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# 47° CONGRESSO NAZIONALE

SOCIETÀ ITALIANA DI MICROBIOLOGIA

18-21 SETTEMBRE 2019  
ANGELICUM CONGRESS CENTRE

Pontificia Università San Tommaso d'Aquino  
Largo Angelicum 1 – 00184 Roma



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**EUCAST 2019: cosa c'è di nuovo**

*Caso Clinico n.2 Impatto dei cambiamenti sul referto*

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**Introduction:** Since January 2019 EUCAST has decided to change the definitions of the categories S, I and R. The major change regards the “Intermediate” category, that has been replaced with “Susceptible, high exposure”, stressing the curability of infections due to formerly “Intermediate” pathogens in condition of high concentration of the drug at the site of infection. Besides, the uncertainty of results was removed from the definition and moved in a new dedicated field of “area of technical uncertainty” (ATU). The impact of this change is important and is requiring great effort from the laboratories to adapt accordingly.

**Materials and methods:** The impact of the new definitions on the reports will be discussed through the presentation of a clinical case.

**Results:** It will be presented the case of a blood culture positive for *Klebsiella pneumoniae*, which report is challenging, for both microbiologists’ reporting and clinicians’ interpretation. The discussion will go through four major areas: a) how to clearly expound in the report the new concept of “susceptible, high exposure”; b) how to report the ATU (area of technical uncertainty); c) how to deal with “HE” (high exposure) with and without the presence of “susceptible, high exposure” category; d) new EUCAST recommendations for aminoglycosides sensitivity report.

**Discussion and conclusion:** In the new scenario, sensitivity reporting can be problematic. The presentation will aim to cover the more complex cases starting from practical examples, by analyzing the major problems, providing possible solutions and opening discussion to additional issues.

### *Caso Clinico n.3 Personalizzazione del referto*

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**Introduction:** The spread of resistance mechanisms and multi-drug resistant organisms has increased the clinical need to provide reliable and predictive *in vitro* data. For this reason, antimicrobial susceptibility testing (AST) show a continuous trend to become more complicated, as demonstrated by the significant increase of number of documents and criteria required for interpretation. Recently EUCAST has introduced new challenging definitions, including the category called “Susceptible, high exposure”, and the “area of technical uncertainty” (ATU). Moreover the introduction of new generation bacteriological methods able to rapidly identify clinically relevant resistance markers (for example KPC or mec-A/C) has led to the need to express also these results in terms of predictivity of drug susceptibility. This growing complexity necessarily requires the customization of the AST report in order to encourage a correct interpretation of laboratory results by the clinician.

**Materials and methods:** The impact of the customization of antimicrobial susceptibility test reports will be discussed through the presentation of a clinical case.

**Results:** It will be presented the case of a 59 years-old woman, that was admitted to the hospital for an acute liver failure. During the hospitalization the patient developed several infections caused by multi-drug resistant organisms and became colonized by different carbapenemase producing strains. One day after the liver transplantation, she had an episode of severe sepsis. The discussion will clarify how the customized susceptibility reports have guided the clinical management of this patient.

**Discussion and conclusion:** *In vitro* data describing the pattern of antimicrobial resistance of pathogens responsible for serious infections play a crucial clinical role. Reporting of susceptibility testing represents an issue of increasingly complexity, considering the different types of informations that the microbiology lab can transmit to the clinician during the diagnostic workflow. The customization of the report can be considered an instrument of antimicrobial stewardship having a relevant impact on appropriate use of antimicrobials and on clinical outcome of the patient.

## DIAGNOSI E MONITORAGGIO DELLE INFEZIONI SESSUALMENTE TRASMESSE

### *Situazione epidemiologica delle IST in Italia - Epidemiology of STI in Italy*

*Barbara Suligoj*

*Centro Operativo AIDS, Istituto Superiore di Sanità, Roma, Italia*

**Introduction:** Sexually transmitted infections (STI) are widely spread worldwide, affecting millions of people every year.

**Materials and Methods:** In Italy, two sentinel surveillance systems report individual data on STI: 1) the sentinel system based on STI clinics (started in 1991) and reporting data on patients with a symptomatic STI; 2) the sentinel system based on a laboratory network (started in 2009) and reporting data on people tested for *Chlamydia trachomatis* and/or *Trichomonas vaginalis* and/or *Neisseria gonorrhoeae*. The National AIDS Unit (Centro Operativo AIDS, COA) of the Istituto Superiore di Sanità coordinates both surveillance systems. Results obtained from these surveillance systems are presented.

**Results:** The total number of people with a symptomatic STI reported since 1991 is approximately 128,000 cases, whereas the laboratory network tested about 156,000 samples since 2009. The number of symptomatic cases has constantly increased since 2004. The number of MSMs with a symptomatic STI has doubled between 2006 and 2017.

Reported cases of *Chlamydia trachomatis* infection tripled in 2017 compared to 2008, and the highest prevalence was detected among young women aged 15-24 years.

In 2017, the number of gonorrhea cases have roughly doubled compared to 2010; the increase was observed in particular among MSM who accounted for 63% of reported cases in 2017.

The epidemic peak of primary and secondary syphilis detected in 2016 is not exhausted: between 2015 and 2017 a 60% increase was observed.

Anogenital warts is the most frequently reported STI; its trend is stable since 2013.

There is a clear reduction in HIV prevalence from 11.7% in 2016 to 7.7% in 2017; nevertheless, in 2017, it was approximately forty-five times higher than that estimated in the general Italian population.

**Discussion and Conclusions:** STI in Italy are on the rise and deserve urgent interventions at both individual and public health level. A national strategy for the control of STI aimed at enhancing the diagnosis and early treatment of STI is needed. Information on STI, addressed to the general population and target populations (e.g., young people, women, foreigners, MSM), should be implemented to educate on sexual health (safe sex, correct condom use, maintain mental clarity when having sex, etc.). Moreover, all patients with an STI should be actively offered an HIV testing.

## *Diagnosis of STDs: old and new methods*

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**Introduction:** Sexually transmitted diseases (STDs) are a group of infections with a high incidence both in developing countries and in economically favored areas. These infections, if not timely diagnosed and treated with adequate therapy, can lead to serious complications in the urogenital tract. Laboratory diagnosis with sensitive and specific techniques, such as Polymerase Chain Reaction (PCR), is considered crucial for optimal etiological identification. The aim of this study was to compare two multiplex PCR systems, recently released on the market, with standard methods used in our laboratory for the detection of seven sexually transmitted microorganisms in endocervical swabs.

**Materials and Methods:** The study was conducted on 120 endocervical swabs collected during the routine ambulatory activity at the Microbiology Unit of the Perugia General Hospital, in the period January - May 2019. All the samples were analyzed by our standard protocol (Anyplex II STI-5 from Seegene for mycoplasma and *Trichomonas vaginalis* detection; Chlamydia tr Q-PCR Alert kit from Elitech Group for *Chlamydia trachomatis* detection; and Martin Lewis agar plates for *Neisseria gonorrhoeae* culture) and Anyplex II STI-7 Kit (Seegene) to detect simultaneously seven microorganisms (*Ureaplasma urealyticum*, *Ureaplasma parvum*, *Mycoplasma hominis*, *Mycoplasma genitalium*, *C. trachomatis*, *Neisseria gonorrhoeae*, and *T. vaginalis*), or Xpert CT/NG (Cepheid) to detect *C. trachomatis* and *N. gonorrhoeae*. Results from the different methods were compared. All tests were run as per manufacturers' instructions.

**Results:** The STI-7 test showed optimal sensitivity and specificity, compared with standard protocols, with 99.1% concordant results. In a culture negative sample, the test detected *N. gonorrhoeae* DNA. Moreover, the test, allowing the simultaneous detection of all the 7 pathogens, greatly improved laboratory workflow, shortening time to results from 3 to 1 day. The Xpert CT/NG gave concordant results with standard method in 118/120 cases, while in 1 case the test detected *C. trachomatis* DNA not detected by standard method, and in one sample, negative by culture, the test revealed *N. gonorrhoeae* DNA. Interestingly, the same discordance was found for the STI-7 kit.

**Discussion and Conclusions:** STI-7 and Xpert CT/NG are rapid and reliable test for the simultaneous detection of pathogens responsible for STDs in endocervical samples, and permit optimal laboratory workflow, reducing the laboratory processing time.

## *Microbiota and IST*

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**Introduction:** The urogenital microbiota seems play a central role in maintaining a healthy environment. Individual modifiable and non modifiable factors are strongly associated with its composition, although certain microbial community types included determinants that go beyond an individual level.

**Material and methods:** Recent advances in molecular techniques and bio-informatics platforms allow us to profile microbial composition in detail, providing to a better comprehension of the structure and of the relationship with host in terms of reproductive and diseases outcomes including susceptibility to sexually transmitted infection (STIs).

The vaginal flora is characterized by a dynamic structure of a limited number of microbial clusters where their relative amount in the context of a vaginal milieu, can be indicative of dysbiosis and/or an altered function. In particular, microbial species involved, are distinguished for driving specific activity of the metabolic pathways including nucleotide biosynthesis and conversion of pyruvate and methane. Regarding the microbiome interplay with the local immune system, the activation of an inflammatory pathway represents a crucial point in triggering the pathogenetic process or a negative reproductive outcome. It is believed that a link between a given vaginal community state type (CST) and opportunistic pathogens exists, where dysbiosis and lack of *Lactobacillus* spp. could drive *C. trachomatis*, *N. gonorrhoeae*, *M. genitalium* or HPV infections. Conversely, STI pathogens may modify the vaginal microbiota.

