the use of implants for aesthetic or reconstructive reasons, gave informed consent and were included in the study. All the patients received textured implants. Collected pre-disinfection and post-disinfection swabs and anatomical samples were immediately placed into sterile designated containers and sent to university laboratories of Microbiology Unit of Seconda Università degli Studi di Napoli. For each pathogenic strain identified the specific antibiogram was carried out and, then, the bacteria isolated were subjected to biofilm detection.

Results: *Staphylococcus epidermidis* was the leading bacteria isolated followed by *Staphylococcus aureus, Klebsiella pneumoniae, Candida albicans, Staphylococcus lentus* and *Acinetobacter Iwoffii.* Biofilm producing Gram-negative organism was represented by *Klebsiella pneumoniae* and biofilm producing Gram-positive organisms were found to be *Staphylococcus aureus, Staphylococcus epidermidis* and *Staphyloccocus lentus*. It was interesting that *Candida albicans* also produced biofilm. Strong biofilm production was shown by *Staphylococcus aureus.*

Discussion and Conclusions: The goal of the study was to establish which are the bacteria and the mechanisms of infection behind the process of contracture, considering both exogenous and endogenous ways of infection. All this to provide the surgeon a therapeutic and behavioral protocol to minimize the incidence of caspular contraction.

P 083

MONITORING OF ECHINOCOCCOSIS IN CAMPANIA REGION TO PREVENT HUMAN INFECTION

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Introduction: Echinococcosis is one of the most important parasitic zoonotic diseases in the world. Cystic hydatidosis (CE) caused by *Echinococcus granulosus* (EG) and *Echinococcus multilocularis* (EM) have been reported in several Mediterranean countries. These parasites depend on the dog-sheep cycle and is actively transmitted in all pastoral regions where sheep, cattle, buffaloes, pigs predominate. Since data concerning the real spread of Cystic Echinococcosis (CE) are inaccurate, in particular for human infection, the Veterinary Sector of Campania Region, in collaboration with the "Research Unit for the Monitoring of Intestinal parasitosis of Migrants of the Mediterranean area" (URPIM), the Regional Center for Monitoring Parasitic Infections (CREMOPAR) and the Experimental Zoo-prophylactic Institute of Southern Italy, has started a project to control the prevalence of CE in farm animals in the Campania region, in order to reduce animal disease and to prevent human infection.

Materials and Methods: Following monitoring programmes in farm animals, diagnosis of CE in humans at risk of infection (farm workers and their relatives) is performed using serological assays (ELISA and IHA) confirmed by molecular tests (Immunoblotting). The data are evaluated using Geographical Information Systems (GIS).

Results: The elaboration of the data with GIS allows us to select farms to be included in our study. We have analyzed 1096 serum samples at the URPIM lab. Among these 14 samples resulted positive at Elisa test, while only 3 samples at IHA. Positive samples after serological examination were analyzed by immunoblotting. The molecular test confirmed the positivity of samples that were selected by the IHA test.

Discussion and Conclusions: Preliminary data on the prevalence of CE in farm animals confirm the importance of the surveillance upon free ranging dogs and for an accurate early diagnosis of human hydatidosis. The patients resulted positive have been directed to the observation od a clinical department. The results of our study could raise awareness about the prevention of CE among those subjects at risk of infection, in addition to increase of information about this parasitosis.

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P 084

NON-DERMATOPHYTIC ONYCHOMYCOSIS DIAGNOSTIC CRITERIA: AN UNRESOLVED QUESTION

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Introduction: In recent Years, non-dermatophytic molds, (NDMs) have increasingly been recognized as causative agents of onycomycosis. As reported in scientific literature, these moulds are responsible for a percentage between 2 and 17% of total onycomycosis and the principal agents identified are represented by *Scopulariopsis, Scytalidium, Acremonium, Aspergillus* and *Fusarium spp*.
Materials and Methods: The diagnosis of onychomychosis is most often obtained by microscopic observation of nail specimens where fungal elements can be detected and cultured. However direct microscopic examination does not always result positive in NDM onychomycosis, therefore, to performe a correct diagnosis, a mycological culture is often required. The purpose of the present study was to evaluate the role of direct microscopic examination in the NDM onychomycosis diagnosis.
Results: The results show that 57.2% of the specimens from onycomycosis patients could be correctly diagnosed showing positivity to both direct microscopic examination and NDM culture isolation in two or more subsequent inoculations, while 42.8% of analyzed specimens showing a negative direct microscopic examination showed NDM growth.
Discussion and Conclusions: Our study emphasized the importance of culture in the diagnosis of all suspected onychomycosis cases. We point out the need for thoroughly evaluating all the specimens showing cultural growth in at least three subsequent inoculations, whatever the result of the microscopic examination, in order to reduce the false negative rates. This strategy would allow for more accurate diagnosis of this mycosis.

P 085

GENOME-WIDE IDENTIFICATION OF *IN VIVO* INDUCED GENES IN *ENTEROCOCCUS FAECIUM* BY RNA SEQUENCING

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Enterococcus faecium is a commensal of the human gastrointestinal tract and an important cause of health- care associated infections. Few is known about the pathogenicity process and adaptation to the host environment. To get a deeper insight into the infection process, we conducted a whole transcriptome analysis by RNA sequencing on bacteria isolated from an infection site using a mouse peritonitis model. We focus on *in vivo* induce genes in comparison to the transcriptome of cultures grown *in vitro*. For our study, we used the vancomycin-resistant strain Aus0004 belonging to clonal complex 17 which includes the majority of strains isolated in hospitals.
Balb/C mice were infected intra-peritoneal and bacteria were recovered 6 and 24 hours post-infection (p.i.). In parallel, bacteria were grown *in vitro* in brain heart infusion broth to the exponential mid-log phase and stationary growth phase. Total RNA has been extracted for library preparation and sequenced on Illumina HiSeq2000.
Using a threshold as differently expressed of ≥ 4-fold, approximately 300 and 700 genes were up-regulated at 6 and 24 hours of incubation in the peritoneum in comparison to lab conditions, respectively. Candidates of *in vivo* induced genes were classified into functional categories. The most abundant class of genes encodes hypothetical/unknown proteins, followed protein synthesis, transport, metabolism and biosynthesis.
The glycerol pathway is up-regulated suggesting that it is an important substrate for *E. faecium* during the infection. The genes coding protein involved in purine and pyrimidine metabolic pathways are strongly induced at 24h p.i. as well as genes implicated in DNA replication and cell division, this suggests that bacteria are actively replicating inside

the peritoneum.
A mechanism of defence against phagocytic cells is activated, as clearly shown by the expression of genes involved in oxidative stress response; the *dlt* operon, implicated in the response against cation antimicrobial peptides, is strongly induced.
Interestingly, despite the absence of vancomycin, all genes of the *vanB* operon are strongly expressed.
The presented database corresponds to the first global insight of how *E. faecium* adapt its gene expression during the infection. This knowledge could be used to define new therapeutic approaches against these multi-drug resistant pathogens.

P 086

ISOLATION AND CHARACTERIZATION OF *STAPHYLOCOCCUS AUREUS* STRAINS COLONIZING NASAL CAVITIES AND SKIN OF FOOD HANDLERS

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Introduction: *Staphylococcus aureus* is a commensal organism of human skin and nose microbiota. Thus, asymptomatic food handlers carrying S. aureus can contaminate food via manual contact or through respiratory secretions. The present study was aimed to investigate the presence of employees carrying *S. aureus* in a food industry and to evaluate associated risk factors through the genotypic and phenotypic characterization of all collected isolates.
Materials and Methods: Skin and nasal samples of food handlers were collected from February 2013 to June 2014 in a food industry. S. aureus strains were characterized by PCR to detect the genes encoding staphylococcal enterotoxins and the antimicrobial susceptibility was tested by agar disc diffusion method. Macrorestriction patterns were determined using pulsed-field gel electrophoresis and multilocus sequence typing was performed for representative isolates of the major pattern. Further analyses were carried out to investigate biofilm formation ability by Microtiter Plate Method.
Results: Of the 21 employees analysed, 7 (33%) were contaminated with *S. aureus*. Skin carriage was three times less frequent than nasal carriage and more than one strain was isolated for each worker. Although we found significant heterogeneity among enterotoxins profiles, all of 26 strains presented at least the gene *sem* whereas the gene sec was detected in 50% of isolates followed by tst gene (46%). Antibiotic resistance was detected for: penicillin, erythromycin, levofloxacin, fusidic acid and clindamycin. No methicillin-resistant *S. aureus* were observed. The genetic analysis revealed four PFGE types. The predominant type belonged to ST45. Biofilm formation ability of skin isolates was less than nose isolates.
Discussion and conclusions: The presence of multiple strains on skin and nasal mucosa among workers and the different profiles of *S. aureus* typed increase the risks of food contamination. These findings suggest the need of a better knowledge of potential role of food handlers as vehicles of S. aureus to reduce food poisoning cases.

P 087

GENOTYPIC AND PHENOTYPIC HETEROGENEITY IN *STREPTOCOCCUS MUTANS* ISOLATED FROM TYPE II DIABETIC PATIENTS

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Introduction: Dental caries and diabetes are long term health concerns that may have a relation. An immunocompromised state of diabetes inclines affected individuals to numerous infections, where indigenous bacteria such as *Streptococcus mutans* inhabiting the tooth may prove pathogenic. More-

over the frequent use of antibiotics may select for antimicrobial resistance. We focused on the antimicrobial susceptibility, genotypic and phenotypic heterogeneity, and serotype of the *S. mutans* isolated from type II diabetic patients.
Materials and Methods: Twenty-five *S. mutans* were isolated in Rome during 2013 from type II diabetic patients (age 42-68). Dental health was monitored in terms of DMTF and plaque index. Serotype was assessed by PCR and macrorestriction analysis of genomic DNA by PFGE. Biofilm biomass production was measured by the crystal violet microtiter assay. Acidogenesis and co-aggregation were also determined. EUCAST guidelines were adapted to test antimicrobial susceptibility against penicillin, rifampicin, oxacillin, clindamycin, cefoxitin, erythromycin, levofloxacin, linezolid, gentamicin, norfloxacin, tetracycline and vancomycin.
Results: Eighty-two percent of isolates were classified as serotype *c*. No serotype *k* was present. Macrorestriction analysis by PFGE exhibited a clonal diversity that paralleled the phenotypic heterogeneity. Biofilm forming ability was not associated with any particular genetic pattern or aggregation ability and acidogenicity. Isolates were susceptible to all classes of antibiotics.
Discussion and Conclusions: Penicillin resistance was not observed, counter trending the previously recorded 14% penicillin-resistant *S. mutans* isolates from dental patients in Rome. Genotypic variability is well known in *S. mutans* and records suggest 23% gene content divergence through whole genome sequence comparison, which validates the diversity found in our study.
In conclusion, a great genetic heterogeneity and no antimicrobial resistance were apparent in *S. mutans* isolated from diabetic patients. With an increasing average age of individuals and an ascending level of diabetes, it is very essential to focus on oral epidemiology of diabetics. Our study encourages and may act as a reference for future epidemiological studies.

P 088

DETECTION AND MOLECULAR CHARACTERIZATION OF EXTENDED-SPECTRUM-B-LACTAMASES-PRODUCING *ESCHERICHIA COLI* ISOLATED FROM DIVERSE POULTRY FARMS

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Introduction: Broiler production is highly industrialized, concentrated and with limited variation in breeds, nutrition and production methods. Due to public health concerns, a great effort has been made to reduce the use of antimicrobials in this sector. Due to public health concerns, a great effort has been made to reduce the use of antimicrobials. The presence of Extended-Spectrum β-Lactamases (ESBL)-producing *Enterobacteriaceae* in food-producing animals has been reported, however, knowledge about the intestinal carriage of ESBL-producing bacteria by healthy broiler chickens is still scarce.
Materials and Methods: In order to track the spread of antimicrobial resistance in the sector of poultry farming in Portugal, *Escherichia coli* strains were isolated from poultry feces collected from 23 poultry farms of the center and north regions of Portugal. Overall, 163 strains were isolated and identified and subsequently analyzed through the antimicrobial susceptibility testing against 19 antimicrobial agents. The isolates that presented an ESBL phenotype were further characterized for the genetic determinants conferring resistance to β-lactams, through PCR and sequencing.
Results: Among all *E. coli* isolates (n = 163), 34 presenting an ESBL phenotype, were multidrug-resistant and all showed resistance to ampicillin, cefotaxime and cephalotin. Moreover those 34 isolates presented one or more β-lactamase genes.

Isolates from different poultry farms had the same phenotype of resistance as well as the same ESBL genotype. The most frequent genotypes found were CTX-M-1, CTX-M-9 and TEM + CTX-M-1.
Discussion and Conclusions: There is a strong epidemiological interconnection between the broiler farms, despite they are located far away from each other. This fact could explain the similarity in the resistance profiles and the domination of the β-lactamase types CTX-M-1 and CTX M-9 in *E. coli* isolated from different poultry farms. Despite strict sanitation programs and biosecurity measures, all these logistical interdependences can act as pathways and sources of multidrug-resistant bacteria.

P 089

MALIGNANT EDEMA IN POSTPARTUM BUFFALOES

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Introduction: *Clostidium septicum* produces alpha toxin, the main toxin factor of this organism, which is necrotizing and hemolytic, causing increased capillary permeability and penetrates through lesions of the skin and mucosa, causing myonecrosis (malignant edema, gangrene), which may be rapidly fatal. This microorganism has been reported to be the cause of malignant edema in cattle (Wayt 1951, Odani and others 2009), sheep, pig (Hungerford 1990) and other species. We describe four cases of postparturient malignant edema due to *Cl. septicum* in buffaloes belonged to a herd of 1000 animals in southern Italy.
Materials and methods: Four buffaloes presented swelling of perineal and perivulvar areas, fever and agalactia a few hours after calving. Two died within 20 hours of calving. The other two developed edema in the skeletal muscles of one leg and were treated 24 h postpartum by means of lavage and curettage of the affected area with hydrogen peroxide mixed with betadine, and i.m. administration of 10% ketoprofen for 3 days and sulfadiazine-trimethoprim for 10 days. Aseptic samples of exudate and tissue samples were taken from the edematous area of the vulva and thigh and they underwent bacteriological and molecular examination. The isolates suggestive of the genus *Clostridium* were identified by means of biochemical techniques (Vitek 2) and colonies underwent DNA extraction. Two primers (F 5'CGGCTGGATCACCTCCTTTC-3'and R 5'-ATCACGTCCTTCATCGGCTC-3') were designed to amplify a 460-bp PCR (accession n° AB040716).
Results: This study identified the isolation of *Cl. septicum* through bacteriological investigations performed on the species *Bubalus bubalis*. This is the first case described in Italy highlighting the importance of the welfare health and hygiene conditions of the farms.
Discussion and Conclusions: In the prevention of malignant edema, it is very important to prevent the entry of germs through lesions; great care should therefore be taken in situations in which the integrity of the skin or mucosa may be breached. Moreover, it should be borne in mind that Clostridia are more abundant in alluvial areas where the land is poorly drained and marshy and that the incidence of the disease is higher during periods of heavy rain intense.

P 090

DRAFT WHOLE GENOME SEQUENCING OF A VIM-1- AND SHV-12-PRODUCING *AEROMONAS CAVIAE* CAUSING A NEWBORN BLOODSTREAM INFECTION

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Introduction: Aeromonads are ubiquitous species that rarely cause bloodstream infections which can be associated to high mortality rates. *Aeromonas caviae* does not have a resident metallo-β-lactamase (MBL) gene of the *cphA* lineage, but recently

some *A. caviae* strains with acquired MBLs (VIM-1 or VIM-35) have been reported from rectal surveillance cultures of inpatients in two Israeli hospitals.
Materials and Methods: *A. caviae* AOUC-AA14 was isolated in October 2014 from the blood culture of a one day-old newborn inpatient at Meyer Children's University Hospital of Florence.
The isolate was identified by MALDI-TOF (Vitek MS) as *A. caviae/hydrophila*. Draft whole genome sequencing (WGS) of AOUC-AA14 was performed using an Illumina MiSeq system and a paired-end approach (2 x 250 bp). *De novo* assembly was performed using the ABySS software.
Results: MICs of AOUC-AA14 revealed an MDR phenotype including resistance to penicillins and extended spectrum cephalosporins and altered susceptibility to carbapenems. Multi-Locus Sequence Analysis of data from WGS allowed to identify AOUC-AA14 as A. caviae. WGS data also revealed the presence of two acquired beta-lactamase genes, namely bla_{VIM-1} and bla_{SHV-12} in addition to the resident class D β-lactamase gene, and the resident ampC-type gene of the CMY-1/MOX lineage. S1 nuclease analysis and hybridization with bla_{VIM-1} specific probe revealed that the MBL gene was carried on a ~150 kb plasmid. The plasmid co-harbored the bla_{SHV-12} gene, could be transferred by conjugation to *Escherichia coli* J53 Azi^R and belonged to the IncA/C type. The genetic context of the bla_{VIM-1} gene showed that it was associated with a novel integron platform, named In1164.
Conclusions and Discussion: This work confirm the circulation of *A. caviae* strains with acquired VIM-type MBLs in the mediterranean area, which can also cause invasive infections. In this case, bla_{VIM-1} and bla_{SHV-12} genes were carried on a transferable plasmid that could easily be transferred by conjugation, underscoring the potential role that similar strains could acquire in the dissemination of relevant resistance determinants in clinical and environmental settings.

P 091

DIFFERENTIAL INFLAMMATORY RESPONSE INDUCED BY THE TWO CLADES OF THE PANDEMIC ST258 *KLEBSIELLA PNEUMONIAE*

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Introduction: Strains of *Klebsiella pneumoniae* (KP) of sequence type 258 (ST258) are mainly responsible for the global spread of KPC-type carbapenemases. Apart from the multiresistant phenotype, the basis for the success of this clone are largely not determined. Two distinct clades, mainly differing in capsular composition, were reported in ST258 KP clone. Clade 2 is the most prevalent worldwide. To investigate whether peculiar host-pathogen interaction might explain the success of ST258 clone, we studied the ability of ST258 clades to activate cells of innate and adaptive immunity in comparison with CIP 52.145, a highly virulent KP strain.
Materials and Methods: KK207-1 and KKBO-1, as representatives of ST258 KP clade 1 and clade 2 respectively, and CIP 52.145 KP strains were used live or 95°C heat-inactivated to stimulate peripheral blood mononuclear cells (PBMC) or myeloid dendritic cells (MDC). $TLR4^+$HEK293 cells were used for TLR4 assay. Cytokine measurement and T-lymphocyte effector response were assessed by immunoplex array and cytofluorimetry respectively.
Results:
- The ST258 clade 1 and 2 activated TLR4 more than CIP 52.145 and equally induced the differentiation of immature MDC in antigen-presenting cells;
- clade 2-KKBO-1 induced a higher production of IL1β and IL23 by MDC compared either to clade 1-KK207-1 or to the virulent CIP 52.145 strain.
A time-dependent TH17/TH1 response was induced in PBMC by all KP strains, but the ST258 clade 2 was the most potent inducer of TH17 differentiation
Apart from the known differences in the cps gene clusters, the comparative genomic analysis of the two ST258 clades revealed the presence in both clades of the *entB*/*entE* genes encoding entero-

bactins, which can compete with the IL17-induced iron-chelator lipocalin. The *mrkABCDFHIJ* operon, involved in epithelial colonization and coding for proteins with high antigenic property (type 3 fimbriae), was functionally inactivated in clade 1 but not in clade 2. Further investigations are needed to establish whether this genomic region is involved in the immunogenic potential of clade 2.

