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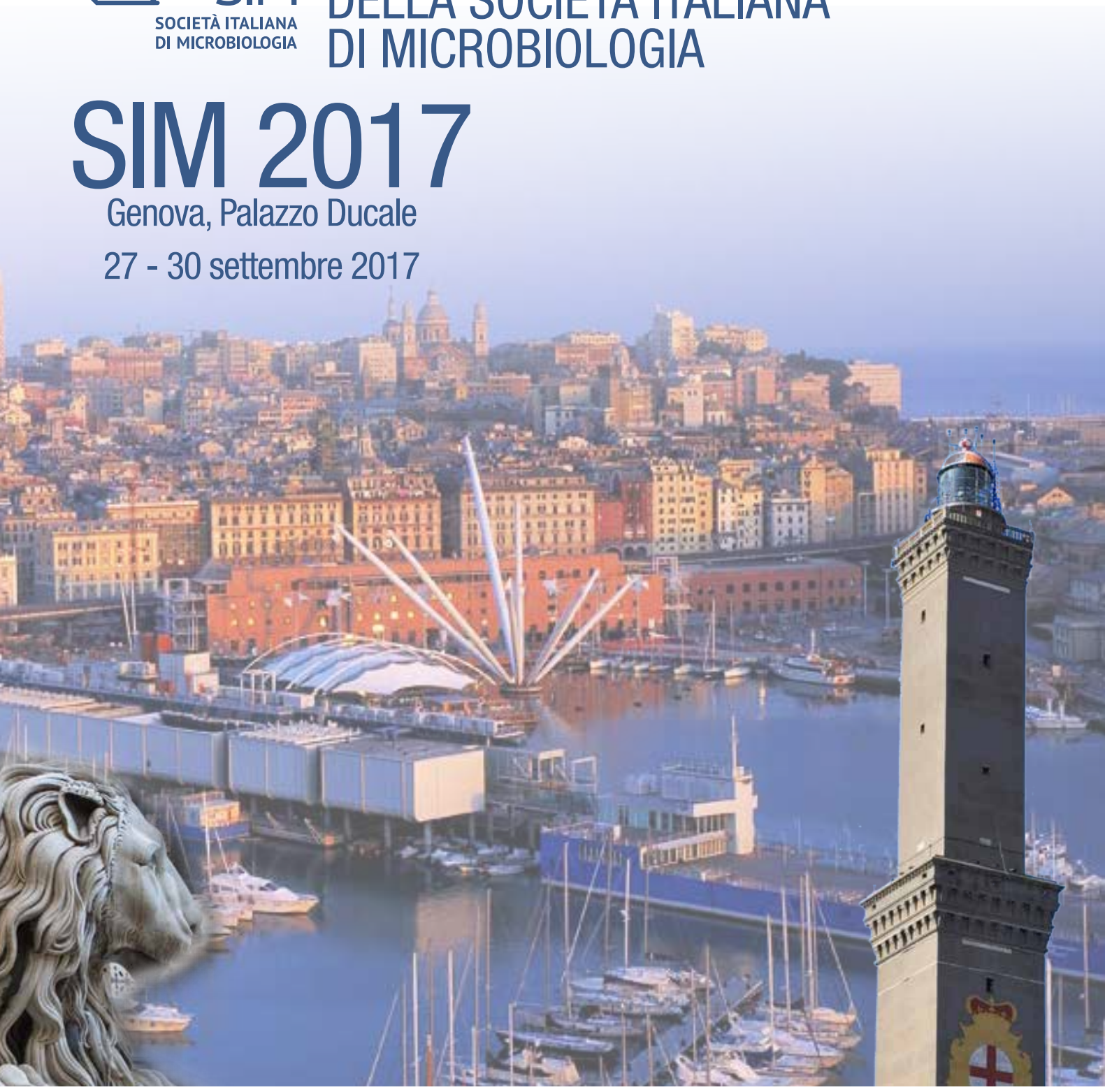

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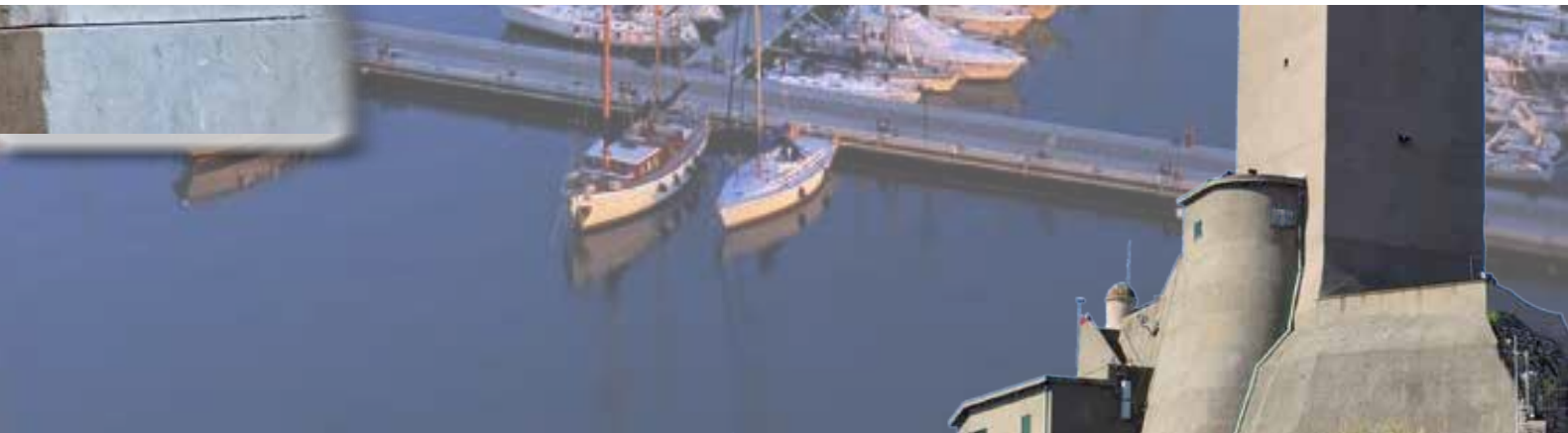

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INVITED LECTURES

PATHOGENS AND AUTOPHAGY

AUTOPHAGY: FROM THE DISCOVERY TO THE NOBEL PRIZE. HISTORICAL INTRODUCTION AND RELEVANCE TO INFECTIONS

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Our knowledge of autophagy is strictly related to the seminal studies of two scientists who were awarded the Nobel Prize in Physiology or Medicine. The first is Christian de Duve, who coined of the term "autophagy" in 1963 for describing the degradation process of cytoplasmic constituents occurring in the lysosome he revealed while studying these cellular organelles, and was awarded in 1974 just for the discovery of the lysosomes. The second is Yoshinori Ohsumi who was awarded in 2016 "for his discoveries of mechanisms for autophagy". In fact, for a long time after the pioneering discovery of C. de Duve, studies failed to furnish significant advances at gene/molecular levels concerning this pathway of degradation. Only after autophagy was discovered in yeast, thanks to Ohsumi and colleagues many of the molecular components of the autophagic machinery, mainly encoded by the autophagy-related genes (ATG), have been elucidated and shown to be conserved from yeast to mammals. Now our knowledge of autophagy and its relevance to different fields of biology and medicine, including microbiology, as well as to health and disease is dramatically expanding. After an essential introduction to history of autophagy, some aspects of specific interest and relevance to infections, such as the complex and apparently contradictory crosstalk between cell death and autophagy that unquestionably determines the overall fate of the cell during infection, will be discussed.

EBV REDUCES AUTOPHAGY IN CELLS IN WHICH IT REPLICATES

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Introduction: Viruses, as obligate intracellular parasites, are totally dependent upon host cells for their persistence or replication. For that reason, they have developed strategies to exploit the physiologic cellular processes for their own purposes. This is particularly true for viruses that persist life time in the infected host such as Epstein-Barr virus (EBV), a herpesvirus strictly associated to several forms of human cancer. EBV has been reported to manipulate cellular processes essential for the regulation of cell survival, including apoptosis and autophagy. The latter is a catabolic process basally activated in all cells and up-regulated in stressful conditions such as nutrient starvation or virus infections. In the course of viral infection autophagy, mainly activated by the unfolded protein response (UPR), plays an important role in the elimination of viruses, especially in the cells of the immune system. Therefore, viruses try hard to counteract or subvert autophagy to avoid their elimination.
Materials and Methods: To monitor autophagy and EBV infection/replication, western blot analysis and immunofluorescence assays were performed. The role of autophagy in EBV infection and replication was investigated by using pharmacological autophagy inhibitors or by silencing autophagy specific genes. FACS analysis was employed to detect ROS production and western blot analysis was used to assess the activation of the anti-oxidant response.
Results: In line with the above reported evidences on virus/autophagy interactions, we have found that EBV dis-regulated autophagy in naturally infected B lymphoma cells induced to in vitro replication by down-regulating Rab7. Moreover, we found that EBV exploited the autophagic vesicles to travel into the cell cytoplasm to promote viral egress. More recently, we are focusing on the manipulation of autophagy by EBV in monocytes. In these studies, we found that EBV established a replicative infection in these cells and reduced Rab7, resulting in autophagy inhibition. Interestingly, this effect also led to an increase of the anti-oxidant response and to a reduction of intracellular ROS, through the activation of p62-Keap1-NRF2 axis. As a

consequence of autophagy inhibition and ROS reduction, the monocyte in vitro differentiation into DCs induced by GM-CSF/IL-4 cocktail, their survival and phagocytic function were severely impaired.

Conclusions: Our studies indicate that autophagy manipulation by EBV plays an essential role in its maintenance in the infected host since it promotes virus replication and reduces the anti-viral immune surveillance through the impairment of DC formation.

IMPACT OF MYCOBACTERIUM TUBERCULOSIS GENETIC VARIABILITY ON VIRULENCE AND MODULATION OF AUTOPHAGY

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Among the most important features that make Mycobacterium tuberculosis (Mtb) one of the most successful human pathogen is the ability to subvert the intracellular antimicrobial activities of phagocytes. Autophagy, an ancient evolutionary conserved process involved in cellular homeostasis, has been shown to play an important role in the elimination of invasive microbes and experimental evidences suggest that Mtb employs a number of mechanisms to avoid autophagic degradation. A key step in this process is the ability of Mtb to avoid maturation of the autophagosome to evade killing. Interestingly, Mtb possesses specific proteins directly implicated in this process, the most important of which are proteins secreted through the ESX-1 secretion system. Nevertheless, the cellular and molecular events used by Mtb to avoid killing and block the autophagic process have been only partially clarified.

Although Mtb is a monomorphic bacterium, the little genetic variability has been shown to significantly impact the pathogenetic properties of the Mtb strains isolated in different geographic regions. Indeed, ancients Mtb strains have been shown to be less virulent than modern Mtb strains, that emerged in the last 10 thousand years and have been and still are responsible for the most devastating pandemics. We have investigated the impact of the genetic variability of Mtb on the autophagic process by infecting human primary macrophages with Mtb strains belonging to different phylogeographic lineages. The results indicate that the modern Mtb strains show enhanced intracellular replication in macrophages despite the fact that these strains are able to stimulate the autophagic process, suggesting that the most successful Mtb strains do not simply block autophagy but rather manipulate the autophagic process to avoid bacterial killing and at the same time prevent macrophage death by apoptosis. The implications of these results will be discussed in the context of Mtb virulence and evolution. Moreover, the understanding of the cellular and molecular mechanisms involved in this process may open new and unanticipated opportunities to develop host directed therapies against tuberculosis and experimental evidences showing the potential of this strategy will be presented and discussed.

THE MICROBIOTA AND ITS APPLICATIONS: COMPARISON BETWEEN ACADEMY AND INDUSTRIAL WORLD

THE GUT MICROBIOTA AS AN EMERGING TARGET FOR A HEALTHY AGEING

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Introduction: Age is one of major intrinsic variables impacting on the gut microbiota composition and function. Indeed, it is well known how the gut microbiota describes an adaptive trajectory along human aging in which microbiome changes provide the host with ecological services calibrated for each stage of life from infancy to old age. The relationship between microbiome and longevity is a very young research field and quite limited is the number of papers which have studied the microbiota of centenarians by NGS, contributing to the build up of a international dataset which allows the comparison of the microbiota profiles in centenarians living in different countries.
Materials and Methods: We explored the age-related differences in the microbiome of young adults, elderly, centenarians and super-centenarians using NGS approaches.
Results: We observed that the microbial composition and diversity of the microbiota of young adults and seventy-years old people was unexpectedly similar. Indeed, elderly presented a biodiverse intestinal ecosystem but started to show also some of the age-associated features observed in centenarians. Centenarians showed taxonomic changes in the structure of the gut microbiota characterized by a low bacterial diversity, a reduction of ecosystem resilience and stability. A decrease of SCFAs producers such as Roseburia, Faecalibacterium and Bifidobacterium and an increase of pathobionts with high pro-inflammatory potential sustaining immune activation and inflammaging was observed. Moving from centenarians to semi-supercentenarians, we demonstrated that, even if the age gap is very small, 6 years only in average, these 2 groups are significantly separated, suggesting that differences were present between the microbiota structures of these two groups. Extremely long-living people seem to experience a parallel increase in several health-associated taxa, such as Christensenellaceae, Akkermansia and Bifidobacterium, known to promote immunomodulation, protect against inflammation, and promote a healthy metabolic homeostasis.
Conclusions: The taxonomic evidences found in the different age-groups affected the functional distribution of the age-related metagenomes, allowing us to identify clusters of specific taxa and genes characteristic of the extreme ageing. In particular, Christensenellaceae might represent a signature of the ecosystem of extremely longevous people.

OMICS TECHNOLOGIES: WHAT CAN WE LEARN ABOUT MICROBIAL COMMUNITIES AND THEIR FUNCTIONS?

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Introduction: The human gut is colonized by complex microbial populations, collectively known as gut microbiota (GM). GMs change dynamically the relative abundance of their members and/or their metabolic and functional features, in response to food intake, immunological effectors, gut enzymes, metabolites, and neurohumoral control of the gastrointestinal motility. Not surprisingly, a variety of diets and diseases cause a modified and unhealthy GM (so-called dysbiosis). Thus, the deep characterization of GM taxonomy and its functions in health and disease, is of paramount importance and requires a continuous improvement of analytical methods for GM DNA sequencing (metagenomics), quantification of the expressed functional features (metaproteomics) and integrated metaproteogenomics pipelines. To these aims, we developed an innovative pipeline for GM analysis and we recently applied it to investigate: i) the GM taxonomic and functional features actually expressed in a healthy human population, ii) the differential microbiota traits in multiple sclerosis (MS) and, iii) in experimental models of caloric restriction (CR) e other dietary interventions.
Materials and Methods: Human samples were obtained from a selected cohort of 15 healthy subjects from a native and highly monitored Sardinian population in Ogliastra, Sardinia (Italy); fecal samples from 15 MS patients were collected at the Ospedale Binaghi in Cagliari (Italy); Fisher 344 rats fecal samples (groups of 4-6 animals) were collected at different time points during CR or other dietary interventions. Extracted DNAs were subjected to either 16S rDNA and/or shotgun Metagenome sequencing on Illumina DNA sequencers. For functional analyses, fecal proteins were extracted and digested with the FASP protocol. Peptide mixtures were analyzed by LC-MS/MS using an LTQ-Orbitrap Velos mass spectrometer coupled with a nanoLC system (Thermo).
Results: We have applied an optimized metaproteogenomic analysis pipeline that enables to gather detailed information on relative abundance of GM's taxa, active functions and pathways. We were able to profile the potential and active functions in the GM of a healthy human cohort and to identify functional features that are differentially expressed in MS patients. The GMs of rats subjected to CR and other different diet interventions revealed significant changes according to the diet regimens.
Conclusions: Our results provide detailed information on taxa, functions and pathways actively working in healthy GMs. Further, we demonstrate that a considerable divergence exists between microbial functional potential and real expression both in healthy and diseased subjects. Finally, our data provide evidences that specific members of the GM might serve as markers of dietary intervention in experimental animal models.

CLINICAL APPLICATIONS OF THE MICROBIOTA

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Introduction: The study of the human gut microbiota has been revived by metagenomic analyses that have revolutionized the understanding of its relations with human health and diseases. Disruption of the intestinal microbiota, in particular, is believed to promote a range of bacterial infections, often as a result of broad-spectrum antibiotic treatment that increases the susceptibility to these infections. An intact microbiota can exclude invading bacteria by direct and indirect mechanisms that, together, provide "colonization resistance". So, administration of live bacteria can compensate for loss of commensal microbes and colonization resistance after antibiotic treatment.

Materials and Methods: The aim of this talk is to summarize recent significant advances and ongoing challenges in the area of clinical applications of the intestinal microbiota, taking advantage of growing evidence obtained from literature searches, clinical laboratory experience, and the author's opinions.

Results: Extensive analyses of the intestinal microbiota have revealed the impact of distinct commensal bacterial populations on the development of obesity, metabolic syndrome, intestinal inflammation, and autoimmunity. Clinical studies dealing with transplantation of stool (i.e., fecal transplant, intestinal microbiota transplantation, fecal bacteriotherapy) show that it is possible to repopulate the human gut with commensal bacterial species by infusion of fecal material via nasogastric tube or via colonoscopy or enema. Recently, capsules containing spores isolated from human feces have been administered orally and have effectively treated recurrent *Clostridium difficile* infection. Experimental studies identifying protective commensal bacterial species that can induce high level of colonization resistance to vulnerable individuals are also presented. Finally, current research is undertaken to map the human gut microbiome in order to develop products that restore or preserve beneficial indigenous bacteria and prevent colonization with harmful bacteria (e.g., probiotics, prebiotics, or synthetic microbiota).

Conclusions: Reestablishment of normal intestinal (colonic) microflora is an intuitive therapeutic approach. However, the effective role in treating and preventing bacterial infections in clinical practice needs to be elucidated in future studies.

ROLE OF PROBIOTICS

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Despite microbiome composition can widely vary across individuals, massive changes in the microbiota are associated with a large number of disease states. Scientific evidence supports probiotics, live microorganisms which confer a health benefit on the host, as therapeutic option for the normalization of the perturbed microbiota. A common assumption is that probiotics work by influencing the microbiota composition. However, recent literature indicate that these microbiota modifications are not so evident in humans. Most probably, probiotics induce bacterial metabolic shifts that can be responsible for their demonstrated clinical effects.

Probiotics have been found useful in reducing the symptoms of antibiotic-associated diarrhea, ulcerative colitis and necrotizing enterocolitis, in favorably modifying the major metabolic outcomes in different metabolic diseases, in reducing the number of respiratory tract infection episodes and in the treatment of allergic rhinitis.

Probiotics may introduce beneficial functions, interact with host immune cells, inhibit pathogens that are not dominant members of the microbiota, promote faster return to the microbial homeostasis, stimulate epithelial cell proliferation and fortify the mucosal barrier. The mechanisms of probiosis, which considerably vary depending on the used microorganisms, will be discussed.

FOCUS ON THE RESEARCH IN MICROBIOLOGY FOR SAFETY AND EFFICACY OF THERMAL WATERS

THE ADMINISTRATIVE REGULATIONS OF THERMAL WATERS

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Introduction: From a public health point of view, thermal waters show several issues in common with recreational waters, including coastal and fresh waters. They can represent a resource for wellness and rehabilitation application, and require appropriate hygiene management, depending also on the specific chemical composition and user typology. The peculiar and typical composition of thermal waters represents an interesting property, but may imply difficulties in management, monitoring and microbiological characterisation. At the moment thermal waters are governed by various and special regulations.
Materials and Methods: The hygienic evaluation criteria of thermal waters, from a regulatory point of view, is not easy. Thus a review of the regulations to which thermal waters may be subjected is presented. The search was performed by visiting institutional archives and/or querying on line databanks.
Results: This presentation evidences main critical points related to the general frame of the European and national regulations on thermal waters. The main Italian law concerns the qualification of thermal water based on a series of scientific analysis. Recognition for the inclusion in the national list of thermal waters is given by the Ministry of Health. The first law taking into account also thermal waters is the Legge 16 July 1916, n. 947 - "Miscellaneous provisions on public health" which however does not deal to establish the hygienic properties of thermal waters. Several international guidelines and recommendations are available, and the World Health Organization Guidelines for Recreational Waters should represent a milestone in the field.
Conclusions: It is essential to consider the several needs for developing new and appropriate criteria in evaluating safety and quality of thermal waters. Application of advanced technologies to thermal waters may represent a challenge that can provide new insights for addressing hygiene and safety performance in implementing safety, quality and other technical goals related to public health issues.

FREE LIVING AMOEBAE IN THERMAL WATERS

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Introduction: Free-living amoebae (FLA) are protozoa ubiquitous in Nature, isolated from a variety of environments worldwide. In addition to their natural distribution, some species have been found to be pathogenic to humans.
Materials and Methods: In the present study a survey was conducted in order to evaluate the presence and to characterize at molecular level the isolates of amoebic organisms collected from different water sources in Italy. A total of 160 water samples were analyzed by culture and microscopic examination.
Results: FLA were found in 46 (28.7%) of the investigated water samples. Groundwater, well waters, and ornamental fountain waters were the sources with higher prevalence rates (85.7%, 50.0%, and 45.9%, respectively). Identification of FLA species/genotypes, based on the 18S rDNA regions, allowed to identify 18 (39.1%) Acanthamoeba isolates (genotypes T4 and T15) and 21 (45.6%) Vermamoeba vermiformis isolates. Other FLA species, including Vahlkampfia sp. and Naegleria spp., previously reported in Italy, were not recovered.
Conclusions: The occurrence of potentially pathogenic free-living amoebae in habitats related to human population, as reported in the present study, supports the relevance of FLA as a potential health threat to humans.

MICROFLORA THERMARUM ATLAS AND QUALITY CONTROLS IN SPA WATERS

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Introduction: Natural properties of thermal spring waters are related to their composition and thus are characterized by specific chemical, physical and biological features. Thermal springs are natural environments present all over the world and exploited since ancient times for medical and wellness applications. Such ecological niches are characterized by delicate physical-chemical and microbiological balances within their unique microflora biodiversity. Thus, knowledge regarding biotic and abiotic components is essential to characterize water properties and support appropriate management and quality controls.
Materials and Methods: Waters and sediments were collected from Italian springs and their microbiota defined by metagenomic analysis of mfDNA. Classical microbiological, chemical and physical parameters were collected by standard methods. Thermophilic species were grown on agar plate with medium D modified at > 50°C and metabolically characterized by phenotype microarrays. Full genome sequencing was performed on selected strains and deposited at DDBJ/EMBL/GenBank after assembly by bioinformatic analysis; isolates underwent submission to the BCCM/LMG collection repository. Microflora profiles were mapped by Geographic Information System.
Results: Thermal spring waters have a specific genetic fingerprint related to the heterogeneous composition of the bacterial microflora. Comparison of phylogenic microbial profiles allowed to study and map biodiversity throughout different areas of Italy. Statistical analyses revealed significant correlations between the relative abundance of specific bacterial taxa and chemical-physical variables. Selected species were isolated and characterized, showing potentials for further studies and applications. This natural microflora is mainly unclassified and unculturable, but the microbiota can be studied by genetic analysis, showing biological signatures of interest for tracing and monitoring water quality. Geographical mapping in an Atlas database (www.mfatlas.it) contributed to understanding of the natural microbial communities harboring thermal springs, unraveling the impact of contaminants or treatments, the influence of pipeline management, the role of chemical, physical, hydrogeological

factors. Thermal waters can be considered as vital biological fluids and promising resources for science and technology advancements.

Conclusions: Biodiversity remains an underestimated property of thermal springs, yet it is a key element in several SPA applications. The Atlas progress is shedding light on biotic and abiotic components in these ecological niches, providing insight for the development of further guidelines for appropriate use, monitoring and management of the quality and safety of thermal waters.

SCIENTIFIC RESEARCH IN BALNEOTHERAPY

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Introduction: Spa medical therapy or balneotherapy is a complex of practices that use water with different mineral components and physical properties to alleviate or cure the symptoms of various pathologies, mainly of the respiratory, digestive and urinary tract, of the skin and middle ear, as well as of rheumatologic diseases. Balneotherapy has reached our age as a medical discipline scarcely regulated by scientific standards; only in the last century the improved knowledge of chemistry and physics has added a rationale in classifying the different waters based on their chemical and physical properties. One could say that balneotherapy wouldn't have lasted 2,000 years or more if it was useless: however, nobody would doubt that a long history cannot be taken per se as scientific proof of efficacy. Moreover, even in the recent resurging of the spa thermal practices in Europe, it is sometimes difficult to distinguish between therapy and leisure (9,10). The concept of spa itself is very different from United States – were spas are essentially dedicated to leisure – and Europe, were they also have a prominent role in non-acute patient therapy and clinical recovery. This therapeutic role has been challenged for years because of the lack of a rigorous scientific basis for balneotherapy.

Materials and Methods: In the recent years both Italy and France have created granting agencies that fund rasearch in field. FoRST is active in Italy, implementing national grant programs with an international peer review system of the projects, with a rigid control of scientific quality and money allocation, as well as grants selectively dedicated to specific pathologies, also in collaboration with other major national granting agencies.

Results: Allocating grants upon scientific merit, several groups of scientists started studying the clinical applications of balneotherapy with modern standard methodologies of evidence-based medicine; in parallel, basic science in this field is making relevant progresses, demonstrating the biological effects of waters' active principles from the molecular point of view. The most significant success in this aspect, has been the Hydroglobe report, a study promoted by FoRST, the World Federation of Hydrotherapy (FEMTEC) and the World Health Organization (WHO), that included balneotherapy in its strategies to 2023

Conclusion: With this approach, science in this field is rapidly recovering its delay. However, more collaboration is expected between the International Organizations that are representing the field, with an emphasis on protocols definition and clinical indications. A lot has still to be done and strong research programs are needed at the national and European level to foster the scientific basis of balneotherapy.

FOCUS ON UPDATE ON EMERGING VIRUSES

EPIDEMICS: GLOBAL RISKS IN AN INTERCONNECTED WORD

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At the beginning of the third millennium communicable diseases continue to cause worldwide -mostly in developing countries- the death of several million people each year with the greatest impact on morbidity and life expectancy. Communicable diseases do not respect national borders and the failure of control measures in one country can put neighbours and global health at risk.
In the recent years the concern for unplanned and unexpected emergence or reemergence of severe infectious diseases is increasing. Not just Ebola, but also Lassa, Zika, pandemic flu, Dengue).
There is evidence that the impact of these diseases is increasing due to continuing and worsening levels of poverty, the effect of population growth, the increase in man-made and natural disasters resulting in displacement of populations, the behavioural changes, the emergence of resistance to anti-infectives, climatic changes and deteriorating sanitation. Infectious diseases can be partially controlled through improved living conditions and effective public health and education systems.
There is a need of a coherent, comprehensive and accelerated response to communicable diseases, securing adequate resources to produce better health outcomes towards a sector-wide approach, setting a broad policy framework and establishing longer-term partnerships.
Examples related to viral infections, with economic, social and political implications will be presented.

THE HEPATITIS VIRUSES TRANSMITTED BY FECAL-ORAL ROUTE. NEW EPIDEMIOLOGICAL TRENDS?

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Hepatitis A virus (HAV) and hepatitis E virus (HEV) are the most common causes of acute hepatitis worldwide and tens of millions of individuals are estimated to become infected each year.
Both viruses are trasmitted by the fecal-oral route and usually cause self-limiting infections, although HEV clinical course is, on average, more severe and almost 70,000 persons each year are expected to suffer a HEV related death.
HAV is transmitted via ingestion of contaminated food/water or through direct contact with an infectious person. The incidence rate is strongly correlated with socioeconomic indicators: as incomes rise and access to clean water increases, HAV incidence decreases. This means that fewer countries remain highly endemic.
The global distribution of HEV has distinct epidemiological patterns based not only on socioeconomic factors but also on ecology. Analogously to HAV, HEV is responsible for large-scale waterborne epidemics and can spread through person-to-person in resource-poor countries. However, in Western world, the initial perception that HEV is just a travel-related disease has recently changed since autochthonous cases are reported every year: most infections in industrialized countries are due to zoonoses with a wide animal reservoir. Consequently HEV epidemiological picture has changed, risk factors being: older age; consumption of undercooked meat from infected animals, seafood, contaminated vegetables or field-grown strawberries; underlying disease; sexual habits and blood transfusion (albeit rarely observed). Moreover, HEV infection has been clearly associated to chronic liver disease in immunosuppressed individuals
Thus in Italy, the epidemiology of HEV infection has recently been clarified, while that of HAV infection, due to improved sanitation and living standards, has changed in the last decades. Sero-epidemiological studies on Italian military recruits in 1981, 1990 and 2003 showed a drop in anti-HAV prevalence from 66.3%, to 29.4% and to 5.3% respectively. This decreased general population immunity has a series of consequences: the burden of disease is shifting towards adolescents; infections are more frequently symptomatic; people at risk include not only travellers to endemic countries, but also people which become infected following localized outbreaks, occurring with particular frequency in men who have sex with men and in injecting drug users, besides the general population.
HEV seroprevalence rates in Italy and Europe show a high variability, ranging from 0.6% to 52.5%.
As both HAV and HEV are characterized by a peculiar geographical distribution of viral genotypes, the molecular surveillance of circulating viruses could provide valuable information about the possibility of importing new strains with contaminated food.

MERS: EMERGING OR ESTABLISHED ZOONOSIS?

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Ancona

Middle East Respiratory syndrome(MERS) was identified as a new clinical syndrome associated to a previously unknown coronavirus (MERS-CoV) in 2012. All cases reported to the World Health Organization have been linked to the Arabian Peninsula, and camels (Camelus dromedarius) were soon identified as a reservoir for the virus. MERS-CoV infection can exhibit a wide range of clinical manifestations, including mild or limited symptoms, however it can cause severe respiratory illness in humans, with deaths recorded in 35%-40% of cases reported globally. Although prolonged viral shedding from the respiratory tract of symptomless subjects has been demonstrated, human transmission has been documented only among close contacts of symptomatic cases, primarily following healthcare-associated exposures and, to a lesser degree, household exposures. There is no definitive evidence of sustained human-to-human transmission in the community. Serosurveys in humans in the Arabian Peninsula indicate low to very low prevalence of seropositivity. Serosurveys in camels, by contrast, suggest a well-established endemicity (at least back to the last decades of the 20th century) of the infection from West Africa through Pakistan, which peculiarly excludes camels in Australia (imported from Arabia in the 17th century), suggesting a relatively young (decades-centuries) immission in camels. Phylogenetic analyses of MERS-CoV sequences obtained to date suggest an epidemiologic pattern based on ongoing periodic introductions of the virus into human populations, presumably from animal reservoirs, with sporadic short and auto-extinguishing chains of transmission in households and healthcare settings ($R_0 < 1$). Despite the virus currently poses no threat of generating a human pandemic, the health burden carried by Arabian populations (and potentially other dry zone inhabitants in Africa and Asia) will be significant until a vaccine will be developed.

VACCINES: STATE OF THE ART AND NEW APPROACHES

MOLECULAR EPIDEMIOLOGY OF VACCINE PREVENTABLE PEDIATRIC INFECTIONS

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Introduction: Molecular epidemiology is an important tool to understand epidemic dynamics of viruses and bacteria, which may be usefully applied to vaccine preventable iseases
Methods: Data from outbreaks of vaccine preventable pediatric infectious diseases were collected and analyzed to describe their trends over time and geographical distribution. Molecular variability of the pathogens was also taking into account to study sub-epidemic dynamics.
Results: Two major outbreaks of vaccine preventable diseases were considered. The first one was the meningococcal C invasive disease outbreak occurring in Tuscany in 2015.2016, and the second one the current measles outbreak reported in Italy in 2017. In the meningococcal C outbreak, a unique cc11 ST11 meningococcal C strain was identified. Its characteristics, in terms of hypervirulence and capacity of rapid spread, may explain the large number of cases with relatively high disease severity. A mass vaccination campaign was implemented to control the outbreak. With regard to measles, which has caused more than 4500 cases since the beginning of 2017 in our Country alone, genotype B3, D8 and H1 were identified. Of them, the B3 and D8 genotypes can be considered endemic, while H1 has been identified only in a small outbreak occurred at the beginning of the year. A vaccination campaign is ongoing, reinforced by a recent national law on mandatory immunization.
Conclusions: Molecular epidemiology may improve our knowledge on the spread of vaccine preventable diseases, guide intervention in a more effective way, and allow a better evaluation of vaccination plans.

THE CURRENT NATIONAL IMMUNISATION PROGRAM

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Introduction: In Italy, immunization programs are managed within the National Health Service; the new National Immunization Prevention Plan (PNPV) 2017–19 was approved on February 18th 2017 by publishing on the Italian Official Gazette.
Furthermore, on May 19, 2017, the Italian Parliament approved the Decree-Law Containing Urgent Measures on the Compulsory Vaccination of Children, which makes vaccinations mandatory for children as a condition of school registration. Italy's Parliament has given final approval last July 28th (Law 119/17).
Materials and Methods: As PNPV 2017-19 is about to be launched, the aims of the conference talk will be to: show the innovative aspects such as the introduction of new vaccinations; present the immunization schedules included in National Plan; indicate the recommended and mandatory vaccines; display the coverage data for vaccines included in the PNPV.
Results: PNPV added new vaccinations compared to the previous Plan: meningococcus B, rotavirus and varicella for children; the quadrivalent meningococcal and HPV vaccines extended to males in adolescence; zoster and pneumococcal for elder and at risk populations. Nevertheless since 2012-13, vaccination coverage rates have been decreasing and this phenomenon has led to a fall in vaccine coverage below 95%, the threshold recommended by the World Health Organization for achieving so called "herd immunity". For example, vaccine coverage data for measles and rubella indicate a 5% decrease between 2013 and 2015, ranging from 90.4% to 85.3%. The result was a worrying increase in the number of cases of measles as well as the reoccurrence of illnesses found to date only episodically and in less serious forms than in the past (such as diphtheria), and an increase in cases of infectious diseases in different age ranges from classical ones, with more serious clinical pictures and greater recourse to hospitalization. For this reason, the Law 119 was issued in order to ensure homogeneous national activities for the prevention, containment and reduction of risks to public health; mandatory vaccinations go from 4 (diphtheria, tetanus, polio, hepatitis b) to 10 (pertussis, haemophilus influenzae, measles, mumps, rubella and varicella). Furthermore, the decree-law provides that other vaccines are also offered free of charge and actively, according to the specific indications of PNPV: meningococcal B, meningococcal C, pneumococcal and rotavirus.
Conclusions: The Italian PNPV can be considered one of the most modern and updated immunization schedule on the European scene.
The rationale of the decree-law is to make vaccinations against diseases of high contagion and at risk of epidemics compulsory.
The challenge for the future is to achieve the goals of the PNPV to improve the health of the population.

ANIMAL MODELS AND DEVELOPMENT OF ANTI-MENINGOCOCCAL VACCINE

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Neisseria meningitidis (meningococcus) is a leading cause of life-threatening diseases worldwide in humans, such as sepsis and meningitis. Invasive disease is preceded by asymptomatic nasopharyngeal colonization occurring in up to 10-20% of the normal population. In some individuals this common transitory colonizer is able to breach the mucosal barrier, get into bloodstream and multiply uncontrollably, and finally cross the blood-brain barrier to cause meningitis with major long-term sequelae. Both host and bacteria factors seem to be involved in this switch from harmless transitory colonization to invasive disease.
Progress in understanding the pathogenesis of meningococcal disease and developing effective vaccines has been delayed by the inability to reproduce the disease in an animal model as described in the human host due to the narrow host range and the high degree of genetic variation of the surface structures of this microorganism. Indeed it is known that some candidate meningococcal vaccine antigens are only expressed in vivo. Therefore approaches that rely on antigens from bacteria grown *in vitro* could fail to find potentially important new candidates.
To date a wide range of vaccines is available for preventing life-threatening diseases caused by meningococcus. Capsule polysaccharide-conjugate vaccines are successful prophylactics for main serogroup MenA, MenC, MenW and MenY infections and outer membrane vesicle vaccines have been used successfully for controlling clonal serogroup MenB infections. Although several studies indicate that such vaccines provide protection and reduce the number of asymptomatic carriage, little is known about the real impact on the carriers and the duration of the protection.
In this context, experimental animal models of invasive meningococcal meningitis could represent a critical tool for the selection of novel meningococcal vaccine antigens, the evaluation of immunogenicity, and the assessment of the effective induced protection in different population groups. In this talk, it will present and discuss current research data concerning lights and shadows on experimental animal model employed for the development of anti-meningococcal vaccine.

NEXT GENERATION SEQUENCING (NGS) IN THE LABORATORY OF MICROBIOLOGY: OPPORTUNITY AND CHALLANGES

RATIONALE AND PERSPECTIVES OF NEXT GENERATION SEQUENCING APPLICATIONIN MICROBIOLOGY

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Introduction: Recent advances in nucleic acid sequencing technologies, referred to as "next-generation" sequencing (NGS), have produced a true revolution and opened new perspectives for esearch and diagnostic applications in microbiology.
Methods: NGS allows whole genome sequencing of numerous pathogens in one sequence run, either from microbial isolates from different sources, or from multiple species present in a single sample. Hence, the composition of a microbial and viral communities in individual patients (microbiome/virome analysis) can be studied; in addition, data on resistance and virulence, as well as information for typing is obtained, useful for outbreak investigation. In fact, comparison of genome wide sequence data from clinical isolates allows to identify mutational markers that link cases each other, reconstructing the pathway of transmission into a community.
Results: Applications of NGS in the clinical setting include outbreak management, molecular case finding, characterization and surveillance of pathogens, rapid identification of bacteria using the 16S-23S rRNA region, taxonomy, metagenomics, and the determination of transmission pathways of zoonotic micro-organisms from animals to humans. In addition, metagenomics-based strategies are currently applied for the discovery of novel viruses. Further applications include the study of viral genome variability, useful to track viral dynamic. This is relevant for viruses, such as HIV, HBV and HCV, whose error-prone replication machinery, combined with high replication rate, results in the formation of many genetically related viral variants (quasi-species) in each patient. Hence, NGS is a powerful tool with which to investigate

previously inaccessible aspects of viral dynamics, such as contribution of different viral reservoirs to replicating virus in the natural history of the infection, compartmentalization of viral quasispecies in different anatomic sites, co-receptor usage in minority viral populations harboured by different cell lineages, dynamics of development of drug resistance, and re-emergence of hidden genomes after treatment interruption.

Conclusions: The role of NGS in medical microbiology laboratories will increase during next years, not only for research, but also for molecular diagnostics, infection prevention, outbreak investigation, characterization and surveillance of pathogens, track genetic changes, detection of novel resistance genes and application of a metagenomics to clinical samples. Equipment simplification, methods standardization, development of user friendly bioinformatic tools represent urgent advancements in order to render fully accessible to clinical microbiology the new, potent tool represented by NGS.

DIAGNOSTIC STEWARDSHIP AS PART OF INTERVENTIONAL MICROBIOLOGY

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In hospital care today, the vast majority of disease caused by microorganisms are healthcare-associated infections rather than classical infectious diseases. This means that patients seek care and during healthcare infections occur. Major drivers are clinical conditions due to underlying disease, frequent use of invasive medical devices, environmental but also behavioural factors and the presence of specific microorganisms. Especially, in case of the spread of antimicrobial variants of microorganisms of resistance genes between bacteria, more patients will be colonized and more infections will occur caused by these subtypes. In consequence, probability of optimal treatment of such infections become limited. As frequency of healthcare is expected to increase in the coming decades due to an eldering society and innovations in healthcare using an increasing number of invasive devices, healthcare-associated infections will be increasing, also with optimal infection prevention measures implemented. Without proper antibiotic treatment, modern healthcare may become a risk for patient care. Major efforts are needed to reduce the spread of antibiotic resistance and maintain optimal antibiotic therapy. Next to the reduction of the selective pressure by implementing antimicrobial stewardship programs, diagnostic stewardship will be implemented in the coming years. Responsive, regional and proactive service will lead to interventional microbiology for gatekeeping the indication for diagnostic, producing rapid results proper for the patient population served and using diagnostic results for antimicrobial stewardship and infection prevention&control. Results from microbiological and molecular epidemiological diagnostic (Zhou et al. 2015), as well as mathematical modeling show us today the transmission dynamics of nosocomial pathogens, especially of multiple drug-resistant bacteria (MDRO), such as MRSA, Klebsiella pneumonia ESBL). Data show that their spread cannot be prevented by one single hospital only. Transmission follow a complex pattern comparable to systemic network connections. Hospitals might implement correctly a bundle of infection control measures, they fail to reduce the prevalence of MDRO. Here, the implementation of regional hub&spoke-laboratory network allows diagnostic stewardship and create an interventional and more proactive microbiological care and help to substantially reduce the spread of antimicrobial resistance.

ADVANCES IN THE APPLICATION OF NEXT GENERATION SEQUENCING TO THE STUDY OF VIRUSES

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Novel DNA sequencing techniques, referred to as "Next Generation sequencing" (NGS), provide high speed and throughput that can produce an enormous volume of sequences in a single run at relatively low cost.
This development has facilitated enormously the direct examination of clinical and environmental samples. Today different workstations and improved platforms of NGS are commercially available to sequencing molecules of DNA from hundreds of Mb (millions of bases) to Gb (billions of bases) in only one test depending on the type of technology. In this way, strains present at frequencies lower than 0.1% can be detected, while with the Sanger methodology only strains with a frequency greater than 20%. This is of particular interest in virology since the high genetic variation observed in many viruses results in the establishment of a swarm of genetically related but distinct viral variants within the host, named quasispecies. The assessment of viral genome variability within the host and detection of low-abundance antiviral drug-resistance mutations using NGS approaches have been the foundation of the new era of the molecular epidemiology of viral diseases, by investigating previously inaccessible aspects of viral dynamics and evolution with an exceptional resolution. Overall, NGS can be attractive tools for broad-based pathogen discovery because all infectious bio-agents, with the sole exception of prions contain either RNA or DNA, and are thus amenable to nucleic acid-based detection. NGS is capable of comprehensively identifying all potential pathogens in clinical samples from humans and animals. In the field of virology, indeed NGS technologies have been widely analyzed for: whole genome sequencing, investigation of genome diversity such as compartmentalization and minority quasispecies, drug-resistance, metagenomics, discovery of new viruses and characterization of viral communities in different setting. All these aspects will be discussed.

MASSIME PARALLEL SEQUENCING AND BIOINFORMATICS ANALYSIS OF FUNGAL GENOMES OF PATHOGENIC *SPOROTHRIX* SPECIES

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Introduction: Sporotrichosis, is a (sub)cutaneous infection of humans and felines that tends to occurs in the form of epidemics, sometimes with thousands of individuals. This disease is caused by a group of phylogenetically related *Sporothrix* species (*S. schenckii, S. brasiliensis, S. luriei, S. globosa*) which constitute the so-called "pathogenic clade" while occasional opportunists (*S. pallida, S. mexicana*), are found elsewhere in the genus. Most cases occur in (sub)tropical regions whereas in Europe, this disease is generally considered as rare although recent data argue against this assumption. However, despite sporotrichosis is an emerging global health problem, the study of these fungi is still in its infancy and very little is known about the evolution of drug resistance, virulence-associated genes and their regulation in pathogenic processes. Here, three genomes of pathogenic and environmental *Sporothrix* species were sequenced with the aim to facilitate future studies for understanding the basic biology, evolution and virulence of these dimorphic fungi.
Materials and Methods: The whole genomes of two *S. globosa* strains (SS01 and CBS120340) and one *S. pallida* strain (SPA8) were sequenced using Illumina Hiseq 2000 technology. A total of three different insert size libraries (200bp, 500bp, 6kb) were constructed for each strain and paired-end sequenced. Raw reads were initially cleaned by removing adapters and sequences with low Phred-scores (cutoff: ≥ 20) and then assembled using SOAPdenovo program. Protein-encoding genes and tRNAs were predicted *ab-initio* by AUGUSTUS and tRNAscan-SE programs respectively. Mitochondrial genomes were extracted and identified from the sequencing data using the GRABb program.
Results: The genomes of the two *S. globosa* strains were assembled in 19 (SS01) and 24 (CBS120340) large scaffolds covering over 33,4 Mbp (G+C: 54.37%). A total of 126 putative tRNAs and over 7,700 protein-encoding genes were identified in both genomes. For *S. pallida*, the assembled genome resulted in 432 contigs (length > 37.8 Mbp;

G+C: 52.8%). A total of 11,356 protein-encoding genes and 151 tRNAs were found in this species. The *S. globosa* mitochondrial genomes consisted of a circular DNA molecule (> 26 Kbp) and were about 9 Kbp smaller than the circular mitogenome of *S. pallida* (> 35 Kbp). The whole-genome sequences were deposited in GenBank under accession numbers: LVYW00000000, LVYX00000000 and JNEX02000000.

Conclusions: The release of the whole genome sequences of *S. globosa* and *S. pallida* represents an important milestone for *Sporothrix* research because it offers the possibility to compare the genomes of pathogenic and non-pathogenic species in order to understand what makes the members of the "pathogenic clade" so unique among all other *Sporothrix* species.

FOCUS ON INNOVATION IN DIAGNOSTICS

LATEST UPDATES FOR THE DIAGNOSIS OF VIRAL GASTROENTERITIS

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Introduction: In industrialized countries, acute gastroenteritis (AGE) is a major cause of morbidity in infants and young children and constitutes a substantial burden in terms of medical and indirect costs. Group A Rotavirus (RVA), Adenovirus (AdV) type 40-41, Norovirus (NoV) and Astrovirus (AstV) are major agents of viral GE. RVA, NoV and AstV are highly variable and continuously evolving both antigenically and genetically. The identification of the causative agents of AGE is important for a correct management of the patients, in order to limit the burden of nosocomial infections, facilitate protection of susceptible patients and reduce the costs of inappropriate antibiotic therapies. Several commercial tests based on classical antigen detection or biomolecular assays have been developed for the diagnosis of viral GE.

Materials and Methods: The prevalence of RVA, AdV, NoV and AstV in children hospitalized with AGE was investigated on 4480 stool samples collected over more than 15 years of uninterrupted surveillance for enteric viruses. The positive samples were genotyped by amplification and sequence analyses. Moreover, the performance of commercially available assays for RVA and NoV detection antigens were retrospectively evaluated on different sets on samples using Real-time PCR as reference test.

Results: The prevalence of enteric virus infections among AGE patients was 63.8% from 2000 to 2016, with detection rates of RVA, AdV, NoV and AstV in 32.5%, 10.36%, 18.04 % and 2.86%, respectively. Genotyping showed a great genetic variability within each group: G1P[8] RVAs were occasionally overcome by G9P[8], G4P[8] or G2P[4]; NoV GII.4 circulated in different variants and a novel GII.17 type emerged in 2016; different lineages of AstV-1

genotype were cyclically replaced over time. Retrospective evaluation of two commercially available immunochromatographic assays (ICTs) used for the diagnosis of RVA infections showed an accuracy of 90% for both tests when compared to Real-Time PCR, independently of the genotype involved. Commercial ICTs for NoV antigen were scarcely performant both in outbreak settings, where a single genotype was involved, and sporadic cases with seasonal substitution of predominant variants.
Conclusions: Enteric viruses represents the main cause of hospitalization for AGE in children and show continuous genetic/antigenic evolution possibly responsible for their uninterrupted circulation in the pediatric population, but also challenging the diagnostic performance of laboratory tests. Although the ICTs for RVA tested show a satisfactory efficacy as diagnostic tools, further efforts should be made to increase the level of sensitivity for NoV antigen detection assays and provide affordable results regardless NoV patterns of evolution. Biomolecular techniques should be recommended for fast and reliable diagnosis and epidemiological purposes.

ACCURACY IN THEME OF BACTERIAL IDENTIFICATION

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Accurate and timely characterization of bacterial species is strongly mandatory. Bacterial identification methodologies can be divided into the rapid identification methods directly from clinical samples, mainly molecular methods, and identification methods from subcultures. MALDI TOF technology is accurate, rapid and economic per sample analysis, that has been established in clinical microbiology laboratories and tends to be considered as the standard identification tool. However, several misidentifications still remain, mainly in specific groups of bacteria, such as streptococci, that need to be resolved.
In this work we focused on the current methods (conventional, molecular, and proteomic techniques) used to identify streptococcal species from colonies and to evaluate their level of accuracy. Fan et al. conducted a systematic review to evaluate the use of MALDI TOF for rapid identification of streptococci by including 3,540 clinical isolates, and they found that MALDI TOF correctly identified 96% of the streptococcal species, with an overall difference of the performance between the two major MALDI TOF platforms, Bruker biotyper and Vitek MS. An excellent performance was observed for both systems to identify β-haemolytic streptococci and *S. pneumoniae*, but accuracy of the identification of species belonging to some non β-haemolytic streptococci, such as *Streptococcus mitis/oralis* (SMO) group vs *Streptococcus pneumoniae*, and *Streptococcus bovis/Streptococcus equinus* complex (SBSEC), needs to be improved.
SMO species can be misidentified as *S. pneumoniae* and a combined use of conventional tests, such as optochin sensitivity and bile solubility, and MALDI TOF. With Bruker biotyper the use of updated databases and implementation of MALDI TOF peak analysis should be recommended. Molecular methods should be performed in some situations for ID confirmation. For identification of species/subspecies of SBSEC, that are associated with specific clinical manifestations, no gold standard method is available. Currently, the two MALDI TOF methods are not convincing to accurately identify all SBSEC species/subspecies, therefore additional optimization of the available databases or identification algorithms is needed, and only a combination of MALDI TOF and molecular methods can provide a correct identification. Among *S. anginosus* group

accurate results are obtained for *S. anginosus* and *S. constellatus*; however, *S. intermedius* is poorly identified to the species level by MALDI TOF and conventional tests, therefore PCR and sequencing-based methods are recommended.
In conclusion, MALDI TOF MS is rapid and accurate method for identification of the majority of streptococci. Nevertheless, some drawbacks still remain mainly for some α-haemolytic streptococci, and a combination of different methods are requested. The introduction of next generation sequencing technologies with information on multiple locus sequences could solve these problems.

HOW AND WHEN TO USE INNOVATIONS IN MYCOLOGICAL DIAGNOSIS?

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Invasive fungal disease (IFD) remains a significant cause of morbidity and mortality in immunocompromised patients despite the availability of effective therapies. Timely and accurate diagnosis is essential to reduce the high mortality associated with IFD. Blood cultures remain the mainstay for the diagnosis of candidemia, although sensitivity is not optimal and the time from collection to the microbiological response of growing yeast is long. New laboratory tests have been developed to make an early diagnosis of IFD in critically ill patients. Modern diagnostic efforts aim at detection of fungal cell wall components or nucleic acids. The use of single or combined biomarkers, such as (1 → 3)-beta-D-glucan, *Candida albicans* germ tube antibody, mannan antigen, anti-mannan antibodies and polymerase chain reaction (PCR) detection of *Candida* DNA has received increasing attention. A combined approach of these methods improve the diagnostic performance of the current methodologic repertoire.

FOCUS ON RETROVIRUS AND OTHER PERSISTENT VIRUS INFECTIONS

IS INTEGRATION OF THE VIRAL GENOME INTO THE HOST CELL GENOME A SOLE BENEFIT FOR THE VIRUS?

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Introduction: Integration of viral genomes into host cell genomes is considered a clever way for viruses to perpetuate their life and hitchhike cells to disseminate infection within and between hosts. The benefit may not be so unilateral as traditionally thought, a wealth of recent evidences show that integration of the viral genetic material is one of the many ways virus-host interact and that the cell itself may tweak the viral genome at its own advantage.
Materials and Methods: Next generation sequencing and other cutting-edge biomolecular techniques demonstrated that integration of viral genome into host cell genome, once considered unique feature of retroviruses, occurs in fact for many viruses, even those that entirely replicate in the cytoplasm and have a RNA genome which is not converted into DNA during replication. Molecular studies also showed that integration eventually causes a net loss for virus ability to disseminate infection.
Results: Integration of retroviral genome, a hitherto presumed well-known mechanism, is governed by a number of cell and viral factors. It has been shown, for example, that site depends on accessibility of host cell DNA, flanking genes, possible interference with adjacent cellular promoters, etc. Integration is not a mere matter of viral persistence either; proviruses undergo rearrangements and loss of genetic material that progressively undermine replication capacity of the virus, a phenomenon called endogenization. Further, endogenized retroviruses and endogenous retroviral elements confer the host cell resistance to superinfection by same or similar viruses and likely played an important role in evolution and speciation of vertebrates. Similar hypotheses have been put forward to explain integration of single-stranded RNA viruses (bornaviruses, filoviruses, flaviviruses, orthomixoviruses, picornaviruses, rabdoviruses) and single stranded DNA viruses (e.g. circoviruses and parvoviruses).
Conclusion: With today techniques and technologies, we will likely unveil the mechanistic model of integration for non-retroviruses soon. Why this occurs? To answer, we need to be open-minded and consider that it is not a mere matter of persistence but a novel facet of virus-host relationship with various and unexpected advantages to the host cell.

INTRINSIC HOST RESTRICTION FACTORS AGAINST VIRAL INFECTIONS AND MECHANISMS OF ESCAPE

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Viral replication is the result of complex interactions between host and viral proteins. In the course of evolution, mammalian immune systems have evolved to response through an array of cellular defense mechanisms, which include both innate and adaptive immune responses, designed to protect against and remove invading pathogens. The innate immune system, mediated by specialized cells such as natural killer cells (NK), dendritic cells, and macrophages, is the first to respond, but lacks specificity and does not lead to a long-lasting memory of the response to the pathogen. A specific kind of innate immunity, termed intrinsic immunity, has also been identified of late, thus generating a third branch of the immune system that was previously considered a bipartite system. Intrinsic immunity involves a set of defense mechanisms working on the cellular level, realized by cellular proteins known as restriction factors (RFs), as they can interfere with various steps of the virus replication cycle. The word restriction factor was first coined by research groups studying the murine immune response to retroviruses. Retroviruses consequently became a model system for investigating intrinsic immunity and have been instrumental in deepening our knowledge of the interaction between viruses and their hosts. Over time, the notion of intrinsic immunity get up from the finding that the cells attacked by primate lentiviruses are able to resist infection, despite the fact that no signaling event appeared to be necessary for this form of defense, and from the finding that these cells constitutively express prototype human antiretroviral RFs, including the APOBEC3 family of cytidine deaminases, TRIM5a, tetherin, and SAMHD1. In the last years, new classes of RFs have been identified, able to sense and control the replication of different family of both RNA and DNA viruses. Among the others, the absent in melanoma 2 (AIM2)-like receptors (ALRs), namely IFI16 and AIM2, are a new class of pathogen recognition receptors (PRRs) involved in cytosolic and nuclear pathogen DNA recognition. A wealth of evidence now supports a key role of IFI16 in the activation of innate immunity and viral restriction against herpesviruses and lentiviruses, such that the definition of IFI16 as a RF is now widely accepted.

In conclusion, RFs are thus germline-encoded proteins mediating the intrinsic cellular immune response against viral replication. Over time viruses have learned how to antagonize RFs through mechanisms that are specific for each virus. In this presentation, we review our current understanding of how viral replication is sensed and then inhibited by well known and newly described RFs, and, most important, the viral strategies employed to defeat this host defense mechanism.

HIV DRUG RESISTANT VARIANTS IN LATENT RESERVOIRS: ROLE IN VIROLOGICAL REBOUND

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Since the advent of antiretroviral therapy (ART), morbidity and mortality rates in individuals infected with human immunodeficiency virus type 1 (HIV-1) have been significantly reduced. However, HIV-1 is known to persist in several types of cells and tissues, and will usually return to pretreatment levels when therapy is stopped, even in those patients who have been on suppressive ART for a long time. The discovery of drug sanctuaries and viral reservoirs in the body, in which HIV may persist, has helped to explain why therapeutic eradication of HIV-1 has proved so difficult.

Several studies have indicated that the latent reservoir is an archive, composed of a mixture of wild-type and drug-resistant strains. It has been shown that archived drug resistant associated mutations (DRAMs) can still be detectable in the cellular reservoir for lengthy periods of time, i.e. up to 6 years, thereby precluding the successful recycling of any drug towards which resistance has arisen. The DRAMs detected in plasma do not necessarily reflect those found in cell-associated compartment, and may not be the same as those in different anatomical compartments. This could, indirectly, imply that PBMCs as well as body compartments may constitute a reservoir for drug-resistant variants and might replenish plasma with drug-resistant HIV-1 variants in certain circumstances. Although assessment of drug resistance in plasma is of direct and immediate importance for treatment, several authors have underlined the value of pro-viral DNA as an additional source of information on the total burden of resistance in an individual. Particularly, the evaluation of drug resistance in proviral-DNA may be helpful in clinical practice to plan drug switches for toxicity, intolerance, or simplification in suppressed HIV-1 infected patients. It has been reported that a population of HIV-1-positive patients who were fully responsive to ART, showed an association between the presence of mutations in pro-viral DNA and the occurrence of virological failure over the subsequent 2- year period. On the contrary, other authors suggested that, in multidrug-resistant patients treated with salvage therapy, the archived drug-resistant viral variants may change during suppressive ART but this does not affect virological success.

To date the clinical relevance of archived DRAMs remains to be defined, genotypic resistance testing in PBMCs appears to be an important tool that may allow a better disease management but prospective studies should be conducted to verify the predictive capacity of the HIV-1 DNA genotype and its value in clinical practice.

CLINICAL APPLICATION OF QUANTITATIVE HIV-1 DNA

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Modern antiretroviral therapy (ART) allows optimal control of HIV-1 replication in the vast majority of patients, as shown by persistently undetectable levels of viral RNA in plasma. However, HIV-1 remains latent in a small but long-lived pool of cells and typically relapses upon ART discontinuation, posing a formidable obstacle to virus eradication. The inability to measure HIV-1 RNA in patients under effective ART has redirected the focus on HIV-1 DNA which can be instead monitored in virtually all patients. Actually, HIV-1 DNA is made of different forms such as integrated, circular and linear unintegrated and includes both replication competent and defective sequences. Although the relative proportion, significance and evolution of the different HIV-1 DNA species is a matter of intense investigation in pathogenesis studies, the rough total HIV-1 DNA level measured in blood cells is being explored as a clinically relevant marker of patient status to inform treatment decisions. Indeed, total HIV-1 DNA has been shown to be a predictor of disease progression both in the natural course of HIV-1 infection and in patients under treatment. Most importantly, there is evidence that total HIV-1 DNA levels reflect the size of the latent HIV reservoir and predict the success of ART simplification strategies. In principle, total HIV-1 DNA load could also help select the ideal candidates for pilot eradication studies, as suggested by the association between lower HIV-1 DNA levels and longer time to rebounding HIV-1 RNA following ART discontinuation, particularly in patients with early ART start. Despite growing interest in HIV-1 DNA quantification, quite surprisingly commercial kits have not yet been developed by the major molecular diagnostics companies. The set-up of an accurate assay must proceed through obligate steps including (i) appropriate choice of the target region and experimental design, (ii) inclusion of an internal control ensuring the quality of the procedure, (iii) thorough evaluation of precision, accuracy, specificity and sensitivity based on international standards and (iv) analysis of robustness through repeated analysis of clinical samples by different operators and laboratories. External quality assurance programs are then mandatory and laboratory networks using the same validated protocol are highly advisable to generate large amounts of data that can be appropriately used for clinical investigation and consolidation of the role of total HIV-1 DNA quantification in the clinical setting.

SYNERGISM BETWEEN CRISPR/CAS9 AND LONG ACTING ART LEADS TO ELIMINATION OF HIV-1 WITH NO VIRAL REBOUND IN HUMANIZED MICE

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Introduction: Advances in gene editing technology especially CRISPR/Cas9 and its in vivo delivery by viral vectors (Temple University, Philadelphia) and cell-based nanotechnology for long acting slow effective release antiretroviral therapy, LASER ART, and humanized mice (University of Nebraska, Omaha) were joined to facilitate eradication of HIV-1.
Methods: First, optimal medicinal and polymer chemistry modified antiretroviral drug delivery to ensure maximal restriction of viral growth, biodistribution and pharmacokinetic through the generation of hydrophilic drug crystals. Second, the bioavailability/activity of the CRISPR/Cas9 designed for excision of intra-chromosomal HIV-1 DNA sequences were realized in tests of transgenic mice and rats. Intravenous administration of AAV9-CRISPR/Cas9 resulted in excision of a DNA fragment in various tissues and caused a substantial decrease in the level of viral gene expression in blood cells. Third, a humanized mouse model of HIV-1 disease was made whereas 17 week-old CD34 NOD/Scid-IL-2 RYcnull mice were infected with HIV-1NL4-3.
Results: After assessment of the immune profile reconstitution and stable viral load measures, animals were treated with LASER-ART and after seven weeks when virus was undetectable, fourteen animals (7 mice/group) were randomly chosen to receive no additional therapy or CRISPR/Cas9. Five weeks later, peripheral blood viral load, immune cell profile, measures of HIV-1 DNA/RNA, viral genotyping/sequencing were performed to evaluate the efficiency of viral excision and gene editing. Resurgence of virus in a range of 1.8x105-1.5x106 RNA copies/ml was observed in a group of animals receiving LASER ART or the additional AAV9-CRISPR/Cas9 control despite viral excision in the latter group. No excision was seen in the LASER-ART. In the group who received the combination therapy, two mice showed complete restoration of CD4+ T cells and no viral rebound. Results from RT-PCR, ddPCR, RNA/DNA scope failed to reveal any evidence for the presence of full length viral

DNA. Examination of total genomic DNA for the presence of viral DNA showed robust excision of the viral DNA by CRISPR/Cas9 at the expected locations with respect to the signature PAM domain. **Conclusion:** This is a proof-of-concept study indicates LASER ART and CRISPR/Cas9 performed in synergy is able to eliminate replication-competent HIV in an infected animal.

OCEANS AND HEALTH: THE MICROORGANISMS AND THEIR PRODUCTS

MARINE SEDIMENT AS A RESERVOIR OF ANTIBIOTIC RESISTANCE AND VIRULENCE GENES: FOCUS ON ENTEROCOCCI

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Ancona

Antibiotic resistance (AR) is a serious threat to human health worldwide. The environmental "resistoma" and Horizontal Genetic Transfer (HGT) are major players in AR evolution and spread. The marine environment, impacted by different anthropogenic activities, is a sink for microorganisms of different origins and for pollutants, including antibiotics, that can select AR clones. Biotic and abiotic factors can accumulate in marine sediment, providing an ideal setting for HGT between autochthonous and allochthonous bacteria, which can eventually contribute to the emergence of multidrug-resistant (MDR) pathogenic strains. We analyzed sediment samples collected in diverse coastal areas of the Adriatic Sea - close to aquaculture plants, beaches, river estuaries and an oil refinery - and clams from natural beds. Antibiotic-resistant enterococci (ARE) were counted, identified, and typed using traditional microbiological and molecular methods (Real-time PCR, MLST). Susceptibility tests were performed following EUCAST guidelines; AR and virulence genes were detected by PCR and mobile genetic elements (MGE) were characterized by PCR mapping and sequencing, hybridization and WGS assays.

The enterococcal microbiota of coastal sediment showed an overall low prevalence of *E. faecalis,* which was far outnumbered by *E. faecium* and other *Enterococcus* spp. Strains highly resistant to erythromycin, tetracycline, ampicillin, gentamycin and/or streptomycin were frequent in all sampling areas while resistance to quinupristin/dalfopristin and borderline susceptibility to daptomycin was observed in ARE from clams. AR genes were sought directly in samples and in the bacterial isolates. The frequent occurrence of ARE in the absence of the relevant AR genes (ARG) indicates the possible in-

volvement of uncommon or even unknown ARG. The detection of ARG and the increase in benthic enterococci cultured in antibiotic-supplemented rich broth suggest that sediments can be reservoirs of dormant ARE, which can revive in presence of optimal growth conditions and exploit a selective growth advantage due to drug pressure. Transfer assays to human strains highlighted the co-presence and co-transferability of antibiotic and heavy metal resistance genes and the mobilization of non-conjugative AR elements through the generation of cointegrates promoted by IS-mediated transposition. A new MDR mobilizable plasmid (pLAG) was characterized.
In conclusion, coastal marine sediments are an underestimated reservoir of ARE of diverse species, which can reach humans through contaminated seafood and recreational waters, and of uncommon ARG transferable to human strains. Actions to control the spread of AR and the risk for human health should take this into consideration.

EVOLUTION OF THE HUMAN PATHOGEN VIBRIO CHOLERAE IN AQUATIC HABITATS

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Introduction: Vibrio cholerae is considered an important model organism for examining aspects of bacterial pathogenesis including biofilm formation, quorum sensing, and virulence factor production and function. However, compared to its pathogenic potential in humans, much less is known about the bacterium's lifestyle in its primary habitat, the aquatic environment. Such environmental habitats often contribute to pathogen emergence, which is frequently accomplished through the acquisition of novel genetic information by means of horizontal gene transfer (HGT). Natural competence for transformation as a mode of HGT plays a key role in bacterial evolution and V. cholerae enters the competence state within biofilms that are formed on the chitinous exoskeletons of zooplankton [1].
Methods and Results: In this talk, I will show genetic and imaging-based data that illustrate that the type VI secretion system (T6SS) of pandemic V. cholerae strains is part of the competence regulon and co-induced with the DNA-uptake complex upon growth on chitin. T6SS is a molecular killing device [2], and it fosters HGT by the deliberate killing of neighboring bacteria followed by the absorption of their genetic material [3]. The extent of such DNA transfer events has not been investigated in the past. We have addressed this lack of knowledge through whole genome sequencing. These sequencing data provide evidence for the transfer of chromosomal stretches of significant length.
Conclusion: I will conclude my presentation with the hypothesis that competence-induced kindiscriminated neighbor predation serves as a DNA acquisition strategy and might represent a common biological theme of naturally competent bacteria [4].

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ECOLOGY AND SPREAD OF HUMAN PATHOGENIC VIBRIO IN THE MARINE ENVIRONMENT AND LINK WITH CLIMATE CHANGE

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Introduction: In the marine environment, vibrios are found attached to chitin-containing organisms, especially zooplankton, which represent one of the most important environmental reservoirs of these bacteria in nature. *Vibrio*-related infections are increasing worldwide both in humans and aquatic animals. Rise in global sea surface temperature (SST), which is approximately 1°C higher now than 140 years ago and is one of the primary physical impacts of global warming, has been linked to such increase. To investigate if rising SST is associated to an increased spread of *Vibrio* bacteria in the ocean we performed a retrospective molecular analysis of marine *Vibrio* populations on historical samples collected by the Continuous Plankton Recorder (CPR) survey.

Materials and Methods: Using microbiological molecular techniques applied on archived formalin preserved plankton samples collected by the CPR survey (CPRs) over the past half a century (1958-2011), we assessed retrospectively the relative abundance of vibrios, including the human pathogenic species *Vibrio cholerae, Vibrio vulnificus* and *Vibrio parahaemolyticus*, in nine areas of the North Atlantic and North Sea and investigated correlation with climate and plankton changes.

Results: We were able to recover environmental DNA from CPR samples that had been stored for up to ~50 years in a formalin-fixed format, which is suitable for molecular analyses of the associated prokaryotic community. An unbiased molecular index of abundance for *Vibrio* spp. named "*Vibrio* relative Abundance Index (VAI)" was developed and assessed on CPR samples. Results showed that in the majority of investigated areas VAI is increased during the past half-century. Generalized additive models revealed that long-term increase in *Vibrio* abundance is promoted by increasing sea surface temperatures (up to 1.5 °C over the past 54 years) and is positively correlated with the Northern Hemisphere Temperature (NHT) and Atlantic Multidecadal Oscillation (AMO) climatic indices ($p < 0.001$). Such increases are associated with an unprecedented occurrence of environmentally acquired *Vibrio* infections in the human population of Northern Europe and the Atlantic coast of the United States in recent years.

Conclusions: This study provide evidence, for the first time to our knowledge, that a warming trend in sea surface temperature is strongly associated with spread of vibrios, an important group of marine prokaryotes, and emergence of human diseases caused by these pathogens

BACILLI FROM SHALLOW HYDROTHERMAL VENTS OF EOLIAN ISLAND (ITALY) AND THEIR BIOTECHNOLOGICAL POTENTIAL

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Shallow hydrothermal vents of the Eolian Islands (Italy) represent a rich source of *extremophiles* able to produce novel biomolecules having useful functions in situ and a wide variety of properties with potential applications in biotechnology.
New thermophilic and thermotolerant bacteria isolated from these sites offer several advantages for biotechnological purposes, as they are fast growing and their biomolecules can be produced faster than their mesophilic counterparts. Novel species of *Bacillus* and *Geobacillus* genera were described from Eolian vents, and some of them were able to produce new biopolymers under laboratory conditions, such as exopolysaccharides (EPSs) and poly-glutamic acids (PGAs). These biopolymers possess unique chemical composition and physicochemical properties, as well as attractive characteristics (thermostability, water-solubility, noncytotoxicity, anti-cytotoxicity, biodegradability and ecological acceptability), that make them useful in responding to the increasing demand of novel bioproducts in different applicative sectors and biotechnological perspectives for human health. The new EPSs produced by *G. thermodenitrificans* B3-72, *Bacillus licheniformis* strains B3-15 and T14, and the PGA from *B. horneckiae* APA, have been reported among the few compounds until now derived from marine bacteria with antiviral and immunomodulatory activities. As stimulators of Th1 cell-mediated immunity, these biopolymers could represent potential novel therapeutics in immunocompromised hosts. Some EPSs were also able to prevent biofilm formation by multi-resistant pathogenic bacteria, without affecting bacterial vitality, but acting as anti-adhesives they interfere with the settlement of the pathogens on abiotic surfaces. Therefore, the use of these biopolymers may represent a promising therapeutic strategy for combating bacterial biofilm-associated infections.
With ongoing research on physical properties and their related biological activities, the number of possible applications of the biopolymers from Eolian bacilli increases rapidly, ranging from medical and nonmedical purposes, such as food additives, biomedical agents in dental care and ophthalmic solutions, anti-aging in cosmetics, and in environmental applications as antifouling, bioflocculants and heavy metals adsorbers for bioremediation.

FOCUS ON "ANTIBIOTIC STEWARDSHIP": FROM DIAGNOSTICS TO CLINIC

MICOBIOLOGY FAST TRACK DIAGNOSIS IN AN ERA OF MULTI-DRUG-RESISTANT PATHOGENS

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Early availability of information on bacterial pathogens and their antimicrobial susceptibility is of key importance for the management of infectious diseases patients. Until microbiological results are available, broad empirical antimicrobial coverage is used to ensure adequate treatment and optimal survival in critically ill patients. However, this antibiotic overuse in the hospital setting, results in continuous pressure towards selection of resistance .During last two decades, global selective pressure due to antimicrobial overuse has driven the emergence and spread of multi-drug-resistant (MDR) microorganisms such as methicillin-resistant Staphylococcus aureus, extended-spectrum beta-lactamase-producing Enterobacteriaceae and carbapenem-resistant gram-negative rods.

Because rapidity and acuity of microbiological diagnosis may impact significantly on the patient outcome as well as on antibiotic consumption, many efforts have been taken during last years to improve microbiological tests.

Currently, using traditional approaches, it usually takes at least 24 hours for obtaining growth from clinical specimens, and an additional 24 hours for identification and susceptibility testing of bacterial pathogens.

The improvement of available techniques (e.g. for susceptibility testing, DNA amplification assays), and introduction of novel technologies (e.g. MALDI-TOF) has fundamentally changed approaches towards pathogen identification and characterization. Importantly, these techniques offer increased diagnostic resolution while at the same time shorten the time-to-result, and are thus of obvious importance for antimicrobial stewardship to control emergence and spread of MDR microorganisms.

MALDI-TOF mass spectrometry fingerprinting has now been widely adopted by clinical microbiology laboratories for rapid identification of cultured microorganisms. Compared to other conventional (e.g.biochemical) identification strategies, the time-to-results is typically reduced by at least one working day up to several days for fastidious species or isolates that require additional tests for definite identification. Because of its performances MALDI-TOF mass spectrometry fingerprinting must be regarded as a major advance in the field of clinical microbiology.

Other new diagnostic technologies with the potential of shortening identification and antimicrobial susceptibility times compared to conventional microbiology include rapid immunochromatography, molecular biology and automated time-lapse microscopy. All these technologies offer significant opportunities to further reduce the turn around time for pathogen identification and information on key resistance traits and when next generation sequencing will be implemented into routine diagnostic procedures, additional improvements will be expected.