**Discussion and conclusion:** Efforts in understanding the relationship between STIs and vaginal microbiota could be crucial to propose a comprehensive approach to sexual and reproductive health. The next generation sequencing era has contextualized the personalized medicine also in STI clinical and public health setting. Characterizing the vaginal microbiota will pave the way to new prevention and screening strategies, diagnostic tools and therapeutic solutions based on individual's predispositions to infection.

## *The importance of microbiological investigations in a patient with risk of preterm birth, in a bichorial-biamniotic twin pregnancy.*

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**Introduction:** A 28 years old women was referred to gynecological service of Azienda Ospedaliera Universitaria Pisana (AOUP) from Livorno hospital for risk of preterm delivery in a 24 weeks and 6 days bichorial-biamniotic twin spontaneous pregnancy. She had three pregnant voluntary interruptions in her past. She was a smoker with bad socioeconomic conditions. She didn't do any obstetric visit during this pregnancy.

**Materials and methods:** At the first admission to the hospital, obstetric examination and ultrasound, PROM (Premature Rupture Of Membrane) test, cardiotocographic and clinical-laboratory monitoring were performed. At the speculum pervia cervix with a protruding amniotic sac in the vagina was founded. At the obstetric examination, the cervix was very shortened, in the process of centralisation, open to one finger. At the transabdominal ultrasound the first and the second fetus were described as breeches, respectively on the right and on the left side of the patient, vital, posterior placenta. Regular amniotic fluid and biometry in both. Screening tests to Syphilis, HIV, HBV, HCV were negative. The patient was immune to rubella and toxoplasmosis. Urine culture and two sets of vaginal swabs were also performed, respectively at 24 weeks and 6 days and at 26 weeks and 5 days. Tocolytic therapy associated with Mg-sulphate, progesterone, folic acid, iron, corticosteroid therapy for the neonatal RDS prophylaxis, antibiotics and trichomonicide were provided. The insertion of cervical Arabin pessary was realized.

**Results:** According to the laboratory procedures the urine culture was positive for *Escherichia coli* and antibiogram confirm that strain was ESBL producer. Both vaginal swabs were positive for *Trichomonas vaginalis*, the first one was also positive for *Escherichia coli* and the second one was positive for *Klebsiella pneumoniae*. Histopathological examination of the placenta reveals diffuse chorioamnionitis, associated with spotted necrotic areas.

**Discussions and conclusion:** After 4 weeks from the first admission to the hospital, against medical advice, the patient leaves the hospital at the gestational age of 28 weeks and 6 days. At 30 weeks and 1 day the patient was re-admitted to the hospital for Preterm-PROM (pPROM). Emergency Cesarean section was performed and two newborns were born, respectively I° female (1180 gr) and II° male (1300 gr). Six days after delivery patient went home with antibiotic therapy, uterotonic therapy and anticoagulant prophylaxis. At the obstetric visit the uterus was contract, the cervix, the suture and the spontaneous urination were regular. Low compliance and low adherence to microbiological investigation during pregnancy reveals how microbiological infection are associated with increased risk of adverse pregnancy outcome.

**VACCINI CONTRO STREPTOCOCCUS PNEUMONIAE E NEISSERIA MENINGITIDIS:  
DISCUTIAMO DEGLI ASPETTI MICROBIOLOGICI**

*Impatto della vaccinazione sulla storia naturale dell'infezione da batteri capsulati*

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*Streptococcus pneumoniae, Neisseria meningitidis and Haemophilus influenzae* are the main causative agents of bacterial meningitis. The main virulence factor of these bacteria is the polysaccharidic capsule surrounding them, which is protective against the host immune responses. *S. pneumoniae, N. meningitidis* and *H. influenzae* reside in the human nasopharynx as commensals, from there they can invade other districts and cause a range of diseases. The natural history of infections sustained by these bacteria therefore begins with the colonization of the nasopharynx upon airborne transmission from other individuals, eventually followed by invasion of tissues and disease. Currently used vaccines against *S. pneumoniae, N. meningitidis* and *H. influenzae*, with the notable exception of serogroup B *N. meningitidis* vaccine, contain the bacterial capsular polysaccharide as an antigen. Vaccinated subjects develop anti-capsule antibodies which are the effectors of protection against the disease. Recently, experimental evidence has accumulated suggesting that anti-capsule antibodies also mediate protection from carriage both in animal models of pneumococcal infection and in human pneumococcal experimental carriage. These experiments will be discussed together with specific microbial interactions which happen in the nasopharynx ecological niche, with a focus on *S. pneumoniae*.

## *Serotypes of Streptococcus pneumoniae in the vaccination era: open issues*

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The introduction of glycoconjugate vaccines has profoundly changed the epidemiology of pneumococcal invasive diseases (IPD) and carriage of *Streptococcus pneumoniae*. Starting from 2000, three glycoconjugate vaccines (PCVs) were introduced for children immunization, targeting seven, ten and thirteen serotypes from the more than 95 serotypes of *S. pneumoniae*. Use of PCV7 (targeting serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F) has substantially decreased the incidence of PCV7-type invasive pneumococcal disease not only in vaccinated children but also in older unvaccinated individuals, due to a reduction in carriage of vaccine serotypes that led to herd protection. Conversely, an increase in the prevalence of non-PCV7 types was documented in most countries where the vaccine was used, both in IPD and carriage, the so-called serotypes replacement phenomenon. The two higher-valency vaccines, which were subsequently introduced, extended the PCV7-type coverage to the additional serotypes 1, 5, and 7F (PCV10) and to the additional serotypes 1, 3, 5, 6A, 7F, and 19A (PCV13), respectively. Also these vaccines were effective in reducing respective vaccine-type IPD and carriage, but their success was impaired by a progressive replacement of vaccine-types with non vaccine-types. Serotypes replacement is a complex phenomenon that can involve different non-vaccine serotypes depending on the geographical areas, the population under surveillance, the surveillance practices, the transmission dynamics and the pathogen evolution. In Italy data regarding IPD are available through the National Surveillance of Invasive Bacterial Diseases (<https://www.iss.it/mabi/>). Analysis of IPD cases between 2010 and 2017 confirmed a reduction in vaccine-type IPD, but this was counterbalanced by the increase of non-vaccine types. In particular, in children under 5 years old the percentage of PCV13-type IPD decreased from 68.4% in 2010-2012 to 12.6% in 2016-2017, while non PCV13- type IPD increased from 31.6% to 87.4% in the same periods of time.

An additional indirect positive effect of PCVs use was the decrease of antibiotic resistance rates. In fact, several vaccine serotypes were associated with resistance to penicillin and/or erythromycin, therefore their reduction led to an overall antibiotic resistance decrease in pneumococcus.

The surveillance of pneumococcal serotypes epidemiology both in invasive diseases and carriage is essential to monitor changes promptly, also in view of new higher-valent vaccines under development.

## 18/09/2019 LETTURA INAUGURALE

### *Le nuove sfide della vaccinologia*

Rino Rappuoli

Vaccination has been the most effective medical intervention in the history of mankind. Infectious diseases that used to kill or cause disability in millions of people annually such as diphtheria, tetanus, smallpox, polio, measles, mumps, and rubella were conquered during the last century with the first wave of vaccines. The second wave of vaccination started during the 1980s and consisted of vaccines that were made possible by the new technologies such as recombinant DNA, conjugation, genomics, that allowed the development of vaccines against Hepatitis B, papillomavirus, Haemophilus influenzae, pneumococcus, and meningococcus. Thanks to the advances in understanding of the antigens structure and their epitopes and how they interact with the human immune system, we are now entering the third wave of vaccine development, characterized by optimal design antigens, adjuvants and delivery systems. This new phase is expected to tackle diseases such as respiratory syncytial virus, tuberculosis, malaria, and HIV that have, so far, been refractory to vaccine development. In addition, the new technologies will help vaccines to address new emerging threats such as antimicrobial resistance which, if untreated, may have an impact on our planet as big as climate change.

## FARMACI BIOLOGICI E INFEZIONI

### *Rilevanza clinica delle infezioni in corso di terapie con farmaci biologici*

Emanuele Durante Mangoni

*Università della Campania "Luigi Vanvitelli" AORN dei Colli - Ospedale Monaldi*

Biologic agents (BA) are a large, increasingly expanding class of drugs that mostly target a specific step among one of the several pathways governing our cell and system biology. Many BA impact directly or indirectly on immune function, putting a growing population at risk for opportunistic or newer infections.

One major challenge today is represented by the common lack of knowledge on the clinical presentation, diagnosis and treatment of BA-associated infections among the diverse medical subspecialists that prescribe BA. These encompass Dermatologists (for psoriasis, pemphigo, pyoderma gangrenosum, hidradenitis suppurativa, scleroderma, dermatomyositis), Gastroenterologists (for inflammatory bowel diseases [Crohn, UC], eosinophilic esophagitis), Oncologists, Respiratory physicians (for asthma, COPD, sarcoidosis, ILD), and mostly Rheumatologists, who may prescribe BA for the majority of rheumatic diseases (RA, SLE, CTDs, vasculitides).