Discussion and conclusions: Based on these results we hypothesize that ST258 strains, and clade 2 in particular might have evolved mechanisms to resist in the strong TH17-induced inflammatory environment, and they could advantage of the TH17-induced anti-microbial response to compete with endogenous flora microbiota at the gut level.

P 092

RAPID DETECTION OF FLUCONAZOLE AND ANIDULAFUNGIN RESISTANCE IN *CANDIDA GLABRATA* ISOLATES USING A MALDI-TOF MS-BASED ASSAY

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Introduction: *Candida glabrata* is an important cause of bloodstream infection. This infection is very difficult to treat due to its tendency to easily develop azole (e.g., fluconazole [FLC]) resistance. Although echinocandins (e.g., anidulafungin [AND]) are recommended as a first-line therapy, resistance also emerged against this drug. So, early detection of azole- or echinocandin- resistant isolates of *C. glabrata* has become an essential prerequisite for establishing appropriate antifungal therapy. For this reason we sought to use of MALDI-TOF analysis for mass spectrometry (ms)-based antifungal susceptibility testing (AFST) to the *C. glabrata* and rapid detection of FLC- or AND- resistant isolates.

Materials and Methods: We studied 98 *C. glabrata* isolates, 69 molecularly well-characterized strains and 19 freshly collected routine clinical isolates. For all the isolates, conventional AFST assay was performed by the broth microdilution method in accordance with the Clinical and Laboratory Standards Institute (CLSI) document M27-A3. For the ms-AFST assay, yeasts were grown in RPMI medium with intermediate (16 mg/L for FLC and 0.06 mg/L for AND), maximum (256 mg/L for FLC and 32 mg/L for AND) drug concentrations or with RPMI alone, for 3 h at 37°C. After protein extraction, MALDI-TOF analysis was performed .Spectra at the null (0 mg/L), intermediate, or maximum concentration of drug were used to create individual composite correlation index (CCI) matrices for each isolate; then, the isolate was classified as susceptible or resistant to FLC (or AND) if the CCI value obtained by matching the spectra at intermediate and maximum concentrations was respectively higher or lower than the CCI value obtained by matching the spectra at intermediate and null concentrations.

Results: Results obtained with ms-AFST and conventional AFST assays were compared to reference molecular-based analyses. Among 66 isolates tested against FLC, 36 of 38 isolates were correctly classified as FLC-resistant and 28 of 28 isolates as FLC-susceptible by ms-AFST. Among 32 isolates tested against AND, 24 of 25 isolates were correctly identified as AND-susceptible and 6 of 7 isolates as AND-resistant by ms-AFST. With regards to the conventional AFST, FLC results were fully concordant with the molecular analyses, whereas one wild-type isolate was classified as AND-resistant (also in accordance with ms-AFST) and one fks1 mutant was categorized as AND-intermediate (this isolate was classified as susceptible with ms-AFST).

Discussion and Conclusions: Our MALDI-TOF MS-based AFST method seems to be very promising for the rapid and accurate detection of antifungal resistance in relevant pathogenic yeasts.

P 093

THE IMPORTANCE OF *POSTMORTEM* MICROBIOLOGICAL EXAMINATION FOR THE EX POST DIAGNOSIS OF INFECTION: FATAL SEPSIS BY *KLEBSIELLA PNEUMONIAE* IN A PATIENT WITH SYSTEMIC LUPUS ERYTHEMATOSUS

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Introduction: *Postmortem* microbiology can be regarded as a useful tool contributing to the forensic and medico-legal elucidations of death due to infection. Herein, we describe a case of fatal sepsis in a patient with systemic lupus erythematosus (SLE) where *postmortem* culture allowed to define the etiology of sepsis, also revealing the inadequateness of antimicrobial therapy.
Materials and Methods: A 29-year old male presented with persistent fever and 7-day history of cough and sore throat, treated with ceftriaxone. He previously had an incision of a gluteal abscess, without microbiological evaluation. Due to the initial suspicion of infective endocarditis, an antimicrobial therapy was started (days 5-14: amikacin, teicoplanin, and levofloxacin). Because the patient remained febrile, therapy was modified but rapidly suspended because of a high suspect for SLE (days 15-17; piperacillin/tazobactam, metronidazole, and amikacin). On day 22, an acute heart failure required aortic valve replacement and venoarterial extracorporeal membrane oxygenation. On day 23, increased levels of PCT, CRP, and LDH led clinicians to start a new antibiotic therapy (linezolid, piperacillin/tazobactam, and fluconazole) until day 33 when patient passed away because of multi-organ failure. At autopsy, cardiac tissue and valves, liver tissue, cerebrospinal fluid, and blood samples were collected for microbiologic analyses.
Results: *Antemortem* culture analysis of pericardic liquid, urine, CVC, and blood samples – the last ones collected on days 1, 14, 15, and 24 - was negative for both bacteria and fungi. Molecular and serological detection for several viruses and protozoa was negative as well. *Klebsiella pneumoniae* was instead cultured from all autoptic samples. PFGE revealed that all strains belonged to the same clone. Susceptibility tests revealed the same MDR phenotype for all isolates, susceptible to carbapenems, colistin and fosfomycin only. All *K. pneumoniae* isolates were positive for ESBL, but negative for carbapenemase.
Discussion and Conclusions: This case clearly showed that *postmortem* microbiology represents a useful tool in forensic autopsy, and to evaluate the adequateness and effectiveness of therapy. Particularly, our *postmortem* findings justify the use of carbapenems as first choice in patients admitted to centers with high incidence of ESBL-producing species.

P 094

A COMPARISON OF FAECAL EGG COUNT TECHNIQUES FOR THE DETECTION OF TAENIIDAE EGGS IN DOGS

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Introduction: Taeniidae family includes many zoonotic tapeworms as *Echinococcus granulosus,* whose life cycle includes canids as definitive hosts and a wide range of domestic and wild mammals and humans as intermediate hosts. The aim of this study was to evaluate the best copromicroscopic technique for the detection of Taeniidae eggs in dogs in endemic areas as southern Italy for the subsequent recognition of species using molecular techniques.
Materials and Methods: Four pooled samples with 4 different level of Taeniidae eggs per gram (epg) of faeces were used: 4, 15, 150 and 350 epg. For each pooled sample 9 replicates of the following 5 different techniques were permorfed: (i) FLOTAC basic technique with zinc sulfate (specific gravity,s.g. =

1,350); (ii) flotation in centrifuge with zinc sulfate (s.g.= 1,200); (iii) flotation in centrifuge with Breza solution (s.g. = 1,300); (iv) flotation in centrifuge with Breza solution (s.g.= 1,400); (v) flotation in centrifuge with zinc chloride (s.g.=1,450).
Results: The FLOTAC technique (method i) produced positive results for all the replicates of all the 4 levels of epg and the mean epg was significantly higher ($p < 0.05$) than that obtained by all the other 4 techniques, namely methods (ii), (iii), (iv) and (v). Moreover the simple flotation with zinc chloride solution (method v) gave negative results for all the replicates at level of 4 epg.
Discussion and Conclusions: The findings of the present study showed new insights into copromicroscopic diagnosis of Taeniidae. The best method for the diagnosis of Taeniidae in dogs resulted the FLOTAC basic technique using zinc sulphate, s.g. = 1,350.

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P 095

DIAGNOSIS OF *ECHINOCOCCUS GRANULOSUS* IN DOGS: NEW INSIGHTS

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Introduction: *Echinococcus granulosus* is a tapeworm of the Taeniidae family and its life cycle includes canids as definitive hosts and a wide range of domestic and wild mammals and humans as intermediate hosts. Disability Adjusted Life Years (DALYs) resulting from human cystic echinococcosis have been calculated as high as 1 million, similar to Dengue, Chagas Disease and Trypanosomiasis. The aim of this study was to develop and standardize innovative diagnostic tools to control *E. granulosus* in endemic areas as southern Italy.
Materials and Methods: Fifty faecal samples collected from farm dogs infected by Taeniidae were used for the study. To examine each sample, four different protocols of DNA extraction were compared and standardized for the diagnosis of *E. granulosus*: (i) QIAamp Tissue Kit (Qiagen) from eggs, (ii) QIAamp Stool (Qiagen) from eggs, (iii) QIAamp Stool (Qiagen) from faeces, (iv) Wizard Magnetic Purification System for Food (Promega) from faeces. DNA extraction was followed by PCR amplification and sequencing of mitochondrial cytochrome C oxidase subunit 1. The FLOTAC device was used to isolate the Taeniidae eggs for the methods (i) and (ii).
Results: The most efficient kit for DNA extraction resulted the QIAamp Stool from eggs (method ii) providing the highest number of positive samples for Taeniidae, i.e. 47/50 (94.0%; 95% Confidence Interval [CI] = 82.5-98.4%). The samples resulted negative had very low faecal egg counts (2 eggs per gram of faeces). The less efficient method was the Wizard Magnetic Purification System for Food (method iv). This method produced only 7/50 samples (14%; 95% CI = 6.3-27.4) positive for Taeniidae. After sequencing, 9/50 samples (18.0%; 95% CI = 9.1-31.9) were identified as *E.granulosus.*
Discussion and conclusions: The best method for the diagnosis of Taeniidae and *E. granulosus* in dogs resulted the QIAamp Stool (Qiagen) using the eggs floated in the FLOTAC device.
This technique could be very useful for the control of *E. granulosus* in animals and humans by an efficient diagnosis in dogs in endemic areas as the Campania region of southern Italy.

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P 096

KENNEL DOGS AND HELMINTHS INFECTIONS IN THE CAMPANIA REGION

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Introduction: Intestinal parasites are common in dogs worldwide, and their importance has recently increased for a renewed awareness on the public health relevance of some of them. Dogs can harbour several helminths, i.e. ascarids, ancylostomatids and tapeworms (e.g. *Dipylidium caninum, Echinococcus* spp. and *Taenia* spp.), most of which able to infect also humans. Kennel dog can serve as sentinels and/or reservoirs of diseases of zoonotic interest because they have often roamed free and lived outdoors, thus being exposed to pathogens. Moreover there is the possibility of infection both for technicians that work with these dogs and families that adopt them.

The aim of the present cross-sectional copromicroscopic survey was to evaluate the presence and distribution of helminth infections in kennels dogs in the Campania region (southern Italy) and assessing sanitary risks for humans.

Materials and Methods: The survey was conducted in 68 kennels distributed on the whole territory of the Campania region. In each kennel 20 boxes were examined; if the boxes were less, all the boxes were sampled. Fresh faecal samples were collected from the ground of each box (composite sample) and preserved in formalin 5%. Each faecal sample was then examined using the *FLOTAC dual technique*, using two flotation solutions on the same faecal composite, namely a sodium chloride based solution (FS2; density = 1.200), and a zinc sulphate based solution (FS3; density = 1.200).

Results: Helminth infections were found in 100% of the 68 studied kennels as follows:

- *Trichuris vulpis* (63/68; 92.6%);
- Ancylostomatidae (46/68; 67.6%);
- *Toxocara canis* (52/68; 76.5%);
- *Toxascaris leonina* (13/68; 19.1%);
- *Crenosoma vulpis* (9/68; 13.2%);
- *Angiostrongylus vasorum* (9/68; 13.2%);
- *Oslerus osleri* (2/68; 2.9%);
- *Dipylidium caninum* (34/68; 50.0%).

Discussion and Conclusions: The findings of the present survey show a high prevalence of helminths (including many zoonotic agents) in kennel dogs from southern Italy despite the regular use of anthelmintic treatments. This situation has important consequences on different issues concerning animal health and welfare, treatment and control, and public health. Among the zoonotic parasites, the most frequent species found were *T. vulpis, T. canis*, Ancylostomatidae and *D. caninum*. In particular, *T. canis* and Ancylostomatidae have an high zoonotic potential and cause *visceral larva migrans* and *cutaneous larva migrans*, respectively. Due to the impact of these parasites on public health, there is the need to improve preventive measures currently in place for kennel dogs, regular parasitological surveillance, appropriate treatment strategies and high quality standard of hygiene.

P 097

LACTOBACILLUS RHAMNOSUS INFLUENCES THE SHOALING IN ZEBRAFISH (*DANIO RERIO*)

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Introduction: Zebrafish is increasingly utilized in biomedical research. Shoaling, a group forming behavior, is best defined as aggregation behavior that leads to conspecifics being distributed closer to each other. Shoaling has been observed both in nature and in the laboratory. Here we evaluate the effect of *Lactobacillus rhamnosus* on zebrafish shoaling behavior.

Materials and Methods: Two experimental groups were evaluated: a control group (CTRL) fed with a commercial diet, and a probiotic-treated group (PROBIO) fed with a commercial diet and *L. rhamnosus*, 10^6 colony-forming units/g for 28 days. Twelve zebrafish were evaluated for each tank. Fish swimming behavior was recorded on the first day of probiotic treatment (T0) and successively at 7 days intervals (T1-T4). Recording was performed for 6 min, acquired and analysed with 2D video tracking tool (Tracker) and ImageJ 1.49 software to determine: Average Distance (AD); Distance Variance (DV); Nearest Distance (ND); Occupied Area (or Shoal size area) (OA) and Water column position (CP).

Results: Zebrafish fed with probiotics shoaled differently by T2 until T4. Two way ANOVA was used as statistical analysis. In particular the AD is not statistically significant. DV changes from the second week of treatment with a significant difference. The PROBIO was more uniform and showed a bigger and constant homogeneity of the distribution of fish within the shoal throughout the whole period of treatment. The ND displayed significant dependence from both treatment ($p < 0.05$) and time ($p < 0.01$). The AO was significantly affected by both treatment ($p < 0.01$) and time ($p < 0.01$). About CP, CTRL group spent most of the time in the upper side of the tank while PROBIO preferred the medium/deeper part of the tank occupying most of area of tank.
Discussion and Conclusions: The lower value of DV in PROBIO, meaning more homogeneity of the distribution of fish within the shoal. Regarding ND, the fishes in CTRL swam closer than PROBIO. It may be associated to more anxiety/fear that causes the shoal to "tighten" (the fish swim closer together). The larger OA of PROBIO suggests an increased exploration area with a preference in the middle/deeper part of the water column of the tank. These differences might be explained with an increasing of attention or possibly alert in the PROBIO group. In this study a probiotic strain modulated the zebrafish shoaling behavior and it provides a basis for further studies on the gut-brain axis in *Danio rerio*.

P 098

PHENOTYPIC AND MOLECULAR CHARACTERIZATION OF *ACINETOBACTER BAUMANNII* STRAINS

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Introduction: The human opportunistic pathogen *Acinetobacter baumannii* has emerged in recent decades as a leading cause of nosocomial infections associated with elevated morbidity and mortality, especially in intensive care units (ICUs). This bacterium has the ability to form biofilms and to adhere to, colonize and invade human epithelial cells. Moreover, the high plasticity of the *A. baumannii* genome allows the development of numerous antibiotic resistance mechanisms, giving rise to multidrug resistance (MDR) *A. baumannii* strains. Remarkably, the vast majority of *A. baumannii* epidemics are caused by a limited number of strains worldwide, regarded to as International clonal lineages I, II and III. Therefore, the present study was undertaken to investigate the epidemiology of MDR *A. baumannii* strains isolated in an ICU during a three-year period (2010-2012).
Materials and Methods: Strains were isolated from respiratory specimen of ICU patients. Identification and antibiotic resistance profiles were achieved with the Vitek2 system (bioMerieux). Strain clustering and biofilm-forming ability were performed using Pulsed Field Gel Electrophoresis (PFGE) and crystal violet staining, respectively.
Results: In the period examined, a total of 103 *A. baumannii* strains were isolated and identified. PFGE analysis patterns clustered three different clones (referred to as A, B and D), associated with a temporal distribution during epidemics. In particular, clone A was found to be responsible for the epidemic in 2010, clone D in 2011, and clone B in 2012. All clones were able to form biofilm on abiotic surfaces. Antibiotic resistance profiles revealed that all isolates were MDR. Remarkably, a significant rise in gentamicin and trimethoprim-sulfamethoxazole resistance was observed and one of the isolates was found to be colistin-resistant.
Discussion and Conclusions: In line with worldwide reports, all isolates showed an increase in antibiotic resistances. The high biofilm-forming capacity on abiotic surfaces seen in all isolated strains might have favored the spread and persistence in the ICU environment of *A. baumannii* strains. Genotyping data of bacterial isolates showed the prevalence of three different clonal populations, responsible of ICU epidemic. Finally, a multilocus sequence typing (MLST) according to the Pasteur's scheme for *A. baumannii* will be performed. MLST analysis will corroborate PFGE clonality studies and will allow international comparison of the temporal evolution and spatial distribution of Italian clones.

P 099

AN UNUSUAL CASE OF *ASPERGILLUS* INFECTION COMPLICATING LUNG CANCER

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Introduction: *Aspergillus* is an ubiquitous saprophytic mould that can cause severe syndromes expecially in immunocompromised individuals . Aspergilloma is a non-invasive form of pulmonary Aspergillosis usually presenting as a clump of mold in pre-existing cavitatory lung disease. Cases of pulmonary aspergilloma without any pre-existing underlying cavity, are rarely reported. We present here a case of pulmonary aspergillosis after chemotherapy and radiotherapy treatment for lung squamous cell carcinoma in which cavity formation occurred after the establishment of *Aspergillus* infection.
Materials and Methods: A 72 year old male with a history of smoking, affected by type 2 diabetes mellitus, coronaropathy and vasculopathy was admitted to the Division of Medical Oncology of the "S. Vincenzo" hospital in Taormina. The total body CT showed the presence of a primary bronchogenic tumor and the subsequent biopsy was compatible with a poorly differentiated squamous cell carcinoma (clinical stage IIIB), because of likely infiltration of the pulmonary artery. The lesion was treated with radiotherapy after a chemotherapy cycle. In December 2014 a new CT showed a posterior pulmonary cavity closely resembling aspergilloma and a sputum sample was sent to the laboratory of Micology of the University Polyclinic of Messina.
Results: *Aspergillus* flavus was isolated from sputum sample on both Sabouraud Dextrose Agar (SDA) and Potate Dextrose Agar (PDA). The sputum was negative for acid-fast bacilli and mycobacteria and Quantiferon yelded negative results. Our isolate was sensitive to the triazoles and treatment with fluconazole was replaced by voriconazole and itraconazole.
Discussion and Conclusion: Aspergilloma occurres principally in the upper lobes of the lungs of patients with previously formed cavities formed by preexistent illness. Although pulmonary aspergilloma without any pre-existing underlying lesions of the lung has been reported, our case is unusual because of the extension of the cavitary lesion. Indeed, cavitation extended from the upper to the mid-lower lobe of the right lung and occurred after the establishment of infection. Moreover, the sputum culture has been important to confirm diagnosis and guide treatment since the patient was severely immune compromised and it was not possible to perform lung biopsy. Microbiological identification was essential for a targeted therapy, allowing a significant improvement of clinical conditions.