Thanks to new technologies, in the next future we are going to opening an era in which medical microbiology will be able to substantially support also the earlier stages of clinical decision making.

This approach should successfully limit selection of resistance by reducing overuse and inappropriate antimicrobial therapy.

"OLD" MOLECULES AND ANTIMICROBIAL STEWARDSHIP

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The Antimicrobial Stewardship project conducted in our institution, in strict collaboration with the Hospital Pharmacy, is based both on educational activities and on a semi-restrictive intervention. Educational activities included a series of front lessons on the proper use of antibiotics, while the semi-restrictive intervention has focused on the control of usage of some "crucial" antibiotics. The semi-restrictive intervention is based on a computerized approach using a software which allows individualized prescription and administration of drugs (Sofia®, SANTALUCIA PHARMA APPS©, Località Gragnanino, PC, Italy). Briefly, for each prescription of antimicrobials deemed to have a major impact on antibiotic resistance and/or routinely used in the treatment of resistant infections (i.e., vancomycin, teicoplanin, daptomycin, ceftaroline, ceftobiprole, meropenem, ertapenem, imipenem, linezolid, tigecycline, colistin, voriconazole, posaconazole, liposomal amphotericin B, micafungin, caspofungin, anidulafungin) a request for approval is automatically generated by the software, to be evaluated by an Infectious Disease Physician (IDP). After prescription, the IDP is allowed a maximum of 48 h (72 h if the prescription is made during weekend days) for approval , rejection or changes in dosage and/or duration. Discussion and feed-back with the original prescriber is the rule, to reach consensus. Pending the IDP's decision, the prescribed drug continues to be administered. In absence of intervention within 48/72 h, the prescriber is anyway allowed to administer the drug to avoid the omission of doses, but a warning is automatically issued by the system, followed by a phone call by the Pharmacy, in order to remind the IDP to evaluate the case. The "new" molecules (ceftolozane-tazobactam (C-T), dalbavancin, ceftazidime-avibactam, isavuconazole, can be prescribed only by the IDP, according to a rule issued by the Italian Medicine Agency.

In terms of antimicrobial stewardship, the main intervention, at least in our country, is to limit as much as possible, the use of carbapenems, giving the ongoing epidemics of infections due to Carbapenam Resistant Enterobacteriaceae. The problem is, therefore, to agree on the indications for carbapenems and the main guidelines for carbapenem-sparing strategies.

In targeted therapy, indications for the use of carbapenems (meropenem e imipenem) include severe infections due to III-generation cephalosporin and piperacillin-tazobactam (PT)-resistant Gram-negative rods. In empirical therapy, at least in our country, carbapenems are indicated as first line therapy of hospital infections in high risk patients, in severe clinical conditions, pending microbiological results. These situations include complicated abdominal infections, complicated urinary tract infections in heavily pre-treated patients, necrotizing pancreatitis, Cystic Fibrosis in acute relapse, nosocomial bacterial meningitis and fever in neutropenia. In practice, all those situations in which ESBL-producing Enterobacteriaceae are highly probable and the patient is too sick for waiting for microbiological results.

Escluding tygecicline, aminoglycosides anf fosfomycin, which may have a role in combination therapy, carbanemem-sparing procedures include day 2 or 3 de-escalation, as recommended by international guidelines in hematology (Averbuck et al, Haematologica, 2013) and the use of C-T (budget-permitting and according to approved indications). Whether or not P-T is safe and effective against infections due to ESBL-producers is a matter of long debate. Until the results of an ongoing RCT will be available, we can probably conclude that P-T can be used in infections due to ESBL-producers, provided the drug is in-vitro active against the isolated pathogen (controversies on the best cut-off exist), is used at the highest possible dosage (18 g), in a patient not in critical conditions. Combining and aminoglycoside might be prudent, at least in the first days, depending on the patient's renal function.

ANTIMICROBIAL STEWARDSHIP IN FUNGAL INFECTIONS: ROLE OF THE MICROBIOLOGIST

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Introduction: The use of antifungal drugs poses a daily challenge for hospital professionals of many different specialties, including microbiology. Clinical symptoms of invasive fungal diseases (IFDs) may be non-specific, diagnostic tests are far from perfect, and patients requiring antifungal agents often have comorbidities that increase the risk of toxicity and severe drug-drug interactions. Despite sharing of the aim of antimicrobial stewardship that is to preserve the future effectiveness of antimicrobial agents and to improve patient outcomes, antifungal stewardship (AFS) has peculiar features. These include, among others, high case-fatality rates, high drug costs, the development of antifungal resistance, and the complexity of IFDs in different patient populations. This poses the need for a multidisciplinary team encompassing the necessary expertise in the management of IFDs to develop and implement the AFS.

Materials and Methods: The aim of this talk is to summarize recent significant evidence obtained from literature searches, clinical laboratory experience, and the author's opinions, that outline how AFS programmes have been implemented in different clinical settings worldwide. The key issues discussed focus on how surveillance and diagnostics can be used to optimize antifungal management strategies.

Results: The potential activities of the clinical microbiologist as a member of the AFS team are reviewed, with particular emphasis to issues concerning the diagnostic test delivery and interpretation, the antifungal susceptibility testing, and the antifungal drug selection. Relevant studies underscore the importance of assessing the clinical utility of a diagnostic test, i.e., how the result will determine a treatment strategy and potentially influence patient management and outcome. Adequate risk assessment is an important element for the interpretation of test results. Hence, different approaches may need to be used in different patient groups to maximize diagnostic accuracy. In particular, clinical microbiologists are asked to i) provide rapid information regarding microbiological test results ii) optimize diagnostic pathways and implement innovative diagnostic tools; iii) assist with the interpretation of test results; iv) advise on environmental control; v) detect outbreaks or unexpected cases; and vi) advise on therapeutic drug monitoring to allow dosage optimization.

Conclusions: AFS programmes delivered by experienced teams can be efficacious and cost effective. However, barriers to the success of such programmes often include lack of investment in new diagnostic tools.

YOUNG SCIENTIST SESSION: CURRENT AND FUTURE IDEAS

FIRST STEP OF THE SIM GIOVANI

Francesco d'Aleo
Specialist in Microbiology e Virology

Introduction: The success of modern scientific society is mostly based on the investment on the young talents. About one year ago the Italian Society of Microbiology wagered on a new group of microbiologists for relaunch the spread of the microbiological science beetween the mostly young. The objective of the presentation is to review the main objectives pursued during the last year, those achieved and those in the pipeline.
Materials and Methods: The "Gruppo Giovani SIM" is a "toponym" that show a new lymph of our scientific society. The group has worked for improved the spread of microbiological science across a new methods of communication: a) a new internet platform for exchange of information; b) a special number of "Italian Biologist" journal where all contributs were wrote by young microbiologists; c) We have sponsored a course of Sexual-Infections diseases.
Results: Across our initiatives we have offered the improve of professional training at the young colleagues. The new internet platform is consolidate for the exchange of information, over more thousand messages was posted. Eleven microbiologist has wrote on the national journal "Italian Biologists" for improve and spread the their researchs. During the year the our policies have increase the number of subscribers at the Italian Society for Microbiology.
Conclusions: In conclusion this is the first step for a increase the interest about for microbiology between the young italian scientists. We hope that further ideas shall be carry forward in near future.

MICROBIOLOGY IN THE PHARMACEUTICAL FIELD: A FIGURE, MULTIPLE POSSIBILITIES

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Microbiology is well established for its role in investigating the relationship between infectious agents and their hosts that has a crucial impact on human health. This is particularly true not only in a hospital setting, but also in the pharmaceutical industry, where the role of the microbiologist is relevant to avoid the presence of microorganisms in the manufacturers of pharmaceuticals that could have a severe impact on public health. Moreover, during the last years we have experienced a rapid and increasing development of new anti-viral and anti-bacterial agents where the microbiologist can act both in the in vitro setting, during the drug design phase, but also in the commercialization one. The scientific communication, for example, is now requiring strong scientific skills so that biologist are the predominant candidates, as well as for the medical devices (included in vitro diagnostics-IVD) field. Therefore, what we are experiencing now is that not only the laboratory practical skills are requested by the pharma industry but also the scientific knowledge and the ability to properly communicate and this opens up to new job possibilities where biologists can find their positioning. The personal carrier experience I would like to share describes how a strong scientific curiosity led to a job position that covers several aspects of science from microbiology, through the oncology field to rare diseases.

PHD IN ITALY: WILL WE SURVIVE?

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Introduction: A PhD (Doctor of Philosophy) is a postgraduate degree that is awarded after the completion of challenging coursework and research. Many programs take around three to four years to complete, and graduates often find careers in research or academia. In Italy, the PhD aims to provide students with a comprehensive understanding of the scientific research methodology. The course continues for a period of time depending upon the topic of research, grants, donations and the infrastructure of the institution which you have chosen. The doctorate is attained with extensive documentation of information, practical experimentation and a final dissertation. Positions are restricted to a limited number of applicants, not necessarily Italian. They may receive a PhD scholarship and may pay student fee to access the PhD program.
Materials and Method: To find all PhD course we conducted an research using web site of Ministero dell'Istruzione dell'Universita e della Ricerca (http://cercauniversita.cineca.it/php5/dottorati/cerca.php). We considered, only PhD program that include as scientific discipline (S.D.), MED/07, BIO/19, AGR/16 and VET/06 and used the following criteria: cycle, Ateneo, and area.
Results: In our research we considered XXX, (university year 2014/15), XXXI (university year 2015/16), and XXXII (university year 2016/17), cycles of PhD. Considering all S.D., Ateneo and area were activate for XXX cycle in n° 897 of PhD program, for XXXI cycle in n° 909 of PhD program and finally for XXXII cycle in n° 913.
S.D. AGR/16, MED/07, BIO/19 and VET/06 were present in n°147 of PhD program for XXX cycle, in n°152 of PhD program for XXXI cycle and finally in n° 142 for XXXII cycle. Only three (0,3%) PhD programs reported the term "microbiology" in title.
Conclusion: PhD candidates in Italy are considered as students at the highest level of their education.
Our analysis showed that the microbiology science is present in about 16% of PhD programs activated in the last three years. In addition in the last year, it possible valuates a light reduction of the presence of the microbiology sciences in PhD programs.
Even more, we found that specific PhD programs on microbiology sciences were used in 0,3% only.

ORAL COMMUNICATIONS

WORKING GROUPS
VIRUS-HOST INTERACTIONS

INTRODUCTION VIRUS AND HOST INNATE RESPONSES

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Introduction: Natural killer (NK) cells, as part of the innate immune system, play a key role in host defense against viral infections. Recent advances have indicated that NK cell activation and function are regulated by the interplay between inhibitory and activating signals.
Materials and Methods: To investigate the possible effect of herpesvirus (HHVs) infections on NK cell response, two models will be presented: i) HHVs infection in Multiple sclerosis patients; ii) HHVs infection at the NK/endometrium interface.
Results: We have recently shown that NK cells from a subgroup of MS patients have an impaired response to Human Herpesvirus (HHV) infection (Rizzo et al. J Neuroimmunol. 2016) and are characterized by the expression of KIR (Killer Ig-like receptors) 2DL2 inhibitory receptor. NK92 cells were engineered to switch on and off KIR2DL2 expression by demethylation and transfection. The turning on of KIR2DL2 resulted in NK cell inability to control HHV (HSV-1, HHV-6A and 6B, EBV) infection, with a 3 log increase in viral production. The KIR2DL2 switch off re-established NK cell activation towards HHV infected cells, to levels detected in parental cells. We have recently shown the presence of HHV-6A DNA in 43% of endometrial biopsies from primary idiopathic infertile women, but not in fertile women (Marci R et al. PlosOne 2016). The presence of HHV-6A infection seems to affect endometrial specific CD56brightCD16- NK cells percentage and activation, with infertile HHV-6A positive women with a decrease in specific endometrial resident NK cells, a different expression of activating and inhibitory receptors and a hampered DNA sensor STING-STAT6 signaling.
Conclusions: For the first time, we show the mechanisms responsible for NK cell activation and function in the control of HHVs infections. These data will help the development of antiviral and NK cell-based therapies.

C1

HPV18 PERSISTENCE IMPAIRS BOTH BASAL AND DNA LIGAND-MEDIATED IFN-B AND IFN-Λ1 PRODUCTION THROUGH TRANSCRIPTIONAL REPRESSION OF MULTIPLE DOWNSTREAM EFFECTORS OF PATTERN RECOGNITION RECEPTOR SIGNALING

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Introduction: Upon virus infection, host cells mount a concerted innate immune response involving both type I and III interferons (IFNs) and pro-inflammatory cytokines to enable the elimination of pathogens. Escape from innate immune surveillance appears to be the hallmark of human papillomavirus (HPV) infection. Since undifferentiated keratinocytes express several pattern recognition receptors (PRRs), which are able to sense viral pathogens and promote the innate immune response, it is highly likely that high-risk HPVs have developed effective strategies to evade the innate immunity by inhibiting PRR downstream signaling.
Materials and Methods: In order to better recapitulate the HPV impact on its natural target cells, we have assessed the innate immune response in NIKSmcHPV18 cells carrying high numbers of episomal viral genome copies. These cells were used when the E6 and E7 transcripts were higher than those of E2, an expression pattern typical of persistent HPV infection. For comparison, we also included HeLa cells, which are transformed cells harboring integrated DNA and characterized by overexpression of E6 and E7 oncogenes.
Results: We demonstrated that HeLa and NIKS cells harboring multiple copies of episomal HPV18 genome fail to produce both type I and III interferons not only under differentiating conditions, but also following exposure to either salmon sperm DNA or poly(dA:dT), two potent inducers of PRR signaling. Furthermore, cGAS, IFI16, STING, and RIG-I proteins were all poorly expressed or almost undetectable in both NIKSmcHPV18 and HeLa

cells when compared to parental NIKS cells. Their suppression mainly occurred at the mRNA rather than the protein level. This inhibitory activity seems to be irreversible in the case of the cGAS-STING pathway, as we did not find any recovery of these proteins even after treatment with exogenous DNA, while RIG-I protein expression was induced in response to poly(dA:dT) transfection, and it likely mediated the residual IFN production observed in both HPV-positive cells.

Conclusions: Overall, our findings provide novel insights into HPV18 immune escape mechanisms with possible implications in cervical carcinogenesis.

C2

FROM DESIGN OF A GENOME SEQUENCE TO GENERATION OF A SYNTHETIC VIRUS: PARVOVIRUS B19 REVERSE GENETICS

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Introduction: Parvovirus B19 (B19V) is a human pathogenic virus, characterized by a selective tropism for erythroid progenitor cells (EPCs) in the bone marrow, and responsible for a wide range of clinical manifestations. Central to genetic studies for B19V is the availability of genomic clones possessing full competence and ability to generate infectious virus.

Materials and Methods: In our study, we established a new model genetic system for B19V. A synthetic approach was followed, by design of a reference genome sequence, by generation of a corresponding artificial construct and its molecular cloning in a complete and functional form, and by setup of an efficient strategy to generate infectious virus, via transfection in UT7/EpoS1 cells and successive rounds of amplification in EPCs.

Results: A reference genome sequence was designed as a consensus, possibly ancestral sequence for B19V. Following transfection in UT7/EpoS1 and amplification in EPCs, the synthetic genome was able to generate virus with biological properties paralleling those of native virus, its infectious activity being dependent on the preservation of self-complementarity and sequence heterogeneity within the terminal regions. Studies of serial passages of the synthetic virus in EPCs indicate that a complete replicative cycle is achieved in 72 hours, can yield a 4 Log increase in the amount of virus replicating within cells and a 3 Log increase in the release of virus in cell culture medium.

Conclusion: Our study provides the first achievement of reverse genetics for B19V, leading from the design of a genome sequence to generation of a synthetic virus. A virus of defined genome sequence, obtained from controlled cell culture conditions, can constitute a reference tool for investigation of the structural and functional characteristics of the virus and the development of antiviral strategies.

C3

THE HUMAN CYTOMEGALOVIRUS TEGUMENT PROTEIN PP65 (PUL83) DAMPENS TYPE I INTERFERON PRODUCTION BY INACTIVATING THE DNA SENSOR CGAS WITHOUT AFFECTING STING

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Introduction: The innate immune response plays a pivotal role during human Cytomegalovirus (HCMV) primary infection. Indeed, HCMV infection of primary fibroblasts rapidly triggers strong induction of type I interferons (IFN-I) accompanied by proinflammatory cytokine release.
Materials and Methods: Here, we show that primary human foreskin fibroblasts (HFFs) infected with a mutant HCMV TB40/E strain unable to express UL83-encoded pp65 (v65Stop) produce significantly higher IFNI levels than HFFs infected with the wild type TB40/E strain (v65Rev), suggesting that the tegument protein pp65 may dampen IFNI production.
Results: To clarify the mechanisms through which pp65 inhibits IFNI production, we analyzed the activation of the cGAS/STING/IRF3 axis in HFFs infected with either v65Rev or the pp65-deficient mutant v65Stop. We found that pp65 selectively binds to cGAS and prevents its interaction with STING, thus inactivating the signaling pathway through the cGAS/STING/IRF3 axis. Consistently, addition of exogenous cGAMP to v65Rev infected cells triggered the production of IFN-I levels similar to those observed with v65Stop infected cells confirming that pp65 inactivation of IFN-I production occurs at the cGAS level. Notably, within the first 24 hours of HCMV infection, STING undergoes proteasome degradation independent of the presence or absence of pp65.
Conclusions: Collectively, our data provide mechanistic insight into the interplay between HCMV pp65 and cGAS, leading to subsequent immune evasion by this prominent DNA virus.

C4

THE FATE OF HIV-1 PROVIRUS AFTER CRISPR/CAS9 ABLATION

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The HIV-1 provirus is efficiently excised by CRISPR/Cas-9 from the host genome but it persists as episome for prolonged period of time and shows minimal expression activity in the infected cells
Introduction: The highly active anti-retroviral therapy (HAART) controls the HIV-1 replication and has transformed the infection from a deadly disease to a chronic illness, but it does not provide a cure. Indeed, the latent proviral DNA is neither affected by drug treatment nor blocked from reactivation. Infection poses a threat and can easily re-emerge upon development of drug resistance. Recently developed gene-editing tools have the ability to precisely manipulate the cell genome and have been used as novel therapeutic strategy to cut out the proviral genome of HIV-1 from host cell genome. Despite great potential and providing an approach to cure cells from infection, little is known about the fate of excised proviruses. The aim of this work is to monitor what happens to HIV-1 genome once excised by CRISPR/Cas9 from cell genome.
Materials and Methods: We have generated a CRISPR/Cas-9 system targeting 5'- and 3'LTRs to cut out the integrated pNL4.3 HIV-1 genome and monitored its persistence, and recombination and rearrangement events in the host cell. To test the ability of excised provirus to recombine, we generated two replication-competent pNL4.3 HIV-1 vectors, one bearing the Kanamycin resistance gene (pNL4.3 KAN), the second with a bacterial origin of replication (pNL4.3 ORI). 293T cells were first co-transduced with pNL4.3 KAN and pNL4.3 ORI then, 3 days after, further transfected with the CRISPR/Cas9 system cutting in the LTRs. The excised proviruses were ligated to generate a plasmid-like structure containing pNL4.3 KAN e pNL4.3 ORI and that was rescued by transforming competent *E. coli*. The recovered concatamer was then examined for transcription and translation ability *in vitro*.
Results: We found that the excised HIV provirus persists in linear form or as episome for several weeks and has the capacity to recombine. Recombination occurs more frequently in sensus-sensus orientation and can partially or totally rescue LTR sequence. Once transfected in 293T cells the concatamer shows an imperceptible (but not negative) transcriptional activity. Notably,

expression is strongly enhanced in the presence of Rev provided in trans and the concatamer has the potential to generate infective virions in the presence of a helper virus.

Conclusion: Even if de novo integration or recombination of circularized single genome or concatamers is unlikely, the pre-integration complex is disassembled, we have shown that these molecules persist for several weeks and may be rescued by a second infection. These results, albeit preliminary and awaiting further confirmation, do not undermine the extreme potential of gene-editing to eradicate infection and disclosed an issue that has to take into account before deploying the strategy on a full clinical basis.

C5

THE HUMAN ENDOGENOUS RETROVIRUS H IS OVER EXPRESSED AND HYPOMETHYLATED IN THE TISSUES OF COLORECTAL CANCER PATIENTS

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Background/Aim: Human Endogenous Retroviruses (HERVs) are remnants of ancient exogenous retroviral infections of the humans, representing about 8% of the human genome. Several reports have shown the existence of a relationship between the HERVs expression and tumors, based on the mRNA expression profile of HERVs in normal and cancer tissues, but conclusive evidence is still lacking.

Methods: The expressions of the env genomic region of HERV-H, HERV-K, HERV-P, and HERV-R were evaluated in the peripheral blood mononuclear cells (PBMCs), in the tumor and the adjacent normal tissues of 30 colorectal cancer patients. A group of control composed by PBMCs of 46 healthy subjects (HC) was also included in the study. RNA was isolated from the clinical specimens, reverse transcripted, and subjected to relative quantitative Real Time PCR. The env expressions were related to the expression of the housekeeping GAPDH gene. The quantification was conducted using comparative Ct method and the difference between the levels of env gene expression in the different specimens was given by fold difference. Fold difference values were relative to a calibrator, the PBMCs of patients first and the PBMCs of the healthy subjects, then Methylation status of the HERV- H, -K and -P LTRs was evaluated by means of bisulfite-PCR and pyrosequencing.

Results: Higher levels of expression of HERV-H, HERV-K and HERV-P were found in tumor tissues, as compared to adjacent normal tissues (5.0, 4.0

and 4.1 increasing folds, respectively) and PBMCs (5.7, 6.6, and 12.5 increasing folds respectively). The expressions of HERV-H, HERV-K and HERV-P env were also increased in the tumor tissues compared to the HC PBMCs (8.3, 9.4 and 4.4 folds respectively). No differences were observed in the expression of HERVs env among HC PBMCs, PBMCs and normal adjacent tissues of patients. HERV-H LTR was hypomethylated in the tumor tissues (56.1%) compared to normal adjacent tissues (73.1%) and PBMCs (86.5%).

Conclusions: Increased expression of HERV-H env in association with the demethylation of its LTR might support the hypothesis of specific liberation of HERV-H LTRs from epigenetic controls in colon cancer.

C6

RELEVANCE OF GLIA-NEURON CROSSTALK IN HSV-1-INDUCED NEURONAL DAMAGE

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Introduction: Our previous data show that HSV-1 infection of primary neurons leads to accumulation of neurotoxic fragments of Amyloid Precursor Proteins (APP) (De Chiara et al, 2010; Civitelli et al, 2015), key players in Alzheimer's disease (AD) pathogenesis. Among them, there are Aß peptides that are known to activate glia surronding neurons and to induce pro-inflammatory gene expression, which in turn potentially contribute to neuronal dysfunction and cell death. Here, the crosstalk between neurons and glia during HSV-1 infection and the resulting appearance of inflammatory/neurodegenerative markers, including pro-inflammatory cytokines, APP fragments and hyper-phosphorylated tau (p-tau) protein, were analyzed in order to investigate the cause/effect relationship between the viral infection and AD.

Methods: HSV-1-infected human glia-neuron co-cultures were used as experimental model. HSV-1 infections were titrated by standard plaque assay and by "In-Cell Western™" Assay, as recently described (Fabiani et al, 2017). APP amyloidogenic processing and the production of its neurotoxic fragments as well as p-tau were investigated by western blotting (wb). Pro-inflammatory mediators (chemokines, cytokines, reactive oxygen species ROS) were quantified by ELISA, FACS analysis and Multiplex assay technology. Secreted extracellular vesicles (produced at different stages of HSV-1 infection, were isolated by ultracentrifugation and analyzed by wb to study glia-neuron communication.

Results: Wb analysis on cellular lysates harvested 20 hrs after HSV-1 infection (pi) highlighted increased levels of p-tau and Aβ oligomers in glia-neuron co-cultures, compared to those found in single cultures. These levels significantly increased in neuronal cells infected with HSV-1 for 20 hrs in the presence of cellular supernatants from HSV-1-

infected glia, suggesting a possible involvement of some mediators secreted from these cells. Among them, we revealed elevated levels of MCP-1 in glial supernatants at different time pi, particularly significant 20 hrs pi. Moreover we found increased levels of IL-6 and Rantes in supernatant from HSV-1-infected glia-neuron co-cultures. In addition, we revealed increased levels of ROS and lipid peroxidation products starting from 4h pi, especially in neuronal cells. Finally, experiments carried out on exosomes secreted from HSV-1-infected neuronal cells demonstrated an increased secretion of tau protein (both phosphorylated and non-phosphorylated form).

Conclusions: Overall, these findings suggest that HSV-1 infection in the brain influences the cross-talk between glia-neurons, inducing a dangerous link between neuroinflammation and neurodegeneration.

C7

ANTI-HERPETIC ACTIVITY OF TEMPORIN PEPTIDES CONJUGATED WITH LIPID MOIETES

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Introduction: Skin secretions of frogs contain many different types of antibacterial peptides. For example, temporins are peptides isolated from the skin secretion of *Rana temporaria*. The temporins belong to a family of short (8–17 amino acids), hydrophobic, C-terminally α-amidated peptides with antibacterial and antifungal properties. They adopt an α-helical conformation in hydrophobic environments and have the ability to perturb the integrity of target cell membranes. Not all temporins are cationic, but the number of positively charged amino acids correlates with antimicrobial potency. Temporins are mostly effective against Gram-positive bacteria, but some are also active against Gram-negative bacteria. Previously, we have demonstrated the powerful anti-herpetic activity of temporin 47 (Phe-Val-Pro-Trp-Phe-Ser-Lys-Phe-DLeu-Lys-Arg-Ile-Leu) and its low cytotoxicity. So we have decided to conjugate this native peptide with cholesterol and fatty acids (palmitic acid, tridecanoic, pentadecanoic and undecanoic acids, aminocaproic acid) at amino-terminal (N) or carboxyl-terminal (C) ends in order to improve its biological activity.

Materials and Methods: The peptides are synthesized using solid-phase Fmoc chemistry method, followed by purification by reversed phase HPLC. The cytotoxic activity was determined via MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. The antiviral activity was evaluated against Herpes Simplex Virus type 1 (HSV-1) through co-treatment, cell pre-treatment, virus pre-treatment and post-treatment assays in a range of concentrations between 100 and 0.1 μM.

Results: The peptides have been modified in order to increase the peptide stability and antiviral activity. Among the peptides generated, the majorities have shown a low toxicity, in particular those conjugated

with cholesterol. Instead, the counterparts without cholesterol showed a greater inhibition of HSV-1 infectivity together with the peptides modified with fatty acids. In details, they work in an extracellular phase, probably in viral attachment and entry steps. **Conclusions:** Our results show novel possible applications of TL47 derived peptides in the field of antivirals. Further studies will be focused on their specific mechanism of action in order to understand the viral target on which the peptides act.

ORAL COMMUNICATIONS

WORKING GROUPS
ANTIBIOTIC RESISTANCE IN THE “ONE-HEALTH” APPROACH

C8

SPREAD OF COLISTIN RESISTANCE GENE MCR-1 IN ITALY: FIRST DETECTION OF THE ALLELIC VARIANT MCR-1.2 IN *ESCHERICHIA COLI*

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Introduction: The transferable colistin resistance gene mcr-1, encoding a phosphoethanolamine transferase, was first reported in China and has subsequently shown to have worldwide distribution. To date, two new different genes (*mcr-2* and *mcr-3*) and six allelic variants of mcr-1 (from -1.2 to -1.7) have been described.Here we report the first detection of the *mcr-1.2* variant, carried by a conjugative plasmid, in colistin-resistant *Escherichia coli*, against a background of increasing circulation of the *mcr-1*-mediated colistin resistance in Italy.

Materials and Methods: Two colistin-resistant blood culture isolates of *E. coli* were investigated to gain insight into the genetic basis of colistin resistance. The presence of *mcr-1* was detected by PCR and its transferability was explored by conjugation.The plasmid harbouring *mcr-1*, identified both in the donor and in the transconjugants, was analyzed by S1-PFGE, Southern blot and hybridization assays, and then sequenced. The genetic relatedness was determined by XbaI-PFGE and multilocus sequence typing (MLST). Finally, the *mcr-1* fitness cost was evaluated by growth assays.

Results: Since the two colistin-resistant isolates were indistinguishable - identical XbaI-PFGE pulsotype, identical sequence type (ST354) -, only one isolate was investigated further for molecular traits. The sequencing results demonstrated that the *mcr-1* gene (i) was identical to the allelic variant *mcr-1.2* recently described in Italy in *Klebsiella pneumoniae* and (ii) was carried by an IncX4-type plasmid of 33,293 bp (designated pMCR1.2-IT-Ec). Mating experiments showed that pMCR1.2-IT-Ec was transferable to *E. coli* and *K. pneumoniae* recipients and its acquisition did not cause any significant fitness cost.

Conclusions: This is the first time that the allelic variant *mcr-1.2* is found in *E. coli*, specifically in an ST354 blood isolate where it is carried by an IncX4 plasmid, confirming the ongoing spread of *mcr-1*-mediated colistin resistance in Italy.

C9

DETECTION OF AN OXA-48 AND NDM-1 MDR KLEBSIELLA PNEUMONIAE OF SEQUENCE TYPE (ST) 101 FROM BLOOD, ITALY

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Introduction: The aim of the study was to characterize at molecular level a carbapenem resistant MDR *Klebsiella pneumoniae* strain collected from both blood and rectal swab of a patient with a history of previous hospitalization in Egypt.
Materials and Methods: Identification and susceptibility tests were performed by MALDI-TOF-MS and Vitek-2 bioMérieux; confirmed by AutoScan4 System (Beckman Coulter). Rectal swabs screening on ChromID agar bioMérieux, GeneXpertCarbaR (Cepheid), CT103XL Microarray Analysis (Check-Points) and genome sequencing were accomplished.
Results: On March 21th 2016, a 62 years old woman underwent surgery due to aneurysm rupture and cerebral hemorrhage at Cairo Hospital, during a holiday period. In early April, after the discharge from the Cairo Hospital ICU, the patient has been transferred at the neurosurgery ward of AO S. Croce and Carle, Cuneo, Italy. During the stay, a rectal colonization by a meropenem resistant strain was detected. The patient developed sepsis by both carbapenem resistant *K. pneumoniae* and *Acinetobacter baumannii*. On May 17th the patient was transferred to the Rehabilitation Unit of SS Trinità Fossano Hospital ASLCN1. An OXA-48/NDM-type *K. pneumoniae* strain was detected by both ChromID agar and Gene XpertCarbaR from the rectal swab specimen collected at the patient hospital admission. The above isolate multi-drug resistance and susceptibility to colistin, tigecycline and fosfomycin only, was confirmed by AutoScan4 at Pavia University. Microarray and genome analysis allowed the identification of $bla_{TEM1a, CTX-M-14b, CTX-M-15, OXA-9, OXA-48}$ and bla_{NDM-1} genes. The aminoglycosides, tetracyclines, fluoroquinolones and trimethoprim/sulfonamides resistance was due to aadA1, strA/B; tet(D); qnrS1; dfrA5/sul1 genes, respectively. FIIK, L, R and ColE replicon types were detected in the *K. pneumoniae* clinical strain, finally assigned to Sequence Type (ST) 101. On May the 18th, due to worsening of patient conditions, three blood culture sets were collected; two of these resulted positive for a blaOXA-48 Enterobacter cloacae by Gene XpertCarbaR. On the bases of the sensitivity testing data (IMP S = 2 mg/L e COL S ≤ 0.5 mg/L), an imipenem/colistin combination therapy was administrated. Subsequently to the improvement of the conditions and despite the persistance of the OXA-48 *E. cloacae* intestinal colonization, the patient was discharged on November the 3th and transferred to a Long Term Facility of Cuneo.
Conclusions: This is the first report on a ST 101 OXA-48 and NDM-1 MDR *K. pneumoniae* from blood, in Italy. Due to the well known ability of ST 101 *K. pneumoniae* lineage (and harboured plasmids) for efficient transmission, these findings highlight the importance to improve surveillance at the molecular level.

C10

NEW DESIGNED PGP INHIBITORS AS USEFUL CHEMICAL PROBE TO INVESTIGATE DRUG RESISTANCE IN TUBERCULOSIS AND NON TUBERCULAR MYCOBACTERIA

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Introduction: Tuberculosis (TB) is one of the most common infectious diseases worldwide, with about one-third of world population infected with Mycobacterium tuberculosis (M.Tb.). More frightening is the recent emergence of multi-drug and extensively drug resistant M.Tb. strains (MDR-TB and XDR-TB, respectively). Actually, Europe face a TB recrudescence due to the new human migration from TB high-incidence countries. Clearly, there is an urgent need to improve treatment by introducing new drugs able to reduce treatment duration, have an acceptable tolerability profile, be active against MDR/XDR TB, be of use in HIV-infected patients with TB, and be active against latent TB. The development of mutations in the genes associated with the resistance to the anti-tuberculosis drugs have long been considered the sole cause of resistance in tuberculosis. Nevertheless, M.Tb also presents intrinsic drug resistance, attributed to the unusual structure of its cell wall combined with effective efflux mechanism. Numerous studies show that the activity of antibiotics subjects to efflux can be enhanced by the combined use of efflux inhibitors (EIs). In this work, we evaluated the combinations between antituberculosis drugs and several EIs, designed by us, against clinical isolates of M.Tb and Non Tubercular Mycobacteria (NTM).

Materials and Methods: Using the resazurin microplate-based assay, 10 clinical strains of M. tuberculosis, H37Rv M. tuberculosis reference strain and 4 NTM clinical strains, were characterized using first and second line antituberculosis drugs both in presence and absence of a series of quinoxaline derivatives (EIs), previously identified as able to potentiate the antiproliferative activity of several anticancer drugs.

Results: The different resistance levels founded in the clinical strains have been reduced by efflux inhibitors and in some cases the susceptibility was completely restored. The fractional inhibitory concentrations (FIC) indicate synergistic activity for the interactions between different antibiotics and the EIs. The FICs ranged from 0.5, indicating a two-fold reduction on the Minimum Inhibitory Concentrations (MIC), to 0.015, indicating a 64-fold reduction.

Conclusions: The results obtained in this study indicated that the intrinsic efflux activity significantly contributes to the overall resistance in resistant clinical isolates of M. tuberculosis and NTM, and that the inhibition of efflux pumps by the EIs can enhance the clinical effect of antibiotics that are their substrates. The use of quinoxaline derivatives (EIs) as adjuvants of the antitubercular treatment may be a promise for the development of new and shorter therapeutic strategies against tuberculosis.

C11

WHOLE GENOME SEQUENCE ANALYSIS OF MULTI-DRUG RESISTANT *KLEBSIELLA PNEUMONIAE* ISOLATES AT PISA UNIVERSITY HOSPITAL

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Introduction: In KPC-producing *Klebsiella pneumoniae* (KPC-KP) isolates, colistin-resistance has been described as plasmidic (*mcr-1*) or chromosomically mediated. One frequently described mechanism is the disruption of *mgrB* gene by insertion sequences, for instance ISL3. In this study, the resistance profile of KPC-KPs was characterized and the localization and targets of ISL3 sequences within the core and the accessory genome of common KPC-KP lineages were identified.

Materials and Methods: Thirty clinical KPC-KPs isolated from patients hospitalized in the Azienda Ospedaliero-Universitaria Pisana were randomly selected during 2015-2016. Whole genome sequences (WGS) were analysed for resistance genes, plasmids, and insertion sequences. Phylogenetic analysis were performed to detect the spread of KPC-KPs in the hospital.

Results: Two isolates belonged to ST307, 3 isolates to ST37, and 25 isolates to ST512. Twenty-eight isolates carried *bla*KPC-3; among these, two had also *bla*VIM-1 and two others had *bla*CTX-M-15. One *bla*KPC-3 strain carried *mcr1.2*. The plasmid structures harboring the above-mentioned genes were reconstructed. ISL3, a transposable type I restriction modification system, was detected in 28 out of 30 isolates. ISL3s were localized on pKpQIL plasmids: exclusively in the two ISL3-negative isolates, pKpQIL plasmids were not present. In two ST512 isolates, an identical copy of the plasmid ISL3 was inserted in *mgrB* nucleotide position 133.

Conclusions: pKpQIL plasmids harbored the *bla*KPC-3 gene and the ISL3 insertion sequence, conferring both carbapenems and colistin resistance. These plasmids are omnipresent in KPC-KPs, representing a threat in the evolution of pan-resistant isolates. The ISL3 carrying plasmids play an important role in the rising of horizontal dissemination of colistin-resistance among KPC-KPs. In addition, the other plasmids analyzed harbored a huge list of genes coding for virulence factors and antimicrobial resistances, for instance aminoglycosides, quinolones and sulfonamides.

C12
CHALLENGES AND OPPORTUNITIES BY BRIDGING HUMAN AND ANIMAL SURVEILLANCE TO TACKLE ANTIMICROBIAL RESISTANCE

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Introduction: Antimicrobial resistance (AMR) requires a global and multidisciplinary approach to improve awareness, understand dynamics and find preventive solutions. In this framework we set up a one-health tailored pilot surveillance network to monitor occurrence of *Escherichia coli* ESBL in humans and food producing animals FPA, to investigate on dissemination paths of ESBL resistant E. coli in human healthcare units and to plan and develop educational modules for prudent use of antimicrobials in human and animals. With the project network in place we could promptly investigate on the emergence of the plasmid-mediated colistin resistance gene *mcr-1* that has been recently reported in *E. coli* of both animal and human origin.

Materials and Methods: According to objectives: i) we set up a cross-sectional study (2016-2017) by collecting representative *E. coli* ESBL+ isolates from human communities and FPA industrial holdings in six Italian Regions; ii) we searched for available data sources for descriptors and parameters to develop dynamic mathematical models to describe *E. coli* ESBL+ dissemination paths between health care units and iii) we explored the learning needs to tackle AMR so to identify knowledge gaps to be addressed by specific educational proposals. In this frame, colistin resistance was investigated among *E. coli* ESBL+ isolates from both animal and human sources. MIC was determined by broth microdilution method and interpreted according to EUCAST breakpoints. *mcr-1* gene was detected by PCR and sequencing.

Results: Halfway through the project 827 *E. coli* ESBL+ isolates (277 were from FPA) were collected. A post graduate course and a distance learning courses were identified to fulfill learning needs of clinicians, veterinarians and microbiologists. Few *E. coli* ESBL+ of human origin were tested for colistin resistance so far and found all susceptible. On the contrary among colistin resistant *E. coli* ESBL+ isolates of animal origin *mcr-1* was detected in 5% of poultry and 10% of bovines and of swine isolates. Data from antimicrobial susceptibility test (AMS) of patients from a high geographical coverage and statistics from health care units is feeding and providing parameters for mathematic dynamic models.

Conclusions: A challenge in a one-health-based surveillance of opportunistic bacteria is the different reference population that require a wide animal sample to detect the targeted human pathogens. So far, our experience recommends to stick on strong case definitions of both clinical cases and isolates to be included in the study. Although the data herein reported is preliminary the proportions of *mcr-1* carriers *E. coli* ESBL+ detected in FPA represents a serious public health threat that requires strict surveillance.

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C13

DETECTION OF MULTI-DRUG RESISTANT ESCHERICHIA COLI AND *SALMONELLA INFANTIS* ISOLATES FROM POULTRY

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Introduction: The development of antimicrobial resistance in *Enterobacteriaceae* has become a global problem in veterinary and in human medicine. Multiresistant gram–negative bacteria can reach humans through the food chain. The present study was carried out to detect phenotypically the susceptibility to various antibiotics and to investigate the presence of resistance genes in *E. coli* and *Salmonella infantis* isolated in healthy chickens in industrial farms and at slaughter.

Materials and Methods: One hundred-two *E. coli* and 25 *Salmonella infantis* isolates were collected from cloacal swabs and from skin samples in farms and in slaughterhouses respectively. Susceptibilities to antimicrobials (penicillins, tetracyclines, quinolones, fluoroquinolones, trimethoprim/sulfamethoxazole, beta-lactam and carbapenems) were evaluated by the agar diffusion method. Susceptibility to colistin was evaluated by broth microdilution method. Screening for ESBL-phenotypes was carried out by double-disk synergy test (DDST) with cefotaxime, ceftazidime and amoxicillin -clavulanic acid disks. The beta-lactamase genes (bla_{TEM}, bla_{TEM-1}, bla_{CTX-M}, $bla_{CTX-M-1}$) and the plasmid-mediated *mcr-1/mcr-2* genes responsible for colistin resistance were investigated by PCR.

Results: A multiple–antibiotic resistance phenotype was detected in 73 of 102 *E.coli* isolates (72%); in particular 59 (81%) were resistant to 4 classes of antimicrobials (penicillins, quinolones, tetracycline and trimethoprim/sulfamethoxazole), and 14 (19%) were resistant to 3 (penicillins, quinolones, tetracycline). Moreover 15 (15%) of isolates exhibited ESBL phenotype carrying bla_{TEM-1} and bla_{CTX-M}. Four colistin-susceptible isolates (MIC 0.5-1 µg/mL) were positive for *mcr-1* gene. Four colistin-resistant (MIC 4 µg/ml) isolates were *mcr-1* negative. The *mcr-1* gene was detected in two colistin-resistant isolates (MIC > 8 µg/ml). All *Salmonella infantis* isolates showed a multi-resistance to 4 classes of antimicrobials and an ESBL phenotype, whereas they displayed different resistance genotype patterns. Ten (40%) colistin-resistant (MIC 4-32 µg/mL) isolates were *mcr-1* negative.

Conclusions: Our results confirmed that chicken farms constitute a reservoir of multiresistant and ESBL *E. coli* and *Salmonella infantis* isolates reflecting a high antibiotic pressure for selection of resistant bacteria in the ecosystem. In this scenario drug-resistant bacteria can disseminate in healthy chickens and spread to humans through consumption of contaminated food, transferring genes coding for antimicrobial resistance. In this point of view the *mcr-1* detection especially in *E. coli* colistin susceptible isolates, not only in *E. coli* colistin resistant ones can be a threat to public health as the gene could silently and extensively spread.

ORAL COMMUNICATIONS

WORKING GROUPS
MECHANISMS OF MICROBIAL PATHOGENICITY

INTRODUCTION *TRICHOMONAS* AND FRIENDS: MICROBIAL "PATHOGROUPS" AND INFECTIONS

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While microbial symbiosis between free-living protozoa and different prokaryotes are well described and quite common in nature, the role of symbiosis between protists and microorganisms in human infection has received little attention. The relationship between Legionella pneumophila, the causative agent of Legionnaire's disease, and environmental amoebae is the most intensively studied. The first reported example of symbiosis between two obligate human pathogens is the association between *Trichomonas vaginalis* and *Mycoplasma hominis.*

T. vaginalis is a flagellate anaerobic protist, responsible for the most prevalent nonviral sexually-transmitted infection in humans: it causes over 220 million cases of trichomoniasis each year worldwide. Trichomoniasis has been associated with invasive cervical and prostate cancers, and in females the genital infection is associated with severe pregnancy and postpartum complications, including abortion, endometritis, preterm delivery, and low birth weight.

M. hominis is a pathogenic bacterium able to colonize human urogenital tract in both sexes. Like the other mycoplasmas is characterized by small size, by a small genome of about 650 kilobases, and by the absence of a cell wall. Very recently, by using a next-generation sequencing approach to the study of the vaginal microbiome, a new species of unculturable mycoplasma, named *Candidatus Mycoplasma girerdii*, has been described as strictly and specifically associated with T.vaginalis infection in a high number of trichomoniasis patients.

Intestingly, *T.vaginalis* is also parasitized by a group of double-stranded RNA viruses (TVV) belonging to family of Totiviridae

The complex interactions between *T.vaginalis* and *M.hominis, Ca. M.girerdii*, and TVV describes a model in which the symbiosis between intracellular symbionts and protists influences the pathobiology of all microorganisms, and modulates host immune response during infection.

Endosymbionts of *T.vaginalis* represent a "pathogroup": a combination of bacterial species that alone are not pathogenic, but together, produce a disease state. Thus, we aim to study how *T. vaginalis* acts in concert with symbiotic bacterial and viral species to form units of pathogenicity or 'pathogroups', in order to understand how these interactions contribute to adverse outcomes linked with T.vaginalis infection.

C14

FUNCTIONAL DISSECTION OF LARGE SURFACE PROTEINS IN *ACINETOBACTER* SPP

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Introduction: Surface components have a major role in the life style of pathogenic bacteria of the genus *Acinetobacter*. In this genus large proteins ranging in size from 1800 to 5160 aa, such as the protein BAP (biofilm-associated protein) and the filamentous hemagglutinins (FHAs), are highly heterogeneous. FHA is an important adhesin that is secreted by the two-partner secretion (TPS) system. Peculiar TPS systems known as CDI (contact dependent growth inhibition) systems allow cells to touch and inhibit each other using toxic domain CdiA proteins (CdiA-CT). Small immunity (CdiI) proteins bind the CdiA-CT domain, that protects the CDI+ cells from autoinhibition. CdiA shares a number of features with FHA.

Materials and Methods: The two *A. baylyi* FHA genes have been mutated by inserting the kanamicin gene cassette in the region encoding the NH2 terminus of the corresponding proteins (DN2784 and DN0940 strains) and the COOH terminus of long FHA (DN0940 strain). By the same approach, a mutant in which the entire BAP-like protein encoded by *A. baylyi* (D2866).

Results: *In silico* analyses revealed that in *Acinetobacter* spp. FHA proteins come in two main size variants. Some strains produce only short type FHAs, others both short and long size variants. Short (ACIAD 2784) and long (ACIAD 0940) FHAs are encoded also by the *A. baylyi* ADP1 strain. Each system is made up by 3 genes, encoding an activator protein, the FHA protein and a immunity protein neutralizing toxin activity of the COOH terminus of FHA. We have mutagenized the two *A. baylyi* FHA and BAP genes, and the deletion mutants were analyzed for some phenotypes. Mutations introduced did not influence cell growth. All strains showed same round converse colony morphology, with exception of DN0940 strain that showed a irregular flat one. Mutating the short FHA had a limited, negligible effect on biofilm and motility. In contrast, inactivation of the long FHA significantly enhanced biofilm formation. The Δ2866 strain was fully impaired in biofilm formation and showed significantly lower motility than the wt. Wt and mutant *A. baylyi* strains had been challenged in their ability to interact with human cells. Mutating the small FHA protein had no effect on cell adhesion. In contrast, the removal of the NH2 terminus of the large FHA had a significant effect on cell adhesion. No change were observed when the COOH terminus of the protein was deleted. Competition assays showed that the wt inhibited the growth of ΔC0940 target bacteria lacking the COOH terminus and the immunity protein.

Conclusion: *A. baylyi* CDI systems are implicated in biofilm formation and provide an advantage in competition assays. The result also supports the notion that the BAP protein is a major component of biofilm formation in *A. baylyi* strain.

C15

CROSS SECTIONAL EVALUATION OF THE GUT-MICROBIOME METABOLOME AXIS IN AN ITALIAN COHORT OF IBD PATIENTS

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Introduction: Inflammatory bowel disease (IBD) is a chronic inflammatory disease of the gastrointestinal tract, whose etiology and pathogenesis are still under debate. IBD includes ulcerative colitis (UC) and Crohn's disease (CD), both diseases of the digestive tract with similar clinical, pathological and epidemiological features. Recent metagenomics studies suggest that the composition of gut microbiota may change in IBD affected individuals, but it is not yet entirely clear whether dysbiosis is the cause or the consequence of inflammatory processes in the intestinal tissue.

Materials and Methods: In this study, the composition of the microbiota and the metabolites in fecal extracts of 183 subjects (82 UC, 50 CD, and 51 healthy controls) were determined. Barcoded amplicon libraries for the analysis on the Illumina MiSeq platform were generated using degenerate primers targeting the V3 and V4 hypervariable region of the bacterial 16S rRNA gene. Analysis of the data generated on the Miseq System was carried out using the BaseSpace 16S Metagenomics App (Illumina), whereas operational taxonomic unit (OTU) mapping to the Greengenes database (V.13.8) were performed using the Quantitative Insights Into Microbial Ecology (QIIME) platform (V.1.8.0). Furthermore, frozen feces were extracted and the extract was divided in 3 aliquots for GC-MS, ^{1}H-NMR and LC-QTOF-MS analysis.

Results: The analysis showed a clear separation between CD and UC, and healthy individuals, and revealed significant differences in the metabolites content and the microbiological profiles. Microbiota differences between IBD patients and healthy subjects were evident at several taxonomic levels. At phylum level, there was a prevalence of Firmicutes and Actinobacteria in UC patients, and at genus level a relative abundance of Escherichia, Faecalibacterium, Streptococcus, Sutterella and Veillonella genera was seen in both CD and UC. Various metabolites including biogenic amines, amino acids, lipids and others, were significantly increased in both CD and UC, while other compounds, such as two B group vitamins, were decreased in the two pathological classes compared to healthy subjects. Spearman correlations were used to find out statistically significant metabolite-microbial relationship, showing an interesting interplay between metabolites and microbiota profiles in IBD patients.

Conclusions: This study underlines the potential role of an inter-omics approach in the understanding the metabolic pathways involved in IBD. The results emphasize that the combined evaluation of metabolites and fecal microbiome can be useful to discriminate between healthy subjects and patients with CD or UC.

C16

THE IMMUNE RESPONSE ASSOCIATED WITH THE MICROBIAL PERTURBATION IN VAGINAL DYSBIOSIS

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Introduction: Bacterial vaginosis (BV) is one of the most threatening subclinical issues during a woman's reproductive life. Progresses have been achieved toward the management of BV but many roads are still to be taken. The female reproductive tract is colonized by commensal microorganisms and the human vaginal mucosa harbours an immunologically unique microenvironment that differs from other tissues. The cross-talk between the microbial perturbation and the vaginal immunity response may be crucial for protection against adverse outcomes associated with BV.

Material and Methods: We profiled the vaginal microbiome and identified the potential role of specific bacteria in modulating the production of local immune mediators in selected women of reproductive age grouped according to the Nugent's criteria (NC). After informed consent, cervical-vaginal lavages were obtained from the women enrolled in the study. The V3-16S rRNA gene region was sequenced by the Ion Torrent PGM platform. Data analysis was performed by the QIIME 1.8.0 software, using the Vaginal 16S rDNA reference database. The quantification of 48 soluble immune mediators was carried out by the Luminex Multiplex Platform. Data analysis was performed by the GraphPad Prism (v. 5) software.

Results: Microbial and immune patterns diverged between healthy and dysbiotic NC status. G. vaginalis and B. breve dominated in the intermediate NC status while BVAB1 in vaginosis NC status. The perturbation of the resident Lactobacilli, Gardnerella and Ureaplasma were significantly associated with the increase of the IL5 and IL13, which are secreted by Th2 cells. Both the intermediate and vaginosis dysbiotic NC status differed from the healthy one for the increase of IL1ra and IL2 and the decrease of FGFβ and GMCSF. A specific pattern of pro-inflammatory cytokines, including IL1α, IL1β, and chemokines, such as IL8, MIG, MIP1α and RANTES, distinguished the intermediate NC status from the vaginosis NC status.

Conclusions: We postulate that the immune responses in the vagina could be orchestrated by the functional diversity of resident bacteria. Microbial products may bias the individual subset-driven immune responses, foremost by influencing the balance of Th1- vs. Th2-type responses. This study supports new in vivo insights into the cross-talk between the commensal bacteria and the immune compartment in modulating the vaginal microenvironment.

C17

EVALUATION OF *BACTEROIDES FRAGILIS* PROTECTIVE ROLE AGAINST BARTONELLA HENSELAE LIVER DAMAGE IN IMMUNOCOMPROMISED MURINE MODEL

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Introduction: *Bartonella henselae* is a gram-negative facultative intracellular bacterium and is the causative agent of cat-scratch disease. Our previous data have established that *Bacteroides fragilis* colonization is able to prevent *B. henselae* damages through the polysaccharide A (PSA) in an experimental murine model.
Material and methods: In order to determine whether the PSA is essential for the protection against pathogenic effects of *B. henselae* in immunocompromised hosts, SCID mice were co-infected with *B. fragilis* wild type or its mutant *B. fragilis* ΔPSA and the effects of infection on murine tissues have been observed by High-Frequency Ultrasound (HFUS), histopathological examination, and Transmission Electron Microscopy (TEM).
Results: For the first time, echostructure, hepatic lobes length, vascular alterations, and indirect signs of hepatic dysfunctions, routinely used as signs of disease in humans, have been analyzed in an immunocompromised murine model. Our findings showed echostructural alterations in all infected mice compared with the Phosphate Buffer Solution (PBS) control group; further, those infected with *B. henselae* and co-infected with *B. henselae/B. fragilis* ΔPSA presented the major echostructural alterations. The echogenicity score of co-infected mice with *B. henselae/B. fragilis* ΔPSA differed significantly compared with the PBS control group ($p < 0.05$). Moreover the inflammation score of the histopathological evaluation was fairly concordant with ultrasound findings. Ultrastructural analysis performed by TEM revealed no significant alterations in liver samples of SCID mice infected with *B. fragilis* wild type while those infected with *B. fragilis* ΔPSA showed the presence of collagen around the main vessels compared with the PBS control group. The liver samples of mice infected with *B. henselae* showed macro-areas rich in collagen, stellate cells, and histiocytic cells.
Conclusions: Interestingly, our data demonstrated that immunocompromised SCID mice infected with B. henselae and co-infected with *B. henselae/B.fragilis* ΔPSA showed the most severe morpho-structural liver damage. In addition, these results suggests that the HFUS together with histopathological evaluation could be considered good imaging approach to evaluate hepatic alterations.

C18

EFFECTS OF THE MUSCARINIC AGONIST PILOCARPINE ON THE PATHOGENESIS OF SYSTEMIC *CANDIDA ALBICANS* INFECTIONS

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Introduction: Acetylcholine (ACh) can directly affect *Candida albicans* filamentation and biofilm formation, modulating its pathogenicity, and promote an effective *Galleria mellonella* cellular immune response to *C. albicans* infection. Therefore it was hypothesized that C. albicans possesses a putative cholinergic receptor and that cholinergic receptors can also coordinate cellular immune responses in the *G. mellonella* infection model. The aims of this study were to determine the cholinergic receptor subtype responsible for the modulation of biofilm formation by C. albicans and to investigate the role of cholinergic receptors on *G. mellonella* cellular immunity.

Materials and Methods: The effect of cholinergic-receptor agonists - Pilocarpine (PLC, non-selective muscarinic agonist), and SIB1508Y maleate (SIB, non-selective nicotinic agonist) - on *C. albicans* biofilm formation was assessed using crystal violet and XTT assays, and by measuring cell surface hydrophobicity (CSH). ACh and PLC modulation of host immunity to *C. albicans* infection was determined using haemocyte analysis, and larval histology.

Results: PLC, but not SIB, inhibited *C. albicans* biofilm formation and decreased *C. albicans* cell surface hydrophobicity. Like ACh, PLC was also found to protect *G. mellonella* larvae from *C. albicans* induced mortality through inhibition of *C. albicans* biofilm formation *in vivo* and manipulation of cellular immunity. However, PLC and ACh differentially modulated *G. mellonella* hemocyte responses to *C. albicans*, with ACh promoting a stronger granulocytes activation.

Conclusion: Our data demonstrate that a muscarinic-type receptor modulates *C. albicans* yeast to hyphae transition, filamentation and biofilm formation. In addition, the data suggests that haemocyte subsets of G. mellonella possess differing repertoires of cholinergic receptors that can modulate their differentiation and function. However, nicotinic rather than muscarinic receptors seem to play a more important role in immune regulation in the host.

C19

HOST-GUT MICROBIOTA MARKS IN CYSTIC FIBROSIS: NEW POTENTIAL CLINICAL CUES

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Introduction: Cystic fibrosis (CF) is the most common autosomal recessive disease among Caucasians, affecting more than 30,000 individuals in the United States and more than 70,000 individuals worldwide. Abnormal expression of the cystic fibrosis transmembrane conductance regulator (CFTR) gene, leads to dysregulation of epithelial fluid transport in the lungs, pancreas, and other organs. Ionic imbalance disregulates intestinal homeostasis and affects gut microbiota eubiosis. This altered microbial ecosystem impairs the digestion of nutrients as well as host immunity and supports the growth of potentially pathogenic microbes The investigation of gut microbiota metacommunities and their functions by targeted-metagenomics (MG) may define CF gut microbiota enterogradients. The metabolite network produced by the gut microbial-host co-metabolism displays distinct "metabotypes", which contribute to the resolution of disease-driven shifts in host-microbiota interplay. The aim of this study was to provide a predictive and functional model of the gut microbiota enterophenotype of pediatric patients affected by Cystic Fibrosis (CF) naïve phenotypes.
Materials and Methods: Thirty-one fecal samples, collected from CF patients and healthy children (age range, 1-6 years), were analysed using targeted-MG and metabolomics (MB) to characterize the ecology and metabolism of CF-linked gut microbiota. The multidimensional data were low fused and processed by chemometric classification analysis.
Results: The fused MG and MB based gut microbiota profile was characterized by a high level of *Propionibacterium, Staphylococcus* and Clostridiaceae, including *Clostridium difficile*, slightly higher in patients with no antibiotic administration, and a low level of *Eggerthella, Eubacterium, Ruminococcus, Dorea, Faecalibacterium prausnitzii,* and Lachnospiraceae, associated with overexpression of 4-aminobutyrate (GABA), choline, ethanol, propylbutyrate, and pyridine and low levels of sarcosine, 4-methylphenol, uracil, glucose, acetate, phenol, benzaldehyde, and methylacetate. The CF gut microbiota pattern revealed an enterophenotype intrinsically linked to CFTR gene impairment, regardless of age, and with dysbiosis uninduced by pancreatic status phenotype and only partially related to oral antibiotic administration or lung colonization/infection.
Conclusion: Childhood microbiota profiling characterizing the naïve enterophenotype of CF gut microbiota, together with endogenous and bacterial CF biomarkers, directly reflected disease functional alterations at the intestinal level. This new understanding of CF host-gut microbiota interactions may be helpful to rationalize novel clinical interventions to improve the affected children's nutritional status and intestinal function.

C20

A SYNTHETIC KILLER PEPTIDE IMPAIRS *CANDIDA ALBICANS* BIOFILM FORMATION AND PERSISTENCE IN VITRO

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Introduction: *Candida* spp. colonize human skin and mucosae of healthy subjects, behaving as harmless commensals. Nevertheless, in susceptible patients, they behave as opportunistic pathogens also due to their capacity to form biofilm on host mucosae or medical device surfaces. When embedded in a biofilm, *Candida* exhibits enhanced tolerance to common disinfectants and most antifungals, including azoles. Thus, there is an urgent need to identify novel therapeutic molecules. Recently, several antibody-derived peptides proved to exert antimicrobial, antiviral, immunomodulatory and antitumor activity *in vitro* and *in vivo*. The aim of this study was to investigate the effects of a synthetic killer peptide (KP) on the formation and persistence of *Candida* biofilm.

Materials and Methods: *C. albicans* reference strain SC5314, two fluconazole-resistant and two fluconazole-susceptible *C. albicans* isolates were used. The activity of KP (AKVTMTCSAS) together with a scrambled peptide (negative control), was tested against *Candida* biofilms at different stages of development by microscopy, crystal violet and tetrazolium salt reduction assays. qRT-PCr was used to evaluate the effect of KP on biofilm related genes.

Results: KP strongly influenced *C. albicans* capacity of to form biofilm and significantly impaired mature biofilm. In particular, KP treatment induced *Candida* oxidative stress response, altered fungal cell membrane permeability and markedly impaired biofilm-related gene expression. Similar inhibitory effects were observed against all the yeast strains tested, irrespective of their resistance or susceptibility to fluconazole. Interestingly, the KP-mediated inhibitory effect was confirmed against a catheter-associated *C. albicans* biofilm.

Conclusions: These results provide the first evidence for the efficacy of KP against *C. albicans* biofilm, suggesting that this peptide may represent a novel potential molecule for treatment and prevention of biofilm-related *C. albicans* infections.

POSTERS

VIROLOGY

P1

DIFFERENTIAL ANTI-HERPETIC ACTIVITY OF KILLER PEPTIDES

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Introduction: Human antibody fragments proved to have an antimicrobial and antiviral wide spectrum activity *in vitro, ex vivo* and/or *in vivo*, mediated by different mechanisms of action. Parental killer peptide KP (AKVTMTCSAS) proved to be active against HIV, by downregulation of CCR5 coreceptor expression and/or steric block of gp120, and Influenza viruses, by inhibition of the late phase of viral multiplication. The aim of the present study was to investigate the activity of KP against Herpes Simplex-1 and 2 Viruses (HSV-1, HSV-2). Two other decapeptides were employed: K10S, a KP-derivative with a substitution of the first amino acid (KKVTMTCSAS), and a Scrambled Peptide (SP) with the same amino acids as KP but in a different sequence (MSTAVSKCAT), the last used as a negative control.

Materials and Methods: KP cytotoxicity on VERO cells was evaluated by MTT assay, while the antiviral activity was assessed by a viral yield reduction assay. Several protocols were then applied to study at which stage of the viral replication cycle KP was able to exert its antiviral activity (pre-treatment of VERO cells, pre-incubation KP-HSV-1, inhibition of adsorption and of penetration, and replication kinetic).

Results: KP showed strong antiviral activity in the range of 50 μg/ml to 100 μg/ml against both HSV-1 and HSV-2. HSV-1 virus yield was reduced of about 4 Logs at both concentrations; the same inhibition was obtained only at 100 μg/ml for HSV-2. K10S showed a lower HSV-1 inhibition (0,9 Log, at 100 μg/ml), while SP had no activity. Inhibition of virus replication was increased by pre-incubating the viral inoculum with KP for 1h at 37°C. With this treatment, the antiviral activity was remarkable (4 Logs) even at 10 μg/ml. The adsorption and penetration assay and the replication kinetic showed that KP exerts its activity directly on the free virions and during the initial phases of the replication cycle.

Conclusions: This *in vitro* study provides evidence of KP's activity against HSV-1. The increase in antiviral activity after pre-incubation of KP with HSV-1 inoculum suggests a direct activity against HSV-1 free particles. Moreover, also adsorption and penetration were inhibited, though at a lower extent. These results, together with those related to other viruses, open to the possibility that KP can offer a broad spectrum therapeutic approach to important viral infections.

P2

ANTIVIRAL AND ANTIOXIDANT ACTIVITY OF A HYDROALCOHOLIC EXTRACT FROM *HUMULUS LUPULUS* L.

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Introduction: Female strobilus inflorescences from *Humulus lupulus* L., commonly named hop cones or hops, are widely used in the brewing industry as preservative and flavouring additives, as well as in traditional medicine. Hop constituents include polyphenolic compounds, mainly prenylflavonoids (viz. xanthohumol) and their metabolites, which possess a variety of interesting biological properties, including antioxidant, anti-inflammatory, chemopreventive, antimicrobial and antiviral. Experimental evidences suggest that herbal medicines, likely due to their polyphenolic composition, can represent alternative or integrative strategies for fighting viral infections, including influenza.

The aim of this study was the evaluation of anti-influenza activity of a hydroalcoholic extract from female inflorescences of *H. lupulus* standardized to contain 0.4% of flavonoids.

Materials and Methods: Two epithelial cells (MDCK and A549), known to be permissive to influenza virus replication, were infected with influenza A/Puerto Rico/8/34 H1N1 (PR8) strain and treated with different concentrations and for different times with the extract. Cytotoxicity of the treatment was evaluated by the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-2H-tetrazoliumbromide (MTT) assay. Viral titer was measured by the hemagglutination assay. The viral protein expression was analysed by western blotting. In *in vitro* assay, the extract was able to scavenge different radicals and to interfere with lipoperoxidation. Being virus-induced oxidative stress useful for viral replication, the effect of the extract on the intracellular glutathione levels were evaluated by colorimetric assay in untreated and treated infected cells.

Results: In the range of the concentrations tested (10-140 μg/ml), the *H. lupulus* extract did not exhibit cytotoxicity on none of the cell lines. The addition of the extract to infected cells significantly inhibited viral replication, reaching about 80% inhibition at concentration of 140 μg/ml. In these conditions a significant inhibition of viral proteins was also highlighted, along with increased levels of intracellular GSH into infected cells, suggesting that the antiviral activity of the extract might be due to a restoration of the intracellular redox conditions. Viral replication was partly inhibited also when the virus was incubated with the extract before infection, suggesting also a virucidal activity.

Conclusions: *H. lupulus* extract exhibited antiviral activity when added at different steps of viral replication. Further studies are needed in order to hypothesize its possible application as anti-influenza dietary supplement.

P3

INHIBITION OF HUMAN HERPESVIRUS 8 BY SULFONE DERIVATIVES

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Introduction: The Human Herpesvirus 8 (HHV8) is the causative agent of Kaposi sarcoma, a malignant angiosarcoma that is frequent in immunosuppressed subjects and transplant recipients. After a short lytic infection, HHV8 enters into a latent phase inside the cells,where it can remain as a viral episome for the cell's entire life-span. HHV8 infections can be treated with common anti-cancer drugs, which are able to control disease severity, but do not clear the virus episome from the cell nucleus. We have recently demonstrated that some sulfonamides are able to inhibit latent HHV8 in primary human cells [1]. In this work we present data about the possibility of clearing latent HHV8 from permanently infected lymphoblastic BC3 cells by the use of some other sulfone derivatives, in particular di-amino-sulfones and sulfanilureae.

Materials and Methods: Human primary endothelial cells (HUVEC) were cultured in M200 medium with LSG supplement. Permanently infected lymphoblastic BC3 cells were used to produce stock amounts of purified HHV8 virus. An RT-PCR technique was used to detect HHV8 virus DNA in the cell nucleus. A cytofluorometry method was employed for detecting the LANA latent-viral antigen in the cell.

Results. We have already shown that the most active anti-HHV8 sulfonamide was the sulfathiazole compound. Similarly, glibenclamide and gliclazide were the most active drugs among sulfanilureae, clearing about 80-86% of viral DNA and 33-60% of LANA antigen from BC3 cells after 6 days of culture in M200 medium with LSG supplement at the concentration between 10 and 100 uM. Tolbutamide was found to be less active. Studies on the mechanism of action revealed that sulfone derivatives are able to interfere with the formation of the MDM2-p53 complex, which is at the base of HHV8 episome-latency and is also responsible for cell transformation.

Conclusions: Sulfone derivatives and sulfanilureae can be a new potential tool for the therapy of HHV8 induced pathologies, such as Kaposi sarcoma, primary effusion lymphoma (PEL) and Castleman's disease. Moreover, MDM2-p53 is a new original target discovered for sulfonamide derivatives. Interestingly, sulfanilureae are also common ipoglycemic anti-diabetic compounds; this property strengthens the possible association between HHV8 infection and diabetes mellitus type 2 [2].

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P4

NOVEL N1-ARYL-2-ARYLTHIOACETAMIDO-BENZIMIDAZOLES WERE EFFECTIVE AGAINST HERPES SIMPLEX VIRUS 1 (HSV-1) REPLICATION *IN VITRO*

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Introduction: A series of N_1-aryl-2-arylthioacetamido-benzimidazoles (NAAB) were synthesized and previously evaluated as inhibitors of human immunodeficiency virus type-1. Some of them proved to be effective in inhibiting HIV-1 replication at submicromolar and nanomolar concentration acting as HIV-1 non-nucleoside RT inhibitors, with low cytotoxicity [1]. The aim of the present work was to investigate the effectiveness of the same molecules against herpes simplex virus 1 (HSV-1).

Materials and Methods: NAABs derivatives were obtained in good yields by a previously published standard protocol [1]. The biological activity was performed in VERO cell lines (American Type Culture Collection) propagated in minimal essential medium (EMEM) supplemented with 6% fetal bovine serum (FBS) (Lonza, Belgium) at 37°C under 5% CO_2. The cell viability was determined with a cytotoxicity bioassay kit using the following NAAB concentrations: 0.1 mg/ml, 0.05 mg/ml and 0.01 mg/ml. To verify the antiviral activity of NAABs, the standard plaques reduction assay was performed. Therefore, Vero cells were infected with HSV-1 at the multiplicity of infection MOI of 1 for 1 hours at 37°C and, after the incubation time, were treated with different concentration of NAABs. Viral stocks were propagated and then tritered in Vero cells [2].

Results: Results of cell viability indicated NAABs exhibit different citotoxicity effect, which depends on the tested concentrations and the chemical structure. However, NAABs not exhibiting citotoxicty demonstrated a statistically significant decrease in the viral replication at the concentration ranging between 0.01 and 0.05 mg/ml.

Conclusions: NAABs were effective against HSV-1 and their use could be further explored to study the mechanisms involved in the negative regulation of viral replication.

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P5

ANTI - MEASLES ACTIVITY OF TEMPORIN ANALOGS

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Introduction: Nowadays is well established the role of antimicrobial peptides (AMPs) in the innate immune response. AMPs constitute the first line of defense against different pathogens. They are produced by several tissues and cell types in plants and in animal species, such as insects, amphibians, vertebrate, etc. The intrinsic characteristic of the amino acid sequences are useful to confer bactericidal properties. Several evidences have shown that the bacterial membrane is one of their preferred targets. In this scenario, this study focused on a specific AMPs class called "temporins" (TLs). In order to understand the potential of temporins as antiviral compounds we extended our previously study, which analysed anti-herpetic activities of TLs analogs, and performed antiviral experiments against measles virus.

Materials and Methods: The peptides were synthesized using solid-phase Fmoc chemistry method, followed by purification by reversed phase HPLC. The cytotoxic activity was determined via MTT 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. The antiviral activity was evaluated against measles virus through inhibition infectivity assays in a range of concentrations between 50 and 1 μM.

Results: A marked antibioviral activity against measles was observed for some TL analogs with a dose-response activity. TL analogs displayed a low hemolytic effect at the active concentrations and, overall negligible levels of cytotoxicity toward mammalian cells.

Conclusions: Measles virus remains one of the most important causes of worldwide morbidity and mortality in children, despite the availability of a safe and effective live attenuated vaccine. An essential step in the measles virus life cycle is viral entry into host cells, mediated by virus-cell membrane fusion thus membranotropic peptides can be an attractive tool for therapeutic development. Collectively, the results obtained demonstrated that subtle changes in the primary sequence of TL may provide TL analogs that exhibit promising features against measles virus.

P6

IN VITRO INFECTIVITY INHIBITION BY HSV SYNTHETIC PEPTIDES DERIVED FROM GH/GL HETERODIMER

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Introduction: Herpes simplex virus type 1 (HSV-1) is a human pathogen that infects epithelial cells and is characterised by a latent phase in neuron ganglia. The virus enters cells via fusion of the viral envelope with the cellular membrane involving multiple interactions between different HSV glycoproteins and host cell receptors. The fusion machinery is conserved in all Herpesviridae subfamilies, and it is composed by the glycoproteins B (gB), H (gH), L (gL) and D (gD). In details, the binding of gD to a cell receptor enables it to somehow transmit a signal to gH/gL heterodimer, which, in turn, activates gB. gH-gL is an unusually tight complex with a unique architecture that, unexpectedly, does not resemble any known viral fusogen.

Materials and Methods: Two library of overlapping peptides spanning the entire ectodomains of gH and gL glycoproteins were prepared and screened for their inhibitory activity against HSV-1 infection. Solid phase-9-fluorenyl-methoxycarbonyl (Fmoc) on an automatic synthesizer SYRO multisyntech standard method for peptide synthesis was used. The peptides have been firstly tested for their cytotoxicity through lactate dehydrogenase assay and then their anti-herpetic activity was evaluated through a co-treatment assay.

Results: No peptide toxicity was observed by lactate dehydrogenase assay at the concentrations used in the experimental conditions. Twenty-four of the gH peptides at a concentration of 150 μM reached the 50% of inhibition cut-off. Interestingly, they are mainly located in the gH carboxy-terminal domain. None of the gL peptides had a clear inhibiting effect.