The risk of infection varies with the specific BA and its molecular target. Moreover, the specific infection syndromes that may be observed also vary according to the BA used. Often, infection is the result of a cumulative effect of both BA and drugs received before. In many cases, steroids are used in association with BA to attenuate their toxicity or improve patient tolerability. The net state of immunosuppression, accounting for both BA effect and the current clinical conditions, finally determines infection risk in individual patients. Primary care physicians, internal medicine specialists and infectious disease specialists must know how to recognize and diagnose BA-associated infections.

## *Gestione della tubercolosi nei pazienti trattati con farmaci biologici*

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Immune-mediated inflammatory diseases comprising rheumatic diseases, chronic intestinal diseases and dermatological diseases, may require treatment with biological agents targeting different cytokines and cells of immune response, such as TNF  $\alpha$ , IL1, IL6, IL12/IL23, IL17, CD20 and CD28 lymphocytes. Therapy with biological agents has been associated with an increased risk of infections. In particular, among the biological agents, the inhibition of TNF has been associated with the highest risk to reactivation of infection and progression to active tuberculosis. Extrapulmonary localization of tuberculosis are more frequent in these patients and the clinical features are particularly severe. Therefore, screening for latent tuberculosis infection is mandatory in candidates to biological therapy. A description of the screening procedures and the preventive therapy for latent tuberculosis infection available will be provided. We will discuss also new insights on experimental tests for detection of latent tuberculosis infection and risk of progression to tuberculosis.

19/09/2019 – FOCUS ON

## MICROORGANISMI E DISORDINI NEUROLOGICI

*Microbiota, immunità e cervello: dall'autismo alla sclerosi multipla*

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The human gut microbiota plays a crucial role in the functioning of the gastrointestinal tract and its alteration can lead to gastrointestinal abnormalities and inflammation. Additionally, the gut microbiota modulates central nervous system (CNS) activities affecting several aspect of host physiology. Motivated by the increasing evidences of the role of the gut microbiota in the complex set of interactions connecting the gut and the CNS, known as gut-brain axis, we will discuss whether the gastrointestinal abnormalities and inflammation commonly associated with neuro-behavioral disorders such as Autism and neuroinflammatory disorders, such as Multiple Sclerosis (MS), could be related to alterations of the bacterial and fungal intestinal microbiota.

In particular, since only few reports have explored the fungal component of the gut microbiota in health and disease, we will describe the characterization of the gut mycobiota and microbiota of three cohorts of healthy, autistic and MS subjects to investigate if these neurological disorders harbor alterations of the gut microbiota.

Culture-based and metataxonomics analysis of the faecal fungal populations of healthy volunteers revealed that the gut mycobiota differs in function of individuals' life stage in a gender-related fashion. Autistic subjects are characterized by a reduced incidence of Bacteroidetes and that Collinsella, Corynebacterium, Dorea, Haemophilus and Lactobacillus were the taxa predominating in the gut microbiota of autistic subjects. No consistent bacterial species patterns were found altered in MS, both in our study and in meta analyses. Both Autistic and MS subjects showed increased alteration in the cell count and species patterns of the fungal mycobiota, in particular with an overgrowth of strains of *Candida sp.* and other species often associated with fermented foods. Culture based analysis allowed association of the strains isolated with specific immune responses in patients. We conclude that strain level analyses of the gut mycobiota in larger cohorts, in patients during remission and relapse, could lead to understanding of the causal relationships between the gut microbiome and the onset of disease.

## *Human endogenous retroviruses in autism*

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Autism spectrum disorder (ASD) is a heterogeneous group of neurodevelopmental disorders, clinically diagnosed since the lack of reliable biomarkers. Autism aetiology is probably attributable to the combination of genetic vulnerability and exposure to environmental risk factors, and recently, maternal immune activation has been linked to derailed neurological development, resulting in ASD in the offspring. Human endogenous retroviruses (HERVs) are relics of ancestral infections, stably integrated in the human DNA. Given the HERV persistence in the genome, some of HERVs have been co-opted for physiological functions during evolution, while their reactivation has been associated with several pathological conditions, including cancer, autoimmune, neurological and psychiatric disorders. Particularly, due to their intrinsic responsiveness to external stimuli, HERVs can modulate the host immune response and in turn HERVs can be activated by the immune effectors.

In previous works we demonstrated high expression levels of HERV-H family in blood of autistic patients, closely related with the severity of the disease. Moreover, in a preclinical ASD model we proved changes of expression of several ERV families and cytokines from the intrauterine life to the adulthood and across generations *via* maternal lineage.

Based on our previous findings we also analyzed the expression profiles of several HERV families and cytokines in blood from individuals belonging to families with autistic children and individuals enrolled as healthy controls, to look for a common molecular trait within family members. Results showed that ASD patients and their mothers share altered expression of HERV-H and of the human endogenous MER34 (medium-reiteration-frequency-family-34) ORF, envelope gene (HEMO) and of cytokines such as TNF- $\alpha$ , IFN- $\gamma$ , IL-10, suggesting a mother-child association by HERVs and cytokines expression. The present data support the involvement of HERVs in ASD and suggest HERVs and cytokines as ASD-associated traits, highlighting the need to study the family members as a unit. Moreover, considering that ASD is a heterogeneous group of neurodevelopmental disorders, a single determinant alone could not be enough to account for the multifaceted nature of ASD. If our findings will be confirmed in larger studies, the expression of selected HERVs and cytokines could be considered as ASD-associated biological traits in a set of biomarkers, easily detectable in blood and potentially useful for an early diagnosis.

## *Herpes simplex virus-1 (HSV-1) and Alzheimer's disease*

*Giovanna De Chiara<sup>1</sup>, Maria Elena Marcocci<sup>2</sup>, Giorgia Napoletani<sup>2</sup>, Virginia Protto<sup>1</sup>, Marco Fabiani<sup>2</sup>, Dolores Limongi<sup>3</sup>, Roberto Piacentini<sup>4</sup>, Domenica D. Li Puma<sup>4</sup>, Claudio Grassi<sup>4</sup>, Anna Teresa Palamara,<sup>3</sup>*

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Several epidemiological and experimental evidence suggested potential links between HSV-1 and Alzheimer's disease (AD), a neurodegenerative disorder characterized by a progressive decline in cognitive function and the accumulation in the brain of amyloid- $\beta$  peptides (A $\beta$ s), hyperphosphorylated forms of tau protein (pTau), that aggregate in neurofibrillary tangles, high levels of oxidative stress markers and neuroinflammation. However, these links have been considered merely associative throughout neuroscientists so far, since a causal relationship between HSV-1 and this disorder has yet to be definitely proved. Recently, an impressive analysis of postmortem brains points out herpes viruses as key agents in accelerating brains towards AD (Readhead et al, 2018), and another excellent study revealed how HSV-1 could speed up A $\beta$  deposition as well as related amyloid plaque accumulation (Ezzat et al, 2019). Other authors highlighted the anti-microbial property of A $\beta$ s, providing in vivo evidence on the capability of A $\beta$  fibrils to entrap and neutralize herpesviruses (Eimer et al, 2018). Thus, same authors proposed that AD may result from an aberrant brain innate response to pathogen invasion, especially for HSV-1. Indeed, this peculiar virus is able to establish a life-long infection in humans, characterized by multiple reactivations that may also involve the brain. In this line, we recently provided novel evidence that multiple HSV-1 reactivations trigger in mouse brain accumulation of A $\beta$ s, pTau, neuroinflammation and oxidative damages, that are paralleled by increasing cognitive decline (De Chiara et al 2019). We then investigated how the virus may promote its own spreading among neurons as well as the propagation of some virus-induced AD hallmarks, demonstrating that HSV-1 preserve the function of CRMP2, a protein involved in microtubule stabilization, to move inside neurons, and exploits extracellular vesicles (EVs) to infect and damage neighbor cells. We also explored the virus capability to affect neuron physiology, by altering the epigenetic mechanisms, demonstrating that it is able to accelerate the normal brain aging. Altogether, our findings support the view that multiple HSV-1 reactivations, causing mild but repeated viral spreading and replication in the central nervous system, may be a risk factor for AD.

## TRAPIANTI E INFEZIONI

### *Monitoring of viral infections in transplantation*

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**Introduction:** Viral reactivations are a major cause of morbidity and mortality for recipients of solid organ transplantation. In kidney transplantation, BK virus (BKV) and cytomegalovirus (CMV) are major pathogens. These viruses are very common in healthy population, with an approximate prevalence of 80% and 60%, respectively. Primary infection usually occurs during childhood, but the virus stays latent and asymptomatic under normal conditions. Individuals with compromised immune systems, after a solid organ transplantation, are prone to both primary infection and reactivations with clinically relevant symptoms. Thus, the role of viral and immunological monitoring is crucial in the control of BKV and HCMV infections and reactivations.

**Materials and Methods:** 328 patients were analyzed by EliSPOT assay: 201 prospectively monitored in the first year post kidney transplant, 127 with a single determination at >1 year. Clinical features, including occurrence of CMV-DNAemia, were evaluated. For BKV, 560 kidney transplants in adult patients were performed at the AOU Città della Salute e della Scienza di Torino. Some of these needed to perform a renal biopsy which allowed to diagnose and classify BKV-induced nephropathy. Virological monitoring for each patient was performed at least once a month from the time of transplantation and in conjunction with the biopsy.