P 100

DISTRIBUTION AND SUSCEPTIBILITY PROFILE OF *CANDIDA* SPECIES IN INTENSIVE CARE UNIT OF POLYCLINIC "G. MARTINO". A RETROSPECTIVE ANALYSIS 2011-2014

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Introduction: *Candida* species and are most important causes of worldwide hospital fungal infections. The aim of our study was to determine the distribution of candidiasis and antifungal susceptibility of *Candida* isolate in Intensive Care Unit of the Messina Polyclinic "G. Martino", Italy. This study was carried out as a retrospective analysis since 2011 to 2014.
Material and Methods: In our study, we evaluated the patients admitted at the Intensive Care Unit and a review of positives specimens between 2001-2014 was performed. After the identification of yeasts, a germ-tube test for presumptive identify of *Candida albicans* was performed while the organisms that were not positives for germ-tube test were identified using rapid biochemistry tests. Antifungal susceptibly testing was performed using broth microdiluition methods.
Results: A total of 142 *Candida* isolates were obtained: *C. albicans* was the predominant species (53%), while among 77 non-albicans species (47%)

C. parapsilosis (54%) was the most frequent followed by *C. tropicalis* n = 8 (12%), *C. guillermondii* n = 8 (12%), *C. glabrata* n = 5 (8%), *C. krusei* n = 4 (6%), *C. lusitaniae* n=2 (3%), *C. famata* n = 2 (3%), *C. zeylanoides* n = 1 (1%), *C. lambica* n = 1 (1%). Among 142 clinical isolates, n = 9 (7%) were resistant to the most commonly used azoles.
Conclusion: Our retrospective study shows that although *C. albicans* remains the most frequent etiological agent of infection, there is an increasing of incidence of infections caused by non-albicans species. *C. parapsilosis* is the second most common isolated (54% of non-albicans species). Our data confirm that there is a increasing trend in the isolation of non-albicans species. *Candida* spp. showed increased resistance to different azoles.

P 101

CLOSTRIDIUM DIFFICILE: COMPARISON BETWEEN TWO ALGORITHMS IN LABORATORY DIAGNOSIS

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Introduction: *Clostridium difficile* infection (CDI) is related to older age, recent hospitalization, longer hospitalization, use of proton pump inhibitors, chemotherapy, chronic kidney disease, and prolunged antibiotic therapy, in many industrialised countries. Increasing of severity infection was noted in 1999. This emergence occurred in North American and European to extend to Western Australia, South Korea, Hong Kong, and Costa Rica during 1999-2010 period Particularly, outbreaks related to iper-virulent strain *C. difficile*, PCR ribotype 027/North American pulse-field type that caused severe colitis with an highest grade of mortality than other ribotypes. Different alghorithms have been proposed to improve the laboratory diagnosis.
Material and Methods: We compared two algorithms during two years period. In 2013, 3112 fecal were analyzed by detection of toxin A/B and nucleic acid amplification test (NAAT) to detect *C. difficile*. In 2014, we received 3536 fecal samples. In this year, the diagnosis was carried out using GDH-Test as screening, toxin A/B immunoassay for positive samples and NAAT to solve any doubts.
Results and Discussion: Results on positive patients don't shown a significant difference: 212/1964 (10.8%) vs 264/1964 (13.4%) p value = 0.14.

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COMPARISON OF TWO IMMUNOLOGICAL METHODS IN ALLERGIC ANISAKIASIS (*ANISAKIS PEGREFFII*) DIAGNOSIS

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Aim: This study aimed to compare two immunological methods (namely, immunoCAP = iCAP and Western Blot = WB) to detect **Anisakis** specific IgE recognizing the two major antigens/allergens of the parasite, i.e. *Ani s1* and *Ani s7*, in order to define their significancein diagnosing the possible sensitization to the parasite antigens/allergens.
Patients and Methods: Human sera (N = 105) from patients having a history of raw fish consumption and/or allergic manifestation, or resulted positive to the larval detection of *Anisakis pegreffii* at gastric-intestinal level, were analysed by iCAP and WB. The sensitivity and specificity of the two methods were calculated. Specific antigens/allergens to be used in WB analysis were obtained from excretory/secretory antigens of *A. pegreffii* larvae cultured *"in vitro"*.
Results: 68 of the 105 sera tested by iCAP resulted positive (p4 > 0.35KUA/L) for *Anisakis*-IgE. However, among them, only 22 confirmed their positivity also at the WB, to both the antigens *Ani s1* and *Ani s7*. Conversely, 37 sera resulted negative by using both iCAP and WB. The remaining 8 sera resulted IgE positive only to *Ani s 7*. The two methods had a correspondence of 56%.
Discussion: Overall, the average specificity was around the 44,6% for the iCAP; whilst it resulted of the 100% in the WB. The recorded sensitivity was

optimal for both iCAP and WB.
Two main reasons might explain the incongruence found between the two methods: i) cross-reactive antibodies directed against non-parasite related epitopes; in this case, the iCAP usually shows a pronounced reactivity against panallergenic protein; ii) an underlying atopic state of the patients who might have, among polyclonal IgE, some directed against *Anisakis*. In these cases usually a diffuse reactivity against several bands in the WB is present, without reaction against specific Ani s 1 or Ani s 7, which indicates the lack of sensitization.

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P 103

LIPID COMPOSITION OF LUNG TISSUE AND ALVEOLAR SURFACTANT ARE ALTERED IN EXPERIMENTAL MALARIA ASSOCIATED - ACUTE RESPIRATORY DISTRESS SYNDROME

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Introduction: Malaria-associated acute lung injury (MA-ALI) and its more severe form malaria-associated acute respiratory distress syndrome (MA-ARDS) are common, often fatal complications of severe malaria infections. However, little is known about their pathogenesis.
Materials and Methods: C57BL/6J mice were infected with *Plasmodium berghei* NK65 to induce lethal MA-ARDS, or with *Plasmodium chabaudi* AS, a parasite strain that does not induce lung pathology. Biochemical alterations of the lipid composition of the lungs were investigated as a possible mechanism contributing to the severity of murine MA-ALI/ARDS.
Results: The lipid profile of the lung tissue from mice infected with *Plasmodium berghei* NK65 developing MA-ALI/ARDS, but not those from mice without lung pathology or controls, is characterized by high levels of phospholipids -mainly phosphatidylcholine- and esterified cholesterol. The high levels of polyunsaturated fatty acids and the linoleic/oleic fatty acid ratio of the latter reflect the fatty acid composition of plasma cholesterol esters. In spite of the increased total polyunsaturated fatty acid pool, which increases the relative oxidability of the lung membranes, and the presence of hemozoin, a known pro-oxidant, no excess oxidative stress was detected in the lungs of *Plasmodium berghei* NK65 infected mice. The bronchoalveolar lavage (BAL) fluid of *Plasmodium berghei* NK65 infected mice was characterized by high levels of plasma proteins whereas the phospholipid profile of BAL large and small aggregate fractions was changed as well, with a significant increase in the amounts of sphingomyelin and lysophosphatidylcholine- the hydrolysis product of dipalmitoylphosphatidylcholine - and the decrease in phosphatidylglycerol. Both the increase of proteins and lysophosphatidylcholine are known to decrease the intrinsic surface activity of surfactant.
Conclusions: Together, these data indicate that an altered lipid composition of lung tissue and BAL fluid, partially ascribed to oedema and lipoprotein infiltration, is a feature characteristic of murine MA-ALI/ARDS and may contribute to lung dysfunction.

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TOXINOGENIC *CLOSTRIDIUM DIFFICILE* AND *CANDIDA*: COLONIZATION OR MICROBIAL SYNERGISM?

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Introduction: Toxinogenic *Clostridium difficile* infection (CDI) is recognized as one of the major cause of infectious diarrhea developing in high-risk patients such as subjects treated with long lasting antibacterial and antacid therapy, older age and immune-compromisation. Our recent observation

evidenced a link between CDI and *Candida* colonization of the gut,and others correlated CDI to candidemia. Aim of the present study was to investigate whether the biofilm production by *Candida* strains colonizing the gut of CDI patients could be linked, and considered a pathogenic co-factor, into the development of the disease.
Materials and Methods: Consecutive diarrheic stool specimens (n° = 267) were sampled at the teaching hospital Policlinico "Umberto I" in Rome between September 2014 and May 2015 and tested for the presence of both toxin A/B of *C. difficile* and *Candida* (> 105 CFU/ml). All the isolated yeasts were tested for their capacity of biofilm production through a semi-quantitative measurement by XTT reduction assay. *Candida* biofilm production was correlated to CDI. Pearson $\chi 2$ test was used for statistical analysis.
Results: Toxins A/B of *C. difficile* were detected in the stools of 157 patients, while 111 were from CDI-negative subjects. *Candida* colonization resulted statistically more frequent in the CDI positive patients ($\chi 2 = 20.9$, $p = 0.002$); and *Candida albicans* was the species more frequently associated to CDI (64% , $p = 0.001$). Of the CDI positive patients, 37,5% were infected by the hypertoxic strain BI/NAP/027, and once again *C. albicans* was the species more frequently associated (60%; $p = 0.03$). Data on biofilm production indicated that *C. albicans* strains from CDI positive patients were mostly (90.63%) high producers. However, an overall correlation between the biofilm production by the different species of *Candida* and CDI could not be observed, since biofilm produced yeast species isolated from CDI positive and negative patients are equally distributed in the two groups ($p = 0.6$).
Discussion and Conclusions: The results of our study confirmed the association between *Candida* colonization and CDI and evidenced that the biofilm production could be strongly related to the development of CDI depending on the colonizing species, being *C. albicans* the species with the higher attitude in the biofilm production in CDI patients.

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MOLECULAR DETERMINANTS OF INACTIVATION OF THE RESUSCITATION PROMOTING FACTOR B FROM *MYCOBACTERIUM TUBERCULOSIS*

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Introduction: Inactivation of revival of *Mycobacterium tuberculosis* (Mtb) from dormancy is one of the main goals of the WHO Global Plan to stop Tuberculosis 2011-2015, given the huge reservoir of latently infected individuals. This process requires a pool of Ser/Thr kinases [1] and Resuscitation promoting factors (Rpfs) [2-3]. Of these, RpfB is the sole member indispensable for resuscitation *in vivo*.
Materials and Methods: We cloned, expressed and purified several variants of RpfB for both structural and functional characterization.
Results: The structural work provided key information, which is being used for the development of inhibitors [3].
Discussion and Conclusions: By combining computational and experimental approaches, we successfully identified the key interactions of RpfB with its substrates, aimed at the development of molecules therapeutic interest for Tuberculosis eradication.

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POSTERS

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HIGH CONCENTRATION OF CYTOKINES PROMOTING ANGIOGENESIS AND CELL PROLIFERATION SIGNIFICANTLY ASSOCIATED WITH HR-HPV INFECTION IN YOUNG WOMEN

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Introduction: The *in vivo* interplay between Human Papillomavirus (HPV) and the cervical local immune response, almost from the start of the infectious cycle and before malignant alteration of the infected cell, seems to be the hallmark of HPV-induced disease. The causal interpretation of local cytokines expression is complex and poorly defined, basically due to the delicate balance existing between pro-inflammatory and anti-inflammatory cytokines network influenced by HPV and host risk factors. The aims of this study are to explore the local cytokines network in the early phase of HR-HPV infection and their role in HR-HPV–induced cytological damage.
Materials and Methods: The cytokines classified as Th1/Th2, T-reg, Th17 cells, Th9 and growth factors were explored in a selected cohort of 100 young immunocompetent women HR-HPV infected but negative for additional cervical cancer risk cofactors, including demographic, behavior, and sexually transmitted diseases (STD) infections and multiple HPV infections. Eighty women, negative for HPV and intraepithelial lesions (NIL) were enrolled as control group.
HPV genotyping was performed by Linear Array HPV genotyping test, while the soluble concentration of 48 cytokines was analyzed by pro-human cytokine Bio-Plex assay. The software Stata (v. 13.1) and Graph Pad Prism (v. 5) were used for statistical data analysis.
Results: The prevalence of HR-HPV was of 68.8%: 30% in NIL women, 100% in LSIL and 100% in HSIL. A panel of 10 cytokines, the interleukins IL-6, IL-3, IL-12p40, IL-16, IL-18, IL-2Ra and the growth factors SDF-1α,VEGF, LIF and IFN-α_2 significantly associated to HR-HPV ($p < 0.001$), showed an increased level of concentration in women with precancerous lesions independently of the grade of severity, when compared to controls. The growth factor GM-CSF was the unique protein positively associated with cytology.
Discussion and Conclusion: In the current *in vivo* study the intrinsic ability of HR-HPV to modulate the immune response leading both to the inhibition of a pro-inflammatory response and the enhancement of growth factors triggering the oncogenic process, was documented in the early phase of infection. Moreover, the input to the initial cell proliferating phase inducing intraepithelial damage seems to be driven by the same cytokines network assuming a key role in disease progression.

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A ROLE FOR SECRETORY ASPARTYL PROTEINASES OF *CANDIDA ALBICANS* IN MOUSE VAGINAL INFLAMMATION AND VAGINAL CANDIDIASIS

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Introduction: Secretory aspartyl proteinases (Sap) of *Candida albicans (C. albicans)* are considered to play important roles in the pathogenicity of this fungus, in particular have been suggested to play a major role in *C. albicans*-induced vaginal inflammation (vaginitis). In this work we sought for mechanisms by which Sap2, a dominantly expressed member of the Sap family, exerts its role in an original, *Candida* low-burden, high- inflammatory murine experimental model of vaginal candidiasis.
Materials and methods: Pro-inflammatory cytokines production and neutrophil influx in vaginal washes obtained from CD1 mice injected with Sap2 were analyzed by ELISA assays and flow cytome-

try. Sap2-induced inflammasome in vaginal epithelial cells was also assessed by western blot analysis. Finally, real-time *in vivo* imaging technique was used to monitor the course of vaginal infection in mice treated with anti-Sap2 reagents.
Results: Injection of a low dose (0.5 μg) full-length, recombinant Sap2 (and its close homologs Sap1 and Sap3) into the mouse vagina caused early (1 day) vaginal inflammation as witnessed by local neutrophil influx and accumulation of the inflammasome-dependent interleukin (IL)-1β. This effect compared with that of 50 ≠g LPS and required Sap2 enzymatic activity as shown by the inability to cause inflammation by a N-terminus-truncated, enzymatically-inactive tSap2 as well as the inflammation blockade by enzyme inhibitory anti-Sap2 human recombinant Fab (HuCal) and pepstatin A. Importantly, HuCal and pepstatin A strongly inhibited neutrophil influx and cytokine production early during experimental vaginal infection by *C. albicans* although being ineffective on vaginal fungal burden. The Sap2-induced vaginitis was mediated by the activation of an inflammasome sensor, likely the NLRP3, as inferred by the expression of activated caspase-1 in murine vaginal epithelial cells and in a human vaginal epithelial cell line, and the observation that caspase-1 inhibition down-regulated IL-1β and IL-18 production by the vaginal epithelial cells.
Discussion and Conclusions: Overall, the data demonstrate that Sap2 is a critical determinant of the acute inflammatory response by the epithelial cells in vaginal candidiasis, and support the notion that vaccine-induced or passively administered anti- Sap antibodies could help control vaginal disease.

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RIFAXIMIN PREVENTS ALTERATIONS OF ENTERIC BACTERIA IN A RAT MODEL OF DICLOFENAC-INDUCED ENTEROPATHY

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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), (50mg/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 bacterial total load and quantitative analysis of phyla, via 16S real-time PCR.
Results: Ileal specimens from control animals were found to contain a total bacterial load of $4.66 \pm 1.01 \times 10^{10}$ (copies/g). In particular, the Bacteroidetes phylum was $0.14 \pm 0.05 \times 10^{10}$, the Firmicutes phylum was $0.26 \pm 0.03 \times 10^{10}$ and the Proteobacteria phylum was $8.86 \pm 6.29 \times 10^{9}$. All these bacterial loads increased after treatment with diclofenac, with the only exception of Proteobacteria. In rats treated with diclofenac plus Rifaximin-EIR, the bacterial total load decreased by 89%, with a significant reduction of all phyla evaluated.
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].

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P 107A

RESTRICTION OF HPV18 REPLICATION IN THE NUCLEUS BY IFI16: WHAT ABOUT INNATE SENSING?

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Objectives: Intrinsic immunity is mediated by cellular restriction factors that are constitutively expressed and active even before a pathogen enters the cell. The host nuclear factor IFI16 acts as a sensor of foreign DNA and antiviral restriction factor as demonstrated by our groups for human cytomegalovirus (HCMV) and herpes simplex virus (HSV-1). In our study, we demonstrate that IFI16 has also a profound effect on HPV18 replication.
Methods and Results: IFI16 affected HPV18 replication in human keratinocytes (NIKS cells transfected with religated HPV18 genome) and the osteosarcoma cell line U2OS, transfected by electroporation with HPV18 minicircles. IFI16 knockdown increased HPV18 replication 7-fold and its overexpression reduced viral yield by over 5-fold. This activity has been also observed in permanent IFI16-negative U2OS cells, where HPV replication was higher and maintained at higher levels during passages in culture compared with the parental cell line. ChIP studies demonstrated that IFI16 promotes the addition of heterochromatin marks and the reduction of euchromatin marks on viral chromatin. Type I interferon response was not involved in the observed phenotypes. Combined immunofluorescence and FISH analysis performed in monolayer and HPV18 raft cultures showed that in HPV18FISH-positive nuclei IFI16 disappeared from the nucleus as already reported for herpesviruses. IFI16-HPV colocalization was not evident by this costaining. Work is in progress with EdU-labelled minicircles.
Conclusion: These results argue that IFI16 restricts chromatinised HPV DNA through epigenetic modifications and executes a broad surveillance role against viral DNA in the nucleus that is not restricted to Herpesviruses.