Conclusions: HSV-1 therapy is based on acyclovir treatment, but some resistant strains are emerging. In this scenario, innovative approaches for HSV-1 treatment are necessary. In this study, we have described an alternative approach for identifying

functionally important regions of HSV-1 gH and gL, showing for the first time the HSV-1 inhibition activity of unreported peptides derived from gH protein. These peptides may be useful for probing gH activity during membrane fusion, but their major use could be as direct antiviral molecules. Further analysis and appropriate modifications may be convenient to improve their efficiency.

P7

IN VITRO ANTIVIRAL ACTIVITY OF METHANOLIC EXTRACT OF *PSEUDOCOCCOMYXA SIMPLEX*, A FRESHWATER AND TERRESTRIAL ALGA

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Introduction: Microalgae are eukaryotic primitive microscopic plants with photosynthetic metabolism. Microalgae occur widely in freshwaters, marine environments and brackish water. *Pseudococcomyxa simplex* is a Genus of single-cell green algae belonging to the phylum Chlorophyta. Its growth occurs in a wide habitat range. In Europe, it has been found in aquatic system as well as in grasslandsoils, on a variety of mosses and in volcanic soils too. Microalgae have for long been used with therapeutic purposes. In the last decade, microalgae have become the focus of extensive research efforts, aimed to finding novel compounds that might lead to therapeutically useful agents. The antimicrobial activity of microalgae has been attributed to compounds belonging to several chemical classes, for example the sulfated polysaccharides. The ability of these compounds to inhibit the replication of enveloped viruses is well established and is due to the ability to mask the positive charge of host cell surfaces and to a complex interplay of structural features including distribution of sulfate groups along the polysaccharide backbone, molecular weight, sugar residue composition, and stereochemistry.

Materials and Methods: *P. simplex* was gently provided from the Biological Science Departmentof Federico II University of Naples. Microalgal cultures were prepared according to recommendations by the culture collections. The BBM medium was inoculated with a 5% inoculum of exponentially growing cells. The microalgae were grown in 500-mL flasks that contained 200 mL medium in batch mode at 26°C with and continuous shaking and illumination (60 μE/m2 s). A methanolic extraction was obtained and the antiviral activity was assayed against HSV-1 using a co-treatment assay at the concentrations from 10 ug/ml to 100 ug/ml.

In vitro cytotoxicity assays (MTT assay,FACS

assay, apoptosis assay) have been also performed.
Results: The methanolic extract of *P. simplex* has a strong antiviral activity against HSV-1 at the concentrations evaluated, while the effects against Gram-negatives and Gram-positive bacteria are minimal. Interestingly, the inhibitory effect is preserved at lower concentration. The extract did not show any toxicity.
Conclusions: We reported for the first time the antiviral activity of *P. simplex* methanol extract against HSV-1. Future investigations will be directed to the isolation of the active molecules and the identification of the mechanism responsible of the antiviral activity and finally the evaluation of antifungal and anti-inflammatory activity of the same extracts.

P8

ANTI-HERPES SIMPLEX VIRUS 1 AND IMMUNOMODULATORY ACTIVITIES OF A POLY-Γ-GLUTAMIC ACID FROM *BACILLUS HORNECKIAE* STRAIN APA OF SHALLOW VENT ORIGIN

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Introduction: Herpes simplex virus type 1 (HSV-1) is responsible of common and widespread viral infections in humans, through the world, and of rare, but extremely severe, clinical syndromes in the central nervous system. The emergence of resistant strains to drugs actually in use encourages the searching for novel antiviral compounds, including those of natural origin. In this study, the recently described poly-γ- glutamic acid (γ-PGA-APA), produced by the marine thermotolerant *Bacillus horneckiae* strain APA, and previously shown to possess biological and antiviral activity, was evaluated for its anti-HSV-1 and immunomodulatory properties.
Materials and Methods: Different concentrations of APA-PGA were added to HEp-2 monolayers cells or monocytic U937 cells and cell viability was evaluated by trypan blue exclusion test. To address the antiviral activity of biopolymer, HEp-2 cells were incubated with medium containing, or not, APA-PGA for 2 hours prior to and during infection with HSV-1 (strain "F") at a multiplicity of infection (MOI) of 5 PFU/cell. Following virus adsorption (1 h at 37°C), virus inoculum was removed and, at the end of incubation time, cultures were processed for evaluation of i) infectious viral particle production using a classical plaque assay on Vero cells; ii) expression of gD-HSV-1 glicoprotein by immunofluorescence assay and iii) expression of IE ICP0 and late gD viral proteins by Western blot analysis. After APA-PGA treatment, mRNA levels of TNF-α, IL-1β, IL-6, IFN-α were assessed by RT-qPCR analysis and nuclear NF-kB binding activity was evaluated by EMSA.
Results: The biopolymer hindered the HSV-1 infection in the very early phase of virus replication. In addition, the γ-PGA-APA was

shown to exert low cytotoxicity and noticeable immunomodulatory activities towards TNF-α and IL-1β gene expression. Moreover, the capacity to positively modulate the transcriptional activity of the cytokine genes was paired with increased level of activation of the transcription factor NF-kB by γ-PGA-APA.
Conclusions: Overall, as non-cytotoxic biopolymer able to contribute in the antiviral defence against HSV-1, γ-PGA-APA could lead to the development of novel natural drugs for alternative therapies.

P9

ANTIVIRAL ACTIVITY OF ZINC IN ROTAVIRUS INFECTION: EFFECT ON VIRUS MORPHOGENESIS

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Introduction: Rotaviruses are recognized as the leading cause of gastroenteritis-associated severe morbidity and mortality in children. No effective antiviral therapy for rotavirus infection exists and at the present time the mainstay of therapy is oral rehydration. Zinc supplementation is known to reduce the duration and severity of the disease; however, the mechanism responsible for the beneficial effect of zinc in rotavirus infection is not known. Herein we investigated the effect of zinc on rotavirus replication, and explored the mechanism of the antiviral action *in vitro*.
Materials and Methods: A/SA11-G3P[2] and Wa-G1P[8] rotaviruses were grown in monkey MA104 cells, and human Caco-2 or HT-29 gut-derived cells. Virus yield was determined by hemagglutinin titration or infectivity assay. Viral protein synthesis was analyzed by SDS-PAGE/autoradiography after ^{35}S-methionine-labeling, Western blot, and immunofluorescence analysis. Activity of Nuclear Factor-kB (NF-kB) was determined by EMSA, and TNF-a mRNA levels were detected by RT-PCR and real-time PCR.
Results: Zinc-treatment inhibits rotavirus replication in cultured cells. This effect is independent of the host cell type. Zinc acts at post-translational level by hindering NSP5/NSP2 protein interaction and inhibiting viroplasm formation. Interestingly zinc also inhibits rotavirus-induced NF-kB activation and NF-kB-dependent TNF-a expression in infected cells.
Conclusions: Altogether the results identify zinc as an inhibitor of rotavirus replication, and suggest that zinc antiviral activity, together with its ability to suppress NF-kB-dependent proinflammatory cytokine production by infected cells, may contribute to the beneficial effects of zinc supplementation to oral rehydration therapy during rotavirus disease.

P10

ENDOGENOUS OXYSTEROLS POTENTLY INHIBIT HUMAN ROTAVIRUS INFECTION BY SEQUESTERING VIRUS PARTICLES INTO LATE ENDOSOMES

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Introduction: Oxysterols represent an important family of 27-carbon molecules originated from cholesterol by either enzymatic and non enzymatic mechanisms. These pleiotropic molecules play roles in several physiologic processes, namely bile acid synthesis, steroid hormone biosynthesis, sterol transport, and gene regulation. Recently, one member of the family (25-hydroxycholesterol, 25HC) has been indicated as a sterol-lipid effector of innate immunity against viral infections and a growing body of evidence shows that other oxysterols (mainly of enzymatic origin) are endowed with a wide spectrum antiviral activity against several pathogenically relevant enveloped and non-enveloped viruses. While the mechanisms of action of oxysterols against enveloped viruses are extensively studied, the antiviral mechanisms against non-enveloped viruses are still elusive. In the light of these emerging evidences, we tested the antiviral activity of oxysterols against human rotavirus (HRoV) as a model of pathogenically relevant non-enveloped virus and identified a totally novel antiviral mechanism of action.

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

Results: 25HC and 27HC can block the infectivity of different HRoV strains at 50% inhibitory concentrations (EC_{50}) in the low micromolar range, and were characterized by SIs above 100. Both oxysterols can block the earliest steps of HRoV infection: while virus-cell attachment is not impaired, virus-cell penetration (i.e. virus endocytosis and HRoV excape from endocytic vescicles) is totally inhibited. The antiviral activity of 25HC and 27HC is ascribable to their ability to interact with the oxysterol binding protein (OSBP) and preventing its association to the vesicle-associated membrane protein-associated protein-A (VAP-A). By altering the activity of these cellular mediators, oxysterols disturb the recycling of cholesterol between late endosomes and endoplasmic reticulum and induce a substantial accumulation of cholesterol in the late endosomal compartment, thereby sequestering viral particles inside these vesicles.

Conclusions: These findings suggest that oxysterols of endogenous origin might be a primary host strategy to counteract HRoV infection and indicate lipid involvement during viral infections as an emerging field which holds great promise to unravel novel antiviral mechanisms

P11

ANTIVIRAL ACTIVITY OF COLOSTRUM FROM MOTHERS OF PRETERM INFANTS AND ROLE OF COLOSTRUM-DERIVED EXOSOMES

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Introduction: Transmission of some viral pathogens by breast-feeding is an important risk for preterm infants since they are more susceptible to develop severe clinical symptoms. Colostrum is a pre-milk substance produced from 1 to 7 days after the lactation has started. It is a very important source of fats, sugars, proteins and secretory IgAs. The aim of this study is to test the antiviral activity of human colostrum from mothers of preterm newborns against three viruses clinically relevant for infants, namely human cytomegalovirus (HCMV), respiratory syncitial virus (RSV) and human rotavirus (HRV). In search of non immunological components of human colostrum, we explored the antiviral potential of subcellular vescicles – also known as exosomes - secreted in human colostrum.

Materials and Methods: Different samples of colostrum were collected from mothers of preterm newborns and exosomes were purified and characterized by western blot for the expression of specific antigenic markers. The *in vitro* antiviral activity of exosomes was tested against HCMV, RSV and HRV and compared with the one of colostrum by standard plaque assays or focus reduction assays. The putative step of viral replicative cycle inhibited by colostrums-purified exosomes was investigated with specific antiviral assays.

Results: Each sample of colostrum and colostrum-derived exosomes showed a significant antiviral activity with a wide interpersonal variability (EC_{50} of colostrum ranging from 65 to 933 µg/ml of proteins and EC_{50} of exosomes ranging from 8 to 434 µg/ml of proteins). Mechanism of action studies demonstrated that exosomes do not directly inactivate viral particles, but they hamper the early steps of replicative cycle.

Conclusions: Breast-feeding represents a main maternal-infant transmission of several infections, but colostrum and mature milk contain also protective factors against infectious diseases. Immune and non-immune factors, including maternal antibodies, lactoferrin, lysozyme, cytokines and lipid compounds have been reported to protect newborns from viral infections. The results of this study provide the demonstration of the wide spectrum antiviral activity of human colostrum and they disclose the antiviral activity of colostrum-derived exosomes against HCMV, RSV, HRV. These data clarify, for the first time, the role and contribution of a non immunological component of human colostrum in mediating its protective effect against viral infections.

P12

QUINOXALINE DERIVATIVES AS NEW INHIBITORS OF COXSACKIEVIRUS B5

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Introduction: Enteroviruses are among the most common and important human pathogens for which there are no specific antiviral agents approved by the US Food and Drug Administration so far. Particularly, coxsackievirus infections have a worldwide distribution and can cause many important diseases. We here report the synthesis of new 14 quinoxaline derivatives and the evaluation of their cytotoxicity and antiviral activity against representatives of ssRNA, dsRNA and dsDNA viruses.
Materials and Methods: The antiviral activity and the study of mechanism of action of the most interesting compounds was determined with a combination of experimental techniques (i.e. virucidal activity, time of drug addition and adsorption assays) and in silico modeling studies.
Results: Promisingly, three compounds showed a very potent and selective antiviral activity against Coxsackievirus B5, with EC50 in the sub-micromolar range (0.3 - 0.06 μM).
Conclusions: Our studies show that the present new quinoxaline derivatives, possessing high activity and selectivity accompanied with very low cytotoxicity may serve as a good starting point for the development of novel drugs for the treatment of infections by Enterovirus.

P13

HOST DEFENSE TEMPORIN-34 ANALOGUES AND THEIR POTENTIAL AS ANTIVIRAL AGENTS

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Introduction: Antimicrobial peptides (AMPs) are widespread in all kingdoms of life and have been shown to exert a direct antimicrobial activity on a large number of invading microorganisms. They represent the most ancient and fast-acting elements of the host's innate defence system against microbial pathogens. In this scenario, AMPs have emerged as good candidates for the generation of new anti-infective agents. While the antibacterial activity has been deeply investigated, the notions available for antiviral effect are still underexplored. Furthermore, due to their chemical and biological instability, only few AMPs are currently in clinical trials as antimicrobial agents. The amphibian temporins represent one of the largest families (more than 100 members) and are among the smallest-sized AMPs (10-16 amino acids) found in nature to date. Temporins are known to be active particularly against Gram-positive bacteria, with minimal inhibitory concentrations ranging from 2.5 μM to 20 μM. In details, temporins which belong to L group, show a wide range of action and a greater antibacterial effect but a high hemolytic activity. For this purpose, the selected temporin-34 (TL34 sequence: Phe-Val-Gln-Trp-Phe-Ser-Lys-Phe-Leu-Gly-Arg-Ile-Leu) has been modified producing different analogues.
Materials and Methods: TL34 has been modified at glycine residue in position 10. Analogs are synthesized using solid-phase Fmoc chemistry method, followed by purification by reversed phase HPLC. The cytotoxic activity was determined via MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. The antiviral activity was evaluated against Herpes Simplex Virus type 1 (HSV-1) through co-treatment, cell pre-treatment, virus pre-treatment and post-treatment assays in a range of concentrations between 100 and 1 μM.
Results: Those peptides have been modified in order

to increase the peptide stability and antimicrobial activity. Among the peptides generated, the majorities have shown a high stability in solution, low toxicity and a significant inhibition of HSV-1 infectivity. In details, they work in an extracellular phase, probably during viral attachment and penetration.

Conclusions: Actually the gold 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 approaches for HSV-1 treatment are necessary. Our results show novel possible applications of TL34 analogues in the field of antivirals.

P14

EFFECT OF DIFFERENT ANTIRETROVIRAL STRATEGIES ON CELLULAR HIV-DNA LEVEL IN VIROLOGICALLY SUPPRESSED HIV-1 INFECTED PATIENTS

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Introduction: Total HIV-DNA load in peripheral blood cell (PBMCs) reflects the global viral reservoir that seems to be not affect by antiretroviral treatment. However, some studies reported a different permeability of some drugs, such non nucleoside inhibitors (NNRTI), protease inhibitors (PI) and integrase inhibitors Raltegravir (INI, RAL) in cellular compartment. So, it is reasonable to assume that antiretroviral therapy impacts on the viral persistence and might have a direct effect on HIV-1 DNA.

Materials and Methods: The study enrolled HIV+ patients on stable combination antiretroviral therapy (cART) for over 4 years: patients were divided respect to ART composition in four groups, three containing a backbone plus nevirapine (NVP) or darunavir/ritonavir (PI/r) or another NNRTI as third drug, one containing Raltegravir plus darunavir/r. Cellular HIV DNA was measured in PBMCs by a quantitative real time PCR assay, targeting the 5'Long Terminal Repeat region and was expressed as HIV-DNA copies/10^6 PBMCs.

Results: We studied 161 subjects with a median baseline HIV-RNA of 4.7 log copies/ml (IQR,4.1-5.2), median CD4+ nadir of 222 cells/mm^3 (IQR, 108-366 cells/mm^3), median CD4+ of 688 cells/mm^3 (IQR, 564-879 cells/mm^3). The four groups were homogeneous for the main characteristics. Univariate analysis confirmed that PBMCs HIV-1 DNA level is related to pre-ART immuno-virological parameters (plasma HIV-1 RNA zenith and CD4 cell count nadir). The median values of DNA load were heterogeneous in four different groups, ranging from 358 copies/10^6 PBMCs (IQR, 148-800) to 260 copies/10^6 PBMCs (IQR, 159-787), but not significantly differences were found. The only predictive markers of successful therapy (smaller reservoir size) were a baseline HIV-RNA

< 3 log and CD4+ > 200 cells/mm^3, and, fixed this reference, we found a lower level of HIV-DNA in the Raltegravir-treated group respect to NNRTIs-treated group (95 vs 174 copies/10^{6}PBMCs, p < 0.03).

Conclusion: In this cohort of virologically suppressed HIV-infected subjects, the amount of HIV DNA was comparable in patients receiving different type of NRTI-based cART including non nucleoside analogues and PI/r, ruling out that one or more antiretroviral regimens could be related to a major efficacy on achieving low level of proviral DNA amount. However, total HIV-DNA was significantly lower in Raltegravir plus PI/r treated group. So, this finding requires larger investigations to carefully select patients for NRTI-sparing regimen, to avoid a potentially "re-seeding" of viral reservoir in well-controlled patients.

P15

HEPATITIS C VIRUS NS5A REGION: RESISTANCE-ASSOCIATED SUBSTITUTIONS (RAS) PLUS POLYMORPHISMS ANALYSIS BY MOLECULAR MODELING

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Introduction: Hepatitis C Virus (HCV) can develop resistance-associated substitutions (RASs) after exposure to direct acting antiviral (DAA) agents during treatment. In clinical trials and real-life data, failures occurred in patients with NS5A 93H RAS at retreatment baseline. Herein, computational study was designed on NS5A protein along the known mutation 93H and the other polymorphisms, observed during patient's therapy follow-up, in order to evaluate the drug-resistance profile of ombitasvir (OMV) targeting the NS5A phosphoprotein.

Materials and Methods: Serum samples from one HCV1b chronic infected patient, failed NS5A inhibitor containing regimen, were collected at different time points of therapy. NS5A and NS5B regions were sequenced by population sequencing (cut-off >15%) at baseline, at week 12 post treatment and at week 32 post treatment follow up. HCV typing was performed by phylogenetic analysis using Neighbor-Joining (NJ) tree method, and by subtyping tools. RASs and polymorphisms on target gene were analyzed aligning newly sequences to reference sequences and by Geno2pheno tool.

Computational analysis started from the closed conformation of NS5A model deposited in Protein Data Bank. For each isolate, the mutated enzymes were generated by single-residue replacement and submitted to Glide Standard Precision docking simulations.

Results: Concatenate genomic regions NJ tree showed that isolates at different time points from patient closely clustered with each other (bootstrap support 100%), clearly identifying a viral relapse rather than a reinfection. NS5A baseline isolate, at least more than 15% of viral population, showed 6R, 34V, 44R, 61V, 78R and 138L polymorphisms. Isolates at weeks 12 and 32 post treatment carried the same baseline polymorphisms plus the acquired 93H daclatasvir, elbasvir, ledipasvir, OMV and velpatasvir RAS. At week 32, viral population additionally displayed K substitution on 108 position, showing an aa change respect to previous time points. In agreement with literature data, docking analysis showed that the presence of 93H RAS decreased OMV binding-affinity for NS5A. On the contrary, the introduction of 108K in the 93H-mutated NS5A structure increased the NS5A binding-affinity for OMV.

Conclusions: Phylogenetic analysis is important to confirm HCV1b subtype and to identify a relapse of infection. NS5A RASs, selected under therapy, generally persist longer than resistances in the other target genes and may impact treatment outcome. According to our data, molecular modeling approach is a powerful method to evaluate the impact of these RASs plus specific aa changes on phosphoprotein. Therefore, computational study could guide choice of second line regimen, in view of the first line failures coming out in real-life.

P16

HIV DRUG RESISTANCE REMAINS A RELEVANT CLINICAL CONCERN, DESPITE ITS DRAMATIC DROP OVER THE YEARS

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Introduction: Dynamics of HIV-resistance has profoundly changed over time; however, it is still a concern for the long-term success of antiretroviral treatment (ART). Thus, resistance monitoring is crucial to preserve future therapeutic uated according to the genotypic susceptibility score (HIVdb algorithm 8.3).

Results: 14189 GRTs from 6051 ART-experienced pts were analyzed. Overall, 40% of GRTs showed no resistance, while the prevalence of resistance to 1, 2 and ≥ 3 classes was 21%, 25% and 14%, respectively. Prevalence of resistance to NRTI, NNRTI, PI and INI was 47%, 38%, 26% and 10%, respectively. Resistance significantly decreased from 1999 to 2010 (2 class-resistance: from 42% to 14%; ≥ 3 class-resistance: from 30% to 6%, $p < 0.0001$), in conjunction with a remarkable increase of GRTs without resistance (from 13% to 58%, $p < 0.0001$). An increase of one1 class resistance was also found (from 14% to 22%, $p < 0.0001$). Beyond 2010, resistance prevalence remained stable from 2011 to 2016. The proportion of GRTs with a fully susceptible virus increased over time settling in 2016 at > 80% for NRTI, > 74% for NNRTI, > 90% for PI ($p < 0.0001$). Regarding INI, the proportion of GRTs with a fully susceptible virus was stable from 2008,

settling in 2016 at > 88% and 95% for raltegravir/elvitegravir and dolutegravir, respectively. After 2008, INI-resistance contributed to resistance mostly in GRTs with ≥ 3 class-resistance (1 class: 9.2%; 2 classes: 16.7%; ≥ 3 classes 41.6%, p < 0.0001). This contribution roughly increased over time especially in the case of GRTs with 1 (2008: 0%; 2016: 6.8%, p = 0.036) and 2 drug classes (2008: 0%; 2016: 20%, p = 0.034).

Conclusions: A dramatic drop of drug resistance has been achieved, confirming a good clinical practice and ensuring a high number of treatment options for failing patients. However, in the last 5 years drug resistance is stable, and resistance to ≥3 classes remains a clinical relevant issue. For the management of multi-class resistance an effort in the use of diagnostic tools is mandatory to ensure the efficacy of the currently available drugs.

P17

HUMAN POLYOMAVIRUS JC REPLICATION IN IMMORTALIZED COS-7 AND GLIAL SVGP12 CELL LINES: AN IN VITRO MODEL OF INFECTION

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Introduction: JC virus (JCV) is a human Polyomavirus that causes Progressive Multifocal Leukoencephalopathy (PML). Primary infection occurs in childhood and it establishes latency in the kidney. Upon reactivation it causes PML in AIDS patients and in patients treated with monoclonal antibodies. JCV genome is a double-stranded circular DNA, composed of an early and a late region physically separated by the non-coding control region (NCCR). Two different forms of NCCR exist: the archetype non-pathogenic CY and the prototype which results from a rearrangement of the archetype sequence isolated from patients with PML. There is no animal model for PML. To date, the cell culture systems to propagate JCV have been very limited. The aim of the work was to produce an *in vitro* model in Cos-7 and SVGp12 cell lines to study JCV DNA replication, analyze NCCR rearrangements and define NCCR rearrangements as possible viral biomarkers for an early PML diagnosis.

Materials and Methods: Cos-7 and SVGp12 cell lines were maintained in medium supplemented with 10% fetal bovine serum (FBS) and incubated at 37°C at 5% of CO2. Both cell lines were transfected with CY DNA using the Xfect™ Transfection Reagent kit. Two times a week until 35 days post-transfection, cells and supernatants were harvested. Viral DNA was extracted from 1x106 Cos-7 and SVGp12 cells and used in a quantitative PCR (Q-PCR) for JCV TAg in order to evaluate the JCV replication. Supernatants was directly used in Q-PCR. Hemagglutination assay (HA) was used to determine virus titer. The sample was mixed

with 50 µl of 0.5% human type O erythrocytes and incubated. Virus replication was demonstrated by Western Blot for JCV VP1 expression. Protein extract from both cell lines were separated by SDS-PAGE, transferred to PVDF and blocked with NFDM 5% (non-fat dry milk). The membranes was incubated with a monoclonal mouse antiserum directed against JCV VP1. As secondary antibody, conjugated with fluorescent dye were used. NCCR region, was amplified and sequenced.

Results: An efficiently JCV replication was observed by progressive increase of viral load. Virion particle production during the time of transfection was confirmed by HA assay and Western Blot. JCV NCCR showed an archetype CY-structural organization during viral growth, although two characteristic point mutations were detected after 28 days of transfection.

Conclusions: Since the importance of NCCR rearrangements in the onset of PML has been demonstrated by the discovery of rearranged sequences in patients with this condition, this *in vitro* model could be useful to study the possibility of JCV NCCR rearrangement in sites of viral persistence, to assess the molecular pathways of viral infection and to test drugs that can inhibit viral replication.

P18

STUDY OF HIV-1 MATURATION, PRODUCTION AND INFECTIVITY FOLLOWING AMPRENAVIR WITHDRAWAL IN CHRONICALLY INFECTED MONOCYTES/MACROPHAGES

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Introduction: Paucity of information is so far available on the *in vitro* activity of protease inhibitors (PI) in chronically infected Monocyte-derived Macrophages (M/M) and on the kinetics of viral rebound after PI removal from culture sups.

Material and Methods: To evaluate the in-vitro activity of AMP in HIV-1 infected M/M, human primary M/M were challenged with 300 $TCID_{50/ml}$ of HIV-1_{BaL} and after 14 days from infection (time point, when the chronic infection is stablished) they were treated with amprenavir (AMP) at a concentration of 20, 4, 0.8 and 0.16 µM. The p24 production was quantified every day up to 12 days after drug administration. To evaluate kinetics of viral rebound AMP was removed from culture sups after 7 days from drug administration and p24 production was quantified at 12h, 1, 2, 3 e 7 days after drug removal. The ratio p24/p55 was determined at 1 day after drug removal by WB to investigate AMP ability to affect viral particle production.

Results: The first step of this study was to evaluate the antiviral activity of AMP in HIV-1 chronically infected M/M. AMP, used at concentration of (4 and 20) µM, determined a drastic decrease in p24 amount in sups since day 2 after drug treatment; this decreased was maintained stable up to 12 days after drug administration. The second step was to evaluate the kinetics of viral rebound in HIV-infected M/M, after AMP removal. At 12h after drug removal, there was an increase in p24 production for all concentrations of AMP tested. As expected, for the lowest concentration of AMP

(0.16 μM), p24 production overtime reached levels observed for untreated M/M. A different behavior was observed for the highest concentrations of AMP (4 and 20 μM). Indeed, after an initial increase, p24 production overtime never reached the level observed for untreated M/M. Notably, for AMP 20 μM, p24 production at 3 and 7 days after drug removal was 55% lower than that observed for untreated M/M. Similarly, for AMP 4 p24 production at 3 and 7 days after drug removal was 48 and 18% lower than in untreated M/M. This suggest a persistent intracellular activity of the drug. This was supported also by analyzing the p24/p55 ratio. Indeed the p24/p55 ratio, after the removal of AMP 4 and 20 μM, was lower than that observed for untreated controls.

Conclusions: AMP shows high efficacy in blocking HIV-1 replication in M/M. This highlights the role of this protease inhibitor in controlling the establishment and enlargement of this important HIV-1 reservoir. Due to the role of M/M in disseminating HIV in different body compartments, this is also critical to efficiently hit HIV-1 replication in all anatomical compartments where the virus hides and replicates.

P19

PARTICULATE MATTER 10 AND 2.5 (PM10/PM2.5) EXPOSURE INDUCES JC POLYOMAVIRUS REPLICATION

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Introduction: Human Polyomaviruses (HPyVs) are small non enveloped DNA viruses that asymptomatically infect an high percentage of human population during childhood and then establish latency in the host. Reactivation can occur when the immune system is impaired. Air pollution exposure is a major problem worldwide and has been linked to many diseases. Particulate Matter $(PM)_{10}$ is one of the components of air pollution. Studies assessing the relationship between PM_{10}/$PM_{2.5}$ exposure and the replication of the HPyVs are completely lacking.

Materials and Methods: A Chemical Transport Model was used to estimate daily PM_{10} concentrations at municipality resolution, whereas monitoring stations were used to estimate daily $PM_{2.5}$ concentration of 50 healthy adult subjects. For each subject, a fast urine sample and an EDTA tube of blood to separate plasma fraction were collected. DNA was isolated from the urine samples and multiplex Real Time PCR were conducted to quantify HPyVs (JCPyV, BKPyV, MCPyV, HPyV6, HPyV7 and HPyV9) loads in the urine. Extracellular vesicles (EVs) were purified from plasma and concentrated by ultracentrifugation: miRNAs were isolated, reverse transcripted and the levels of JCPyV 5p miRNA were quantified by means of specific Real Time PCR assay.

Results: HPyVs DNA was detected in 58% ($4.9*10^5$ copies/ml) of urine samples. JCPyV genome was the most prevalent (48%) with a mean viral load of $6.6*10^5$ copies/ml (range: $1.82*10^3$ - $6.72*10^6$ copies/ml). JCPyV 5p miRNA was expressed in 7 EVs samples (14%), 4 of which collected from patients who did not show viruria. An increase of 10 $\mu g/m^3$ of PM_{10}/$PM_{2.5}$ measured 6 days before the samples collection was associated with an increased risk to be positive for JCPyV genome in urine of 51% and 52% respectively (p-values < 0.05).

Conclusions: An increase of 10 $\mu g/m^3$ of PM_{10}/$PM_{2.5}$ can affect the replication of JCPyV which is

urinary excreted after 6 days from exposure. The circulating JCPyV miRNA may have a role in the downregulation of the viral replication.

P20

DNA SENSOR SIGNALING IN NK CELLS DURING HHV-6A AND HHV-6B INFECTION

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Introduction: The host DNA sensor proteins (e.g. IFI16, STING) have been identified as central signaling molecules in the innate immune response to cytosolic nucleic acids. Here we propose to investigate how Roseolavirus (HHV-6A, HHV-6B) infections engage DNA sensor signaling in Natural Killer (NK) cells.
Materials and Methods: We infected NK cells with cell-free inocula of HHV-6 and HHV-6B and analyzed DNA sensors by immunofluorescence and flow cytometry, 24 and 48 hours post-infection.
Results: NK cells were promptly infected by both HHV-6A an HHV-6B, as shown by the analysis of viral proteins (early U94, late gp160). At RNA and protein levels, we observed an increase in STING and IFI16 espression during HHV-6A infection, while HHV-6B induced only STING expression. STAT6 expression was increased by both HHV-6A and HHV-6B infection, while IRF3 expression was up-regulated only by HHV-6B. When we looked at the phosphorylation status, we observed a ten fold increase in STAT6 phosphorylation post HHV-6A infection, while HHV-6B infection induced only a two fold increase in STAT6 phosphorylation. We analyzed cytokine/chemokine secretion in the media of infected NK cells. HHV-6A induced IL-13, IL-4 and IL-5, that have a positive feedback effect on STING-STAT6 signaling, supporting a NK cell secretory profile. On the contrary, HHV-6B increased IFN-alpha and –beta and Fas Ligand, suggestive of a NK cell cytotoxic profile.
Conclusions: In conclusion, we demonstrate, for the first time, a different engagement of DNA sensor molecules during HHV-6A and HHV-6B infection of NK cells. HHV-6A engages STING-STAT6 and IFI16 signaling and immune cell chemotaxis, while HHV-6B induces STING-IRF3 signaling, NK cell activation and resistance to viral infection. These results demonstrate the biological and functional difference between HHV-6A and HHV-6B viruses.

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P21

APOBEC3G HOTSPOTS ARE LIMITED IN HUMAN CYTOMEGALOVIRUS GENOME ENCODING ESSENTIAL GENES

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Introduction: The Apolipoprotein B Editing Enzyme Catalytic subunit 3 (APOBEC3) is a family of DNA cytosine deaminases that mutate and inactivate viral genomes by single-strand DNA editing, with important roles in innate immunity and cancer. It has been demonstrated that APOBEC3 interferes with viral replication of RNA and DNA viruses, but its role during Human Cytomegalovirus (HCMV) replication has never been investigated. APOBEC3 is able to counteract viral replication increasing mutation rates in viral genome. On the other hand, HCMV displays high variability limited to distinct parts of the genome.
Matherials and Methods: In this study we analysed the impact of HCMV infection on APOBEC3 expression and activity. We also focused on effects of APOBEC3 action on HCMV genome with a bioinformatic approach.
Results: We observed that, among all APOBEC3 family members, only APOBEC3G (A3G) is strongly modulated by HCMV infection in primary Human Foreskin Fibroblast (HFFs), at both mRNA and protein level. Consistent with previous reports, we also demonstrated that the induction of A3G expression upon HCMV infection is mediated by IFN-type I. A3G preferentially deaminates the 3' cytosine within CCC hotspots in single-stranded DNA. We assessed the representation of these hotspot motifs in the HCMV and observed that the CCC motif is strongly under-represented in long genomic regions, broadly correspondent to essential ORFs.
Conclusions: During HCMV infection, HFFs upregulate the levels of A3G to exert a selective pressure on HCMV genome. On the opposite site, the virus has evolved to limit A3G hotspots in essential genes to counteract its restriction activity.

P22

SUMOYLATION OF HIV-1 INTEGRASE MIGHT BE ASSOCIATED WITH VIRO-IMMUNOLOGICAL PARAMETERS AND INTEGRASE RESISTANCE: A PROOF OF CONCEPT STUDY

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Introduction: Recent findings showed that novel molecules inhibit HIV-1 infectivity by perturbing integrase (IN) sumoylation, a post-translational modification that occurs at Lysine (K) residues when the ψ-K-x-D/E aminoacidic motif is present. Three sumoylation consensus sites (SCSc) are present in IN (K46-K136-K244). However, no data about these sites are available from clinical samples. Thus, the aim of this study is to characterize IN SCSs and their potential association with viro-immunological parameters.
Materials and Methods: IN HIV-1 subtype B sequences with contextual therapy information were collected. The presence of major (MRMs) and accessory resistance mutations (ARMs) was evaluated according to Stanford list 2017. Prediction of SCSs was obtained through GPS-SUMO tool (v.2.0). Levels of viremia, CD4 count and IN-resistance were evaluated according to SCSc variability.
Results: 2224 IN sequences were analyzed (from

981 drug-naïve, 913 drug-experienced INI-naïve and 330 INI-treated patients). At least one SCS was present in all sequences analyzed. In particular, SCS at position 244 was present in 99.4% of sequences, while SCS at positions 136 and 46 were present in 87.5% and 87.1% of sequences, respectively. The majority of sequences (76.3%) had three SCSs, while 21.4% and only 2.4% had two and one SCS, respectively. No significant differences in the number of SCSs was observed according to treatment. Among sequences from drug-naïve patients, a lower CD4 count was found when < 3 SCSs were present (< 3 vs. 3 SCSs: median [IQR]: 302 [124-472] vs. 348 [185-512], cells/mm^3, p = 0.016). Differently, among sequences from drug-experienced INI-naïve patients, when < 3 SCSs were present a significantly lower CD4 count (< 3 vs. 3 SCSs: median [IQR]: 239 [114-220] vs. 299 [137-525], cells/mm^3, p = 0.002) and higher viremia (< 3 vs. 3 SCSs: 4.4 [3.4-5.0] vs. 4.1 [3.2-4.8], $\log_{10}$ copies/mL, p = 0.006) were found. Sequences from INI-treated patients with < 3 SCSs showed a higher median [IQR] viremia (3.9 [3.0-5.1] $\log_{10}$ copies/mL) compared to those with all 3 SCSs (3.5 [2.5-4.5] $\log_{10}$ copies/mL, p = 0.038), while no significant differences were found in CD4 count. 38.1% and 7.3% of INI-treated patients showed ≥ 1 MRM and ≥ 1 MRM+ ≥ 2 ARMs, respectively. In particular, by decreasing the number of SCSs detected, the proportion of patients harboring ≥ 1 MRM+ ≥ 2 ARMs significantly increased (three SCSs: 4.1%; two SCSs: 14.0%; one SCS: 42.9%; p < 0.001).

Conclusions: SCSs are highly conserved in HIV-1 clinical specimens. Losing SCSc seems to be associated with lower CD4 count and higher viremia. A high level of INI resistance is gathered among INI-treated patients with only one SCS. These findings reinforce the interest in new therapeutic strategies targeting cellular sumoylation.

P23

GAIN OF POSITIVELY CHARGED AMINO ACIDS AT SPECIFIC POSITIONS OF HBSAG C-TERMINUS TIGHTLY CORRELATES WITH HBV-INDUCED HEPATOCELLULAR CARCINOMA BY ALTERING THE STRUCTURAL FOLDING OF THIS DOMAIN

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Introduction: Acquisition of positively charged amino acids (aa) can affect the folding of a transmembrane protein domain. HBsAg C-terminus is a hydrophobic transmembrane domain, composed by alpha-helices, critical for a proper HBsAg secretion. Altered HBsAg folding in endoplasmic reticulum (ER) membrane can affect HBsAg secretion and in turn promote HBV-induced hepatocellular carcinoma (HCC). The role of mutations associated with gain of charged aa in HBsAg C-terminus on HBV-induced HCC onset is not known.

Materials and Methods: This study includes 807 HBV chronically infected patients from

routine clinical practice: 28 with HCC (78.6% D; 21.4% A), and 779 patients without HCC (79.8% D; 20.2% A). Mutations associated with gain of charged aa in HBsAg C-terminus (aa189-226) are analyzed. Association of mutations with HCC is assessed by multivariable logistic regression model. Hydrophobicity profiles of HBsAg C-terminus are constructed to predict the stability of a domain in a membrane (Black, 1991). I-Tasser is used to predict three-dimensional HBsAg structures (aa:1-226) and their stability ($\Delta\Delta G$[wt-mutated] < 0 indicating reduced stability in presence of mutation based on Quan, 2016).

Results: The gain of ≥ 1 positively charged aa at HBsAg C-terminus positions 204, 207, and 210 tightly correlates with HCC (71.4% with HCC vs 30.2% without HCC, $p < 0.001$). Multivariable analysis confirms this correlation correcting for patients' demographics, HBV genotype, serum HBV-DNA and anti-HBV drugs use (OR[95%CI]:6.3[2.6-15.3], $p < 0.001$). The gain of positively charged aa derives from mutations S204R, S207R and S210R present in 14.3%, 28.6% and 28.6% of HCC-patients. S204R, S207R and S210R determine a reduction in hydrophobicity index of HBsAg C-terminus compared to wt (S204R:16.0, S207R:16.0, S210R:16.2 vs wt:16.4), and in $\Delta\Delta G$ values ($\Delta\Delta G$[S204R-wt] = -0.27; $\Delta\Delta G$[S207R-wt] = -0.11; $\Delta\Delta G$[S210R-wt] = -0.14). Moreover, S204R, S207R and S210R determine a shortening of membrane-spanning alpha-helix motif compared to wt (predicted alpha-helix length: aa209-224 for S204R, S207R and S210R vs 205-225 for wt). Overall, this suggests an impaired HBsAg C-terminus stability in presence of these mutations.

Conclusions: Gain of positively charged aa at specific HBsAg C-terminus positions tightly correlates with HCC, by altering the proper folding of this domain in ER membrane. These mutations might affect HBsAg secretion and in turn contribute to the initiation of HBV-related tumorigenesis. Their role in identifying patients at higher HCC-risk deserves further investigation.

P24

MEASLES CNS INFECTION AND ITS INHIBITION

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Introduction: Measles (MV) causes disease worldwide despite efforts towards eradication by vaccination, largely because it is spread so readily between people. MV elimination is currently hindered by the endemic nature of MV in developing countries and the decreasing rate of vaccination in developed countries. MV disease is generally self-limited but can lead to life-threatening complications related to the transient immune suppression and to central nervous system (CNS) invasion. CNS manifestations after MV infection may occur early after infection, in the case of acute encephalomyelitis. A second form of MV-induced CNS disease, a progressive infectious encephalitis known as measles inclusion body encephalitis (MIBE), occurs in immune-suppressed patients from 1 to 6 months after MV infection. This lethal sequela is relatively common in the growing population of immune-compromised patients who cannot receive or respond to MV vaccination. A third form of MV-induced neurological disease – subacute sclerosing panencephalitis (SSPE) – leads to fatal outcomes years after infection even in the presence of neutralizing antibodies. In recent US outbreaks the incidence of SSPE was as high as 1 every 600 infected infants, highlighting the significance of this disease in the immune competent population as well as persons with impaired immunity. There is no specific therapy for acute or persistent CNS manifestations of measles.

Results: We have applied the results of fundamental research to develop a new antiviral strategy for MV CNS infection, based on inhibiting membrane fusion during MV entry. We generated MV-specific peptides, derived from the C-terminal heptad repeat region of the MV fusion protein, that we proved to be potent inhibitors of MV entry, spreading and MV-induced cell fusion. Moreover, we show here that our antiviral strategy effectively blocks not only WT but also MIBE derived viruses

Conclusion: Our results suggest that fusion inhibitors represent a promising antiviral strategy for the prophylaxis of MV infection in unvaccinated and immune-compromised people, as well as potential for the treatment of grave neurological complications of measles.

P25

IN VITRO MODULATION OF GENE EXPRESSION IN HUMAN HERPES VIRUS 8 (HHV8) INFECTED LYMPHOID AND EPITHELIAL CELLS

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The pattern of expression of cellular genes involved in inflammation and apoptotic processes was investigated in HEK293 and PBMCs infected with A1 and C3 strains at time 24 and 48 h after infection with DualChip ® Microarray, Eppendorf.
Obtained results describe a comprehensive picture of global gene changes soon after HHV8 active infection. Host cell expression changed after only 24 hours of infection apparently with non-overlapping list of genes in all of two viral subtypes analyzed and HHV-8-induced transcriptional profiles in the epithelial and PBMC were closely different when a criteria of > 4-fold gene induction as significant was set.
The replicative capacity of the two different strains in the two cell types and the modulation of cell involved in the inflammatory process would seem to indicate that the genotypic difference could be translated into a different virus-host relationship, also attributable to a different pathogenetic mechanism. In addition our findings identified novel candidate genes that are not yet known in HHV8 biology. For example, up-regulated genes involved in inflammation (AGT, CCR8, CXCR4, IL-6, IL-9, IL10RA, IL-17, IL-23A), signal transduction (FGF5, FGF7, MAP3K1, MAPk9, TGFBR1, TRAF2), cycle cellular (IGFBP1), immune response (IL-16, TLR4), Apoptosis (TNFSF4, TNFSF11). The roles of these molecules in HHV-8 infection and KS development remain to be studied.

P26

PERSISTENT AND LATENT HIV-1 INFECTION: ANALYSIS OF THE EXPRESSION OF HOST RESTRICTION FACTORS AND THEIR CORRELATION IN ACTIVATION/DEACTIVATION OF VIRAL RESERVOIR

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Introduction: HIV-1 infection is of global importance, and still causes substantial morbidity and mortality. One approach to a cure is to use natural mechanisms by which the host restricts HIV-1. HIV-1 Restriction Factors (RFs) are diverse set of host proteins that limit HIV-1 replication at multiple levels. Generally, HIV-1 evades RFs inhibitory activities in human cells, thereby allowing efficient virus replication. Therefore, a better understanding is required of how HIV-1 persists despite host cell restriction. This study was focused on investigating the role of RFs in regulation of HIV-1 reactivation, replication rate, reservoir size, and their relevance in regulating innate and adaptive immunity in HIV subjects with recent and chronic infection.
Materials and Methods: The experiments were conducted on plasma samples and PBMCs (Peripheral Blood Mononuclear Cells) obtained from healthy donors and HIV-1 infected patients of the S.Orsola-Malpighi Hospital in Bologna. PBMCs were isolated from fresh blood using centrifugation in Ficoll-Paque and cultured in RPMI 1640 containing 2mM L-Glutamine, 10% FBS and 1X PS antibiotic solution. Equal amounts of cell cultures were collected and processed in order to obtain a total nucleic acid fraction. Real-time PCR and RT-PCR were carried out by using the RotorGene 6000 system (Corbett Reaserch) and SybrGreen detection of amplification products for evaluating the levels of HIV-DNA. The qRT-PCR−RT2 Profiler PCR Arrays (Qiagen) were used for analyzing the expression of a focused panel of specific RFs. In parallel, RFs expression was analyzed by flow cytometry using a FACScalibur

flow cytometer (Becton-Dickinson, Palo Alto, CA) and data were analyzed using CellQuest software (Becton-Dickinson).

Results: Pathway-focused gene expression analysis were performed to detect individual genes, like APOBEC3G, TRIM5α, Tetherin (BST-2), SAMHD1, MX2, SERINC3, IRF3, pIRF3, IFI16 and STING, in the same RNA sample. The PBMCs from healthy donors expressed a basal level of RFs. Protein and gene expression analysis different levels of RFs in PBMCs and HIV-1 infected patients. Correlation between RFs and amount of HIV DNA load in infected patients are in progress and will be presented.

Conclusions: Our preliminary results suggest that RFs are different expressed in HIV infected patients and might play a crucial role in HIV-1 reservoir size and control of the infection.

P27

MULTIPLE HERPES SIMPLEX VIRUS-1 (HSV-1) REACTIVATIONS INDUCE NEURODEGENERATIVE AND OXIDATIVE DAMAGES IN MOUSE BRAINS

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Introduction: Several evidences support the role of oxidative stress in Alzheimer disease (AD) pathogenesis, a neurodegenerative disorder characterized by the accumulation in the brain of b-amyloid peptides (Abs) and neurofibrillary tangles (mainly composed by hyperphosphorylated tau), high levels of oxidative stress markers and neuroinflammation. In particular, many redox proteomics studies on AD cerebral tissues led to the identification of oxidatively modified proteins that were consistent with biochemical or pathological alterations of the disease (Nunomura et al, 2001; Zhu et al, 2004; Smith et al, 2007; Droge et al, 2007). Interestingly, Herpes Simplex Virus-1 (HSV-1), a neurotropic virus able to establish a lifelong latent infection in trigeminal ganglion followed by periodic reactivations, has been reported linked both to AD (De Chiara et al, 2012; Piacentini et al, 2014) and to oxidative stress conditions (Nucci et al, 2000; Palamara et al, 1995). Herein, we design *in vivo* studies to investigate whether multiple HSV-1 reactivations induced in the brain the accumulation of oxidative stress hallmarks, particularly those correlated to AD.

Methods: BALB/c mice were inoculated via snout abrasion with HSV-1, virus reactivation was periodically induced by thermal stress, and virus replication in the brain was verified through PCR and RT-PCR analysis of viral TK gene and ICP4 mRNA. These mice showed several signs of neurodegeneration (De Chiara et al, 2017 in preparation). Oxidative stress marker levels, i.e. 4-hydroxynonenal (HNE, marker of lipid peroxidation), 3-nitrotyrosine (3NT, marker of protein nytrosylation) and carbonylated proteins were measured in brains of mice undergone multiple

HSV-1 reactivations by dot-blot. In addition, redox proteomic was used to identify those HNE-modified proteins mostly modulated by recurrent HSV-1 reactivations into the brain.

Results: Following several cycles of viral reactivation, we found in mouse brains: 1) increased levels of HNE, 3-NT, and protein carbonylation, indicating generalized conditions of oxidative stress; 2) thirteen HNE-modified proteins whose levels were significantly modulated in the cortex of HSV-1 infected mice compared to control mice. Interestingly, all these proteins are involved in important cellular processes, such as energy metabolism, protein folding, cell structure, and signal transduction, suggesting that their oxidative modification may affect brain physiology. Some of these proteins are reported to be significantly HNE-modified in AD brains compared to matched controls.

Conclusions: Overall, these data support the hypothesis that repeated HSV-1 reactivations into the brain may concur to neurodegeneration also inducing oxidative damages.

P28

PHYLODINAMIC ANALYSIS OF HCV GENOTYPE 4D IN SICILY

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Introduction: HCV genotype 4 (HCV/4) represents 12-15% of total global HCV infections and it's endemic in North-Central Africa and the Middle East regions. HCV/4 is classified into 17 confirmed subtypes whose prevalence varies geographically. Some reports the spread of HCV-4a and 4d in Southern European countries due to the immigration and the movement of men who have sex men (MSM) and intravenous drug users (IDUs) across European borders. In this study, the epidemic history of HCV/4 in Sicily was investigated by Bayesian phylogenetic approaches.

Materials and Methods: HCV/4 isolates were obtained from a cohort of 63 patients, age mean 50, M/F: 1.5, 7/63 HIV-coinfected. The time of most Recent Common Ancestor (tMRCA) was investigated in a datasets of NS3 and NS5b sequences, using dated tips corresponding to the sequence sampling times (1998-2015), by Markov Chain Montecarlo (MCMC) analysis and run for 50 million generations in BEAST 2 (Bayesian Evolutionary Analisys by Sampling Trees) software. Demographic models of population growth as constant size, exponential growth and a Bayesian skyline (BSP) were compared by evaluation of MLE (Marginal Likelihood Estimation). All BEAST out-put log files were analyzed with TRACER v1.6.0 until Effective Sample Size numbers were >250. A maximum clade credibility (MCC) tree was obtained by summarizing the trees using Tree Annotator. The phylogenetic trees were visualized in FigTree program.

Results: Bayesian analysis conducted on HCV/4d isolates studied using exponential growth model showed the clustering of NS3 and NS5b sequences into two distinct clades A e B. The concatenated alignment of NS3 plus NS5b fragments (a total of 928 bp) increased the number of parsimony informative sites and allowed to estimate the date of PA_HCV/4d common ancestor as 1855 with a wide 95% HPD credible region (1784-1910). Moreover, clade A showed a lower genetic variability than clade B (5% vs. 7.8%) and a posterior probability value of 0.99. Interestingly, clade A isolates were obtained from 34 sicilian patients, 27/34 (79%) males, aged mean 42 years (26-61 years) and 7 of

them resulted HIV-coinfected. Clade A sequences resulted phylogenetically related to many strains obtained from people belonging to high risk groups (MSM and IDUs) lived in other European countries. **Conclusion:** HCV-4d showed a low prevalence (< 4%) in Sicily and the phylodinamic analysis conducted traced the origin of Sicilian 4d isolates more than 150 years ago. This finding could be explain by the multiple introduction of HCV/4d strains from Mediterranean endemic areas through the continuous migration flows arriving in Sicily. We demonstrated the spread of phylogenetically -related HCV/4d strains in young men some of them known as MSM and IDUs.

P29

HEPATITIS C VIRUS (HCV) DISTRIBUTION IN THE METROPOLITAN AREA OF NAPLES, ITALY, IN THE ERA OF INTERFERON-FREE REGIMENS

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Introduction: The World Health Organization (WHO) estimates that 130–170 million people are infected with hepatitis C virus (HCV) worldwide and that more than 350,000 people die each year of HCV-related liver diseases. HCV presents high level of variability, which translates into the existence of 7 major HCV genotypes, 67 subtypes and 9 recombinant forms. Since HCV genotypes and subtypes remain cornerstones in the management of chronic HCV infection even in the directly acting antivirals (DAA) era, our aim is to evaluated the distribution of HCV genotypes and subtypes from 2010 to 2015 from patients living in the metropolitan area of Naples, Italy.

Material and Methods: The study was carried out on 1,221 anti-HCV/HCV-RNA-positive plasma samples collected between April 2010 and December 2015 at Azienda Ospedaliera Universitaria – Università della Campania "Luigi Vanvitelli". In order to compare the prevalence of the HCV genotypes according to genre and age , patients were categorized in male and female, and into five age groups (< 41, 41-50, 51-60, 61-70 and > 70 years).

Results: Of the 1,221 patients enrolled, 633 (51.9%) were males and 588 (48.1%) females, with a mean age of 60 (± standard deviation, SD, 13) years. The most frequent HCV genotype observed was genotype 1 (68.1%; 1b in 55.3% and 1a in 9.5%); HCV genotype 2 was found in 289 samples (23.67%); genotype 3 in 6.47%; genotype 4 in only 19 samples, and only two samples were classified as genotype 5. The mean age of the patients with genotype 1a or 3 was lower (age mean ± SD: 51 ± 12 and 49 ± 12, respectively) than those with genotype 1b (62 ± 11, $p < 0.0001$ for both) or 2 (62 ± 14, $p < 0.0001$ for both).

Conclusions: The data emerging from the present study seem to suggest that there have been no

substantial changes in the overall epidemiology of the HCV genotypes in the metropolitan area of Naples over the last six years, suggesting that HCV genotype 1b remains the most prevalent in this area, followed by genotype 2, 1a and 3a. Considering the age distribution, genotypes 1b and 2 were considerably more frequent in older patients, while 1a and 3 were particularly common in younger patients. New subtypes may "emerge" to modify the future epidemiology.

P30

A NOROVIRUS OUTBREAK OCCURRING AT SCHOOL STRESSES NEED OF HYGIENE MAINTENANCE IN AT RISK SETTINGS

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I**ntroduction:** Noroviruses (NoVs) are cause of acute gastroenteritis (AGE) outbreaks in isolated settings such as nursing homes, hospitals, day care centers, cruise ships, restaurants. Excretion in symptomless persons and long environment persistence play a key role in NoVs diffusion. Animal reservoirs were investigated yet a zoonotic role not proved, still food from animals (e.g. shellfish) may act as carrier. Outbreaks occur when peculiar circumstances with heavy exposure, or/and low natural or acquired immunity (as in children, elderly or immunocompromised persons), or new viral strains are involved.
Material and Methods: A longitudinal study was performed on November 2016 after reporting (day zero) of AGE in children from a school with classes from kindergarten to seven grade and pupils from 8 months to 12 years aged. All students were traced back and parents/tutors interviewed. Information on time onset, symptoms type and severity, therapy details and whether other family members were affected was collected by questionnaire. A case was defined as a person school linked and with at least one of following symptoms: diarrhea, vomiting, headache, abdominal pain and asthenia within 2 days before day zero. All clinical cases were asked to provide a fecal sample for microbiological testing. Norovirus was investigated by EIA and real time RT-PCR. Amplification products of a 314 bp region in capsid gene were sequenced. Environment

investigation was performed

Results: All enrolled students and all school personnel were contacted. After the media coverage of the outbreak two AGE cases not school related yet linked to same catering service at the university canteen were detected. Overall 197 persons were interviewed, 156 (among them 79% of school enrolled students) were considered exposed: 115 and 41 persons were classified as cases and not cases, respectively. Within classes attending the canteen between 44% and 100% of exposed students were affected, one case among five interviewed toddlers from the kindergarten displayed AGE. According to the epidemic curve the incubation time ranged 24-42 hours, and the mean symptomatic period was 14.6 hours. NoV was detected in 37 fecal specimen out of 59 tested, 11 virus strains were genotyped and all isolates belonged to the GII.2 genotype, including one isolate from the University canteen. The environment investigation detected an important lack of hygiene in the management of drinking water battles within and between meals

Conclusions: Genotyping provided evidence of no emergent NoV strain involvement. Hygiene management was traced back as the main risk factor for outbreak onset after a student was identified as index case. The catering service was the suspect link between the school and the university canteens.

P31

LATE PRESENTERS AMONG NEW HIV-1 DIAGNOSIS IN EMILIA ROMAGNA REGION (ITALY) DURING 2013-2017 PERIOD

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Introduction: In 2011 the European Late Presenter Group (ELPG), published a consensus definition of Late Presenter (LP): "*Persons presenting for care with a CD4 count below 350 cells/mm^3 or presenting with an AIDS defining event, regardless of the CD4 cell count*". Within Emilia Romagna surveillance system, samples collected from patients with a first positive fourth generation HIV test were divided in two groups, on the basis of CD4 cell count (less or more than 350 cells/ mm^3, late and not late presenters respectively), according to a consensus definition. We also compared socio-demographic and immune-virological parameters used, to highlight differences between late presenters (LPs) and not-late presenters (NLPs) patients.

Materials and methods: Data for newly HIV-1 infection diagnosed in Emilia Romagna in 2013-2017 period were analysed. These data included

socio-demographical factors (age, nationality, gender and risk transmission factor) and immuno-virological parameters (western blot positivity, RNA viral load, CD4 cell count and CD4/CD8 ratio and) at diagnosis. Samples from patients with a first positivity to ELISA test were confirmed by a Western Blot according to and immunoblot strips were read and interpreted by a specific software. Peripheral blood CD4 and CD8 lymphocytes were counted by flow cytometry. For HIV-1 RNA quantification, all the whole blood samples were analyzed by COBAS AmpliPrep/COBAS TaqMan HIV-1 Test.

Results: Among 1059 new infected patients 53% were classified as LPs by a consensus definition, confirming data from European CDC and remaining 47% as NLPs. Not significant differences between median age and gender were recorded.On the basis of CD4 cell count median, as expected significantly higher in NLPs, serum and virological parameters were considered. In particular, serum reactivity by CMIA showed lower OD values in late presents even if the reactivity to a Western Blot assay showed a comparable reactivity. Finally the amount of HIV-1 RNA was slightly lower in NLPs versus LPs.

Conclusion: More than 50% of the newly diagnosis HIV-1 infected patients is represented by subject that arrive at diagnosis with a first CD4 cells count < 350 cells/mL. Only 43% of new HIV-1 infections arrive at diagnosis in time, with a CD4 cell count > 350 cells/mL and so, only these patients, can obtain the maximal benefits from cART treatment. In fact, an early start of the therapy reduces viral load and the probability of HIV-1 transmission. Several factors contribute to the late presentation for HIV-infection: the first is represented by the patients, for example that not consider themselves to be at risk of HIV infection; another factor is the health care professional, as the overall decision about when and in which patients to perform screening for HIV-1 infection.

P32

IS SICILIAN POPULATION PROTECTED FROM POLIOVIRUS? RESULTS OF A SEROPREVALENCE SURVEY IN PALERMO

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Introduction: Although the European region was declared polio-free in 2002, migration flows and lowering vaccine coverage put Italy at risk of importing wild (WPV) or circulating vaccine derived (cVDPV) poliovirus from endemic areas. Sicily, due to its geographical position in the Mediterranean, represents the preferred destination of many immigrants reaching Europe from Africa and Middle East. Periodical assessments of population susceptibility to polioviruses (PVs) is essential for evaluating population protection and planning appropriate vaccination strategies. In accordance with the suggestion of the "Polio Eradication and Endgame Strategic Plan 2013–2018" a seroprevalence survey was conducted in Palermo to assess serological protective titers against all three polioviruses in the general population, representing the best defense against the risk of neurovirulent PV reintroduction.

Materials and Methods: The evaluation of immunity against PV1, 2 and 3 was carried out on a population of 725 immunocompetent subjects (1 - 90 years old). Serum samples were collected in 2009 (318 samples) and in 2015 (407 samples) from the "P. Giaccone" University Hospital of Palermo during routine general health and immunological checkups. Neutralizing antibodies titers were determined by micro-neutralization tests at the Department of Health Promotion Sciences and Mother and Child Care "Giuseppe D'Alessandro", University of Palermo, according to the WHO protocols (WHO 1993). Samples were considered protective if neutralizing antibodies were detected at dilutions ≥ 1:8.

Results: The immune coverage for PV1, PV2 and PV3 in Palermo was, respectively, 59.2%, 68.4% and 42.7% in 2009 and 73.7%, 81.6% and 52.6% in 2015. In 2009, an insufficient immune protection against PV3 was already visible in the adolescents (48% 11-15 y unprotected), while protection against PV1 was low in adults (54.4% 16-30 y and 56.8% 31-45 y unprotected). In 2015, protection against PV3 declined later, in the 17-30 y age group (55.7% unprotected), while the lowest immunity against PV1 (47.4% unprotected) was in the 31-45 y old adults. The protective titers of neutralizing antibodies were generally higher in 2015 and in children up to 14 years of age. Several subjects were found seronegative for all three PV serotypes, 30% and 12.4% of the sampling in 2009 and 2015, respectively.
Conclusions: Our results support the recent change in the regional vaccination schedule with the introduction of the fifth IPV dose in adolescence, in order to obtain a longer-lasting immunity in the population. This seroprevalence study provided valuable information to evaluate the levels of protection against PV and suggests to maintain a state of attention in a country with strong migration pressures, such as Italy.

P33

A CASE OF BREAST MILK ACQUIRED HCMV POST-NATAL INFECTION IN A PREMATURE INFANT

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Introduction: *Cytomegalovirus* (hCMV), a member of *Herpesviridae* family, is one of the most important human pathogens responsible of congenital and postnatal infections. The mother-to-child transmission of hCMV infection is congenitally transmitted through the placenta virus penetration or by reinfection of a new strain or endogenous reactivation of latent virus transmitted to the newborn by breast milk. hCMV post-natal infection may also be acquired by the newborn throught blood transfusions or following the exposure of maternal genital infected secretions during the delivery. Postnatal acquired infection is not so common in full-term infants. We report a case of hCMV postnatal infection through mother breastfeeding acquired following endogenous maternal reactivation.
Materials and Methods: Our patient was a female born prematurely at 28 weeks of gestational age, with a birth weight of about 1000g. The newborn recovered in Neonatology showed fever and was immediately submitted to laboratory examinations which revealed neutrophils 39.3% and lymphocytes 50.6%. Blood cultures were performed and it turned out negative for bacteria and yeasts. After five weeks lower values of red blood cells 2.58 $\times 10^6$/µl and HGB 8.7 gr/dL were found and irradiated packed red blood cells transfused. After two weeks the newborn showed a typical viral respiratory symptomatology and a serum sample was sent to the laboratory. Serological tests revealed IgG anti-hCMV 160.3 AU/ml e IgM anti-hCMV 27 Index. The urine sampling showed a positivity of 99300 hCMV DNA copies/mL. To exclude the possibility of an emotrasfusional infection the donor was contacted, we performed molecular and serological tests that turned out negative. The mother serum sample tested showed negative anti-hCMV IgM and hCMV DNA on blood and urine not detected, but breast milk revealed 2360 hCMV DNA copies/mL so it was possible to diagnose hCMV primary postnatal infection.
Results: The mother breastfeeding for about a month is the cause of newborn's infection followed

by endogenous maternal latent virus reactivation. Mother-to-preterm newborn transmission of breast milk infection had a high risk of developing postnatal symptomatic infection.
Conclusions: Even if breast milk is considered the ideal food for newborns it is also a dangerous vehicle for transmission of infections. To prevent hCMV transmission the breast milk should have to be pasteurizated, but this procedure tends to alter the milk properties in term of nutritional value and anti-infectious components.

P34

INFECTIOUS BOVINE RHINOTRACHEITIS (IBR) IN MEDITERRANEAN WATER BUFFALO (BUBALUS BUBALIS): PREVALENCE OF BUBALINE HERPESVIRUS 1

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Introduction: Bovine herpesvirus 1 (BoHV1) and bubaline herpesvirus 1 (BuHV1) are ruminant alphaherpesviruses. BoHV-1 can provoke infectious bovine rhinotracheitis (IBR), genital disorders, conjunctivitis, abortions, encephalitis or immune suppression which may lead to secondary bacterial infections and cause pneumonia. Hence, BoHV-1 infection might cause substantial economic loss and trade restrictions in cattle industry. IBR control and eradication programs have been implemented in many European countries. Recent serological surveys described a prevalence of antibodies against BuHV-1 in water buffalo farms in Campania Region.
Objectives: The aim of this study was to compare the results of two different serological surveys, performed respectively in 2009 and in 2016, to evaluate the prevalence of BoHV-1 and BuHV-1 infections in water buffalo farms in the Province of Caserta, Campania Region (Italy), where IBR control programs were recently implemented.
Animals and Methods: In both surveys, serum samples were collected from buffalo farms (precisely, 862 animals/28 farms in 2009 and 309/16 farms in 2016). To detect antibodies for IBR, two commercial ELISA kits were used, and, precisely, in 2009 IBR-gB and IBR-gE (IDEXX), in 2016 Eradikit Discrimination BoHV-1/BuHV-1 (IN3 diagnostic). Both tests provide the most correct diagnosis on serum samples (Nogarol et al., 2014; Tignon et al., 2017).
Results and Discussion: In 2009, a seroprevalence of BuHV-1 (49.55%) vs. BoHV-1 (23.06%) was found. In 2016, 57.60% of buffaloes were positive to BuHV-1 vs. BoHV-1 (1.62%), as previously detected (Caruso et al 2016). Overall, we detected

a time-dependent increase in BuHV-1 prevalence and a significant decrease in BoHV-1 infection rates between the two surveys. To explain the difference in the decrease of BoHV-1, we suppose that it could be in the existence of antigenic cross-reactions between these viruses and in their ability to cross the species barrier. Indeed, both BoHV-1 and BuHV-1 have been reported to cross the species barrier (Thiry et al 2006).

Conclusion: Currently, the high percentage of sera reactive to BuHV-1 indicates that BuHV-1 may be the main circulating alphaherpesvirus infection in Mediterranean water buffalo.

P35

HCV LAB MAPPING/NETWORK: A COMPREHENSIVE RESOURCE TO IMPROVE CURRENT TESTING LANDSCAPE

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Introduction: Hepatitis C is a major cause of chronic liver disease. In 2015 an estimated 71 million people had the chronic Hepatitis C virus (HCV) infection which is usually asymptomatic or accompanied by non-specific symptoms. The therapy for HCV infection has greatly improved over recent years, especially with the introduction of direct acting antivirals (DAAs) that are able to achieve high cure rates, although the cost of treatment still remains a barrier in many countries. Importantly, a rapid, efficient and affordable diagnosis is fundamental to allow treatment access in order to contain HCV infection spread that is still high in Europe.

Method: Data from the Diaceutics database, which contains testing information from a range of international laboratories, was used to understand the current HCV testing landscape in Italy. This study was part of a more extended European analysis, with a particular focus on the EU5 countries.

Results: Data within this report, for the period of 2016, provides insights into HCV in Europe (EU), including Italy. The Italian diagnostic landscape is characterized by a large number of regional testing centers. Nevertheless, there is an increasing tendency to centralize testing, observed notably in the north. Antibody (AB) and RNA testing positivity rates increased in 2016 from the north to the south of Italy, an area recognized as hyperendemic because of the proximity with North African countries. In all EU5 countries there is a higher growth in RNA testing when compared to AB testing, possibly correlating with larger follow-up testing for infection activity and treatment monitoring. The turnaround time (TAT) required to perform and report an HCV AB test in Italy is one of the lowest among the EU5 countries.

Conclusions: A better understanding of the testing landscape is beneficial in a number of ways such as epidemiology studies, monitoring the annual laboratory testing activity and budget, and developing specific campaigns to improve the number of patients tested, as recommended by WHO global HCV guidelines. Moreover, testing quality assessment programs, as well as pharma initiatives, can also be developed to support laboratory testing

P36

A NOVEL METHOD TO TITRATE HERPES SIMPLEX VIRUS-1 (HSV-1) USING A LASER-BASED SCANNING OF NEAR-INFRARED FLUOROPHORES CONJUGATED ANTIBODIES

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Introduction: Among several strategies used for Herpes simplex virus (HSV) detection in biological specimens, standard plaque assay (SPA) remains the most reliable method to evaluate virus infectivity and quantify viral replication. However, it is a manual procedure, thereby affected by operator subjectivity, and it may be particularly laborious for multiple sample analysis. Here we describe an innovative method to perform the titration of HSV type 1 (HSV-1) in different samples, using the "In-Cell Western" Assay (ICW) from LI-COR, a quantitative immunofluorescence assay that exploits laser-based scanning of near infrared (NIR).

Methods: NIR-immunodetection of HSV-1 proteins (both late protein as glycoprotein B, gB, and early protein as Infected Cell Protein 4, ICP4) was used to monitor foci of virus infection in Vero cells following incubation with HSV-1 samples (HSV1-S). In parallel, a standard curve was build up with serial dilutions of an HSV-1 stock and exploited to interpolate the HSV1-S titre. Similarly, ICW was used to test HSV-1 titre in Vero cells infected in the presence of different concentration of Acyclovir (ACV), the main inhibitor of HSV-1 replication.

Results: By assessing HSV-1 titre in different samples, we found that ICW produced similar and superimposable values compared to SPA, but it is faster (24h vs 72h for SPA) and can be performed in 96 well plate, thus allowing to easily and quickly analyse and quantify many samples in parallel. These features make our method particularly suitable for the screening and characterization of antiviral compounds, as we demonstrated by testing ACV. In particular, ACV IC50 was calculated by both gB and ICP4 staining resulting in superimposable values (0.018 mg/ml for ICP4 staining, 0.025 mg/ml for gB staining). Moreover, we developed a new data analysis system, exploiting Image-J software, that allowed to overcome potential bias due to unspecific florescence signals, thus improving data reproducibility.

Conclusions: Overall, these results strongly support ICW as suitable technique for HSV-1 titration in biological samples and antiviral drug screening, and make it as a useful tool for virologists.

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IN SILICO EVALUATION OF RELEVANT MOLECULAR ASSAYS FOR CCHFV DETECTION

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Introduction: Crimean-Congo hemorrhagic fever virus (CCHFV) can cause lethal disease in humans and is considered one of the major emerging infectious threats. Molecular tests are the methods of choice for laboratory diagnosis of CCHF during acute phase of infection, but their sensitivity can be strongly affected by CCHFV sequence diversity. In fact, the genetic evolution of CCHFV is characterized by a substitution rate of 0.8, 1.3 x 10^{-4} and a differentiation in 6 geographically distinct clades. We provide an in silico evaluation of currently used molecular assays targeting S segment to estimate the potential sensitivity of different assays in detecting CCHFV, with a special focus on strains circulating in different geographic areas.

Material and Methods: All available CCHFV genomes by May 10th 2017 were retrieved from NCBI Nucleotide database. CCHFV strains with complete S segment were selected for phylogenetic analysis. Primers/probe sequences of published in-house assays were collected and aligned to all CCHV strains. Using a homemade python script, all variants vs primers and probe sequences were identified for each molecular assay, and the percentage of viral strains in each clade fully matching (100% nucleotide identity) primers and probes was calculated. To consider a molecular assay suitable in detecting a CCHFV clade, two different parameters were considered: 1) if at least 50% of CCHFV strains belonging to the clade fully match the molecular assay, or 2) if no more than 4 mismatches contemporary occur between the assay and any CCHFV strain belonging to the clade.

Results: Fourteen molecular assays published from 1997 to 2014 were analyzed: 8 end point RT-PCR (3 single round, 5 nested) and 6 Real Time RT-PCR. A total of 126 CCHFV sequences were analyzed for phylogenesis. In agreement with previous works, six different clades, associated with different geographic areas, were recognized by phylogenetic analysis. Primers and probes of each assay show a wide range of nucleotide identity with CCHFV strains (between 63% and 100%), with a median value around 91%.

Conclusions: With few exceptions, most assays seem to perform unevenly with respect of the 6 CCHFV clades, being optimal for only 1 or 2 clades, while primers/probe combinations showed a dramatic increase of mismatches when aligned to other clades. In general, analyzed tests seem to be more suitable for detection of clades III (Africa), IV (Asia) and V (Europe). These findings suggest the adoption of more than one test in diagnostic algorithm, and previous availability of epidemiological information as a guide for the selection of the most appropriate molecular assay.

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FIELD EVALUATION OF THE EBOLA AG K-SET RAPID TEST PERFORMANCE IN SIERRA LEONE

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Introduction: The recent resurgence of Ebola virus Disease (EVD) in the north of the Democratic Republic of Congo (DRC) showed that Ebola virus (EBOV) still poses a relevant threat and the International community must remain on high alert and ready to respond. One of the main takeaways from the 2013-2016 EVD epidemic in West Africa is that the efficient interruption of EVD transmission chains critically depends on rapid and reliable diagnosis. EVD diagnosis is currently performed using reverse transcription-PCR (RT-PCR) method, which is highly sensitive and specific but requires skilled laboratory personnel and advanced equipment. New rapid diagnostic tests (RDT), performed as point-of-care (POC) or in resources-poor settings are still needed. This study aimed to preliminarily evaluate in a field setting a new RDT, EBOLA Ag K-SeT, developed by Coris BioConcept (Gembloux, Belgium) in the framework of the EbolaMoDRAD project.
Material and Methods: The study was performed in the EVD laboratory established at the Princess Christian Maternity Hospital (PCMH) in Freetown (Sierra Leone) run by Emergency NGO and the Italian National Institute for Infectious Diseases (INMI). Residual aliquots of plasma samples (n = 210, EVD prevalence 50%) collected from patients hospitalized at the Emergency NGO's Ebola Treatment Centre in Goderich (Sierra Leone) from 12 December 2014 to 21 June 2015 and stored at -20°C, were anonymized and retrospectively used for the evaluation of the RDT. EBOLA Ag K-SeT is a lateral flow immunoassay which detects Ebola virus Zaire strain Vp40 antigen and which is based on immunochromatography in a sandwich format. The diagnostic efficacy of the RDT was only evaluated for Ebola virus Zaire strain, comparing the clinical results with those obtained with the RT-PCR test (RealStar® Filovirus Screen RT-PCR Kit 1.0, Altona Diagnostics) as reference method.
Results: Overall, the sensitivity of the EBOLA Ag K-SeT RDT was 88.6% (95% Confidence Interval [CI]: 82.5-94.7) and the corresponding specificity was 98.1% (95% CI: 95.5-100.7). The positive and negative predictive values were 97.9% (95% CI: 95.0-100.8) and 89.6% (95% CI: 84-95.2), respectively. The sensitivity strongly increased up to 98.7% (95% CI: 96.1-101.2) for those samples with low Ct values (≤ 25).
Conclusions: Although further investigation needs to be carried out to fully evaluate the assay, our study showed that the EBOLA Ag K-SeT RDT represents a promising candidate as a reliable and fast POC, which may be of great help in future EVD outbreaks to limit the burden on health care systems, control disease transmission and improve patient outcomes in otherwise resource-constrained setting.