**Results:** In the ELISPOT assay a significantly higher prevalence of DNAemia values >105copies/mL was found in non-responders versus responders (9/110, 8.2% versus 5/218, 2.3%, respectively,  $p < 0.05$ ). In responders, episodes of infection were characterized by low level CMV-DNAemia and short duration. In the BKV study, at the first biopsy two patients presented PVAN-A, eleven PVAN-B1, four PVAN-B2, one PVAN-B3 and one PVAN-C, with viremia values between 1000 and >5000000 copies/ml. Reducing the immunosuppression some patients showed a regression and resolution of the pathology, others developed a progression of the pathology with subsequent reduction in viral load and loss of renal parenchyma and development of fibrotic tissue.

**Discussion and Conclusions:** Viro-immunological routine monitoring of CMV evidenced that restoration of specific T-cell response is frequently and stably achieved within few months post transplantation and is associated to a favorable outcome in terms of reactivation risk. The results of BKV analysis suggest that, despite the use of targeted therapeutic strategies, in rare patients the progression of the PVAN pathology is not arrestable, thus leading to the loss of renal function and transplant failure. This is probably due to determinants in the host, in the target organ, and in the virus, which are subject to dynamic modulators that need to be investigated.

## LO SCREENING EGLI ENTEROBATTERI PRODUTTORI DI CARBAPENEMASI (CPE) E LA PREVENZIONE DELLE INFEZIONI

### *Diagnostic methodologies and preliminary results*

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**Introduction:** The recent emergence of carbapenem-resistance among Enterobacterales is a major threat for hospitalized patients, and effective strategies are needed. This investigation was carried out to study CRE rectal colonization of patients admitted to different risk units in 7 Italian hospitals, following an agreed upon protocol.

**Materials and methods:** The active rectal swab surveillance program, CCM 2017, was started in September 2018 and included the use of rectal screening at admission and then weekly thereafter of patients hospitalized in different risk units. These rectal swab samples were placed in brain-heart infusion broth with a disk of ertapenem or streaked onto MacConkey agar plates with carbapenem discs, or the chromogenic medium and incubated overnight at 35 °C in ambient air. Bacterial identification and antimicrobial susceptibility testing were carried out by using Automated Microbiology System (e.g. Vitek2, BD Phoenix™, BD). All CRE isolates were evaluated, by both phenotypic and genotypic methods, for carbapenemase production (KPC, VIM, NDM, OXA-48 type).

**Results:** During the intervention period, the rate of carbapenem-resistant *K.pneumoniae* rectal colonization was higher at admission and after 7 days, which decreased gradually each week during the first month.

The majority of patients were positive for KPC-*K.pneumoniae*; NDM-1, OXA-48, and NDM carbapenemases were also detected. Some isolates co-produced two carbapenemases such as KPC/OXA-48 or KPC/VIM.

**Discussion and conclusions:** The preliminary results of our study highlight the prevalence of KPC-*K.pneumoniae* in colonized patients. However, VIM, NDM and OXA-48 carbapenemases are present as well as the isolates co-carrying double genes with a high spreading propensity and dramatic consequences for the epidemiology of antibiotic-resistance.

**CCM 2017 Participants:** Palermo (Prof. A.Giammanco); Catania (Prof. S.Stefani); Firenze (Prof. G.M.Rossolini); Torino (Prof. R.Cavallo); Genova (Prof. A.Marchese); Bologna (dott. S.Ambretti); Napoli (Prof. R.Catania)

## 19/09/2019 - APPROCCIO SINDROMICO ALLE MALATTIE SESSUALMENTE TRASMESSE: CONFRONTO TRA MICROBIOLOGO E CLINICO

### *Il laboratorio di microbiologia nella diagnosi delle malattie sessualmente trasmesse*

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Sexually transmitted infections (STIs), caused by a wide variety of bacteria, viruses, and parasites, are spread primarily through sexual contact and represent a major cause of morbidity and mortality worldwide. Among bacterial pathogens, *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG) are the most common causes of STIs. *Mycoplasma spp.* and *Ureaplasma spp.* are associated with genital tract infections, being potential agents of maternal, fetal and neonatal infections. Among members of genital Mycoplasmas, *Mycoplasma genitalium* (MG) and *Mycoplasma hominis* (MH) are emerging as important causative agents of sexually transmitted infections in both males and females, leading to infertility. Currently, *Ureaplasma spp.* are separated into two species, *Ureaplasma urealyticum* (UU) and *Ureaplasma parvum* (UP), both considered commensals of the uro-genital tract, although it has been reported that UU is associated with some diseases including non-gonococcal urethritis, pregnancy complications and prenatal infections and UP is an overlooked pathogen which may perturb homeostasis in the genital tract providing a survival advantage for CT. Finally, the protozoan *Trichomonas vaginalis* (TV) causes trichomoniasis, the most common non-viral sexually transmitted infection all over the world.

Generally, STIs are often asymptomatic or responsible for non-specific symptoms. Therefore, if undiagnosed, they will lead to reproductive sequelae or complications in the upper genital tract.

Laboratory testing plays a major role in the diagnosis and treatment of STIs and clinical laboratorians should be familiar with the current guidelines and methods for testing.

Hence, since the quick and accurate identification of responsible agents for STIs is crucial to administer appropriate antimicrobial therapy and to interrupt the cycle of disease transmission, high sensitive methods to diagnose these diseases are required. New molecular tools have been developed in the past few years in order to improve STIs diagnosis. Herein, we report the experience of the Microbiology and Virology Unit of the Sapienza University “Policlinico Umberto I” (Rome) that, in the last months, is evaluating multiplex q-PCR Allplex™ (Seegene, Korea) for the diagnosis of STIs. Preliminary results confirm that the multiplex q-PCR Allplex™ is a robust tool for the STIs’ diagnosis. Its ease of use and processing could allow it to be incorporated into the day to day laboratory work ensuring a high sensitivity and allowing the detection of more pathogens simultaneously with a Turn Around Time (TAT) of less than 5 hr.

## *The importance of the vaginale ecosystem in sexually transmitted diseases*

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“Microbiota” is a term that was first introduced 10 years ago, substituting the term Bacterial flora. It indicates the community of microorganisms (bacteria, protozoa, fungi and viruses) with which we share our everyday environment and without which humans wouldn't exist in the form we recognise today. “Microbiota” has been defined as the human being's “sixth sense”. Human microbiota consists of 10 to 100 trillion symbiotic cells. One hundred thousand million of bacteria and billions of viruses live in or on each human body .For every cell in the human body there are 10 bacteria; for every bacteria there are 10 viruses and most of them are as yet still unknown to medical scientific.

The “Microbiome” represents the genes of our microbial symbionts (microbiota) and allows our body to enhance and multiply our genetic potential, controlling the relationship between genetic factors and environmental factors such as life style, nutrition and drug consumption.

Every area of our body contains a specific microbioma, each one with the common aim of reaching “Eubiosis”, i.e. the balance between different species.

“Dysbiosis” is the breakdown of that balance, which can lead to a Pathobiosis process involving one of more organs, manifesting itself with a variety of symptoms .

The most recent discoveries in molecular biology have introduced the new concept of “omics” science, including transcriptomics, proteomics and metabolomics. The implication of these concepts in medical practice may mean the ability to provide a preventive diagnosis, rather than just an early diagnosis. Identifying the conditions that could potentially impair Eubiosis, thereby preventing with minimal medical intervention the onset of the pathobiosis process .

The interface between human tissue cells and the external environment consists of a barrier colonized by microorganism swchich form the microbiota, dynamically exchanging information between our DNA and microorganism genes .

Vaginal microbiota, the first microbiota to be studied and known, is dominated by Lactobacillus species including L.Crispatus, L. Gasserii, L. Jensenii and L.Iners. The predominance of Lattobacillus Crispatus indicates the ideal vaginal eubiosis status, expressing a prefect symbiosis between vaginal tissue and mictobiota. Any factor causing an alteration of this equilibrium, causes overgrowth of L. Crispatus and activates the citolysis process in order to restore the eubiosis status. The presence of an imbalanced microbiota and the onset of pathobiosis characterized by the substitution of L. Crispatus with L. Gasseri and Jensenii and the prevalence of Candida with alteration of pH.

Further protection of the normal vaginal microbiota is the dominance of L. Iner, indicating the evolution towards pathobiosis. L. Iner is able to survive in abnormal pH and to aggregate anaerobic bacteria resistant to metronidazole. The alteration of this last defence leads to a breakdown of the eubiosis and the prevalence of bacteria or viruses .

Bacterial vaginosis shows the gradual reduction of lactobacillus species and at the same time an increased prevalence of anaerobic species. This is one of the most frequent gynaecological disorders among fertile age women, with an incidence of 29% among women between 14 and 49 years old.

There is a widely understood correlation between bacterial vaginosis and sexually transmitted diseases such as HIV, HPV and HSV and this association can be explained by the lactobacillae species imbalance. Dysbiosis condition can cause a predisposition to increased levels of bacteria or viruses, with uncontrolled expression of genetic information (DNA or RNA) and atypical growth leading to preneoplastic or neoplastic lesions .

The current pharmacological treatments, including bacteriostatic and bactericidal antibiotics, are not always effective in preventing a recurrent pathogenic event and represent the main cause of bacterial antibiotic resistance. OMS therefore recommends their limited use, also because they are not able to reconstitute the perfect eubiotic balance, and may provoke a predisposition to relapses or recurrence of disease.