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E. COLI INFECTION IMPAIRS INSULIN SECRETION THROUGH A PLA_2-DEPENDENT MECHANISM

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Introduction: Microbial infections are suspected to take part in the pathogenesis of diabetes mellitus type 1 (T1DM). Glucose stimulation of b cells is accompained by release of free arachidonic acid (AA) mainly by the action of cytosolic and calcium independent phospholipases A_2 ($cPLA_2$ and $iPLA_2$). *E. coli* infection also activates AA release: the exact relationship between *E. coli* infection and insulin secretion has been a matter of speculation.
Materials and Methods: Insulinoma cell line (INS-1E) was infected with enterohemorrhagic *E. coli* (EHEC). Invasion assay, Scanning Electron Microscopy and Transmission Electron Microscopy were used to demonstrate the capacity of EHEC to enter cells. Glucose-induced insulin secretion was evaluated after acute (6h) and chronic (72h) infection and after transfection of INS-1E with specific $cPLA_2$- or $iPLA_2$-*si*RNAs. PLA_2s activities in presence of specific inhibitors and Western blot analysis were performed.
Results: After acute infection, the insulin release was very similar to control cells but after chronic infection the insulin release significantly decreased. PLA_2s activities were significantly activated and the presence of EDTA ($cPLA_2$ inhibitor) or BEL ($iPLA_2$ inhibitor) demonstrated that $cPLA_2$ activity is mainly responsible for the AA production. Western blot analysis showed an increase of $cPLA_2$, $iPLA_2$, phospho-$cPLA_2$, and COX-2 expressions after acute and, even more, after chronic *E. coli* infection. After acute infection, the silencing of the two isoforms of PLA_2s, with specific $cPLA_2$- or $iPLA_2$-*si*RNAs, reduced the insulin secretion. After chronic infection, the silencing of the PLA_2s determined a rise in the insulin release.
Discussion and Conclusions: The results obtained demonstrated that $cPLA_2$ and $iPLA_2$ play a key role in the process of insulin secretion after EHEC infection, probably because a high enzymatic activa-

tion leads to the release of AA. This polyunsaturated fatty acid per se or its metabolites, since it is the substrate of cyclooxygenase, which could produce a high amount of prostaglandins, could be responsible for the reduced secretion of insulin. The understanding of the molecular mechanisms activated by the bacterial infection is a necessary requirement in order to develop new therapeutic approaches in patients suffering from an imbalance in the secretion of insulin in response to bacterial infection.

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POTENTIAL EFFECTS OF ACETYLCHOLINE IN THE PATHOGENESIS, BIOFILM FORMATION AND OUTCOME OF *CANDIDA ALBICANS* EXPERIMENTAL INFECTIONS

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Introduction: *Candida albicans (CA)* is the fourth most common organism isolated from total bloodstream infections and continues to carry a high mortality, despite available antifungal treatment. *Candida* 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.

Both neuronal and non-neuronal acetylcholine has been demonstrated to modulate inflammatory responses during bacterial infection. The role of acetylcholine (ACh) in the pathogenesis of fungal infections is unknown. Therefore, the aim of this study was to determine whether acetylcholine plays a role in fungal biofilm formation and the pathogenesis of *CA* infection.

Materials & Method: The effect of ACh on CA biofilm formation and metabolism *in vitro* was assessed using a crystal violet assay. ACh administration on CA infection outcome, fungal burden and biofilm formation were also investigated *in vivo* using a *Galleria mellonella* infection model. ACh modulation of host immunity to CA infection was determined *in vivo* using haemocyte counts, cytospin analysis, and larval histology, and real time PCR on antimicrobial peptides.

Results: ACh was shown to inhibit *CA* biofilm formation *in vitro* and *in vivo*. Crystal violet assays revealed a dose-dependent decrease in CA biofilm biomass with a maximum reduction with 50 µg/mL of ACh. In addition, ACh (50 µg/larva) protected *G. mellonella* larvae from *CA* infection mortality, with a 75% reduction of mortality at 48h. Histology analyses showed that the *in vivo* protection occurred through ACh enhancing the function of haemocytes whilst at the same time inhibiting *CA* biofilm formation. Furthermore, acetylcholine also inhibited inflammation-induced damage to internal organs.

Discussion & Conclusion: This is the first demonstration of a role for acetylcholine in protection against fungal infections. Our data assign two independent roles for ACh in *CA* pathogenesis: (i) as an inhibitor of *C. albicans* biofilm formation and pathogenicity and (ii) as a regulator of host cellular immune responses to facilitate rapid clearance of *CA*. The novel findings described in this study therefore suggest that ACh may be an adjunctive therapeutic to prevent or treat potentially fatal fungal infections.

P 109

DEGRADATION OF POLYOMAVIRUS JC T-ANTIGEN BY STRESS INVOLVES THE LIP ISOFORM OF C/EBP BETA

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Introduction: Endoplasmic reticulum (ER) stress

is caused by the accumulation of misfolded or unfolded proteins in the lumen of the endoplasmic reticulum. CCAAT/enhancer-binding protein beta (C/EBPb) is one of the cellular proteins whose expression is upregulated during ER stress. Translation of the C/EBPb mRNA from different initiation codons leads to the synthesis of two transcriptional activators (LAP-1 and -2) and a transcriptional repressor (LIP). Previously, we have identified C/EBPb LIP as a negative regulator of polyomavirus JC (JCV), the causative agent of the demyelinating disease progressive multifocal leukoencephalopathy (PML). In this study, we examine the effect of C/EBPb LIP on JCV large T antigen (T-Ag).

Material and Methods: BsB8 cells were originated from JCV-transgenic mouse tumor cell line expressing T-Ag. The cells were plated for 24 h and transfected with LIP expression plasmid, in concentration-depended manner, or transduced with adenoviral vectors containing LIP siRNA. These cells were treated with or without 35mM thapsigargin (an ER stress-inducer) in DMSO vehicle for 6, 12 and 24 h, or overnight with MG115 (a proteasome inhibitor). Total cell extracts were prepared and analyzed by Western blot. In Immunoprecipitation (IP)/Western blot, the cells were transfected with LIP expression plasmid and T-Ag was immunoprecipitated followed by Western blot for LIP or vice versa for the reciprocal IP/Western blot.

Results: Here, we show that the induction of ER stress by thapsigargin increase the expression of endogenous LIP and at the same time the degradation of JCV T-antigen in BsB8 cells. Our results also revealed that overexpression of LIP significantly reduced the level of T-Ag and this effect is reversed upon siRNA-mediated silencing of LIP. Immunoprecipitation/Western blot experiments indicated that LIP interacts with T-antigen directly. Treatment of cells that overexpress LIP with MG115 partially rescued LIP-mediated degradation of T-antigen.

Discussion and Conclusions: Our observations point to a role of LIP in ER stress regulation of T-antigen stability and may open a new avenue to study host-virus interaction during ER stress.

P 109A

INVESTIGATING THE MICROBIOTA-GUT-BRAIN AXIS IN ANOREXIA NERVOSA

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Introduction: Anorexia Nervosa (AN) is a severe eating disorder with unknown etiology and a high mortality, often co-occurring with anxiety disorders. Both environment and innate molecular determinants seems to play a role. The hypothesis of a possible involvement of microbiota shift in the contribution to AN is supported by studies, focused on other pathological conditions such as gastrointestinal diseases, showing that an aberrant gut microbiota is sometimes associated with psychiatric disorders.

Materials and Methods: We collected 8 stool samples from 4 AN patients (one with a strong amelioration in BMI) and 4 NW female subjects (one of them with a very low calories intake). Investigation of microbial communities has been performed by Next Generation Sequencing using 16S rRNA (V3-V4) genomic region on Illumina MiSeq platform with a 250PE protocol. Real time PCR on specific bacterial species was also made.

Results: The characteristics of enrolled subjects are: for AN, BMI = 14 ± 2.01 (mean ± SD), mean age 22 ± 1.7; for NW, BMI = 21 ± 0.47 and mean age 22 ± 0.98.

We found, despite the low number of analyzed subjects and inter-individual differences, a clusterization in two distinct groups. All the AN patients but the one (AN2 with a BMI higher that 16) grouped together. Similarly, all NW subjects but the one (NW2, with a very low calories intake) clustered in the second group. Indeed, AN2 was more similar to the NW group, and NW2 more similar to the AN group.

The *Bacteroidetes/Firmicutes* ratio was 47/45 and 41/52 for AN and NW, respectively. No significant or minor differences were seen at phylum level. However, we found some species to be present only in AN group, such as *Meganonas* spp. and *Prevotel-*

la copri, or in a higher percentage, such as *Desulfovibrio* spp. and *Bacteroides caccae* and *B. fragilis*. NW subjects were characterized by the presence of *Megasphaera* spp. and *B. uniformis*, and a higher rate of *Enterobacteriaceae* and *Bifidobacterium adolescentis*.

Discussion and Conclusions: Although the low number of subjects enrolled, our preliminary data suggest alterations of the microbial communities in AN patients. A dysbiotic intestinal microbiota might thus contribute to or worsen several AN traits (weight regulation, anxiety, and depression), and might represent a possible marker.

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CANDIDA ALBICANS ISOLATES WITH DIFFERENT GENOMIC BACKGROUNDS SHOW DIVERSE HOST ADAPTATION AND DIFFERENTIAL SUSCEPTIBILITY TO MICROGLIAL CELL-MEDIATED DEFENSES

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Introduction: *C. albicans* behaves as an opportunistic pathogen causing infections in susceptible hosts, while acting as a harmless commensal in healthy conditions. A complex interplay between host immunity and fungal virulence accounts for the outcome of infection. To this regard, we have previously shown that microglia, the brain phagocytes, plays a crucial role in preventing experimental meningoencephalitis by *C. albicans*, while a diverse adaptation of *C. albicans in vitro* and *in vivo* has been demonstrated. Such capacity largely depends on both the genome of the strains and their ability to adapt to host environment. In this study, we investigated the susceptibility of two *C. albicans* isolates with different genetic backgrounds to microglial cell-mediated defenses.

Materials and Methods: Two *C. albicans* clinical isolates, YL1 and YQ2, were used in our *in vitro* infection model, employing the BV2 microglial cell line. The whole genome analysis of YL1 and YQ2 showed an extreme variability/divergence between them. Thus, such strains were evaluated for their susceptibility to phagocytosis and killing by microglia.

Results: Although comparable in their susceptibility to phagocytosis, YL1 and YQ2 showed marked differences in term of intracellular survival. In particular, the YL1, unlike YQ2 isolate, resisted to intracellular killing and eventually replicated inside microglial cells; moreover, the percentage of YL1-containing acidic phagosomes was significantly higher than that observed in YQ2-infected BV2 cells.

Discussion and Conclusions: These results indicate that the genetic background of *C. albicans* may greatly affect its behaviour in terms of *in vitro* susceptibility to immune effector cells. Based on this approach, a more in depth understanding of fungal genetic makeup is achievable, thus allowing to establish the virulence potential of a given isolate and possibly to predict the outcome of *C. albicans* infection.

P 110A

REGULATION OF PROTEIN KINASE R DURING HSV-1 REPLICATION: INVOLVEMENT OF VIRAL PROTEINS Us3 AND UL13

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Introduction: Protein kinase R (PKR) is an antiviral protein of the innate immune response activated after binding to viral *dsRNAs*. Once active, as a kinase, PKR phosphorylates eIF2α kinases and blocks synthesis of both cellular or viral proteins.

Therefore, PKR activation during the viral infection is an efficient way to inhibit virus replication. Viruses have evolved a number of strategies to prevent host anti-viral defenses, thus they express viral proteins capable to block to different levels the antiviral action of the PKR. Particularly, the herpes simplex virus 1 (HSV-1) virion host shutoff (VHS) RNase protein block the activation of PKR most likely by degrading the viral RNA. However it is not clear whether VHS can directly block PKR or other viral proteins are involved in this regulatory mechanism. Here we investigated the involvement of tegument proteins US3 and UL13, two Ser/Thr kinases respectively expressed with early and kinetics in the productive growth cycle, in facilitating VHS in the control of PKR activation during HSV replication.

Materials and Methods: HEp-2 cells were infected with 10 PFU/cell with HSV-1 (F) wild-type virus, R2621(ΔUL41), R7356 (ΔUL13) or R7041 (ΔU_S3) mutant viruses. 293T cells were transfected with the plasmids designed as follow pVHS, pU_S3 and pUL13. Infected or transfected cells were subjected to proteins extraction for Western blot analysis and probed with polyclonal antibodies directed to p-PKR (thr446), total PKR and GAPDH.

Results: The results showed that p-PKR was reduced in cells infected with UL13-deficient virus (R7356) as well as the Us3-deficient virus (R7041) if compared to the VHS-deficient virus (R2621). The expression on U_S3, UL13 and VHS in a transient transfection experiments confirmed the suppressions of phospho-PKR accumulation in HEp-2 cells.

Discussion and Conclusions: The key findings reported here complement and extend the studies reported by Sciortino and collaborators. In particular in this study we shows for the first time, that during HSV-I replication, UL13 and U_S3 collaborate with VHS to regulate PKR activation. The block mediated by the virus of this specific host protein reflects the importance of the innate response as a threat to virus replication and dissemination.

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TLR2 ACTIVATION IN CORNEAL EPITHELIAL CELLS BY SECRETED GLYCOPROTEIN B OF HERPES SIMPLEX VIRUS TYPE 1 IN AN *EX VIVO* KERATITIS MODEL

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Introduction: Herpes simplex virus (HSV) is a human pathogen infecting the majority of world's population. While oral and genital lesions are the most common manifestations of infection, HSV type 1 (HSV-1) can cause disease in ocular tissues, including the lids, conjunctiva, cornea, uveal tract and retina. HSV-1 corneal infections have been studied in a variety of animal models, where rabbits display many of the features of HSV-1 ocular disease in humans. Accordingly to several studies HSV-1 clearance from the cornea during primary infection is largely due to innate immune response. Corneal epithelial cells can initiate immune response to HSV-1 through expression of toll like receptors (TLR). It has been reported that TLR2, 4, 7 and 9 may be implicated in the pathogenesis of active HSV infection in the cornea. However, the possible mechanisms by which TLRs recognition of HSV constituent leads to increase inflammation and promotes the subsequent development of inflammation-associated lesions are not well defined. Recent studies have shown that HSV glycoproteins gH/gL and gB are able to interact with TLR2 and activate NF-kB inducing the production of pro-inflammatory cytokines. The aim of this study was to investigate the expression and function of TLR2 of corneal epithelial cells in an *ex vivo* keratitis model induced by secreted protein gB1 (gB1s) of HSV-1.

Methods: gB1s was obtained from human 293 cells constitutively expressing the secreted form of the glycoprotein and subsequently purified by affinity chromatography. Rabbit corneas with sclera rims,

maintained in culture at an air-liquid interface, were assigned to two groups: (a) abraded corneas and (b) abraded corneas exposed to gB1s. The epithelial cells acquired by impression cytology were examined by quantitative real-time RT-PCR to determine TLR2 mRNA e IL-8 mRNA.
Results: The results demonstrated that TLR2 and IL-8 mRNA were significantly expressed in the epithelial cells of the group exposed to gB1s. Moreover, each of the corneas exposed to gB1s developed pupillary dilation, a clinical sign of HSV keratouveitis.
Conclusions: Our results demonstrate that TLR2 ligand activity of HSV is a property of gB and play a role in the induction of an immunopathological response in the cornea.

P 111A

TRANSCRIPTIONAL ACTIVITY OF DIFFERENT ENDOGENOUS RETROVIRUS FAMILIES AND PRO-INFLAMMATORY CYTOKINES IN A MOUSE MODEL OF AUTISM

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Introduction: Human Endogenous Retroviruses (HERVs) are ancient remnants of germline integrations, as provirus, in chromosomal DNA, of exogenous infectious retroviruses and have been implicated in many neurological and neuropsychiatric disorders. In a previous study, we demonstrated a different expression profile of three HERV families in peripheral blood mononuclear cells (PBMCs) from Autistic Spectrum Disorders (ASD) patients. To deeply understand the role of HERVs in ASD, we used a mouse model of autism, consisting in CD-1 outbred mice, prenatally exposed to valproic acid (VPA). VPA is an inhibitor of the histone deacetylase and when administered during pregnancy represents an ASD risk factor for humans and triggers an ASD like phenotype in rodents. In this mouse model, we evaluated the expression of several ERVs and pro-inflammatory cytokines, since in the etiology of ASD an immune component, on which ERVs could act, was recognized.
Materials and Methods: We evaluated at different times the transcriptional activity of several ERVs (ETnI, ETnIIα, ETnIIβ, ETnIIγ, MusD, IAP) and pro-inflammatory cytokines (IL-1β, IL-6, TNF-α) in embryos and in blood and brain samples from offspring of VPA-treated and untreated mice. RNA was extracted and retro-transcribed for the Real Time PCR analysis.
Results: Embryos, blood and brain from VPA treated mice showed high levels of ERVs. In particular, in brain samples the expression of ERVs was high and stable, while in blood high levels of ERVs were detectable only at the first observation times and over time tended to reach the levels of untreated mice. Interestingly, also in embryos from VPA treated dams, ERVs were present at high levels. Finally, high expression of pro-inflammatory cytokines was observed in embryos and brain from offspring of VPA treated dams. No difference between treated and untreated mice, were highlighted in the blood samples.
Discussion and Conclusions: Data from this mouse model confirm those obtained by us in humans, proposing the use of ERVs as early biomarkers for ASD. These also support the hypothesis that early epigenetic changes contribute to the mechanism of VPA-induced neurobehavioral alterations, and candidate ERVs as downstream effectors. The correlation between modifications of the pattern of inflammation and the expression of ERV suggests a possible link between ERVs dysregulation and immune component in ASD.