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PRELIMINARY EVALUATION OF A RAPID AND SENSITIVE RT-PCR ASSAY FOR THE DETECTION OF EBOLA VIRUS

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Background: The reappearance of Ebola virus in the north of the Democratic Republic of Congo (DRC) highlighted the need to remain on high alert and to be prepared to face the virus. The efficient interruption of the Ebola virus disease (EVD) transmission chains remains the best mean to efficiently face EBOV spread. Interruption of transmission critically depends on reliable and rapid diagnosis. Currently, EBOV diagnosis relies on reverse transcription-PCR (RT-PCR), highly specific and sensitive, but requiring skilled personnel and sophisticated equipment. During the 2013/2016 Ebola virus disease (EVD) outbreak in West Africa and in the framework of the EbolaMoDRAD project, CLONIT S.r.l. and STMicroelectronics S.r.l. developed a prototype of a fast, easy-to-use and reliable qRT-PCR assay for EBOV detection, time-to-diagnosis of 1 hour. We evaluated its performance during the outbreak and in further studies in the EVD laboratory established at the Princess Christian Maternity Hospital (PCMH) in Freetown (Sierra Leone) run by Emergency NGO and the Italian National Institute for Infectious Diseases (INMI).

Material and Methods: Residual aliquots of clinical samples (n = 116, 66 plasma, 13 oral swab and 8 urine samples; EVD prevalence 36%) collected from patients admitted at the Ebola Treatment Center in Goderich, Freetown, Sierra Leone and stored at -20°C were used for the evaluation of the qRT-PCR assay. The PCR assay allowed the target reverse-transcription, amplification and detection of the extracted RNA in a One-Step fast reaction (Clonit), performed on ready to use silicon based chips. The analysis was performed using an innovative, portable system (Q3 plus, STMicroelectronics). The diagnostic efficacy of the new qRT-PCR was compared to the results obtained with the reference test Altona Filovirus Screen RT-PCR-Kit 1.0 (Altona Diagnostics) performed on a SmartCycler® (Cepheid) PCR instrument.

Results: Overall, the test was very easy to use with a time-to-diagnosis of one hour (from RNA to quantitative PCR result). The instrument was robust and reliable in field settings. The sensitivity of the RT-PCR assay was 100% and the specificity 98.6%, with a negative predictive value of 100% and a positive predictive value of 97.7%.

Conclusions: Although further tests are needed, the preliminarily evaluation of the qRT-PCR assay developed by Clonit S.r.l. and STMicroelectronics S.r.l. showed a better performance in sensitivity compared to the reference test, indicating its promising applicability for laboratory diagnosis, especially in resource-limited settings.

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ENHANCED *IN VITRO* REPLICATION OF VIRUSES USING A THREE-DIMENSIONAL CELL CULTURE SYSTEM

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Introduction: *In vitro* isolation has been progressively replaced by fast and more sensitive diagnostic methods. Despite this, cultivation of viruses has been improved thanks to the recent development of three-dimensional (3D) cell cultures that better reproduce complexity of *in vivo* microenvironment compared to two-dimensional cell cultures (2D). With the aim to create an efficient system for viral isolation, we set up 3D cultures with cell lines routinely used in laboratory virology and compared them to traditional 2D cell cultures.

Materials and Methods: The cellular lines used were: A-549, ML, Vero, CaCo2, HUH-7, KB, and MRC-5; 2D cultures were produced using the "shell vial" technique (SV), 3D cultures were generated with the following scaffold-free systems: hanging drop microplates, direct centrifugation of cells in microplates, and inhibition of cell adhesion by coating microplates with poly-hydroxyethyl methacrylate (pHEMA). Susceptibility to viral infection was tested with primary strains of Adenovirus (ADV), Herpes Simplex Virus type-1 (HSV-1), and Cytomegalovirus (CMV) prototype strain AD169. Viral growth and release was assessed by examining infected cells with direct immunofluorescence and culture supernatants with Real-Time PCR. Analyses were performed at 24, 48, and 72 hours post-infection and, as regards 3D cultures, with spheroids obtained with pHEMA method.

Results: All cell lines were amenable to 2D cultures. In contrast, 3D cultures were obtained will all cells except f ML cells. We were able to obtain spheroids of various size with all the three methods tested but we eventually choose the pHEMA protocol as it allowed to generate larger spheroids in shorter time compared to the other two methods. The 2D and 3D methods were compared for suitability to viral infection and propagation with particular reference to their applicability and reliability in routine diagnosis. Preliminary results indicate that viral isolation and yield are more efficient with 3D compared to 2D cell cultures and show that a streamlined pHEMA protocol can be easily adapted to day-to-day activity. Further studies to evaluate the breadth of susceptibility to infection with other clinical isolates and samples are in progress.

Conclusion: The development of sensitive and robust 3D culture systems is crucial to improve viral isolation, in particular for viruses that are difficult to cultivate. The 3D cell culture is an emerging technology that closely mimics morphological and biochemical conditions found *in vivo* and provides therefore a potential tool for studying viral infection, pathogenesis, and virus-cell host interaction.

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COPY-YEARS VIREMIA AS A VIROLOGICAL FAILURE PREDICTOR IN HIV POSITIVE PATIENTS TREATED FOR A LONG TIME

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Introduction: The goal of antiretroviral therapy (ART) is to maintain plasma viremia below detectable levels of HIV RNA assay. However, patients on prolonged effective ART, may show very low levels of HIV RNA defined residual viremia (RV). It has been demonstrated that patients with basal RV showed a higher incidence of virological failure (VF) compared to patients with undetectable basal viremia. This prediction is based on one single measurement of viral load (VL) and few studies analyzed the impact of cumulative viremia over time on early failure in ART treated HIV infected patients. To detect the cumulative exposure to HIV a parameter, viremia copy-years (VCY) was used. VCY is defined as the area under a patient's longitudinal VL curve and combine the level and the duration of this replication.
In the light of above consideration, the aim of this study was to evaluate the impact of cumulative HIV VL on VF in treated HIV+ patients monitored for a long time.

Methods: Eight-hundred ninety HIV-1 infected patients from Policlinico Umberto I University Hospital were retrospectively examined for 48 months. The population included 636 males (71%) and 254 females (29%) with a median age of 53 years (IQ 47-55). HIV RNA was quantified using the kPCR molecular system (Siemens Healthcare Diagnostics). For each patient, VCY was determined calculating area under the plasma HIV VL time series using the trapezoidal rule. Cox proportional hazards analysis was used to calculate the adjusted relative hazards of viral failure for age, gender, expression of CD41, groups, type of drug, HIV regimen and area under VL quartile.

Results: To evaluate risk of VF, patients were grouped either according to T0 (start of the study) VL (group1 patients with undetectable viremia; group2 patients with levels of viremia < 37 copies/ml detectable; and group3 patients with VL between 37 and 200 copies/ml) or into quartiles according to VCY value (< 0.82 log copy-years/mL; 0.82 to 4.1 log copy-years/mL; > 4.1 log copy-years/mL). Cox regression proved that both grouped patients with higher levels of cumulative burden > 4.1 log copy-years/mL and basal levels of viremia value between 37 and 200 copies/ml are independently associated with early risk of virological failure. Interestingly, 34% of patients with undetectable viremia at T0, showed a cumulative burden > 4.1 log copy-years/mL. Only 12% of patients with levels of viremia < 37 copies/ml and 14% of patients with viremia value between 37 and 200 copies/ml had a cumulative burden > 4.1 log copy-years/mL ($p < 0.0001$).

Conclusion: These data suggest that VCY may provide prognostic information beyond single measurement of VL, and further underline that a sustained complete suppression of viral replication is needed to reduce the risk of VF.

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DETECTION OF MERKEL CELL POLYOMAVIRUS (MCPYV) INFECTION IN FIBROBLASTS DERIVED FROM THE NON-NEOPLASTIC DERMIS SURROUNDING MERKEL CELL CARCINOMAS (MCCS)

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Introduction: Merkel cell polyomavirus (MCPyV) is an abundant virus that, in elderly and immunosuppressed individuals, can lead to a lethal form of skin cancer, Merkel cell carcinoma (MCC). Despite the well-defined function of the viral oncogenes in cellular transformation, many aspects of its infectious life cycle are still obscure, including its target cells and those of tumour origin in MCC. Jianxin You and coworkers recently identified, by *in vitro* model of infection, the human dermal fibroblasts as the cell type that supports productive MCPyV replication cycle. However, infection of dermal fibroblasts in human tissues remains to be established.

Methods and Results: Here, we demonstrate the presence of MCPyV infection in the fibroblasts obtained from the surrounding healthy tissues of one patient with relapsing MCPyV-positive MCC. Infection was demonstrated by PCR and FISH analysis. These fibroblasts have lost the virus after the sixth passage in culture. Moreover, for the same patient, tumourgraft in a nude mouse was obtained, and half of it was used to establish MCC cell cultures. From this tumourgraft and other three MCCs (1 MCPyV-positive and 2 MCPyV-negative), we generated cells that are growing either as suspension or adherent cultures. The cells derived from MCPyV-positive MCCs are all virus-positive by PCR (still after the sixth passage), but have lost the neuroendocrine markers. The adherent cells display fibroblast markers (e.g. vimentin). Vimentin-positive cells were already abundant in the MCC tumourgraft.

Conclusion: Together, we provide compelling evidence that the fibroblasts are the natural target cells for MCPyV infection.

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THE DIASORIN LIAISON® XL MUREX ANTI-HDV: A DIAGNOSTIC INNOVATION FOR HDV SCREENING

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Introduction: Hepatitis Delta Virus (HDV) is a satellite of the Hepatitis B Virus (HBV). Despite of HBV/HDV co- or superinfection leads to more severe liver disease, the HDV infection is no routinely investigated and probably the infected population is underestimate. HDV diagnosis relies on the detection of total Anti-HDV antibodies (Anti-HDV) and HDV serology is currently investigated by time consuming-and labour demanding-Enzyme ImmunoAssay (EIA).The aim of this study, was to investigated the performance of a new fully automated LIAISON® XL Murex Anti-HDV test and to assess its use in our setting.

Materials and Methods: Serum samples from inpatients with a medical order for the laboratory diagnosis of HDV were screened for Hepatitis B surface Antigen (HBsAg) and then processed by HDV serology when positive. The Anti-HDV evaluation was carried out using EIA ETI-AB-DELTAK-2 (DiaSorin S.p.A., Italy), currently in use in the laboratory and defined as reference method, and new Murex Anti-HDV (DiaSorin S.p.A., Italy), a chemiluminescent immunoassay for the qualitative determination of Anti-HDV on the fully automated LIAISON® XL Analyzer. When requested, HDV-RNA was evaluated. The results obtained were analysed by statistical analysis.

Results: During the study period, 105 samples resulted HBsAg-positive and were also evaluated for the presence of Anti-HDV. Thirty one (29.5%) and 74 (70.5%) specimens showed Anti-HDV-positive and –negative results respectively by EIA test. The overall concordance between the reference method and new Murex Anti-HDV test was 97.1%, with 31 (29.5%) positives and 71 (67.6%) negatives samples. All 3 discordant specimens (2.9%), were positive with EIA and negative with Murex Anti-HDV. Nineteen samples were also evaluated for HDV-RNA. Ten (53%) resulted Anti-HDV positive and showed high levels of HDV-RNA. Three (16%) resulted positive for Anti-HDV without HDV-RNA and the remaining 6 samples (32%) were negative for Anti-HDV and HDV-RNA. In comparison with the reference method, the use of Murex Anti-HDV allowed a more rapid diagnosis of HDV infection with a remarkable reduction of turn around time.

Conclusions: LIAISON® XL Murex Anti-HDV assay showed an excellent concordance with the reference method. The fully automation of the assay on LIAISON® XL Analyzer assures the standardization of the results and a rapid response to the clinicians, allowing for the optimal management of infected patients as well as organ and tissue donations. This new accurate diagnostic tool could be used for a more efficient approach to the HDV diagnosis and evaluation of HDV epidemiology.

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THE POLYOMAVIRUSES MICRORNA TRANSCRIPTOME IN SALIVA FLUID IS A POTENTIAL VIRAL TRAIT TO ASSESS INFECTIOUS STATUS IN ORAL CAVITY

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Background: Evidence suggested Polyomaviruses (PyVs) microRNA (miRNA) in circulation in biological fluids as relevant trait for understand viral persistence. The purpose of this study is to evaluate saliva samples as a biological fluid where PyVs miRNA expression in exosomes can be checked to assess the viral prevalence/persistence in the respiratory tract.

Materials and Methods: The study involved the anonymous analysis of 100 coupled saliva and plasma samples collected from HIV infected patients and of 50 coupled saliva and plasma samples collected from adult healthy subjects, as controls. DNA extracted from saliva and plasma samples was amplified with a Polyomavirus JC (JCPyV), Polyomavirus BK (BKPyV), Merkel cell polyomavirus (MCPyV) and Simian virus 40 (SV40) specific droplet digital PCR absolute quantification assay by using primer and probes targeting Large T antigen and Vp1 antigen coding region. Exosomes were extracted from plasma with NORGEN Exosome Purification kit and characterized by Nanoparticle tracking analysis (NTA) and by western blot (WB) for presence of tetraspanin CD63 protein. Total RNA was isolated from the exosomes of saliva and plasma and PyVs miRNA expression was analysed and quantified with the specific JCPyV (jcv-miR-J1-5p), BKPyV (bkv-miR-B1-5p), MCPyV (mcv-miR-M1-5p) and SV40 (sv40-miR-S1-5p) miRNA-5p quantitative stem-loop RT-PCR MiRNA assay.

Results: PyVs DNA infectious status in saliva and plasma of HIV patients exhibited a prevalence of 25% (12% JCPyV, 8% MCPyV, 5% SV40, 3% BKPyV) and 36% (22% JCPyV, 9% SV40, 1% BKPyV and absence of MCPyV), respectively. PyVs DNA infectious status in saliva and plasma of healthy subjects exhibited a prevalence of 22% (14% JCPyV, 4% BKPyV and SV40 and absence of MCPyV) and 4% (4% SV40, and absence of JCPyV, BKPyV and MCPyV), respectively. Investigating PyVs miRNA expression in well characterized exosomes derived from saliva and plasma significant high positivity were obtained compare to DNA status: in HIV patients 76% (61% BKPyV, 21% JCPyV, 32% SV40 and 14% MCPyV) and 36% (16% BKPyV, 10% JCPyV and SV40 and 9% MCPyV), respectively, in Healthy subjects 84% (70% BKPyV, 40% SV40, 30% MCPyV and 10% JCPyV) and 68% (36% MCPyV, 30% BKPyV, 10% JCPyV, 4% SV40), respectively. Noteworthy, more than one specific PyV miRNA was observed in many saliva samples. Moreover, when PyV DNA and miRNA status in saliva and plasma was stratified according age, HIV time of infection and viral load and CD4 cell counts, it was showed only a clear dependence from age.

Conclusion: Collectively, high PyV miRNA expression in exosomes of saliva confirm viral persistence in oral cavity and suggests additional investigation may be of warranty to divulge their potential implication as cofactors in oral diseases.

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TRANSCRIPTOMICS OF THE IMMUNE RESPONSE: COMPARISON BETWEEN TWO DIFFERENT SOFTWARES FOR ANALYSIS OF DATA OBTAINED IN A VACCINE CLINICAL TRIAL

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Introduction: RNA sequencing is becoming increasingly important to study gene expression changes through differential analysis between different groups of samples. Among the bioinformatic tools available for identification of differentially expressed (DE) genes, two R packages, *edgeR* and *DESeq2*, are the most commonly used. Aim of this work was to compare the performance of these two packages.

Materials and Methods: Analyses were performed on transcriptomic data of 381 RNA samples from 64 volunteers vaccinated with recombinant vesicular stomatitis virus–vectored Zaire ebolavirus(rVSV-ZEBOV); blood samples were taken at different time points after vaccine injection and RNA was extracted from whole blood. Targeted transcriptome sequencing was performed on an Ion Proton instrument. *edgeR* uses the generalized linear model (*glm*) approach to identify differentially expressed genes. *DESeq2* performs differential analysis of gene expression using logarithmic expression approach to normalize data and to smooth over the outliers read counts; for each sample, it calculates a scaling factor which considers both read depth and library composition. Samples from each study day were compared against baseline samples (Day 0) to assess the number of DE genes at each time point after vaccination. Analysis was performed on vaccinated volunteers applying a filter to exclude genes with low or no expression: *DESeq2* has a default filter that excludes genes with less than 1 count, while in *edgeR* genes with at least 1 count per million in 10 libraries were included. Concordance was calculated as the sum of DE and not-DE genes identified by both programs divided by the number of expressed genes.

Results: The average concordance between *edgeR* and *DEseq2* obtained across all contrasts was 80%. Concordance ranged from 45.6% in the contrast Day 1 vs Day 0, to 99.9% in the contrast Day 35 vs Day 0, increasing with time after vaccine administration. Comparison of *edgeR* and *DEseq2* results identified a core of 5462 DE genes at Day 1, which corresponded to 38.6% of the expressed genes. The number of DE genes decreased over time with both programs, identifying just 3 common DE genes at Day 35 after vaccination. While the number of DE genes identified was similar for both programs at each time point, the shared DE genes between *edgeR* and *DEseq2* ranged from 3.4% at Day 14 to 65% at Day 35.

Conclusions: The number of DE genes identified by *edgeR* and *DEseq2* had a peak at Day 1 after vaccination and decreased over time. The concordance between the programs was higher at late time points because most genes were identified as non-DE by both *edgeR* and *DEseq2*. Functional evaluation of DE genes identified will be performed assessing differences in the activation of the blood transcription modules.

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EPIDEMIOLOGICAL AND SOCIO-ECONOMIC IMPACT OF ROTAVIRUS VACCINATION IN SICILY

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Introduction: Rotaviruses (RVs) are the most common cause of severe diarrhoea among children < 5 years of age. Group A RVs are commonly classified based on outer capsid proteins, defining G (for VP7) and P (for VP4) genotypes. Although rarely fatal in industrialized countries, RVs are a major cause of hospitalization in children, thus producing substantial healthcare costs. Newborns RV vaccination with monovalent (RV1, G1/P[8]) Rotarix® vaccine was introduced in Sicily in November 2012. In this study, the prevalence, age-distribution and seasonality of RV infections, as well as circulating genotypes, were compared in pre- and post-vaccination periods to evaluate the epidemiological and socio-economic impact of RV vaccination in Palermo, Sicily.

Material and Methods: During 2011-2016, a total of 5765 fecal samples were collected from all children hospitalized for gastroenteritis (GE) at the "G. Di Cristina" Pediatric Hospital in Palermo, Sicily. All samples were screened for RV infection using an immunochromatographic test and RV-positive samples were G/P genotyped according to EuroRotaNet surveillance network protocols. For the socio-economic survey, both direct (hospitalization, ER and Pediatrician visits) and indirect (nappies, medications/diet and lost working days) healthcare costs have been assessed. Vaccination cost was estimated from coverage rates and demographic data.

Results: In Palermo, vaccine coverage (infants receiving at least one dose of RV1) progressively increased, from 44.6% in 2013 to 72.1% in 2015 but declined to 58,1% in 2016. Upon four years of universal vaccination, a steady decline in the prevalence of RV GEs was observed (10.6% in 2016 vs. 21.9-29.3% in 2011-12). A reduction in the number of cases in the age group 0-4 years and the fading of the typical seasonal infection pattern was also noticed. Comparing the genotypes of circulating strains, in 2016 genotype G1P[8], included in R1 vaccine, was down to 1.7% of cases vs. 72-61% in 2011-2012, while an increase in circulation of G9P[8] (68.3% vs. 0.5%) and G12P[8] (13.3% vs. 5%) genotypes was observed. In 2016, vaccination generated public health benefits in the pediatric population of Palermo in terms of reduction of GE hospitalizations and RV-related healthcare costs (reduction of total RVGE costs from € 806,180 in 2011-2012 to € 440,762 in 2016, including vaccination costs).

Conclusions: The reduction of GE hospitalizations and RV-related healthcare costs observed after the introduction of RV vaccination has generated public health benefits in the paediatric population of Palermo. Increase in vaccine coverage and a desirable lowering of vaccination costs would further improve the cost-effectiveness of the anti-RV vaccine in Sicily.

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INCREASING MIGRATION FLOWS AND THE RISK OF POLIOVIRUS REINTRODUCTION: THE ROLE OF SICILY IN THE POLIO ERADICATION PLAN

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Introduction: The poliomyelitis eradication initiative, launched in 1988 by the WHO, is based on immunization activities, syndromic and environmental surveillance. Due to migration flows and lowering vaccine coverage, Italy can be considered at risk of importing wild (WPV) or circulating vaccine derived (cVDPV) poliovirus from endemic areas. Sicily, due to its geographical position, represents the preferred destination of many immigrants reaching Europe from Africa and Middle East across the Mediterranean. Following national and WHO recommendations, the Sicilian Regional Reference Center (RRC) monitors Acute Flaccid Paralysis (AFP) cases in 0-14 years old children and investigates the presence of (WPV) and (cVDPV) in sewage (environmental surveillance). Furthermore, in accordance with the suggestion of the "Polio Eradication and Endgame Strategic Plan 2013–2018" a seroprevalence survey was conducted to investigate the immune coverage levels of the local population, representing the best defense against reintroduction.

Materials and Methods: Since 1991, active AFP surveillance was carried out in Sicily by the sub-national laboratory of CRR following the National Institute of Health (ISS) and the Italian Ministry of Health guidelines. Environmental surveillance was performed according to WHO/V&B/03.03 protocol on wastewater samples collected from 2013 to 2015 from 7 immigrants detention centers (IDCs) with an hosting capacity of 200-2000 people. The level of immunity in the population was evaluated by micro-neutralization tests on 725 sera from Sicilian patients (1 - 90 years old), collected in 2009 and in 2015.

Results: Poliovirus etiology was excluded for all the 103 AFP cases reported in Sicily from 2002 to 2016, according to fecal samples cell-culture tests. Guillain-Barré syndrome was the main cause of AFP in Sicily. All patients showed a protective immunity as a result of vaccination. No wild polioviruses were isolated from the 64 sewage samples analyzed during environmental surveillance in IDCs. Only in four sewage samples, collected in December 2013 in IDCs in the districts of Caltanissetta, Trapani, Ragusa and Siracusa, the Sabin-like PV2 was isolated. Seroprevalence studies showed an insufficient immune protection against PV3, already visible in the adolescents, and PV1 in adults.

Conclusions: The continuous and increasing flow of refugees from countries where polio is still endemic or has been recently circulated expose Sicily to the risk of reintroduction of neurovirulent strains. Therefore, the maintenance of surveillance activities will be mandatory until the goal of global polio eradication is achieved.

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A COMPARISON OF HEMAGGLUTINATION INHIBITION AND NEUTRALIZATION ASSAY FOR CHARACTERIZING THE ANTIBODY RESPONSE TO INFLUENZA VACCINATION IN ELDERLY (60-75 YEARS OLD) AND VERY ELDERLY (> 75 YEARS OLD) INSTITUTIONALIZED PEOPLE

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Introduction and Aim: Several studies have explored whether the antibody response to influenza vaccination in elderly adults is as strong as it is in young adults. Results vary, but tend to indicate lower post-vaccination antibody titers in the elderly, supporting the concept of immunosenescence – the weakening of the immunological response related to age. Our previous results showed a lack of difference in the response to the influenza vaccine between elderly and very elderly (below or over 75 years) when the antibody response was evaluated by hemagglutination inhibition assay (HI). Recent results obtained investigating the relationship between the age and the function of B lymphocytes suggest that the quality of antibodies seems to be affected by the patient's age rather than the quantity. For more information on this aspect, we analyzed the antibody response in elderly (60-75 years old) and very elderly (> 75 years old) institutionalized subjects after 2016/17 influenza vaccine administration using two different serological techniques: HI and virus neutralization assay (VN). In comparison to HI, VN identifies a wide range of neutralizing antibody bacause it detects antibodies that neutralize the virus via entry/replication inhibition in mammalian cells whereas HI only measures antibodies directed against haemagglutinin that act by preventing erythrocite agglutination.

Materials and Methods: Sera samples were collected from 81 study participants (38 elderly and 43 very elderly). All subjects were vaccinated with influenza vaccine (Fluad) commercially available for the 2016-17 influenza season. A double blood sample was collected (before and 1 month after vaccination). HI and VN assays were performed for the B vaccine component (B/Brisbane/60/08). HI antibody titres were determined in RDE treated serum samples by a standard microtiter method using 0.5% turkey erythrocytes. NT tests were carried out by mixing serially diluted sera with 100 $TCID_{50}$ of egg-adapted virus and added to a MDCK cell monolayer. Cytopathic effect was read and hemagglutination assays performed to detect the presence of viral replication.

Results: The data obtained by HI test showed a similar response in the two age groups, evaluating both the general population and the volunteers no protected before vaccination (HI < 40). On the other hand, the results obtained by VN assay showed a better response in the elderly (60-75 years) rather than in the very elderly (> 75 years) group.

Conclusions: The measurement of neutralizing antibodies provided new information about the antibody response in the elderly following influenza vaccination. The antibody response of the elderly (60-75 years) were quantitatively similar to those of people over 75 years but functionally more effective.

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TIME COURSE ANALYSIS OF GENE EXPRESSION IN BLOOD CELLS FOLLOWING VACCINATION WITH A LIVE RECOMBINANT VESICULAR STOMATITIS VIRUS EXPRESSING THE GLYCOPROTEIN OF ZAIRE EBOLAVIRUS (RVSV-ZEBOV)

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Introduction: In recent years with the advent of Next Generation Sequencing technologies, RNA sequencing has become a widely used method for identify genes that are differentially expressed (DE) between two or more biological conditions. Blood transcriptomics could be very informative about early events of the immune response. We sequenced whole-blood transcripts from volunteers vaccinated with recombinant vesicular stomatitis virus–vectored Zaire ebolavirus (rVSV-ZEBOV), the candidate vaccine against Ebola. Goal of this work was to perform a time course analysis fitting, for each gene, an impulse model designed to describe a two-sigmoid behavior with 6 parameters. Parameters include expression at time 0 (h0), peak expression (h1), final expression (h2), time of first transition (t1), time of second transition (t2), and slope (β).

Materials and methods: Samples were collected from 64 volunteers (51 received vaccine and 13 placebo) at day 0, 1, 3, 7, 14, and 28 after vaccination. The PAXgene Blood RNA system was used to collect and store the blood samples and to purify RNA. Then RNA was sequenced with the Ion Proton technology, using the targeted Ion AmpliSeq™ Transcriptome Human Gene Expression Kit. Differential expression analysis was performed with the R package edgeR following the generalized linear models (GLM) pipeline (glmLRT function). The time course analysis was performed with *impulseDE,* following the one-course analysis workflow. The expression levels of 40 randomly sorted vaccinees (training set) were used to fit an impulse model for each gene. Results were then validated with the remaining 11 vaccinees (validation set).

Results: We found that 9127 genes (64% of total expressed genes) were DE at day 1 (early DE genes). According to *impuseDE* results, the expression level of most early DE genes abruptly returned to baseline level within day 3, while only 76 genes remained DE until day 14. A group of 24 genes showed an expression peak between day 7 and day 14 (late DE genes). For each gene, we obtained the 6 impulse model parameters. The model could fit late DE genes while for most of the other genes impulse model parameters did not yield biologically plausible values.

Conclusions: Vaccination with rVSV-ZEBOV activated many early DE genes and few late DE genes. Our results showed that the impulse model could only fit genes with similar kinetics of activation and deactivation. This is probably due to the unique slope parameter (β) which could not accurately describe gene expression transition with different activation and deactivation times. Implementation of new models with two independent transition slopes as parameters could improve accuracy of description of gene expression time series.

POSTERS

MICROORGANISM / HOST INTERACTIONS

P50)

RHEUMATOID ARTHRITIS PATIENTS HIGHLY RECOGNIZE ANTIBODIES AGAINST IL-2 IN THE IMMUNE RESPONSE PATHWAY INVOLVING IRF5 AND EBV ANTIGENS

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Introduction: Rheumatoid arthritis (RA) is an autoimmune disease of complex pathogenesis caused by risk allels acting in concert with largely unknown environmental factors among which major role is attributed to viral infections. Past contacts with Epstein-Barr virus (EBV) and human endogenous retrovirus K (HERV-K) are supposed to promote RA onset due to molecular mimicry between viral and self proteins leading to the loss of immune tolerance in predisposed hosts. Moreover, transcription of HERV-K remnants in human genome may be activated by exogenous viruses such as EBV resulting in the production of antigenic peptides. Interferon regulatory factor 5 (IRF5) is known to mediate virus-induced immune responses including expression of proinflammatory cytokines such as interleukin-2 (IL-2). IL-2 is crucial for function, expansion and survival of regulatory T cells (Treg) and recently anti-IL-2 autoantibodies have been detected in RA patients.

Materials and Methods: We evaluated humoral responses against two peptides of IL-2 protein ($IL\text{-}2_{6\text{-}20KK}$ and $IL\text{-}2_{56\text{-}70}$), $Herv\text{-}Kenv_{19\text{-}37}$ as well as $IRF5_{424\text{-}434}$ and its homologs derived from EBV inner tegument protein $BOLF1_{305\text{-}320}$ and *Mycobacterium avium* subsp. *paratuberculosis* (MAP) $MAP\_4027_{18\text{-}32}$ in sera of 140 RA Sardinian patients and 137 healthy controls (HC).

Results: The highest antibody (Ab) responses among RA patients were detected for IRF5 (56%, $p < 0.0001$) and BOLF1 (44%, $p < 0.0001$) compared to 9% in HC for both peptides. The two IL-2 epitopes elicited different reactivity established at 39% for $IL\text{-}2_{6\text{-}20KK}$ ($p < 0.0001$) and 23% for $IL\text{-}2_{56\text{-}70}$ ($p = 0.0031$) in RA subjects, while respective Ab were identified in 7% and 8% of HC. HERV-K and MAP antigens showed lower but still highly significant Ab prevalence among RA patients when compared to HC (24% vs. 9%, $p = 0.0012$ and 21% vs. 9%, $p = 0.0076$, respectively). The highest correlation coefficients were obtained for the homologous epitopes BOLF1, MAP and IRF5 in pairwise plots, while IL-2 correlated moderately with HERV-K. RA patients displayed major prevalence of strongly significant multiple seropositivity between BOLF1, IL-2 and IRF5.

Conslusions: High responses of RA patients to IRF5, EBV and IL-2, together with a good correlation of IRF5 with homologous BOLF1 and MAP epitopes point at the imbalance in the viral-induced immunity pathway and at cross-reactivity with the assessed environmental agents that may lead to the loss of self-tolerance. The contribution of HERV-K, putatively transactivated by EBV, is suggested by a moderate correlation with anti-IL-2 Abs. Further investigation is necessary to shed light on the efficacy of IL-2 therapy to establish the homeostasis and correct functioning of Treg cells in RA as demonstrated for type 1 diabetes.

P51

HHV-6A/6B INFECTION OF NK CELLS MODULATES THE EXPRESSION OF MIRNAS AND TRANSCRIPTIONAL FACTORS POTENTIALLY ASSOCIATED TO IMPAIRED NK ACTIVITY

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Objectives: Natural killer (NK) cells have a critical role in controlling virus infections, and virus presence can result in alterations of their intracellular environment finally leading to an impairment of their functions, as observed for several viruses as a part of their immune evasion mechanisms. Since we recently showed that HHV-6 *in vitro* infection is accompanied by the modulation of many miRNAs in human T cells and thyrocytes, possibly associated to alterations in immune response, the present work was aimed to study the impact of HHV-6A and -6B acute infection on the intracellular factors that might be directly correlated to NK functionality.

Methods: To this aim, we infected a human NK cell line (NK-92, ATCC CRL-2407) with cell-free HHV-6A or 6B inocula, and analyzed daily both the expression of transcription factors and miRNA for one week after infection. Virus infection was verified by analyzing virus DNA, RNA and antigen expression inside infected cells. In parallel, human transcription factors and miRNAs expression were studied by quantitative PCR microarrays and individual assays.

Results: The results confirmed that both virus species can promptly infect NK cells, where establish a productive cycle. Virus DNA was detectable at all days post infection (d.p.i.), as well as virus lytic transcripts and antigen expression.

As hypothesized, both viruses induced significant alterations in the expression of NK miRNAs, with clearly distinct early and late effects and species differences. In particular, we observed a 6-fold down modulation of miRNAs known for their role in NK development and maturation (miR-let-7, miR-181, miR-155), a further 10-fold decrease of miRNAs not yet directly associated to NK functions but with recognized roles in immune response (miR-23, miR-340), and a 12-fold increase of inhibitory miRNAs (miR-301, miR-223, miR-146) ($p < 0.001$, Student t test). Similarly, also the expression of transcription factors was significantly influenced by virus infection, with an increase of factors impairing NK functions and a simultaneous of factors promoting NK cell activation. In particular, HHV-6B mainly induced an early 8-fold increase of ATF3 and FOXO1, whereas HHV-6A induced a 15-fold decrease of POU2AF1 and a 4-fold decrease of ESR1.

Conclusions: Our data show that HHV-6A and -6B infections have a powerful effect on the intracellular signaling based on expression of miRNAs and transcription factors. This might induce NK function impairment, which could be important for HHV-6A/6B escaping and potentially affect viruses-related pathologies.

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FROM EMBRYONIC LIFE TO ADULTHOOD: THE PARALLEL MODULATION OF THE INFLAMMATORY PROFILE AND ENDOGENOUS RETROVIRUSES EXPRESSION IN THE IDIOPATHIC MOUSE MODEL OF AUTISM

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Introduction: Autism Spectrum Disorders (ASD) is a multifactorial disease for which the most etiopathogenic model proposed recognizes the interplay between environmental, epigenetic and genetic factors to determine the phenotype (Zhubi et al., 2014). Among environmental factors, viruses have been associated with ASD but, given the huge variety identified, immunological imbalance in general might be an underlying risk factor for ASD. Indeed, it has been demonstrated that during the pregnancy, maternal infection could alter the immune status of the fetal brain and the entire fetal immune system (Pardo et al., 2005), as supported by the ASD mouse model induced by the maternal immune activation (MIA) (Mazina et al, 2015). Endogenous Retroviruses (ERVs), based on their ability to be modulated under specific stimuli, could represent the link between environmental, genetic and epigenetic factors and immune system in the acquisition of ASD phenotype. In our previous study, we demonstrated in the BTBR T+ tf/J (BTBR) inbred mice, an idiopathic model of ASD, that the inflammatory profile and ERV transcriptional activity were higher in whole embryos of BTBR mice in comparison to C57 control mice, maintained also after the birth, both in blood and brain samples. Our results demonstrated the persistent activation of both immune system and ERVs, suggesting that they could have an effect on brain functions throughout the life span.

Aim of this study is to evaluate the differential expression profile of several pro-inflammatory cytokines and ERVs expression in dissected embryos and in separated from maternal tissues, in order to understand in which specific area the immune dysregulation and ERV overexpression occur.

Materials and Methods: Whole embryos from inbred BTBR T+tf/J (BTBR) mice were explanted at 10.5 gestational day and dissected and separated from maternal tissues in order to obtain: cephalic and non-cephalic embryonic tissues, maternal decidua and extra-embryonic tissues in which the expression of pro-inflammatory cytokines (IL-1β, IL-6 and TNF-α), Toll-like receptors (TLR3 and TLR4) and several ERV families (ETnI, ETnIIα, ETnIIβ, ETnIIγ, MusD and IAP), were assessed by Real-time PCR.

Results: The results demonstrated the dysregulated expression of the pro-inflammatory cytokines, TLRs and ERV genes in BTBR dissected embryos in comparison to controls, and the tissue depending differential expression; in particular the highest levels were found in cephalic tissue from embryos and maternal decidua from BTBR mice.

Conclusions: Current results support the hypothesis that changes in the inflammatory transcriptional profile could be related to ERVs expression, or at least they proceed in parallel with the acquisition of the ASD phenotype.

P53

ROLE OF GENETIC POLYMORPHISMS OF INNATE IMMUNITY GENES IN WEST NILE VIRUS INFECTION

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Introduction: West Nile virus (WNV) is a mosquito-borne, RNA virus belonging to the genus Flavivirus. Despite the majority of WNV infections in humans are asymptomatic (80%), some infected patients develop West Nile fever (WNF, 20%) and a small subset (< 1%) develops severe neuroinvasive disease (WNND). Summer outbreaks of WNV meningoencephalitis occur in Italy since 2008. No targeted treatment is available for this infection and management of infected patients is complicated by the lack of knowledge on individual susceptibility to infection and on risk factors predisposing to severe disease evolution. Considering that only a small proportion of people infected with WNV develops severe disease and that the risk factors, except for older age, are not well defined, there is a strong rationale to suspect a genetic predisposition to severe clinical forms of WNV infection. Aim of the study is to define the role of polymorphisms in key genes of the innate immunity in determining severity of WNV infection.

Materials and Methods: Blood samples collected during 2013-2016 from WNV-infected patients, and healthy controls were employed to perform real time PCR assays to test the presence of single nucleotide polymorphisms (SNPs) in key genes of the innate immune system (Toll-like receptor (TLR3) rs3775291; TLR7 rs179008; TLR8 rs3764879 e rs3764880; RIG-I rs1081383; IRF3 rs2304207; IL-28B rs12979860 and rs8099917). The frequency of the SNPs was evaluated in three study groups: n. 138 WNV-seronegative blood donors (healthy controls), n.34 WNF- and n. 42 WNND- patients. The Fisher's exact test was used to evaluate differences in the genotypic frequencies of SNPs among the study groups.

Results: For each SNP examined, the genotype frequency was compared in the different study groups in order to identify SNP association with clinical severity of WNV infection. Since TLR7 and TLR8 are located on the X chromosome, differences in genotype frequency in TLR7 and TLR8 SNP were separately assessed in males and females. There were no significant associations among different groups for most of the SNP analysed. Interestingly, a significant association was observed between TLR8 rs3764879 (-129G>C) and WNV infection outcome in women. Among female patients, the heterozygous genotype was more frequent in the WNF group than in the WNND group (84.6% versus 38.5%; $p = 0.04$)

Conclusions: We can hypothesize that the heterozygous TLR8 rs3764879 genotype may protect female patients from developing the neuroinvasive form of WNV infection. This result, even if preliminary, suggests that TLR8 variants may have a functional relevance in the setting of WNV infection for assessing individual patient's risk profiles.

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AGE-DEPENDENT DYSREGULATION OF TYPE I IFN AND RETROVIRAL RESTRICTION FACTORS IN HIV-1 INFECTED PATIENTS ON LONG-TERM ANTIRETROVIRAL THERAPY

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Background: In order to understand how aging influences the IFN mediated immunity during HIV infection, the profile of expression of type I IFNs and the retroviral restriction factors, APOBEC3G, SAMDH1 and ISG15/UBP43, were compared among two age groups (30-50 and over 60-80 years) of treated HIV-1+ patients with stable virologic suppression. Furthermore, the functional integrity of IFN-signaling pathway was evaluated.

Methods: PBMC from 150 long-term ART-treated HIV-1+ patients and from 65 gender and age matched healthy individuals were collected at the Sapienza University Hospital. Both the HIV-1+ patients and healthy subjects were divided into two groups: middle aged (age 30-50 years) and elderly (age 60-80 years). Levels of type I IFNs, ISG15, UBP43, APOBEC3G, SAMDH1-mRNAs were evaluated by Real Time RT-PCR assays. The same analysis was performed in lymphocytes CD4+ and monocytes CD14+ collected from two subgroups [middle aged (n = 12), old (n = 10)] of HIV-1 positive patients and in PBMC collected from two subgroups [middle age (n = 5), old (n = 5)] of HIV-1 positive patients after 24 hours of stimulation with IFNα or Poly:IC.

Results: In the healthy individuals, the levels of all the type I IFNs analysed were comparable between the two age groups, while the levels of all the restriction factors were increased in the old group compared to the middle aged. In the HIV-1+ patients the trend was different: a decreased expression of the IFN alpha/beta subtypes, ISG15 and UBP43 was observed in HIV-1+ elderly patients compared to the middle aged ($p < 0.01$ for both genes). In contrast, both APOBEC3G and SAMDH1 levels tend to remain unchanged. Moreover, an increased expression of IFNs, ISG15 and UBP43 was recorded in lymphocytes compared to monocytes in the old group of HIV-1+ patients ($p < 0.05$). No such differences were observed for APOBEC3G and SAMDH1. Lastly, we found that aging did not affect the ability to induce an IFN response after *in vitro* PBMC stimulation by Poly:IC and IFNα.

Conclusions: Our results indicate that the expression of type I IFNs and the host restriction factors was differentially affected by ageing, being sustained in part by an IFN age-dysfunction in monocytes, and was not associated with a loss of functional integrity of IFN signaling pathway.

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A PROSPECTIVE OBSERVATIONAL STUDY ON THE ORIGIN AND PATTERN OF HUMAN POLYOMAVIRUSES REPLICATION AFTER KIDNEY TRANSPLANTATION

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Introduction: Human Polyomaviruses (HPyVs) are ubiquitous DNA viruses establishing latent infections in the host. Immunosuppression is a recognized risk factor for HPyVs reactivation. The most extensively studied HPyV in kidney transplantation (KTx) is BK virus (BKV) but other HPyVs such as JC virus (JCV), Merkel Cell PyV (MCPyV), and Polyomavirus 9 (HPyV9) have been detected in KTx. Origin, natural history, and clinical significance of these HPyVs remain unclear.

Materials and Methods: Urine and blood samples from 31 KTx donor/recipient pairs were collected immediately before KTx (donors and recipients) and on day 1 (T1), 15 (T2), 30 (T3), 60 (T4), 90 (T5), 180 (T6) post KTx (only recipients). Samples were tested for BKV, JCV, MCPyV, Polyomavirus 7 (HPyV7), and HPyV9 genomeS by virus-specific DNA duplex TaqMan Real Time PCR. Molecular characterization of the amplified viral strains was conducted by automated sequencing.

Results: No HPyVs viremia was observed whereas HPyVs viruria was detected in 15/31 (48.4%) donors and 17/31 (54.8%) recipients. Identical HPyV strains were isolated in 9/31 (29.0%) donor/recipient pairs. Eight out of nine recipients did not show JCV viruria before the transplant (T0), but became positive immediately after (from T1). JCV strains detected in the recipients were identical to those amplified in the paired donors. MCPyV DNA was detected in 4 recipients at T2 and in 4 recipients from T0. BKV genome was detected in 3 recipients from T3. Four recipients experienced concomitant replication of JCV and MCPyV. No relationship between HPyV replication and clinical course was identified during the first six months of follow up.

Conclusions: Our data confirm that replicating JCV is frequently observed in organ donors and that JCV replication is common in the early post KTx phase. We demonstrated that JCV early post KTx infections are caused by viral strains transmitted by the donors. MCPyV and BKV post-transplant replications observed in this series were likely due to reactivation of recipient strains. Extended follow up is needed to rule out clinical impact of early JCV infection after KTx.

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RUBELLA IN PREGNANT WOMEN: EPIDEMIOLOGY AND EFFECTS OF THE DEL PIANO NAZIONALE DI ELIMINAZIONE DEL MORBILLO E DELLA ROSOLIA CONGENITA (PNEMORC) NEL 2016

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Introduction: Rubella is an acute febrile disease, charachterized by eruption with limphoadenopathy, usually with a benign development. Attention must be placed on female patients who become infected during pregnancy, especially in the first trimester. The most common adverse events in these cases are represented by severe fetal alterations and mind retardation. The fundamental element to control Rubella infection is vaccination. From 2004, Piedmont is screening IgG serum level of all women who have child and those who want to have an abortion, proposing vaccination to the susceptible patients, according to PNEMoRoC (Piano Nazionale per l'Eradicazione del Morbillo e della Rosolia Congeniti). The purpose of this work was to find the seroprevalence of the Rubella virus in fertile women led to Azienda Ospedaliero-Universitaria Città della Salute e della Scienza di Torino in 2016.

Materials and methods: Immune status of fertile women (age ranging from 15 to 49 years), led to S. Anna hospital in 2016, has been evaluated with titration of IgG and IgM vs. Rubella with chemiluminescence method LIAISON Rubella IgG and LIAISON Rubella IgM (DiaSorin).

Results: Over a total population of 6648 women evaluated for Rubella immunoglobulins titre led to S. Anna hospital, we found a prevalence of 6162 immune women (87.3%); among those who had child and those who wanted to have an abortion, we found 92.5% immune women. Of the last group, to the susceptible ones (7.5%) has been proposed vaccination, and 72.4% has been actually vaccinated.

Conclusions: From this study emerged that the prevalence of immunity of the population evaluated overlaps with the National data. Taking into account only who had child and those who wanted to have an abortion, the prevalence is higher: maybe this population, based on its characteristics of fertile age and the consequent presumable ostetric precedents, had, in part at least, already benefit from vaccination strategies planned in the eradication plan in the last fifteen years, although the sub-optimal acceptance of the vaccination proposal (196 vaccination done over 264 susceptible patients), as seen in 2016. It would be desirable to improve the percentage of women who accept vaccination among those who had child and those who wanted to have an abortion, in order to reach the cutoff value of 95%, objective of the eradication plan.

P57

CHARACTERIZATION OF THE PRIMARY AND SECONDARY T AND B RESPONSES TO A M. TUBERCULOSIS VACCINE ANTIGEN FOLLOWING DIFFERENT PRIME-BOOST INTERVALS

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Introduction: The quality and amount of memory T and B cells is set during antigen driven primary immune responses, and the time for boosting can deeply influence the secondary immune response. In preclinical experimentation of model vaccines, short intervals between priming and boosting are generally used to obtain a rapid rise in antibody titers but the optimal timing for boosting should be carefully determined in order to avoid suboptimal responses. In order to optimize prime-boost regimens, we have characterized the kinetic of primary T and B cell responses up to 4 months after a single immunization, and analyzed the effect of the interval between the first dose and the booster injection on the B and T cell memory responses.

Materials and Methods: To this aim C57BL/6 mice were primed with the tuberculosis vaccine antigen alone or combined with different adjuvants and boosted with the vaccine antigen, one or four months after primary immunization. The antigen-specific antibody response and their antigen affinity have been monitored every 2 weeks for all the duration of the experiments. The frequency of plasmacells and germinal centre B cells, together with antigen-specific CD4+ T cell response, have been measured both in draining lymph nodes and spleen at different time points following priming or boosting, while the induction of antibody-secreting cells has been analyzed ten days after boosting into the bone marrow.

Results: Our data show that the primary activation of B cells and antibody secretion are still increasing a month after priming, suggesting that this time point is not optimal for booster immunization. Indeed the fold-increase of H56-specifc IgG titres observed in mice boosted 4 months after priming was significantly higher compared to mice boosted a month after. The duration of the germinal center reaction varied depending on the nature of the vaccine formulation, and in the absence of adjuvant it was not promoted. Also H56-specific CD4+ T cells were much more responsive to booster immunization when it was performed four months after priming.

Conclusion: These data suggest that an interval of several weeks between the prime and the boost is necessary to obtain stronger secondary B and T cell responses, providing an important contribution to the rational development of prime-boost vaccine immunization protocols.

P58

INDUCTION OF DIFFERENT APOPTOSIS PATHWAYS BY TWO PROTEUS MIRABILIS CLINICAL ISOLATES STRAINS IN PROSTATIC EPITHELIAL CELLS

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Introduction: With age, men acquire structural and functional abnormalities of the urinary tract that impair normal functioning among which the most common is benign prostatic hyperplasia. Bacterial prostatitis is believed to be the leading cause of recurrent urinary tract infections (UTIs) in men under 50 years of age and it occurs both as an acute febrile disease responsive to antibiotic treatment and as a chronic infection often unresponsive to antibiotic treatment and is believed to involve bacterial biofilm formation. *Proteus mirabilis* is more commonly associated with UTIs in these abnormalities, especially in catheterized patients. This pathogen is capable of colonizing and causing disease due to his virulence factors including fimbriae, flagellar motility, immune avoidance, host damaging factors and the ability to form crystalline biofilms. In addition, *Proteus* lipid A may have apoptotic activity and induce desquamation of epithelial cells. It's known there are two main apoptotic pathways: the extrinsic signaling or death receptor pathway that involve transmembrane receptor-mediated interactions, and the intrinsic signaling pathway that involve non-receptor-mediated stimuli that cause changes in the inner mitochondrial membrane resulting in release of pro-apoptotic proteins. In our previous work we have shown that two clinically isolated strains of *Proteus mirabilis* phenotypically different, named PM1 of PM2 respectively, are able to induce a different type of immune response in bladder epithelial cells. In particular, PM1 produces a chronic infection by forming a larger amount of biofilm, while PM2 produces an acute infection with a strong inflammatory response. The aim of this work was to evaluate the ability of PM1 and PM2 to induce apoptosis in human prostatic adenocarcinoma PC-3 cells.

Materials and Methods: *Bacterial strains*: PM1 and PM2 were cultured in LB broth at 37 °C. *Cell culture and infection*: PC-3 semiconfluent monolayers were infected with exponentially-growing PM1 and PM2 for 6 and 18 hours at 37°C in 5% CO_2. *Real-time* PCR: the expression levels of apoptosis factors (BAX, Bcl-2,TNFR1, FasL, FasR, caspase-8 and caspase-3) were evaluated using LightCycler software. *MTT assay*: after 18 hours of infection, spectrophotometric absorbance was measured a 570 nm.

Results: PM1 upregulates the expression of the genes involved in the intrinsic pathway of apoptosis and induces 50% of cell death after 18 hours of infection. PM2 upregulates the expression of genes involved in the extrinsic pathway of apoptosis and induces 80% of cell death.

Conclusions: The identification and understanding of relations between the microorganism and host may provide the basis for new solutions to these clinical problems as regards diagnosis and therapy.

P59

MICROBUBBLES AND EPIGENETIC MODULATORS FOR PERSONALIZED LIVER CANCER MEDICINE

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Introduction: Microbubbles sonoporation is a leading technology that allows physician to increase the resolution of liver anatomical parenchyma. Nowadays is emerging the role of those microbubble as delivery system for liver genetic therapy. In our vision, we hypothesized the possibility to achieve a liver cancer treatment with the combination of genetic and epigenetic approach trough microbubble sonoporation. Liver cancer can be the result of viral infections, metabolic disorder, genetic variation/mutation and epigenetic deregulation. Among those phenomena evidence of oncosuppressor gene silencing like p53 and TRAIL represent the major class of liver cancer patients. Aim of the project is the re-activation of silenced apoptotic pathway in liver cancer models, plasmidic gene delivery and epigenetic treatment.

Material and Methods: HepG2 cell line from ATCC was selected for all the experiments. Lipofectamine 2000 (Invitrogen) was used for pEGFP-TRAIL and pEGFP-p53 (Addgene plasmids) and the respective control were selected and propagated in LB broth in order to obtain the necessary amount. Plasmid were purified with Invitrogen PureLink (Thermo) kit. GFP (Green Fluorescent Protein) was acquired via FACS excalibur DB analysis. MS.275 (HDACi calss I) was acquired from selleckchem. Cell cycle software used: Cell-Quest and ModIFit

Results: HepG2 cells transfected with TRAIL-GFP and pEGFP-p53 recombinant protein were used for the cell cycle analysis. Data shown the re-expression of selected recombinant proteins in over than 30% cells post 24h from transfection. The transfected cells were treated post 24h with MS-275 for other 8h and the cells were collected. The total protein extract was analysed by western blot and the apoptosis pathways were evaluated via caspase activation proteins. In details we detect the relative bands for capase 8 and caspase 9 full lengths and activated form in transfected cells and post MS-275 treatment.

Conclusion: Results showed the possibility o restore the expression of pro-apoptotic gene TRAIL and p53 in a liver cancer model HepG2. Moreover, the treatment with epigenetic modulators MS-275 enhanced the pro-apoptotic effect mediated by the re-expression of those silenced genes.

P60

ACTIVITY OF TRANSGLUTAMINASE TYPE-2 INHIBITORS AGAINST *MYCOBACTERIA*

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Introduction: Host-directed therapies are emerging as a promising area of research and are opening new avenues to fight tuberculosis (TB). Previously, we demonstrate that in the absence of transglutaminase type 2 (TG2), virulent *Mycobacterium tubercolosis* (Mtb) shows a reduced capacity to survive intracellularly in murine macrophages both in vitro and in vivo. This phenomenon is characterized by the reduced capacity of Mtb to hide inside LC3-II positive vesicles and is paralleled by a significant increase in cells viability. Interestingly, the same inhibitory effect can be pharmacologically reproduced in vitro by using a highly specific inhibitor of TG2's transamidating activity. In the present study, we show that pharmacological inhibition of (TG2) with a TG2 inhibitor already tested in preclinical studies, leads to significative reduction of the replicative capacity both in human monocytic cell line (THP-1) and human derived macrophages (hMDM) of Mtb and *Mycobacterium abscessus* (Mabs), a pathogenic, rapidly growing mycobacterium responsible for pulmonary and cutaneous infections in immunocompetent patients and in patients with Mendelian disorders, such as cystic fibrosis (CF).

Materials and Methods: To further investigate the potential usefulness of TG2 as a target for anti-mycobacteria regimens, the human monocytic cell line (THP-1) and human monocyte-derived macrophages (hMDM) extracted from different donors were infected with Mtb and Mabs and four hours later infected macrophages were incubated with several drugs and let it work for 2 days until harvesting for CFU counting.

Results: A statistically significant reduction in intracellular CFUs were observed for the TG2 inhibitors, which warranted an anti-mycobacterial activity similar to that achieved by rapamycin and most importantly by the second line anti-TB drug capreomycin. Combined use of inhibitors did not warrant a synergistic effect, as expected for drugs sharing the same target. These results provide further evidences supporting TG2 as a potential target for host-directed therapies against virulent mycobacteria.

Conclusions: These data open a new avenue in the possible treatment of TB and Mabs infection since many specific inhibitors of TG2 are in clinical trials and they can also be applied for the treatment of virulent mycobacteria infections alone or in combination with the available therapies.

P61

ESCHERICHIA COLI COLONIZES COLORECTAL ADENOMATOUS POLYPS: INSIGHTS INTO GENOTYPIC AND PHENOTYPIC FEATURES

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Introduction: The composition of the gut microbiota has been implicated as a potential etiologic factor of colorectal cancer (CRC). *Escherichia coli* is the most well adapted bacterium within the human gut. A role for *E. coli* in CRC development is emerging especially for *E. coli* that possess a high carriage of virulence factors. *E. coli can be an opportunistic pathogen causing several diseases when it extensively colonizes the intestine*. According to the acquisition of different virulence traits *E. coli* can be grouped into seven different phylogenetic groups (A, B1, B2, C, D, E, and F). B2 *E. coli* strains have highest capacity to colonize the human gut, due to the expression of many virulence factors. Adenomatous polyp is a major risk factor for the development of sporadic which was found to be associated with specific subtype of B2 *E. coli*. Therefore, the aim of this study was to characterize the *E. coli*-associated with adenomatous polyps. The phylogenetic background and the presence of major virulence factors, such as biofilm formation, motility and the presence of common cytotoxins, were evaluated.

Materials and Methods: A total of 600 *E. coli* isolates were obtained from specimens of 20 patients presenting adenomatous polyp and 10 subjects presenting normal mucosa. Ethical approval was granted by Hospital "Umberto I" of Rome Research Ethics Committee. Biopsies were washed with 500 µL of cold phosphate buffered saline pH 7.4, lysed with Triton-X 100 0.1% and plated on *E. coli* chromogenic agar chrom ID CPS3. Ten *E. coli* colonies per biopsy sample were analyzed. Phylogrouping was assessed. Soft agar, Congo red agar, ESBL and blood agar plates were used to assay bacterial motility, biofilm, antibiotic susceptibility and cytotoxins.

Results: The isolates recovered from biopsy samples of normal mucosa and polypoid lesions were mainly grouped into phylogroup A, that includes commensal *E. coli*. The second most represented phylogroup was B2, which was mainly recovered from the polypoid lesions compared to the mucosa from healthy subjects. The phenotypic characterization showed that the majority of isolates were able to swarm, possibly facilitating the spread and the establishment within the host. The high percentage of beta-lactamase susceptible isolates indicates that gut resident *E. coli* do not possess acquired resistance mechanisms. Finally, a high percentage of biofilm forming isolates indicates a possible connection between biofilm formation and the persistence of these isolates as resident flora within the host.

Conclusion: This study extend the literature data regarding the genotypic and phenotypic characterization of *E. coli*-associated with adenomatous polyps.

P62

STUDY ON THE INVOLVEMENT OF HUMAN ENDOGENOUS RETROVIRUS IN THE STEMNESS FEATURES OF DIFFERENT TYPE OF TUMOURS

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Introduction: The involvement of retroelements, such as human endogenous retroviruses (HERVs), is recognized to play a role in the carcinogenesis process. Indeed, HERVs deregulation contributes to the onset, progression and acquisition of aggressiveness features in tumour cells. Numerous experimental evidence have also shown that tumour aggressiveness is associated with the presence of a subpopulation of cells with distinctive plasticity features, known as Cancer Stem Cells (CSCs). These cells have been characterized in different types of tumours, and present different surface markers, among which the best known are CD44, CD90 and CD133. The CD133 marker was also associated with metastasis formation and resistance to pharmacological treatments. Recently, we demonstrated that in human melanoma cells the change in the microenvironment results in an increased expression of HERV-K and the generation of a CD133+ subpopulation with typical features of CSCs, high aggressiveness and dependence on HERV- K the preservation under stress condition. Therefore, the objective of the study was to analyse HERV-K and HERV-H expression in different type of tumour cells, such as from melanoma, hepatocellular carcinoma and colon cancer, subjected to microenvironment modifications and evaluate their correlation with the CD133 marker and the maintenance of the CSC subpopulations.

Materials and Methods: TVM-A12, Hep-G2 and Caco-2 cell lines were cultivated in standard or stem cell media. Phenotypic modifications were assessed by flow cytometry. The expression of HERV-K, HERV-H and CD133 were evaluated by RT-Real time PCR.

Results: Depending on changes in culture conditions, all the cell lines showed modifications in cellular morphology, with generation of non-adherent cell aggregates similar to spheres, when cultivated in stem cell medium. Flow cytometry analysis demonstrated the presence of a CD133+ subpopulation in all the cell lines assessed. Moreover, microenvironment modifications induce an increase of HERV-K and HERV-H expression, associated with an increased expression of the CD133 marker.

Conclusions: Our findings support the hypothesis that HERVs activation may represent a mechanism common to different types of tumour, needed to respond to stress due to changes in the tumor microenvironment, and, since it correlates with the acquisition of stemness and aggressiveness features, suggests HERVs as noteworthy therapeutic targets.

P63

EFFECT OF 1,064 NM Q-SWITCHED ND:YAG LASER ON INVASIVENESS AND INNATE IMMUNE RESPONSE IN KERATINOCYTES INFECTED WITH *CANDIDA ALBICANS*

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Candida albicans is an opportunistic pathogen commensal in the oral cavity, gastrointestinal tract, vagina, and skin of healthy individuals. Common therapeutic options for fungal infections are topical or systemic antifungal drugs, with long and expensive treatment times. Recently in cutaneous pathologies lasers and light-based treatments have emerged showing few contraindications and minimal side effects.

The Q-switched (Nd-YAG) laser at a wavelength of 1064 nm has been shown to be useful in dermatology, dentistry, and some other medical specialties. It is used to treat onychomycoses and warts, wounds and in some other treatments.

In our study we analyzed the effect of Q-switched (Nd-YAG) laser 1064 nm on human keratinocytes infected with *C. albicans*. In particular we evaluated the effect of laser on invasiveness of *C. albicans* and on inflammatory and protective response of HaCaT cells infected.

The results obtained did not show any inhibitory, fungicidal or fungistatic effects of laser on C. albicans, in addition laser did not affect HaCaT vitality.

HaCaT cells infected with *Candida albicans* and irradiated with Q-switched (Nd-YAG) laser 1064 nm showed a reduction of invasiveness of TNF-α and IL8 gene expression and an increasing of immunomodulatory cytokines such as TGFβ. Furthermore Q-switched Nd:YAG laser induces a significant over-expression of HSP70B(heat shock protein) and of HBD-2 (Human β defensin-2) in HaCaT infected with *C. albicans*, compared to the untreated control.

In conclusion the use of Q-switched Nd:YAG laser in skin mycosis caused by *C. albicans* reduces yeast invasiveness in keratinocytes, downregulates inflammatory activities and facilitates cytoprotection, tissue healing and antimicrobial defence. Our results offer a promising therapeutic strategy in the management of skin candidiasis, also in combination with conventional therapies, with aim to reduce treatment times, pharmacological doses, antifungal resistence and side effects. This constitutes the first study to report on the relationship between Q-switched Nd:YAG laser irradiation and modulation of *Candida albicans* infectiveness on human keratinocytes.

P64
THYMUS VULGARIS L. (THYME) EXTRACT AND PHYTOCOMPOUND THYMOL ASSIST MURINE MACROPHAGES (RAW 264.7) IN THE CONTROL OF *IN VITRO* INFECTION BY *STAPHYLOCOCCUS AUREUS*

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Introduction: During an infection, microorganisms can inactivate some functions of the host's immune system and provide their penetration and proliferation in the invaded tissue. Including, synthesis of cytotoxic proteins, alteration of surface molecules, proteins to modify the acidity of phagosomes and proteins to block chemotaxis. In this study, the ability of plant products, such as thyme extract and phytocompound thymol, to assist murine macrophages (RAW 264.7) to control *in vitro* infection by *S. aureus* was verified.
Material and Methods: Firstly, the minimum inhibitory concentration (MIC) of the plant products for *S. aureus* (ATCC 6538) was determined by broth microdilution method. Macrophages were cultured (37°C and 5% CO_2) in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin (PS) for 24 h, in 24-well plates (10^5 cells/well). The infection was induced in the ratio of 1:5 (macrophage:yeast) for 30 min (37°C and 5% CO_2). For this, the MIC of the plant products was separately added in DMEM containing microbial suspension (10^7 CFU/mL - colony-forming units per milliliter), however free of antimicrobial and FBS. This solution was added on the macrophages. Pure DMEM and PS were used as controls (n = 6/group). The macrophages were lysed, the suspension was seeded on Brain Heart Infusion (BHI) agar and after 24 h incubation the CFU/mL was determined. The viability of infected macrophages was analyzed in a 96-well plate by neutral red (NR) assay to verify the lysosomal activity of the macrophages.
Results: There was a significant reduction in CFU/mL in the treated groups with thyme (43 ± 9%), thymol (100 ± 0.1%) and PS (78 ± 3%), in relation to the control group. The macrophages were viable in all experimental groups, however, it was verified that they were more active during the infection and in the treatments with the vegetal products.
Conclusion: According to the results, thyme and thymol effectively aided the macrophages to control infection by S. aureus, reducing the microbial concentration after phagocytosis. Additionally, the lysosomal activity of the macrophages was more active during infection and treatments with the plant products.

P65

COMPARATIVE EFFECT OF TEA TREE OIL AND ANIDULAFUNGIN ON HUMAN POLYMORPHONUCLEAR LEUKOCYTES FUNCTIONS AGAINST *CANDIDA KRUSEI*

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Introduction: During the last three decades the number of fungal infections caused by yeasts of *Candida* spp. has increased dramatically, mainly due to the rise in the number of immunocompromised patients. Moreover,the increasing number of clinical isolates resistant to current antifungal therapies highlight the need for search for new therapeutic alternatives. Many studies have been conducted on the antifungal activity of natural products (include essential oils) against *Candida* spp. involved in fungal infections. Tea tree oil (TTO) is an essential oil that is obtained by steam distillation of the leaves and terminal branches of *Melaleuca alternifolia (Myrtaceae)*. TTO is used as a local agent for treating various diseases, predominantly dermatoses (e.g., recurrent herpes labialis, acne,pustules,dandruff, and rash). It has a broad spectrum of antimicrobial activity against a wide range of bacteria, viruses, and fungi, as well as microorganisms that are resistant to conventional drugs. However, clinical experience showed that the efficacy of antimicrobial drugs depends both on their direct effect on microorganisms and the activity of the host immune system. Hence, the purpose of this study was to evaluate the influence of TTO, at subinhibitory concentrations,on intracellular killing by human polymorphonuclear leukocytes (PMNs) against *Candida krusei*, in comparison with anidulafungin (AND),one of the common antifungal drugs used in candidiasis management.

Material and Methods: A clinical *C. krusei* strain was used.The EO was purchased from Primavera/Flora,Pisa,Italy, and analysed by GC (Drug Science and Technology Dept).AND (Pfizer) was provided by Prof. Milici (University of Palermo). Susceptibility testing was based on the CLSI M27-A3 method, with some modifications. Intracellular killing was investigated by incubating yeasts (10^6cfu/mL) and PMNs (10^6cells/mL) at 37°C for 30, 60,90 min in presence of TTO subinhibitory concentrations and 1/2MIC of AND. Killing values were expressed as Survival Index. EO/AND-free controls were included. Statistical evaluation of the differences between test and control results was performed by Tukey's test. The cytotoxicity of various concentrations of TTO was evaluated with MTT test assay.

Results: Results showed that TTO at higher concentrations(i.e.1/4MIC)was toxic, as it could interfere with the PMN functionality. On the contrary, TTO at 1/8 MIC significantly enhanced intracellular killing of *C. krusei* by PMNs, with killing values higher in comparison with those observed in free-EO systems and in presence of AND, indicating that the decreasing concentrations did not cause lower candidacidal activity.

Conclusions: These data show a promising potential application of TTO against *C. krusei*, often resistant to conventional drugs.

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HYDROALCOHOLIC EXTRACT FROM ORIGANUM VULGARE PROMOTES *IN VITRO* INNATE ANTIMYCOBACTERIAL ACTIVITY

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Introduction: Plants are an invaluable source of molecules with possible therapeutic value against infectious diseases and still today represent the main health care source for most of human population. In this study, we analysed the ability of hydroalcoholic extract gained by *Origanum vulgare* (Ov), a plant known for its anti-inflammatory properties, to improve antimycobacterial response in *in vitro* models of infection with Bacillus Calmette-Guerin (BCG).

Materials and Methods: Hydroalcoholic extract of Ov (HyE-Ov) was tested on BCG infected dendritic cells (DC), type-1 macrophages (M1) and type-2 macrophages (M2), differentiated from peripheral monocytes, derived by healthy donors, by *in vitro* stimulation with GM-CSF plus IL-4, GM-CSF and M-CSF, respectively. The effect of HyE-Ov on anti-mycobacterial response was evaluated in the three cell types in terms of intracellular mycobacterial viability, by a BCG-lux based luminescence assay, phagolysosome maturation and ROS generation, both assessed by fluorometry.

Results: Our results indicate that HyE-Ov did not exert any toxic effect on DC, M1 and M2 at the doses used in the experiments and did not directly affect BCG viability. The stimulation of BCG-infected DC, M1 and M2 with HyE-Ov significantly reduced intracellular mycobacterial viability by a ROS dependent mechanism, as the addition of catalase completely restored intracellular BCG growth in the three cell types. Moreover, the reduction of intracellular mycobacterial viability observed following HyE-Ov stimulation was associated with enhanced phagosome acidification in M1 and M2, only, suggesting a possible involvement of mitochondrial ROS in HyE-Ov induced anti-mycobacterial response of DC. Finally, in agreement with its anti-inflammatory properties, the stimulation of DC with HyE-Ov significantly reduced lipopolysaccharide induced Tumor Necrosis Factor-a production.

Conclusions: These results show that the hydroalcoholic extract of *O. vulgare* promotes antimycobacterial innate immunity and suggest that this plant may contain potential immunomodulatory molecules usable for novel host-directed therapeutic approaches against Tuberculosis. HPLC fractionation is currently in progress to identify the molecule/s responsible for the observed biological effects.

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IN VITRO DALBAVANCIN ACTIVITY AGAINST CLINICAL ISOLATES OF STAPHYLOCOCCUS SPP. AND SUBSEQUENT INTERACTION WITH HUMAN PHAGOCYTES

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Introduction: Dalbavancin is a semisynthetic long-acting lipoglycopeptide antibiotic recently approved in the United States by the Federal Drug Administration (FDA) for treatment of adult patients with acute bacterial skin and skin structure infections caused by susceptible Gram-positive bacteria. Among the Gram-positive bacteria, dalbavancin has a broad spectrum of action against most microorganisms, including those resistant to other antimicrobial families. The aims of this study were to evaluate the activity of dalbavancin against *Staphylococcus aureus* and *Staphylococcus epidermidis* clinical strains and to determine the straightforward performance of dalbavancin upon the binomial antibiotic resistant/susceptible bacterium and host defenses, to establish its potential immunomodulating activity.

Materials and Methods: Twenty-five isolates of methicillin-susceptible *S. aureus* (MSSA), 25 isolates of methicillin-resistant *S. aureus* (MRSA), 25 isolates of methicillin-resistant and teicoplanin susceptible *S. epidermidis* (MRSE-Teico S), 25 isolates of methicillin-resistant and teicoplanin resistant *S. epidermidis* (MRSE-Teico R) were collected from various clinical sources and studied. Minimum inhibitory concentrations (MICs) of dalbavancin were determined by broth microdilution according to Clinical and Laboratory Standards Institute guidelines. Additionally the human polymorphonuclear cells (PMNs) functional activity [(microbicidal activity and cytokine release profile (TNF-α, IL-1β, IL-10)] was investigated by incubating MRSA and phagocytes for 30, 60, 90 and 120 minutes with dalbavancin at MIC level.

Results: Dalbavancin demonstrated *in vitro* activity against all isolates tested with MICs ≤ 0.12 μg/mL, the FDA susceptible breakpoint for *S. aureus*. The normal MIC distribution among *S. aureus* (both MSSA and MRSA) and *S. epidermidis* (both MRSE-Teico S and MRSE-Teico R) isolates was between 0.03-0.12 μg/mL, with a clear modal MIC of 0.06 μg/mL for MSSA, MRSA and MRSE-Teico S, and 0.12 μg/mL for MRSE-Teico R. Moreover, the data on PMN activity showed that dalbavancin at MIC concentration had effect on both MRSA intracellular killing and cytokine release, in comparison with dalbavancin-free controls, for the entire period of observation.

Conclusions: The need for new agents with potent activity against staphylococci is acute due to increased resistance to most common currently available antibiotics. Our results confirm and extent previous literature data on the antistaphylococcal activity of dalbavancin: it has activity not only in fighting staphylococcal resistant strains, but also in influencing PMN microbicidal activity. These data support the continued clinical study of dalbavancin in infections where staphylococci are prevalent.