Over the last few years new therapeutic strategies have been introduced, being used either as preventive tool, either alongside traditional agents or on their own in order to restore the eubiosis of the microbiota. These include the use of probiotics, which can be generic, or targeted against a specific microorganism, aiming to induce a selective response.

**Methods:** This is a retrospective-observational study performed between January 2007 and December 2018. All women presenting to outpatient clinic of Sexually Transmitted Disease (STD) of Policlinico Umberto I, Sapienza University of Rome, were evaluated with bacterioscopic examination investigated for possible coinfections, trying to identify correlations between different germs development. *C. trachomatis*, *N. Gonorrhoeae*, *Mycoplasma* spp., yeast and HPV were tested based on patients' clinical characteristics.

**Results:** Overall, 2110 patients were screened. Of those 1299 patients ( 61.5%) presented alterations of vaginal microflora while 811 (38% ) had a vaginal eubiosis. In 824 patients with microflora alteration identified by bacterioscopic exam (mixed bacterial flora or bacterial vaginosis) a STD was detected. In 811 patients who did not present an alteration of the vaginal ecosystem there was no associated co-infection.

**Discussion:** Often, even slight alterations of vaginal microenvironment and flora can promote good bacterial overgrowth but mainly might further co-infection with other pathogens, giving an higher predisposition to STD. But by better re-establishing eubiosis in vaginal ecosystem, bacterial and viral predominance could be reduced, leading to a lower susceptibility to co-infections.

## 19/09/2019 – COMUNICAZIONI ORALI SUL TEMA STRATEGIE ANTIMICROBICHE INNOVATIVE E ANTIMICROBICO RESISTENZA

### *Opportunism and resistance in nosocomial Gram-negative bacteria*

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We recognize bacteria as microorganisms of enormous adaptability and capacity to adjust to diverse conditions. Bacterial tolerance and resistance to antibiotics are the inevitable consequence of positive selection imposed by the use of antimicrobials. Several gram-negative bacterial species produce nosocomial infections, among which *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* stand out as opportunist pathogens with the capacity to develop multiresistance to first-choice antibiotics. The worldwide emergence of epidemic strains, linked to increased disease severity and mortality and extreme drug-resistance are growing concerns in the nosocomial environment and resulted in greater research efforts toward determining the resistance factors and pathogenesis mechanisms. Epidemiological, clinical and microbiological research greatly contributes to improve knowledge on factors influencing the emergence and spread of antimicrobial resistance. Accelerated dispersion of resistance mechanisms across bacteria has been observed as the consequence of selection of bacteria that have acquired those mechanisms.

**ID 013 - Effective elimination of Staphylococcal contamination from hospital surfaces by a bacteriophage-probiotic sanitation strategy.**

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**Introduction.** Persistent contamination of hospital surfaces and antimicrobial resistance (AMR) are recognized major causes of healthcare-associated infections (HAI). The control of pathogen contamination has been approached so far by conventional chemical-based sanitation, which however does not prevent recontamination, has a high environmental impact and can favor the selection of resistant strains. In the search for effective methods, we recently showed that a probiotic-based sanitation (PCHS) can stably decrease surface pathogens, significantly reducing also AMR and HAI incidence. However, PCHS action is slow and non-specific. By contrast, bacteriophages have been proposed as a decontamination method due to their ability to kill bacterial targets very rapidly and specifically, but their routine application has never been tested. We thus analyzed the feasibility and effectiveness of phage addition to PCHS sanitation, aiming to obtain a both rapid and stable abatement of specific pathogens in the hospital environment.

**Materials and Methods.** The study was performed in a private hospital located in Ferrara (Italy), after approval by local Ethics Committee. Phage decontamination potential was tested in 8 bathrooms of the General Medicine wards, against *Staphylococcus* spp., being bathrooms the most contaminated areas and Staphylococci the most prevalent bacteria in such settings, as detected by preliminary tests. Four rooms were treated with PCHS alone (controls) and 4 rooms with phage nebulization combined with PCHS. Sanitation procedures were applied daily, and Staphylococcal contamination was analyzed by culture-based methods and molecular real-time PCR analyses.

**Results.** The characterization of Staphylococcal contamination showed the presence of four prevalent species, namely *S. epidermidis*, *S. haemolyticus*, *S. cohnii*, *S. simulans*. A daily phage application by nebulization was associated with a rapid and significant decrease of *Staphylococcus* spp. load on treated surfaces, up to 97% more than PCHS alone ( $p < 0.001$ ). The bacterial decrease was paralleled by an increase of phage load on treated surfaces, as detected by specific real-time PCR. No variations were observed in other bacterial genera, confirming a specific action of phages.

**Discussion and Conclusions.** Collected data suggest that the use of a probiotic-phage sanitation system is associated with a stable and specific decontamination, that would be highly desirable in the hospital environment, as it would prevent infections associated to the persistence of specific pathogens on hospital surfaces. Such system might be thus considered as a part of infection prevention and control strategies, to counteract outbreaks of specific pathogens and prevent associated infections.

## **ID 184 - Lactoferrin: iron and inflammatory homeostasis in cystic fibrosis infection**

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**Introduction** Cystic fibrosis (CF) is a genetic disorder affecting several organs including airways. Bacterial infection, inflammation and iron dysbalance play a major role in the chronicity and severity of the lung pathology. Recently, lactoferrin (Lf), a multifunctional cationic iron-chelating glycoprotein, is emerging as a pivotal actor of innate immunity in iron and inflammatory homeostasis through the modulation of the synthesis of ferroportin (Fpn), intracellular ferritin (Ftn), transferrin receptor (TfR)-1, ceruloplasmin and interleukin (IL)-6. Here, we investigated the role of Lf in CF human cells and in mice affected by acute and chronic *Pseudomonas aeruginosa* lung infection.

**Materials and Methods** Lf was checked for purity, integrity, iron saturation rate, iron binding ability and lipopolysaccharide contamination. *P. aeruginosa* PAO1 (ATCC 15692) and MDR-RP73, a clinical strain isolated at the late stage of chronic lung disease from a CF patient, were used to mimic the acute and chronic infections, respectively. In in vitro models, CF bronchial epithelial (CFBE) cells, homozygous for the DF508-CFTR mutation, were infected with *P. aeruginosa* PAO1. In in vivo models, anesthetized mice were intra-tracheally infected with  $1.0 \times 10^6$  *P. aeruginosa* PAO1 to establish acute lung infection or with  $1.0 \times 10^6$  agar beads suspensions of *P. aeruginosa* MDR-RP73 for the chronic infection. In acute infection, the mice received only one treatment with 100 or 200  $\mu$ g of aerosolized Lf or saline solutions and sacrificed after 6h, while in chronic lung infection, mice received 200 $\mu$ g of aerosolized Lf or saline daily over seven days.

**Results** In CFBE cell line, Lf did not affect *P. aeruginosa* PAO1 adhesion, but significantly reduced the number of intracellular bacteria. Interestingly, the high levels of pro-inflammatory cytokines in infected CFBE were reduced by Lf. In WT mice model, aerosolized Lf treatments reduced bacterial load in both acute and chronic infection models compared to the vehicle group. Regarding inflammatory response induced by infection, mice treated with Lf showed a significant decrease of several pro-inflammatory cytokines in both acute and chronic infections compared with untreated ones. Of note, in chronic infection WT and CF mice model, Lf reduced also pulmonary iron overload as well as decreased both the iron exporter Fpn and iron storage Ftn.

**Conclusions** Overall, Lf acts as a potent multi-targeting agent able to break the vicious cycle induced by *P. aeruginosa*, inflammation and iron dysbalance, thus mitigating the severity of CF-related pathology and sequelae.

**ID 129 - Bovine lactoferrin: a novel potentiator for restoring the susceptibility of resistant *Helicobacter pylori* strains. An *in vitro* and *in vivo* study**

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**Introduction.** The increase of antimicrobial resistance in *Helicobacter pylori* and the consequent failure of therapeutic regimens underlines the need to find novel strategies to enhance the eradication rate. Lactoferrin, a glycoprotein with multiple antimicrobial, antiviral and antifungal properties, has been shown to have an antibacterial property against *H. pylori* *in vitro* and *in vivo*. The aim of this study has been twofold: first to analyze the *in vitro* antimicrobial/anti-virulence action of bovine lactoferrin (BLF) and its capability to synergize with levofloxacin (LEVO) against resistant or multi-drugs resistant (MDR) *H. pylori* strains and second, to evaluate whether the inclusion of BLF in a triple therapy containing LEVO, amoxicillin (AMOX) and esomeprazole (ESO) at full dosage for first-line treatment of *H. pylori* infection could increase the eradication rate.

**Materials and Methods.** *In vitro* - The BLF antimicrobial/anti-virulence effect was analyzed by MIC/MBC determination and twitching motility against 6 clinical *H. pylori* and a reference strain. The synergism was evaluated using the checkerboard assay. *In vivo* - The prospective therapeutic trial was carried out on two separate patient groups: A) treated with ESO/AMOXI/LEVO; B) treated with ESO/AMOXI/LEVO/BLF. Treatment outcome was determined by <sup>13</sup>C Urea Breath test.

**Results.** *In vitro* BLF inhibited the growth of 50% of strains at 10 mg/mL and expressed 50% of bactericidal effect at 40 mg/mL. Combined with LEVO, BLF displayed synergistic effect for all strains with a MIC reduction until 16 and 32 fold, for LEVO and BLF, respectively. BLF at 1/4 MIC reduced the microbial motility significantly for all detected strains.