P 112

IN VITRO INTERACTION OF *STENOTROPHOMONAS MALTOPHILIA* WITH HUMAN MONOCYTE-DERIVED DENDRITIC CELLS

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Introduction: *Stenotrophomonas maltophilia* (SM) is increasingly identified as an opportunistic pathogen in immunocompromised, cancer and cystic fibrosis (CF) patients. Knowledge on innate immune responses to SM and its potential modulation is poor. The present work investigated the ability of SM strains from CF and non-CF patients to survive in human monocyte-derived dendritic cells (HMoDC) and the effects of the bacteria on maturation of and cytokine secretion by DCs.
Materials and Methods: The ability of 12 clinical strains (5 CF strains, 7 non-CF strains) and 1 environmental strain to survive inside HMoDCs was investigated. Immature DCs (iDCs) were generated from leukocyte-enriched buffy coat. iDCs (10^6 cells/ml) were exposed to bacteria at a MOI of 20:1 for 1h to allow bacterial internalization. Uninfected iDCs in RPMI were used as a control. Then, the extracellular bacteria were killed by gentamycin assay and DCs were lysed to quantify intracellular bacteria. To measure intracellular replication, after treatment of infected DCs with antibiotics, cells were washed and incubated with fresh RPMI up to 18h. DC maturation was analized measuring CD86 and CD80 expression by flow cytometry. For TNF-α expression, cells were infected in the presence of Brefeldin A and stained with anti-TNFα antibody. IL-12p70 production by DCs was measured by ELISA.
Results: The analyzed strains presented a high degree of heterogeneity in internalization and intracellular replication efficiencies: some were able to escape DCs uptake, others were internalized and able to replicate in DCs. All tested strains were able to induce the production of TNFα and IL-12 in DCs and most of them induced a significant increase in the expression of DC surface maturation markers. No significant difference was observed in the maturation of iDCs stimulated with SM strains from CF and NCF group.
Discussion and Conclusions: The data suggested that internalization, intracellular survival and immunostimulatory properties of S. maltophilia are strain-dependent. This is in accord with studies showing that different SM strains replicate and persist in the murine lungs to significantly different degrees. The observed heterogeneity is not surprising, in light of the high genetic diversity exhibited by SM isolates.

P 112A

INTERACTION BETWEEN THE HYPOXIA INDUCIBLE FACTOR 1 ALPHA AND THE HUMAN POLYOMAVIRUS BK: A RISK FACTOR FOR THE DEVELOPMENT OF POLYOMAVIRUS ASSOCIATED NEPHROPATHY

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Introduction: BK polyomavirus (BKV) has a worldwide seroprevalence of about 90%. After the primary infection, it establishes a lifelong latency within the urogenital tract. The severe immunological impairment occurring in transplant kidney recipients leads to BKV reactivation that may result in the polyomavirus associated nephropathy (PVAN). During transplantation, the kidneys are subjected to hypoxic conditions, driven by the action of Hypoxia Inducible Factor (HIF). It has been proved that HIF isoform 1-α (HIF-1α) may

interact with several viruses, but till now the scientific literature does not present any information regarding the interaction between BKV and HIF-1α. The aim of the study is to investigate the possible interaction between HIF-1α and BKV, and its potential effect on the pathogenesis of PVAN.
Materials and Methods: The expression of HIF-1α was evaluated at the mRNA level in 17 kidney paraffin-embedded tissue samples, from BKV+ PVAN, BKV+ NOT PVAN, and BKV- patients, by means of RNA retrotranscription and subsequent specific real time PCR. Luciferase and Cromathin Immunoprecipitation (ChIP) assays were performed on BKV transfected VERO cells, to verify the interaction between the viral promoter and HIF-1α. The effect of hypoxia on BKV replication was assessed by evaluating BKV load in BKV infected VERO cells, treated and not treated with Cobalt Chloride (CoCl2).
Results: HIF-1α expression was 13.6 folds higher in BKV+ PVAN tissues and 0.7 folds higher in BKV+ NOT PVAN tissues compared to BKV- tissues. Luciferase activity was higher ranging from 2 to 6 fold ($p < 0.05$) when VERO cells were transfected with both BKV and HIF-1α, compared to those transfected only with BKV; ChIP analysis showed the structural binding between the viral promoter and HIF-1α. BKV load was 5 fold increased in infected cells treated with CoCl2 treatment compared to infected cells without CoCl2 treatment.
Discussion and Conclusion: The interaction between BKV and HIF-1α was observed both *in vitro* and ex vivo. HIF-1α, that is stabilized by the hypoxia conditions, occurring during the transplantation, is able to bind the BKV promoter and to enhance BKV replication. Consequently, the hypoxia represents a risk factor for the PVAN developing in kidney transplant recipients.

P 113

HUMAN ENDOGENOUS RETROVIRUS EXPRESSION IN A COHORT OF AUTISM SPECTRUM DISORDER YOUNG PATIENTS AND THEIR PARENTS

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Introduction: Autism spectrum disorder (ASD) is a neurodevelopmental disorder, characterized by impairment in social interaction and communication, with restricted, repetitive or stereotyped patterns of behaviour, interest and activities. During the last decades the prevalence of ASD has increased rapidly, suggesting the involvement of environmental factors in the etiopathogenesis of the disease. In fact, 10–20% of ASD patients have an identified genetic etiology, supporting the hypothesis that ASD is the result of a complex interaction between genetic and environmental factors, were epigenetic events could represent this link. In a previous study on a cohort of ASD young patients, we have demonstrated that HERV-H was highly expressed, negative correlated with the age of patients and higher levels were observed in patients with severe impairment in communication and motor development. On the basis of obtained data, we extended the study to peripheral blood mononuclear cells (PBMCs) from parents of ASD patients, in order to evaluate i) the expression of HERV-H in the previous generation and ii) any change in the levels of HERV-H after *in vitro* treatment with chemical agents such as valproic acid (VPA) and non-nucleoside reverse transcriptase inhibitors (NNRTIs) and iii) apoptosis levels.
Materials and Methods: PBMCs from ASD young patients and their parents were analysed immediately after collection or post stimulation in culture, in presence of VPA or NNRTIs. Flow cytometry analysis after nuclei staining, were performed to evaluate apoptosis levels.
Results: High levels of HERV-H in PBMCs from ASD children and their mothers at the baseline and a significant increase of expression after *in vitro* stimulation were observed. In fathers, HERV-H basal level of expression was low and a minimum

increase after *in vitro* stimulation was detected. Pharmacological treatments modified HERV-H expression in PBMCs from ASD young patients and their mothers. Modification was minimally extended also in fathers. The levels of spontaneous apoptosis in ASD patients were higher than in PBMCs of parents and didn't change after stimulation.
Discussion and Conclusion: The results, although preliminary, encourage us to further investigate the maternal features, supported by the belief that the intrauterine development and the alteration of the delicate balance between mother and fetus have a key role in the development of the autistic phenotype.

P 113A

IN VITRO AND *IN VIVO* TRANSMISSION BLOCKING EFFECTS OF *AZADIRACHTA INDICA* LIMONOIDS ON THE MURINE MALARIA PARASITE *PLASMODIUM BERGHEI*

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Introduction: *Azadirachta indica* (Meliaceae) possesses numerous bioactive limonoid secondary metabolites. Azadirone, nimbin and azadirachtin A (AzaA) exhibit activity against *Plasmodium* asexual blood stages *in vitro*. NeemAzal® an AzaA rich commercial kernel extract completely blocked transmission of *P. berghei* to *Anopheles stephensi* when administered to mosquitoes by blood meal on mice treated at 75 mg/kg. The present studies aimed at assessing *Plasmodium* early sporogonic stage (ESS) specific effects of *A. indica* fruit fractions and bioavailability of a fraction rich in AzaA compared to the isolated AzaA molecule.
Materials and Methods: *In vivo* and *in vitro* assays were performed with *P. berghei* ANKA strain and *P. berghei* CTRPp.GFP. Fractions and molecules were obtained from *A. indica* seeds (Burkina Faso) and from NeemAzal® by column chromatography. Constituents were identified by NMR spectroscopy.
Results: AzaA, Azadirone, NeemAzal®, *A. indica* unripe fruit kernel fraction 12 (almost pure nimbin) and 13 (nimbin with other limonoids) revealed inhibitory activity on ESS *in vitro* at ≤ 50 µg/ml. Azadirone, isolated from the epical part of the fruit, showed a 50% inhibitory activity on ESS of 27.3 µM (CI_{95} 17.8 ± 42.0) *in vitro* but no effects on sporogonic development in mosquitoes. An *in vitro* IC_{50} value of 6.8 µg/ml (CI_{95} 5.95 ± 7.86) was determined for NeemAzal® and of 12.4 µg/ml (CI_{95} 11 ± 14) for pure AzaA. Bioavailability of AzaA in the commercial neem extract compared to pure AzaA appeared to be increased. From biological response tests emerged a half life of anti-plasmodial compounds of up to 7 hours when NeemAzal® was i.p. administered to mice at an AzaA dosage of 150 mg/kg.
Discussion: Accumulated evidences on bioavailability and anti-plasmodial activity of limonoids against *Plasmodium* stages developing in the human and mosquito host suggest *A. indica* to be a valid resource for the design of limonoid dosed, preventive-transmission blocking phytomedicines.

P 114

ANALYSIS OF ANTI-INFLAMMATORY EFFECTS OF CHITIN NANOFIBER/ LIGNIN IN HUMAN KERATINOCYTES

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Introduction: In the last few years many studies focused their attention on different potential biomedical application for biocompatibile polymers especially nanopolimers, nanofibers and nanoparti-

cles. Polimer nanocomposites arising from different chemistries and construction, they includes aliphatic polyester such as polylactide (PLA) and poly (DL-lactic-cd-glycolic acid) (PLGA) and natural biopolymers such as chitin, chitosan and lignin. The increasing attention to the environment has brought about an in-depth study of the biological activity of natural polymers or artificial polymers that respect the characteristics of biodegradability and biocompatibility. Our research group are testing *in vitro* a novel combination of chitin nanofiber/lignin in different ratios for their anti-inflammatory and wound repair activity in experimental models of human keratinocytes. For the evaluation of the anti-inflammatory effect of chitin nanofibers/lignin, the inflammatory cytokine expression has been evaluated on human keratinocytes treated with Lipopolysaccharide (LPS) of *P. aeruginosa.* We tested the expression of the beta-defensin 2 (hBD-2) and metalloproteinases 2 and 9 (MMP-2 and -9) too, molecules involved in the mechanisms of tissue regeneration.

Material and methods: *Polymers*: chitin nanofibers/lignin were provided by MAVI SUD s.r.l. *Cell culture and treatment:* the HaCaT, an immortalized human keratinocyte cell line, were cultured in DMEM supplemented with 10% FBS at 37°C in a 5% CO_2 atmosphere. Semiconfluent monolayers were treated or not with the substances of interest in presence or not of LPS 10 µg/ml. *Real time PCR:* total RNA was isolated and two hundred nanograms of this were reverse-transcribed into complementary DNA (cDNA) according to the manufacturer's instructions. Real time PCR was carried out using 2 ml of cDNA.

Results: The results showed that the polymers tested significantly reduced the IL-8, IL-1α, and TNF-α expression induced by LPS in human keratinocytes. They also up regulated the expression of MMPs and hBD-2, genes involved in the process of tissue repair.

Conclusion: Our data suggest that the novel combination of chitin nanofiber/lignin significantly reduce the inflammatory response induced by LPS in human keratinocytes. In addition, these polymers are able to actively contribute to the wounds healing process, regulating the expression of metalloproteinase and HBD-2.

P 114A

ROLE OF CASPASE-8 IN HSV-1-INDUCED CELL DEATH

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Introduction: We recently reported that the BH3-only protein Puma is the major mediator of HSV-1-induced Bax/Bak activation and MOMP induction, leading to caspase-3-dependent apoptosis in cells in which this form of cell death is not adequately prevented, and that death receptors and corresponding ligands are not involved in this phenomenon. Intriguingly, however, other results indirectly suggest that caspase-8 could be involved in apoptosis triggered by HSV-1. We, then carried out a series of experiments to discern the possible role of caspase-8 in this phenomenon.

Materials and Methods: Caspase-8 knockout (C8 KO), Bax/Bak knockout/caspase-8 knockdown (DKO-C8KD), FADD knockdown (FADD-KD) MEFs were generated and utilized together with wt MEF cells. Cells were infected with HSV-1 at a MOI of 10 PFU/cell. Cells were kept in culture up to 72 h p.i. and subjected to detection of cell death by different techniques and various experimental procedures.

Results: The results showed that caspase-8 was clearly cleaved in HSV-1-infected MEF cells undergoing apoptosis. Moreover, Bax/Bak DKO cells, as expected, were resistant to HSV-1- induced cell death in comparison with wt cells. Interestingly, however, also C8 KO were remarkably protected from HSV-1 induced cell death. Protection from death conferred by both caspase-8 and Bax/Bak absence was very effective at 24 h p.i. and less effective at 72 h p.i., when cells started to die. Nevertheless, DKO-C8KD cells showed the higher resistance to HSV-1-induced cell death. FADD-KD cells showed partial resistance to HSV-1 induced apoptosis. Surprisingly, however caspase-8 was still cleaved in response to HSV-1 infection in FADD-KD cells, but not in Bax/Bak DKO cells, excluding the involvement of FADD in HSV-1-induced caspase-8 activation as well as a role for caspase-8 cleavage as an upstream signal in HSV-1-induced apoptosis.

Discussion and Conclusions: Here we demon-

strate that caspase-8 activation participates in HSV-1-induced apoptosis. Intriguingly, however, results show that this role does not fit with the canonical role of caspase-8 as early, positive signal in the apoptotic cascade, but, rather, are compatible with a down-stream regulatory role to counteract apoptosis prevention. Further studies are necessary to verify this hypothesis.

P 115

SERRATIOPEPTIDASE EFFECT ON STAPHYLOCOCCI: STUDY *IN VITRO* ON CELL INVASION AND CELL PROLIFERATION

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Introduction: *Staphylococcus aureus* is a Gram positive pathogen frequently isolated from community-acquired and nosocomial infections. *Staphylococcus epidermidis* is now recognized as an important opportunistic pathogen responsible for numerous nosocomial infections. The use of medical devices is associated with a significant risk of infection by bacteria possessing virulence factors and proteins that promote invasion of host cells. This study aims at determining the ability of *S. aureus* 6538P and *S. epidermidis* O47 to invade the human osteoblastic cell line MG-63; we evaluated the anti-infective effect of a secreted protein obtained from *Serratia marcescens*, serratiopeptidase (SPEP), on invasion and on proliferation of eukaryotic cells. Finally we analyze the ability of osteoblasts to release a monocyte chemoattractant protein-1 (MCP1), chemokine responsible in selectively recruiting monocytes, neutrophils and lymphocytes, when eukaryotic cells are exposed to bacterial species.
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 37°C. 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: Significant differences of osteoblast invasion rate were found between *S. aureus* and *S. epidermidis* at a MOI 1:300 ($p = 0.017$) but no significant difference were found at a MOI 1:30. In presence of SPEP, we observed a significant decrease of invasion rate for *S. aureus* ($p = 0.045$), while a increase was found for *S. epidermidis* at a MOI 1:300 ($p = 0.017$). Significant increase of osteoblast proliferation was observed between cells alone and cells incubated with *S. aureus* ($p = 0.029$); SPEP did not affect this result. No significant differences on cell proliferation was found when cells were incubated with *S. epidermidis*.
Discussion and Conclusions: Our study shows that SPEP could be developed as a potential anti-infective agent to prevent the invasion of these bacteria in bone cells. The use of SPEP may be a therapeutic strategy for preventing orthopedic infection and bone disease.

P 115A

ADHERENT-INVASIVE *ESCHERICHIA COLI* (AIEC) AND HOST CELL NUCLEUS: ROLE OF BACTERIAL AND CELLULAR EPIGENETIC MECHANISMS INVOLVED IN CELL DNA DAMAGE AND REPAIR

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Introduction: Crohn's disease (CD) leads to enhanced gut mucosal inflammation and higher risk of colorectal cancer development. A new adherent-invasive *E. coli* pathovar (AIEC) was found in 100% of early CD lesions and was linked to megalocytosis. LF82, the AIEC prototype, induces double-strand breaks (DSBs) in host DNA allowing a

cell cycle block in G2/M phase. Interestingly, LF82 uses its outer membrane vesicles (OMVs) to gain access to epithelial lining cells: OMVs are usually employed by bacteria for cell-to-cell signals or delivery of toxins to host eukaryotic cells. Our preliminary data indicate that LF82 could also modulate DNA damage repair (DDR) enzymes to increase its own intracellular survival, but such a hypothesis has to be fully investigated.

Materials and Methods: Western blot and qPCR were used to investigate LF82-mediated regulation of epigenetic enzymes involved in DDR (HDAC1, HDAC2) in Caco2 and THP1 cells upon six hours of infection. In order to evaluate the potential involvement of these enzymes on AIEC intracellular survival and replication, Caco2 and THP1 cells were infected after 12-hours pre-treatment with specific HDAC inhibitors trichostatin A (TSA) or sodium butyrate (NaBu). OMVs were isolated from LF82 and the wild-type non-pathogenic MG1655 strain.

Results: In a qPCR survey of 84 genes related to DDR pathway, after 6 hours of LF82 challenge on Caco2 cells, a significant 2-fold decrease in expression levels of ataxia-telangiectasia-mutated (ATM) gene, and a parallel 3-fold decrease of HDAC1 and HDAC2 mRNAs, were observed. Interestingly, other genes related to base excision repair (ERCC2, XPA), homologous recombination repair (XRCC3), and mismatch repair (MLH3) were also down-regulated by LF82 infection. LF82 down-regulated by 50% both HDACs nuclear protein levels in Caco2, but not in THP1 cells. TSA and NaBu enhanced intracellular survival of LF82 by 80% in Caco2, but not significantly in THP1. At 4h, LF82-derived OMVs were able to down-regulate HDAC1, while enhancing the phosphorylation of the Ser139 residue on H2AX histone variant, a marker of DSBs.

Discussion and Conclusions: HDACs enzymes play a role in LF82 intracellular survival/replication, suggesting that still unknown LF82 effectors, delivered by OMVs, are probably involved in their cell type-dependent modulation.

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TOLL-LIKE RECEPTOR 9 (TLR9) AND VIRAL PERSISTENCE

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Introduction: Both human parvovirus B19 (B19V) and human papillomaviruses (HPVs) may cause acute and persistent infections with various clinical consequences. The mechanisms which allow the viruses to persist even lifelong are poorly understood. Viral and host immunological factors have been investigated at this purpose. Here the role of innate immunity and, in particular of TLR9 response to viral DNA, was investigated.

Material and Methods: HEK-293 cells expressing huTLR9 and stably transfected with the NF-kB-inducible reporter gene, pNIFty2-Luc, were stimulated with different amounts of vector-bearing cloned viral DNA in the presence or absence of suboptimal concentrations of CpG-ODN. Lipofectamin was used to help viral or vector DNA entry within the cells. NF-kB activation was considered as measure of TLR9 activation. The viral genomes used were: B19V genotype 1 from acute and from persistent infection and genotype 2 from persistent infection; High Risk (HR)-HPV16, Low Risk (LR)-HPV11 and cutaneous HPV38.