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INFLAMMATORY RESPONSE DURING HUMAN VAGINAL INFECTION WITH *CANDIDA ALBICANS*

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Introduction: The microbiological, pathological and clinical factors determining vaginal candidiasis and recurrent vaginal candidiasis have long been studied, particularly using rodent models. The validity of which for understanding the pathogenesis of disease in women has been questioned. The most prevalent agent is critically determined by activation of microbial and host factors leading to persistent vaginal inflammation coupled to the inability of the inflammatory cells to resolve the fungal infection. Here we studied the activation of inflammasome complex neutrophil-recruiting and activating cytokines in the vaginal secretion of women with clinically established vaginal candidiasis.
Materials and Methods: In human vaginal samples positive for *C. albicans* with vaginal candidiasis (n = 20) and carriage (n = 15), infiltration of neutrophils ,inflammatory mediators such as IL-8 and IL-1β, activation of inflammasome complex and expression of aspartyl proteases (SAPs) were examined.
Results: In vaginal swabs of patients with vaginal candidiasis we found: i) consistent recruitment of neutrophils; ii) appreciable level of IL-8 and IL-1β; iii) activation of inflammasome complex; iv) consistent expression of *SAP2, SAP5* and *SAP6.*
Conclusions: These results show that immunopathogenesis of vaginal candidiasis is mediated by local recruitment of neutrophils, inflammatory cytokines secretion and inflammasome activation that mirror the upregulation of *SAP2, SAP5* and *SAP6* gene expression.

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BETA-DEFENSIN-2 AND -3 REDUCE INTESTINAL DAMAGE CAUSED BY *SALMONELLA TYPHIMURIUM* INFECTION

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Introduction: The human intestine hosts a large and diverse microbial community estimated to contain approximatively 400-1000 different species of bacteria, virus and fungi. These microbes are collectively referred to as the microbiota. Among the main functions of the intestinal microbiota are the synthesis of essential amino acids and vitamins (K, B2, B1, folic acid, biotin, and pantothenic acid) and extraction of energy from components in the diet as some are not digestible polysaccharides of plant origin. Moreover, it is involved in the absorption of calcium, magnesium and iron, and contributes to maintaining the integrity of the intestinal wall, modulating responses to pathogenic noxae, and representing a key factor in the maturation of the immune system. In fact, it plays an active role in the intestinal immune response through the secretion of inflammatory cytokines, chemokines, and antimicrobial peptides such as b-defensins. Between these, human beta-defensin-2 and -3 (hBD-2 and hBD-3) are expressed at mucosal sites and exhibit broad antimicrobial activity against Gram-positive and Gram-negative bacteria, mycobacteria, fungi, and viruses. In addition to direct antimicrobial properties, hBD-2 and hBD-3 recruit innate and adaptive effector cells to sites of inflammation, induce cytokines and mast cell degranulation, and aid in wound healing. The aim of this study it was to create a line of intestinal epithelial cells expressing high concentrations of the antimicrobial peptides hBD-2 and hBD-3, and to assess their role in the host inflammatory response resulting from bacterial infections.

Materials and Methods: *Cloning and transfection*: genes coding for antimicrobial peptides HBD-2 and HBD-3 were cloned into the pEF/V5-HIS TOPO vector then transfected into Caco-2 cells previously subcultured for 21 days to obtain their full differentiation. *Infection and Real-Time PCR:* the transfected cells were subsequently infected with *S. typhimurium* and the modulation of expression

levels of proinflammatory cytokines IL-6, IL-8, TNF-α, IL-1α and IL-1β and anti-inflammatory cytokine TGF-b compared to untransfected cells was evaluated by Real-Time PCR.

Results: The results obtained show that the inflammatory response in hBD-2 and hBD-3-transfected cells is modified with respect to untransfected cells, since the expression of proinflammatory cytokines is greatly reduced, while the expression of anti-inflammatory cytokine is increased.

Conclusions: These data indicate that the invasive and inflammatory potential of *S. typhimurium* is significantly reduced in the presence of antimicrobial peptides. This system will allow us in the future to better clarify the mechanisms underlying their role in defense of host against intestinal pathogenic bacteria.

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THE DNA SENSOR-ALARMIN IFI16 PROMOTES INFLAMMATION UPON LPS BINDING

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Introduction: Lipopolysaccharide (LPS) is the main cause of gram-negative bacterial sepsis. LPS consists of a lipid A component, a sugar moiety that forms the core, and an O-polysaccharide of variable length. When LPS is introduced into the bloodstream, LPS binding protein (LBP) recognizes the LPS molecules and transfers LPS to CD14, which in turn transfers LPS to the TLR4-MD2 receptor. However, other proteins than LBP have been revealed for their ability to bind to and amplify the LPS-mediated pro-inflammatory process. The IFI16 protein is a nuclear phosphoprotein that is physiologically limited to vascular endothelial cells, keratinocytes, and hematopoietic cells. IFI16 acts as viral restriction factor and as DNA sensor in inflammasome signaling. Crystallographic analysis demonstrated that IFI16 contains two oligosaccharide/nucleotide-binding (OB) folds with a unique DNA-binding surface. Moreover, we have previously demonstrated that: i) the occurrence of IFI16 in sera of systemic-autoimmune patients hampers biological activity of endothelia through high-affinity membrane binding; ii) the extracellular IFI16 protein alone or by synergy with lipopolysaccharide (LPS) acts like damage-associated molecular patterns propagating "danger signals" through MyD88-dependent TLR-pathway, amplifying the secretion of IL-6 and IL-8. In this work, we have characterized the nature of the interaction between IFI16 and LPS by using different experimental approaches.

Materials and Methods: In order to test the direct interaction between IFI16 and LPS, we have performed: i) ELISA assays, using the recombinant IFI16 protein, LPS from different sources, or different genera of gram negative bacteria as solid phase antigens; ii) pull down assays with biotinylated LPS. To study the effects of extracellular IFI16 and/or LPS, we have treated different cell lines with the two molecules, alone or

in combination, at different concentration and time. The induction of inflammatory cytokines has been evaluated with ELISA assays.

Results: Our results show that IFI16 binds to LPS in a concentration-dependent manner, that the binding is stronger to lipid A moiety than to the polysaccharide moiety of LPS, and that the IFI16 OB folds could not be involved in LPS binding. Finally, the effect of the IFI16/LPS interaction is strongly dependent on the cellular target.

Conclusions: Our data suggest that IFI16, a well known intracellular receptor for viral DNAs, following extracellular exposure may also be involved in the recognition and binding of other microbial structures, such as gram-negative LPS. Moreover, IFI16 is able to differentially tune the inflammatory property of LPS depending on the cellular model considered.

POSTERS

BACTERIOLOGY / MYCOLOGY / PARASITOLOGY / VETERINARY

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ANTIMICROBIAL ACTIVITY OF THE ESSENTIAL OIL FROM SATUREJA MONTANA, *L. LAMIACEAE*

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Introduction: Many essential oils (EOs) possess antibacterial properties and have been screened as potential sources of novel antimicrobial compounds. An important characteristic of EOs and their components is their hydrophobicity, which enables them to partition with the lipids of the bacterial cell membrane, interfering with the bacterial cell permeability. EOs of the genus *Satureja* are known to have several biological properties, such as antimicrobial, fungicidal and anti-HIV-1. This study aims to analyze the antibacterial properties of essential oil of commercial *Satureja montana* (SGG) and to evaluate its activity in addition to antibiotics towards *Staphylococcus aureus* strains. Further studies to assess its antibiofilm and anti-adhesive potentials against Gram positive were performed.

Materials and Methods: The SGG (Talia, Rome) compositional analysis was evaluated by gas chromatography and mass spectrometry (GC-MS). Identification and susceptibility tests of *Staphylococcus aureus* ATCC 25923, ATCC 6538P and one clinical isolate *S. aureus* (PGSA) were performed by automated VITEK-2 System. The combined antimicrobial activity of EO and gentamicin was reported as the fractional inhibitory concentration index (FICI). Biofilm formation was evaluated in microtiter plate by crystal violet staining.

Results: In the *Satureja montana* essential oil, the prevalent components are carvacrol (51%) cymene (13%), tymol (11%) and terpinene (5.5%). Nevertheless, it has been considered that the presence of minor components could be important to determine the biological effect. The results obtained show a good antibacterial activity of SGG with MBC of 0.78 mg/ml towards all tested bacterial strains. FICI value show a synergic effect for gentamicin with SGG, for reference strains at the lower tested concentrations con FIC Index values ≤ 0.5 at SGG 1/4,1/8, 1/16 MIC values, while for clinical isolate PGSA, FIC Index highlighted an additive effect. SGG oil influences bacterial adhesion to abiotic surfaces.

Conclusions: The results of the present study support data that the essential oil of SGG was effective against Gram positive bacteria such as *S. aureus* strains. The main activity was bactericidal. Furthermore, SGG was able to inhibit the biofilm formation of *S. aureus* ATCC strains *"in vitro"* assays.

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IN VITRO ANTI-*HELICOBACTER PYLORI* ACTIVITY OF A NEW ARTEMISININ DERIVATIVE

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Introduction: *Helicobacter pylori* (*H. pylori*) is a microaerophilic spiral bacterium that is associated with the pathogenesis of active and chronic gastritis, peptic ulcer, and gastric carcinoma. *H. pylori* eradication therapy is compromised by the increasing drug resistance. This is particularly alarming since *in vivo* studies demonstrate that antibiotic therapy targeted at eliminating the microbe, may also contribute to the prevention of stomach cancer in humans. The aim of this study was to evaluate the *in vitro* activity of a new artemisinin derivative, GC012 alone and in combination with two antibiotics commonly used in anti-*H. pylori* therapy. Moreover, since the antibiotics degradation can occur in an acidic environment, the stability of the tested compound was investigated at different pH values.

Materials and Methods: Twenty-four clinical strains of *H. pylori* with different antibiotic susceptibility pattern, were used in this study. A reference strain of *H. pylori* ATCC 43504 was used as control. The Minimal Inhibitory Concentrations (MICs) and Minimal Bactericidal Concentrations (MBCs) were performed by modified broth dilution method; the same method was used for MICs and MBCs determination at different pH values (2.5; 5.5 and 7.5).

The bactericidal activity of GC012 was evaluated by time-to-kill assay. *H. pylori* ATCC 43504 and a clinical isolate resistant to clarithromycin (CAM), were used to test the efficacy of GC012 at 0.5 (0.5X), 1 (1X) and 2 times (2X) the MIC value.

The combination effect of standard drugs and GC012 was examined by using the checkerboard method against *H. pylori* ATCC 45304 and five clinical isolates having a different antimicrobial susceptibility pattern against CAM and metronidazole (MNZ). Of these, *H. pylori* ATCC 45304 was MNZ resistant, one was MNZ and CAM resistant, one was CLR resistant, and the other two were CLR, AMX and MNZ susceptible.

Results: *H. pylori* activity was assessed in term of bacteriostatic and bactericidal effect. GC012 showed good *in vitro* antimicrobial activity, with MIC_{50} of 0.06 mg/L, MIC_{90} of 0.125 mg/L, and MBC_{50} of 0.125 mg/L. Interestingly, the pH variation did not alter the activity of GC012 in all the strains tested. Killing kinetic data demonstrated that GC012 at 1X MIC (0,125 µg/ml for both strains) had bactericidal effect on both the reference and clinical strains, leading to a total killing after 48 h.

Using the fractional inhibitory concentration index, GC012 showed synergism with amoxicillin and metronidazole in 80% of the strains, and with clarithromycin in 60%.

Conclusion: GC012 shows excellent antibacterial activity against *H. pylori*, no alteration of MIC value was detected under acid conditions. So, GC012 may be useful for future development in the treatment of *H. pylori* infection.

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ENHANCEMENT OF ANTIMICROBIAL ACTIVITY IN DRUG ASSOCIATION EXPERIMENTS WITH EFFLUX PUMP INHIBITORS

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Introduction: The rapid spread of bacteria expressing multidrug resistance (MDR) has necessitated the discovery of new antibacterials and resistance-modifying agents. Various mechanisms provide bacteria with resistance to antibiotics; these include target-site modification and antibiotic inactivation. To become multidrug resistant a bacterium must acquire multiple mechanisms, and whilst many species have done so the spectra of resistances vary. Many efflux pumps, ABC (ATP-Bindig Cassette) trasporters, are encoded chromosomally and their presence enhances resistance mediated by these individual mechanisms. In this study we have used a new Quinoxaline derivatives (Qd) as ABC trasporters inhibitor against several microorganisms to evaluate the efficacy.

Materials and Methods: The Qd inhibitor was assayed on 22 strains of *Candida* species, Gram positive and Gram negative bacteria, isolated from different infection sites, by determining the value of the Minimal fungicidal concentration/Minimal bactericidal concentration (MFC/MBC). The tested microorganisms included: *C. albicans, C. parapsilosis, C. tropicalis, C. glabrata, C. krusei, Staphylococcus aureus, Pseudomonas aeruginosa, Enterobacter aerogenes, Proteus mirabilis, Citrobacter freundii, Providencia stuartii*, and *Acinetobacter baumannii.* Subsequently, an additional assay was performed in association with a drug to verify a possible decrease in the MFC/MBC values. The design, synthesis and purification of Qd were performed starting from the commercially available 2-nitro-4-methyl-aniline following the standard procedure.

Results: The data obtained shows that the best performance was found against *Candida* strains followed by *P. aeruginosa* strains with MFC/MBC values between 1000 and 500 µg/mL. The Qd-drug association also gave the best results against *Candida* species and *P. aeruginosa* strains with a 50% reduction in MFC/MBC values.

Conclusions: The problems of resistant Gram-positive and Gram-negative bacteria highlight the urgent need for new drugs with new modes of action and/or combination therapy to treat infections caused by resistant human pathogens such as *S. aureus, Pseudomonas aeruginosa* and *Mycobacterium tuberculosis*. Novel antibiotics with new modes of action are urgently required to suppress the rise of MDR bacteria. An alternative approach would be to identify molecules that can interfere with the process of efflux.

P74

BACTERIA MEET GRAPHENE: MODULATION OF GRAPHENE OXIDE NANOSHEET INTERACTION WITH HUMAN PATHOGENS

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Introduction: Antibiotic ineffectiveness represents a global health issue that demands the development of drugs circumventing microbial resistance mechanisms and attacking new bacterial targets. Graphene oxide (GO) can represent the nanotechnology answer being economical and easy to produce and to degrade and having multitarget specificity against bacteria. Three possible antibacterial mechanisms have been observed: (i) GO sheets cutting bacteria membranes, (ii) inducing oxidative stress, and/ or (iii) wrapping and isolating bacteria from the external environment and nutrients. Unfortunately, controversial results from inhibition to bacterial growth enhancement have been reported. The main difference among all experimental evidence relies on the environmental conditions adopted to study the bacteria-GO interaction. In our work, we demonstrate that by modulating the GO stability in solution, the antibacterial or growth enhancement effect can be controlled on *S. aureus* and *E. coli*.

Materials and Methods: We expose *S. aureus* and *E. coli* to different concentrations of GO ranging from 3 to 200 μg/ml, in different incubation conditions (ultrapure water, PBS, NaCl, MgCl2 and CaCl2). We analyze the growth of microorganisms and characterize GO effects on bacteria cells with Atomic Force Microscopy and Colony Forming Units Assay.

Results: Our data indicate that any buffer solution utilized during the GO-bacteria interaction alters specifically the GO surface zeta potential and the consequent GO clusters size and structure. The GO stability influences antimicrobial activity and, while at low concentration, the sheets cut microorganisms membranes and, at high concentration, complexes between pathogens and aggregates inhibit or enhance bacteria growth in a surface potential-dependent manner

Conclusions: The main consequence of our results is the surprising possibility to finely modulate the GO effects on bacteria and to produce versatile applications of GO materials in the environmental and medical sciences from treatments against multidrug resistant bacteria to water remediation systems and probiotic therapies. With the framework defined in this study, the clinical application of GO gets closer, and controversial results in literature can be explained.

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NOVEL ANTIBACTERIAL COMPOUNDS FROM ACTINOMYCETES

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Introduction: The continuous rise of antibiotic-resistant pathogens requires discovery and development of novel antibacterial agents. Actinomycetes are prolific producers of bioactive metabolites, including several antibiotics in clinical use. Starting from Naicons proprietary actinomycete library, comprising ca 45000 strains (ca 75% of which belonging to uncommon genera), we have identified several antibacterial molecules belonging to novel or unexploited classes (e.g. allocyclinones, paramagnetoquinones, NAI-108). We will focus here on the latest additions, Pseudouridimycin and Enduracyclinones.

Materials and Methods: Actinomycete strains from Naicons collection were cultured in various media, metabolites were recovered from cultures using different procedures, including solvent extraction and binding to resins. Extract bioactivity was assayed by growth inhibition tests or target-based *in vitro* assays. Determination of MIC of purified molecules was performed according to CLSI protocols. Active extracts were fractionated by HPLC and active fractions characterized by LC-MS. NMR was applied to purified samples for structural determination.

Results: Screening of extracts from liquid cultures of actinomycete strains led to the identification of antibacterial substances which properties are being evaluated. Antibiotic-resistant isolates were used to discard molecules showing cross-resistance with several known classes of antibacterials, focusing on potentially novel products. Among these, a group of related molecules possessed extremely potent activity (MIC 0.004 to 0.125 μg/ml) against *S. aureus*, including MRSA and GISA strains. They share a common, unprecedented structure in which the non-proteinogenic aminoacid enduracididine is fused to a polycyclic polyketide scaffold, the molecules were therefore named Enduracyclinones.

Pseudouridymicin (PUM) was identified by an *in vitro* assay for RNA-polymerase inhibitors. PUM is a novel nucleoside analog, selectively inhibiting bacterial RNA polymerase, which does not show cross-resistance with rifamycin and other RNA polymerase inhibitors. PUM is active also *in vivo* in a mouse experimental infection model.

Conclusions: Pseudouridimycin and Enduracyclinones, antimicrobials belonging to two novel classes, have been isolated from actinomycete cultures. Our results show that, when proper methods such as screening of underexploited strains, early activity-based dereplication and early chemical characterization (looking for structural novelty) are applied, these microorganisms still represent a valuable source of antibiotics.

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BACTERICIDAL ACTIVITY OF CEFTOPIBROLE COMBINED WITH OTHER ANTIBIOTICS AGAINST RESPIRATORY PATHOGENS

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Introduction: Ceftobiprole is a new broad-spectrum cephalosporin active against a wide range of Gram-positive and Gram-negative pathogens, which provides a potential therapeutic option for difficult-to-treat infections. The purpose of this study is to evaluate the synergistic activity of ceftobiprole with other antibiotics against Gram-positive and Gram-negative clinical isolates from low-respiratory tract infections (LRTIs).

Materials and methods: Thirty Gram-negative strains (ESBL-producers; carbapenem and COL-R *K. pneumoniae* and *P. aeruginosa*) and forty-six Gram-positive strains (MSSA, hVISA/VISA-MRSA, and linezolid-resistant CoNS; VRE and MDR enterococci) with different antibiotic-resistance profiles were included in this study. The antibiotic combinations of ceftobiprole with diverse antibiotics commonly used in the treatment of LRTIs sustained either by Gram-negative or Gram-positive isolates, were evaluated by gradient-test and time-kill curve analysis.

Results: Gradient-test analysis demonstrated that ceftobiprole plus piperacillin/tazobactam was very active against all ESBL *E.coli* and *K. pneumoniae* isolates showing synergic and additive activity. Almost all ceftobiprole combinations were found inactive against *K. pneumoniae* and *P. aeruginosa*, with only few exceptions. Overall, ceftobiprole synergistic and additive activity was found against 40-60% of MSSA and MDR-MRSA/VSSA, when tested in combination with the antibiotics in study. Ceftobiprole plus daptomycin, linezolid and rifampicin represented the most potent combinations against MDR-MRSA/hVISA. Ceftobiprole was synergic or additive plus linezolid, rifampicin and beta-lactams against *E. faecalis* susceptible to penicillin, and one *E. faecium* beta-lactamase producer. Time-kill curves for representative isolates confirmed the synergistic activity of ceftobiprole in combination with amikacin, levofloxacin and colistin against *K. pneumoniae* COL-R, KPC and OXA-48 producer, *E. coli* ESBL-producer and *P. aeruginosa* ceftazidime-resistant. Ceftobiprole plus daptomycin represented the most potent combination with a 2-fold decrease in MIC and synergy against representative isolates of MSSA, MRSA-VSSA/hVISA and MRSA carrying PBP2a mutation. Antagonism was not observed.

Conclusions: The use of combination therapy with ceftobiprole may provide a needed addition for the treatment of Gram-positive and Gram-negative infections sustained by difficult-to-treat MDR isolates. Ceftobiprole demonstrated a good activity against this varied sample of clinical pathogens, with different responses related to the specific MDR classes analyzed.

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IN VITRO STUDY ON ANTIBACTERIAL ACTIVITY OF THE PROBIOTIC STRAIN *LACTOBACILLUS RHAMNOSUS* HN001

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Introduction: The genus of *Lactobacillus* includes safety strains frequently used as probiotic; some of them are able to produce active metabolites which could show antimicrobial activity.
Materials and Methods: The inhibitory activity assay was performed by agar diffusion and broth dilution method using the supernatant obtained from the probiotic strain *Lactobacillus rhamnosus* HN001 against *Escherichia coli* ATCC25922, *Escherichia coli* ATCC35218, *Klebsiella pneumoniae* ATCC700603, *Pseudomonas aeruginosa* ATCC27853, *Streptococcus agalactiae* DSMZ2134, *Enterococcus faecalis* ATCC29212 *Staphylococcus aureus* ATCC29213. The brothcolture of *L. rhamnosus* HN001 was obtained at 37°C in aerobic conditions using de Man Rogosa and Sharpe medium added with cysteine (0,25%). The agar diffusion assay was performed depositing the cells free supernatant of the brothculture, obtained by centrifugation and filtration, in wells set up in Mueller Hinton agar plates, where the standard strains were spread. For the broth dilution method, an inoculum of each strain was inoculated in each single well containing the supernatant of *L. rhamnosus* HN001 diluted in Mueller Hinton, broth using serial twofold dilutions.
Results: The supernatant obtained from the probiotic strain *L. rhamnosus* HN001 showed a good inhibitory activity against all tested strains, using both assay methods. In particular, a very good inhibitory activity was observed against *Pseudomonas aeruginosa* ATCC27853.
Conclusions: This preliminary study shows a good inhibitory activity of *L. rhamnosus* HN001 against both Gram-negative and Gram-positive strains. The inhibitory activity should be related to acid compounds or small proteins.

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ANTIMICROBIAL ACTIVITY OF NATURAL PRODUCTS AGAINST *HELICOBACTER PYLORI*

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Introduction: The relationship between *Helicobacter pylori* and acquired resistance to various drugs from conventional therapy is of worldwide concern. Therefore, it is extremely important to search for new therapeutic sources. In this study the anti-*Helicobacter pylori* activity of some molecules including epigallocatechin gallate (EGCG), capsaicin, propolis, clorogenic acid, N-acetyl-L-cysteine (NAC), and extracts of fruits of "Maqui-berry" were examined. EGCG was tested in combination with all other compounds.
Materials and Methods: *Helicobacter pylori* ATCC 43504 was used for susceptibility tests. Minimum inhibitory concentration (MIC) was performed with agar dilution method in Mueller Hinton agar supplemented with 5% defibrinated sheep blood according to recommendations of the Clinical and Laboratory Standards Institute (CLSI; document M45). Bacteria were suspended in sterile saline solution to obtain a turbidity equivalent to 2.0 McFarland standard. After bacterial inoculation, plates were incubated in jars under microaerophilic conditions for 72 h (GENbag microaer; bioMérieux – Marcy l'Etoile France). EGCG was tested in combination with all other compounds in agar plates following the checkerboard scheme. The effects of combinations (synergism, partial synergism, additive effect, indifference and antagonism) were evaluated calculating the Fractional Inhibitory Concentrations (FIC) index. Synergism was present if the FIC index was ≤ 0.5, partial synergism if the FIC index resulted in the range of 0.51 to 0.75, additive effect if the FIC index was 0.76-1. Indifference was represented by an index including > 1-4, while antagonism was the result when the index values exceed 4.
Results: Against *H. pylori*, EGCG and propolis showed MIC values equal to 32 μg/mL. The most

effective molecules were capsaicin (MIC 4 µg/mL), followed by clorogenic acid (MIC 16 µg/mL), while Maqui and NAC showed the lowest anti-H. pylori activity with MIC values of 2048 µg/mL. All combinations tested showed indifference with FIC index values higher than 1.

Conclusions: The use of natural compounds against H. pylori has acquired popularity in scientific research because of their broad flexibility and low toxicity. In our study the combination of different molecules was not a successful approach, however single compound, such as capsaicin, showed a promising activity. Further studies are required to establish the beneficial effects of natural compounds in human healthcare.

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IN VITRO ACTIVITY OF CEFTAZIDIME-AVIBACTAM AGAINST SELECTED MULTI-RESISTANT GRAM NEGATIVE STRAINS

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Introduction: Ceftazidime-avibactam (Avycaz™; Allergan, Inc., Irvine, CA, USA) is a third generation cephalosporin and betalactamase inhibitor combination that was approved on the 25th of February, 2015 for the treatment of complicated intra-abdominal infections, in combination with metronidazole, caused by the following susceptible Gram-negative microorganisms: *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Enterobacter cloacae, Klebsiella oxytoca, Citrobacter freundii* complex, and *Pseudomonas aeruginosa* in patients 18 years or older. Ceftazidime-avibactam has the same spectrum of activity of ceftazidime (e.g. against Enterobacteriaceae and *P. aeruginosa*) but the addition of avibactam expands its activity against many resistant strains carrying certain beta lactamases, KPC carbapenemase including. In this study the *in vitro* activity of ceftazidime-avibactam was evaluated against clinical isolates from patients admitted to San Martino Hospital, Genoa, Italy.

Materials and Methods: A total of 22 Gram-negative isolates, including extended spectrum β-lactamase (ESBL) producers, carbapenem-resistant Enterobacteriaceae (CRE) and *P. aeruginosa*, (15 *K. pneumoniae*, 4 *P. aeruginosa*, 1 *E. coli*, 1 *K. oxytoca* and 1 *Enterobacter* spp.) were collected from patients admitted to San Martino Hospital, Genoa, Italy, from April 2016 to June 2017. Bacteria were isolated from different clinical specimens including blood (11; 50%), sputum (4; 18.2%), rectal swab (3; 13.6%), urine (2; 9%), pus (2; 9%), and only one isolate per patient infection episode was included in this study. Bacteria were identified using Vitek 2 automated identification system (BioMerieux, France). Isolates were tested for *in vitro* susceptibility to ceftazidime-avibactam using disk diffusion method (Becton Dickinson, Italy) according to manufacturer's recommendations. The European

Committee on Antimicrobial susceptibility Testing (EUCAST) ceftazidime-avibactam breakpoints of ≤ 13 mm susceptible, ≥13 mm resistant and ≤ 17 mm susceptible, ≥ 17 mm resistant were used for Enterobacteriaceae and *P. aeruginosa*, respectively.

Results: Twenty one (95.5%) isolates were susceptible to ceftazidime-avibactam (halo diameters ranging from 16 to 40 mm and from 19 to 27 mm for Enterobacteriaceae and *P. aeruginosa*, respectively, whereas only 1 (4.5%) *P. aeruginosa* isolate was resistant (halo diameter: 15 mm) because carrying the metallo β-lactamase VIM.

Conclusions: These data suggest that ceftazidime-avibactam may be a valid therapeutic option for the treatment of serious infectious caused by multi-drug resistant pathogens.

P80

IN VITRO ACTIVITY OF TIGECYCLINE AMONG IMPORTANT CLINICAL PATHOGENS ISOLATED IN A LARGE ITALIAN HOSPITAL, DURING 2014-2016

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Introduction: In this study, the *in vitro* activity of tigecycline, synthetic analogue of tetracycline and member of the class of the glycylcyclines, was evaluated against clinical isolates collected in San Martino Hospital.

Materials and Methods: In this study the microorganisms (species and number of isolates) to be collected and evaluated were defined by the Tigecycline Evaluation and Surveillance Trials (TEST) program study design. A total of 571 clinical pathogens (derived from blood, respiratory tract, urine, skin, wound, body fluids and other defined sources) were collected and identified using Vitek 2 automated identification system (BioMerieux, France) during 2014-2016. Minimum inhibitory concentrations (MICs) of the antimicrobial agents were determined by the broth microdilution method recommended by the Clinical and Laboratory Standards Institute (CLSI) in 2013. Quality controls were performed using the following strains: *Escherichia coli* ATCC 25922, *Haemophilus influenzae* ATCC 49766, *H. influenzae* ATCC 49247, *Pseudomonas aeruginosa* ATCC 27853, *Enterococcus faecalis* ATCC 29212, *Streptococcus pneumoniae* ATCC 49619 and *Staphylococcus aureus* ATCC 29213.

Results: Globally 194 Gram-positive (75 *S. aureus*, 44 *S. pneumoniae*, 30 *S. agalactiae*, 45 *Enterococcus* spp.) and 377 Gram-negative (75 *Klebsiella* spp., 60 *P. aeruginosa*, 30 *Serratia marcescens*, 18 *H. influenzae*, 44 *Acinetobacter baumannii*, 75 *E. coli*, 75 *Enterobacter* spp.) strains were collected and evaluated. Tigecycline demonstrated excellent inhibitory activity against *S. aureus, Enterococcus* spp., *H. influenzae, Enterobacter* spp., *A. baumannii, E. coli* with $MIC_{90} \leq 1$mg/L, while against *Klebsiella* spp. showed limited *in vitro* activity with $MIC_{90} \geq 8$mg/L and against *S. agalactiae* and *S. pneumoniae* with $MIC_{90} \geq 4$mg/L.

Conclusions: Tigecycline confirmed a potent *in vitro* antibacterial activity (comparable to or greater than those of most commonly employed antimicrobials) against both Gram-positive and Gram-negative clinical pathogens and those data underline that this drug is a suitable antimicrobial agent for empiric treatment of serious infections sustained by some of the commonly encountered pathogens. In an era of multi-drug resistant microorganisms this antimicrobial agents could represent an interesting choice.

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IN VITRO ACTIVITY OF CEFTOLOZANE-TAZOBACTAM AGAINST SELECTED MICRORGANISMS

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Introduction: Ceftolozane-tazobactam is a new combination endowed with broad activity against many aerobic and facultative anaerobic Gram-negative bacteria including the Enterobacteriaceae family and *Pseudomonas aeruginosa*. Ceftolozane-tazobactam (Zerbaxa™; Merck & Co., Kenilworth, NJ, USA) was approved on the 19th of December, 2014 for the treatment of complicated intra-abdominal infections (in combination with metronidazole) and complicated urinary tract infections, including pyelonephritis but have not activity against carbapenemases such as the well known KPC and VIM. In this study, the *in vitro* activity of ceftolozane-tazobactam was evaluated against clinical isolates from selected patients admitted to San Martino Hospital, Genoa, Italy.

Materials and Methods: A total of 18 multi-resistant Gram-negative isolates, including extended spectrum β-lactamase (ESBL) producers, carbapenem-resistant Enterobacteriaceae (CRE) and *P. aeruginosa*, (13 *P. aeruginosa*, 2 *E. coli*, 1 *K. pneumoniae*, 1 *K. oxytoca* and 1 Enterobacter spp.) were collected from patients admitted to San Martino Hospital, Genoa, Italy, from April 2016 to June 2017. Bacteria were isolated from different clinical specimens including blood (6; 33.3%), sputum (4; 22.2%), urine (2; 11.1%), pharyngeal swab (2; 11.1%), pus (2; 11.1%), rectal swab (1; 5.5%) and bronchoalveolar lavage (1; 5.5%) and only one isolate per patient infection episode was included in this study. Bacteria were identified using Vitek 2 automated identification system (BioMerieux, France). Isolates were tested for *in vitro* susceptibility to ceftolozane-tazobactam using E-test method (Liofilchem, Italy) according to manufacturer's recommendations. The European Committee on Antimicrobial susceptibility Testing (EUCAST) ceftolozane-tazobactam breakpoints of ≤ 1 mg/L susceptible, > 1 mg/L resistant and ≤ 4 mg/L susceptible, > 4 mg/L resistant were used for Enterobacteriaceae and *P. aeruginosa*, respectively.

Results: Fourteen (77.7%) isolates were susceptible to ceftolozane-tazobactam (MIC range 0.13-0.5 mg/L for Enterobacteriaceae and MIC range 0.5-4 mg/L for *P. aeruginosa*), whereas 3 (16.6%) *P. aeruginosa* isolate (MIC values > 256 mg/L) and only 1 Enterobacter spp. strain (MIC value > 256 mg/L) were resistant. *P. aeruginosa* and Enterobacter spp. resistant to ceftolozane-tazobactam carried carbapenemases.
Conclusions: These data suggest that ceftolozane-tazobactam may be an important treatment option for the selected microorganisms causing serious infectious caused by clinically relevant pathogens.

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EPIGENETIC DRUG UVI5008 ACTIVITY AGAINST METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS*

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Introduction: Antibiotic resistance is a global health emergency that diffuses rapidly all over the world. Last World Health Organization (WHO) epidemiology reports show that over than 450.000 person die each year due to multi-resistant microorganism. One of themost widespread bacterial infection is represented by multi-resistant *Staphylococcus aureus* (MRSA), responsible of thousands of septic infections and deaths in hospitals throughout the world. The efforts of scientific community are focused on new antibiotics therapies in order to overcomethe bacteria multi-resistant problem. For this intent we screened a panel of drugs known to be epigenetic modulators. We found several drugs able to inhibit the Gram-positive bacteria growth. Among them we selected the UVI5008, previously described as pan histone deacethylase inhibitor.
Methods: We have evaluated antibacterial activity of selected compounds by broth micro-dilution method.The antibacterial activity was determined against four pathogenic bacterial strains including Gram-positive (*S. aureus* and *Enterococcus faecalis*) and Gram-negative bacteria (*Escherichia coli* and *Klebsiella pneumonia*).To understand the mechanism of UVI5008 action was performed the tryptic in-situ digestion of Blue Coomassie stained bands of SDS-PAGE of treated *S. aureus* samples. Esi iontrap LC-MS/MS experiment wereexecuted. MGF file were analyzed by MASCOT.
Results: The results show that UVI5008 is active against Gram-positive bacteria in a specific manner as well as in MRSA.The UVI5008 minimum inhibitory concentration (MIC) in multi-sensible *S. aureus* (MSSA) was registered at 7 μM; the compound has a bacteriostatic action. Synergic effect was evaluated in combination with ampicillin

on MRSA. Our results show a high synergistic activity on MRSA by using the UVI5008 and ampicillin treatment. In-silico structural studies predicted the potential interactionof UVI5008 with *S. aureus* DNA gyrase protein in a similar manner of Psammaplin A. Moreover,we used the SEM electro microscopy that shows the structural *S. aureus* modifications post stimulation with UVI5008. The full database of identified protein was analyzed and results were summarized in the table1. The major different protein expressedfollowing treatment with UVI5008 wereidentified using gene ontology analysis. Several proteins showed a specific pattern that is of key importance in bacteria homeostasis.

Conclusions: Among the tested drugs, UVI5008 could be an alternative approach to fight the MRSA diffusion.

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IN VITRO ACTIVITY OF CEFTAZIDIME-AVIBACTAM AGAINST CARBAPENEMASE-PRODUCING *ENTEROBACTERIACEAE* FROM URINE SPECIMENS, ITALY

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Introduction: The emergence of carbapenem-resistant *Enterobacteriaceae* (CRE) represents a serious threat to public health worldwide. Indeed, due to the limited number of available therapeutic options, novel compounds are urgently needed. Ceftazidime-avibactam (CAZ-AVI) is a novel β-lactam/β-lactamase inhibitor combination with promising activity against CRE including those producing class A, class C, and some class D carbapenemases such as OXA-48. The aim of this study was to assess the *in vitro* antibacterial activity of CAZ-AVI against a recent Italian collection of CRE isolated from urine specimens.

Materials and Methods: A total of 9408 Gram-negatives from urine specimens were screened in five centers distributed across the Italian territory from October 2016 to February 2017. All isolates were subcultured onto chrome ID® CARBA SMART agar selective plates and each colony morphotype was identified using MALDI-TOF mass spectrometry (bioMérieux). Any isolate identified as a member of *Enterobacteriaceae* was considered as CRE. Antimicrobial susceptibility testing was performed by reference broth microdilution (BMD) using custom frozen plates (Thermo Fisher Scientific). CAZ-AVI and meropenem were also tested, in each center, using E-test (bioMérieux). Results were interpreted according to the EUCAST breakpoints.

All CRE were subjected to real-time PCR targeting $bla_{KPC\text{-}type}$, $bla_{VIM\text{-}type}$, $bla_{NDM\text{-}type}$, $bla_{OXA\text{-}48\text{-}type}$ carbapenemase genes.

Results: Overall, 318 of non-replicate CRE were collected, including 236 *K. pneumoniae*, 72 *E. coli*, five *P. mirabilis*, three *Enterobacter* spp. and two *S. marcescens*. MICs results showed that CAZ-AVI was the most powerful compound (94.3% of susceptible isolates) followed by tigecycline (83.7% of susceptible isolates) and colistin (78.1% of susceptible isolates). Among β-lactams, the second most effective molecule after CAZ-AVI was meropenem (42.1% of susceptible isolates). Comparison of results from E-test with those from BMD showed high level of category agreement (99.7% and 83% for CAZ-AVI and meropenem, respectively). Molecular analysis revealed that 209 isolates were positive for a carbapenemase gene and among these, 92.3% were positive for $bla_{KPC\text{-}type}$, 4.8% for $bla_{OXA\text{-}48\text{-}type}$, 3.3% for $bla_{NDM\text{-}type}$, and 1.9% for $bla_{VIM\text{-}type}$. All class B β-lactamase-producers were resistant to CAZ-AVI while OXA-48- and KPC-producers were susceptible, with the exception of seven KPC-producing-isolates (4.2%), which tested resistant to the drug.

Conclusion: CAZ-AVI showed potent *in vitro* activity against a recent Italian collection of CRE from urine, including 91.4% of carbapenemase producers.

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ANTIMICROBIAL ACTIVITY OF NOVEL DUAL BACTERIAL DNA TOPOISOMERASE INHIBITORS AGAINST ESKAPE BACTERIA

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Introduction: In the last decade the incidence of resistance to quinolones has rapidly increased, justifying efforts to discover new agents to overcome resistance to this class of antibiotics. An extensive hit optimization program aimed to discover novel dual bacterial topoisomerase II inhibitors (NBTIs) led to the selection of optimized hit compounds.

Materials and Methods: *In vitro* activity of compounds, identified by using a computational approach, was verified by functional enzymatic assays and then confirmed by MIC determination against a collection of reference strains and clinical isolates according to CLSI guidelines. Mutant strains of ciprofloxacin-resistant *Staphylococcus aureus* ATCC BAA-1720, resistant to two of our NBTIs, were selected by the single-step mutation method. S. aureus resistant mutants were analysed for the presence of point mutations in *gyrA, gyrB, grlA* and *grlB* genes and the sequences were then compared versus the four gene sequences of the wild-type strain. The activity of one NBTI was verified in the *in vivo* peritonitis infection mouse model.

Results: We have identified a series of compounds with a potent equivalent dual targeting activity against two clinically validated bacterial targets, DNA gyrase and topoisomerase IV enzymes from both *S. aureus* and *E. coli* (IC_{50}s in the range of <0.1 - 2.25 μM). Consistently, compounds confirmed strong activity against *S. aureus* and *E. coli* yielding MIC values ≤ 0.5 mg/L. They showed antimicrobial activity also against ciprofloxacin-resistant strains. The NBTIs mechanism of action, different from that of fluoroquinolones, was confirmed in the *S. aureus* mutant strains by the occurrence of point mutations in DNA gyrase encoding genes, outside the Quinolone Resistance Determining Region. The X-ray structure of *S. aureus* DNA gyrase with the DNA and co-crystallized with one of our hits was used to further optimize the new NBTIs and to confirm structurally their different site of interaction. In agreement with their mechanism of action,

compounds maintained relevant antimicrobial activity against quinolone-resistant clinical isolates of *S. aureus*, Van-A and Van-B *Enterococci* spp., *E. coli, Acinetobacter baumannii* and *Neisseria gonorrheae* with MIC_{90} values in the range 0.06-2 mg/L. The antimicrobial activity was confirmed in the peritonitis infection mouse model, in which after 4-hours from subcutaneous treatments a significant decrease of CFU/ml of MRSA strain was observed in the peritoneal fluid ($p < 0.05$ vs vehicle group).
Conclusions: Overall, the potent antibacterial profile of new molecules against a wide range of bacteria, including several ESKAPE pathogens, is clinically relevant. The discovery of this new class of compounds may have therapeutically relevant implications paving the way to the development of new broad spectrum antimicrobial agents.

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THE ESSENTIAL OIL FROM *PISTACIA VERA* L. VARIETY BRONTE HULL IS ACTIVE AGAINST CLINICAL ISOLATES OF *CANDIDA* SPP

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Introduction: Essential oils are a complex mixture of hydrocarbons and oxygenated hydrocarbons arising from the isoprenoid pathways and secreted by glandular trichomes, which are mainly disseminated onto the surface of plant organs. Our previous data showed that the essential oil from hulls of *Pistacia vera* L. variety Bronte (PEO) has an antioxidant, cytoprotective and antimicrobial properties against *Staphylococcus aureus* and *Escherichia coli*. The aim of the present study was to investigate the effectiveness of PEO against clinical isolates of *Candida spp.*

Materials and Methods: PEO was extracted from ripe pistachio hulls (*Pistacia vera* L., variety Bronte) by hydrodistillation, dried on Na_2SO_4 and stored in a sealed vial under N_2 until analysis. The major components identified were 4-Carene (31.7%), a-Pinene (23.6%), D-Limonene (8.0%), and 3-Carene (7.7%). PEO was tested against 3 clinical isolates of *Candida glabrata*, 3 clinical isolates of *Candida parapsilopsis* and 4 clinical isolates of *Candida albicans*. Strains were isolated at the IRCCS (Centro Neurolesi "Bonino Puleio"), Messina. The MIC and MFC determinations were performed according to the EUCAST guidelines. The association between PEO and caspofungin, voriconazol and fluconazole were also tested.
Results: PEO was bactericidal against all tested isolates at a concentration ranging between 0.25 and 1.0%. The overall association results demonstrated an antagonistic effect (FIC index > 4) between PEO and the antifungal compound against the selected strains.
Conclusions: PEO was effective against clinical isolates of Candida spp. and its used could potentially be explored *in vivo* as topical agent. The effectiveness of PEO in combination with existing drugs could also be further explored.

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EVALUATION OF THE ANTIFUNGAL ACTIVITY OF MENTHA OF PANCALIERI (TURIN, ITALY) ESSENTIAL OIL AND SYNERGISTIC INTERACTION WITH AZOLES

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Introduction: With the wide use of synthetic and semi-synthetic antimicrobial drugs, pros and cons have been so far experienced, including the spread of drug resistant pathogens, and have directed research back to natural products as useful antimicrobial resources. Currently, there is evidence that essential oils (EOs) may exert remarkable biological activities against viruses, bacteria, fungi and parasites. The antimicrobial activity of EOs is due to a number of small terpenoids and phenol compounds. Several EOs are generally recognized as safe, do not accumulate in the liver or kidneys, can stimulate the immune system, and cause no resistance, since microbes are unable to adapt to their heterogeneous structure. The promising antimicrobial activity of EOs has led researchers to use them in combination with available antimicrobial drugs, in order to reduce drug toxicity and side effects, as well as to overcome drug resistance with single agents.

Mentha x piperita L. (peppermint) EO is one of the most widely produced and consumed essential oils. Literature data have shown that peppermint EO and its main components (menthol and menthone) display antimicrobial properties, but their mechanism of action is still not clear. Near Torino, in Pancalieri, there is a typical local production of *M. piperita* [(Huds) species OFFICINALIS (Sole),RUBESCENS (Camus)kind], worldwide known as "Menta di Pancalieri". This EO, thanks to its high quality, is actually considered by experts to be as one of the best peppermint EO in the world. In this study, we evaluated the antifungal activity of "Menta di Pancalieri" EO either alone or in combination with azole drugs against a wide panel of yeast and dermatophyte clinical isolates.

Material and Methods: The EO was analysed by GC-MS at Drug Science and Technology Dept., and its antifungal activity (MIC,MFC) was evaluated against 16 Candida spp.,15 non-Candida spp.,and 5 dermatophyte strains *(Microsporum canis, M. gypseum, Trichophyton mentagrophytes)* according to the CLSI guidelines, with some modifications. The interaction of peppermint EO with azole drugs (fluconazole, itraconazole, ketoconazole) was evaluated through the chequerboard and isobologram methods.

Results: The phytochemical composition of peppermint of Pancalieri EO was in agreement with the European Pharmacopoeia. Susceptibility test results suggest this EO exerts a fungicidal activity against yeasts, and a fungistatic activity against dermatophytes. Interaction studies with azole drugs indicate mainly synergistic profiles between itraconazole and peppermint EO vs. Candida spp., Cryptococcus neoformans and T.mentagrophytes.

Conclusions: Peppermint of Pancalieri EO may act as a potential antifungal agent and may serve as a natural adjuvant for fungal infection treatment.

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MODE OF ACTION OF A KILLER PEPTIDE AGAINST *TOXOPLASMA GONDII*

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Introduction: *Toxoplasma gondii* is a protozoan of significant human and veterinary interest. Killer peptide (KP) is a synthetic decapeptide derived from the variable region of a recombinant yeast killer toxin-like microbicidal single-chain antibody. KP proved to exert significant activities against diverse microbial and viral pathogens through different mechanisms of action, but little is known on its effect on apicomplexan protozoa. A previous *in vitro* study showed that KP treatment reduced the number of *T. gondii* tachyzoites able to invade host cells and the parasite intracellular proliferation. Aim of the present study was to explore the mechanism of action of KP against *T. gondii.*

Materials and Methods: Tachyzoites of RH strain, Type I, cultured in Vero cells were harvested and incubated with 50 μg/ml or 100 μg/ml KP for 3, 24, or 48 h. Externalization of phosphatidylserine (PS) to the surface of tachizoites and DNA strand breaks labelled *in situ* were analysed by flow cytometry. Changes in mitochondrial membrane potential (ΔΨm) in tachyzoites treated with KP were evaluated by confocal microscopy in time-lapse experiments. The effect of peptide treatment on *T. gondii* structure was studied by transmission electron microscopy (TEM).

Results: Flow cytometry studies showed a rapid and dose dependent induction of PS externalization in *T. gondii* tachyzoites after 3 h treatment with KP. Increase of reactivity for DNA strand breaks was detected in tachyzoites treated with 50 μg/ml KP starting from 24 h. Time course confocal microscopy analysis of live *T. gondii* extracellular tachyzoites revealed a partial loss of ΔΨm, starting 10 min after the addition of 50 μg/ml KP. In mitochondria of extracellular parasites incubated with 100 μg/ml KP, a complete collapse of ΔΨm was recorded after 15 minutes. TEM analysis revealed structural damages in T. gondii incubated with KP, showing characteristic morphological changes previously associated with cells undergoing apoptosis.

Conclusions: Pathways that regulate apoptosis in protozoa differ from those described in mammals. Morphological observation and analysis of apoptotic markers of protozoan parasites showed that KP may trigger a form of apoptosis-like cell death in *T. gondii* tachyzoites. Overall, our results indicate that KP could be a promising candidate for the development of new drugs for the treatment of toxoplasmosis.

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ANTIMICROBIAL AND ANTI-BIOFILM ACTIVITY OF CRYPTIC PEPTIDES PRESENT IN THE HUMAN STOMACH

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Introduction: Due to the persistent emergence of bacterial strains multi drug resistant (MDR) to traditional antibiotics, there is an urgent need for novel antimicrobial compounds to fight off microbial infections. Antimicrobial Peptides (AMPs) are key components of the innate immunity response after exposure to microbial attack. Exploration of other human secreted proteins resulted in the discovery of novel cryptic AMPs, derived from secreted precursors. Among these, Human pepsinogen, inactive forms of gastric proteases, was converted into active enzymes by the acidic pH of stomach with the cleavage of the long N-terminal peptide. The aim of this study was to investigate the possibility that human pepsinogen A3 prosegment (PAP-A3) and its two shorter peptides (IMY25, FLK22) might exhibit additional functions involved in microbial surveillance of the upper digestive tract.

Materials and Methods: The MIC values of human pepsinogen A3 derived peptides against planktonic bacteria were examined by broth microdilution method. The anti-biofilm activity of the compounds, was assessed by quantifying both the biomass, using the crystal violet staining method, and the metabolic activity by XTT assay. The effects of peptides against mature biofilm were visualized also by confocal scanning laser microscopy (CSLM).

Results: PAP-A3, IMY25 and FLK22 exhibited an important antimicrobial activity against all tested strains with the exception of two *Pseudomonas aeruginosa* and *Listeria monocitogenes* clinical isolates. At concentration of 6,25 mM, IMY25 significantly reduced biomass and viability of PAO1 biofilm; the biofilm disruption was confirmed by CLSM. In contrast, the PAP-A3 and FLK22 did not shown anti-biofilm activity. None of the three peptides reduced *S. aureus* biofilm biomass while CLSM micrographs revealed that cell viability in pre-established biofilms was impaired after 24 h of IMY25 treatment. Both PAP-A3 and FLK22 were found ineffective in killing mature biofilm cells.

Conclusion: Although the role of pepsin prosegments in the activation pathways have been well described, beyond safe storage and secretion of the protein, no other significant functions have been attributed to these peptides. Our study revealed that human pepsinogen A3 derived peptides have a broad-spectrum antimicrobial activity against all tested bacterial strains and an anti-biofilm activity against S. aureus and PAO1 mature biofilm. These results suggest that Pepsinogen derived peptides might exhibit additional functions involved in microbial surveillance of the upper digestive tract.

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ANTIBACTERIAL AND ANTIBIOFILM ACTIVITY OF ESSENTIAL OILS FROM DIFFERENT PLANTS AGAINST *STAPHYLOCOCCUS SPP.* AND *PSEUDOMONAS AERUGINOSA* BACTERIAL STRAINS

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Introduction: Several drugs for treating a variety of diseases have been discovered via screening of natural compounds obtained from animals, microorganisms, marine organisms, and plants. These drugs can be natural products per se or semi-synthetic analogs. Although there are a large number of plant species, only approximately 10% produce mixtures of odorous and volatile compounds, collectively called essential or volatile oils (EOs) (1). From a chemical standpoint, EOs are typically composed of hydrocarbons and oxygenated monoterpenes, sesquiterpenes and diterpenes, aromatic compounds, and low molecular weight aliphatic compounds. Some EOs play an important role in protecting plants against insects, fungi, bacteria and viruses (1). Within the same variety, the essential oil composition can vary according to geographical region and seasonality. EOs from a variety of plants are also endowed with antibacterial activities (2-4) as well as anti-inflammatory and antioxidant properties (5). In this work we investigate the antibacterial and antibiofilm effect of EOs from *Calamintha nepeta, Foeniculum vulgare* and *Ridolfia segetum against Staphylococcus aureus, Staphylococcus epidermidis and Pseudomonas aeruginosa.*

Materials and Methods: *S. aureus* 6538P and 25923, *S. epidermidis* RP62A and O-47, *P. aeruginosa* PaO1 strains were used. EOs were obtained from plants harvested in different seasons and conditions so as to obtain a total of about 100 different samples. The chemical composition of each EO was determined. Determination of MIC for each EO on the bacterial strains was carried out. The action on biofilm formation was assessed by crystal violet and the IC50 of biofilm inhibition (BIC50) was calculated.

Results: Reported results demonstrate that EOs with different antimicrobial and antibiofilm features were selected. Some of them inhibit bacterial growth at high concentration and possess good antibiofilm activity at sub-MIC concentration showing a very low BIC50 (ng). Other EOs were able to destabilize biofilm structure without killing cells.

Conclusion: Our data suggest that some of the studied EOs could be proposed as antibacterial drugs against *Staphylococcus spp.* and *P. aeruginosa.* Their action on sessile phenotype renders them particularly interesting for possible applications in biofilm infections and disinfection procedures.

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HYDROPHOBIN COATED SURFACES PREVENT *S. EPIDERMIDIS* BIOFILM FORMATION

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Introduction: *Staphylococcus epidermidis* normally colonizes the human skin and mucous membranes and represents a major component of the normal bacterial flora of this habitat. In predisposed hosts, usually with an indwelling medical device, *S. epidermidis* has become a significant nosocomial pathogen. The major virulence factor associated with this organism is the ability to cause infections is dependent on adherence to medical devices and formation of a biofilm. Microbial adhesion to surfaces has been shown to be a complex process, involving physicochemical, protein and polysaccharide factors. Protein-functionalized surfaces are a hot topic in life sciences and are becoming more and more popular also in the medical field such as protein-mediated tissue engineering.
In this work, the impact of hydrophobin coated surfaces on *S. epidermidis* biofilm formation was studied. Hydrophobins (HFBs) are small proteins produced by fungi, able to self-assemble into amphipathic membranes at hydrophobic-hydrophilic interfaces. Beyond their natural functions, hydrophobins show very peculiar properties, which are of interest for biotechnological applications.

Materials and Methods: Two hydrophobins were extracted from mycelium of *Pleurotus ostreatus* and from culture broth of *Acremonium sclerotigenum* (1, 2). Purified hydrophobins were used to functionalize surfaces by deposition. The presence of hydrophobin layers on different surfaces were detected by Water Contact Angle (WCA). *S. epidermidis* RP62A and O-47strains were used. Biofilm formation was assessed by crystal violet method and by Confocal laser scanning microscopy (CLSM).

Results: The inhibition of *S. epidermidis* biofilm formation on surfaces coated by two hydrophobins was analysed. Both proteins were able to reduce the biofilm formed in polystyrene multiwell plates, as measured by crystal violet assay. Then, the reduction of the biofilm thickness and the preservation of cell vitality, in presence of HFBs, were demonstrated through CLSM analysis. This reduction was related to the increase of the protein concentrations used to coat the wells. Different surfaces (glass, steel, titanium and PTFE) were coated by HFB and characterized by WCA analysis, showing a change of surface wettability. Finally, the anti-biofilm efficiency of HFB coated supports was evaluated.

Conclusion: This study reports the capability of hydrophobin coated surfaces to prevent *S. epidermidis* biofilm formation; this is the first reports on anti-biofilm activity of these *Pleurotus ostreatus* proteins.

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EFFECTS OF DIFFERENT MOUTHWASHES ON BIOFILM FORMATION BY ORAL STREPTOCOCCI

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Introduction: Oral microbiota is an extremely complex and dynamic system including bacteria, archea and fungi. Different locations of the oral cavity host a large variety of microbiota mostly organized as biofilm, characterized by spatial and temporal differences in their distribution. Several bacteria belonging to the genus *Streptococcus* act as early colonizers, binding to the adhesive pellicle and providing a substrate for the attachment of late colonizers bacteria. The latter, in turn, may be responsible of severe pathologies, such as carious lesions, gingivitis and periodontal lesions. Therefore, we evaluated the effects of commercial mouthwashes (MoWs) on biofilm (BF) formation/ persistence of early colonizers Streptococci.

Materials and methods: Fourteen isolates belonging to 5 different species of oral streptococci (*S. salivarius, S. mitis/oralis, S. sanguinis, S. parasanguinis, S. vestibularis*) and 3 isolates of *Enterococcus faecalis* were obtained from pharyngeal swabs and employed for the present study. All the bacteria were incubated for 1 minute with 6 commercial MoWs, 4 with (MoWs 1, 2, 3 and 7) and 2 without (MoWs 4 and 5) chlorhexidine digluconate (CHX), which is known to exert antibacterial and antifungal activity. Control groups of each strain were treated for 1 minute with PBS and used as negative controls. After treatment with MoWs or PBS, bacteria were seeded in 96-well plates and allowed to form BF for up to 48 hours. BF formation was assessed by crystal violet assay. The capacity to form BF was expressed as the optical density (OD) percentage of each MoW-treated strain, as compared to the OD of the PBS-treated strain, which was considered as 100%. Statistical analyses were carried out by one-way ANOVA test with Bonferroni post-hoc test.

Results: CHX-containing MoWs were capable to inhibit BF formation in most of the cases. In detail, CHX-containing MoWs significantly reduced BF formation by all the *S. salivarius* and *E. faecalis* isolates, while only several *S. parasanguinis, S. mitis/oralis, S. sanguinis* and *S. vestibularis* isolates were affected. One of the 2 CHX-free MoWs was capable to significantly inhibit BF formation by *S. salivarius* and *S. vestibularis*, whereas the remaining CHX-free MoW did not prove to be effective in impairing BF formation by any of the bacterial isolates assessed.

Discussion and conclusions: Similarly to *Candida albicans* (1), BF formation by oral streptococci is affected by MoWs, provided that they include CHX in their formulation. Since the streptococci used in the present study act as early colonizers in the multispecies microbial BF of the dental surface, special attention should be used when choosing MoWs for prevention and/or treatment of oral pathologies of microbial origin.

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ANTIBIOFILM EFFECT OF CURCUMIN AGAINST A MULTIRESISTANT CLINICAL ISOLATE OF *MYCOBACTERIUM ABSCESSUS*

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Introduction: Curcumin, a phenolic compound extracted from *Curcuma longa*, exerts multiple pharmacological effects, including an antimicrobial action. *Mycobacterium abscessus*, an environmental, non-tuberculous, rapidly growing mycobacterium (RGM), is an emerging human pathogen causing serious lung infections and one of the most difficult to treat, due to its multidrug resistance and biofilm-forming ability. The aim of the present study was to evaluate the antimicrobial and antibiofilm activity of curcumin and its ability to synergize with amikacin against a clinical *M. abscessus* isolate.

Materials and methods: *M. abscessus* 29904, isolated from the bronchoaspirate of a 66-year-old woman admitted to hospital for suspected tuberculosis was used throughout the study. The susceptibility to amikacin, curcumin, and their combinations were performed by the microdilution and chequerboard assay. The ability of *M. abscessus* 29904 to form biofilm was evaluated in terms of produced biomass at 2, 4, 8 days and the cellular viability among clustered cells was detected by BacLight Live/Dead Viability Kit. The antibiofilm properties of curcumin associated with amikacin were evaluated on mature biofilm by BIC and BEC quantifications and measuring the biomass.

Results: Curcumin (MIC=128 mg/L) exhibited synergistic activity with amikacin (FIC index value = 0.18) to which strain 29904 showed intermediate susceptibility. *M. abscessus* 29904 produced more biofilm at 8 days but with less live bacteria than 4-day-biofilm. Curcumin at 4×MIC completely inhibited 4- and 8-day mature biofilms. Synergistic combinations of curcumin and amikacin induced a general reduction in microbial aggregates and substantial loss in cell viability. The BIC values were twice the curcumin MIC and overlapped with amikacin MIC; instead, the BEC values were higher than 4×MIC of Curcumin and 8×MIC of amikacin. Disruption of 4- and 8-day biofilms was the main effect detected when curcumin was the predominant compound.

Conclusions: The present findings support evidence that curcumin is a compound capable of restoring the effectiveness of failing antibiotics by reducing their MICs. The strong effect of curcumin and amikacin on mature biofilm emphasizes the importance of their synergistic association suggesting that, curcumin combined with antibiotics could provide a novel strategy to combat the antibiotic resistance of *M. abscessus*.

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A NEW LIGNAN-LIKE COMPOUND INHIBITS *STAPHYLOCOCCUS AUREUS* AND S. EPIDERMIDIS GROWTH EXHIBITING ANTIBIOFILM PROPERTIES

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Introduction: *Staphylococcus aureus* and *S. epidermidis* are bacteria most frequently associated to infections of skin and soft tissue lesions resulting from trauma, burns or surgery, as well as to vascular catheter-related bloodstream infections. Biofilms play an important role in medical device infections, and in the recent years their relevance has been recognized in burns, grazes or surgical wounds infections as well. Since most antimicrobial cannot penetrate the extracellular matrix of biofilm it is fundamental that an antimicrobial could act both on the planktonic and sessile bacteria. To provide alternative molecular scaffolds with anti-biofilm activity, we have turned to an anti-microbial class of natural products, the lignans, as a source for structural insight to guide molecular design. The synthetic arylfurans and lignan-like arylbenzylfurans, were tested against Gram-negative and Gram-positive bacteria.

Materials and methods: Minimal inhibitory concentrations (MIC) of the compounds were determined in LB and TSB medium against all bacterial strains by micro-dilution assay. The activity against biofilm formation and preformed biofilm were evaluated by Stepanovic method and XTT assay and by confocal laser scanning microscopy (CLSM).

Results: Compound 11 was the only active against *S. aureus* and *S. epidermidis* with a MIC_{50} value of 4 μg/ml. None of the compounds tested could affect *Pseudomonas aeruginosa* and *Escherichia coli* growth. The antimicrobial activity of compound 11 was further investigated against methicillin resistant *S. aureus* (MRSA) and *S. epidermidis* (MRSE) resulting in a 80% decrease of MRSA and MRSE growth. Compound 11 didn't result cytotoxic on HaCat cells. Regard the anti-biofilm activity, compound 11 was not able to influence biofilm formation, whereas it significantly decreased the viability of preformed biofilm. This activity against *S. aureus* and *S. epidermidis* preformed biofilm was dose-dependent and already visible at the value of 2x MIC (8 μg/ml). The minimal concentration which eradicated the 90% ($MBEC_{90}$) of *S. aureus* and *S. epidermidis* preformed biofilm resulted 32 μg/ml and 8μg/ml, respectively. The anti-biofilm property of compound 11 was investigated by CLSM showing that *S. aureus* and *S. epidermidis* biofilms vitality was strongly reduced following treatment with compound 11 at 8x MIC (32μg/ml).

Conclusion: This is the first study demonstrating the antimicrobial properties of a synthetic lignan-like furan. Although the small structural differences, only one of the tested furans showed anti-microbial properties, thus confirming the importance of synthetizing libraries of compounds to be tested to find new molecules with improved biological activity.

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EFFICACY OF PHAGES AND ANTIBIOTIC-PHAGE COMBINATIONS AGAINST BIOFILM BY *PSEUDOMONAS AERUGINOSA* FROM CYSTIC FIBROSIS PATIENTS

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Introduction: *Pseudomonas aeruginosa* is the main cause of mortality of many cystic fibrosis (CF) patients. Inherited resistance is not the only reason antibiotic treatment fails. In fact, in CF lung *P. aeruginosa* is able to grow as biofilm communities resistant to antibiotics and host response. P. aeruginosa can be killed by an abundance of phages isolated from a variety of sources, including sewage. While there are clear limitations to the use of phage therapy per se for treating bacterial infections, there is increasing evidence that bacterial viruses may be an effective adjunct to antibiotic treatment. In this investigation, we evaluated the efficacy of selected phages, tested alone and in combination with antibiotics, against biofilm formed by *P. aeruginosa* strains from CF patients.

Materials and Methods: Five phages (Φ4, Φ9, Φ14, Φ17, Φ19), isolated from a local sewage treatment plant, were assayed against 24h-preformed biofilms by 33 *P. aeruginosa* strains collected from CF patients at different stages of infection (first, early, chronic). The five most effective phages were also tested in combination with antibiotics (tobramycin, TOB; amikacin, AK; meropenem, MPM), each tested at the MIC value, as assessed by broth microdilution technique. Control (CTRL) biofilms were exposed neither phage nor antibiotic. The dispersion of biofilm caused by exposure to phages, antibiotics or their combinations was spectrophotometrically measured by crystal violet stain.

Results: All strains were able to form biofilm, although striking differences in biofilm biomass formation were observed (OD_{492} range: 0.055-5.459). No relationship was observed among biofilm formation and stage of infection. Phages Φ14 and Φ19 exhibited higher efficacy against preformed biofilms, causing significant (at least 30% vs CTRL; $p < 0.05$) dispersion in 13.6% and 12.5% of cases, respectively. TOB and AK caused biofilm dispersion in 3 out of 5 (60%) cases, whereas MPM in 80%. Phages showed higher activity than antibiotics in 2 cases for TOB (Φ4, Φ9), and once (Φ4) for AK. Exposure to both phage and antibiotic always caused a dispersion comparable to phage or antibiotic tested alone. Exposure to antibiotic caused significant increase in biofilm biomass by strains Pa12 (TOB) and Pa21 (AK).

Conclusions: The dispersion of preformed *P. aeruginosa* biofilm is phage- and strain-dependent. Some phages showed higher activity compared to antibiotics commonly used for treatment of CF lung infections. The phage-antibiotic combinations tested could not cause a biofilm dispersion higher than either agent alone. Further work is ongoing to test other combinations.

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IN VITRO ACTIVITY OF MULTI-BRANCHED PEPTIDES AGAINST PLANKTONIC AND BIOFILM CELLS OF *PSEUDOMONAS AERUGINOSA* FROM CYSTIC FIBROSIS PATIENTS

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Introduction: Treatment of cystic fibrosis (CF)-associated lung infections is hampered by the presence of multidrug resistant pathogens, many of which are also strong biofilm producers such as *Pseudomonas aeruginosa*. Antimicrobial peptides (AMPs) represent a vast class of antibiotic compounds potentially suitable to address multidrug resistant bacteria. Here we evaluated previously reported antimicrobial peptide dendrimers (AMPDs) and related AMPs in comparison with DJK5 recently reported by the Hancock group, against both planktonic and biofilm lifestyles of *P. aeruginosa* strains from CF patients.

Materials and Methods: Four *P. aeruginosa* strains collected from CF patients (PaPh13, PaPh14, PaPh29, PaPh32) were selected because strong-biofilm producers. MIC and MBC values were measured by microdilution broth technique according to EUCAST guidelines. The effect of each peptide at sub-inhibitory concentrations (sub-MICs) on biofilm formation was spectrophotometrically evaluated following crystal violet stain. Control (CTRL) biofilms were not exposed to peptides.

Results: We identified several potent AMPDs against *P. aeruginosa* cells showing MIC values ranging from 8 to 16 µg/ml. MBC values were equal or 1-$\log_2$ dilution higher than relative MIC values, thus suggesting a bactericidal mechanism of action. All peptides tested affected biofilm formation, although at different extent. Two peptides were most active at sub-MICs against biofilm formation causing a significant, concentration-dependent, reduction of biofilm biomass (compared to CTRL) up to 80 %. DJK5 was the least effective causing reduction of biofilm biomass in 25% of cases.

Conclusions: The activity shown by second-generation dendrimers peptides against planktonic cells and biofilm formation makes them promising "lead compounds" for future development of novel drugs for therapeutic treatment of CF lung disease. Ongoing work is investigating the activity of AMPs against higher number of strains.

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STREPTOCOCCUS MUTANS X-PROLYL DIPEPTIDYL PEPTIDASE AS A TARGET AGAINST BIOFILM FORMATION UNRAVELLED BY ANTI-HUMAN DPP IV DRUGS: A NEW PARADIGM FOR THE SYNTHESIS OF INNOVATIVE ANTI-CARIES AGENTS

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Introduction: Dental caries, a multifactorial global disease caused mainly by *Streptococcus mutans* may exhibit higher morbidity in diabetic patients with poor glycemic control. We speculated that gliptins, commonly used as anti-dipeptidyl peptidase (DPPIV) drugs in diabetics, may interfere with *S. mutans*.

Materials and Methods: We have determined the *ex vivo* antimicrobial and anti-biofilm activity on *S. mutans* (reference strain UA159) of three gliptins, namely saxagliptin, sitagliptin, and vildagliptin. The bacterial dipeptidyl peptidase (Sm-DPP IV) was over-expressed, purified by affinity chromatography, characterized and evaluated for its inhibition by the same drugs. Also isogenic deletion mutants of the gene expressing the Sm-DPP IV, *pepX*, were obtained and used to perform CLSM studies aimed at evaluating the biofilm structure and viability formed at different stages of development. Concurrently, we performed modeling and docking studies in parallel with synthesizing new molecules, which may be effective against the bacterial enzyme and not against the human homologue.

Results: Saxagliptin was quite effective in inhibiting biofilm at 128 µg/mL, a concentration close to the inhibition constant of Sm-DPP IV (K_i = 129µM ± 16), while vildagliptin and sitagliptin inhibited at higher concentrations (~256 µg/mL). In CLSM studies, biofilm of *pepX* isogenic mutant was comparable to that formed in presence of saxagliptin, establishing a probable role of this enzyme in sucrose independent biofilm formation by *S. mutans*. The purified enzyme has shown to be strongly inhibited by the known DPP IV inhibitor valine-pyrrolidide. In view of these promising outcomes, a series of compounds, designed by modeling and docking experiments on the computed structure of the Sm-DPP IV, were synthesized and tested for their inhibition of the Sm-DPP IV. Among them some lead compounds have been identified to foster the development of additional, more potent and selective inhibitors of the streptococcal biofilm.

Conclusions: In an era of multidrug resistant pathogens, it is urgent to look for new anti-virulence drug targets. Sm-DPP IV might be one of them. Our work is now waiting this paradigm to be extended to other streptococci. Additionally, this study added evidence to the concept that routinely used non-antibiotic medicinal drugs might affect the human microbiota.

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ANTIFUNGAL PROFILE OF CANDIDA SPECIES AND RELATIONSHIP TO *C. ALBICANS* AND *C. GLABRATA* BIOFILM PRODUCTION

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Introduction: *Candida* species are commensals of the skin, the mucosa or gastrointestinal tract of 30–50% of healthy subjects but they can also represent opportunistic pathogens able to express severe infections involving major body organs. The well documented rapid development of resistance to antifungals, such as azoles or echinocandins, contributes to the interest against *C. albicans* and other species such as non *Candida albicans Candida* (NCAC) and in particular *C. glabrata* that has been detected as nosocomial pathogen in mucosal and systemic infection. In this study, we collected samples from different body sites to characterize *Candida* species for antifungal pattern and to analyze the biofilm produced by *C. albicans* and *C. glabrata* isolates.

Materials and Methods: A total of 81 strains were isolated from 77 positive patients for *Candida* spp. with clinical signs of infections from the Microbiology Laboratory of Pescara Hospital, in Central Italy (Abruzzo) a period of four months. The antifungal susceptibility test of *Candida* species was determined by using ASTYS01 kit on Vitek 2 system. The Vitek 2 cards contained serial twofold dilutions of flucytosine–5FC (from 1 to 64 μg/ml), fluconazole–FLC (from 1 to 64 μg/ml), voriconazole–VOR (from 0.12 to 8 μg/ml), micafungin–MFG (from 0.06 to 4 μg/ml) and amphotericin B–AMB (from 0.25 to 16 μg/ml). The analysis of the biofilm formation was performed at 24 and 48 hours comparing resistant and susceptible strains of *C. albicans* to resistant and susceptible strains of *C. glabrata.*

Results: From the 81 isolated strains, the most isolated species were *C. albicans* (44), *C. glabrata* (13) and *C. parapsilosis* (13) mainly from Hematology, Infectious Diseases, Medicine, Neonatology and Oncology divisions; the majority of biological samples were swabs (44) and blood cultures (16).

Candida albicans has a greater ability to form biofilm compared to *C. glabrata*, both in the susceptible and resistant strains reaching maturity after 24 hours with a complex structure composed of blastospores, pseudohyphae and hyphae embedded in a matrix. On the contrary, *C. glabrata* biofilm was composed exclusively of blastospores that in the resistant strain after 24 hours, was organized in a compact multilayer different to the discontinuous structure observed in the susceptible analyzed strains.

Conclusion: The changing of *Candida* species epidemiology together with the increase of resistant strains and their ability to form biofilm, underline the need to monitor the incidence of new emerging *Candida* species and the antifungal susceptibility profile for a correct management of the infections.

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THE N-TERMINUS OF HUMAN LACTOFERRIN DISPLAYS ANTI-BIOFILM ACTIVITY ON *CANDIDA PARAPSILOSIS* IN LUMEN CATHETERS

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Introduction: *Candida parapsilosis* is a major cause of hospital-acquired infection, often related to parenteral nutrition administered via catheters and hand colonization of health care workers, and its peculiar biofilm formation ability on plastic surfaces. The mortality rate of 30% points to the pressing need for new antifungal drugs. The present study aimed at analyzing the inhibitory activity of the N-terminal lactoferrin-derived peptide, further referred to as hLF 1-11, against biofilms produced by clinical isolates of *C. parapsilosis* characterized for their biofilm forming ability and fluconazole susceptibility.
Methods: hLF 1-11 anti-biofilm activity was assessed in terms of reduction of biofilm biomass, metabolic activity, and observation of sessile cell morphology on polystyrene microtiter plates and using an *in vitro* model of catheter-associated *C. parapsilosis* biofilm production. Moreover, fluctuation in transcription levels of genes related to cell adhesion, hyphal development and extracellular matrix production upon peptide exposure were evaluated by quantitative real time RT-PCR.
Results: The results revealed that hLF 1-11 exhibits an inhibitory effect on biofilm formation by all the *C. parapsilosis* isolates tested, in a dose-dependent manner, regardless of their fluconazole susceptibility. In addition, hLF 1-11 induced a statistically significant dose-dependent reduction of preformed-biofilm cellular density and metabolic activity at high peptide concentrations only. Interestingly, when assessed in a catheter lumen, hLF 1-11 was able to induce a 2-log reduction of sessile cell viability at both the peptide concentrations used in RPMI diluted in NaPB. A more pronounced anti-biofilm effect was observed (3.5-log reduction) when a 10% glucose solution was used as experimental condition on both early and preformed *C. parapsilosis* biofilm. Quantitative real time RT-PCR experiments confirmed that hLF 1-11 down-regulates key biofilm related genes.
Conclusions: The overall findings suggest hLF 1-11 as a promising candidate for the prevention of *C. parapsilosis* biofilm formation and to treatment of mature catheter-related *C. parapsilosis* biofilm formation.