In the *in vivo* study, six failures out of 24 patients recruited were recorded in the group treated with ESO/AMOXI/LEVO (75% of eradication rate) and in the group with ESO/AMOXI/LEVO/BLF, two failures were recorded out of 53 patients recruited (96.07% of eradication rate). BLF achieves a therapeutic gain of 21% when added to a triple therapy with a proton pump inhibitor.

**Discussion and conclusions.** This study demonstrates that BLF *in vitro* inhibits the growth and motility of *H. pylori*. When used in combination with LEVO on *H. pylori* strains resistant to LEVO and other antibiotics, BLF in sub-MIC values restores the effectiveness of the antibiotic through a synergistic action. BLF can improve the potency of traditional antimicrobial schemes fighting the antimicrobial resistance through the reduction of flagellar motility and consequently the microbial colonization. BLF can be considered a novel potentiator for restoring the susceptibility in resistant *H. pylori* strains. BLF added to a triple therapy in first-line treatment potentiates the therapeutic effect.

## **ID 109 - Phage therapy: treatment and prevention of methicillin-resistant *Staphylococcus aureus* in *Galleria mellonella* model of implant-associated infection**

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**Introduction:** Renewed interest has arisen in potential applications of bacteriophages for bacterial contamination control of medical devices and antimicrobial treatment of persistent infections. Staphylococcal bacteriophage Sb-1 is a therapeutically effective phage formulation suitable for the treatment of *Staphylococcus aureus* infection which exhibits *in vitro* antibiofilm properties. In this study, we developed an *in vivo* model of implant-associated infection in *Galleria mellonella* larvae implanted with an orthopaedic Kirschner-wire and investigated the ability of Sb-1, either alone or in combination with antibiotics, to prevent/treat implant-associated infection due to methicillin-resistant *S. aureus* (MRSA).

**Materials and Methods:** The stability of Sb-1 in *G. mellonella* larvae was investigated by injecting a phage titer of  $10^8$  PFU and evaluating the presence of Sb-1 in hemolymph at different time points by plaque assay. For infection experiments, cut pieces of sterile stainless-steel K-wires (4 mm, 0.6 mm Ø) were implanted into the last proleg of larvae. After 2 days, larvae were infected with MRSA ATCC 43300 ( $1 \times 10^5$  CFU) and incubated at 37 °C for further 2 days. Implanted larvae were treated 3□/day with 10 µL of Sb-1 ( $10^7$  PFU), Daptomycin (4 mg/kg), PBS (24h) / Daptomycin (24h) and Sb-1(24h) / Daptomycin (24h). An untreated control was also added. For prevention experiments, a prophylaxis based on phages or vancomycin (10 mg/kg) administration, followed by MRSA infection, was performed on the same day of the implantation. Either 2 days post-infection or post-treatment, larvae were dissected for K-wire explanting and the material was sonicated and plated for colony counting.

**Results:** The tested titer of Sb-1 resulted stable in hemolymph of *G. mellonella* larvae for 6-8 h post-administration. Therefore, the administration of phages was performed 3□/day. Two days post-infection of K-wire implanted larvae,  $\approx 5 \times 10^7$  CFU/ml MRSA were found on the material. K-wires from larvae treated with Sb-1 or Daptomycin showed a *S. aureus* CFU/ml reduction of 1 log compared to the CFU/ml values of the untreated control. The staggered administration Sb-1/Daptomycin determined higher CFU reduction ( $\approx 3.5$  logs). Prophylaxis with Sb-1 prevented the infection in 7 out of 10 larvae in the 5<sup>th</sup> day post-infection similarly to vancomycin.

**Discussion and Conclusions:** A suitable and easy model to test antibiofilm formulation *in vivo* on implant in *G. mellonella* larvae was developed. Sb-1 phage, administered as prophylaxis, was able to prevent biofilm-associated infection due to MRSA in larvae and it might be used as alternative to vancomycin. Sequential combination of Sb-1 and Daptomycin strongly reduced biofilm formed on implanted K-wire in comparison to the mono-therapy based on either phage or antibiotic alone.

## ***ID 150 - Analysis of KPC carbapenemase gene in ceftazidime/avibactam resistant K. pneumoniae clinical isolates***

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**Introduction.** KPC producing bacteria showing rapid spread. Genetic analysis of *bla<sub>KPC</sub>* genes, have usually been identified in plasmids carrying also ESBL genes as *bla<sub>CTX-M1</sub>*. Avibactam is a non- $\beta$ -lactam  $\beta$ -lactamase inhibitor, actives also against KPC enzyme. approved, in 2015, in combination with ceftazidime. Ceftazidime-avibactam (CZA) resistance is an emerging threat. Porins mutations, efflux overexpression usually account for resistance in clinical isolates. Recently we reported a clinical isolate with a deletion of two aminoacids in the *KPC* sequence.

**Material and Methods.** 8 CZA resistant *Klebsiella pneumoniae* KPC carbapenemase producer strains were included in the study. These strains were isolated from purulent specimens in the period march 2018-march 2019. Antimicrobial susceptibility tests were performed by Etest and/or broth microdilution methods. Carbapenems and third generation cephalosporins were tested other than CZA. The last was tested in broth microdilution with a 2 mg/L fixed concentration of avibactam. ESBL (CTX-M groups, TEM and SHV) and carbapenemases (KPC, VIM, NDM, IMP and OXA-48) presence was checked by PCR. KPC amplicons were sequenced and analysed. Carbapenemase production was checked by immune assay and carbapenemase activity by CarbaNP and hydrolysis assays.

**Results.** CZA resistance was confirmed both by Etest and microdilution methods. All strains showed resistance to third generation of cephalosporins, 6 out 8 was resistant also to carbapemems. 2 out 8 showed carbapenems low susceptibility (MIC 1mg/L for imipenem and 8 mg/L for meropenem). No CTX-M group ESBL were detected, only TEM or SHV. All strains amplified the *KPC* gene and no other carbapenemases. 6 out of 8 strains showed no mutations in the *KPC-3* gene, while the two strains with low carbapenems MICs showed the same deletion of 6 nucleotides in position 498-503, corresponding a deletion of a glutamic acid and leucine in position 167 and 168, as we recently reported. All 8 strains showed KPC production by immune assay, but only 6 showed carbapenemase activity. The two isolates showing deletion in the *KPC* gene had no carbapenems hydrolysis activity.

**Discussion and Conclusions.** CZA resistance in *k. pneumoniae* KPC producer strains may had different origin. 6 out of 8 *K. pneumoniae* isolates had no mutations in KPC gene showing an KPC-3 variant. These strains need further investigation on porins mutations and efflux system overexpression. 2 out of 8 isolates confirmed a CZA resistance due a deletion of two amino-acids in position 167-168, other than the first isolate that we already described. The amino-acid in position 167 is involved in the proton acceptor active site. CZA susceptibility would be tested and monitored at any time.

## **ID 049 - IP-10 contributes to the inhibition of mycobacterial growth in an ex vivo whole blood assay**

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**Introduction:** Interferon- $\gamma$  inducible protein 10 (IP-10), is a potent chemoattractant that promotes migration of monocytes and activated T-cells to inflammation foci. IP-10 binds and activates CXCR3, expressed on activated T cells but also on NK cells, epithelial cells, fibroblasts and even granulocytes. Interestingly, CXCR3 signalling can be inhibited by an antagonist form of IP-10 which lacks two amino acids (IP-10<sub>3-77</sub>) in the N-terminal end of the mature protein (IP-10<sub>1-77</sub>), this truncation is due by the enzyme DPP4. To date, we lack a clear understanding of the potential role of IP-10, and primarily of the antagonist form IP-10<sub>3-77</sub>, in TB pathogenesis, and we do not know whether the virulent *Mtb* can differentially modulate IP-10 compared to non-tuberculous mycobacteria (NTM), or whether *Mtb* strains belonging to different phylogeographic lineages show a differential ability to elicit IP-10. In this study, we started to answer some of these questions by first implementing an *ex vivo* infection model based on the use of whole blood from healthy subjects.

**Material and methods:** We investigated the impact of IP-10 and the role of DPP-IV on mycobacteria replication using the *ex vivo* model of human whole-blood (WB) assay. In particular, we compared the levels of IP-10 upon infection with different *Mtb* clinical strains and species of non-tuberculous mycobacteria (NTM) and evaluated how IP-10 may contain bacterial replication. To study the role of IP-10 isoforms, we expressed a recombinant form of IP-10 (rIP-10) in eukaryotic cells by transfecting rhabdomyosarcoma (RD) cells with the plasmid pUNO1-hIP-10. Transfected cells were cultured in antibiotic-free media, in the presence or absence of DPP-IV inhibitor, the sitagliptin, and supernatants collected at day 5 have been used to condition WB infections.

**Results:** Infection with a panel of *Mtb* clinical strains belonging to different phylogeographic lineages showed that concentrations of IP-10 were different between the *Mtb* clinical strains evaluated and did not directly or inversely correlate with virulence in WB. Inhibition of DPP-IV in infected WB consistently enhanced the killing of *Mtb* or other NTM, though the extent of the anti-mycobacterial activity differed depending on the *Mtb* strain or the NTM species. In line with these findings, WB infected in the presence of sitagliptin, a clinically approved drug for the treatment of type 2 diabetes, showed enhanced and dose-dependent anti-mycobacterial activity; as well as the addition of rIP-10.