Results: B19V DNA from acute infection induced a slight but significant TLR9 activation compared to either not stimulated or vector-stimulated cells while any NFkB activity was induced by viral DNA from persistent infection. When suboptimal concentrations of CpG ligands were used to activate TLR9, the addition of B19V DNA with acute infection caused a dose-dependent increase of CpG-induced NFkB activation. In contrast, viral DNA from persistent infection (either by genotype 1 or genotype 2, significantly decreased the CpG-induced NFkB activation in a dose-dependent manner.

DNA of cutaneous HPV38, LR-HPV11 and HR-HPV16 and of the relative vectors were tested for TLR9 activation in the same assay. In the absence of CpG-ODN, poor NFkB activation was induced by HPV16, HPV11 and HPV38. No significant differences were recorded even when strains were compared to each other or to the relative vector. When suboptimal concentrations of CpG ligands were used to activate TLR9, a dose-dependent in-

crease of CpG-induced NFkB activation was elicited by LR-HPV11 and by cutaneous HPV38 DNA which showed an half-maximal activity significantly lower compared to the relative vector. In contrast HR-HPV16 did not induce a significant additive effect of CpG-induced TLR9 activation.
Conclusion: On the whole these data underscore the relevant role of TLR9 in innate immunity to viral infections and suggest that viruses more able to persist within the cells might be evolved to escape or to interfere with TLR9 activation.

P 116A

BACTERIAL OUTER MEMBRANE VESICLES AND HOST INFLAMMATORY RESPONSES

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Introduction: A wide variety of Gram-negative bacteria, including *Escherichia coli* and *Klebsiella pneumoniae*, constitutively secrete outer membrane vesicles (OMVs). OMVs are small spherical, bilayered proteolipids sized from 20 to 250 nm. Previous biochemical and proteomic studies have revealed that bacterial OMVs are composed of outer membrane proteins, LPS, phospholipids, periplasmic proteins, DNA, RNA, and other factors associated with virulence. It was recently revealed that released OMVs play diverse roles by enhancing bacterial survival, killing competing bacteria, transferring genetic materials and proteins, delivering toxins into host cells, and modulating the immune response in host environments.
The objective of this study was to evaluate the ability of OMVs, derived from *E. coli* and *K. pneumoniae,* to induce the inflammatory cascade.
Materials and Methods: *E. coli* and *K. pneumoniae*, ATCC and clinical isolated, were grown in LB liquid medium until OD_{600} equal to 1 and the corresponding OMVs purified through serial centrifugations and filtrations. The vesicles were quantified by determining the protein concentration. The SDS-PAGE has allowed to identify the presence of the vesicles, observing the OmpA and OmpC porins of 34 KDa and 36KDa, respectively. To test the cytotoxic activity of OMVs, an LDH assay on monocytes (THP1) stimulated with OMVs was performed. In order to verify the involvement of the OMVs in the inflammation process, monocyte were stimulated *in vitro* whit OMVs and the proinflammatory cytokine production was assessed by ELISA.
Results: From analysis of the production data of the OMVs is possible to observe a distinct yield in different strains and greater release of vesicles from clinical isolates compared to the corresponding ATCC. Through the LDH assay a direct proportionality between the stimulus used and the percentage of cytotoxicity was detected. These preliminary data led to verify the action of OMV in inflammation. The assays of proinflammatory cytochine release from monocytes stimulation with OMVs are still in progress.
Discussion and conclusion: The study showed that the discrepancy in the production of OMV between the strains used could be due to the different degree of virulence of the standard strain and its nosocomial counterpart.

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MUCOSA-ASSOCIATED MICROBIOTA STRUCTURE AND METHYLATION STATUS OF GENES INVOLVED IN THE INFLAMMATION RESPONSE IN CHRON'S DISEASE

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Introduction: Inflammatory Bowel Diseases (IBD), encompassing Crohn's disease (CD) and Ul-

cerative Colitis (UC), are chronic inflammatory disorders of the gastrointestinal tract, where a deregulated immune response to the intestinal microbiota exists. A dysbiotic gut microbiota is considered a feature triggering the underlying inflammatory response, while a parallel examination of methylation levels of genes involved in the host inflammatory response remains to be determined. The project is aimed at evaluating the mucosa-associated microbiota structure and methylation levels of genes involved in the inflammation response in Chron's disease.

Material and method: Total DNA was extracted from CD ileal biopsies and healthy subjects, and analyzed for: i) mucosa-associated microbiota through 16S rDNA metagenomics (MiSeq); ii) methylation level of 22 promoter genes whose involvement in inflammation through dedicated qPCR arrays (QIAGEN).

Results: Preliminary results confirmed a mucosal dysbiosis in CD patients (Parsimony test, $p < 0.001$), with higher relative abundance of *Pseudomonas* genus in CD ($p = 0.00047$) and *Veillonella* in controls ($p = 0.00019$). Biodiversity index (ACE) was diminished in CD ($p = 0.033$). A significant decrease in methylation levels of *IL13RA1* ($p = 0.0193$) and *InhA* ($p = 0.0303$) promoters was observed. IL-13RA1 is a receptor for IL-13 and IL-4 cytokines, whose activation leads to an increased epithelial colonic permeability and matrix metallo-proteinases production. InhA is a protein belonging to the TGF-β super-family involved in cell proliferation.

Conclusion: A microbial dysbiotic status and a decreased biodiversity were found in CD mucosal biopsies. Differential methylation levels of *IL13RA1* and *InhA* promoters in CD patients, probably related to an increased mRNA expression of corresponding genes, could represent a new connection among gut microbiota and inflammatory response in CD.

P 117A

IFNL4 rs368234815 TT/ΔG POLYMORPHISM IS NOT ASSOCIATED WITH THE EXPRESSION LEVELS OF INTERFERON STIMULATED GENES AND THE CLINICAL OUTCOME OF HIV-1 INFECTION

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Introduction: Recently, a new member of IFNL family was discovered, called IFNL4, opening a new scenario. Indeed, IFNL4 expression is controlled by a dinucleotide polymorphism, called IFNL4 rs368234815 (ΔG/TT), located in exon 1 of IFNL4 and IFNλ4 protein can be produced only by individuals carrying the functional genetic variant IFNL4-ΔG allele. Interestingly, IFNL4 rs368234815 polymorphism is strongly linked with rs12979860 SNP and the IFNL4-ΔG/ΔG genotype represents the strongest known pre-treatment host predictor of HCV clearance. The possibility that IFNL4 also plays an important role in infectious disease other than HCV hepatitis has not been fully investigated. Then, we plan to evaluate the effect of the IFNL4 dinucleotide polymorphism rs368234815 on the clinical course of HIV-1 infection and on the mRNA levels of well-known ISGs (MXA,ISG15, P56, Apobec 3F/3G) measured in two groups of HIV-1 positive patients, i.e. naïve for antiretroviral treatment and HAART-treated subjects HIV-1 positive patients.

Matherial and Methods: PBMC from 112 HIV-1-infected patients were collected at the "Sapienza" University Hospital. The IFNL4 dinucleotide polymorphism rs368234815 (TT/ΔG) and ISGs will be evaluated with taqman assays.

Results: The observed IFNL4 genotypes frequencies in HIV-1 infected patients were the following: ΔG/ΔG = 9%; TT/ΔG = 14.1%; TT/TT = 76.9%. No statistically significant differences in viral load ,in CD4+ T cell count and integrated HIV DNA

levels related to IFNL4 polymorphism were observed. Then, we analyzed mRNA levels of well-known ISGs (Mxa, ISG15, P56, Apobec3F/3G) in both naïve and treated HIV-1 positive patients. When the mRNA levels of the above-mentioned ISGs between patients carrying different IFNL4 rs368234815 polymorphism genotypes were compared, no significant differences in both naïve and treated HIV-1 positive patients were found.
Discussion and Conclusions: Overall, our results showed that IFNL4 rs368234815 TT/ΔG polymorphism is not associated with the viro-immunological markers of HIV-1 infection.We showed, for the first time to our knowledge, that the production of IFNλ4 does not affect expression levels of ISGs in HIV-1 positive patients.

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EXOCRINE PANCREATIC INSUFFICIENCY (EPI) IN CYSTIC FIBROSIS PATIENTS: A 'FUNCTIONAL METAGENOMICS' APPROACH

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Introduction: Cystic fibrosis (CF) is an autosomal recessive disorder affecting the exocrine glands throughout the body, characterized by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. In about 80-90% of CF patients the pancreas is affected, leading to exocrine pancreatic insufficiency (EPI). Pancreas ducts are obstructed and pancreatic enzymes may not reach the intestines to digest food, and gut bacteria could be affected in their levels. The aim of the present study was to evaluate the correlation between severity of pancreas symptoms, fecal microbiota composition and fecal metabolomics profiles.
Materials and Methods: Thirty-six FC patients (16 with pancreatic insufficiency, PI, and 20 with pancreatic sufficiency, PS) underwent fecal sampling. Microbiota composition was analyzed by 16S rDNA sequencing (MiSeq), while fecal metabolites were profiled by SPME-GC/MS technique.
Results: Preliminary results obtained indicated that among CF patients exocrine pancreatic insufficiency condition strongly influence the composition of the fecal microbiota, and consequently the fecal metabolite profiles. The microbiota separation between the two studied groups is significant (Unifrac Weighted $p < 0.001$). In particularly the pancreatic insufficient group was characterized by the genera Acidaminococcus, a Firmicutes member recently associated to children with chronic malnutrition, while the genera Barnesiella and Alistipes, both anaerobic obliged bacteria belonging to Bacteroidetes, are genera characterizing the pancreatic sufficient group, already reported as decreasing genera after antibiotic treatment.
Discussion and Conclusion: Preliminary results indicate that within Cystic Fibrosis pathology the exocrine pancreatic insufficiency strongly effect the microbiota composition, and consequently all the related microbiota influenced factors should be affected too. This will have consequences in the severity of the pathology, consequences that could be avoided by interventions on the microbiota composition.

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EFFECTS OF TOTAL ANTIGENS OF *DICROCOELIUM DENDRITICUM* ON HUMAN HEPATOCARCINOMA CELLS

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Introduction: The relationship between *Dicrocoelium dendriticum*, a zoonotic helminth, and cancer has been poorly investigated so far, but a large amount of findings suggest that other trematodes can favour cancer in both animals and humans. The choice of *Dicrocoelium* as model for the present study is related to both the poor knowledge on its pathogenesis, very often masked by the frequent poliparasitism in ruminants, and to its high prevalence in sheep, goats, cattle and buffaloes of southern Italy and other regions of the Mediterranea area. The objective of this study is to investigate whether the trematode D. dendriticum may be involved in the neoplasia onset.
Materials and methods: In this study, the effects of *D. dendriticum* on cell proliferation, cell death mechanisms and oxidative stress induction were evaluated in hepatocellular carcinoma (HCC) cell lines (HepG2 and HuH7). Adults of *D. dendriticum* were removed from livers of sheep naturally infected by the trematode. Total antigenic extracts were obtained from adult worms. Analysis of cell proliferation was performed in the presence of somatic antigens of *D. dendriticum* by the MTT assay. The apoptosis, autophagy and oxidative stress was evaluated by flow cytometry analysis.
Results: Results showed that short time exposure to low concentrations of somatic antigens from D. dendriticum caused slight proliferation in both HepG2 and HuH7 cells while high concentrations and long exposure time to extracts from *D. dendriticum* caused a significant growth inhibition. This effect was, however, not paralleled by apoptosis but it occurred with an about 40% increase of the formation of autophagic vacuoles. In the same experimental conditions, a strong oxidative stress was recorded with an about 100% increase of the intracellular O^{2-}.
Discussion and conclusions: These data show the occurrence of an escape anti-apoptotic mechanism in HCC cells. In conclusion, these results suggest a role for D. dendriticum in the chronic oxidative stress and in the regulation of transformation processes in HCC warranting additional investigations in this specific area of research.

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INTESTINAL COLONIZATION BY KPC-PRODUCING *KLEBSIELLA PNEUMONIAE*: INVESTIGATION OF THE RELATIONSHIPS BETWEEN HUMAN MICROBIOME, COLONIZATION AND ANTIBIOTIC THERAPY

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Introduction: The human microbiome is responsible for a number of critical functions within the host, including resistance to colonization by pathogens. Infection by multidrug resistant pathogens is often preceded by gut colonization and carriers are at increased risk of infection. The recent dissemination of KPC carbapenemase-producing *Klebsiella pneumoniae* (KPC-Kpn) is a matter of major concern due to the limited treatment options and high mortality rates. Carriers play a major role in the dissemination of this pathogen and are a major target for infection control practices. At present, little is known about the connection between the gut microbiota and colonization by KPC-Kpn. Here we report on the analysis of microbiome structure in relation to KPC-Kpn gut colonization in a cohort of critically ill patients from an intensive care ward.
Materials and Methods: DNA was extracted from 17 fecal samples using the MoBio Powersoil DNA Isolation Kit. Amplicons of the V3-V4 bacterial 16S rRNA region were amplified and sequenced using an Illumina MiSeq platform according to Illumina specific protocols. Sequences were analysed using the QIIME pipeline (version 1.9.1). Microbiome diversity was estimated using QIIME default parameters and similarity between different categories of samples was evaluated on UniFrac distance matrix using the ANOSIM test.
Results: A high degree of variability was observed

among the patients gut microbiome composition. When compared on the basis of antibiotic treatment, changes in the overall diversity and structure of the gut microbiome could be observed in both KPC-Kpn colonized and KPC-negative patients; an increase in the relative abundance of *Enterobacteriales* was observed in patient subjected to antibiotic treatment, with *K. pneumoniae* proliferation playing a relevant role in KPC-Kpn colonized patients. The microbiome structure of treated and untreated patients was apparently also affected by KPC-Kpn colonization, resulting in different clustering of samples.
Discussion and Conclusions: Although only a limited number of samples were analysed, modifications of the overall microbiome structure are apparently associated with KPC-Kpn colonization.

P 119A

PEPTIDE-SPECIFIC T HELPER CELLS IDENTIFIED BY MHC CLASS II TETRAMERS DIFFERENTIATE INTO SEVERAL SUBTYPES UPON IMMUNIZATION WITH CAF01 ADJUVANTED H56 TUBERCULOSIS VACCINE FORMULATION

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Introduction: CD4+ T-cell priming is an essential step in vaccination due to the key role of T helper cells in driving both effector and memory immune responses. Here we have characterized the antigen-specific T helper cell activation and subtype differentiation upon subcutaneous immunization with the tuberculosis vaccine antigen H56 plus the adjuvant CAF01.
Materials and Methods: C57BL/6 mice were primed and boosted by the subcutaneous route with H56 plus CAF01 and the antigen-specific CD4+ T cell response was studied by using MHC class II tetramers. CD4+ T cell responses elicited by primary immunization or recalled by booster inoculation were characterized in draining lymph nodes, spleens and lungs, and differentiation of tetramer-positive T cells into different T helper subtypes was performed by multiparameter flow cytometric analysis.
Results: Tetramer-specific CD4+ T cells differentiated into several T helper subtypes upon antigen encounter and the frequency of subpopulations differed according to their localization. Th1 (CXCR3+T-bet+), Tfh (CXCR5+PD-1+Bcl-6+) and RORgt+ cells were induced in draining lymph nodes, while Th1 cells were the predominant subtype in the spleen. In addition, CD4+ T cells co-expressing multiple T-cell lineage-specifying transcription factors were also detected. In the lungs, most of the tetramer-binding T cells were RORgt+, while Tfh and Th1 cells were absent. After boosting, a higher frequency of tetramer-binding cells co-expressing the memory markers CD44 and CD127 was detected compared to primed cells, and tetramer-positive T cells showed a prevalent Th1 phenotype in both lymph nodes and spleens, while Tfh cells were significantly reduced.
Discussion and Conclusions: These data demonstrate that parenteral immunization with H56 and CAF01 elicits a distribution of antigen-specific CD4+ T cells in both lymphoid tissues and lungs, and gives rise to multiple T helper subtypes, that differ depending on localization and following reactivation.

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BOVINE LACTOFERRIN AFFECTS INTRACELLULAR SURVIVAL OF ADHERENT-INVASIVE *ESCHERICHIA COLI* IN CACO-2 CELLS

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Introduction: The iron-binding glycoprotein lacto-

ferrin (Lf) has many biological functions including antimicrobial, anti-cancer, antioxidant and immunomodulatory effects from inhibition to promotion of inflammation. High levels of cytokines, such as interferon (IFN)-γ and an increased expression of inducible nitric oxide synthase (iNOS) were showed in Crohn's disease (CD) , an inflammatory bowel disease characterized by deregulate mucosal immune response to gut microbiota. It has been suggested that nitric oxide (NO) and/or its redox products are an important component of the intestinal epithelial cell response to microbial infection and up-regulation of iNOS by enteroinvasive bacteria can modulate chloride secretion and barrier function in intestinal epithelial cells. In a previous study we have demonstrated that bovine lactoferrin (bLf), added during the infection period, inhibited the intracellular survival of adherent-invasive *Escherichia coli* (AIEC) LF82, a new pathotype detected more frequently within mucosal lesions of patients with Crohn's disease (CD). In order to clarify a possible mechanism of action of bLf, in this study we evaluated the relationship among bLf , apoptosis, NO production and AIEC LF82 intracellular survival on Caco-2 cells.

Materials and Methods: Differentiate Caco-2 were pre-stimulated or not with bLf and IFN-γ at 12h and at 48h respectively, and infected at multiplicity of infection of 10:1. After infection apoptosis, NO levels and cytokine concentrations were evaluated by fluorescence microscopy, Griess reaction and ELISA assay respectively.

Results: We found that bLf pretreatment of cell monolayers significantly reduced the LF82 invasion and survival ability maintaining this effect over time ,without inducing apoptosis. Moreover bLf was not able to modulate significantly NO production. Regarding cytokines production, we found that pretreatment cells with bLf significantly reduced IL-6 and IL-8 only in synergy with INF-γ.

Conclusions: Our preliminary data suggest that bLf, if added 12h before infection, is able to activate intracellular antimicrobial mechanism(s) independent from NO production.

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"FUNCTIONAL METAGENOMICS" APPROACH IN CIRRHOTIC HEPATITIS PATIENTS

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Introduction: Liver and gut are interconnected by the hepatic portal system, which accounts for the 75% of blood reaching the liver. Such a highway collects metabolites and bioproducts deriving from the resident gut microbiota, which, in turn, could exert an effect on liver physiology. Cirrhotic Hepatitis (CH) patients present both an alteration of intestinal motility and pH, which, together with the reduction of antimicrobial activity of bile acids, may lead to an imbalance of the intestinal microbiota. Dysbiosis is always linked to altered metabolic profiles. The present study was aimed at evaluating the mucosal and fecal microbiota and metabolic profiles in CH patients, compared to controls.