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EFFICACY OF POLY(LACTIC ACID)/CARVACROL ELECTROSPUN MEMBRANES AGAINST PLANKTONIC AND SESSILE GROWTH OF *STAPHYLOCOCCUS AUREUS* AND *CANDIDA ALBICANS*

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Introduction: Carvacrol (CAR) is one of the most promising antimicrobial components of the essential oils. New technologies are aimed to incorporate this molecule into carrier matrix. Currently, there is a renewed interest in the development of ultrafine fiber membranes by electrospinning. The remarkable properties of nanofibers such as high flexibility, porosity and surface-to-volume ratio make them ideal matrices to be used in a wide variety of biomedical applications including wound dressing. The aim of this study was to investigate the feasibility of electrospinning CAR into nanofibers of poly(lactic acid) (PLA) and to evaluate the release profile, the mechanical properties and the antimicrobial efficacy against planktonic and sessile growth of *S. aureus* and *C. albicans*.

Materials and Methods: A conventional electrospinning equipment was used to prepare 28 wt% PLA/CAR nanofiber membranes. The CAR release kinetic and the morphological and mechanical properties of the nanofibers were evaluated by spectroscopic methods, electronic scanning microscopy (SEM) and dynamometer measures, respectively. PLA/CAR membranes at various weight (3, 2, 1 mg) were placed in 24-well plates, seeded with *S. aureus* ATCC 6538 and *C. albicans* ATCC 10231, either in single or in mixed culture, and incubated at 37 °C for 24 up to 144 h. At the time intervals, the cell number and biofilm biomass were evaluated by plate count and absorbance measures. The effects on established 24 h- and 48h-biofilms were estimated by cell count and metabolic activity using the Cell Proliferation Kit II XTT.

Results: The release kinetic showed that about 85% of CAR was released in the medium. The results of morphological and mechanical properties proved a slight increase in the fibers diameter and a marked rise either in deformation from 7.8% to 115% and in elastic modulus from 57 MPa to 98 MPa. The killing curves indicated a greater efficacy of PLA/CAR membranes 3 and 2 mg. In particular, PLA/CAR 3 mg determined a strong decrease of cell number within 24 h, after which cell death continued at a much slower rate up to 144 h. A significant bactericidal effect for *S. aureus* and *C. albicans*, in single (5 and 4.3 log reductions) and mixed culture (4.5 and 3.7 log reductions) was achieved. A marked inhibition of biofilm formation (> 85%) and a considerable reduction of cell count (≥ 4 log) and metabolic activity (70-80%) of established 24 h- and 48h-biofilms were observed.

Conclusions. The improved physicochemical properties of PLA/CAR membranes and their antimicrobial/antibiofilm efficacy against *S. aureus* and *C. albicans* also in mixed colture open up interesting perspectives in previously limited biomedical areas.

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EFFECTS OF DIFFERENT MOUTHWASHES ON *CANDIDA ALBICANS* ADHESION, SUSCEPTIBILITY TO PHAGOCYTIC CELLS AND CAPACITY TO ELICIT PRO-INFLAMMATORY CYTOKINE RESPONSE

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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, including the daily use of toothbrush and mouthwashes (MoWs). Among several virulence factors, *C. albicans* has the ability to adhere to epithelial surfaces, to avoid phagocytosis and/or intracellular killing and to elicit proinflammatory cytokines production. We have previously demonstrated that both *C. albicans* hyphal development and biofilm formation/ persistence are affected by MoWs, provided that they contain chlorhexidine digluconate (1). Therefore, in this study we aim to expand our knowledge on MoWs effects by investigating the behaviour of MoWs-treated *C. albicans,* in terms of adhesion to both abiotic and biotic surfaces, susceptibility to phagocytosis and capacity to elicit pro-inflammatory immune responses.

Materials and Methods: *C. albicans* SC5314 and 6 commercial MoWs have been employed: 4 with and 2 without chlorhexidine digluconate (CHX), a component known to have antibacterial and antifungal activity. Adhesion was assessed by a bioluminescent strain of *C. albicans* SC5314; MoWs-treated and PBS-treated fungal cells were incubated in 96-well plates containing or not a monolayer of TR-146 oral epithelial cell line; after 60 min, plates were washed and the residual bioluminescent signal recorded. Susceptibility to phagocytosis was assessed by exposing MoWs-treated and PBS-treated *C. albicans* to phagocytic cell line BV2 (effector:target = 1:2). Following 24 hours incubation of TR-146 cells with MoWs-treated and PBS-treated *C. albicans*, cytokine levels in supernatants were measured.

Results: Adhesion of MoWs-treated *C. albicans* to abiotic surfaces was significantly lower than PBS-treated Candida. Adhesion of MoWs-treated *C. albicans* to TR-146 cells was significantly lower than PBS-treated Candida, in all but MoW 4. No differences could be highlighted in terms of susceptibility to phagocytosis (percent phagocytic cells and phagocytosis index) between MoWs-treated and PBS-treated Candida. On the contrary, significantly higher acidic phagolysosomes percentages were recorded from Candida treated with 4 out of 6 MoWs, with respect to PBS-treated fungi. Finally, Candida pretreatment with 4 out of 6 MoWs and 5 out of 6 MoWs impaired the production of IL-1a and IL-1b, respectively.

Discussion and Conclusions: *C. albicans* adhesion, susceptibility to phagocytosis and capacity to elicit pro-inflammatory cytokine response are affected by MoWs, especially those containing CHX. Thus, special attention should be used when choosing MoWs whether prevention and/or treatment of Candida-associated oral pathologies was intended.

Reference

1. Paulone et al. New Microbiol 2017.

P101

ANTIBACTERIAL ACTIVITY OF CHITOSAN-SHELLED OXYGEN-LOADED NANOBUBBLES AND NANODROPLETS AGAINST MRSA IN CHRONIC WOUNDS

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Introduction: Hypoxia, inflammation and microbial infections, usually associated with *Staphylococcus aureus*, in particular methicillin-resistant *S. aureus* (MRSA) strains, are common features of chronic wounds. The limited solubility, short half-life, and variable adsorptions of drugs along with the limited efficacy of transdermal drug delivery are driving the attention towards new therapeutic approaches. The known benefits of nanotechnology combined with the emerging advantages of using natural antimicrobial polysaccharides, such as chitosan, have paved the way to develop nonconventional and innovative adjuvant therapies for infected wounds. Chitosan-shelled oxygen-loaded nanobubble (OLNB) and chitosan-shelled oxygen-loaded nanodroplet (OLND) potential, as treatments for infected wounds, was here assessed by investigating their antibacterial activity against MRSA along with toxicity on human keratinocytes.
Materials and Methods: Medium molecular weight (MW) chitosan-shelled OLNBs/OLNDs were prepared and characterized for morphology, size, and avarage diameter. Confocal microscopy analyses was performed to study the physical interaction between OLNBs/OLNDs and cell walls. Cytostatic activity was evaluated by incubating MRSA alone or with 10% v/v OLNBs/OLNDs within 24 h. Toxicity on human skin cells was studied by lactate dehydrogenase assay after incubating HaCaT keratinocytes alone or with 10% v/v OLNBs/OLNDs either in normoxia and hypoxia for 24 h.
Results: Both OLNBs and OLNDs adhered on staphylococcal cell walls and showed shortterm (up to 4 h) antibacterial activity against MRSA, due to chitosan presence as antimicrobial compound in the shell. OLNBs, as well as OLNDs were not toxic to keratinocytes, whereas oxygenfree nanobubbles (OFNBs) and oxygenfree nanodroplets (OFNDs) slightly affected hypoxic cell viability, thus suggesting a protective role for oxygen on human skin cells. Nanodroplets are able to release from the core more oxygen than nanobubbles, so we focused attention on this nanocarrier. A new OLND formulation was prepared using low molecular weight (LW) chitosan for the shell manufacturing. Interestingly, LW OFNDs, compared with OFNDs, were not toxic to keratinocytes and LW OLNDs (in contrast with OLNDs and OFNDs), as well LW OFNDs, are internalized by MRSA.
Conclusions: Chitosan-shelled OLNBs and OLNDs development has the potential to be a highly innovative, cost-effective and non-toxic product for the concurrent treatment of hypoxic infected tissues. Such treatment could represent an innovative therapy of infected chronic wounds improving the patient quality of life, especially the elderly. Based on these data, future studies on chitosan nanodroplets, especially manufactured with LW chitosan, are heavily encouraged.

P102

INNOVATIVE FORMULATION FOR TOPIC ANTIMICROBIAL DRUG RELEASE AGAINST GRAM-NEGATIVE BACTERIA

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Introduction: Skin infectious diseases represent a global issue costing millions of dollars per year in developed countries. Antibacterial therapies are generally considered the gold standard to treat overt infections. However, the limited efficacy of transdermal drug delivery are currently driving the attention of researchers towards new approaches to improve the topical release of active principles. In this context, an interdisciplinary study (Politecnico of Torino, Drug Science and Technology Dept., Public Health & Pediatrics Dept.) was aimed to develop innovative eudermic formulations based on reservoir of antimicrobial drugs (i.e. amikacin, AMK), allowing a sustained and prolonged release. The study is based on the use of porous biocompatible particles (i.e. silica), inside of which the active antimicrobial drug is incorporated. The active ingredient is released from the pores of the particles with a mechanism similar to the drug dissolution in solution. The technology is protected by a European patent (Eudermic compositions,WO2012/007906) owned to the Politecnico of Torino. The role of Politecnico of Torino and the Drug Science and Technology Dept. was the engineering of the reservoir that is the synthesis of porous particles, the incorporation of the drug and the characterization of the final system. The role of the research group of the Public Health & Pediatrics Dept. was to determine the antimicrobial efficacy and the prolonged release time of the new formulation (silica particles coupled to 5% AMK) in comparison with the commercial product Dramigel 5% AMK (Morgan, VI).

Materials and Methods: The antimicrobial activity of AMK saturated formulation, and Dramigel was evaluated by MIC and MBC towards the main Gram negative bacteria involved in skin infections.

In vitro AMK release from the formulation and Dramigel was determined in Franz diffusion cells. The receptor fluid was removed at specific times until 48h. AMK concentration in the receptor fluids was determined by a microbiologic agar-diffusion inhibition assay of the growth of test bacteria to obtain a linear relationship between diameter of the inhibition halo and AMK amount.

Results: The release of AMK from the new formulation was considerably more prolonged (48h) than that observed for Dramigel (24h), with AMK amount more than double in comparison with Dramigel at the same observation time.

Conclusions: The approach of surface reservoirs of drug in the topical administration will be an interesting alternative to the existing therapeutic strategies. The achievement of a constant concentration of active ingredient on the skin for a controlled and prolonged time would allow a reduction in applications of the dermatological product, with benefits in the treatment and patient compliance.

P103

IN VIVO AND EX VIVO COMPARISON OF ANTIMICROBIAL EFFECTIVENESS OF PHOTON-INDUCED PHOTOACUSTIC STREAMING (PIPS) ON ROOT CANAL DISINFECTION WITH Er:YAG LIGHTWALKER® LASER DEVICE

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Introduction: Apical periodontitis is most commonly caused by an infected root canal system subsequent to pulp necrosis. The primary goal of endodontic therapy is to attempt to disinfect the root canal, thus providing an environment for apical healing. One important aspect of successful treatment involves the irrigant selected as well as how it is delivered and agitated. Laser-activated irrigation has been found to enhance the irrigation efficacy of NaClO. The Er:YAG(Erbium-doped solid-state laser system) has become an established tool in dentistry: LightWalker's engineering has made it the world's fastest-cutting Erbium laser with an improved clinical efficacy. PIPS (photon-induced photoacoustic streaming) harnesses the power of the Er:YAG laser to create photoacoustic shockwaves within the cleaning and debriding solutions introduced in the canal by enhancing their effectiveness. The aim of this study was to evaluate through *ex vivo* and *in vivo* assays the antibacterial effectiveness of PIPS/ Er:YAG laser LightWalker compared with standard irrigation.

Materials and method: *Ex vivo* study: 22 human single-root teeth with fully formed apex were infected with *Enterococcus faecalis* for 3 weeks to allow penetration into dentinal tubules. Then, all root canals were filled with irrigant solutions and either exposed to laser irradiation by an Er:YAG laser LightWalker (PG, PIPS group) or only treated with standard needle irrigation (CP, Control group). The residual bacterial count was then evaluated. *In vivo* study: 28 patients diagnosed with pulp necrosis and apical periodontitis were recruited to this study and randomly assigned to PG or CP. Bacterial samples collected before and after irrigation treatment were cultured on selective and differential plates to detect viable microorganisms. After anaerobic (2 weeks) or aerobic (24-48 h) incubation, the colonies were counted and Gram-stained. Kolmogorov-Smirnov test for normality was used to analyze normally distributed data. Differences among groups were analyzed with Mann-Whitney test ($p < 0.05$).

Results: Pips endodontic irrigation appears to be more effective in reducing bacterial load in the root canal compared with standard irrigation, both *ex vivo* and *in vivo* models, above all for Gram negative obligate anaerobe bacteria isolated *in vivo* study ($p = 0.04$).

Conclusion: The results of this study showed that the PIPS method performed with Er: YAG laser LightWalker's engineering could inhibit bacterial growth and could be a promising endodontic tool for root canal disinfection, in order to simplify operating protocols and reduce instrumentation times.

P104

IN VITRO PHOTO-KILLING OF HELICOBACTER PYLORI WITH AN INNOVATIVE LED-BASED DEVICE

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Introduction: Nowadays, the antibiotic treatment of *Helicobacter pylori* infection has become increasingly ineffective due to the onset of antibiotic resistance. To overcome this issue, antimicrobial Photodynamic Therapy (aPDT) represents a promising therapeutic strategy. aPDT uses harmless light sources with a photosensitizing dye for the killing of several pathogens by producing cytotoxicity via generation of reactive oxygen species. The advantage of aPDT for *H. pylori* eradication is that this bacterium naturally produces endogenous porphyrins as photosensitizers. In the framework of the project "CapsuLight", devoted to the realization of an ingestible LED-based robotic pill able to perform in situ irradiation without invasive endoscopic apparatus, the effectiveness of H. pylori photo-killing were evaluated. Moreover, the main components of *H. pylori* porphyrin content responsible of its photosensitivity were identified.

Materials and Methods: Two *H. pylori* strains (ATCC 43504 and the virulent ATCC 700824) were used. From frozen stocks, both strains were grown in Brucella Broth (BB) with 10% heat-inactivated fetal bovine serum (FBS) and incubated at 37°C in the dark under microaerophilic condition. For the photo-killing assays, an aliquot of a bacterial suspension was transferred to a 35 mm Petri plate, and subjected to illumination by means of a device equipped with LEDs (at $\lambda_{405\ nm}$ or $\lambda_{625\ nm}$) for different time intervals in microaerophilic atmosphere. The illumination efficacy compared to the dark control was assessed by plating serial dilutions of each sample onto Brucella agar plates with 10% FBS and by viable cell counting after 5 days of incubation. Compositional analysis of *H. pylori* endogenous porphyrins was performed by HPLC-MS (High Performance Liquid Chromatography-Mass Spectrometry) on bacterial extracts.

Results: In both *H. pylori* strains, the illumination for 20 minutes with LEDs at 405 nm determined a reduction of about 2 log in bacterial count (CFU/ml), compared to the dark control, whereas no decrease was observed after 10 minutes. Conversely, the treatment with LEDs at 625 nm induced a similar bactericidal effect after 1 hour of exposure. We also identified three different porphyrins as the main components of the raw extracts from both *H. pylori* strains and other compounds of porphyrinic nature in smaller amounts.

Conclusions: Our data suggest that the exposure to harmless levels of visible light using a novel LED-based device induced a bactericidal effect against both tested strains of *H. pylori,* including the virulent one. Hence, aPDT could be a valid alternative to conventional antibiotic therapy for *H. pylori* infection.

P105

A COMBINED PHAGE-ANTIBIOTIC THERAPY AS A PROMISING APPROACH TO ERADICATE STAPHYLOCOCCUS AUREUS BIOFILM

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Introduction: The increasing prevalence of antimicrobial resistance reduces treatment options for biofilm-associated infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) thus fueling the challenging research for novel antibiofilm therapies. Phago-therapy, as a potential adjunct to conventional antibiotics, represents a valid alternative to the antibiotic monotherapy for the treatment of bacterial biofilm. Lytic bacteriophages are promising due to their rapid bactericidal activity, the ability to degrade biofilm matrix and lytic activity also against multi-drug resistant bacteria and persisters cells. In this study, we investigated the synergistic activity of a commercially available lytic bacteriophage Sb-1 in combination with different antibiotics against MRSA biofilm.

Material and methods: Rifampin, fosfomycin, vancomycin and daptomycin were tested alone and in combination with *S. aureus* bacteriophage Sb-1, against MRSA ATCC 43300. MRSA biofilm formed on porous glass beads for 24h, was sequentially exposed, first to different titers of bacteriophages, ranging from 10^2 to10^5 plaque-forming units (pfu)/ml for 24h and then, it was exposed to subinhibitory concentrations of antibiotics. Isothermal microcalorimetry was used to evaluate the presence of bacteria still viable in biofilm after the incubation with phages and antibiotics. The minimal biofilm eradication concentration (MBEC) defined as the lowest antimicrobial concentration required to eradicate the biofilm (0 cfu/bead on plate counts), was evaluated by isothermal microcalorimetry and CFU/ml counting.

Results: Higher values of MBECs were observed when rifampin, fosfomycin, daptomycin and vancomycin were tested against MRSA biofilm (256 µg/ml, > 4096 µg/ml, 128 µg/ml and 2048 µg/ml, respectively). While, the obtained Sb-1 MBEC was 10^7 pfu/ml. A synergistic effect was observed when 10^5 Sb-1 was added to subinhibitory concentrations of vancomycin, rifampicin, fosfomycin and daptomycin (32, 16, 64 and 4 µg/ml, respectively). Interestingly, this effect was observed only when a sequential exposure (first phages and then antibiotics) was used to treat MRSA biofilm. No synergistic effect was observed when phages and bacteria where co-incubated at the same time with the biofilm.

Conclusions: While MBECs of antibiotics against MRSA biofilm were above drug concentrations achievable in clinical practice, the co-administration of Sb-1 and antibiotics in a sequential exposure strongly reduced the antibiotic doses needed to eradicate MRSA biofilm. Therefore, the use of bacteriophages and antibiotics in combination might represent an effective strategy to treat biofilm-associated infections.

P106

BACTERIOPHAGE EFFECTIVENESS IN HARD SURFACES DECONTAMINATION

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Objectives: Persistently contaminated hospital surfaces represent a reservoir of infectious pathogens, hence contributing to the development of healthcare associated infections (HAI), which are often sustained by antibiotic-resistant pathogens. So far, decontamination of hospital surfaces was performed by conventional disinfectants, that cannot prevent recontamination phenomena, which occur in about 30 minutes. Previous studies suggested that specific bacteriophage mixtures could be used to reduce bacterial viability on food, thus the present study was aimed to determine the effect of phage treatment as decontaminating agents on hard surfaces, specifically targeting hospital pathogens.

Methods: Both Gram positive and Gram negative bacteria were included in the study, focusing on strains most frequently detected on hospital surfaces, based on previous observations [Caselli E. et al., PLoS One 2016]. Bacterial strains were applied on sterile ceramic tiles and plastic surfaces, at a concentration similar to what detected on hospital surfaces. Namely, cultures of *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), and *Proteus mirabilis* (ATCC 29906) were separately seeded on surfaces at 10^2 or 10^3 CFU per 24 cm^2 (corresponding to 4,000 or 40,000 CFU/m^2), and dried. Phage mixtures (*Staphylococcal phage* and *Pyophage*; Eliava Institute) were then applied uniformly on surfaces after dilution in saline at 1, 10, 100 and 1000 multiplicity of infection (m.o.i.; phage PFU/bacterial CFU ratio), and incubated at room temperature for 0.5, 1, 3, 6 and 24 hours. Bacterial survival was determined by standard CFU count after applying surfaces contact Rodac plates containing the selective media for the analyzed bacterial strain. All assays were also repeated by diluting phage preparations in conventional detergents at work dilution, to ascertain their usability during the usual sanitation procedures.

Results: Both used phage mixtures reduced bacterial CFU on artificially contaminated surfaces 70-90%, depending on the used m.o.i., after 1 hour of incubation, compared to what detected on control surfaces (treated with phage diluent only). After 6 hours, almost no survivors were detected, and the results were maintained in the subsequent 24 hours. Phages activity was dose-dependent, but no statistically significant differences were observed between 100 and 1,000 m.o.i. The results were similar for all the tested bacteria. Notably, phages retained their 100% activity when diluted in detergents commonly used for hospital surface sanitation.

Conclusions: Our data indicate that bacteriophages are active in decontaminating *in vitro* dry hard surfaces, acting against pathogens levels similar to those detected *on field* on hospital surfaces. They are active within 1 hour at room temperature, and maintain their full activity when suspended in conventional detergents at work dilution. These features render phage mixtures suitable for use as sanitizing agents, and, especially in consideration of the high proportion of antibiotic-resistant isolates on hospital surfaces, open the way to the development of innovative products for the effective elimination of nosocomial pathogens *on field*.

P107

CHARACTERIZATION OF PERSISTER CELLS OF *STAPHYLOCOCCUS AUREUS* AND *PSEUDOMONAS AERUGINOSA* GENERATED BY CHEMICAL TREATMENT

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Introduction: Persister cells (PCs) are a small subpopulation of non-growing, metabolically dormant bacteria able to survive lethal doses of antibiotics. Generation of PCs *in vivo* accounts for the recalcitrance of most chronic infections to antimicrobial treatment and demands for the identification of novel therapeutic strategies. Unfortunately, the low frequency of PCs makes it difficult to isolate the persister population from bacterial cultures and complicates the study of persistence and the development of anti-persister drugs. Aim of the present study was to generate *in vitro* PCs of *Pseudomonas aeruginosa* and *Staphylococcus aureus* at high efficiency by treatment with the uncoupling agent carbonyl cyanide *m*-chlorophenylhydrazone (CCCP), and to characterize in detail their metabolic profile and susceptibility to antibiotics.

Materials and methods: Stationary-phase cultures of *S. aureus ATCC 33591* and *P. aeruginosa ATCC 27853* were exposed to CCCP to induce persistence. Amount of CCCP-induced persisters was determined based on their survival to antibiotic treatments. Metabolic profile of CCCP-pretreated cells was characterized by measuring bacterial heat production through isothermal microcalorimetry and by evaluating oxidoreductase activity through flow cytometry. Time required for the revival of CCCP-pretreated cells was assessed by monitoring bacterial heat production and growth upon CCCP removal and introduction of fresh nutrients. Finally, susceptibility of CCCP-induced persisters to different antibiotics was evaluated after the revival in terms of MIC and MBC values.

Results: Exposure to optimized concentrations of CCCP significantly increased the tolerance of *S. aureus* and *P. aeruginosa* to different classes of antibiotics with only a minor effect on cell viability. CCCP-induced persisters displayed a global reduction in metabolic activity as stated by the substantial decrease in heat production and the lower reductase activity compared to the untreated control. After CCCP removal, induced persisters exhibited a delay in heat production and a lag phase before resuming replication at a normal rate. Resumption of normal growth coincided with their reversion to an antibiotic-sensitive phenotype, indicating a correlation between loss of CCCP effect and sensitization to the action of antibiotics.

Conclusions: High antibiotic-tolerance, reduction in metabolism and reversion to a normal-growing, antibiotic-sensitive phenotype after CCCP removal confirmed the development of the persister status in CCCP-pretreated bacteria. CCCP treatment emerged as a suitable method to induce persistence at high efficiency in *S. aureus* and *P. aeruginosa* to be exploited for the evaluation of the anti-persister properties of novel antimicrobials.

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P108

A DUAL ROLE OF ACTIN-LIKE FTSA IN BOTH PERIPHERAL AND SEPTAL PEPTIDOGLYCAN SYNTHESIS IN STREPTOCOCCUS PNEUMONIAE

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Introduction: *Streptococcus pneumoniae* (*pneumococcus*) is a clinically important oval-shaped Gram-positive human pathogen for which new therapies against unexploited essential targets, such as growth and division, are needed. Similar to the rod-shaped model organisms *Escherichia coli* and *Bacillus subtilis*, pneumococcus grows by alternating cycles of peripheral and septal peptidoglycan (PG) synthesis. In the model rods, these processes are organized by two specialized actin-like proteins, MreB and FtsA, that organize the PG biosynthetic complexes required for lateral elongation and cell division, respectively. It is unknown how cocci, which lack MreB, drive their lateral elongation and coordinate it with cell division. In this work we characterized the phenotypic effects of FtsA inactivation in *S. pneumoniae*, confirming its essentiality for viability. In contrast to the model rod-shaped bacteria, inactivation of FtsA results in cell lysis rather than cell filamentation, indicating a role of FtsA in both growth and division and validating FtsA as an antibacterial target.

Materials and Methods: *ftsA* conditional lethal mutants were generated by error-prone PCR and insertion/deletion mutagenesis. Fusions to GFP and other fluorescent proteins were generated in the pJWV25 plasmid, inserted by transformation at the ectopic chromosomal *bgaA* locus, expressed under the P_{Zn} inducible promoter and visualized by fluorescence microscopy. Sites for PG synthesis were determined by fluorescent D-ala-D-ala staining. Protein-protein interactions were detected using the bacterial two-hybrid system.

Results: Partial depletion of FtsA perturbed septum synthesis and resulted in elongated cells with multiple FtsZ rings that fail to complete septation, while complete depletion of FtsA resulted in delocalization of FtsZ rings, dispersion of PG synthesis, and ultimately cell ballooning and lysis. Overproduction of FtsA stimulated septation and suppressed division defects caused by inactivation of other cell division proteins, SepF and GpsB, under some conditions, supporting the notion that FtsA shares overlapping functions with both proteins during later cell division stages. Heterologous expression of MreB fully suppressed the enlarged cell phenotype of an *ftsA* thermosensitive mutant at the permissive temperature (28˚C), and partially suppressed the cell rounding and lysis phenotype at the nonpermissive temperature (40˚C).

Conclusions: Our results support a model in which peripheral and septal PG synthesis complexes in *S. pneumoniae* are both coordinated by FtsA at midcell. This dual function in cell growth and division explains how, in oval-shaped cocci like *S. pneumoniae*, *FtsA* likely functionally replaces MreB, which was lost during the transition from rod to ovococcal shape.

P109

MICROBIOLOGICAL CHARACTERISATION OF CANDIDA SPP. FROM BLOOD CULTURES OVER SIX YEARS

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Introduction: Candida is an important cause of bloodstream infections (BSI) in nosocomial settings causing significant mortality and morbidity. Moreover, inadequate initial antifungal treatment has been associated with increased mortality in patients with candidemia . The most recent guidelines for treatment of fungal infections suggest echinocandins as first line drugs in case of candidemia. We assessed whether Candida isolates from blood were sensitive to fluconazole, thus precluding this choice as empirical therapy.

Materials and methods: Candida isolates from blood obtained from 2011 to 2016 were reviewed. A descriptive statistics was applied. The isolates were identified using the Vitek-2 System (bioMeríeux, Italia) and by Maldi-TOF MS (Matrix-assisted Laser Desorption Ionization Time-of-Flight/Mass Spectrometry) (bioMeríeux, Italia). Antifungal susceptibility was performed by using the Vitek-2 System (bioMeríeux, Italia).

Results: 45 patients displayed *C. albicans*, 14 *C. parapsilosis*, 3 *C. tropicalis*, 5 *C. glabrata* (12 during the year 2011, 9 in 2012, 21 in 2013, 13 in 2014, 8 in 2015, 11 in 2016). The majority of isolates were obtained at the intensive care unit in 40/64 (62%) patients. Only in one case, MIC for fluconazole was 4µg/ml. In all the remaining cases the isolates (any species) were sensitive to fluconazole.

Conclusions: Since isolates obtained over recent years were mostly sensitive to fluconazole, sparing echinocandins for empirical treatment of candidemia could be a viable option. Analysis of all species for the remaining drugs using different Minimum Inibitory Concentraton (MIC) cut-offs is ongoing.

P110

A NEW METHOD FOR SEMEN CULTURE

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Introduction: Infertility is becoming health worldwide problem. The social, economic and psychological impact regard about 20% of Italian couples. This *phenomenon* is due to a plethora of different causes. Among them, it is estimated that genital tract infections are associated to about 8-35% of male infertility cases. Semen culture is the gold standard as diagnostic tool for infection as a probable cause of infertility. Due to the possibility of diagnosis failure in terms of infection evolution, a prompt detection is mandatory. In order to improve the quality of semen culture investigations, we combined the standard microbiological semen culture method with an alternative new experimental microbiological procedure standardized in our laboratory.

Materials and methods: Semen samples of 540 infertile men, with age between 30 and 45 years, with corresponding sterile urine cultures, where simultaneously analyzed using the standard microbiological method and an alternative one. Semen samples were diluted with sterile saline solution at 1:10 and centrifuged at 1500 rpm for 15 min. The sediment (10 µl) was seeded on Columbia CNA Agar with 5% Sheep Blood, MacConkey agar, Thayer-Martin agar, Gardnerella Selective Agar with 5% Human Blood, Chocolate agar and Sabouraud agar. Media were incubated for 24 to 48 h in atmosphere supplemented with 5% of CO_2 at 37°C. Our technique requires an extra step in the semen processing which consists in enriching an aliquot of the semen pellet (250 µl) in Brain Heart Infusion broth, BHI, (500 µl) and incubate at 36° ± 1° C for 24 hours. After 24 hours, the turbid broths are plated as described above and incubated at the same temperature in aerobic/microaerobic conditions, while if the broth is still clear it needs to be re-incubated for further 24 hours and then eventually cultured. The bacterial isolates were identified and characterized using Vitek II.

Results: Out of the 540 semen specimens cultured, when BHI media was used to enrich the microbiological culture of the semen samples, a

total of 220 (40,7%) specimens resulted positive respect to 58 (11%) obtained with the standard method. Among the 540 specimens, 262 samples did not yield any bacterial growth. In agreement with the literature, our findings show that between Gram positive bacteria responsible of semen infections, the most frequently isolated is *E. faecalis* (21.5% by the alternative method vs. 7% by the standard method), while among the Gram negative bacteria is E. coli (10.7% by the alternative method vs 3.3% by the standard method).

Conclusions: Our results show that the application of our protocol to detect bacteria from semen samples confer a higher sensitivity and specificity compared to the standard one.

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FOSFOMYCIN MINIMAL INHIBITORY CONCENTRATION (M.I.C.) EVALUATION IN *PSEUDOMONAS AERUGINOSA*: VITEK2 SYSTEM AND GRADIENT TEST COMPARING

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Background and Objectives: Fosfomycin is a bactericidal antimicrobial agent active against a range of Gram-negative bacteria, including multidrug-resistant species (MDR). For this reason the fosfomycin is re-evaluated as a treatment of multidrug-resistant bacteria infections. However, M.I.C. differences have been described among different susceptibility testing. VITEK2 System (Bio-Merieux) is a semi-automated microbiology system utilizing growth-based technology and it is a widely used commercial antimicrobial susceptibility test system. Gradient Test is a M.I.C. evaluation method by diffusion in solid culture medium. The aim of the study was to evaluate the susceptibility to fosfomycin of *Pseudomonas aeruginosa* isolates and to compare performance of the automated Vitek2 system and Gradient Test (Liophilchem).

Materials and Methods: We collected twenty *P. aeruginosa* strains isolated from different samples of patients attending to Policlinico Umberto I Hospital of Rome. Some strains exibited pattern of resistance to antimicrobials (MDRs) as showed by M.I.C. determined by Vitek2 System. Bacterial isolates were used to compare M.I.C. value to fosfomycin by Vitek2 System and gradient test performed on Mueller Hinton Agar with antibiotic (fosfomycin) and glucose-6-phophate. The M.I.C. values of the two methods are compared and analysed by regression test.

Results: Results showed that two utilized methods are in agreement: infact in all tested strains the M.I.C. values of fosfomycin presented small discrepancies without very major error based on sensitivity and resistance ranges established by the Clinical and Laboratory Standards Institute (CLSI) for Pseudomonas aeruginosa. Comparison between the two methods revealed a number of differences

in cost, preparation, reliability, and timing for results. Moreover, from the data emerged a frequently susceptible to fosfomycin among MDR *P. aeruginosa* strains.

Conclusion: Preliminary results obtained by Vitek2 system provided comparable results to gradient test with quick turnaround for the testing of *P. aeruginosa* susceptibilities. Moreover fosfomycin represents a useful antibiotic not only in MDR Enterobacteriaceae strains but also in *P. aeruginosa* MDR strains. Results show that there is a need to upgrade automatic systems to establish fosfomycin M.I.C and the gradient test can be considered an alternative.

P112

USE OF SINGLE BLOOD CULTURES IN POST-MORTEM MICROBIOLOGY INVESTIGATIONS

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Introduction: Post-mortem microbiology is a useful tool to determine the cause and manner of death. This is particularly helpful in difficult forensic cases such as sudden unexpected death or death caused by healthcare-associated infections. The heart blood and cerebrospinal fluid are the most useful specimens for postmortem microbial cultures, although other organs as spleen or liver are used for microbiological investigations. The bacteremia is detected when two or more blood cultures obtained from different sites are both positive. The aim of this study is to determine whether a post-mortem single blood culture can be used to detect true bacteremia.

Material and Methods: We investigated 25 autopsy cases performed with standard techniques and a single blood sample collected as soon as the thoracic cavity was opened. A 10 ml syringe has been introduced, depending on cases, in major vessel or directly in the cardiac cavity. Then blood has been aspirated. BacTAlert 3D bottle for aerobic and anaerobic bacteria has been filled with the cadaveric blood. Sub-cultures have been carried out in every positive bottles, and we have considered negative results after one week of incubation. For each of the sample a Gram staining has been performed before and after the cultures.

Results: We analyzed data about age, gender, ante-mortem diagnosis and post-mortem cultures results, for every single case of those 25 considered. The mean age of all patients was 54.6 years, two cases regarded intrauterine death. Gender ratio was M:F = 20:5. All the cases with negative post-mortem cultures didn't have any pre-mortem evidences of infection. Eleven post-mortem cultures were found positive and, among these, only two subjects had a pre-mortem diagnosis of sepsis. In six positive cases

was highlighted a polymicrobial growth. Finally, five cases without pre-mortem diagnosis of sepsis showed the growth of a single microbe species.
Discussion: This is the very first study to investigate the usefulness of forensic microbiological analysis of a single cardiac blood sample. Our preliminary data showed that a post-mortem single blood sample cultures might provide useful information in order to evaluate the presence of microbial growth, especially in cases of healthcare-associated infections. Obviously, the interpretation of these results must be integrated by circumstantial, clinical autopsy, and histological findings. Moreover, it is important to analyze critically the microbiological results taking into account possible bacterial contamination. In this regard we highlight that it is crucial to perform post-mortem microbiological analysis on a road range of samples.

P113

INTEGRATED MICROBIOLOGICAL APPROACH FOR BLOODSTREAM INFECTION DIAGNOSIS AND ANTIMICROBIAL MANAGEMENT

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Introduction: The incidence of bloodstream infections (BSI) is progressively increasing with high morbidity and mortality rates, configuring BSI as one of the most important Public Health issues worldwide. The initial BSI antimicrobial treatment is empirical, therapy should be revalued after Gram stain results, microorganism's identification and, after 24-48h with conventional phenotypic methods, on its of antimicrobial susceptibility profile. Delays in both bacteria identification and antimicrobial susceptibility profile may impact on patient's outcome leading to an inappropriate or unnecessary antibiotic use. We have evaluated an integrated microbiological approach that combine rapid identification of microorganisms, epidemiological antimicrobial susceptibility profile and active surveillance cultures in the management of patients with BSI in attendance for definitive identification and antimicrobial susceptibility testing (AST) results from conventional methods. **Materials and Methods:** From November 2016 to January 2017, positive monomicrobial blood cultures for bacteria only were selected at S.C. Microbiologia e Virologia U., Città della Salute e della Scienza di Torino. Rapid identification was performed by MALDI-TOF MS after short incubation on solid medium. Epidemiological antimicrobial susceptibility profile was obtained for the 3 most frequent isolated microorganisms through Mercurio software data extraction of the previous 10 months from Laboratory Information System. Patient's multidrug-resistant (MDR) bacteria colonization was obtained from rectal surveillance swab in the previous 3 months. **Results:** 316 monomicrobial blood culture were selected from 134 patients. The 3 most frequent isolated bacteria were *E. coli, S. aureus, K. pneumoniae*. Rapid identification protocol concordance with the reference method was globally of 94% at species level. 503 AST were extracted and gathered on the basis of the requiring

wards to reach more reliable data. 137 surveillance cultures from rectal swab from 55 patients were analysed: a statistically significant association between MDR colonization status and BSI caused by MDR bacteria was observed. Combining these microbiological data > 99% of patients received crucial information for antimicrobial therapy management.

Conclusions: Bacteria rapid identification combined with epidemiological AST and patient's MDR bacteria colonization status are powerful and easily extractable tools for BSI antimicrobial therapy management in the expectation of definitive phenotypic identification and AST. However, Gram stain results and the number of blood culture sets sent to the laboratory are still fundamental data for BSI microbiological results interpretation.

P114

EVALUATION OF THE ALLPLEX™ GASTROINTESTINAL PANEL ASSAY FOR THE DETECTION OF ENTERIC PATHOGENS

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According to WHO globally every year, about 2 billion people suffers from diarrhea due to gastrointestinal infections and infectious diarrhea is the second leading cause of deaths in children under 5 yrs old. Conventional culture or microscopy based diagnosis, highly variable, insensitive and labor-intensive does not identify 80% of pathogens. In this study a total of 234 stool samples have been analyzed using the Gastrointestinal Panel Assay (GIP, Seegene, Korea), that detects and identifies 25 GI pathogens including 6 viruses, 13 bacteria and 6 parasites simultaneously. Parassitic infections have been also evaluated by microscopic analysis.

The detected pathogens have been, *Campylobacter* (2,9%), *C. difficile* (2,5%), Salmonella (1,7%), Yersinia (1,2%), enteropathogenic *E. coli* (1,7%), enterotoxigenic *E. coli* (0,4%), enteroaggregative *E. coli* (2,1%), *Giardia lambia* (0,4%), *Blastocystis hominis* (8,5%), *Dientamoeba fragilis* (7,2%), Norovirus (0,8%), Rotavirus (0,8%); 11 samples showed a coinfection with *Blastocystis* and *Dientamoeba* (4,7%), and one sample a coinfection with *Campylobacter* and enteropathogenic *E.coli*. The microscopic analysis identified only 30% of the parassitic infections and none coinfection.

Overall GIP showed high detection rates of GI pathogens, including coinfections. This sensitive and convenient system will prove to be an invaluable tool for the rapid diagnosis of most GI.

P115

RAPID METHOD FOR THE IDENTIFICATION AND ANTIFUNGAL SUSCEPTIBILITY TESTING OF YEAST FROM POSITIVE BLOOD CULTURES

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Introduction: *Candida* spp. are the fourth most commonly isolated microorganisms from blood cultures (BC). Different rapid methods have been recently described that are able to directly identify yeast from positive BC, showing concordant identification results with those obtained after growth on solid media in a percentage ranging from 56 to 95.9%. Despite identification results can provide indication about empirical antifungal treatment, there is a pressing need for a rapid antifungal susceptibility test. For this purpose, this study aimed at assessing a rapid and reliable method for the identification and antifungal susceptibility testing (AFST) of yeast from positive BC bottles.

Materials and Methods: One hundred and twenty four positive BC collected at the SD Universitaria di Microbiologia of Pisa University Hospital were analysed. For each patient, only the first positive BC that resulted monomicrobial for yeast after Gram staining was included in this study. An aliquot of the pellet of properly treated blood samples was laid on the MALDI-TOF mass spectrometer (MS) target plate, exposed to a protein extraction protocol, and subjected to identification. All the identification results with at least 4 identical matches in the pathogens list were considered correct. Next, the pellet was used for assessing the AFST. Identification and AFST results by the rapid method were compared with those obtained by MALDI-TOF and AFST on isolated colonies. Differences in MICs within ± 1 two-fold dilution were considered concordant.

Results: All the collected samples were analyzed both for identification and AFST. The identification results obtained with the rapid method were concordant with the results obtained from yeast grown on solid media for 109 of 124 (87.9%) blood samples. Results were not reliable for 13/124 (10.5%) samples, for which no identification was obtained. In addition, in two cases (1.6%), yeast were concordantly identified only at genus level. In the latter two cases, score values were lower than 1.3. Yeast were identified with scores ranging from 1.300 ≤ 1.699 for 41 (33.1%), 1.700 ≤ 1.899 for 43 (33.9%) and ≥ 1.900 for 25 (20.2%) by the rapid method. Rapid AFST gave 100% concordant results with those obtained from yeast isolated on solid media, among which the 25.8% differ from colony susceptibility profiles for only one two-fold dilution.

Conclusions: The rapid method for identification and AFST herein described provided concordant identification and susceptibility profile for 88% of the isolates in 24 hours upon BC positivity. Furthermore, this method yielded correct AFST results even when the rapid MALDI-TOF MS results were not obtained.

P116

A NEW, FAST AND RELIABLE TECHNIQUE FOR QUANTIFICATION OF INTRACELLULAR BACTERIA BY IN-CELL WESTERN ODYSSEY ASSAY

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Introduction: Several pathogens including *Shigella* spp., *Salmonella enterica, Listeria monocytogenes, Mycobacterium tuberculosis* and Uropathogenic *Escherichia coli* (UPEC) are known to invade and colonize host cells, escaping from host cell defense mechanisms. These infections can be challenging to treat, since their intracellular life-style often protects pathogens from immune system effectors. To facilitate fast quantification of bacterial invasion and intracellular proliferation, an high-throughput screening method to replace the traditional colony forming units (CFU/ml) counting is needed. Our study was aimed at adapting the "In-Cell Western™" assay to quantify intracellular bacterial pathogens.
Materials and Methods: *Shigella flexneri* 5a M90T strain was chosen to set up the technique using the Odyssey CLx Infrared Imaging System (LI-COR). Sera against *S. flexneri* type V and labeled antibody IRDye 680 RD Goat Anti-Rabbit were used as primary and secondary antibodies, respectively. The conventional counting procedures, CFU/ml, were performed.
Results: A well-established bacterial infection model was chosen to set up this technique: *S. flexneri* 5a str. M90T in the classic HeLa cell invasion model. In this time course experimental model, Hela cells at the 70% of confluency are infected with the M90T strain at a multiplicity of infection (MOI) of 100. Different time points after the addition of gentamicin were measured. Multi-well plates were immunostained and the CFU/ml were recorded. To maximize the specific signal and minimize the background, different concentrations of both primary and secondary antibodies were tested. At the end of the experiment, the plates were scanned at low and at the highest resolution by Odyssey Infrared Imager, and the numerical integrated fluorescence intensity values of each well, expressed as arbitrary units (a.u.), were recorded by LI-COR Image Studio Software. To eliminate the background, the fluorescence intensity values of uninfected cells were subtracted. Currently, we are defining the formula to correlate the fluorescence a.u. versus its relative traditional CFU/ml counting.
Conclusions: The method described in this study provides new insights into the quantification of intracellular bacteria by offering several benefits such as accuracy, sensitivity, specificity, processivity and speed. This method will allow the enumeration of facultative intracellular bacteria, characterized by high and low replication rates, as well as of obligate intracellular bacteria, even in different forms of the cell cycle. Overall, our method is a useful approach for both clinical and research investigations.

P117

SCREENING OF AZOLE RESISTANCE IN BLOOD CULTURE ISOLATES OF *CANDIDA* SPP.: COMPARISON AMONG DIFFERENT DIAGNOSTIC SYSTEM VS GOLD STANDARD METHODS

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Introduction: *Candida* species are ubiquitous yeasts; they are found on many plants and are members of the normal flora of the alimentary tract of mammals and mucocutaneous membranes of humans. *Candida* sp. are regarded as a human symbiont; in fact, they can be isolated routinely from the oral cavity, genitourinary and respiratory tracts of most individuals. As an agent of serious infection, it has been associated with endocarditis, meningitis and, multifocal, disseminated disease. Acquired and /or intrinsic resistance to azole antifungal agents has been described and represents an increasing problem in Europe, USA and China. Fluconazole, due to its broad spectrum, high efficiency, good bioavailability and safety profile, is recommended by the Infectious Disease Society of America Candidiasis treatment guidelines (2016) for treating Candida infections. However, long-term or repeated treatment with fluconazole can lead to resistance, possibly leading to azole cross-resistance, which could seriously hamper the clinical treatment of candidiasis.
Aim of this work was to screen azole resistance in blood culture isolates belonging to different Candida sp. and compare the results obtained by using commercial diagnostic system or two different reference methods.
Materials and Method: Azole resistance in nineteen blood culture isolates (identified both by Vitek2 and MALDI-TOF) belonging to different Candida sp. was evaluated using Fluconazole and Voriconazole. The analysis has been performed by two commercial diagnostic systems (Etest and Vitek2) and two reference methods (EUCAST E.DEF 7.3.1 and NCCLS M44-A).
Results: Nineteen *Candida* sp. isolated from blood culture were identified as follows: seven strains of *C. albicans*, six of *C. glabrata*, three of *C. parapsilosis*, two of *C. krusei* and one *C. tropicalis*. Based on the results obtained by the standard methods, 70% of *C. albicans*, 66% of *C. glabrata* and *C. tropicalis* (only one) strains resulted resistant to both the azoles considered. These results were only partly overlapped with those obtained by using commercial method.
Conclusions: This work underline the low level of sensitivity of the commercial methods and highlight t the necessity to develop new tools in order to characterize this important traits of Candida isolates, especially those resposnsible for systemic infection.

P118

UPDATE OF PCR-BASED REPLICON TYPING (PBRT) SCHEME AND AUTOMATIC AMPLICON DETECTION

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Introduction: Plasmids are the main vectors of antimicrobial resistance genes in Enterobacteriaceae and plasmid typing is basic for the study of evolution, epidemiology and spread of antibacterial resistance. The PCR-Based Replicon Typing (PBRT) is a method for plasmid identification and typing in Enterobacteriaceae that has been updated from 2005 to date (Carattoli et al. 2005, J. Microbiol. Methods 63:219-228; Carloni et al. 2017, Plasmid 90:10-14). A PBRT-KIT is now commercially available (Diatheva, Fano, Italy) and includes all the most relevant modifications, detecting 28 different replicons (HI1, HI2, I1-α, M, N, I2, B/O, FIB, FIA, W, L, P, X3, I1-γ, T, A/C, FIIS, U, X1, R, FIIK, Y, X2, FIC, K, HIB-M, FIB-M, and FII) in 8 multiplex PCRs. Recent studies showed the epidemiological relevance of new replicons and therefore the need of an additional update of the PBRT scheme. The aims of this work were: a) the PBRT scheme implementation and validation; b) the PBRT method improvement, in terms of time consuming, using a new high-throughput instrument for the automatic amplicon detection and analysis of results.

Materials and methods: Primers were designed to target new replicons (P1, N2, FIB-KN, FIB-KQ, X4) and multiplex PCR conditions were optimized. The new PBRT scheme has been validated for specificity and sensitivity testing 37 Enterobacteriaceae strains provided by the Istituto Superiore di Sanità (ISS) in Rome, Italy. The amplicons of the new PBRT scheme were analysed both by standard electrophoresis on agarose gel and by the AATI Fragment Analyser instrument (Advanced Analytical, Ankeny, USA).

Results: The new PBRT scheme detected 30 replicons and identified them in 100% of the analysed strains. No cross-reactions, false positive or false negative results were obtained, also in strains carrying different replicons. The analysis of amplicons by AATI Fragment Analyser instrument showed a good resolution of the peaks and the possibility to combine two different multiplex PCRs, reducing the number of loadings. To this aim, T-tail primers were devised to adjust the amplicon sizes. The 8 multiplex reactions could be assembled in 5 capillary electrophoresis runs.

Conclusions: The updated PBRT scheme exhibited a good specificity and sensitivity, detecting new epidemiologically relevant replicons. The proposed method for the amplicon analysis by the AATI Fragment Analyser instrument allows the combinations of two multiplex PCRs in the same lane, resolving up to 8 peaks. This approach reduces the detection time and simplifies the electrophoresis step with an automated workflow.

P119

DETECTION OF PHAGE Φ1207.3 IN *STREPTOCOCCUS PNEUMONIAE* BY IMMUNOASSAYS TARGETING THE MAJOR CAPSID PROTEIN

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Introduction: Φ1207.3 is a prophage found in a clinical strain of *Streptococcus pyogenes* which carries macrolide resistance genes. We previously transferred the phage in *Streptococcus pneumoniae* and showed that it is able to produce mature and complete phage particles with a *Siphoviridae* morphology. The aim of this study was to evaluate the presence of phage particles associated to the host in a bacterial culture of *S. pneumoniae* by immunoassay methods.

Materials and methods: The presence of phage major capsid protein (Orf42) was evaluated by western-blot, flow cytometry and immunogold labeling. Rabbit polyclonal antibody Orf42-1 (anti-phage capsid protein) were generated using peptide antigens designed and optimized using the Optimum Antigen™ Design Tool (Genscript). *S. pneumoniae* FR1, carrying Φ1207.3, was grown to an OD_{590} of 0.1 (about 10^8 cells/ml) and was induced with 100 ng/ml of mitomycin C for 2 hours. Whole bacterial lysate was then assayed by Western Blot with a secondary goat anti-rabbit antibody conjugated with alkaline phosphatase or FITC for flow cytometry. Immunogold labeled anti-rabbit antibodies were used to visualize bacteria in Transmission Electron Microscopy.

Results: The Orf42-1 affinity purified antibody detected a specific 24 KDa peptide in the Western blot assay performed on *S. pneumoniae* FR1 lysate from a mitomycin induced culture. The lower molecular weight of this band compared to the capsid protein (44 KDa) could be due to cleavage by the Clp protease Orf41. Bacterial cultures of *S. pneumoniae* FR1 were stained with FITC-labeled secondary antibody and analyzed by flow cytometric and immune transmission electron microscopy assays. Both methods showed that the phage particles, in early exponential phase of the bacterial growth, were associated to bacterial surface.

Discussion and conclusions: The three immunoassay methods used in this work enabled the detection of Φ1207.3 both inside and on the surface of the pneumococcus. This provides the possibility to follow either phage cell cycle, the adsorption of the phage to the bacterial cell and the transfer of genetic element from one bacterium to another by transduction.

P120

PERFORMANCE OF T2BACTERIA PANEL FOR RAPID IDENTIFICATION OF BACTERIA RESPONSIBLE OF BLOODSTREAM INFECTIONS

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Introduction: Prompt start of antibiotic therapy impacts on the prognosis of patients with bloodstream infections (BSI). Evidence-based data support the use of rapid molecular methods to rapidly identify the microorganisms responsible of BSI and, therefore, to significantly improve timeliness of targeted therapy. However, most of these techniques rely on positive blood cultures (BCs), which require long incubation times and have low sensitivity in patients pre-treated with antimicrobials. Recently a new technology based on magnetic resonance (T2MR) was proposed for rapid identification of leading BSI pathogens (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa and Escherichia coli*) in 3 to 5 hours directly from whole blood. The objective of this prospective observational study was to evaluate the performance of the T2Bacteria Panel compared to BC results of prospectively collected clinical specimens.

Materials and methods: The study was performed at the Fondazione Policlinico Universitario "A. Gemelli", a 1250-bed teaching tertiary care hospital. Before running clinical study 150 spiked samples were analyzed to evaluate performance. Afterwards, patients undergoing BCs for clinical suspect of BSI admitted to the emergency room, infectious diseases wards and intensive care unit were evaluated for additional 4 ml blood collection for T2 analysis. Patients were excluded if < 18 years old, if treated < 30 days with novel investigational drug compound, and in case of BCs of the same patients belonging to the same septic episode. Sensitivity and specificity of T2 results compared to BC results were analyzed.

Results: Results on 150 spiked samples showed 100% sensitivity (69/69, 95% confidence interval 95%-100%) and 97% specificity (774/801, 95% CI 95%-98%). Forty six samples from 46 patients, 59 years-old on average (range 18-86), 24 (52.1%) of whom males, and 100% caucasian race have been included and analyzed. A sensitivity of 60% (3/5, 95% CI 15%-95%) and a specificity of 98% (247/253, 95% CI 95%-99%) were observed. Rapid time to result provides potential for significantly more rapid intervention for determining appropriate therapeutic regimen in positive cases. A preliminary analysis of discrepant results (T2 positive/BC negative) showed potential identification of true positive patients missed by culture for previous exposure to antibiotics.

Conclusions: Results obtained with contrived specimens showed excellent sensitivity and specificity. The interim analysis demonstrated good concordance between T2Bacteria Panel and clinical BC results. More data from additional patient specimens are needed to confirm these preliminary results.

P121

A PAN-GENOMIC APPROACH TO *E. COLI* CLASSIFICATION

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Introduction: *Escherichia coli* is an intestinal commensal bacterium as well as an important pathogen of humans and other warm blooded animals. The classification of E. coli into subtypes is essential to understand pathogenesis of particular clones. Despite the wide knowledge available for *E. coli*, multiple whole genome sequences comparison can enhance our understanding of what constitutes a pathotype. In this study, 147 fully sequenced *E. coli* genomes, belonging to different previously described types, were compared. The analysis of pan and core genome can provide a molecular basis for actually adopted classification or suggest new comprehensive ones within *E. coli* species.

Materials and Methods: The whole genome sequences of 147 *E. coli* were selected from NCBI Genome database. Among these *E. coli* strains 82, 38, 12 and 15 belonged to Intestinal Pathogenic (IPEC), Extraintestinal Pathogenic (ExPEC), Adherent/Invasive (AIEC) and intestinal commensal *E. coli* respectively. Each genome was *de novo* annotated and the total gene set was grouped in homologous clusters (HCs). The pan, core, and accessory genomes were determined for all strains and subgroups and functional annotation was performed. Clusterizations based on core and accessory sequences as well as on classical typing data were carried out.

Results: Starting from 732.094 genes predicted across all genomes, a pan-genome size about 21.016 HCs were determined. The pan-genome showed a stable rise as more genomes were added suggesting its belonging to an "open" type. The core-genome comprised about 10% of HCs and revealed high mean sequence similarity. The functional annotation showed that metabolic processes were mainly codified by core-genome while cellular processes, as well as signaling functions, were significantly more present in the accessory components. Although the analysis of strains, based on core and/or accessory genome, was not sufficient to fully discriminate *E. coli* pathotypes, it showed a higher resolution power respect to classical typing techniques highlighting promising genetic traits.

Conclusion: With the increasing availability of genomic data pan-genome represents a promising tool for better understand the structure of bacterial genomes and its evolution. A typing method based on core and accessory genomes, should promote knowledge on how different varieties of *E. coli* cause disease.

P122

DETECTION AND QUANTIFICATION OF BACTERIAL MEMBRANE VESICLES (MVS) BY USING FLOW CYTOMETRY

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Introduction: Gram-positive and Gram-negative bacteria produce membrane vesicles (MVs) which are involved in several mechanisms such as pathogenesis, biofilm formation, cell-cell communication, bacterial-host interactions and nutrients supply. MVs can transport a wide variety of components included nucleic acids, proteins and polysaccharides. *Helicobacter pylori* and *Lactobacillus reuteri* produce MVs associated with extracellular DNA (eDNA) in the planktonic and biofilm phenotypes. The aim of the present study was to employ the flow cytometry to identify, quantify and characterize the bacterial membrane vesicles as well as the eDNA-MVs associated in the planktonic and biofilm phenotypes of *H. pylori* ATCC 43629 and *L. reuteri* DSM 17938.

Materials and Methods: *H. pylori* ATCC 43629 and *L. reuteri* DSM 17938 biofilms formation was evaluated by using live/dead staining and fluorescence microscopy at 48 and 24 h of growth, respectively. The count of MVs and the detection of eDNA, produced by the two species, were subsequently carried out through flow cytometry on the total samples (cells plus vesicles) and on MVs from the planktonic (pMVs) and biofilm phenotypes (bMVs) isolated through ultracentrifugation. The flow cytometry analysis was performed by using a known size beaded system and two fluorescent dyes, PKH26 which selectively stains the lipids and PicoGreen that selectively stains the DNA.

Results: Fluorescence microscopy showed the formation of biofilms, characterized by the presence of well-structured towers in the case of *H. pylori* and a monolayer in the case of *L. reuteri*. The flow cytometry analysis showed a higher production of MVs by the biofilm phenotype compared to the planktonic phenotype, for both bacterial species. Moreover, the PicoGreen staining showed that most of the generated vesicles were associated with eDNA.

Conclusions: Flow cytometry was a useful tool for analyzing MVs produced by both biofilm and planktonic cultures. Moreover, the detection of the eDNA associated with pMVs and bMVs provides important information to further our understanding of the role of eDNA interactions with MVs in biofilm structures.

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PROJECTING AND BUILDING UP A PALAEOMICROBIOLOGY LAB: THE VERONA EXPERIENCE

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Introduction: Paleomicrobiology is an emerging science aimed at the discovery, identification and characterization of microorganisms in ancient specimens. Numerous data point out that microbial DNA can survive in bone and dental structures for roughly 20,000 years. The introduction of Next Generation Sequencing (NGS) technologies are making it possible to identify a number of infections by effectively overcoming the limitations of pioneering Paleomicrobiology techniques, such as microscopic and immunological methodologies, and PCR. In Italy, several Anthropology departments usually deal with ancient DNA (aDNA) belonging to microorganisms whose genome could, in principle, differ from those isolated nowadays due to either evolution or extinction, thus requiring specifying microbiological expertise. Vice-versa, Paleomicrobiology studies are few and far between in Microbiology labs.

Materials and Methods: Research on aDNA mandates the use of dedicated laboratories to deal with ancient specimens in a virtually contamination-free environment, and spatial isolation of the facilities in which different steps of the analytical procedure are performed is an essential requirement. Extensive literature research and visiting several European laboratories provided us with extensive knowledge about these peculiar features.

Results: Our laboratory consists of three independently thermostated rooms, two of which are endowed with positive pressurization. In addition, the latter two rooms are equipped with UV lamps and bleach-resistant benches in order to minimize any possible contamination. In detail, Room 1 is an entry area with cleanroom mats designated for suitable operator dressing (sterile gowns, gloves, masks, helmets), thus preventing the introduction of contaminated "modern" DNA in the other rooms. Room 2 is assigned to storage, classification, decontamination and pulverization of the ancient specimens. The samples are then transported to Room 3 using a pass-through cabinet equipped with an UV lamp, in order to avoid any cross-contamination. Room 3 is where the extraction and further analysis of aDNA is performed.

Conclusions: Over the last two years a specific Paleomicrobiology laboratory has been conceived and realized in the Microbiology Section of the Department of Diagnostic and Public Health, University of Verona. Although these studies are still not frequent in Italian Microbiology labs, we strongly support the need for a stronger involvement of our discipline in these studies, also in view of the fact that aDNA might often belong to microorganisms whose genome could, in principle, differ from those isolated nowadays due to either evolution or extinction, thus requiring specifying microbiological expertise.

P124

NOCARDIOPSIS ALBA INFECTION/COLONIZATION IN A PEDIATRIC SEVERE COMBINED IMMUNO DEFICIENCIES (SCID) EPISODE FOLLOWING ALLOGENIC BONE MARROW TRANSPLANTATION

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Introduction: Severe Combined Immuno Deficiencies (SCID) are a group of primitive immunodeficiencies that are characterized by the disturbed development of functional T cells and B cells. SCID patients are usually affected by severe bacterial, viral, or fungal infections early in life and often present with interstitial lung disease, chronic diarrhoea, and failure to thrive. We describe a case of *Nocardiopsis alba* infection in a 10 year boy affected of SCID, with recurrent pulmonary infections who had undergone successful hematopoietic stem cell transplantation.

Materials and Methods: Samples of bronchoalveolar lavage (BAL) of the patient were inoculated in Blood agar, MacConkey's agar, chocolate agar, and Sabouraud's dextrose agar (SDA) and incubated in air atmosphere at 30° and 37°C for 21 days. Identification of the isolates was done with Vitek-MS (bioMérieux- France) and it was confirmed by sequencing of the 16s rRNA. Susceptibility testing was performed with Etest.

Results: Patient was characterized by an immunodeficiency caused by mutations in IL-2Rγ, known as X-linked severe combined immunodeficiency. The condition is inherited in an X-linked recessive pattern and its expression causes a near complete failure of the immune system development and functionality, with low or absent T cells and NK cells, and non-functional B cells. BAL culture showed a growth of few colony of *Aspergillus fumigatus* and many colonies of a white aerial mycelium-producing bacterial strain, Gram-positive, aerobic, with long and branched filaments. The colony appeared waxy, and only started to poorly sporulate after 5-7 days of incubation. MALDI Vitek-MS yielded no identification. Molecular identification of the isolate gave 99% of homology with *N. alba.* Susceptibility test showed the followig (MIC mg/L): Amikacina 0.19, Amoxicillin-clavulanic acid 3, Ceftriaxone 1, Ciprofloxacin 1.5, Linezolid 0.5, Cotrimoxazole 0.064 and Meropenem 0.25.

Conclusion: *N. alba* is one of the actinomycetes frequently isolated from the honeybee guts or from bioaerosol of mushrooms compost facilities, but to our best knowledge this is the first report of a *N. alba* isolated from human samples. The role of *N. alba* in health risk for workers who are exposed has been confirmed and its role in supporting allergic symptoms in mushroom compost facility workers is under study. This actinomycete was characterized by the production of a phenazine-like redox-active molecules, reported as having good activity against several Gram-positive bacteria and that could contribute to raw honey antimicrobial properties. Further study are necessary to understand its role in medical microbiology and to find correlation between environmental transmission and pathogenicity and allergic properties of *N. alba*.

P125

BACTERIAL DACRYOCYSTITIS. ANALYSIS OF 639 POSITIVE CASES AT THE OPHTHALMIC HOSPITAL OF TURIN FROM 1990 TO 2013

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Introduction: The dacryocystistis are infections of the excretory lacrimal apparatus. There are three forms :acute, chronic, ad neonatal . The primary feature of acute dacryocystitis is the abscess of the lacrimal sac. The chronic forms are associated with chronic epiphora and conjunctival infections and suppuration. The neonatal conjunctivitis are severe and can result in orbital cellulitis, cerebral abscess, meningitis. There are few systematic studies on bacterial dacryocystitis and topical antibiotics. Our aim is to provide the clinician a diagnostic and therapeutic support.

Materials and Methods: A retrospective analysis of all patients with clinically diagnosed dacryocystitis presenting at the Ophthalmic Hospital of Turin between January 1990 and December 2013 was performed. The ocular specimens were cultured in Chocolate agar, Haemophilus agar in microaerofilia, Blood agar. *In vitro* susceptibility testing was performed with Kirby Bauer disc diffusion method and interpreted using National Committee for Clinica Laboratory Standards (NCCLS).

Results: During the study period , a total of 639 ocular spesimens with culture positive were analyzed : 86 of less than one year old patients, 337 of 1-2 year old patients, 114 of 2 -3 year old patients, 41 of 3-4 year old patients, 14 of 4-5 year old patients, 9 of 5-29 year old patients, 9 of 26-59 year old patients, 29 of 60-80 year old patients. The bacterial isolates were: *Streptococci* 347 (54.4%); *Haemophili* 110 (17.2%); *Moraxellae* 64 (10%); *Staphilococci* 55 (8.6%); *Pseudomonadaceae* 21 (3.28%); *Pasteurellae* 14 (2.2%); *Klebsiellae* 6 (0.9%); *Serratiae* 6 (0.9%).

Streptococci, Haemophili and *Moraxellae* were susceptible to Chloramphenicol (93.9%, 99.1% and 100% respectively); *Staphilococci* to Moxifloxacin, Levofloxacin and Netilmicin (95.5%, 93.2%, 92.7%); *Pseudomonadaceae* to Ciprofloxacin, Levofloxacin (90.5%, 88.9%)

Conclusions: The most common bacterial species isolated in dacryocystitis are *Streptococci*, *Haemophili* and *Moraxelle* and all are sensible to Cloramphenicol. The *Staphilococci* and *Pseudomonadaceae* are more difficult bacteria to treat and only *Fluorichinolones* for *Pseudomonadaceae* and *Fluorochinolones* and Netilmicin for *Staphylococci* are useful antibiotics.

P126

INCIDENCE OF TUBERCULOSIS IN THE CITY OF REGGIO CALABRIA: CONSIDERATIONS ON 2014-2017 EPIDEMIOLOGICAL DATA

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Introduction: Despite notable progress, tuberculosis (TB) is still a public health concern in most of the countries within the European Union (UE), mainly due to migratory flows from Africa and Eastern Europe. Is essential for addressing health interventions and resources more effectively to control this disease to comprehend the temporal trend of endemic TB, both locally and nationally, and to identify the presence of vulnerable population groups, such as people of foreign origin and immunocompromised hosts.

Materials and Methods: Pulmonary TB cases were confirmed by culture (BACTEC MGIT 960, BD) and identification and differentiation of *Mycobacterium tuberculosis* complex (MTC) and nontuberculous mycobacteria (NTM) was performed by molecular genetic assay (GenoType Mycobacterium CM, Hain LifeScience). Identification of resistance to first-line TB drugs was tested both by automated detection system of micobacterial drugs susceptibility in liquid culture (BACTEC MGIT 960, BD) and by molecular genetic assay (GenoType MTB DR, Hain LifeScience).

Results: There were 87 TB cases, starting from 2175 patients examined, between January 2014 and June 2017, by Microbiology O. U. of Reggio Calabria "Bianchi Melacrino Morelli" Hospital. During three-year period, there was an average of 12,6 cases per year of TB, so the incidence of TB has been at about 2,3 cases per year per 100,000 born in Italy and living in Reggio Calabria. 47.1% (n = 41) of TB cases were foreign origin patients versus 52.9% of the Italian' ones (n = 46). The most affected age range is 25-64 years, both in Italian and foreign populations (respectively 52.2% and 73.2%); however, within Italian population the incidence of TB is also high among over-65, while in the foreign population there were many TB cases among young adults of 15-24 years old.

There was a greater incidence of TB cases in the male population than in the female one (66.7% male cases versus 33.3% female cases). On average in over 77% of cases of TB we detected one of the MTC species, while lower was the percentage of isolation of MNTs. The incidence throughout the observed period of multidrug resistant (MDR) mycobacteria is close to zero: we only report one of a strain with only one sensitivity to the tested first-line.

Conclusions: Even if referring to a limited geographical area, our results reflect the current epidemiological situation of TB in Italy: low incidence in the general population (on average less than 10 cases per 100,000 inhabitants) and predominance of TB cases in some risk groups (like immigrant population) and in some age groups (elderly, often chronic bronchopathic). Although the rate of incidence of MDR cases fortunately has been low, as there have been significant migration flows in recent years, in our view it is necessary to keep a close eye on TB epidemiology.

P127

LONGITUDINAL STUDY ON THE EFFECT OF ORAL HYGIENE EDUCATION ON THE SALIVARY COUNT OF SPECIES WITH CARIOGENIC POTENTIAL

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Introduction: Caries of tooth enamel surfaces is particularly common in children and young adults up to the age of 20 years, whereas, in later life, root-surface caries is an increasing problem due to gingival recession exposing the vulnerable cementum to microbial colonization. *Streptococcus mutans* has long been regarded as a specific pathogen of dental caries. Nevertheless, the actual view considers dental caries as the result of a polymicrobial infection where different species synergize in the etiopathogenesis of the disease. Aim of the present study was to evaluate the effect of oral hygiene education on the colonization with 3 microbial species with cariogenic potential: *S. mutans, Lactobacillus* spp. and *Candida albicans* in 3 groups of subjects at time 0 and after 3 months from the oral hygiene education.

Materials and Methods: The study groups (10 subjects each) included children (4-10 yrs), adolescents (11-18 yrs) and adults (> 30 yrs). Paraffin-stimulated saliva was collected from each subject for 3 min. and salivary flow and pH were recorded. Salivas were serially diluted, and plated on Mitis-salivarius bacitracin agar, Rogosa agar and Candida ID agar for selective recovery of *S. mutans, Lactobacillus* spp. and *C. albicans*, respectively. After incubation for 48-72 h at 37°C or 30°C (*C. albicans*), identification was performed based on colony morphology, Gram-stain, MALDI-TOF and/or Strepto system.

Results: At time 0, high colonization percentage by all three microbial species analyzed were recorded in the study groups. The percent of subjects colonized by more than one species was also very high at time 0. Indeed, 50% of the subjects harbored all 3 species in their saliva, 27% harbored 2 species, and 23% only one species. At 3 months, a statistically significant reduction was observed in salivary count of *S. mutans* and *Lactobacillus* spp. The subjects colonized by all three species dropped to 24%. *S. mutans* reduction in saliva inversely correlated with salivary pH. Interestingly, a statistically significant positive correlation was observed between salivary counts of *S. mutans* and *C. albicans* suggesting a synergistic effect between this two microbial species in the colonization of oral cavity/pathogenesis of dental caries.

Conclusions: Overall, the results obtained suggest that large-scale oral hygiene education programs could be beneficial in reducing the salivary counts of species involved in the onset of dental caries. Although *S. mutans* has been largely accepted as the etiologic agent of dental caries, our results support the recent evidence indicating high prevalence for *S. mutans* in dental biofilms where the fungal pathogen *C. albicans* resides, suggesting that the interaction between these diverse species may mediate cariogenic development.

P128

INCREASE IN NON-TUBERCULOUS MYCOBACTERIA ISOLATED FROM HUMANS IN TUSCANY, ITALY, FROM 2004 TO 2016

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Introduction: Non-tuberculous mycobacteria (NTM) include all *Mycobacterium* species other than *Mycobacterium tuberculosis* complex and *Mycobacterium leprae*. NTM are a group of over 150 environmental species; they are generally endowed with low pathogenicity to human, however some species are associated with a variety of human diseases: respiratory tract infection are the most frequent, followed by lymphadenitis in children, disseminated infections in severely immunocompromised patients and skin infections. In Italy, the prevalence of NTM in human infections is largely unknown. The aim of the present survey is to report the epidemiology and recent trend of NTM infections in a region of central Italy, Tuscany, over the last 13 years, and provide a review of the recent literature on NTM isolation rates in different geographic regions.

Materials and Methods: The complete collection of NTM strains isolated from 50.150 clinical specimens at the Laboratory of Clinical Mycobacteriology of Pisa University Hospital, Italy, from 1 January 2004 to 31 December 2016 was included.

Results: In our setting, in the period 2004-2016 a total of 215 patients had cultures positive for NTM. The number of NTM isolates increased considerably from 5 isolates in 2004 to 36 in 2016; a sharp increase occurred in the last 5 years. Overall, 18 NTM species were isolated; the most common were *M. avium, M. intracellulare* and *M. gordonae* detected in respectively in 37.7%, 15.8% and 13.5% of NTM patients. Notably, *M. kansasii*, a pulmonary pathogen not reported before 2012, was repeatedly isolated in the last 5 years, representing 7.1% of total NTM isolates. In general, NTM isolates were largely prevalent in people older than 60 (59.5%); patients aged 1-10 year-old almost exclusively yielded *M. avium* and *M. intracellulare*. Of the 215 NTM clinical isolates, 77.2% were from respiratory specimens, 10.7% from lymph nodes, 2.3% from blood (yielding exclusively *M. avium*), and the remaining 9.8% from other clinical specimens.