**Discussion and Conclusion:** The results of this study support the contribution of IP-10 in restricting *Mtb* replication in host tissues, though no direct activity of IP-10 against mycobacteria was observed. The differential activity of the agonist and antagonist forms of IP-10 can still be critical to control the *Mtb* infection in the intercellular environment. The clinical relevance of IP-10 in mycobacterial pathogenesis and suggest that targeting the IP-10/CXCR3 pathway may open the way for new host directed therapies for the treatment of TB or other NTM infections.

## 19/09/2019 – COMUNICAZIONI ORALI SUL TEMA RAPPORTI VIRUS-OSPITE

### *ID 006 - HPV18 E7 Oncoprotein Subverts Host Innate Immunity Through Concerted Action of SIRT1 and SUV39H1*

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Identification of novel mechanistic insights into high-risk (hr)HPV immune evasion is critical for understanding how these viruses can persistently infect steadily unreactive cells and promote cancer. We showed that HPV18 persistence leads to the inhibition of both type I and type III IFNs in response to DNA ligands, and this effect is mainly due to the suppression of cGAS-STING and RIG-I pathways by epigenetic modifications. In this study, we have identified SUV39H1 as the principal enzyme responsible for the accumulation of H3K9me3 at the promoter regions of the aforementioned genes in keratinocytes transformed by either HPV16 (CaSki cell line) or HPV18 (HeLa cell line). Inhibition of SUV39H1 led to chromatin remodeling at the promoters of cGAS, RIG-I, and STING genes alongside with recovery of both type I and type III IFN production upon exogenous DNA stimulation. The histone deacetylase SIRT1 is known to be upregulated in hrHPV+ cells. Although we didn't find any direct link between SIRT1 and SUV39H1 in HeLa cells, SIRT1 targeting, by either gene silencing or treatment with the pharmacological inhibitor EX527, also resulted in regaining of IFN production upon poly(dA:dT) transfection that was totally RIG-I-dependent.

Gene silencing and overexpression experiments demonstrated that the E7 oncoprotein is mainly responsible for both SUV39H1 and SIRT1 up-regulation. Of note, SIRT1 inhibition restored functional p53 in HeLa cells while dramatically reducing the expression levels of both E6 and E7 oncoproteins. SIRT1-depleted cells showed increased sensitivity to doxorubicin-induced apoptosis. When these cells were co-treated with a sequence-optimized RIG-I agonist (termed M8) and doxorubicin, a significantly increased production of IFN $\beta$  was observed when compared to M8 alone. Collectively, our findings highlight the importance of the histone modifiers SUV39H1 and SIRT1 in driving immune evasion and oncogenesis in hrHPV-transformed cells.

## *ID 304 - Dengue virus exacerbates oxidative stress and inflammation by cleaving Nrf2*

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**Introduction.** Dengue virus (DENV) is a mosquito-borne virus that infects upward of 300 million people annually and has the potential to cause fatal haemorrhagic fever and shock. While the parameters contributing to dengue immunopathogenesis remain unclear, the collapse of redox homeostasis and damage induced by oxidative stress have been correlated with the development of chronic inflammation and progression towards the more severe forms of disease.

**Materials and Methods.** CD14 positive monocytes were obtained from buffy coats and differentiated into immature DCs (MDDCs) by culturing them for 5 days in the presence of GM-CSF and IL-4. We measured ROS levels in DENV-infected MDDC at different time points after infection by incubating cells with the oxidant-sensitive fluorescent detection probe CM-H2DCFDA prior the analysis by flow cytometry. Total RNA and cDNA were prepared and expression of antioxidant, inflammatory and apoptotic genes was performed by using the BioMark HD System (Fluidigm), which enabled quantitative measurement of up to 48 different mRNAs in 48 samples under identical reaction conditions. Finally, a physical interaction between Nrf2 and DENV NS3 protein was assayed by co-immunoprecipitation following western blot analysis.

**Results.** Here, we report the DENV NS3 protease strategically target the transcription factor NF-E2-related factor 2 (Nrf2), a master regulator of redox homeostasis, by inducing its degradation. Subsequently, the impaired expression of antioxidant genes, during the course of infection, progressively elevated ROS levels and exacerbated the transcription of inflammatory and apoptotic genes. Overall, our data demonstrate a mechanism by which DENV induces oxidative stress and inflammation in infected cells.

**Discussion and conclusions.** The mechanisms contributing DENV immunopathogenesis and development of severe disease, including DHF/DSS are complex and multi-factorial, and to date there is no clear delineation of these mechanisms. However, one contributing factor in DENV pathogenesis centers around the interplay between oxidative stress damage and DENV infection. Oxidative stress is a characteristic of several viral infections, including Zika and influenza virus. Several papers have often reported that ROS contribute to the development of virus-induced pathogenesis. In the Flaviviridae family, viruses such as hepatitis C, downregulate Nrf2 activation by impairing its nuclear import, leading to chronic infection, ROS production and inflammation. Thus, it is conceivable to think that a common mechanism might be shared among these viruses that relies on Nrf2 inhibition.

## *ID 173 - Influenza virus modulates G6PD enzyme to control its replication and host response*

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**Introduction:** The oxidative stress, characterized by increase of reactive oxygen species (ROS) production and decrease of intracellular glutathione (GSH) content, is hijacked by viruses to ensure their own replication and/or regulate host response to infection. Glucose-6-phosphate dehydrogenase (G6PD) activity, the first and the rate-limiting enzyme of pentose phosphate pathway, is responsible for the production of reducing equivalents of NADPH, used for regenerating the reduced form of GSH. Our preliminary data showed that in permissive cells influenza virus caused a reduction of G6PD expression and activity. The G6PD activity is regulated by the deacetylase SIRT2, that plays an important role during oxidative stress, suggesting a possible strategy used by virus for the enzyme regulation.

The aim of this study was to deep inside the mechanisms through which influenza modulates G6PD activity and to evaluate the role of G6PD on influenza virus infection in innate immune cells.

**Materials and Methods:** Human epithelial cell line (A549) and human monocytes-derived macrophages cell line (U937) were infected with influenza A PR8/H1N1 virus. At different time of infections, the expression of G6PD protein and mRNA levels were evaluated by western blot and real-time PCR. The enzymatic activity of G6PD was evaluated through a colorimetric assay kit. G6PD-silenced A549, U937 cells and normal cells were subjected to influenza virus infection and the viral titer on infected cells by TCID<sub>50</sub> assay was evaluated. GSH level was evaluated by using a colorimetric assay kit. Cytokines productions were evaluated by ELISA kit and real-time PCR.

**Results:** We found that the expression level of G6PD and activity decreased in infected cells compared to uninfected ones in both cell lines at 24hrs post infection. Epithelial cells and macrophages silenced for G6PD expression and infected with influenza virus showed an increased expression of influenza viral proteins relative to control-infected cells. Furthermore, both cells silenced for G6PD showed a modulation of cytokines production such as TNF $\alpha$  and IL-6. Finally, A549 cells infected with influenza virus showed a reduction of the deacetylase SIRT2 expression suggesting a possible mechanism by which influenza virus modulates G6PD during the infection.

**Discussion and Conclusions:** The results showed a central role of G6PD in influenza virus replication in both cell lines and the importance of this enzyme in affecting virus-induced redox imbalance. The decrease of SIRT2 expression suggests a possible mechanism by which influenza virus modulates G6PD activity. Further studies are in progress to clarify the mechanisms by which G6PD deficiency may promote the spread of viral infection and modulate the host response against virus.

## ***ID 278 - Expression levels of miRNAs in treated HIV-1 patients: relation with different levels of viral suppression***

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**Introduction:** Human cellular miRNAs can modulate HIV replication and latency, miRNA like miR-150, miR-33a-5p, miR-223, miR-382 are able to promote HIV-1 latency in resting CD4+ T cells and miR-324-5p, miR-34a and miR-132 interfere with viral replication at different stages of viral infection. To date, no data exist about the contribution of these molecules to disease control. Here we analyze the levels of different miRNAs in order to evaluate whether an association between levels of miRNA and different levels of viral suppression exists.

**Materials and Methods:** miRNAs levels (miR-33a-5p, miR-34a, miR-132, miR223, miR-150, miR-324-5p, miR382) were measured by RT-Taqman assay in PBMC from 56 antiretroviral therapy treated patients, grouped in 28 patients with a sustained undetectable viremia for at least 3 years (TND group) and 28 patients with at least 3 values of viremia between 37-200 copies /ml (LLV group)). Twenty-three samples from healthy donor (HD) were used as a control group. Kruskal Wallis test was used to evaluate differences in miRNAs levels expression between groups. A regression model for logistic ordinal dependent variables (gender, age, therapy regimen, duration of therapy, stage of disease, years of infection) was built to identify factors associated with different miRNAs expression levels calculated as fold changes and stratified according to the quartiles.

**Results:** Overall, cellular levels of miR-33a-5p, miR-34a, miR-150, and miR-324-5p were significantly higher in HIV infected patients compared to HD. When stratified according to viremia, both TND and LLV group showed significantly higher levels of miR-33a-5p, miR-34a, miR-150 than HD. In addition, a significantly higher expression of mir324-5p was found in TND group than HD. No differences of miRNAs expression between TND and LLV patients was found. A fold change analysis showed a different levels of miRNA expression levels between patients. To evaluate the influence of clinical parameters on miRNAs expression variability, a regression analysis was performed. Specifically, we found that miRNA 34a and 324-5p expression levels included in the fourth quartile of distribution (fold change range: 1.37-2.96  $p=0.027$  and 1.22-2.57  $p=0.0045$ , respectively) were significantly associated with years of HIV-1 infection.