Materials and Methods: CH patients and controls were enrolled and underwent fecal and colonic biopsy sampling. 14 fecal samples from controls, and 31 fecal samples from CH patients were collected. While six biopsy samples were collected from control, and 15 from CH patients. Microbiota structure was studied by 16S rDNA metagenomic sequencing (MiSeq). Fecal metabolites were profiled with NMR. Blood microbiota was also sequenced through MiSeq technology.

Results: Preliminary results confirm an unbalance in the microbiota structure in both feces and biopsy samples, with significant increase of potentially pathogen genera. Also ecological index (Ace, Gini and Margalef) revealed a gut microbiota unbalance. NGS analysis of the blood samples confirmed a bacterial translocation, and blood ELISA assays (TNFa, IL6, IL1b, IL10) showed a positive correlation among IL6 and CH disease indexes.

Discussion and Conclusions: Our results represent

a novel 'functional metagenomics' approach in CH disease, constituting an important step to provide new therapeutic targets to prevent infections and other complications of cirrhosis and it would help to correct / prevent pathophysiological processes.

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ASSESSMENT OF BACTERIAL INFECTION IN CHRONIC WOUNDS OF GERIATRIC PATIENTS

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Introduction: Aerobic and anaerobic bacteria play an active and critical role in wound pathogenesis leading to progression towards chronicity. The debridement is essential for the wounds healing and surgical debridement of the necrotic tissue is still considered the gold standard. However, surgical intervention can be painful and non selective. A variety of mechanical methods for wound debridement are being evaluated and one of the most promising is the hydro-surgery system (VERSAJET). The aim of this study was to evaluate the hydro-surgery VERSAJET system as a suitable alternative to the traditional invasive tissue sampling technique in detecting bacteria and their load in chronic wounds in the elderly.
Materials and Methods: We simultaneously performed on 19 affected patients a deep tissue biopsy and tissue collections by the VERSAJET hydro-surgical system. After local cleaning and anesthesia, a deep biopsy was performed with a punch of 3-4 mm in diameter. Subsequently, three tissue samples were collected by the VERSAJET system: one from the first washing; one from the second washing; one from the third washing as a control procedure. After treatment, all tissue samples were cultured *in vitro* for diagnostic and microbiological assessment.
Results: 19 patients with chronic wounds of the lower limbs were enrolled and concordance between deep tissue biopsy cultures and tissue cultures collected by the VERSAJET system was examined. The deep tissue biopsy cultures showed as follows: 2 patients (11%) for the first washing sample; 10 patients (53%) for the second washing sample; 4 patients (21%) for the third washing sample.
Discussion and Conclusions: In conclusion, this study shows that the hydro-surgery VERSAJET system is sufficiently effective in detecting bacteria and their load in chronic wounds and can be a potential alternative to a biopsy.

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VITAMIN D MODULATES CYTOKINES EXPRESSION IN HUMAN GINGIVAL FIBROBLASTS AND PERIODONTAL LIGAMENT CELLS

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Introduction: *Porphyromonas gingivalis* implicated as a etiologic agent in the initiation and progression of periodontitis, produces several virulence factors that stimulate human gingival fibroblasts (HGF) and human periodontal ligament cells (HPL) to produce various inflammatory mediators. *Streptococcus pyogenes*, colonizer of the mucosal layers in the mouth, nose, and pharynx is able to either inhibit or evade innate host responses. Vitamin D needed to affect the metabolism of calcium and phosphate, has been associated with varied regulatory effects on cell proliferation and differentiation, especially in the immune system.
Materials and methods: In the present study we used an HGF and HPL model infected with *P. gingivalis* and *S. pyogenes* to simulate the *in vivo* conditions such as those found in diseased periodontal sites. We have examined *in vitro* the effects of exogenous active form of vitamin D3(1a,25[OH]2D3) on antibacterial activity against *P. gingivalis* and against *S. pyogenes*. In addition we investigated the

effect of the vitamin D on the inflammatory immune response of cultured HGF and HPL cells infected with bacteria with respect to expression of IL-8, IL-12 and IL-10 by RT-PCR and ELISA assays.
Results: The results showed that vitamin D increased the viability of HGF or HPL cells in culture, as demonstrated by MTT, and inhibited the growth capacity of *P. gingivalis* and *S. pyogenes* on both cultures cell. Our data demonstrated that the pro-inflammatory cytokines IL-8 and IL-12 are produced by HGF and HPL cells infected with *S. pyogenes* and *P. gingivalis* while the pre-treatment with vitamin D strongly reduced their production; while HGF and HPL cells challenged with bacteria showed a low level of anti-inflammatory cytokine IL-10 production, and the expression of this cytokine was up-regulated in cells pre-treated with vitamin D, suggesting that this substance may prevent periodontal tissue destruction, and alveolar bone resumption.
Discussion and Conclusions: We demonstrated the vitamin D treatment improve the inflammatory responses of HGF and HPL cells to *P. gingivalis* and *S. pyogenes* infection. Our data suggest a therapeutic role for vitamin D in clinical conditions in which the role of cytokines is central.

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AUTISM SPECTRUM DISORDERS (ASDS)

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Introduction: Autism Spectrum Disorders (ASDs) are complex neuro-developmental disorders characterized by cognitive defects, social interaction skills impairments and communication, language and behavioural problems. ASDs are thought to arise from the interaction of genetic and environmental factors. Their pathogenesis is still unclear, however several biochemical events were described as associated with ASDs, as well as many gastrointestinal (GI) dysfunctions. Although several studies have been conducted on the bacterial microbiota identification in ASDs, to our knowledge few studies were focalized on fungal/yeast infections in ASDs.
Our main interest was to examine the fecal fungal and bacterial microbiota in autistic children; for this reason we used a culture based approach to evaluate yeast and bacterial composition of ASD subjects and their correlation to intestinal permeability and inflammation.
Materials and Methods: Forty-seven subjects with ASDs (40 boys and 7 girls with a mean age of 6.0 ± 2.8 yr), were recruited in clinical departments of the University and 33 healthy children (24 boys and 9 girls with a mean age of 7.3 ± 3.1 yr) were randomly recruited. In this study, for each fecal sample: a) morphological examination, b) microscopic examination by means of Gram and May-Grünwald Giemsa staining, c) search for toxins A/B of *Clostridium difficile*, d) bacterial/yeast culture and e) biochemical identification of the colonies were performed.
Results: Main results obtained are: a) presence of *Candida* spp in a large percentage (31,9%, high titers) of ASDs as compared to controls (6,1%, low titers); b) absence of *C. difficilis* detected by culture and toxins production in both ASDs and controls; c) *Lactobacillus* spp detection in 15 ASDs patients (31,9%) as compared to controls (66,7%); d) alteration of IP in 42,4% of ASDs as compared to 18,2% of controls ($p = 0,0121$).

Discussion and Conclusions: High frequency of gastrointestinal *C. albicans* infection in ASD subjects was shown. Changes of microbiota composition were shown as well in that ASD stool samples are less rich of microbial species than controls. Low-mild gut inflammation and augmented intestinal permeability were confirmed. The present data provide rationale basis to a possible specific therapeutic intervention in restoring gut microflora in ASDs.

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LACTOBACILLUS CRISPATUS MEDIATES ANTI-INFLAMMATORY CYTOKINE INTERLEUKIN-10 INDUCTION IN RESPONSE TO *CHLAMYDIA TRACHOMATIS* INFECTION *IN VITRO*

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Introduction: *Chlamydia trachomatis* is a known intracellular parasite. It is the most common cause of bacterial sexually transmitted infections in both industrialized and developing countries. Lactobacilli play a crucial role in protecting the genitourinary tract against pathological conditions and can act through the activation of the immune system, but *C. trachomatis* is able to effectively evade immune surveillance in some individuals. *Lactobacillus crispatus* is an important urogenital species that is routinely found in the vagina of healthy women.
Materials and Methods: We investigated the immunomodulatory efficacy of the potential probiotic strain *L. crispatus* in HeLa and J774 cells subjected to *C. trachomatis* infection by studying the expression of the inflammatory cytokines IL-6, IL-8, TNF-α and IL-10 by an enzyme-linked immunosorbent assay (ELISA) and by RT-PCR.
Results: Our results demonstrated the lack of any cytotoxic effect on the epithelial cells and macrophages when treated with *L. crispatus* and its supernatant; but *L. crispatus* and its supernatant were able to inhibit *C. trachomatis* adhesion and infectivity in human epithelial cells and macrophages. Our study also showed that *L. crispatus* and its supernatant reduced IL-6 and IL-8 and TNF-α production in HeLa and J774 cells *C. trachomatis*-infected. In contrast, a significant upregulation of the IL-10 expression in HeLa and J774 cells by *L. crispatus* and supernatant was also demonstrated. Our data indicate that *L. crispatus* specifically enhances the production of the IL-10 anti-inflammatory cytokine in contrast to the inhibitory effect of *L. crispatus* on the pro-inflammatory cytokines.
Discussion and Conclusions: In conclusion, our results clearly indicated that the production of the IL-10 in HeLa and J774 cells enhanced by *L. crispatus* plays a crucial role in the prophylactic effect on *C. trachomatis* infection by inhibiting the expression of IL-6, IL-8 and TNF-α cytokines in the host cells, which suggests a potential mechanism through which *L. crispatus* protects against pathological inflammatory conditions.

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ZOLEDRONIC ACID MODULATE CYTOKINE PRODUCTION AND CYTOTOXIC EFFECTS OF *CHLAMYDIA PNEUMONIAE*

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Introduction: *Chlamydia pneumoniae* is an obligate intracellular pathogen that causes persistent infections with a tendency to chronicize. The bisphosphonates are an emerging class of drugs mostly used in the palliative care of cancer to inhibit proliferation and metastasis of cancer cells but their role in modulating immune responses remains unknown. The bone is a living tissue that is under constant remodeling by osteoclasts and osteoblasts. Recent observations suggest that activated osteoblasts might also serve as a source of soluble inflammatory mediators. The production of high levels of inflammatory mediators may perpetuate inflammation, with consequent bone destruction.
Materials and Methods: We investigated the *in vitro* activity of a highly potent bisphosphonate, zoledronic acid (ZA), on the cytotoxic effects of *C. pneumoniae* in human SaOS-2 osteoblast-like cells and whether this treatment modulates the se-

cretion of interleukin (IL)-6, IL-8, IL-12 and tumor necrosis factor alpha (TNF-α). The concentrations of cytokine secreted were determined in the culture supernatants by an enzyme-linked immunosorbent assay (ELISA) and by RT-PCR.
Results: We have reported that ZA showed a significant antiproliferative effect on SaOS-2 cell line infected by *C. pneumoniae* in a time- and dose-dependent manner. We have also found that ZA exhibited a significant increase of chlamydial growth inhibition. Our data showed that *C. pneumoniae*-infection of SaOS-2 cells induced a significant gene expression of proinflammatory cytokines TNF-α, IL-6, IL-8 and IL-12, detected by RT-PCR, and confirmed by protein release assay. We have for the first time ascribed to ZA an active anti-infective role versus Chlamydia infection, consisting both in a direct inhibition to form inclusions, and in a consequent increased production of pro-inflammatory cytokines, mostly responsible for the innate immune response.
Discussion and Conclusions: The observed anti-chlamydial activity and the potential of ZA as a positive regulator in anti-infection immunity, indicate that ZA can offer a new potential application for the treatment and prophylaxis of *C. pneumoniae* infections, thus reprofiling this traditional anti-tumor drug with an additional therapeutical indication.

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BARTONELLA HENSELAE MODULATES THE SECRETION OF CYTOKINES BY HUMAN MESENCHYMAL STROMAL CELLS

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Introduction: *Bartonella henselae* causes different human diseases, the most prominent being cat scratch disease, a persistent, necrotizing lymphadenitis characterized by a typical granuloma that manifests in immunocompetent patients. Instead, in immunocompromized patients *B. henselae* can cause bacillary peliosis hepatis and bacillary angiomatosis.
Endothelial cell infection represents an important step in the pathogenesis of cat scratch disease and bacillary angiomatosis. Experimental studies in a rat model showed that a brief bacteremia is followed by a 3-5-day bacteremia-free window before resurgence of persistent intraerythrocytic bacteremia that lasts for 8-11 weeks. This model highlights the possible existence of a sanctuary site where the initial infection gets established before persistent intraerythrocytic infection manifests.
Mesenchymal stromal cells (MSC) are multipotent progenitor cells that can differentiate into various cell lineages such as osteocytes, condrocytes, and adipocytes, and have a proven ability to augment the neovascularization processes. Additionally, MSC also have antimicrobial proprieties and recently their role in the persistence of viable non replicating *M. Tuberculosis* has been established.
The aim of this study was to determine the effect of *B. henselae* on the release of pro-angiogenic and pro-inflammatory cytokines by MSC, which could be possible candidates as reservoir cells.
Materials and Methods: Human adipocyte-derived mesenchymal stromal cells (Ad-MSC) isolated from adipose tissue obtained by lipoaspiration from healthy donors were stimulated with different MOI of *B. henselae* for 24-96 hr. A *B. henselae*-conditioned medium (BCM) prepared after cultivation of bacteria for 24 hr in medium without antibiotics was used to stimulate MSC. Desferrioxamine was used as positive control of hypoxia induction. VEGF and IL-8 secretion upon *B. henselae* infection was measured by ELISA kits.
Results: Infection of MSC with *B. henselae* resulted in an increase of VEGF and IL-8 secretion in a dose- and time-dependent manner. Moreover, the BCM increased the cytokine release in a dose-dependent manner.
Discussion and Conclusions: Our results indicate that *B. henselae* induces the production of pro-angiogenic factors in MSC. The effect may be due to secretion of endothelial cell-stimulatory factor(s). These cells could represent a potential habitat of *B. henselae* trough their differentiation into cells with phenotypic and functional features of endothelial cells.

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CHLAMYDIA PNEUMONIAE CLINICAL ISOLATE FROM GINGIVAL CREVICULAR FLUID: A POTENTIAL ATHEROGENIC STRAIN?

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Introduction: *C. pneumoniae*, a respiratory pathogen, has acquired a great importance as a risk factor for cardiovascular disease (CVD), contributing to the atherosclerotic process.
Recently, chronic periodontitis, an inflammatory disorder of the periodontium, has been described as a predisposing factor for atherosclerotic CVD. In fact, the periodontal pathogens responsible for the peridontitis, upon the destruction of the epithelial barrier, may translocate into the systemic circulation where they contribute to endothelial disfunction. Here we isolated and investigated the molecular and phenotypical characteristics of a *C. pneumoniae* strain, for the first time, from the gingival crevicular fluid of a patient with chronic periodontitis.
Materials and Methods: Gingival crevicular fluid sample was analyzed by quantitative real-time PCR and cell culture for the detection of *C. pneumoniae*. The molecular and phenotypical characteristics of clinical isolate were examined by VD4 ompA gene typing and evaluating the intracellular growth in epithelial and macrophage cells. The atherogenic features of clinical isolate were evaluated by macrophage-derived foam cell determination. A reference strain of *C. pneumoniae* (AR-39), associated to CVD, was included for molecular and phenotypical comparison.
Results: An average of 142950 copies of *C. pneumoniae* DNA/µl of sample and 50 chlamydial inclusions were detected in the gingival crevicular fluid. The *C. pneumoniae* clinical isolate showed a 99% similarity in VD4 ompA gene sequence and a similar growth kinetic in epithelial and macrophage cellscompared to the AR-39 strain, as evidenced by the number of inclusions and genomic copies. More importantly, *C. pneumoniae* clinical isolate induced foam cell formation in macrophages, first step of the atherosclerotic process.
Discussion and Conclusions: The isolation of a potential atherogenic strain of *C. pneumoniae*, from the gingival crevicular fluid of a patient with chronic periodontitis, suggests that *C. pneumoniae* may harbor within the oral cavity and participate to the initiation and/or development of chronic periodontitis, leading to tissue damage. Therefore, we can hypothesize that *C. pneumoniae* may disseminate through monocyte-macrophages and localize to the vascular wall, contributing to the atherosclerotic process.

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CHLAMYDIA PNEUMONIAE-MEDIATED INFLAMMATION IN ATHEROSCLEROSIS: A META-ANALYSIS

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Introduction: In the last decades, several infectious agents have been related to the pathogenesis of cardiovascular diseases and current opinion is that the most implicated pathogen is *Chlamydia pneumoniae*, an obligate intracellular microorganism, responsible for respiratory tract infections. *C. pneumoniae*, able to persist within the host for a long time and lead to chronic infection, seems to be responsible for inducing a systemic inflammatory state. Several studies have attempted to relate the *C. pneumoniae*-mediated inflammation with atherosclerotic cardiovascular disease, providing inconsistent results. We performed, therefore, a meta-analysis to clarify whether *C. pneumoniae* may contribute to the pathogenesis of atherosclerotic cardiovascular diseases by the means of enhanced inflammatory state.
Materials and Methods: We performed a systematic search of all articles in journals indexed on the electronic databases PubMed and

Scopus up to December 2014. Odds Ratio with a 95% confidence interval was used to assess the seroprevalence of *C. pneumoniae* and differences between levels of inflammatory markers were assessed by standard mean differences. Potential publication bias was performed, using Egger's linear regression test, to ensure the statistical power. Sensitivity analyses were assessed by deleting each study, in all cases pooled estimates were very stable.
Results: 12 case control, 6 cross-sectional and 7 prospective studies with a total of 10,176 patients have been included in this meta-analysis. High-sensititvity c-reactive protein (hsCRP), fibrinogen, interleukin (IL)-6, tumoral necrosis factor α and interferon γ showed a significant increase in patients with atherosclerosis compared to healthy controls ($p < 0.05$), along with a higher seroprevalence of *C. pneumoniae* (OR of 3.11, 95% CI: 2.88-3.36, $p < 0.001$). More interestingly, hsCRP, IL-6 and fibrinogen levels were significantly higher in *C. pneumoniae* IgA seropositive compared to seronegative atherosclerotic patients ($p < 0.0001$). Publication bias was detected only for meta- analysis of fibrinogen with IgA ($p = 0.012$).
Discussion and Conclusions: Our meta-analysis suggests that *C. pneumoniae* infection may contribute to atherosclerotic cardiovascular diseases by enhancing the inflammatory state, as demonstrated by increased levels of systemic inflammatory markers. Furthermore, seropositivity to *C. pneumoniae* IgA, together with hsCRP, fibrinogen and IL-6, may be predictive of increased atherosclerotic cardiovascular risk.