Conclusions: The present study provides a snapshot of the prevalent NTM species in our setting and shows an increase in NTM isolation rate, which is in keeping with the general increase in NTM infections reported worldwide in the past two decades, although the distribution of the NTM prevalent species differs by geographic region.

P129

ANALYSIS OF A *BACILLUS CEREUS* STRAIN RESPONSIBLE FOR A FOOD-POISONING EPIDEMIC IN PIGS, ITALY, 2015

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Introduction: During January and February 2015, an extraordinary high mortality of pigs was reported in different breeding farms located in northern Italy. Symptoms, histopathological lesions, and epidemiological distribution suggested that a foodborne disease occurred. Analysis of the animal feed revealed the presence of a microorganism presumptively belonging to the *Bacillus cereus* species. Aim of this study was to isolate, quantify, and characterize the microorganism responsible for the outbreak by both molecular and phenotypic analyses.

Materials and methods: The contaminating organism was isolated and quantified from animal feed samples, identified by MALDI-TOF mass spectrometry, and typified by RAPD-PCR. Susceptibility testing against penicillin, ciprofloxacin, vancomycin and tetracycline was performed by E-test. Secretion of virulence factors (phosphatidylcholine-specific phospholipase C [PC-PLC], proteases, hemolysins) was assessed on agar and detection of toxin encoding genes (*sph, bcet, entFM, entS, plcA, nheA, nheB, nheC, cytK, hblC*) by PCR amplification of genomic DNA. *lef, pag, cya, capA* and *capB* encoding *Bacillus anthracis* specific virulence factors were also searched by PCR. Biofilm formation, swimming and swarming motility were tested. *Galleria mellonella* larvae were used as animal model of infection to evaluate bacterial pathogenicity.

Results: Animal feed was found to contain about 10^8 CFU/g of a single *B. cereus* strain (named MM1) that is resistant to penicillin and susceptible to the other tested antimicrobials. *B. cereus* MM1 demonstrated ability to secrete proteases, hemolysins, and a high amount of PC-PLC. The research of toxin encoding genes by PCR highlighted the presence of *sph, entFM, plcA, nheA, nheB* and *nheC*, while none of the *B. anthracis* genes was detected in bacterial genomic DNA. *B. cereus* MM1 was also shown to swim and produce biofilms that can contribute to bacterial virulence and environmental survival. The intra-haemocelic infection of *G. mellonella* larvae revealed that the LD_{50} of *B. cereus* MM1 was about 7 CFU/larva.

Conclusions: *B. cereus* MM1 was the only contaminant of the animal feed. The strain is potentially able to produce many of the *B. cereus* virulence factors and is highly pathogenic in the *G. mellonella* model of infection. The high amount of MM1 in the animal feed and its virulence potential can reasonably explain the histopathological damages that caused the abnormal mortality of pigs.

P130

PREVENTION IN PUBLIC HEALTH: SURVEILLANCE OF THE *LEGIONELLA* DISTRIBUTION IN THE HEALTH WATER SYSTEM

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Introduction: *Legionella* spp. under unfavorable conditions, can develop Viable But Not Culturable (VBNC) state in which it loses ability to grow on culture medium but keeps virulence. The culture method needs long incubation periods and not detecting the VBNC forms may lead to a false-negative result with subsequent underestimation of risk. In a risk assessment approach, our aim was to complement the classic protocol with the molecular procedure that requires short time to detect the presence of the bacterium. We could then outline an early situation about possible contamination and intervene quickly in case of critical situation.

Materials and Methods: *Legionella* spp. was searched on 33 samples of hot water of three hospitals between July and September 2016 using the standard culture method together with molecular analysis. DNA extraction was carried out to detect bacterial cells both live and death. RNA extraction was subsequently transforms into cDNA to detect live bacteria and VBNC forms. The 16S rRNA gene was searched for the determination of *Legionella* spp. and the MIP gene for *Legionella pneumophila* through Polymerase Chain Reaction (PCR).

Results: Data obtained using PCR were compared with those found by the culture method. From a qualitative and quantitative point of view, the data were consistent. In fact, the results obtained with the molecular method were supported by subsequent plate growth. Positive samples for *Legionella* spp. were 26 (78.8%), with all three methods. In 23 samples (79.2%) there was only one species or one serogroup, while in 3 samples (20.8%) several species were identified. Legionella species most frequently isolated was *L. pneumophila* sg 2-14 (72.7%), followed by *L. pneumophila* sg 1 (12.12%). The examined hospitals were all positive for *L. pneumophila* sg 2-14 although with different load. The brightness of the band (indicated by a scale 1 to 3) corresponded to a low or high bacterial load on the plate.

Conclusions: Considering the ubiquity of the bacterium and the importance of risk assessment, prevention and timeliness in the application of remedial measures in the hospital environment are decisive. Our study did not reveal significant differences in the results obtained and it can be deduced that all bacterial cells detected by the molecular method were alive and cultivable and not in VBNC form as originally assumed, given the continuous disinfection of water. Further analyzes are ongoing on a more representative number of hospitals and samples to better assess the diffusion of the bacterium.

P131

HIGH PREVALENCE OF ST131 *ESCHERICHIA COLI* FROM COMMUNITY-ONSET HEALTHCARE-ASSOCIATED INFECTIONS IN PALERMO, SICILY

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Introduction: *Escherichia coli* is the most common causative agent of urinary tract infections. Management of these infections is complicated by the increasing prevalence and spectrum of antimicrobial resistance. *E. coli* ST131 clone has been usually associated with resistance to β-lactams, mostly due to the production of extended-spectrum β-lactamase (ESBL) CTX-M-15, which confers resistance to penicillins, cephalosporins, and monobactams. Resistance to fluoroquinolones is also frequently found in *E. coli* ST131 clones, probably potentiated by the presence of plasmid-mediated quinolone resistance (PMQR), like *qnr* encoding genes or aminoglycoside modifying enzyme AAC(6')-Ib-cr, usually present in ST131 strains. We report here data from a study focused on the virulence factors and antimicrobial resistance in *E. coli* isolates causing urinary tract infections.

Materials and Methods: One hundred and five E. coli strains were collected from three different clinical laboratories located in Palermo, (Sicily) and from patients suffering of acute uncomplicated cystitis. To see whether our strains belonged to ST131 group, we performed a Multiplex PCR for *mdh* and *gyrB* genes. Two different multiplex PCR were conducted to evaluate virulence factors (*kpsMII, papA, sfaS, focG, iutA, papC, hlyD* and *afa* genes) while for quinolone and Cephalosporin resistance the genes *aac, qnrA, qnrB, qnrS*, bla_{OXA}, bla_{TEM}, bla_{SHV} and bla_{CTX-M} were investigated.

Results: We found out that ST131 strains were predominant (66.6% ST131 positive; 33.3% non-ST131) among our samples. 86.7% of our strains carry at least one of these virulence factors *hlyD, afa, iutA* and *papC* genes while 81% of our isolates showed positivity to at least one of *kpsMII, papA, sfaS* and *focG* genes. As for quinolone resistance genes, only 56,2% of our isolates showed positivity to at least one of the genes analysed. We furthermore looked for cephalosporin resistance determinants and 67.6% of our E. coli isolates were positive to at least one of bla_{OXA}, bla_{TEM}, bla_{SHV} genes. Finally, we looked for ESBL CTX-M gene. We found out that 49 of our samples (46,7%) carried CTX-M genes.

Conclusions: The majority (66,6%) of our E. coli strains belonged to ST131 clone, which is in agreement with studies illustrating the dissemination of such strains in community-onset healthcare-associated infections. Similar high percentage of ST131 isolates has been found in a study from a United Kingdom Region. In addition, the molecular profiling we conducted showed that while these bacteria carry different antimicrobic resistance genes, they aren't actually supplied with a heavy set of virulence factors.

P132

LACK OF RESPONSE TO HBHA IN *MYCOBACTERIUM TUBERCULOSIS*-INFECTED CHILDREN IS ASSOCIATED WITH ACTIVE TB DISEASE

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Introduction: Immunological diagnosis of tuberculosis (TB), performed using the Mantoux test (tuberculin skin test, TST) or the Interferon-g release assays (IGRAs), can be very useful to identify TB infection, though these tests are of little help in distinguishing latent TB from active TB disease. Among the several *Mycobacterium tuberculosis* antigens that have been used in IGRAs to serve as a biomarker of TB disease or latent infection, the heparin-binding hemagglutinin (HBHA) was capable of distinguishing latent TB subjects (which do respond to RD1 and HBHA-based IGRAs) and active TB patients (which respond to RD1 but not to HBHA). The aim of the present study was to evaluate HBHA-based IGRAs in children for which a diagnosis of TB infection and/or disease was seek.

Materials and Methods: A prospective study was conducted on children (0–14 years old) evaluated for TB infection or disease. Following clinical assessment, children were tested with the QuantiFERON TB-Gold In tube (QFT-IT) assay and an aliquot of whole-blood was also stimulated with HBHA and IFN-g measured only in QFT-IT positive subjects. Children showing no signs or symptoms and scoring negative to QFT-IT were defined as non-infected, while children with a positive QFT-IT or high clinical suspicion of TB disease underwent radiological and microbiological investigations to confirm or rule out a diagnosis of active TB or LTBI. The study was approved by the Ethical Committee of the Catholic University of Rome (“23866/13”, CE UCSC, Rome) and all enrolled individuals provided written informed consent.

Results: A total of 550 children were evaluated for TB infection and, based on the results at the QFT-IT, 482 (87.6%) resulted non infected TB subjects and 68 (12.4%) scored positive to QFT-IT. Among the TB infected, 45 (66.2%) were diagnosed with latent TB infection (LTBI) and 23 (33.8%) with microbiologically confirmed active TB. The response to the HBHA-based IGRA scored positive in 41 out of 45 children with LTBI and in 6 out of 6 children with asymptomatic active TB at diagnosis ($p = 0.001$) and negative in all the children with symptomatic active TB (13). Five children with LTBI and who had a known high risk of progression to active TB scored negative following stimulation with HBHA; interestingly, following chemoprophylaxis these children were able to properly respond to HBHA.

Conclusions: The results of this study highlight the potential usefulness of HBHA-based IGRAs in distinguishing, even among children, active disease patients from LTBI.

P133

CASE OF *LEGIONELLA PNEUMOPHILA* PNEUMONIA IN A PRISONER IN SICILY: THE IMPORTANCE OF TYPING METHODS TO ESTABLISH THE SOURCE OF CONTAMINATION

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Introduction: Legionella is found in natural and man-made aquatic environments, such as cooling towers and hot water plumbing infrastructures in particular *Legionella pneumophila* serogroup 1 (Lp1) is the most common etiological agent causing Legionnaires' disease, a severe form of pneumonia that affects primarily elderly and immunocompromised individuals. Water systems provide optimal growth conditions for Lp and help its transmission by generating aerosols. Then, the microbiological aspect of an investigation is to seek evidence linking the source of the outbreak to the cases, by comparing Legionella isolates from environmental samples with those from patients. Accurate discrimination among Legionella isolates is important in order to identify cases with a common source of infection and the transmission routes of the microorganism. The current typing method of Lp recommended by European Working Group for Legionella Infections (EWGLI) is a sequence-based typing (SBT). High-resolution genotyping of Lp isolates can be achieved also by multiple-locus variable-number tandem-repeat analysis (MLVA). To the best of our knowledge, we described the origin and typing of Lp in the first case of legionellosis in a prisoner in Sicily.

Materials and Methods: In this study, we analyzed ten different colonies suspected to Legionella strains isolated from sputum of a patient admitted with severe pneumonia, imprisoned in Palermo. However, ten different colonies from water samples were considered. The serological typing of LP was assessed using polyvalent and monovalent antisera. Subsequently, the isolates were typing by Pulsed-field gel electrophoresis (PFGE), SBT and MLVA.

Results: Colonies of presumptive Legionella organisms were confirmed by serotyping and all were identified as Lp 1. The molecular investigation showed that Lp strains from sputum and water samples had the same PFGE, SBT and MLVA profile.

Conclusions: Accurate discrimination among Legionella isolates is important in order to identify cases with a common source of infection and the transmission routes of the microorganism. Phylogenetic analysis in Lp is a problematic task considering the vast genetic differences detected in the genome of this specie. Cases of Legionnaires' disease at prisons have been reported in the last years worldwide. In order to limit the scale and recurrence of outbreaks, Legionella monitoring of water distribution systems should be recommended in prisons, because penitentiary populations could contain vulnerable people. We demonstrated in our case that typing by three methods provides valuable information for epidemiological correlation between clinical and environmental isolates.

P134

ASSESSMENT OF INFECTIOUS RISK DURING RESPIRATORY REHABILITATION: STUDY OF MICROBIAL AND POLYMICROBIAL CONTAMINATION OF OXYGEN SUPPLY

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Introduction: Chronic obstructive pulmonary disease (COPD) is characterized by persistent bronchial obstruction, associated with an increased chronic inflammatory response to the airways and lungs. COPD is cause of morbidity and mortality worldwide and results in an economic and social burden. It includes three nosological entities, chronic bronchitis, pulmonary emphysema and chronic bronchial asthma. Patients with COPD are usually subjected to respiratory rehabilitation during which they perform oxygen therapy. The aim of the study was to evaluate the presence of microorganism in devices used for oxygen therapy in order to prevent transmissibility and to reduce incidence of respiratory tract infections.

Materials and Methods: In this study we enrolled, from October 2016 to May 2017, 23 patients affected by COPD, attending to IRCSS San Raffaele Pisana, Roma, treated with oxygen therapy. Respiratory sputum samples were carried at admission (T0) and at hospital discharge (T3). Devices and water samples were analyzed at T0, after 7 and 14 days (T1, T2) and at T3. Microorganisms isolated from sputum, devices and water contained in the bubbler was identify by biochemical and molecular tests and by mass spectrometry (MALDI-TOF Bruker). Antimicrobial susceptibility of bacterial isolates was performed by semiautomatic method Vitek2 (Bio-Merieux) and by disk diffusion test (Kirby-Bauer method). Congo Red agar was used to detect microorganisms producing biofilm.

Results: The microbiological analysis of oxygen supply show a bacterial contamination at T0 and at T1, T2, T3. Devices are contaminated by different species of Gram positive, Gram negative bacteria and fungi. Most of the bacterial isolates exhibit resistance to several antimicrobial agents. Sputum samples of most patients are infected with pathogenic microorganisms at T0, that exhibit different antibiotics resistance.

Conclusion: Results evidenced, that there is no a correlation between microorganisms isolated from the patient samples and microorganisms isolated from the oxygen supply and in the water. Further studies are necessary on a larger number of enrolled patients to provide more information.

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CHARACTERIZATION OF BIOFILM FORMATION BY *MYROIDES ODORATIMIMUS* ISOLATED FROM POST-TRAUMATIC CALCANEAL RECURRENT ULCER IN DIABETIC PATIENT

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Introduction: *Myroides odoratimimus* is an aerobic, non-motile, non-fermenting, yellow-pigmented Gram-negative rod-shaped bacterium, known for its opportunistic pathogenicity in humans. Widely distributed in nature, it causes infections in immunocompromised patients due to its multi-drug resistance. Selecting the optimal antibiotic therapy for the treatment of *M. odoratimimus* infections is challenging due to limited clinical experience with this micro-organism and its reported multidrug-resistance. In this study, a clinical strain of *M. odoratimimus* isolated from a recurrent calcaneal ulcer of a 65-year-old male diabetic patient was characterized for its ability to form biofilm.

Materials and Methods: The strain was identified as *M. odoratimimus* by both MALDI-TOF and 16SrDNA sequencing. Biofilm formation was evaluated, under different pH (5.5, 7.2, and 8.5) and glucose concentrations (5, 12.5, and 25 mM), on polystyrene (cristal violet) and in a "skin-like" model (viable count). MIC and MBC of levofloxacin (LVX), meropenem (MPM), and tigecycline (TGC) were determined, both on planktonic and 7-day biofilms cells. Activity against 7-day biofilm was assessed both by viable cell count, and confocal (CLSM) and electronic (FIB-SEM) microscopy. The expression of antibiotic resistance-related genes (*MUS-1; gyrA; AcrB*) during biofilm formation was evaluated by RT-PCR.

Results: *M. odoratimimus* produced relevant amount of biofilm biomass, in a time-dependent manner, but regardless pH and glucose concentration. MBC values between planktonic and sessile cells were different for LVX only. None of the tested antibiotics was able to completely eradicate preformed biofilm. Particularly, MPM and LVX caused a significant, although dose-independent, reduction of cell viability, as also confirmed by microscopy. RT-PCR showed that *MUS-1, gyrA* and *AcrB* are over-expressed during the transition from planktonic to sessile (biofilm) lifestyle.

Conclusions: Our results showed, for the first time, that *M. odoratimimus* is able to form relevant amount of inherently antibiotic-resistant biofilm that might play a role in the pathogenesis of chronic ulcer infections. The present study, along with others reported in literature, clearly suggested that significance of *M. odoratimimus* isolation in clinical specimens should always be discussed between the clinical microbiologist and the clinician, and that antimicrobial assay is necessary to guide therapeutic decisions.

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STREPTOCOCCAL VASCULITIS LEADING TO SUDDEN DEATH IN A SUCKLING KITTEN LITTER: A POST-MORTEM DIAGNOSIS

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Introduction: β-hemolytic *Streptococcus* species are considered part of the commensal mucosal bacteria of the oral cavity, upper respiratory tract, genital tract and perianal area in cats, with the most frequent species being *S. canis* (Lancefield group G). Most of the infections are described in young, intensively housed subjects. Up to 70%-100% of young queens in breeding catteries may carry this bacteria in the vagina, with the result of neonatal infection. Here, we describe a case of neonatal septicemia caused by β-hemolytic *Streptococcus* sp. in a kitten, diagnosed with post-mortem examination.

Materials and Methods: A 4-day-old Sphynx kitten from a breeding cattery died with severe cutaneous lesions consisting in suppurative or ulcerated skin nodules of head, feet and tail. It was submitted for necropsy within 1 hour of death. Two kittens of the same litter presented similar lesions, with poor body growth and dehydratation. They were submitted to microbiological sampling prior to their death, occurred 1 day later. The mother and the other 2 kittens of the same litter showed no clinical signs.

Microbiological sampling of the lesions have been performed aseptically during the necroscopy and submitted to bacteriological and mycological cultural analysis. Antimicrobial susceptibility testing was performed by Kirby-Bauer method, according to CLSI guidelines.

Results: Gram-positive, catalase-negative cocci were isolated from the cultures. The strain has been microscopically and biochemically identified as β-hemolytic *Streptococcus* spp. and submitted to further identification with MALDI-TOF MS (ongoing). The strain showed *in vitro* susceptibility to all the antibiotics tested except Clindamycin and Trimethoprim-Sulfamethoxazole. Tissues collected during the necropsy and submitted to histopathologic examination revealed multifocal severe acute dermatitis, with vasculitis and intravascular Gram-positive coccoid bacteria. Suppurative omphalitis and diffuse congestion of liver, spleen and brain were also evident.

Conclusions: An outbreak of streptococcal vasculitis with suppurative or ulcerated skin nodules occurred in a cattery, caused by a β-hemolytic *Streptococcus* sp. To determinate the source of the infection, screening of the maternal milk and vagina has been performed giving negative results for β-hemolytic *Streptococcus* spp. However, omphalitis revealed by histopatological analysis may suggest the umbilical vein as a route of infection. As the infected and the healthy kittens shared the same environment, other factors may have concurred to the emergence of the infection in only 3 out of 5 kittens, such as management, hygienic practices, stress of captivity, level of maternal antibodies, immune response or concurrent viral disease.

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MYCOBACTERIUM AVIUM COMPLEX INFECTION IN A DOG

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Introduction: The genus *Mycobacterium* contains various obligate or opportunistic microorganisms. The latter are mostly members of the *Mycobacterium* avium complex, acquired mainly from environmental sources, for example, soil, water, dust, and feed. *M. avium* complex strains can cause disease in humans and in a wide range of animals, mainly in immunocompromised subjects. Dogs in particular may be infected by *Mycobacterium (M.) tuberculosis*, *M. bovis*, and *M. avium* complex and the clinical signs associated with each of these infections are usually indistinguishable. Rapid speciation of the infecting organism is desirable because of the public health concerns associated with this pathogen.
Material and Methods: A 10-year-old, male cross breed dog living with pet birds in therapy with immunosuppressive glucocorticoid therapy for immunomediated anemia was presented for weight loss, anemia, abdominal pain and vomiting. Hematological, serum biochemical parameters and echographic examination revealed pancreatitis. Dog was hospitalized and recovered from pancreatitis but it showed intermittent fever, lethargy and anorexia with leukopenia anemia. Bone marrow biopsy and Polymerase Chain Reaction (PCR) were performed.
Results: Bone marrow examination revealed the presence of macrophages containing myriads acid-fast positive organisms. Polymerase chain reaction (PCR) analysis identified *M. avium* complex. Despite treatment with Rifampicin the dog's conditions are progressively deteriorating due to neurological symptoms
Conclusion: The present case is valuable due to the low incidence of Mycobacterial infection in dogs and for its zoonotic potential. Only immunocompromised people and children are susceptible to the infection. Further investigation are required to evaluated if the dog of this study was infected by pet birds or by environment, that is actually considered the most probably cause of infection.

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DETECTION OF BIOFILM-PRODUCER STRAINS OF S. EPIDERMIDIS FROM CLINICAL ISOLATES BY MALDI-TOF MASS SPECTROMETRY

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Introduction: The infections of postoperative surgical sites are the most common nosocomial infections and represent a significant cause of postoperative morbidity. The global impact of surgical site infections (SSIs) on health-care systems is considerable and many are related to the formation of a microbial biofilm. Biofilms play a significant role in the pathogenesis of implantable device-related infections and are also important in persistent postoperative infections. *Staphylococcus aureus* and *Staphylococcus epidermidis* are the most common isolates found in device-related infections. The formation of bacterial biofilms can cause serious medical problems since they represent a reservoir of bacteria that can lead to chronic infections. The aim of this study is to screen the biofilm-producers strain of *S. epidermidis* using the MALDI-TOF Mass Spectrometry (Bruker Daltonik).
Materials and Methods: Clinical isolates of *S. epidermidis* from infected sutures and ATCC strains of *S. epidermidis*; 35984 (biofilm-producer) and 12228 (not biofilm-producer), use as internal controls, were cultured in TSB medium at 37°C until forming biofilm in 96-wells microtiter plates. The biofilm production was assessed by Cristal violet assay. MALDI-TOF MS analysis: After formic-acid extraction, two different ATCC Main Spectra Profiles (MSP) were created by the automated function of Biotyper 3.0 software (Biotyper MSP Creation Standard Methods). Clinical isolates of *S. epidermidis* were analysed by MALDI-TOF MS using MALDI Biotyper RTC software and each spectrum were compared to ATCC MSPs by MALDI Biotyper 3.0 software.
Results: Cristal violet assay showed that some strain of *S. epidermidis* are strong biofilm-producers, others moderate biofilm-producers and few not biofilm-producers. By the MALDI-TOF analysis, the strong and moderate biofilm-producers were

associated with the MSP of ATCC 35984, while the not biofilm-producers were associated with MSP of ATCC 12228.
Discussions: This study want to evaluate the future use of MALDI-TOF MS to identify biofilm-positive and biofilm-negative *S. epidermidis* strains, since this can be an effective support to reduce the SSIs complications.

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MOLECULAR TYPING AND ANTIBIOTIC SUSCEPTIBILITY OF *ACINETOBACTER BAUMANNII* STRAINS ISOLATED FROM HOSPITALIZED PATIENTS

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Introduction: Multi-drug-resistant (MDR) *Acinetobacter baumannii* is a serious nosocomial pathogen often associated with outbreaks, causing a variety of infections including respiratory disease, bacteremia and meningitidis. The objective of the study was to investigate the molecular epidemiology of clinical *A. baumannii* isolates from an outbreak occurred in the intensive care unit (ICU) of our hospital.
Materials and methods: Between January and April 2017, 15 MDR *A. baumannii* strains were isolated from clinical samples of 8 different patients. Identification and antimicrobial susceptibility testing were performed using the Vitek-2 System (BioMérieux, France) and Maldi-Tof analysis (Bruker Daltonics, Germany). MIC breackpoints were interpreted according to EUCAST recommendations. Sequence data for analysis were generated by Next Generation Sequencing (NGS) at the Italian Institute for Technologies (ITT), Rome Italy. DNA was extracted and libraries were prepared using a Nextera XT DNA sample preparation kit (Illumina, San Diego, CA), according to manufacturer's instructions. Complete genome sequences were obtained using the Illumina MiSeq next generation sequencer. Plasmid-borne OXA-23 gene carbapenemase was detected by PCR. Molecular typing was performed by repetitive sequence-based PCR (rep-PCR; DiversiLab BioMérieux) and confirmed by a phylogenetic analysis using core genome MLST (cgMLST) approach by the Seqsphere+ software (Ridom GmbH, Germany) with a reference genome to generate an ad hoc scheme. The cgMLST method uses 2690 gene targets to characterize the gene-by-gene allelic profile of *A. baumanniii.*
Results: Thirteen strains were obtained from 7 patients treated in the ICU and 2 strains were from one patient admitted to the surgical ward. Two of the seven ICU patients and the patient in the

surgical ward, were colonised prior to admission, as determined by positive rectal swabs for MDR *A. baumannii*. All isolates showed an MDR profile; all isolates also carried the OXA-23 carbapenemase.
Typing performed by rep-PCR, using a similarity index of ≥ 95% as threshold, revealed that the isolates clustered into 2 distinct groups; the pattern similarity obtained was ≥ 95% for all strains from the ICU, while it was < 90% for the two from the surgical ward. Further experiments, using the cgMLST, highlighted a further cluster in the ICU. The cgMLST method allowed a more in-depth analysis distinguishing.
Conclusion: Outbreaks of *A. baumannii* have been extensively typed using DiversiLab rep-PCR; however our results confirm the validity of gene-by-gene analysis by cgMLST for epidemiological investigations, since it allows a more in-depth analysis, owing to its highly discriminatory ability in determining clonal relatedness.

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RISK FOR *SALMONELLA* TRANSMISSION FROM PET REPTILES TO HUMAN: A SURVEY ON KNOWLEDGE, ATTITUDES AND PRACTICES OF REPTILE-OWNERS RELATED TO REPTILE HUSBANDRY

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Introduction: Reptiles are becoming increasingly popular as pets: it has been estimated that more than 1.3 millions are kept in Italian households (FEDIAF, 2014). Those animals are reservoirs of a wide variety of *Salmonella* serotypes which may be transmitted to warm-blooded animals, including man. Reptile-associated salmonellosis (RAS) is mainly reported in children younger than five years, elderly people or immune-compromised persons. Accordingly, good hygiene practices related to husbandry are important for prevention of infection.
Materials and Methods: A cross-sectional study was conducted among reptile owners, by administration of a detailed questionnaire. In addition, the cloacal swabs of sampled reptiles were screened for *Salmonella* spp. and the husbandry management practices were evaluated in parallel to assess any possible link between the presence of *Salmonella* spp. and the hygiene practices.
Results: The response rate to the questionnaire was 66.6% (100 out of 150 contacted owners). In 26 out of 100 families, members at risk of RAS (children and elderly) were present. One hundred animals were screened for the presence of *Salmonella* spp. The prevalence of *Salmonella* spp. carriers was 57%. Co-habitation of the animals with other reptiles in the same terrarium was associated with a 2-fold increase in the risk of infection by *Salmonella* spp. (Odds ratio = 2.3, CI 1.2;13, p=0.02). The animals handled by owners that did not report washing their hands after cleaning procedures or handling were subjected to a 3-fold increase in the risk of infection (OR= 3.1, CI 1.1;16, p=0.019). When drinking water was not replaced regularly, the animals were 7 times more exposed to infection (OR = 6.8, CI

1.8;25, p = 0.005). When asked about knowledge of the health risks linked to reptile's husbandry, 55 % of owners answered that they were aware, although no one was able to indicate some specific risks.
Conclusions: In the present survey the prototype reptile owner was a person, aware of ethological aspects of reptile husbandry but ignoring some ethical recommendations and poorly informed about the health risks for him or for the other family members. The poor hygiene standards and high prevalence of *Salmonella* spp. in reptiles observed in this survey indicate that prevention of RAS must rely mainly on information and education, with the veterinarian health bodies primarily involved in this difficult task.

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INVASIVE BACTERIAL DISEASE IN EMILIA ROMAGNA REGION: SURVEILLANCE DATA BETWEEN 1ST JANUARY 2015 AND 31ST DECEMBER 2016

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Background: Invasive bacterial infection are characterized by a proved bacterial isolation from blood/CSF, serious complications and high lethality. Rapid diagnosis and targeted treatment are most important for the patients management with severe sepsis/meningitis and for rapid public health intervention. These infections can be prevented by vaccination; it is crucial to monitor their diffusion and characterize all the isolates. In Italy there's an integrated Surveillance System coordinated by Istituto Superiore di Sanità (ISS) that requires the reporting of all cases of bacterial invasive diseases, in particular if caused by *N. meningitidis, S. pneumoniae* and *H.influenzae*. This report provide the surveillance data in Emilia Romagna Region from 1st Jan. 2015 and 31st Dec. 2016, to define the epidemiological trend of these infections. Isolates serotyping was carried out by the Regional Reference Centre for Microbiological Emergencies (CRREM), St Orsola Malpighi Hospital, Bologna, Italy.
Materials and Methods: All strains are isolates from blood/CSF samples from patients who receive an etiological diagnosis of invasive bacterial disease. The isolates typing was performed by latex agglutination testing and/or molecular methods (real time PCR).
Results: In Emilia Romagna Region the surveillance system included serotyping of 270 isolates (Jan-2015 and Dec-2016), of which 199 cases of Invasive Pneumococcal Disease (IPD), 33 from Invasive Meningococcal Disease (IMD) and 38 from Invasive Haemophilus influenzae Disease (IHD). The most frequent *S. pneumoniae* serotypes were 8, 3, 12F, 19A and 7F, included in conjugate

vaccines, with approximately 70.3% of vaccine serotypes. *N. meningitidis* serogroup B (MenB) was the main serogroup isolated, according vaccine introduction with meningococcal C (MCC) vaccine, with 97% vaccinal serotypes. *H. influenzae* type b (Hib) was the major cause of IHD and its percentage of vaccine serotype was 15.8%, with a significant 84.2% non b (a or c-f)/unencapsulated isolates.

Discussion and Conclusion: Although in the European context Italy is a low-incidence country for invasive disease, regional differences in terms of diagnostic methods used, attitudes to notification, sending of isolates to the reference centers, and typing execution can affect the representativeness of these data. It's more important continue to promote the study of serogroups/serotype distribution for the control of possible secondary cases in the immediate and medium/long term to assess over time not only the impact of vaccines currently available. Since epidemiology has been evolving, our data on serotypes distribution in children and elderly persons, non vaccine serotypes and antibiotic- resistant clones will be discussed afterwards.

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A SNAPSHOT OF *MYCOBACTERIUM TUBERCULOSIS* COMPLEX ISOLATES FROM SICILY, ITALY, 2014-2015

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Introduction: Sicily, Italy, is a low-endemic area for tuberculosis (TB). There is a raising concern that the TB epidemiology could be negatively impacted by immigration from high endemic countries. The number of immigrants living in Sicily has raised to about 183,000 in 2015, but their prevalence on the overall regional population is just 3.61% due to the transient stay of most of them in this region. Moreover, before 2011, the vast majority of sea migrants came from Africa, whereas recently, a higher proportion has come from the Middle East and Asia and includes migrants of minor age.

Materials and Methods: 307 MTBC isolates were collected from 307 different patients hospitalized for TB in eight different hospitals in four cities in Sicily, Italy, in 2014 and 2015. All the collected strains were submitted to molecular typing by spoligotyping and the 24 mycobacterial interspersed repetitive unit (MIRU)-variable-number tandem-repeat (VNTR) method typing. Susceptibility testing to streptomycin, isoniazid, rifampin and ethambutol was also performed.

Results: We collected the main characteristics of TB cases: 198/307 were (64,49 %) foreign-born, 209/307 (68,08 %) male gender and the mean age was estimated as 37 years (DS= 18 years). Based upon the association of lineages/clades with nationality, most of the foreign-born TB patients in Sicily were infected prior to their arrival in Italy. This is further supported by the age class distribution where the prevalence of EuroAmerican isolates within the older patients, most of Italian origin, is evident vs. ''foreign-origin'' strains imported from Asia and Africa and substantially prevalent among

younger patients.
Conclusions: The most striking features of TB epidemiology in Sicily are the increasing proportion of cases in the foreign-born population and the wide heterogeneity of MTBC isolates. This very complex and evolving landscape makes it indispensable the contribution of molecular typing to accurately interpret TB epidemiology and implement effective control strategies.

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INCIDENCE AND ANTIMICROBIAL SUSCEPTIBILITY OF UNCOMMON GLUCOSE NON-FERMENTING GRAM NEGATIVE BACILLI IN CYSTIC FIBROSIS PATIENTS

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Introduction: The predisposition of patients with cystic fibrosis (CF) for recurrent pulmonary infections can result in poor prognosis of the disease. Although the clinical significance in CF of microorganisms, such as Staphylococcus aureus, Haemophilus influenzae and Pseudomonas aeruginosa, is well established, the implication of uncommon glucose non-fermenting Gram negative bacilli (UGNF-GNB) in respiratory samples from CF patients is still unclear. The aims of this study were to evaluate the epidemiology and the antibiotic susceptibility of UGNF-GNB in a cohort of Italian patients affected by CF.
Materials and Methods: During 2010-2016, 330 UGNF-GNB isolated from 3089 airway samples of patients, attending the Regional Care CF Centre of Genoa, were collected and stored at -80°C. The species identification were performed in duplicate by matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) using a Vitek MS Spectrometer (bioMérieux). To assess the sensitivity of the isolates to antibiotics, was used the Thermo Scientific™ Sensititre™ Gram Negative MIC Plate. Interpretative criteria for susceptibility to antibiotics were in accordance with Eucast criteria (Pk/Pd).
Results: During the period of study, 4.5% of patients was infected by Stenotrophomonas maltophilia, 3.7% by Achromobacter xylosoxidans. Incidence of *Chryseobcterium* spp., *Ochrobactrum* spp. e *Sphyngobacterium* spp. ranged 1% to 0.4%. Other species were isolated in single patients (*Pandoraea* spp., *Bordetella* spp. e *Rhizobium* spp.). In all strains the aminoglycosides are poorly efficacious and beta-lactams are rarely effective. In Cryseobacterium spp., Sphingobacterium spp. and *Ochrobactrum* spp. quinolones are the most

efficacious. For *S. maltophilia* trimethoprim-sulfamethoxazole remains the drug of choice. A high rate of the strains isolated were multidrug-resistant.
Conclusions: The results confirm previously reported data; in particular, they show an increase isolation of UGNF-GNB in CF Genoa patients. These strains showed a broad-spectrum antimicrobial resistance. To date the exact role of this microorganism in CF lung disease is unknown. For better understanding the possible pathogenic role of UGNF-GNB in CF patients our future objective is improve the results with study of the correlation between clinical and microbiology data.
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ACQUISITION OF ANTIMICROBIAL-RESISTANT *E. COLI* ISOLATED FROM DOGS ADMITTED TO A VETERINARY TEACHING HOSPITAL

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Introduction: The emergence of bacterial nosocomial infections has become a major public health concern both human and veterinary medicine and incidence of these infections is increasing. Commensal *E. coli* are a reservoir of resistance genes, that could be quickly acquired by pathogen bacteria. To monitor intestinal *E. coli* isolated from hospitalized animals could improve strategies to minimize the development of antimicrobial resistance. The aim of this work was: to evaluate the effect of hospitalization on antimicrobial resistance in *E. coli* isolated from dogs admitted to a Veterinary Teaching Hospital; to identify risk factors associated with acquisition and colonization of multidrug-resistant MDR bacteria (such as ESBL *E. coli*).
Materials and Methods: A sample of 27 dogs were included in the study, and anamnestic data were registered (e.g. sex, age, reason for admission, antimicrobial use, length of hospitalization). Fecal swabs were collected at admission (within 24 hours: T0) and during hospitalization (on day 3: T1). Dogs (n = 8), that remained for a long period in the dog park of the hospital, were included as controls. Standard bacterial culture procedures were used to recover *E. coli* from swabs. Identification of *E. coli* , ESBL- *E. coli* and antibiotic sensitivity tests were carried out by BD-Phoenix^{tm}. The antibiotics panel included antimicrobial used in both veterinary and human medicine. Data were analyzed by descriptive statistics. McNemar's test was used to evaluate the change in ESBL *E. coli* over time, and Fisher Exact test to assess the association between risk factors (reason for hospitalization, previous hospitalization, etc.) and the development of antibiotic resistance.
Results: Paired admission (T0) and day 3 (T1) samples were available for 27 dogs, with a total of 54 isolates of enteric *E. coli*. During hospitalization,

ESBL *E. coli* were acquired in 11 (40.5%) animals. The proportion of ESBL *E. coli* increased over time ($p < 0,01$), but it isn't correlated to the use of antibiotics before and during hospitalization. Dogs used as controls showed high susceptibility to antibiotics and their isolates weren't ESBL producers. Antimicrobial sensitivity tests carried out at the admission showed that 37% of animals were already infected with ESBL and 55% were infected with multidrug resistant strains.
Conclusions: Over the years, studies carried out on dogs admitted to hospital showed that 7-10 days of residence is enough in order to carry MDR bacteria. Our work confirm this trend, but notably we observed that animals could acquire resistant *E. coli* in just 3 days. This finding is relevance because, in the routine of veterinary hospitals, most of the dogs are committed for no more than 72 hours.

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PRELIMINARY RESULTS OF A PROSPECTIVE CASE-CONTROL STUDY OF A *SAPROCHAETE CLAVATA* OUTBREAK IN HAEMATOLOGICAL PATIENTS

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Introduction: We describe a cluster of 6 hematological patients developing a sepsis by Saprochaete clavata within 8 months. Three of the 6 patients died. We determined the clonal relatedness of the isolates and investigated possible sources of contamination with a prospective case-control study of the outbreak.
Materials and Methods: From September 2016 to May 2017 we investigated risk factors for Saprochaete infection by starting a case-control study, selecting 5 controls for each case. We collected information on co-morbidities, chemotherapy, antimicrobial therapy, presence of indwelling devices, transfusions and food intake. Identification of the isolates was done with Vitek-MS plus SARAMIS (bioMérieux- France), and with Vitek2, and it was confirmed by sequencing of the D1D2 and ITS1-5.8S-ITS2 regions. Beta-glucan test (BG) and Platelia Aspergillus (GM) were performed on all the patients. The Sensititre YeastOne Y10 was used to perform the susceptibility testing of the isolates. Isavuconazole susceptibility was done by Etest (Liophilchem).Typing by MALDI-TOF analysis was performed.
Results: All 6 patients developed a sepsis by S. clavata. The underlying disease was acute myeloid leukemia in 3 of the patients, and non-Hodgking lymphoma for the others. The mean ages for cases and controls were 43.8 and 47.8, respectively. The only risk factors identified at univariate analysis were the length of neutropenia [for each extra day of neutropenia, OR 1.11, $p = 0.033$] and previous transfusion with platelets [OR 2.61, $p = 0.022$].

Results of microbiological investigations on environment, food sources and unused device were negative. Neither MALDI-TOF MS-Vitek nor Vitek 2 yielded any identification of the six isolates, while SARAMIS identified the isolates as S. clavata. Molecular identification gave 99% of homology with S. clavata in all the isolates. BG was positive in 3 out of the 6 patients (range 159 ≥ 523 pg/ml), GM was always negative. All the isolates were resistant to echinocandins and Fluconazole, susceptible to Flucytosine, Voriconazole and Amphotericin. Isavuconazole tested with Etest evidenced a median MIC of 0.094 mg/L. The correlation-based dendrogram obtained by MALDI-TOF MS analysis clustered the 6 isolates in the same group, thus showing their close relatedness.

Conclusions: Emerging rare yeast infections are often reported in patients with malignant haematological diseases. We describe here the preliminary result of an outbreak of six cases of *S. clavata* sepsis and the case-control study, which evidenced the possibility that *S. clavata* may be transmitted through contaminated medical devices. The intrinsic resistance to echinocandins limits therapeutic options. Susceptibility to Isavuconazole could represent an alternative.

P146

MICROSATELLITES-BASED GENOTYPING OF *CANDIDA PARAPSILOSIS* ISOLATES RECOVERED FROM CLINICAL SAMPLES AND HOSPITAL ENVIRONMENTS

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Introduction: Healthcare associated infections (HAI) affect an estimated 4.1 million patients in the EU annually and cause considerable increases in illness, mortality and costs. An annual (2014) report prepared by the ECDC highlighted the importance of Candida species in HAIs exacerbating a situation already very serious. In fact, the rise of Candida bloodstream infections (BSI) among hospitalized patients is widely confirmed by many studies worldwide. In several EU countries *C. albicans* and non-albicans (NAC) species cause around 50% of BSIs each and among all pathogenic NAC, *C. parapsilosis* has emerged as an important cause of BSI especially in the Mediterranean countries. The aim of this study was to describe the genetic pattern of *C. parapsilosis* strains recovered from clinical samples, hands of healthcare workers and hospital environments (air, medical equipment and various surfaces) in one of the most important structure of South Italy in the treatment of patients with neurological diseases.

Materials and Methods: A total of 48 *C. parapsilosis* strains were obtained from blood samples (n° 12), hospital environments (air, medical equipment and various surfaces; n° 29) and hands of healthcare workers (7). Isolates were initially characterized using conventional mycological phenotypic methods and subsequently identified by sequencing the ITS1-5,8S-ITS2 region of the rDNA. Analysis of hypervariable microsatellite loci was used to evaluate the genetic relatedness among all fungal isolates.

Results: All fungal isolates examined in this study were identified as *C. parapsilosis* and no closely related species, *C. orthopsilosis* or *C. metapsilosis*, were recovered. A total of 14 different genotypes were obtained by microsatellite analysis. Eight blood, 13 environmental and 2 hands strains shared the same allelic profile and were, therefore, considered as identical strains. Most of the *C.*

parapsilosis isolates recovered from hands (5/7; ~71%) showed 4 unrelated genotypes whereas 2 different genotypes were obtained from remaining blood isolates. These latter genotypes were not found among those colonizing the hands of hospital personnel or environments. Sixteen environmental strains were genetically quite similar showing seven different but close related genotypes.
Conclusions: This study confirms that candidaemia in hospitalized patients is caused predominantly by *C. parapsilosis* strains colonizing various hospital surfaces including air, medical devices and hands of healthcare workers and therefore the implementation of surveillance programmes is imperative to prevent the spread of nosocomial fungal infections.

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P147

DRAFT GENOME SEQUENCE OF A NIGERIAN *CRYPTOCOCCUS NEOFORMANS* STRAIN BELONGING TO AN UNCOMMON MULTILOCUS SEQUENCE TYPE (MLST-ST43)

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Introduction: *Cryptococcosis* is an infection of the central nervous system which affects both immunocompromised and healthy individuals. This disease is caused by members of the *Cryptococcus neoformans* and *C. gattii* species complexes which are, genetically, highly divergent and have been previously subdivided into seven haploid (termed VN and VG) and four hybrid genotypes.
C neoformans VNII genotype is globally distributed but it has a low prevalence compared to the high occurrence of the VNI type. A recent study showed that the VNII type is widely distributed in the Nigerian environment, a country where more than 20% of HIV infected patients show a positive cryptococcal antigenemia. Multilocus analysis of Nigerian *C neoformans* VNII isolates revealed that they belong to a uncommon MLST type ST43 which has only been reported from Thailand, Japan, North Carolina, Washington states and Germany. Here, we report the first draft genome assembly of a rare MLST type (ST43) of *C. neoformans* strain (VNII genotype; mating type α), isolated in Nigeria, with the aim to facilitate future comparative studies for better understand the basic biology and the evolution of this important human fungal pathogen.
Materials and Methods: The whole genome of the Nigerian *C. neoformans* EN28 strain was paired-end (2x100 bp) sequenced using the Illumina HiSeq

2500 platform. High-quality genomic DNA of the EN28 strain was used to generate a paired-end library with insert sizes of approximately 300 bp. Before assembling, raw reads were processed using Trimmomatic to remove adapters and sequences with low Phred-scores (cutoff: ≥ 30). IMR-DENOM program was used for assembling the whole fungal genome using the following sequence GCA_000149245.3 as reference. Protein-encoding genes and tRNAs were predicted by AUGUSTUS and tRNAscan-SE programs respectively.

Results: The genome of the *C. neoformans* EN28 strain was assembled in 15 chromosomes (14 nuclear and 1 mitochondrial) covering over 18,9 Mbp (G+C: 48,19%). A total of 261 putative tRNAs and over 7,849 protein-encoding genes were identified in this fungal genome.

Conclusions: The genomic dataset reported in this study will be made publicly available in the Genbank database as a resource for researchers. The release of this assembly will be useful for the scientific community to initiate further molecular biology work related to this important fungal pathogen.

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ARE WE READY FOR *CANDIDA AURIS* CLONES ATTACK? FIRST STEPS TOWARDS IDENTIFICATION AND RESISTANCE DETECTION BY MALDI-TOF MS

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Multidrug resistant clonal strains of *Candida auris* have been isolated in the last five years in many countries. The emergence of *C. auris* is alarming as this yeast, very recently implicated in cases of nosocomial fungemia and deep seated infections, exhibits resistance to azoles, amphotericin B and echinocandins, thus limiting the options of choice for the antifungal treatment and leading to clinical failure for the patients. Therefore, an accurate and rapid identification at the species-level and differentiation between susceptible and resistant clones to antifungals drugs is urgently needed.

Commercial routine systems for yeasts identification commonly misidentify these recently emerged clones and mass spectrometry systems databases lack in *C. auris* reference profiles. The aim of the present study has been the implementation of the Bruker MALDI-TOF MS database with *C. auris* mass profiles and the development of a fast and reproducible assay able to rapidly detect *C. auris* resistance to fluconazole (FLU) and anidulafungin (AFG).

All the mass measurements below mentioned were performed with a Microflex LT mass spectrometer. Briefly, protein extracts from a panel of *C. auris* clones, isolated from an Indian outbreak, have been obtained both with fast formic acid and long ethanol/formic acid extraction procedures. After the MSPs creation, a score-oriented dendrogram was generated from hierarchical cluster analysis using the integrated statistical tool of the Biotyper 3.1 software, including C. lusitaniae, C. famata, C. guillermondii and C. parapsilosis isolates.

To detect *C. auris* resistance to FLU and AFG a three hours incubation antifungal susceptibility test (AFST) was developed, after preliminary experiments aimed to find the breakpoint and

maximum level of FLU or AFG concentrations needed to obtain a correct categorization for the strains. Spectra obtained at null, intermediate or maximum FLU or AFG concentrations were used to create composite correlation index (CCI) matrices for the *C. auris* isolates.
Cluster analysis of MALDI-TOF spectra, obtained for *C. auris* clones and the species with which it is most commonly misidentified, resulted in the correct grouping according to the five species represented and allowed a correct identification at the species-level for the species included in the analysis. Moreover, the isolates categorizations obtained by MS-AFST, relying on 3h incubation only with the antifungal drug, were consistent with the results obtained by Sensititre YeastOne method. Given the diagnostic urgency related to these recently emerged clones of *C. auris*, our approach would provide a rapid tool for this pathogen identification at the species-level and to speed up the initiation of the appropriate antifungal treatment.

P149

PREVALENCE OF SUPERFICIAL AND CUTANEOUS MYCOSES IN PATIENTS ATTENDING MICROBIOLOGY OUTPATIENT FACILITY AT PISA UNIVERSITY HOSPITAL

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Introduction: Fungal infections of the skin and nails form the most numerous and widespread group of all mycoses that affect 10–25% of the world's population, making them one of the most frequent forms of infections. In the present study, we determined the prevalence of superficial and cutaneous mycoses and their etiologic agents in patients attending to microbiology outpatient facility at Pisa University Hospital between May 2014 and December 2015.
Materials and methods: The clinical specimens comprised 295 skin scrapings and 711 nail scales collected with sterilized scalpels and/or scissors from 930 patients with suspected skin mycoses and/or onychomycosis. The specimens were seeded into Sabouraud dextrose agar i) containing chloramphenicol and gentamicin, ii) chloramphenicol, and iii) chloramphenicol and cycloheximide and incubated at 30°C and 37°C for up to 21 days. For cultivation of *Malassezia* spp oil was added to the medium. The identification was performed by gross and microscopic morphology and/or by routine identification systems (VITEK II, Biomerieux; MALDI-TOF Bruker).
Results: Out of the clinical specimens analyzed, 48.8% of the nail and 33.9% of the skin samples resulted positive for fungi. Yeasts were isolated in 61.2% of the positive lesions, followed by dermatophytes in 20.8%, and non-dermatophyte moulds in 18%. *Trichophyton* spp was the most frequently isolated dermatophyte (87.3%), while *Candida* spp represented the vast majority of the yeasts (82.4%). In particular, *Candida* spp was the dominant pathogen in onychomycosis while dermatophytes were isolated from the majority of the skin mycoses.
Conclusions: Dermatophytes, *Trichophyton* spp in particular, are reported to be the primary causative

agents of the skin and nail mycoses. Our results on skin mycoses are in agreement with the literature as *Trichophyton* spp was the most frequently isolated pathogen from skin lesions. However, we found a very high frequency of *Candida* spp in onychomycosis, which is in disagreement with the literature. This could be due to the fact that yeasts are often isolated from previously damaged nail plates and it is sometimes controversial whether they are primary responsible for nail infection or only concomitant pathogens. It is suggested that repeated isolations of yeasts in the absence of other fungal pathogens may be considered as an evidence of nail invasion.

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BIOFILM PRODUCTION BY *C. PARAPSILOSIS* CLINICAL STRAINS RECOVERED FROM SEVERAL WARDS OF AN HOSPITAL IN PALERMO

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Introduction: *C. parapsilosis* is often associated to nosocomial infections, expecially in ill patients including HIV-infected, cancer, transplant patients. Yeast infections are associated with the use of indwelling catheters such as vascular catheters, urinary catheters or artificial heart valves. Most of the infection are, infact, due to exogenous acquisition of the fungus following colonization of hospital environment and hands of health care workers. Although *C. parapsilosis* virulence determinants are not much known, formation of biofilm and secretion of extracellular enzymes seem to play an important role in its pathogenesis. In mucosal and device-associated biofilms the microrganism acquires the ability to tolerate high antifungal concentrations and to evade host immune response. The present study aims to investigate the *in vitro* biofilm production of 64 *C. parapsilosis* clinical strains from different anatomic district of patients hospitalized in different wards of "P. Giaccone" hospital.

Materials and Methods: The 64 strains of *C. parapsilosis*, recovered from High Risk Units (36) and General Medicine Units (28) of hospitalized patients, were isolated from different pathological samples. Biofilm production was assessed using cristal violet (CV) stain assay, for quantification of biomass and carried out in 96-well polystyrene plates. A $\chi 2$ -test was performed using the R statistical software package to verify the significativity ($p < 0.05$) of correlation between the major number of strains producing biofilm and the Units of provenience.

Results: Biofilm was detected in a total of 35 (54.7%) of 64 stains (considering moderate and high values) regard to biomass production. A greater percentage was found in High Risk Units (75%) respect General Medicine Units (28.5%). The difference between the two percentages is

statistically significant (p-value < 0.05).
Conclusions: The vast majority of infections caused by *C. parapsilosis* involve proliferation of a biofilm on artificial devices or biotic surface as human mucosa. In the biofilm, defined as a surface-associated and highly structured community of microrganisms, the cells are enclosed within a protective extracellular matrix and can proliferate protected by drug action and immune response.
In this study CV assay has permitted to analyze biofilm production and to find that in High Risk Units, *C. parapsilosis* strains have a marked capacity to form it *in vitro*, data non found in General Medicine Units . A possible correlation between a greater capacity of strains to produce biofilm in the first units and a predominant genotype, obtained by microsatellite analysis, is being investigated.

P151

MYCOTIC RHINITIS CAUSED BY SCEDOSPORIUM APIOSPERMUM IN A DOG: FIRST REPORT IN ITALY

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Introduction: Fungi belonging to the Scedosporium/Pseudallescheria complex (SPCF) are important human pathogens. Due to a lower incidence of SPCF infections in pets, there has been a lack of awareness by the veterinary community toward this group as a cause of nasal disease in dogs and cats, focusing on the more common *Aspergillus* spp.*Pseudallescheria boydii* (teleomorph state) and *Scedosporium apiospermum* (anamorph state) are the most known pathogens of the complex and are widely distributed in the environment, especially in the soil. This fungi may cause disseminated infections in dogs involving kidneys, heart, blood vessels, lymph nodes, brain and eyes. Infections of the nasal cavity by *S. apiospermum* in dogs have been described in Spain, Australia and New Zealand. To our knowledge, this is the first report of *S. apiospermum* rhinitis in a dog in Italy.
Materials and Methods: A 4-year-old female Bull Terrier was presented with a 20-day history of muco-purulent monolateral nasal discharge. The dog had not responded to antibiotic therapy. Swabs and biopsy specimens were collected from the nasal cavity for histological examinations and mycological cultures. Susceptibility tests on the mycological strain were performed.
Results: From the mycological examination of the nasal tissue biopsies, pure culture of cottony, yeast-like grayish-white colonies were obtained. Microscopically, the fungal isolate presented septate hyaline hyphae, flask-shaped conidiogenous cells and unicellular, oval conidia. According to the macroscopic and microscopic characteristics *S. apiospermum* was identified. The isolate did not produce the sexual state. The strain resulted susceptible *in vitro* to Econazole and Miconazole, intermediate to Enilconazole and Clotrimazole and resistant to Amphotericin B, Fluconazole, Ketoconazole and Itraconazole. Histopathology examination of the biopsies confirmed a mycotic etiology of the infection, revealing septate hyphae and smooth, one-celled, elongate conidia in the lesion.
Discussion: To our knowledge, this is the first description of *S. apiospermum* as etiologic agent of nasal disease in a dog in Italy. Clinical signs

associated with *S. apiospermum* nasal infections are similar to those caused by *Aspergillus* spp. Furthermore, histological sections cannot be used to distinguish between *S. apiospermum* mycoses and aspergillosis, leading to a misinterpretation of the etiological agent and an underrate of *Scedosporium* spp. infection. Cultural methods remains the gold standard for the diagnosis of SPCF infections. Moreover, a proper identification of the fungal agent involved in the lesions is to choose the best therapy, even if antifungal susceptibility tests for filamentous fungi remain unstandardized and *in vitro* results do not always reflect the *in vivo* outcome.

P152

MONITORING AND CONTROL OF ECHINOCOCCOSIS-IDATIDOSIS IN CAMPANIA REGION, ITALY

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Introduction: Echinococcosis is a parasitic zoonotic disease caused by infection with tiny tapeworms of the genus *Echinocococcus*. Cystic echinoccosis (CE), also known as hydatid disease, is caused by infection with the larval stage of *Echinococcus granulosus* (EG) and *Echinococcus multilocularis* (EM). It represents one of the most important parasitic zoonotic disease in the world. CE is widespread in Africa, Europe, Asia, the Middle East, Central and South America, and many cases have been reported in several Mediterranean countries. The parasite is transmitted to dogs when they ingest the organs of other animals that contain hydatid cysts, resulting actively transmitted in all pastoral regions where sheep, cattle, buffaloes, pigs predominate. Although most infections in humans are asymptomatic, CE causes harmful, slowly enlarging cysts in the liver, lungs, and other organs that often grow unnoticed and neglected for years. The Veterinary Sector of Campania Region, togheter with the 'Research Unit for the Monitoring of Intestinal parasitosis of Migrants of the Mediterranean area' (URPIM) of the University of Campania "Luigi Vanvitelli", the Regional Center for Monitoring Parasitic infection (CREMOPAR) and the Experimental Zoo-prophylatic Institute of Southern Italy, has developed a wide project that focused on the monitoring and control of CE in farm animals and in humans at risk of infection in the Campania region.

Materials and methods: A monitoring programmes in farm animals and diagnosis of CE in farm workers and their relatives have been performed using serological assays (ELISA and IHA) and subsequently confirmed by molecular tests (Immunoblotting). Moreover the data are evalueted using Geographical Information System (GIS), that allows us to select farms to be included in our study.

Results: We have analyzed 1211 human serum samples: 14 samples are resulted positive at Elisa test, while only 3 samples at IHA. The positive samples at IHA test have been confermed through

molecular test.
Conclusions: Preliminary data on the prevalence of CE in farm animals confirm the importance of the surveillance upon free ranging dogs and those human subjects at risk of infection, in order to reduce animal diseases and prevent human infection with an accurate early diagnosis of human hydatidosis.

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METHICILLIN RESISTANCE IN *STAPHYLOCOCCUS PSEUDINTERMEDIUS* AND *STAPHYLOCOCCUS AUREUS* STRAINS ISOLATED FROM COMPANION ANIMALS

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Introduction: The increasing interest for staphylococci in Veterinary medicine highlights the need to have an accurate identification of these bacteria. Furthermore, the control of methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) requires a dual approach aimed at reducing antimicrobial consumption and preventing transmission between animals and from animals to humans or viceversa. In this study, we have isolated *Staphylococcus aureus* and *Staphylococcus pseudintermedius* strains from infection sites of companion animals and we evaluated the degree of identity of these isolates comparing different identification methods.
Materials and Methods: The specimens were collected from diseased dogs and cats 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 firstly by Api Staph method. Later, this identification was compared to matrix assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF-MS) identification method. Molecular profiling, using species-specific *nuc* genes, was also performed. Moreover, genetic profiles of methicillin resistance were carried out by PCR to assess the detection of *mec*A and *mec*C genes in all the identified isolates.
Results: 40 clinical isolates were firstly identified as "*Staphylococcus aureus/Staphylococcus intermedius* if of animal origin" by API Staph method. Molecular profiling, using the species-specific *nuc* genes, identified 30 isolates as *S. pseudintermedius* and 10 isolates as *S. aureus* confirming the MALDI-TOF-MS results. Moreover,

genetic profiles of methicillin resistance were also carried out by PCR to assess the detection of *mec*A and *mec*C genes in all the identified isolates. Precisely, 33% of *S. pseudintermedius* and 10% of *S. aureus* isolates harboured *mec*A gene, whereas none of the isolates revealed mecC gene.
Conclusions: We conclude that MALDI-TOF-MS method, which is becoming a standard method for species identification in medical microbiological laboratories, is also in veterinary medicine the most accurate method for the identification of *Staphylococcus pseudintermedius* and *Staphylococcus aureus*. Moreover, the current investigation highlights a significant higher presence of methicillin resistance in *S. pseudintermedius* than *S. aureus* strains.

P154

ANTIBACTERIAL RESISTANCE PATTERNS OF ESCHERICHIA COLI FROM COMMUNITY-ACQUIRED URINARY TRACT INFECTIONS IN GENOA FROM 2014 TO 2016

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Introduction: Urinary tract infections (UTIs) are the most frequent bacterial infections affecting people both in the community and in hospitals. Over 80% of UTIs are caused by *E. coli*. In recent years, there is increased prevalence of antimicrobial resistant (AMR) *E. coli*, resulting in treatment failures and increased healthcare cost. The aim of the study was to evaluate and to characterize the AMR *E. coli* prevalence in outpatients attending to Local Health Units 3 (ASL3) in Genoa.
Materials and Methods: Microbiology records of patients attending to 45 outpatient clinics of ASL3 in Genoa, from January 2014 to December 2016, were reviewed retrospectively. Urine samples were analysed and processed according to the microbiology laboratory standards. Cultures were considered as positive if bacterial growth was $\geq 10^5$ colony forming units (CFUs)/mL. Antimicrobial susceptibility was performed by Vitek2 (Biomerieux Diagnostics). Interpretation was based on EUCAST criteria.
Results: A total of 88,253 urine samples of outpatient were processed at ASL3 Microbiology Laboratory. Of these, 32.5% (No. 28,694) had positive cultures. *E. coli* was the most common isolated microorganism (No. 17,038 samples, 59.4%). Antibiotic sensitivity pattern showed maximum resistance to ciprofloxacin (30.9 - 32.7%), followed by co-trimoxazole (26.6 - 28.1%) and amoxycillin-clavulanate (AMC) (18.6 - 22.8%), while the lowest resistance rates were for meropenem (0,1%), nitrofurantoin (< 1.7%) and fosfomycine (< 2.7%).
Trend analysis showed no significant increase in resistance to any antibiotics in the three years.
Conclusions: This study demonstrates that urinary *E. coli* resistance to commonly prescribed antimicrobials is high in agreement with European Surveillance but is stable over time in our records. Overuse of AMC, cotrimoxazole and ciprofloxacin should be avoided given the high resistance rates

reported in the community. In developing local antimicrobial prescribing guidelines, the antibiotic choice in the empirical treatment of UTIs should be based on setting of acquisition.

P155

THREE-YEAR ANTIMICROBIAL RESISTANCE PATTERNS OF URINARY ESCHERICHIA COLI AT ASL3 HOSPITALS IN GENOA

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Introduction: Urinary tract infections (UTIs) are predominantly bacterial infections affecting people both in the community and in hospitals. Over 80% are caused by *E. coli.* While UTIs are a major infection burden globally, the growing problem of antimicrobial resistance (AMR) can result in treatment failures and increased cost of healthcare. There is evidence to show that the AMR pattern of urinary *E. coli* is increasing. The aim of the study was to evaluate the AMR of urinary *E. coli* in inpatients attending to Local Health Units 3 (ASL3) in Genoa.

Materials and Methods: ASL3 includes 4 hospitals and 39 residential elderly care (RSA) in Genoa. The microbiology records of inpatients attending to these hospitals and RSA from January 2014 to December 2016 were retrospectively reviewed. Urine samples were analysed and processed based on the microbiology laboratory standards. Cultures were considered as positive if bacterial growth was $\geq 10^5$ colony forming units (CFUs)/mL. Antimicrobial susceptibility was performed by Vitek2 (Biomerieux Diagnostics). Interpretation was based on EUCAST criteria.

Results: Total of 21,242 urine samples of inpatients from 2014 to 2016 were processed by ASL3 Microbiology Laboratory. Of these, 43.2% (n =9,176) had positive cultures. *E. coli* was the most common organism isolated: 2,472 (35%) samples from hospitals and 846 (40%) from RSA. The overall 3-year resistance rates to urinary *E. coli* is very different in hospitals and RSA. The highest rates were seen for ciprofloxacin (respectively 38 - 42.7% and 71.3 - 76.8%), co-trimoxazole (32.4 - 36.4% and 38.4 - 49.2%) and amoxycillin-clavulanate (AMC) (30.4 - 32.9% and 46.7-51.1%). In RSA there were also high resistance to gentamicin (28.9 - 30.4%) and piperacillin/tazobactam (20.3 - 22%). The lowest resistance rates were for meropenem (< 1.2%), nitrofurantoin (< 3.5%) and fosfomycine (< 4.6%). Trend analysis showed a weak decrease in resistance to all the antibiotics tested in hospital patients, while the situation in RSA is unvaried in the three years, except for co-trimoxazole which is

improving.
Conclusions: Antimicrobial resistance poses grave concerns for antimicrobial effectiveness in treating infections such as UTI. This study showed that the greater consciousness of resistance emergence allowed a better use of antibiotics in hospital with decrease of resistance rates. In RSA, the presence of comorbidity in elderly patients not allow the same management in the use of antibiotics and the result is a very high rate of antimicrobial resistance.

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EMERGENCE OF FOSKP96 PLASMID-MEDIATED RESISTANCE MECHANISM IN KPC *KLEBSIELLA PNEUMONIAE* CLINICAL STRAINS IN ITALY

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Introduction: The presence of acquired carbapenemases in *Klebsiella pneumoniae* have been systematically investigated, while only few studies evaluated the occurrence of plasmid-encoded fosfomycin (FOS) inactivating enzymes (e.g. fosA3 and fosKP96) in this species. Aim of the study was to detect *fos*-type genes in multi-drug resistant (MDR) and *bla*KPC-positive *K. pneumoniae* (KPC-Kp) strains from an Italian Long Term and Rehabilitation Facility (LTCF) located in Lombardy, Northern Italy.
Material and Methods: A total of 25 ertapenem resistant and MDR KPC-Kp isolates were collected from ASP Golgi-Redaelli, in the period July-October 2016. Identification and antibiotic susceptibilities were obtained by Vitek-2 (bioMèrieux), EUCAST 2016 guidelines. FOS MICs were also determined by the EUCAST recommended agar dilution method (ADM). *bla*KPC, fosA/A3/C2/KP96 and *aac*-(6')-Ib-cr genes were investigated by PCR. Check-MDR CT103XL (Checkpoint) microarray was also used. Molecular characterization of plasmids and strains was accomplished using PBRT Kit (Diatheva), and Pulsed Field Gel Electrophoresis (PFGE) respectively.
Results: The 25 KPC-Kp isolates were from Rehabilitation wards (20/25, 80%), Long Term Care (3/25, 12%), and others (2/25, 8%). The samples were mainly from urine (20/25, 80%); the remaining (5/25, 20%) from blood, sputum, rectal, and urethral swabs. The susceptibility was retained for tigecyclin in 8/25 (32%) cases, trimethoprim-sulphamethoxazole 11/25 (44%), amikacin 12/25 (48%) and colistin 24/25 (96%). All the strains were resistant to ertapenem, and third generation cephalosporins, and at different extent to imipenem

(10/25, 40%) and meropenem (18/25, 72%). The 72% of the isolates (18/25) showed FOS susceptibility by Vitek-2; while with EUCAST recommended ADM the percentage decreased to 56% (n = 14). FOS MICs ranged between > 128 mg/L and 4mg/L by ADM (MIC_{50} = 32mg/l, MIC_{90} ≥ 128 mg/L); by Vitek-2 the range was > 6 4mg/L - ≤ 16 mg/L (MIC_{50} ≤ 16 mg/L, MIC_{90} ≥ 64mg/L). All the KPC-Kp isolates resulted *fos*KP96 positive by PCR; sixteen were also *aac-(6')-Ib-cr* positive. Two strains were ESBL-positive: one *bla*CTX-M-64, one *bla*TEM- and *bla*SHV-type positive, by microarray. The overall rate of KPC and fosKP96 in the study period was 20% (n = 25/126). The plasmid typing revealed the presence of FIIK, A/C, X2, X3, FIB-M, FI-B and P replicons, and PFGE results showed clonal heterogeneity.

Conclusions: This study highlights the emergence of fosKP96-mediated resistance mechanism in multi-clonal MDR KPC-Kp from a LTCF in Italy. The report confirms that i) automated system underestimate the FOS MIC and that ii) molecular investigations have to be implemented to monitor the spread of acquired FOS resistance mechanisms in *K. pneumoniae*.

P157

PRIORITY LIST OF ANTIBIOTIC-RESISTANT GRAM-NEGATIVE BACTERIA OF REGGIO CALABRIA HOSPITAL

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Introduction: In February 2017, The World Health Organization (WHO) developed a global priority pathogens list (global PPL) about antibiotic-resistant bacteria stratified in three priority tiers (critical, high and medium) according to their antibiotic susceptibility. Priority 1 or Critical tier includes only Gram-negative bacteria: *Acinetobacter baumannii* carbapenem-resistant, *Pseudomonas aeruginosa* carbapenem-resistant, *Enterobacteriaceae* carbapenem-resistant and 3rd generation cephalosporin-resistant. The goal of this work was to identify the most important resistant Gram-negative bacteria at local level, for which there is an urgent need in terms of implementation of nosocomial infection control measures, in order to see if it matches with the global situation.

Materials and Methods: Identifications and antibiotic susceptibility tests (AST) were performed by MicroScan WalkAway plus System (Beckman Coulter). Antibiotic-resistant bacteria have also been subjected to Kirby-Bauer disk diffusion susceptibility test on Mueller-Hinton agar and interpreted with the help of the semi-automated reading system Adagio (Did), according to the EUCAST clinical breakpoints.

Results: The data we collected in 2016, compared with those of previous years, show a progressive increase in the incidence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) Gram-negative bacteria, according to the WHO global PPL. Microbiology O.U. of Reggio Calabria "Bianchi Melacrino Morelli" Hospital everyday isolates antibiotic-resistant bacteria from many types of clinical specimens, such as *Enterobacteriaceae* ESBL- and carbapenemase-producers (59.7%), *A. baumannii* colistin-sensitive only (25.1%) or *P. aeruginosa* carbapenem-resistant (15.2%). The most affected ward is intensive care unit (ICU), however unfortunately nosocomial infections due to antibiotic-resistant bacteria are spreading in many other wards (like heart surgery, hematology, nephrology and vascular surgery).

Conclusions: Even if referring to a limited geographical area, the evaluation of our data

reflects the global situation described by the WHO and underlines the essential role of hospital clinical epidemiology into infection control. In this regard antibiotic stewardship programmes should be implemented to support an appropriate use of antibiotics and an adequate long-term planning urgently required. Infections by antibiotic-resistant bacteria are a public health threat that we need to survey and to fight. They may be addressed by interventions such improvement sanitation and implementation of nosocomial infection control measures, not only by the development of new antibiotics, in order to significantly reduce the spread of infections due to antibiotic-resistant bacteria.

P158

EXTRAINTESTINAL PATHOGENIC AND ANTIMICROBIAL-RESISTANT *E. COLI* OF FOOD ORIGIN

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Introduction: Globally, resistance to antibiotics and in particular to quinolones has been dramatically rising among human and also veterinary isolates, likely as a consequence of their large consumption. An increasing number of infections due to extraintestinal pathogenic *E. coli* (ExPEC) is reported, but still no clear reservoir of these pathogens has been unraveled. Recently, food animals, and particularly poultry, have been suggested as a vehicles for transmitting resistant ExPEC. The objective of this study hence was to assess the presence of multidrug resistant *E. coli*, with ExPEC-associated traits, that could pose a risk for consumers.

Materials and Methods: This study analyzed 25 quinolone resistant *E. coli* isolates, obtained from meat sold at retail in Palermo. We investigated their genotypes with relation to their plasmid-mediated quinolone resistance (PMQR) genes, their phylogenetic groups and their virulence profiles. Moreover the strains were typed, in order to screen if they were ST131, and subjected to Enterobacterial Repetitive Intergenic Consensus sequence (ERIC) PCR.

Results: D1 (44%) was the most common phylogenetic group, followed by A1 (28%), A0 (20%), and lastly B1 (8%). All strains were analyzed after ascertaining their resistance to quinolones, but only 5 out of 25 possessed any of the PMQR determinants, while no cephalosporin resistance was detected among all of the strains. Of the 25 isolates, 7 met the criteria to be included in ExPEC, that is having at least two virulence factors (VFs) according to Johnson et al (2005), whereas 8 isolates were found to have only one VF. Finally, ERIC PCR showed a quite high level of heterogeneity, except for two pairs of strains, which shared the same patterns of bands.

Conclusions: These data demonstrate a dramatically

high prevalence of multidrug resistance patterns in Palermo area, with some of the isolates having genetic profiles that suggest the capability to cause extraintestinal infections in humans. Therefore these findings highlight the possibility that food animals, and particularly poultry, may be an important source of resistant bacterial strains, posing a potential zoonotic risk. Hence our data support the already clear necessity of a more prudent usage of antibiotics in livestock production, in order to curb the diffusion of resistance determinants.

P159

CHARACTERIZATION AND ANTIBIOTIC RESISTANCE OF *S. AUREUS* ISOLATED FROM RABBITS (*ORYCTOLAGUS CUNICULUS*)

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Introduction: The epidemiology of *Staphylococcus aureus* (SA) infections has gained interest in the last years for their importance in veterinary medicine, the emergence of some clonal animal lineages, and their increasingly evidenced zoonotic potential. The discovery of emergent *mecC* MRSA in farmed rabbits together with the sporadic reports of MRSA in companion rabbits, including livestock-associated clonal complex 398 MRSA and the Panton-Valentine Leucocidin-positive isolates have raised concern about the SA population in rabbits. Phenotypic and genotypic investigations were carried out on SA strains isolated from skin and lesion swabs of different rabbit categories and farm workers in order to evaluate the prevalence, the zoonotic infection risk, the predominant clonal lineages and the antibiotic resistance profiles.
Materials and Methods: In June 2015, 2200 swabs were collected from 400 rabbits belonging to different categories (young rabbits, adults, reproducers), randomly selected from a large farm in the Lazio region. Ear, nasal, axillary, inguinal, perineum areas, and additional skin lesions were tested for the presence of SA. A random selected number of strains (n = 98) isolated from nasal swabs and skin lesions were characterized in order to assess the presence of *nuc* and *mecA, bbp, selm, flank* genes, to assign the *spa*-type and evaluate the antibiotic resistance by Kirby-Bauer and E-test methods (EUCAST 2017). The statistical analysis was performed by Software STATA version 13.0.
Results: SA was detected with a frequency of 592 isolates (17%, n = 3376). All areas were infected, recording a significant difference between skin areas and lesions ($p < 0.05$), while relevant numbers were detected both in the ear (38%) and the nasal

cavity (41%, p = 0.925). A significant difference was recorded in relation to the age ranges ($p < 0.05$), except for young (5%) and reproducers (4%; p = 0.785). All strains resulted MSSA but showed some multi-resistance profiles, ranging from 3 to 7 antibiotic classes: in particular tetracyclines (96%), macrolides (94%), diterpenes (84%), fluoroquinolones (64%), aminoglycosides B-C grades (48%, 5%), and glycopepetides (teicoplanin: 73%; hVISA: 4%; VISA: 5%). All the strains were negative for the presence of virulence genesand therefore were classified as low virulence strains. Five different *spa*-types were identified, belonging to two different clonal complexes (CC97, CC15). The most frequently recovered *spa*-type has been t2802 (55%), also detected in human samples.

Conclusions: In this study a high frequency of multiresistant SA strains was observed in rabbits, although none could be classified as MRSA. Clonal lineages were not correlated to the sampling site, while an association was recorded with the antibiotic-resistance profiles.

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WITHSTANDING DRUG RESISTANCE IN BACTERIA TARGETING SOS RESPONSE

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Introduction: The World Health Organization considers bacterial resistance among the three main risks for human health, and the development of new strategies for fighting bacterial infections are strongly desirable. In 2014 report, WHO evokes a post-antibiotic era in which "common infections and minor injuries can kill". The emergence of a Multi-Drug Resistant profile in bacteria is mainly due to their extraordinary ability to evolve in response to antibiotics; for instance, the activation of the SOS pathway plays a central role in the evolution of resistance under antibiotic stress. The SOS response is mainly orchestrated by two proteins: RecA and LexA. As a consequence of damage to the DNA, RecA promotes self-cleavage of the transcriptional repressor LexA, inducing the expression of more than 40 genes involved in DNA repair and mutagenesis. The inactivation of the SOS regulators, by deletion of RecA and/or by engineering a noncleavable LexA into bacteria, reduces the acquisition and integration of new resistance genes and the ability of bacteria to mutate, decreasing the appearance of new drug resistance phenotypes. The development of resistant bacteria is delayed at the level of vertical and horizontal transfer of resistance genes. The SOS pathway represents therefore an attractive target in reducing the bacterial ability to evolve antibiotic resistance extending the long term viability of known and new antibiotics. This study is focused on the development of potential inhibitors of the SOS activation, through the inhibition of LexA self-cleavage.

Materials and Methods: The autoproteolysis of LexA was experimentally induced by alkaline pH, or in presence of RecA protein, ATP and ssDNA. A first structure-based *in silico* screening has been performed and few boronic acid derivatives have been identified as potential LexA inhibitors. In our hand, boronic acids represented a potent tool to probe, for the first time, LexA catalytic site. We

have developed a quantitative UPLC-MS method to efficiently follow the kinetics of LexA self-cleavage and the inhibitory effect on autoproteolysis of boronic acids.
Results: While the first order rate constant was calculated from the time course of autohydrolysis, to quantify the "potency" of inhibition a novel kinetic model has been developed. The *in vivo* efficacy of boronic acids was evaluated by observing the reduction of filamentation induced by sub-inhibitory concentrations of levofloxacin in *Escherichia coli.*
Conclusions: The results coming from this first screening were encouraging, providing critical information on the catalytic pocket and on the binding requirements necessary for the improved design of high affinity ligands. Our preliminary data suggest that boronic acids can efficaciously hinder the SOS pathway activation by the inhibition of the RecA-LexA axis.

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CO-OCCURRENCE BETWEEN MULTIPLE ANTIBIOTIC RESISTANCE AND HEAVY-METAL TOLERANCE AMONG *ESCHERICHIA COLI* STRAINS OF AVIAN AND HUMAN ORIGIN IN A CATCHMENT AREA OF LAZIO REGION, ITALY

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Introduction: The massive and unrestrained veterinary use of different antibiotics of the same classes used in human medicine, has contributed to the selection and spread of multidrug resistant bacterial strains in the environment. Extraintestinal *Escherichia coli* (ExPEC) group collects heterogeneous pathotypes isolated from infections outside of the intestinal tract both of humans (uropathogenic and neonatal meningitis strains) and animals, such as avian strains (APEC) responsible of colibacillosis. Poultry has been suggested as a reservoir for multidrug-resistant *E. coli* strains causing extraintestinal infections in humans, so that characterization of strains from both hosts can add useful data for the evaluation of the potential zoonotic risk. Furthermore, co-occurrence of antibiotic and heavy-metal resistance in different habitat has been reported. In order to recognized co-spread of multiple antibiotic resistance and heavy-metal tolerance, in the present study we analyzed *E. coli* strains from fecal samples of healthy poultry and isolates from human extraintestinal infections, collected in a defined geographic area of Lazio region.
Materials and Methods: *E. coli* strains from humans were isolated from extraintestinal sites. Avian *E. coli* strains were isolated from fecal samples of healthy poultry. Antibiotic resistance profile was determined by automated VITEK-2 System or microdilution method. Metal tolerance was evaluated in comparison to reference strains on agar plates at different concentrations. Phylogroup belonging was determined by PCR assay. Plasmids were extracted by alkaline lysis and analyzed by gel electrophoresis.
Results: Concerning *E. coli* isolated from animals, higher antibiotics percentages of

resistance was observed for sulfamethoxazole and aminoglycosides. *E. coli* strains can be classified into four main phylogenetic groups (A, B1, B2 and D); whereas the majority of animal isolates belonged to A and B1, human isolates to B2 or D phylogroups. The multidrug resistance in avian *E. coli* was associated with strains belonging to B1 but also to D phylogroup. Furthermore, a multi-resistance spectrum of antibiotic and heavy metals, such as cadmium and arsenic, was observable. Several studies have demonstrated the coexistence of metal and antibiotic resistance in large plasmids. Only in the avian strains were present plasmids from 220-270 kb size.

Conclusions: The results, based on phenotypic and genotypic characterization, on the prevalence of antimicrobial resistance to antibiotics and metals, suggested a correlation between strains circulating in healthy animals destined for food production and those isolated from human extraintestinal infections.

P162

CROSS-RESISTANCE BY *ASPERGILLUS FUMIGATUS* TO CLINICAL AZOLES FOLLOWING EXPOSURE TO TEBUCONAZOLE, A TRIAZOLE FUNGICIDE

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Introduction: *Aspergillus fumigatus* is the leading pathogen causing invasive mould diseases in humans. Competitive inhibitor of cyp51A such as voriconazole, itraconazole, posaconazole and isovuconazole are the recommended primary terapie for aspergillosis in international guidelines. Cumulative evidence described the emergence and geographical expansion of azole resistant A. fumigatus associated with azole treatment. In addition, an environmental route of resistance development has been suggested through exposure to 14a-demethylase inhibitors (DMIs). The main resistance mechanism consisting a 34- bp tandem repeat in the promotor region of cyp51A in combination of a substitution at codon 98 (TR34/L98H). In this study we investigated if tebuconazole, a agricoltural azole, may induce cross-resistance to clinical azoles in clinical *Aspergillus fumigatus* isolates following *in vitro* exposure.

Materials and Methods: Five strains of A. fumigatus isolated from clinical respiratory speciemens and susceptible to clinical azoles are selected for the study. The isolates were identified as belonging to *A. fumigatus* complex by macroscopic and microscopic morphology and by using MALDI-TOF MS. Induction experiments were performed with the triazole DMI tebuconazole. The five strains were cultivated on Sabouraud agar, for 10 passages, inoculating a solution containing 1x10^6 conidia with a sub-inibitory concentration of the agricoltural azole and were incubated at 35°C until presence of conidiation. As control assay, the same strains were cultivated in the same conditions but without tebuconazole. Antifungal susceptibility testing for voriconazole, itraconazole, posaconazole and isavuconazole was performed with commercial E test method on exposed and not exposed A. fumigatus strains, in time 0 (before the induction assays),1 (after two passages), 2 (after five passages), and 3 (after 10 passages). A multiplex PCR assay was performed to detect TR34/L98H mutations from exposed strains in time 3.

Results: A increment of the minimal inhibitory concentration (MIC) of all azole tested, from time 1 until time 3 was observed. In particular, in time 3 the five isolates had a resistant MIC value of itraconazole, 4 strains had a intermediate susceptibility for posoconazole, and one strain was multiresistent with high MIC values for all azole tested. In addition, a concomitant increase of the sub-inibitory concentration of tebuconazole was also observed during induction assay. Furthermore, tebuconazole exposure was associated to a reduction of speed of growth and a delayed formation of conidia. TR34/L98H was not found in any of the clones that were exposed to tebuconazole.
Conclusion: The relation between the use of the triazole DMIs and cross-resistance to medical triazoles in A. fumigatus has major implications for the assessment of health risks associated with the use of DMIs. Molecule structure similarity and activity of triazole DMIs against A. fumigatus appear to be the key features that cause cross-resistance to medical triazoles. In our induction experiments, tebuconazole exposure induced the development of cross-resistance with posaconazole, itraconazole, voriconazole and isavuconazole but TR34/L98H mutations were not detected in any of the exposed A. fumigatus strains. So, our findings support a fungicide-driven route of azoles resistance development in A. fumigatus and highlights the possible role of other Cyp51A-related mutations linked to clinical azole resistence.

P163

COERG11 Y132F MUTATION INDUCES MULTI-AZOLE RESISTANCE IN CANDIDA ORTHOPSILOSIS

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Introduction: *Candida orthopsilosis* is an opportunistic fungal pathogen responsible for mucosal and systemic infections in humans. Recently, azole resistant *C. orthopsilosis* clinical isolates have been described. The aim of the present study was to unravel molecular mechanisms underlying azole resistance in this emerging species.
Material and Methods: 42 azole resistant *C. orthopsilosis* clinical isolates were collected at the Azienda Ospedaliero-Universitaria Pisana and at the Institute of Microbiology, Università Cattolica del Sacro Cuore, Rome. Sequencing analysis of CoERG11 hot spot regions was performed in all strains to investigate the presence of SNPs leading to non-synonymous amino acidic substitutions in the encoded enzyme. To assess the role of Y132F substitution (SNP-A395T) in the development of azole resistance in *C. orthopsilosis*, a fluconazole susceptible isolate was used as a genomic background for a SAT1 flipper driven transformation aimed at integrating a copy of CoERG11 coding sequence bearing the A395T mutation. Mutant clones were tested for their azole susceptibility and for heterozygosity at the hot spot locus.
Results: Six non-synonymous amino acidic substitutions were found in the panel of clinical isolates tested, of which only Y132F was predicted to be deleterious for Erg11 protein functionality. SAT1 flipper driven transformation successfully produced heterozygous clones bearing the desired mutation and showing a resistant phenotype to all azoles tested.
Conclusions: These findings provide the first evidence that Y132F substitution induced in a heterozygous mutant strains is sufficient to confer multi-azole resistance in *C. orthopsilosis.*

P164

RNA-SEQ AND BIOINFORMATICS FOR A COMPARATIVE STUDY ON THE TRANSCRIPTOME IN DAPTOMYCIN-RESISTANT *STAPHYLOCOCCUS AUREUS*

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Introduction: The spread of Daptomycin-resistant *Staphylococcus aureus* strains represents a major public health issue since Daptomycin (DAP) is the last-resort treatment for heterogeneous Vancomycin-Intermediate-*Staphylococcus aureus* (hVISA) and Vancomycin-Intermediate-*S. aureus* (VISA).

Materials and Methods: We investigated the small-RNA and mRNA comparative transcriptome of two DAP-R (1C and 3B) clinical isogenic isolates against their DAP-S (1A and 3A) counterparts by Illumina RNA-seq, bioinformatics tools and databases, filtering and computational analysis.

Results: Statistically significant filtering analysis of the data to sort differentially expressed small-RNAs and mRNAs based on their up or down-regulation profiles in both DAP-R vs DAP-S strains highlighted: i) 31 antisense small-RNAs targeting genes for 5, 16 and 23S ribosomal RNAs, tRNAs, metabolism, transporters, oxidative stress response and cell-wall biosynthesis; ii) 53 mRNAs encoding proteins for the Two-Component Signal Transduction System YycFG, a cell adhesion related virulence factor, T-Cell Receptors, metabolic enzymes, metabolic and transcriptional regulators, transporters, and others involved in DNA replication process, stress response mechanisms, cell envelope biogenesis and in cell-wall and rRNA biosynthesis.

Conclusions: This is the first study to take a full snapshot of the comparative transcriptome of DAP-R MRSA highlighting their RNAome signatures.

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PHENOTYPIC CHARACTERIZATION OF SUBSTITUTIONS IN POSITION H553 OF RPOB GENE IN RIFAMPICIN RESISTANCE STRAINS OF SEROGROUP A *NEISSERIA MENINGITIDIS*

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Introduction: *Neisseria meningitidis*, an obligate human commensal, is capable of causing invasive disease including sepsis and meningitis. Chemoprophylaxis with Rifampicin is urgently indicated to eradicate nasopharyngeal colonisation of close contacts and usually reduces the carriage status by up to 90%. The mechanism of resistance is linked to point mutations in the *rpoB* gene leading to aminoacid substitutions. The most frequent substitution affects the histidine in position 553. Our previous studies have demonstrated how mutations in RNA polymerase of meningococcal serogroup C strain determine global perturbation affecting bacterial fitness, metabolism and virulence.

Materials and methods: By *in vitro* assay, spontaneuos rifampicin-resistant strains (Rif^R) from the hypervirulent serogroup A strain 205900 were analyzed for the *rpoB* gene sequencing and a number of phenotypic features. We determined the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of Rifampicin for the selected mutants. The fitness of Rif^R mutants has been evaluated by growth and survival assays in complex culture media, in competition with the wild type strain and in differentiated human monocytes (THP-1).

Results: Three distinct mutants characterized by substitution of histidine in position 553 were identified: H553Y, H553R and H553L. Broth microdilution assays highlighted, for selected

mutants, MIC and MBC values greater than the wild type strain. *In vitro*, the mutant H553L exhibited a reduced survival rate compared to the parental strain and to the other mutants analyzed, while the H553Y and H553R mutants showed a trend similar to the wild type isolate. Interestingly, the H553L mutant exhibited a significant increase of intracellular survival in differentiated THP-1 cells, despite other Rif^R and wild type strains.

Conclusions: The meningococcal capsule plays contrasting roles during the infectious cycle and its composition allows the subdivision of meningococcal isolates and reflects their different infectious capabilities. Serogroups in this pathogen differ in immunogenicity and virulence in animals models, and influence the outcome of disease in humans. Previous studies have shown that the fitness cost for Rif^R mutations is influenced not only by the experimental systems used but also by the genetic background. Our preliminary results obtained in Rif^R serogroup A show a different pattern compared to that observed in serogroup C. For the first time, we have characterized the mutant H553L that exhibited greater intracellular survival rate than the wild type strain despite its *in vitro* growth inability.

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MOLECULAR CHARACTERIZATION OF ITALIAN ISOLATES OF FLUOROQUINOLONE-RESISTANT *STREPTOCOCCUS AGALACTIAE* AND RELATIONSHIPS WITH CHLORAMPHENICOL RESISTANCE

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Introduction: *Streptococcus agalactiae* (group B *Streptococcus*, GBS), besides being a frequent colonizer of the intestinal and genitourinary tracts in the human population, is a major cause of neonatal sepsis and infections in pregnant women, and a growing cause of infections in adults. In addition to its increasing clinical relevance, its drug resistance is also becoming a cause for concern. As regards fluoroquinolones (FQs), after the first detection in Japan, FQ-resistant strains have been reported worldwide, first in East Asia, then in North America, and later in Europe. Resistance to FQs primarily results from alterations in target enzymes DNA gyrase and topoisomerase IV, both involved in DNA replication. In the present study, we provide a picture of FQ-resistant group GBS isolates in an Italian region.

Materials and methods: 368 clinical GBS isolates were collected in 2010-2016 from three hospitals of a region of central Italy. FQ-resistant isolates were selected using levofloxacin, and levofloxacin-resistant (LR) strains were characterized for several features. Their FQ resistance was analyzed phenotypically and genotypically using seven additional FQs. Their *gyrA* and *parC* quinolone resistance-determining regions (QRDRs) were sequenced.

Results: Levofloxacin resistance was detected in 11 isolates (2.99%), at a high level in 10 and at a low level in 1. The 10 highly LR isolates exhibited higher MICs also of the other FQs and all shared one amino acid substitution in ParC (Ser79Phe) and one

in GyrA (Ser81Leu); only the former substitution was detected in the low level LR isolate. The LR strains exhibited distinctive relationships between their susceptibilities to non-FQ antibiotics and typing data. Remarkably, in spite of the very rare occurrence of chloramphenicol resistance in *S. agalactiae*, no less than 4 of the 11 isolates selected for levofloxacin resistance were resistant to chloramphenicol (the only ones in the entire collection).

Conclusions: Studies of GBS resistance to FQs in Europe remain scarce, notwithstanding the emergence of multidrug-resistant isolates. Surveillance would improve if FQ susceptibility was tested in all clinical GBS isolates. The intriguing relationships between FQ and chloramphenicol resistance deserve further investigations.

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GENETIC LINKAGE OF OPTRA AND CFR, INTEGRATED INTO AN UNCONVENTIONAL CIRCULARISABLE STRUCTURE, IN *ENTEROCOCCUS FAECIUM*

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Introduction: In enterococci nonmutational resistance to oxazolidinones can be mediated by two mostly plasmid-borne genes: *cfr*, encoding a ribosome-modifying enzyme and conferring resistance to linezolid but not to tedizolid; and *optrA*, encoding a ribosome protection mechanism and conferring resistance to both oxazolidinones. optrA was discovered in China in 2015 in enterococci of human and animal origin, where it was detected in different genetic environments. Since then optrA-positive enterococci have been described worldwide, including our report in Italy - the first outside China - in two blood isolates of *Enterococcus faecium* which were also positive for cfr. In that study we also noticed that, in one of those two *E. faecium* isolates (strain E35048), the genetic contexts of both optrA and cfr were capable of undergoing excision to form minicircles. Now, *E. faecium* E35048 has been subjected to WGS and investigated for the location, characterisation, and transferability of the genetic contexts of *optrA* and *cfr*.

Methods: *E. faecium* E35048 underwent WGS. S1-PFGE, hybridisation assays, and BLASTN analysis were used to determine the location of optrA and cfr. Their transferability was assessed by electrotransformation and conjugation experiments.

Curing assays were performed through several passages in agar without selective pressure.
Results: *E. faecium* E35048 belonged to ST117. Both *optrA* and cfr probes hybridised with the chromosome and with a ~45-kb band. WGS analysis revealed that optrA and *cfr* were linked, 23.1 kb apart, on a fragment of plasmid pRE25 (DNA identity, 96%), which was flanked by two extensive duplication regions (DRs) containing the *erm*(B) gene. Overall, the element exhibited the typical features of an unconventional circularizable structure (UCS). Neither *optrA* nor *cfr* could be transferred to enterococcal recipients by transformation or conjugation. The loss of the *optrA*- and *cfr*-carrying UCS by curing experiments was confirmed by the drop in oxazolidinone MICs and by WGS data showing only one copy of *erm*(B) at the empty excision site.
Conclusions: The presence of the new UCS is consistent with hybridisation assays showing it both integrated into the chromosome and in circular form. The finding of a genetic linkage between *optrA* and *cfr* in *Enterococcus* adds to the novel finding of an UCS containing two regions - the genetic contexts of *optrA* and *cfr* - capable, in turn, of excising to form minicircles. The extensive DRs of this UCS contain *erm*(B), one of the most prevalent and best-conserved antibiotic resistance genes in bacteria. This, in addition to the belonging of *E. faecium* E35048 to ST117, a well-known hospital-adapted clone, might favour the spread of oxazolidinone resistance in the hospital setting.

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PMRA POLYMORPHISM RESPONSIBLE FOR COLISTIN RESISTANCE IN AN *ESCHERICHIA COLI* HIGH RISK CLONE

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Introduction: Recently, the emergence and dissemination of extensively drug-resistant (XDR) Gram-negatives has revamped the interest for colistin, as one of the few classes of drugs that maintains its activity against these pathogens. The colistin-resistant (Col-R) *Klebsiella pneumoniae* producing KPC-type carbapenemases and *Acinetobacter baumanni* strains are particularly challenging pathogens due to their XDR phenotypes. Conversely, colistin resistance has remained uncommon among *Escherichia coli* of clinical origin, while it has been reported more frequently among isolates of animal origin. Here we demonstrated that colistin resistance in two *E. coli* strains belonging to ST131 was associated to a novel allelic variant of PmrA, which selectively activates the *pmrC* gene, encoding for a phophoethanolamine transferase responsible for a lipid A modification that is known to lead to colistin resistance.
Materials and Methods: The colistin resistant *E. coli* LC761/12 and LC009/13 strains were isolated in 2012 and 2013 from urine and blood samples of two patients hospitalized in two different wards, in a Hospital of Northern Italy (Lecco). LC761/12 and LC009/13 were subjected to to whole-genome sequencing by the MiSeq platform (Illumina Inc., San Diego, CA). Complementation experiments were performed with plasmid pACYC-*pmrA*, a pACYC184 derivative carrying a cloned blunt (EcoRV) copy of the wild-type *pmrA* gene of MG1655. Quantitative real-time PCR by delta delta threshold cycle method, was used to measure the expression of the *pmrK* and *pmrC* gene.
Results: Colistin MIC was 8 mg/l for both strains. LC761/12 and LC009/13 were found

to be negative for *mcr*-1. In both strains, the same polymorphism g244a was found in *pmrA*, determining a nonconservative aspartic acid to asparagine substitution at position 82 of the protein in the N-terminal receiver domain. Trans-complementation with the *pmrA* allele from MG1655 restored colistin susceptibility in both strains (MIC = 1 mg/L). LC761/12 and LC009/13, compared to their COL-S trans-complemented strains, showed the same level of expression of the *pmrK* gene, a 4-ammino-arabinose transferase. On the contrary, the *pmrC* gene, encoding for a phoshoethanolamine transferase, was up-regulated in the wild type COL-R strains.

Conclusions: This work demonstrates that *E. coli* can evolve toward colistin resistance through chromosomal mutations, and provides the first formal demonstration that a single substitution in PmrA ($Asp_{82}Asn$) is able to confer a COL-R phenotype, by selective expression of the *pmrC* lipid A modification system.

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FIRST REPORT OF MULTI-DRUG RESISTANT, VEB-6 PRODUCING PROTEUS MIRABILIS ISOLATES FROM ITALY

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Introduction: The wide diffusion of extended spectrum β-lactamases (ESBLs) in *Enterobacteriaceae* is a matter of concern, giving their high prevalence in clinical settings and the frequent association with resistance factors active on other antibiotic classes. Even if the majority of clinically relevant ESBLs are those of the TEM-, SHV- or CTX-M- families, several different ESBLs have been reported. VEB-type ESBLs have been initially detected in 1996 in a clinical isolate of *Escherichia coli* from a Vietnamese infant hospitalized in France. Genes encoding VEB-type enzymes are usually located on integrons, that could explain their diffusion in multiple species including several *Enterobacteriaceae, Pseudomonas aeruginosa, Acinetobacter baumannii* and *Achromobacter xylosoxidans*. In this work we describe the first cases of infections caused by *Proteus mirabilis* producing a VEB-type ESBL in Italy.

Materials and Methods: A total of 37 ESBL+ *P. mirabilis* isolated from blood cultures of patients admitted to the IRCCS Arcispedale Santa Maria Nuova of Reggio Emilia during the period March 2011 – December 2016 have been analysed. Identification at the species level was obtained by conventional methods. Antimicrobial susceptibility testing was performed by the Phoenix automated instrument and confirmed by reference broth micro-dilution method. Genotyping was carried out by using RAPD and PFGE. Whole genome sequencing (WGS) of the VEB+ isolates was performed using MiSeq and a paired-end approach. Antibiotic resistance genes were detected on WGS data using the ResFinder web-tool. Gap-closing

was performed by a PCR approach followed by Sanger sequencing of the obtained amplicons.
Results: Four out of 37 ESBL+ *P. mirabilis* isolates tested positive for a bla_{VEB}-type gene by PCR. These isolates expressed a multi-drug resistant (MDR) phenotype, including penicillins, cephalosporins, fluoroquinolones, aminoglycosides and trimethoprim/sulfamethoxazole. Data from WGS demonstrated that all isolates carried a complex array of resistance genes including *qnrA1, dfrA1, sul1* and *sul2,* several aminoglycoside resistance genes and the bla_{VEB-6} ESBL gene. PCR experiments and Sanger sequencing revealed that in all isolates the bla_{VEB-6} was located on a class I integron embedded in the MDR SGI1-V genomic island. Results from both RAPD and PFGE revealed different patterns, suggesting the presence of a multi-clonal population.
Conclusions: To our best knowledge this is the first report of VEB-producing *P. mirabilis* isolates in Italy. The detection of this β-lactamase in four unrelated isolates obtained in a time window of > 4 years suggests the need to set-up surveillance studies to assess the actual prevalence of this resistance gene in invasive isolates of *P. mirabilis* in our country.

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PROLONGED CHRONIC COLONIZATION OF A CYSTIC FIBROSIS PATIENT BY MULTIDRUG-RESISTANT *ENTEROBACTERIACEAE* SPECIES CO-PRODUCING CTX-M, TEM AND DHA ENZYMES

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Introduction: *Enterobacteriaceae* are considered as transient colonizer of the cystic fibrosis (CF) patient's airways and are thought to play only a minor role in the development of severe disease. Although beta-lactamase-producing *Enterobacteriaceae* represent a major cause of nosocomial and community infections, only few reports describing a chronic colonization sustained by these pathogens in CF patients are present. Here we report on the prolonged chronic airways colonization sustained by different *Enterobacteriaceae* species producing an AmpC-type and an Extended Spectrum Beta-lactamase (ESBL) in a CF patient from Italy.
Materials and Methods: 18 isolates were obtained from the respiratory samples of a CF patient and were analyzed for their susceptibility phenotype and resistance gene carriage. Species identification was obtained by MALDI-TOF spectrometry. Susceptibility testing was performed according to CLSI and interpreted according to EUCAST breakpoints; ESBL production was determined with Etest ESBL. ESBL and AmpC genes characterization was performed by PCR and sequencing. Clonality was investigated by pulsed-field gel electrophoresis and Multi Locus Sequence Typing.
Results: A collection of 18 *Enterobacteriaceae* spp. isolates were obtained from a 16 year old CF patient attending to Bambino Gesù Pediatric Hospital (Rome, Italy) from 08/2011 to 04/2016. The isolates comprised 10 *K. oxytoca* and 7 *K. pneumoniae*, that were isolated during a period of 56 and 24 months respectively, and 1 *E. cloacae.* Other pathogens, frequently including *S. aureus* and *C. albicans*, were co-isolated during the follow-up period. All

the isolates produced the CTX-M-3 and TEM-1 enzymes and, in several strains, the presence of a DHA-1 enzyme was also detected. *K. pneumoniae* isolates belonged in a single clone while two distinct *K. oxytoca* clones, sharing the same resistance gene content, were detected; for both pathogens, clones were divided in subclones, supporting the evidence for an in-patient evolution of the pathogens. *K. pneumoniae* ST20, that was already described as associated with relevant enzymes including ESBL, DHA-1 and metallo enzymes, was recognized among the colonizing isolates.

Conclusion: Our study demonstrate the chronic colonization of a CF patients by different ESBL- and AmpC- producing *Enterobacteriaceae*. Although their impact on disease progression remains to be fully elucidated, a possible role of these pathogens in contributing to chronic airways inflammation and pulmonary exacerbations and to the dissemination in the CF setting of relevant resistance traits should be considered. The persistence of these pathogens in CF airways should be considered during diagnostic procedures and could have an impact on the choice of therapeutic regimens.

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VIRULENCE GENES AND ANTIBIOTIC RESISTANCE IN *ESCHERICHIA COLI* STRAINS FROM POULTRY IN WESTERN ALGERIA

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Introduction: The widespread use of antibiotics in food animal production systems has resulted in the emergence of antibiotic resistant zoonotic bacteria that can be transmitted to humans through the food chain. The aim of this study was to evaluate virulence genes and antibiotic resistance in Escherichia coli strains isolated from healthy broiler chickens and eggs samples in Western Algeria.

Materials and Methods: A total of 47 *Escherichia coli* strains isolated from healthy broiler chickens and eggs, collected from 7 different geographic areas of Western Algeria, were identified by matrix-assisted laser desorption-ionization time-of-flight mass spectrometry (MALDI-TOF-MS). *E. coli* O157:H7 ATCC 43894 was the reference strain. Virulence associates genes of E. coli including Shiga toxins (STEC) (*stx1* and *stx2*), O157-antigen gene (*rfbE*), flagellar gene (*fliC*), and intimin (*eaeA*) were detected by polymerase chain reaction. Susceptibility to 11 antibiotics agents (nalidix acid-NA, ciprofloxacin-CIP, amoxicillin-AML, AML+clavulanic acid-AUG, cefotaxime-CTX, trimethoprim-sulfamethoxazole-SXT, tetracycline-TE, chloramphenicol-C, neomycin-N, colistin-CS and imipenem-IMP) was determined by standards methods. Phenotypic extended-spectrum beta-lactamase (ESBL) detection test was performed by double disc synergy test (DDST) using AUG and CTX.

Results: Among *E. coli* strains, only one was non STEC O-157:H7 (*rfbE* $^{+}$), whereas another was non O-157 STEC (*stx2*$^{+}$). The strains were resistant to some of the antibiotics most frequently used in poultry. In particular, they were resistant to NA (95.74%), N (93.61%), TE (87.23%), CIP (85.10),

AUG (85.10%), SXT (76.59%), AML (70.21%), C (21.27%), CTX (17.02%). Furthermore, 8 (17%) were ESBL producer E. coli strains.
Conclusions: These epidemiological data showed the low incidence of STEC strains isolated from healthy broiler chicken and eggs in Western Algeria. This territory may be considered a significant food safety area. However, it is to be highlighted the high prevalence of multidrug resistant *E. coli* strains that can be transmitted from poultry to humans with serious consequences on public health. Therefore, implementation of more efficient preventive measures of antibiotic resistance in broiler and layer farms should became mandatory.

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SUSCEPTIBILITY TO THE SEMI-SYNTHETIC PEPTIDE LIN-SB056-1 OF ENDOGENOUS PSEUDOMONAS AERUGINOSA STRAINS IN SPUTUM OF PRIMARY CILIARY DYSKINESIA PATIENTS AND CHARACTERIZATION OF THEIR VIRULENCE FACTOR PROFILES

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Introduction: Primary ciliary dyskinesia (PCD) is an autosomal recessive disorder characterized by abnormal ciliary ultrastructure or function leading to impaired mucociliary clearance and recurrent respiratory infections. *P. aeruginosa* is a major opportunistic pathogen frequently involved in chronic infections of the upper and lower airways of PCD patients. During the course of an infection, *P. aeruginosa* often undergoes a phenotypic change from a non-mucoid to a mucoid appearance, which directly correlates with a worsening clinical prognosis. Mucoid conversion is characterized by the overproduction of the polysaccharide alginate, which confers a selective advantage for *P. aeruginosa* in the PCD lung by providing recalcitrance to currently available therapeutics. Therefore, there is a critical need for novel antimicrobial drugs that can effectively suppress bacterial infections in the challenging environment of PCD lung. Aims of this study were to evaluate the bactericidal activity of the semi-synthetic antimicrobial peptide lin-SB056-1, in combination with the cation chelator EDTA, against endogenous *P. aeruginosa* in sputa from PCD patients and to perform a phenotypic characterization of different *P. aeruginosa* isolates for the production of relevant virulence factors.
Methods: Bactericidal assays were performed in sputa (diluted 5-fold) of PCD patients chronically infected with *P. aeruginosa*. After an

exposure of 1.5h to the peptide/EDTAcombination, sputa were serially diluted and plated on a selective medium for *P. aeruginosa* (cetrimide agar), for CFU counting. For the phenotypic characterization, pyocianin, pyoverdin and secreted proteases of *P. aeruginosa* isolates were quantified in supernatants of stationary phase cultures (48h). Moreover, the ability of the clinical isolates to form biofilm was evaluated in standard conditions and in the presence of calcium, an inducer of biofilm formation by mucoid strains of *P. aeruginosa*.

Results: Exposure to the lin-SB056-1/EDTA combination caused 50 to 99.9 percent reduction in the *P. aeruginosa* load in 5 out of 6 sputa analyzed. Furthermore, in 4 out of 5 cases a synergistic effect for peptide+EDTA was observed. Most of the *P. aeruginosa* strains isolated from PCD sputa displayed a mucoid phenotype and exhibited different abilities to produce the virulence factors analyzed. However, their susceptibility to the peptide-EDTA combination was independent from the amount of virulence factors produced.

Conclusions: Overall, the results obtained highlight the potential of the lin-SB056-1/EDTA combination to be translated into new antimicrobial tools for the treatment of P. aeruginosa infection in PCD patients.

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ON THE ROLE OF FLHF AS SIGNAL RECOGNITION PARTICLE IN BACILLUS CEREUS

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Introduction: The functionally enigmatic protein FlhF is the third bacterial signal recognition particle (SRP) GTPase and plays a role in controlling the arrangement of flagella in *Bacillus cereus* (Salvetti et al., Microbiology, 2007; 2542-2552). In this organism, FlhF depletion also causes a significant reduction in the secretion of the L_2 component of hemolysin BL (Mazzantiniet al., Front Microbiol. 2016; 7:1644,). Since FlhF has been shown to form homodimers structurally similar to the heterodimeric (Ffh/FtsY) SRP-targeting complex in *Bacillus subtilis*, we wondered whether *B. cereus* FlhF could form dimers and be involved in the cotranslational transport of L_2 to the membrane.

Materials and Methods: The expression of the L_2-encoding gene was analyzed by quantitative Real-Time (qRT) PCR in *B. cereus* ATCC 14579 (WT) and in its Δ*flhF* mutant. The bacterial adenylate cyclase two-hybrid (BACTH) system was used to test *in vivo* interactions between FlhF and L_2 and FlhF/FlhF. The proteins were fused in frame with the T18 e T25 subunits of adenylate cyclase, obtaining several recombinant proteins. An adenylate-cyclase defective *E. coli* strain was used for protein/protein interaction studies. The recovery of adenylate cyclase following positive protein interactions was evaluated on agar media and by the b-galactosidase assay.

Results: The expression of the L_2-encoding gene was not altered in the Δ*flhF* mutant, indicating that the reduced secretion of L_2 by the strain was not caused by a defect in gene expression. In BACTH screens, *B. cereus* FlhF directly interacted with L_2 and with itself. Both interactions yielded positive results in multiple conformations of the recombinant proteins.

Conclusions: *B. cereus* FlhF dimerizes *in vivo*, potentially self-assembling in a structure similar to the Ffh/FtsY complex. In addition FlhF binds the L_2 protein. This finding, together with the reduced secretion of L_2 in the FlhF defective strain, suggests that FlhF acts as SRP protein involved in the delivery of L_2 to the membrane. Further experiments are still ongoing to confirm FlhF activity and to identify the protein domains involved in protein/protein interaction.

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CHARACTERISATION OF A LYSM DOMAIN-CONTAINING PROTEIN INVOLVED IN CELL DIVISION AND IN THE VIRULENCE OF *ENTEROCOCCUS FAECIUM*

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Introduction: *Enterococcus faecium* has been included in the ESKAPE pathogen group, which comprises gram positive and gram-negative bacteria showing high level of antibiotic resistance. These pathogens are often isolated from Health-Care Associated Infections. Current research, therefore, focuses on the identification of novel drug targets and new therapeutic approach A previously transcriptomic analysis conducted on *E. faecium* AUS0004 strain highlights the expression both *in vitro*, in Brain Heart Infusion Broth, and *in vivo*, during intra-peritoneal infection, of the LysM-domain containing protein EFAU004_01209. The function of the latter is unknown, at present, and it was decided to study its role in *E. faecium* growth cycle and virulence.

Materials and Methods: The knockout EFAU004_01209 mutant (Δ1209) has been constructed by PWS3 plasmid mediated double crossing-over. The wild type strain and Δ1209 have been grown in Brain Infusion Broth at 37°C without agitation. EFAU004_01209 has been cloned into PeTSumo vector (Life Technologies) and the recombinant protein expressed and purified in *Escherichia coli*. Mice were immunized sub-cutaneous with rEFAU004_01209.

Results: No differences in the growth curves have been detected, we noticed that Δ1209 tends to sediment at the bottom of the tube compared to wild type. Subsequent observation using scanning and transmission electron microscopy shows that the morphology of the mutant is irregular, with unseparated daughter cells and anarchic septal formation leading to the formation of mini compartments inside cells. We tested the ability of the wild type and Δ1209 to colonize organs in both systemic and urinary-tract infections mice models. The number of Δ1209 cells recovered from the kidneys in both infection models is significantly lower compared to the wild type. We tested the ability of the mutant to grow in human urine and obtained negative results compared to the wild-type. Immunofluorescence analysis showed that EFAU004_01209 localizes on the cell surface; to confirm the immunofluorescence, AUS0004 wild-type and its isogenic mutant were treated with proteinase K, to shave the cell wall. Immunoblot analysis finds that the 1209 signal disappeared in the treated samples, displaying the surface localisation of the protein.

Conclusion: Protein EFAU004_01209 is implicated in the cell division process but the underlying mechanism, however, is not clarified yet. Co-precipitation experiments are in progress to verify an interaction with other proteins of the cell division machinery. Results from both the *in vivo* animal models and the urine growth assay, suggest that this protein may be a good target for Urinary-Tract Infections caused by multi-drug resistant *E. faecium*.

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THE FATE OF VIBRIO CHOLERAE AND *ESCHERICHIA COLI* STRAINS CARRYING MANNOSE-SENSITIVE LIGANDS INSIDE THE EDIBLE BIVALVE *MYTILUS GALLOPROVINCIALIS*

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Introduction: Due to their filter-feeding habit, bivalves accumulate a rich and diverse microbiota, including human pathogens indigenous to the aquatic environment (*e.g., V. cholerae, V. parahaemolyticus,* and *V. vulnificus*) or shed into the water from animals and humans (*e.g., Salmonella* and *Shigella*). Inside bivalve tissues, these bacteria may reach concentrations dangerous to human health when the mollusks are ingested raw or partially cooked. Studies carried out by our group on the mussel *Mytilus galloprovincialis* Lam., an economically important and appreciated seafood in the Mediterranean area, supported the knowledge that persistence of bacteria in bivalve tissues largely depends on their sensitivity to hemolymph bactericidal activity that results from the combined action of hemocytes and soluble factors. Here we show that the extrapallial protein present in *M. galloprovincialis* serum (named MgEP) works as an opsonin promoting interactions of hemocytes with bacteria of interest for human health carrying D-mannose sensitive (MS) ligands.

Materials and Methods: *V. cholerae* N16961 strain with MSHA pilus, its MSHA negative derivative, *E. coli* MG1655 (CGSC6300) with type 1 fimbriae, and its afimbriated mutant AAEC072 were used. Extraction of hemolymph and preparation of hemocyte monolayers were performed according to Pezzati et al. (Environ Microbiol., 2015). MgEP was purified by affinity chromatography on a ConA sepharose column (Pezzati et al., 2015). Adhesion and killing experiments were performed as described (Pezzati et al., 2015).

Results: In the presence of the purified MgEP, adhesion to and killing by hemocytes of *V. cholerae* and *E. coli* strains carrying MS adhesins (MSHA and type 1 fimbriae, respectively) was 2-6 fold higher than those observed in artificial sea water. Such MgEP-mediated increase was similar to that observed with whole serum and was abolished by addition of D-mannose. No inhibitory effect was observed with other sugars used as negative control. MgEP did not affect adhesion to and killing by hemocytes of MSHA-negative *V. cholerae* and afimbriated *E. coli.*

Conclusions: These data show that MgEP promotes interactions of *V. cholerae* and *E. coli* bacteria with hemocytes by specifically binding MS adhesins. Such information could represent the basis for setting up new and efficient procedures for mussel aquaculture and depuration. For instance, in order to decrease the number of unwanted microorganisms to acceptable levels for human consumption, mussel depuration in controlled waters might be conducted under environmental conditions favoring the production of MgEP.

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PATHOGENICITY POTENTIAL OF *VIBRIO CHOLERAE* AFTER LONG TERM INCUBATION IN WATER FOR HUMAN CONSUMPTION

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Introduction: In the aquatic environment bacteria are subjected to a multitude of biotic and abiotic stressors that may affect their viability and gene expression, and lead to modification of phenotypic traits. In this study, we investigated the effects of long term incubation in water for human consumption under different environmental conditions on *Vibrio cholerae* survival, its capability to activate the "viable but non culturable state" (VBNC) and to express virulence related properties.

Materials and Methods: The used *V. cholerae* strains were: O1 El Tor N16961, O1 classic CD81, O139 VO15, non O1/O139 RC60. Bacteria were incubated in freshwater (filter-sterilized and autoclaved) under different temperature conditions (15 ± 0.5°C, 20 ± 0.5°C, 25 ± 0.5°C) and in the presence of high [1 mM total organic carbon (TOC)] and low (0.1 mM TOC) nutrient concentrations. Bacteria were considered to have entered the VBNC state when colony forming unit counts were < 0.1 ml^{-1}, and about 80% of cells maintained viability (Pruzzo et al., Environ Microbiol. 2003). Evaluation of gene expression and adhesion efficiency toward Caco-2 and Intestine 407 cultured cells were as previously described (Pruzzo et al., Environ Microbiol. 2003; Vezzulli et al., PLoS One. 2015).

Results: Culturability of all tested *V. cholerae* strains remained almost constant during 15 day incubation period and was not greatly affected by incubation temperature. After 40-60 day incubation at 4°C, depending on the strain, bacteria entered the VBNC state. Vibrios incubated in freshwater up to 15 days as well as those in the VBNC state maintained the capability to adhere to cultured Caco-2 and Intestine 407 cells though the efficiency of attachment was reduced by 40-60% compared to that of actively growing bacteria. In all conditions, amplicons of the expected size were obtained by amplification of virulence related genes (*gbpA, mshA* and *tcp*). In all tested strains, temperature played a major role in promoting expression of both *mshA* and *gbpA* adhesins, that significantly increased from 15°C to 25°C. The expression of both genes did not show any significant variation at different nutrient levels. No *tcpA* gene expression was detected in any condition.

Conclusions: Overall, these results show that long term persistence of *V. cholerae* in freshwater does not abolish its virulence potential, and suggest that when these bacteria infect humans via contaminated water and reach the intestinal tract, they can initiate colonization and, if endowed with virulence traits, cause disease.

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OCCURRENCE OF CARBAPENEMASE- AND B-LACTAMASE-PRODUCING AQUATIC BACTERIA IN THE ADRIATIC SEA AS A POSSIBLE RESERVOIR OF BETA-LACTAM ANTIBIOTIC RESISTANCE

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Introduction: Carbapenems, in particular imipenem and meropenem, are often used as lastline antimicrobials to treat Multi Drug Resistant (MDR) Gram negative bacterial infections. The rapid spreading of mobile genetic elements carrying the resistance genes encoding for the Extended Spectrum β-Lactamases (ESBL) and carbapenemases through horizontal gene transfer is a global human health concern. In this study, we are investigating the mobilome and resistome of 11 MDR marine strains using Whole Genome Sequencing (WGS) technology to detect new resistance genes involved to the carbapenem resistance.

Materials and Methods: A total of 41 MDR marine isolates, originating from fish farms and Adriatic Sea sampling, were screened to identify carbapenemase and other Extendend Spectrum β-lactamase (ESBL) producing strains. We used the enzymatic Blue-Carba Test followed by the phenotypic assay based on growth on chromID ESBL medium. Genotyping of putative carbapenemases producers strains was performed by Multiplex PCR using primers targeting bla_{KPC}, bla_{VIM}, bla_{NDM}, bla_{IMP} and bla_{OXA-48} and their variants. Bacterial strains were identified at species level by Matrix Assisted Laser Desorption-Ionization Time of Flight (MALDI-TOF) and subjected to WGS using the Illumina MiSeq platform.

Results: Although the marine isolates tested negative to the Multiplex PCR, 11 strains resulted positive for the enzymatic activity assay on imipenem, thus suggesting the presence of carbapenemases. Furthermore, they all grew on the ESBL medium confirming their ability to hydrolyze β-lactams. The MALDI-TOF analysis revealed that almost all the strains belong to the *Shewanellaceae* family (*S. putrefaciens* and *S. algae*) excepting one which belonging to the fish pathogen species *Vibrio anguillarum*. The WGS data were collected from the first strains under study. In fact, with the intent to identify mobile genetic elements and DNA sequences harboring known or still unknown resistance genes, a whole genome shotgun of the *Vibrio anguillarum* genome was performed along with bioinformatic analyses including genome assembly, gene prediction and genome basic annotation.

Conclusions: Current surveillance protocols based on molecular methods such as Multiplex or end-point PCR and MALDI-TOF, in clinical isolates, are not always sufficient for a correct detection and characterization of antibiotic resistance genes in the marine environment. Conversely, WGS technology represents a fast and more informative tool to monitor the marine microbial communities in order to understand their possible role as reservoirs of resistance genes in the environment constituting a risk for human health.

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MICROPLASTICS INCREASE INT1 ABUNDANCE AND PERSISTENCE OF WASTEWATER-DERIVED MICROBES IN FRESHWATER

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Introduction: Increasing amounts of plastics and microplastic (particles of diameter < 5 mm) in lake and oceans are of major global concern. Wastewater treatment plants (WWTPs) are inefficiently in removing small plastic particles and are thus are a hotspot for their release into the environment. Whereas the impact caused by the pollution by microplastics in the oceans is well documented, much less attention has been devoted to the effect of microplastics in freshwater systems. With this study, we evaluate the impact of different microplastic concentrations on a freshwater bacterial community.

Materials and Methods: Using a 9-vessels continuous culture system, we simulated the inflow of treated wastewaters into a temperate lake implemented by a gradient of microplastic particles (0 to 1600) for a period of 15 days. We assessed the impact of microplastic concentration by measuring the microbial abundance, the phenotypic distribution, the gross community composition (ARISA) of free living and biofilm forming bacteria, and the abundance of the class 1 integrons (gene *int*1, suggested proxy of anthropogenic pollution and of antibiotic resistance genes proliferation) by quantitative real time PCR.

Results: With increasing microplastic concentration *int*1 increased in the plastisphere (biofilm on microplastic, $p = 0.0002$) whereas no changes were detected in the planktonic bacterial community. Similarly, WWTP-derived bacteria were found more prominently in the plastisphere with increasing microplastic concentrations ($p = 0.00173$), but not in the free living bacterial community in the surrounding water.

Conclusions: We found a significant correlation between the microplastic concentration and the abundance of *int*1 in the plastisphere, suggesting a direct role of microplastic particles in promoting the persistence of anthropogenic pollutants in enviornment. The absence of any similar correlation in the surrounding water (within the planktonic microbial community) highlight the process of colonization of microplastic surfaces as the driving factor for the persistence and spread of *int*1 in environment. This is confirmed by the limited phylogenetic overlapping of *int*1 harboring bacteria within the plastisphere and the planktonic ones. Bacteria from the WWTP could survive in the system finding a niche on the biofilm on microplastic particles, and their ecological success was correlated to the concentration of microplastic, suggesting their colonization as survival strategy. Our results demonstrate the important role of microplastic particles in promoting the spread and the persistence of determinants of anthropogenic pollution imposing the application of removal strategies as a priority in wastewater treatment.

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DIETARY INTAKE, GUT MICROBIOTA AND FECAL SHORT CHAIN FATTY ACIDS IN CHILDREN WITH HYPERPHENYLALANINEMIA

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Introduction: Phenylalanine (Phe) restricted diet is the main-stain treatment for phenylketonuria (PKU). Being diet a key factor modulating the composition of gut microbiota, the aim of this work was to compare dietary intake, gut microbiota biodiversity and short chain fatty acids (SCFA) production in children with PKU, on a low-Phe diet, and in children with mild hyperphenylalaninemia (MHP), on an unrestricted diet.

Materials and Methods: We enrolled 21 PKU and 21 MHP children matched for gender, age (4-18 years) and body mass index z-score. We assessed dietary intake, including glycemic index (GI) and glycemic load (GL), and performed fecal microbiota analyses, by means of denaturing gradient gel electrophoresis DGGE) and Real-time PCR. SCFAs were quantified by gas chromatographic analysis.

Results: Although a similar energy intake was observed in the two investigated groups ($p = 0.171$), we observed in PKU children a lower protein (% of total energy, $p < 0.001$), and higher carbohydrate (% of total energy, $p = 0.047$) and fiber intakes (g/1000 kcal, $p = 0.016$) compared with MHP children. Vegetables intake (g/day), daily glycemic index and glycemic load were higher in PKU than MHP children (all $p < 0.001$). DGGE analysis showed distinct clusters for MPH and PKU subjects, the latter characterized by a lower degree in microbial diversity. No differences were observed between the two groups regarding acetate, propionate, iso-butyrate and iso-valerate, whereas butyrate was significantly decreased in PKU children ($p=0.02$). Accordingly, two of the most abundant butyrate-producing genera, *Faecalibacterium* spp. and *Roseburia* spp., were found decreased ($p = 0.02$ and $p = 0.03$, respectively) in PKU microbiota.

Conclusion: The restricted PKU diet, characterized by a higher carbohydrate intake, increases glycemic index and glycemic load, resulting in a different quality of substrate for microbial fermentation. Further analyses, thoroughly evaluating microbial species altered by PKU diet are needed to better investigate gut microbiota in PKU children and to eventually pave the way for pre/probiotic supplementations.

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A STARTER CULTURE ENRICHED IN BIFIDOBACTERIA CHARACTERIZES FETAL MAMMALIAN GUT

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Background: Up to date, womb and fetus have been considered sterile until birth or rupture of the amniotic sac. Recent observations suggested that meconium, placenta and amniotic fluid from healthy pregnancies host microorganisms, challenging the "sterile womb paradigm". Due to the deep implications of a "colonized womb", especially considering the development of clinical practices that prevent microbiome perturbations, it is crucial to exclude potential environmental or procedural contaminations. Here, we present our results conducted on a rodent animal model, to assure sterile sampling, and preliminarily results on tissues from autoptic human fetuses.

Material and methods: To ascertain antenatal microbial colonization in mammals, we analyzed intestinal tissues collected from rodent fetuses under sterile conditions, and tissues from autoptic human fetuses. Total bacterial DNA was extracted from collected samples and analyzed by Next Generation sequencing techniques (NGS). Colonizing microbes were visualized *in situ*, using labeled probes targeting 16S rRNA by fluorescent in situ hybridization (FISH).

Results: The NGS results showed the presence of microorganisms in both rodent and human fetal tissues. FISH analyses confirmed the microbes' existence in the lumen of the developing gut. Microbial communities showed a fetus-dependent clusterization, confirming the high inter-individual variability of commensal microbiota even in the antenatal period. *Firmicutes* (57%) and *Bacteroidetes* were the most abundant phyla in all tissues. Compared to adult and childhood gut microbiota, the developing gut was enriched in *Actinobacteria*, suggesting a maternally provided reservoir of bifidobacteria for assimilation of human milk oligosaccharides.

Conclusions: Our data provide evidence that an antenatal fetal microbial colonization occurs in mammals. These findings have enormous implications for an emerging field of enhancing the management of healthy pregnancies but at the same time, open new questions such as where and when bacteria start this in utero seeding.

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COMPOSITION AND VARIATION OF THE ESOPHAGEAL MICROBIOTA IN REFLUX DISEASES AND BARRETT'S ESOPHAGUS

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Introduction: The human microbiome impacts on a number of critical functions within the host, being able of modulating several developmental, metabolic, and immunological pathways. It has been recently shown that a defined microbiota is also present in the esophagus. Consequently, it has been suggested that perturbation of the mucosal microbiota could play a role in esophageal disease, such as gastro-esophageal reflux diseases (GERD), Barrett's esophagus or esophageal adenocarcinoma, although current knowledge remains limited. The objective of this study was to investigate and compare the composition and structure of the esophageal microbiota in a cohort of patients with diagnoses of GERD and Barrett's esophagus.

Materials and Methods: Total DNA was extracted using the MoBio PowerSoil DNA Isolation Kit from esophageal biopsies of patient with erosive esophagitis (ES, n = 13), Barrett's esophagus (BE, n = 12) and controls (C, n = 7). Amplicons of the V3-V4 bacterial 16S rRNA region were sequenced with the Illumina MiSeq platform. Alpha and beta diversity analyses were performed using QIIME, and biomarkers discovery was evaluated using the LEfSe tool. Statistical analysis was performed using GraphPad Prism 6 software.

Results: Alpha diversity indices revealed few significant differences, in terms of bacterial richness, within the studied samples. Similarly, beta diversity analysis showed a no clear trend in alterations of microbiome composition by patient categories (C, ES, BE). Few taxa, with low relative abundances, were identified as potential biomarkers of BE. Within the ES category, treatment with proton pump inhibitors (PPIs) represented the strongest modifying factor affecting the esophageal microbiota structure.

Discussion and conclusions: Although only a limited number of samples were analysed, modifications of the overall microbiota structure were more related to PPIs treatment than to specific disease states such as ES or BE.

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OPTIMIZATION OF A METAPROTEOGENOMIC ANALYSIS PIPELINE ENABLING IN-DEPTH CHARACTERIZATION OF HUMAN, RODENT AND RUMINANT GUT MICROBIOTA

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Introduction: The study of the gut microbiota (GM) is rapidly moving from the mere description of its taxonomic composition towards its functional characterization. To this aim, the application of metaproteogenomics to profile GM activity, although challenging, deserves growing attention. The metaproteome, indeed, is hypothesized to show a high degree of plasticity and its changes are supposed to be strongly linked to the modifications of the gut environment. Here, we aimed to optimize a metaproteogenomic analysis pipeline, to gain the deepest possible characterization of GM activities and metabolic pathways in different hosts.

Materials and Methods: Fecal samples were collected from healthy human subjects, NOD mice, Fisher 344 rats and Sarda sheep. Cecal contents were also collected from NOD mice. Samples were divided into two portions and both DNA and proteins were extracted. The Nextera XT protocol (Illumina) was used for library preparation, and metagenomes were sequenced using the HiScan sequencer (Illumina). Proteins were subjected to the FASP protocol and the peptide mixtures were analyzed by LC-MS/MS using an LTQ-Orbitrap Velos mass spectrometer coupled with a nanoLC system (Thermo). Metagenomic reads were processed using USEARCH, while mass spectra were subjected to database search using Sequest-HT and Percolator. MEGAN and Unipept were used for taxonomic and functional annotation. Spectral counts were used to estimate protein abundance.

Results: We comparatively evaluated the following variables of a metaproteogenomic analysis pipeline: i) sample type: fecal samples vs cecal contents; ii) enrichment: differential centrifugation vs direct extraction; iii) extraction: bead-beating vs bead-beating/freeze-thawing; iv) peptide fractionation: 1D-LC long-run gradients of several lengths; v) metagenome processing: raw reads vs assembled contigs; vi) taxonomic/functional annotation: MEGAN vs Unipept/SwissProt; vii) database type: UniProt- vs metagenome-based; viii) database search engines: X!Tandem vs Sequest-HT vs Andromeda. At the end, we were able to define an optimized pipeline, enabling the identification of over 15,000 unique peptides per sample, and to identify the main analytical biases to be taken into account. We then applied the pipeline to human, mouse, rat and sheep fecal samples, in order to take a picture of the functions played by the main members of the GM in different hosts, conditions and pathways.

Conclusions: We optimized a metaproteogenomic analysis pipeline able to gather detailed and taxon-specific information regarding functions and pathways of human and animal GMs. These results open the way to the application of this meta-omic approach in complex studies aimed at in-depth characterizing GM activity.

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EXPLORING THE RHIZOSPHERIC MICROBIOME OF *VITIS VINIFERA* CV. PINOT NOIR USING METAGENOMIC AND METAPROTEOMIC APPROACHES: PHYLOGENETIC AND FUNCTIONAL CHARACTERIZATION

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Introduction: *Vitis vinifera* is a Mediterranean crop with relevant impact on the Italian landscape, culture and economy. The rhizosphere is a hotspot where the release of root exudates stimulates bacterial density and diversity. Thanks to culture-independent methods (metagenomics) the complexity of the soil/rhizosphere microbial community has been explored. However, metagenomics doesn't provide information regarding the activity and the molecular interactions between the bacterial communities and roots. Proteins are the drivers of cellular activities encoded by the genome. Therefore, proteomic tools could be useful to gain information about microbial community activity and to better understand the real interactions pathways between roots and soil.
Materials and Methods: A comparison between the microbial community structure in rhizospheric and bulk soil using metagenomics (pyrosequencing of 16S rDNA) and proteomics (MS/MS analysis of the total protein occurring in soil samples) was performed.
Results: Actinobacteria were the dominant class in all the soil samples, followed by Proteobacteria, Gemmatimonadetes and Bacteroidetes. While Actinobacteria and Proteobacteria are well known as dominant in soil, for the first time members belonging to Gemmatimonadetes have been observed in vineyard soils. Bacteria belonging to Streptomyces, Bacillus and Pseudomonas genera were the most active. The identified genera were mainly involved in phosphorus and nitrogen soil metabolism.
Conclusions: Our results underlined the difference between the metagenomic and metaproteomic approaches and the potentiality of proteomics in describing the environmental bacterial communities and their activity.

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MICROBIAL BIOGEOGRAPHY OF THE LAMB GASTROINTESTINAL SYSTEM: A TAXONOMICAND FUNCTIONAL CHARACTERIZATION

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Introduction: Sheep (*Ovis aries*) are exclusively herbivorous mammals employed for meat, milk and wool production. These animals have a typical ruminant gastrointestinal system (GIS) including four fermentation chambers, composed of the rumen, the reticulum, the omasum and the abomasum followed by a small intestine (formed by jejunum, duodenum and ileum) and a large intestine (formed by cecum, colon and rectum). Each anatomical district is expected to be inhabited by specific microorganisms that can differ based on their localization (luminal or mucosal compartment). While the first four fermentation chambers, above all the rumen, have been deeply studied, only few investigations have been focused on the distal gut. In order to characterize the different microbial communities of each region, we employed an integrated, multi-omic strategy, comprising 16S rDNA (V4 region) and shotgun metagenomic sequencing to disclose microbiota structure and genetic potential. Further, metaproteomics was applied to identify and characterize functions and pathways actively expressed in each anatomical district.

Materials and Methods: The GIS of a 30-day-old lamb was divided into 10 sections, specifically rumen, reticulum, omasum, abomasum, jejunum, duodenum, ileum, cecum, colon and rectum. From each GIS tract a luminal (by direct recovery and/or through saline solution washes) and a mucosal (by tissue scraping) sample were obtained. DNA and proteins were extracted from each sample. DNA sequencing was performed with the Illumina MiSeq sequencer, using the paired-end method and 250 cycles of sequencing. OTUs generation was performed using QIIME. Proteins were processed using the FASP method and LC-MS/MS analysis of peptide mixtures was carried out using an LTQ-Orbitrap Velos mass spectrometer and peptide identification was performed using Sequest-HT as search engine. The relative proportions of read counts and spectral counts were used as a quantitative estimation of the abundance.

Results: The extraction protocols used here enabled a high yield in terms of protein (mean: 4.8 µg/mg sample) and DNA (mean: 1.4 ng/mg sample) from all samples, with a sufficient quality to carry out subsequent analysis. This study allowed us to evaluate the bacterial distribution along the GIS of a lamb, drawing, by this way, the microbial biogeography of its anatomical regions.

Conclusions: In this work we present the first complete characterization of the microbiota found along the lamb GIS, both at taxonomic and functional level. These results represent an important groundwork to investigate further how the ruminant microbiome relates to host physiology and pathology, as well as to production variables.

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GUT MICROBIOTA CHANGES ON CANARIES WITH A *MACRORHABDUS ORNITHOGASTER* INFECTION IN AN AVIARY CENTER

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Introduction: Birds have an extreme morphological diversity, diets that vary widely and a complex ecosystem which contains a huge number of microorganisms, dominated by members of the Firmicutes, with *Actinobacteria, Bacteroidetes,* and *Proteobacteria* also commonly observed. In addition, a pathological process or a treatment with antibiotics can vary considerably the intestinal flora. At present there are no works about gastrointestinal (GI) microbiota of canaries. In this study, 16S rRNA gene amplicon target sequencing has been employed to assess the gut microbiota diversity of feacal sample from healthy canaries and birds infected with *Macrorhabdus ornithogaster,* an opportunistic avian gastric yeast (AGY) that cause proventriculitis, ulceration of the digestive tract, atrophy of the pectoral muscle.

Materials and Methods: A total of 36 animals, divided into three groups, coming from a single aviary center, were used for the study: 15 healthy birds (N), 12 symptomatic birds (S) and 9 asymptomatic birds (A). DNA directly extracted from feces of birds were used to assessed the microbiota by 16S amplicon based sequencing on an Illumina MiSeq platform.

Results: Comparing the relative abundance of the main OTUs across samples, it was possible to observe that the main OTUs drove the cluster separation according to the birds status ($p < 0.001$). Differences were further demonstrated by principal component analysis (PCA), based on the relative abundance of the main OTUs, clearly showing that N samples were well separated from S and A samples. Moreover, alpha-diversity also showed that there was a higher level of complexity ($p < 0.05$) in N samples when compared to S.

Comparing the relative abundance of the main OTUs across samples, it was possible to observe that the main OTUs drove the cluster separation. In details Acinetobacter, Pseudomonas, Lactococcus and Leuconostoc were found to be characteristic of N samples (FDR < 0.05), Candidatus arthromitus, Lachnospiraceae and Staphylococcus characteristic from S samples (FDR < 0.05) while Lactobacillus and Streptococcus characteristic for A samples (FDR < 0.05).

Conclusions: Modifications of the intestinal tract due to the presence of AGY can clearly affect the modification of the gut microbiota. Birds were reared together and fed in the same way so probably the seeds can be the source of contamination. It is possible that the yeast chemically modifies the digestive tract environment by pH modification. These data suggest a possible competition between yeast and bacteria. Moreover, the results of this research confirm a link between the amount of AGY and the clinical signs of Megabacteriosis.

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FAECAL SUPERNATANTS FROM RETT SYNDROME PATIENTS WITH DYSBIOTIC MICROBIOTA ALTERATE MARKERS OF HOMEOSTASIS IN DIFFERENTIATED CULTURES OF COLONIC EPITHELIAL CELLS

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Introduction: Gut microbiota, with its respective metabolites, is able to communicate with the host through a series of biochemical and functional pathways that affect host homeostasis and health.
In particular, the gastrointestinal tract communicates with the central nervous system through the gut-brain axis to sustain and maintain neuronal development, therefore intestinal barrier dysfunctions could allow microbes to create a pro-inflammatory state with different, possible repercussions on the brain. The main pathways leading to gut epithelial barrier dysfunction are mediated by serotonin, Toll-like Receptors and SCAFs (short chain fatty acids). Alterations in gut microbial composition have been reported in Rett syndrome patients possibly revealing a role of gut microbiota in various symptoms typically associated with Rett syndrome such as intestinal inflammatory and constipation status. Moreover gut microbiota alteration could directly or indirectly influence brain functions. Rett syndrome is an X chromosome-linked neurodevelopmental disorder that affects approximately 1:10,000 females worldwide. Rett individuals develop typically until 6-18 months, when neurological symptoms, including intellectual disability, autistic features, deficits in motor control and sensory perception, breathing irregularities and epilepsy disorders begin. In the present study we have evaluated the effect of fecal supernatants (FSN) of Rett patients in regulating the expression of target genes in human intestinal epithelial cells including pro-inflammatory cytokines, neurotrophic factor BDNF, TLR4 and Serotin Receptor.
Materials and Methods: FSN preparation: Fecal samples were dissolved and homogenized in 0.9% NaCl solutionon ice. Supernatants were collected after centrifugation and filtered by 0.8μm-sized filters and stored until use at -20°C. Cell treatment: Caco-2 cells, HT29-MTX cells and co-culture of Caco-2/HT29-MTX, (that combined two major cell phenotypes found in the intestine, to provide an epithelial monolayer covered with mucus which better mimics the situation *in vivo*), were treated with FSN of children with Rett syndrome at different times and concentrations. Real-time PCR: the expression levels of several target genes involved in maintenance of intestinal homeostasis were evaluated using LightCycler software.
Results: Preliminary results clearly show a modulation of the expression of the selected genes in differentiated cultures of colonic epithelial cells mediated by FNSs from Rett syndrome patients.
Conclusions: FSN from Rett syndrome patients as product of intestinal alteration both in microbial composition and functionality, together with differentiated epithelial cells and co-cultures may represent a valid starting point to *in vitro* evaluate FSN impact on intestinal epithelium homeostasis.

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GUT MICROBIOME BIOFILM IN OBESE PATIENTS ELIGIBLE FOR BARIATRIC SURGERY

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Introduction: Biofilm formation, thanks to the ability to escape the immune system, represents the basis of many chronic diseases. There is evidence showing that mucosal bacteria of the gut grow in biofilm and that these organisms may be important in modulating the host's immune system and contributing to dysbiosis. Many recent studies focused on the links between gut resident bacteria community and their function in obesity since they create with the host a symbiotic dynamic relationship. It has been seen that certain characteristics of the gut microbiota composition confer metabolic efficiency and its dysbiosis is related to obesity. Bariatric surgery is currently the most effective treatment for obesity improving a dramatic loss of fat mass and basically favoring metabolic improvement. These metabolic effects are due to changes in bile acid metabolism, gut microbiota and central regulation of metabolism. Therefore, the aim of this study was to analyze microbiome composition from obese patient before and after bariatric surgery, and to evaluate the differences with normal weigh patients.

Materials and Methods: Obese subjects eligible for bariatric surgery were included in the study in accordance with the international guidelines: patients with acceptable operative risks, failure of non-surgical treatments, declared compliance to follow lifelong medical surveillance, aged 18 to 65 years, body mass index (BMI) of 40 kg/m^2 or between 35 and 40 kg/m^2 with obesity-related co-morbidities. Healthy patients with normal weigh were enrolled as control group. The microbiota characterization of 12 bacterial taxa was performed by Ion Torrent next-generation high-throughput sequencing of the 16S rRNA gene on fecal samples before surgery and during the first follow-up visit (3 months later).

Results: Intestinal microbiota analysis of obese patients after bariatric surgery showed that Proteobacteria decreased after laparoscopic sleeve gastrectomy (SG) while increased after laparoscopic gastric bypass (LGB). Comparing to normal weight (NW) patients, obese patients who were selected for SG showed an almost equal amount of both phyla and the ratio was not affected by the surgery. The obese patients before LGB showed a predominance of Bacteroidetes, whose amount regained a relative abundance similar to NW patients after surgery. Obese patients before LGB showed the predominance of Bacteroides, which decreased after surgery in favor of Prevotella, bacteria associated to healthy diet.

Conclusion: The characterization of the gut microbial communities and the modality of mucosal colonization represent an important indirect marker for the clinical management of obesity in term of maintenance of good health and the weight loss.

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LACTOBACILLUS SPECIES INCREASE RAPIDLY AND SIGNIFICANTLY THEIR ABUNDANCE IN THE GUT MICROBIOTA OF RATS UNDER CALORIC RESTRICTED DIET

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Introduction: Previous studies indicated that caloric restricted (CR) diet enables to lower significantly the risk of cardiovascular and metabolic diseases. In experimental animal models, life-long lasting CR was demonstrated to induce changes of the intestinal microbiota composition, regardless fat content and/or exercise. In this work, we investigated whether faecal microbiota variation is induced by short-term CR and whether such change persists in long-lasting CR, in a rat model of aging.

Materials and Methods: A total of 46 faecal samples from 22 Fisher 344 rats were collected. Animals were fed with standard chow (3% fat) until the age of 8 weeks. Then two groups of 6 rats each were either continued on the *ad libitum* (AL) diet or given 70% of the AL ratio (CR). Blood samples were drawn after 8 weeks to measure total cholesterol and triglycerides. Faeces were collected after 3 and 8 weeks. In a parallel experiment, 10 rats were fed as above up to their mid-life. Faecal samples from adult rats were collected after 36 weeks of CR treatment (6 rats) and from controls (4 rats) fed AL. Stool samples were subjected to DNA extraction and purification. Full length 16S rRNA genes or the V4 region were amplified and libraries were constructed according to the Illumina Nextera XT protocol. DNA sequencing was performed with the Illumina HiScanSQ sequencer, using the paired-end method and 93 cycles of sequencing. OTUs generation was performed using QIIME. Taxonomy assignment of resulting OTUs was done using the Greengenes 13_8 database.

Results: Rats grown while maintaining the AL diet for 8 weeks showed a decreased of the relative abundance of genus *Prevotella* and a parallel increase of genera *Oscillospira, Ruminococcus, Coprococcus,* and *Desulfovibrio*. These variations were already evident and significant after 3 weeks. Faecal samples from CR treated rats showed similar changes for *Prevotella* and *Desulfovibrio* but none of these genera was significantly varied after 8 weeks of CR. In CR, on the contrary, *Lactobacillus* showed a significant increase in young growing rats after 8 weeks of treatment and its relative abundance was significantly higher in CR vs AL fed animals after 36 weeks of dietary intervention.

Conclusions: *Lactobacillus* species appear to compete well with the other members of the gut microbiota in CR conditions, while their intestinal colonization is lessened when excessive intake of food occurs, even with low fat diet. According to our data we propose that *Lactobacillus* might serve as a marker of dietary intervention in experimental animal models, since its increased relative abundance pairs well the lowering of cholesterol and triglycerides, shortly after dietary restriction, then persists highly abundant after long-term CR treatment.

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CHARACTERIZATION OF POTENTIALLY PROBIOTIC CANDIDATES IN VAGINAL *LACTOBACILLUS SPP.* STRAINS ISOLATED FROM HEALTHY WOMEN

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Introduction: Normal flora of vagina is dominated by *Lactobacillus* species that play an important role in the protection against uro-genital pathogen colonization. This study aims to select vaginal lactobacilli, isolated from healthy women, that exert potentially beneficial properties in order to restore and maintain a vaginal healthy ecosystem.
Material & Methods: The *Lactobacillus spp.* were isolated from vaginal samples of 42 healthy women. The strains were screened by deferred antagonism test onto Man Rogosa and Sharpe (M.R.S.) Agar with 0.1% $CaCO_3$, against the main uro-genital pathogens. Only the strains with antagonistic activity, were later identified by *tuf* gene sequencing and characterized for: i) the presence of bacteriocin-encoding genes by Polymerase Chain Reaction (PCR), ii) the ability to produce hydrogen peroxide (H_2O_2) by Eschenbach method. The antibiotic susceptibility profiles of all strains were evaluated for the safety.
Results: Out of 22 *Lactobacillus spp.*, only 15 (7 *L. gasseri;* 5 *L. crispatus,* 1 *L. fermentum* and 1 *L. delbrueckii* and 1 *Lactobacillus spp.)* showed a total inhibitory activity against *S. agalactiae, E. coli, K. pneumoniae, S. aureus, E. faecalis* and *E. faecium*. 13/15 strains inhibited *C. albicans* while only 3/15 showed a weak activity against *C. glabrata*. 6/15 strains resulted positive for *helveticin J* and *acidocin A*. 14/15 isolates produced hydrogen peroxide at different levels. The antibiotic susceptibility profiles showed full sensitivity to ampicillin, amoxicillin clavulanic, tetracyclines, chloramphenicol, erythromycin and rifampin.
Conclusions: In our study, we detected 15 potentially candidate strains suitable to develop a new probiotic product for vaginal application that may provide an alternative approach in the prevention and treatment of vaginal infections.