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TOWARD OPTIMIZATION OF A BMAP-DERIVED ANTIMICROBIAL PEPTIDE FOR THE TREATMENT OF PULMONARY INFECTIONS CAUSED BY *PSEUDOMONAS AERUGINOSA* IN CYSTIC FIBROSIS PATIENTS

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Introduction: BMAP-27_{1-18} is a shortened form of the α-helical bovine myeloid antimicrobial peptide BMAP-27. It is active against *P. aeruginosa* strains from cystic fibrosis (CF) patients (MIC_{90}: 16 μg/ml, on 15 strains), and it is well tolerated when intra-tracheally administered to healthy mice[1]. Aim of this study was to evaluate the potential of BMAP-27_{1-18} for the development of novel drugs to treat CF lung infections.
Materials and Methods: *In vivo* efficacy of BMAP-27_{1-18} was evaluated in C57BL/6NCrl mice by single intratracheal administration of different concentrations of the peptide immediately after exposure to *P. aeruginosa* RP73 strain. Peptide stability in murine bronchoalveolar lavage (BAL) was evaluated by SDS-PAGE. Peptide synthesis was performed using solid-phase Fmoc chemistry method, followed by purification by reversed phase HPLC. MICs were determined according to EUCAST guidelines.
Results: BMAP-27_{1-18} showed no significant protection against *P. aeruginosa* lung infection, regardless of doses used. This is likely due to the fact that it was rapidly degraded (within 10 minutes) when exposed to BAL. To overcome this problem an all-D isomer of BMAP27_{1-18} was synthesized. This peptide resulted to be significantly more resistant to pulmonary proteases, being not degraded in BAL even after one week, and showed an antimicrobial activity against *P. aeruginosa* (strain RP73; MIC: 4 μg/ml) comparable to the all-L isomer.
Discussion and Conclusions: Despite its relevant *in vitro* antimicrobial activity, BMAP27_{1-18} does not show protective effect in a murine model of lung

infection because it is rapidly degraded by pulmonary host proteases. The D-BMAP-27_{1-18} represents a promising improvement of the original peptide, since it is resistant to proteolytic cleavage although preserving *in vitro* antimicrobial activity. Further *in vitro* and *in vivo* studies are now ongoing to evaluate the protective effect and the safety of D-BMAP-27_{1-18}.

Reference
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GUT MICROBIOTA ANALYSIS IN CHILDREN WITH EARLY AUTISM

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Introduction: Gut microbiota regulates many brain functions and affects behavioural phenotypes. Preliminary evidences showed alterations of gut microbiota in children affected by autism. Using high resolution metagenomic analysis we analysed key features of gut microbiota profiles of autistic children at early diagnosis.
Materials and Methods: Metagenomic analysis was performed with MiSeq, Illumina® platform on stool samples from 7 autistic children (age 2-4 years) and 7 age matched healthy controls. After amplification and sequencing of targeted V3 and V4 regions of r16S gene, the number of sequences and operational taxonomic units (OTUs) for each sample were calculated using Quantitative Insights Into Microbial Ecology (QIIME) and tassonomic classification on Greengenes database was assessed. Species richness and diversity within communities (alpha-diversity) and diversity shared across microbial communities (beta-diversity) were also determined.To identify key OTUs, that discriminated between autistic and control groups, Metastats comparison and Galaxy platform-based LDA Effect Size analysis were used.
Results: A mean of 195.000 reads were obtained for each sample and 61 of the 4.601 OTUs detected across any of the samples were found to discriminate between the two groups. The Weighted UniFrac analysis clearly showed a clusterization of autistic and control groups, revealingmicrobial community differences due to changes in relative taxon abundance.
Discussion and Conclusions: Our results evidenced significant differences in gut microbiota composition in young autistic children. These results pave the way to a better understanding of gut microbiota role in autism pathogenesis, and suggest future diagnostic and therapeutic targets.

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GLUTATHIONYLATED PROTEINS RELEASED IN INFLAMMATION AND INFECTION

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Introduction: Glutathionylation, i.e. formation of mixed disulfides between protein cysteines and glutathione (GSH), is a mechanism by which the redox state of the cell regulates protein functions. So far, most studies on identification of glutathionylated

proteins focused on intracellular proteins.
Materials and Methods: We used a redox proteomic technique based on preloading the cells with biotinylated GSH followed by affinity purification on streptavidin and mass spectrometry, to identify glutathionylated proteins in the secretome of macrophages stimulated with lipopolysaccharide (LPS). We confirmed the LPS-induced secretion of a selected set of proteins by Western blot with specific antibodies. We also used immunoprecipitation followed by Western blot with anti-GSH antibody to confirm that the secreted proteins were glutathionylated.
Results: We found that LPS induced the secretion of peroxiredoxin (PRDX)1, PRDX2, vimentin (VIM), profilin1 (PFN1) and thioredoxin1 (TXN1). Moreover, we were able to confirm that the released PRDXs and TXN1 are glutathionylated. The release of the proteins identified was inhibited by the anti-inflammatory drug, dexamethasone (DEX) and by the thiol antioxidants N-butanoyl GSH derivative (GSH-C4) and N-acetylcysteine (NAC). We found that PRDX1, 2 and TXN1 are also released by pulmonary epithelial cells infected with influenza virus. For PRDX2, we showed that once released, it stimulated macrophages to produce and release TNF-a, acting as a danger signal.
Discussion and Conclusion: Proteins released under this specific form of cysteine oxidation may then have immunomudulatory activities. They could be useful as biomarkers of oxidative stress associated with infection or inflammation.

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ROLE OF REDOX-REGULATED INTRACELLULAR PATHWAYS IN CONTROLLING INFLUENZA VIRUS ENTRY

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Introduction: Several data have reported that specific steps of influenza virus life-cycle are favored by the activation of different redox-sensitive intracellular pathways. In particular, we have recently demonstrated that the nuclear export of nucleoprotein is promoted by reactive oxygen species (ROS) produced by NOX4, a member of NADPH family (NOX). It is known that influenza virus (IV), following receptor binding, enters into the host cells through endocytosis and then the viral envelope fuses with the endosomal membrane for releasing the viral genome into the cytosol. Interestingly, some members of NOX are localized on the membrane of endocytic vesicles. While the importance of virus-cell surface receptor interaction for fusion and entry is established, the role of redox-regulated cell pathways in regulating this process is still not clear. Therefore, in the present study, we investigated in lung epithelial cells, the redox-mediated mechanisms involved in influenza virus endocytosis.
Results: We found that 2 to 10 minutes after viral challenge, IV significantly increased the intracellular levels of ROS. This ROS over-production was efficiently inhibited by addition to infected cells of the NADPH-oxidases inhibitor diphenyl iodonium (DPI), thus indicating a significant contribution of these enzymes to virus-induced oxidative stress. Any significant changes in intracellular GSH content were registered. ROS levels increase was associated with phosphorylation of p38 MAPK and of ERK1-2, while the treatment with DPI strongly inhibited the activation of the kinases thus suggesting that NOX-derived ROS activate p38 MAPK and ERK1-2. No activation of JNK was observed during the viral adsorption period. Importantly, the release of viral particles from infected cells treated with DPI only during the viral challenge was reduced 8 and 24 hours post infection compared to untreated ones.
Conclusion: Overall these data suggest a role for NOX-derived ROS family in activating redox-regulated intracellular pathways controlling influenza virus entry. Therefore, the discovery of molecules affecting host-cell factors essential for viral replication may help design therapies with greater effectiveness towards different virus types and lower probability that the infectious agents develop resistance.

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LOW GLUCOSE IN THE HOST CELL IMPAIRS INFLUENZA VIRUS REPLICATION

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Introduction: Metabolic conditions of host cell may shape viral infection. In fact, viruses use several cell pathways to their own advantage that are sensitive to modification of redox state, decrease of pH, as well as alteration of ATP or glucose levels. For example, the cellular vacuolar-type H^+ ATPase, an enzyme important for early steps of influenza virus replication, being linked to various glycolitic enzymes is regulated by glucose content.
The purpose of the present study was to assess whether and how the different contents of glucose affect influenza virus replication.
Methods: Human lung epithelial cells (A549) were plated at a density of $2x10^5$ in 24 multiwell dishes in DMEM medium containing different concentrations of glucose: DMEM high glucose containing 4500 mg/L glucose and DMEM low glucose containing 1000 mg/L glucose. The culture medium was supplemented with 10% fetal bovine serum. Cells were then infected with influenza virus A/PR8/H1N1 at different multiplicity of infection (m.o.i.) (1-10 mo.i.) and viral titer was measured in the supernatant of infected cells by hemagglutinin (HAU)/assay at various times after infection (24, 48, 72 hours).
Results: In the first set of experiments, cell viability was evaluated in cell maintained in the two different medium for the following 24, 48 and 72 hrs. Cells were detached by Trypsine and counted by Trypan blue assay. While no significant differences were observed after 24 hrs plating, a decrease in the number of vital cells was measurable in cells cultured with low glucose (reaching 80% of mortality at 72 hrs after plating). These results indicated that low glucose caused a slowed metabolism and death of cells in the time. Then, cells cultured for 24 hrs in the two medium were infected with different m.o.i. of influenza virus and viral titer was measured at 24-48 and 72 hrs. Results indicated that viral replication was higher in cells cultured with high glucose compared to that cultured with low glucose, suggesting that a slowed cell metabolism could interfere with some pathways involved in viral replication.
Conclusions: Overall the data obtained indicate that glucose levels affect the ability of influenza virus to replicate in the host cells. Further studies are in progress aimed at evaluating the role of oxidative stress in regulating cell metabolism.

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EVALUATION OF THE ANYPLEX™ PLUS MTB/NTM MDR-TB ASSAY FOR RAPID DETECTION OF *MYCOBACTERIUM TUBERCULOSIS* COMPLEX ISOLATES IN PULMONARY AND EXTRAPULMONARY SPECIMENS

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Introduction: The rapid and accurate diagnosis of symptomatic patients is the cornerstone of global strategies for tuberculosis control. Together with culture as the gold standard, molecular diagnostic methods based on PCR are broadly used for the early diagnosis of tuberculosis. An Italian multicenter study was performed to evaluate the performance of the Seegene Anyplex MTB/NTM kit, a new molecular method, based on multiplex real-time PCR system for the detection of the *Mycobacterium tuberculosis* (MTB) and non-tuberculosis mycobacteria (NTM).
Materials and Methods: A total of 755 samples were collected; 534 respiratory specimens and 221 extra-pulmonary specimens were tested, and the results were compared with those from smears and cultures.
Results: For pulmonary specimens, the sensitivities of Anyplex MTB/NTM and acid-fast smear were 86.4% and 75.0%, respectively. The specificities were 99% and 99.4%, respectively. For extrapulmonary specimens, the sensitivities of Anyplex

MTB/NTM and acid-fast smear were 83.3% and 50.0%, and the specificities were 100% and 100%, respectively. Based on an 11.6% incidence of TB in our population, the positive predictive values of Anyplex MTB/NTM and culture were 88.4% and 100%, respectively, and the negative predictive values were 98.8% and 100%, respectively.

Discussion and Conclusion: These results demonstrated that detection of *M. tuberculosis* complex in clinical specimens by Anyplex MTB/NTM is an efficient and rapid method for the diagnosis of pulmonary and extrapulmonary TB.

YOUNG RESEARCHERS AND MICROBIOLOGY

EPIGENETIC REGULATION OF POLYOMAVIRUS JC INVOLVES ACETYLATION OF SPECIFIC LYSINE RESIDUES IN NF-KB P65

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Introduction: Progressive multifocal leukoencephalopathy (PML) is a fatal demyelinating disease caused by Polyomavirus JC (JCV) lytic infection of CNS glial cells. After primary infection, JCV is controlled by the immune system but the virus persists asymptomatically. When immune function is impaired, it can rarely re-emerge to cause PML. The mechanisms of JCV persistence and reactivation are not well understood, but our earlier work implicated epigenetic control by protein acetylation, because histone deactelylase inhibitors such as trichostatin A (TSA) strongly stimulate JCV transcription. Since both TNF-alpha and TSA activate JCV transcription via the same unique NF-kB p65 binding site in the JCV control region, we investigated a role for acetylation of NF-kB p65 in JCV regulation.
Materials and Methods: A site-directed mutagenesis strategy was employed targeting the known lysine acetylation sites of NF-kB p65: K218, K221 and K310. We individually mutated each lysine (K) to arginine (R), which cannot be acetylated and retains a positive charge like lysine. Mutation of lysine to glutamine (Q) gives mutants with a negative charge like acetyl-lysine. Cell fractionation and gel shift studies were also performed.
Results: K218R and K221R were impaired in transactivation of JCV early promoter transcription either alone or combined with TSA treatment or co-expression of acetyltransferase transcriptional coactivator p300, but K310R was largely without effect. Although K218Q and K221Q showed impaired activity, K310Q showed enhanced transactivation. NF-kB p65 acetylation can regulate several aspects of the process of its activation including complex formation with IkB in the cytoplasm, translocation to the nucleus, DNA binding and transcriptional activation. Cell fractionation studies revealed that the mutants had no defect in translocation to nucleus whereas gel shift studies revealed reduced binding to the JCV NF-kB p65 site.
Discussion and conclusion: Acetylation regulates NF-kB p65 activity at the level of JCV DNA binding and transcriptional activation.

CLINICAL RELEVANCE OF HEPATITIS C VIRUS SEQUENCING FOR GENOTYPE AND SUBTYPE DETERMINATION IN THE ERA OF NEW DIRECT ANTIVIRAL AGENTS

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Introduction: A correct genotype and subtype assignment prior to treatment initiation is mandatory for the selection of the regimen containing Direct Antiviral Agents (DAAs). The aim of this study was to evaluate the concordance between commercial genotyping assays and HCV sequencing in the subtype/genotype assignment.
Materials & Methods: HCV sequencing of NS3-protease and/or NS5A/NS5B was performed by home-made protocols, specific for each genotype. Phylogenetic analysis was performed to eval-

uate appropriate genotype allocation and concordance with previous genotype/subtype assignment. Results: A total of 463 HCV-infected patients (pts) candidate to start a DAA-treatment who performed HCV sequencing test were analyzed to confirm the appropriate genotype allocation. HCV sequencing and commercial assays were concordant in 91.4% of cases. Indeed, HCV sequencing identified 7/463 genotypes discordant with the assignment given by commercial assays (commercial/sequencing: 1a/2c; 1a/3a; 1b/2c; 1b/2c; 1b/4d; 2a-2c/1b; 4/1b) and 14/463 discordant subtype cases (commercial/sequencing: 1a/1g[N=2]; 1a/1b[N=3]; 1b/1a[N=9]). Of interest, among two discordant pts, one, found infected with HCV-1g and carrying baseline NS3-mutation T54S, rapidly failed a boceprevir-containing regimen, while the other one found infected with HCV-4d (previously defined as HCV-1b) failed with resistance to daclatasvir+asunaprevir+ribavirin. Furthermore, 19/463 pts (4.1%) with a previous result of mixed (N=9) or indeterminate (N=2) or HCV-1 without subtype information (N=8) by commercial assays, were instead precisely resolved by HCV sequencing.
Discussion and conclusions: HCV sequencing allows a precise subtype and genotype assignment, along with the evaluation of drug-resistance. The relatively low cost of sequencing (compared to therapy) should encourage studies aimed at better defining the advantage of its use before starting therapy in clinical practice.

FIRST DESCRIPTION OF A HYPERMUCOVISCOUS *KLEBSIELLA QUASIPNEUMONIAE* SUBSP. *QUASIPNEUMONIAE* ISOLATE FROM A BLOODSTREAM INFECTION

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Introduction: *K. pneumoniae* is a major human pathogen causing hospital- and community-acquired infections. The latter also include invasive infections characterized by pyogenic liver abscesses with bacteraemia. Strains causing these infections generally exhibit a "hypermucoviscous" phenotype (Hv). Taxonomic studies have demonstrated that strains formerly classified in *K. pneumoniae* actually belong to two new species: *K. variicola* and *K. quasipneumoniae*. Extensive information are available for *K. pneumoniae* and *K. variicola* but data on virulence of *K. quasipneumoniae* isolates are lacking. We sequenced the genome of a *K. quasipneumoniae* strain characterized by a Hv phenotype. The strain was isolated from a Peruvian patient with biliary tract and bloodstream infection.
Materials and Methods: Genomic DNA was subjected to whole-genome sequencing with the MiSeq platform. In total 2,781,628 reads were obtained and were assembled in 84 scaffolds, using A5-miseq software. Identification at the species level was performed by *fusA, gapA, gyrA, leuS* and *rpoB* genes analysis. A screening for virulence genes present in

the BIGSdb-Kp database was performed using the BLASTn tool.
Results: The isolate was identified as *K. quasipneumoniae* subsp. *quasipneumoniae* and the screening for virulence genes revealed the presence of: i) a *allABCDRS* operon, for the allantoin assimilation; ii) the iron uptake system *kfuABC* and iii) the mannose-resistant *Klebsiella*-like fimbriae cluster, *mrkABCDFHIJ*. Interestingly, the allantoin operon was not present in the genome of the *K. quasipneumoniae* type strain (01A030). The strain possessed a new capsular wzi allele. The rmpA and rmpA2 genes, previously associated with the Hv phenotype in K. pneumoniae strains, were not found.
Discussion and conclusions: To the best of our knowledge this is the first description of a *K. quasipneumoniae* subsp. *quasipneumoniae* isolate with a Hv phenotype and a virulence genes content characteristic of hypervirulent *K. pneumoniae*.

ASPARTYL PROTEINASES OF *CANDIDA ALBICANS* INDUCE CASPASE-11: IMPLICATION IN PROMOTING INFLAMMATORY RESPONSE

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Introduction: We recently demonstrated that the secreted aspartyl proteinases (Sap), Sap2 and Sap6, of *Candida albican*s have the potential to induce the canonical activation of NLRP3- inflammasome leading to the secretion of IL-1β and IL-18 via caspase-1 activation. We also observed that the activation of caspase-1 is partially independent from the NLRP3 activation pathway. In this study, we examined whether Sap2 and Sap6 are also able to activate the noncanonical inflammasome pathway in murine macrophages.
Materials and methods: The activation of proteins involved in the canonical and noncanonical inflammasome pathway were determined by western blot analysis in murine macrophage cell line, RAW264.7, pretreated with specific inhibitors and stimulated with Sap2 and Sap6 for different times. Moreover, we evaluated cytokines production by Elisa assays in culture supernatants.
Results: Our data show that both, Sap2 and Sap6, can activate caspase-11 through type I IFN production. Caspase-11 concurs to activate caspase-1 with subsequent increase of IL-1β secretion. Endocytosis and internalization of Sap are required for the induction of type I IFN production, that is essential for induction of noncanonical inflammasome activation.
Discussion and Conclusions: Our study indicates a sophisticated interplay between caspase-1 and caspase-11 that connects canonical and noncanonical pathways of inflammasome activation in response to *Candida albican*s Sap.

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