**Conclusions:** These data suggest that expression pattern of some miRNA is altered in HIV infected population. Although, no difference of miRNA expression levels between patients with maximal viral suppression and patients with low level viremia was detected, analysis of other transcripts belonging to non-coding RNA, such as long ncRNA, siRNA and snoRNA, is needed to better understand the role of these molecules in maintaining maximal viral suppression.

## **ID 266 - Human transcriptomic response to vaccination with recombinant VSV expressing Ebola virus Glycoprotein**

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**Introduction:** rVSV-ZEBOV is a live-attenuated, recombinant vesicular stomatitis virus vaccine expressing Ebolavirus glycoprotein G and is the only Ebola vaccine with demonstrated clinical efficacy. Here we studied the blood transcriptomic response upon administration of a single dose of vaccine.

**Materials and methods:** Whole blood RNA from 64 healthy volunteers, 51 vaccinated either with  $10^7$  or  $5 \times 10^7$  PFU of rVSV-ZEBOV and 13 with placebo, collected at different time points after vaccination, was analysed by targeted transcriptome sequencing. At each time point, differentially expressed genes (DEGs) were identified with edgeR, ranked by FDR, and used to find biological signatures assessing the activation of 346 blood transcription modules.

**Results:** Between baseline and day 1 after vaccination, 5,469 DEGs were detected. This number decreased over time: at day 28 no DEGs were detected. Functional analysis identified 145 different modules affected by vaccination. Innate immunity pathways were activated from day 1 to day 14. At days 2 and 3, neutrophil modules were downregulated and complement-related modules upregulated. T-cell and cell-cycle associated modules were upregulated at days 7 and 14, while at day 28 no modules remained activated. Correlation analysis of module activation with ZEBOV glycoprotein-specific antibody titres identified seven significant directly correlated modules at day 14 after vaccination, including two related to B cell activation.

**Discussion and conclusions:** Vaccination with rVSV-ZEBOV produced a significant modulation of gene expression over time. This live viral vector induced a strong and durable modulation of genes associated with innate response, with upregulation of T cell- and cell-cycle-associated genes at days 7 and 14. The activation of seven blood transcription modules at day 14 after vaccination could be correlated with the magnitude of antibody response against Ebola glycoprotein at day 28.

## *ID 060 - A new therapeutic approach in HSV-1 infection: synergic effect of YY1 and HDACi*

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**Introduction.** Yin Yang 1 (YY1) is a multifunctional mediator of several signaling processes, such as cell growth, differentiation, tumor development and apoptosis. The name derives from its dual role: it can either activate or inhibit gene expression, depending on the cofactors that it recruits. YY1 recognizes a consensus sequence in promoter gene region characterized by at least one of the two most frequent core binding elements, that are CCAT and ACAT. Different studies showed that YY1 regulates histone acetylation, deacetylation and methylation through the interaction with p300, HDACs, Ezh2, Ezh1 and PRMT1. Herpes Simplex Virus-1 (HSV-1) is a common human pathogen, which can establish productive lytic infection in epithelial cells and latent infection in the nervous system. YY1 plays a role in HSV-1 gene regulation through the transactivation of different viral  $\beta$  and  $\gamma$  genes.

**Materials and Methods.** Vero cells (ATCC CCL-81) were pre-treated with YY1 inhibitor, called NPI-0052 (Sigma SML1916), in combination with several HDAC and Ezh2 inhibitors (valproic acid, trichostatin A, entinostat, vorinostat and GSK126). Subsequently, cell monolayer was infected with HSV-1 carrying a GFP gene fused to the HSV-1 tegument protein VP22. The infection was monitored via plaque assay and fluorescence microscopy. It was also evaluated the putative apoptosis effect through Annexin V assay. Finally, a Chromatin immunoprecipitation (ChIP) followed by a Real-Time PCR was performed using  $\beta$  and  $\gamma$  primers.

**Results.** The data indicate a synergic regulation of HSV-1 infection driven by YY1 inhibitor together with HDAC and Ezh2 inhibitors. Indeed, HSV-1 infection was significantly decreased after the treatment of NPI-0052 in combination with several epigenetic drugs. No apoptosis was revealed after the treatment, supporting that the reduction in HSV-1 infection was referred only to the antiviral activity of the different combinations. The results were supported by ChIP analysis, highlighting that NPI-0052, together with the epigenetic drugs, prevent the recognition and the modulation of HSV-1 genome.

**Discussion and Conclusions.** Acyclovir, penciclovir, and their prodrugs have been widely used during the past two decades for the treatment of herpesvirus infections. Antiviral resistance of HSV has emerged very rapidly and the research of new antiviral drugs is mandatory for the scientific community. The synergism between YY1 inhibitor NPI-0052 and several epigenetic drugs could represent a new and efficient therapeutic approach in the prevention and healing from HSV infection.

**LA RISPOSTA DELL'OSPITE ALLE INFEZIONI: AL CONFINE TRA PROTEZIONE E DANNO**

*The transcription factor NF-kB in viral infections: a double-edged sword*

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The transcription factor nuclear factor-kB (NF-kB) complex consists of five related proteins: p50, p52, p65, c-Rel, and RelB. The NF-kB dimer p50/p65 is involved in the activation of classical NF-kB signalling, while nonclassical NF-kB signalling involves p52/RelB. NF-kB plays a central role in the antiviral innate response, i.e. in the first line of defence against viral pathogens. In fact, the NF-kB signalling pathway, that includes in addition to the NF-kB complex several kinases, is positioned downstream innate response receptors and adaptor proteins, such as toll-like receptors (TLRs), retinoic acid-inducible gene I (RIG-I), tumor necrosis factor (TNF) receptor (TNFR), interleukin 1 receptor type 1 (IL-1R1), the major adaptor proteins are myeloid differentiation primary response gene 88 (MyD88), Toll/IL-1 receptor (TIR)-containing adaptor-inducing IFN (TRIF), mitochondrial antiviral signaling protein (MAVS), and upstream the induction of antiviral genes, such as interferon (IFN) and IFN-stimulated genes (ISG). Additionally, NF-kB also promotes viral gene transcription that is harmful to some viruses' latency. However, long-term coexistence of viruses with their hosts established delicate, but extremely efficient balances between the host innate response and the immune evasion strategies of the viruses. Therefore, data collected by different authors suggest a scenario in which an efficient NF-kB-dependent innate response to viruses could be necessary to limit virus replication as well as to promote it, depending on host immune state, the specific cell type that is infected, and the specific viral infectious cycle. Some representative examples, mainly obtained during personal studies, are reported.

## *Pathoadaptive response of Pseudomonas aeruginosa during chronic infection: implications for antivirulence therapies*

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*Pseudomonas aeruginosa* is an opportunistic bacterial pathogen, generally considered a highvirulent species due to the production of an array of virulence factors that are associated with the

high mortality of acute *P. aeruginosa* infection. To combat the unstoppable increase in *P. aeruginosa* antibiotic resistance, alternative anti-*P. aeruginosa* strategies have been devised, including antivirulence therapy. The underlying concept of antivirulence is to impair the production or activity of bacterial virulence factors without affecting bacterial growth. Since quorum sensing (QS) and iron (siderophore-dependent) global regulatory networks have a profound impact on the expression of *P. aeruginosa* virulence factors, we developed antivirulence strategies targeting QS and pyoverdine (PVD) siderophore signaling in *P. aeruginosa*. By means of a drug repositioning approach we identified FDA-approved compounds that reduce the expression of crucial *P. aeruginosa* virulence-associated phenotypes (release of toxic exoproducts, biofilm production etc.), and proved their efficacy in different animal models of acute infection. Evidence is however emerging that during chronic infection (e.g. in cystic fibrosis) *P. aeruginosa* evolves pathoadaptive changes which enable infection to last for long time, including loss-of-function mutations in the QS and PVD signaling global regulators. Such pathoadaptive evolution results in less virulent and more fit phenotypes that allow the bacterium to persist in the host for decades. Here, I discuss and provide examples of how these pathoadaptive traits can be pharmaceutically exploited for the development of therapeutic strategies against chronic *P. aeruginosa* infection.

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## MICROORGANISMI E MALATTIE CRONICHE RESPIRATORIE

### *Impact of infections on chronic lung diseases*

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The importance of airway colonization for regulation of local immunity is reflected in a large number of studies that have linked airway dysbiosis with infection or disease development. LRT infections in early life have been shown to predispose infants to early allergic sensitization and risk of persistent wheeze in later childhood. To be precise, the infection is not necessary for this relationship, because asymptomatic colonization of taxa such as *Streptococcus pneumoniae*, *Moraxella catarrhalis* and/or *Haemophilus influenzae* are also linked to the development of chronic wheeze. The composition of the nasopharyngeal microbiota at six weeks of age has been shown to be correlated with microbiota stability over the first two years of life, as well as with rates of respiratory infection.

In health, the lung microbiome is determined largely by the balance of immigration and elimination. The presence of lung disease alters both the population dynamics of microbial immigration and elimination and the terrain of the respiratory ecosystem and local growth conditions. Apart from variations in the proinflammatory capacity related to the bacterial composition of the human microbiome, mucosal inflammation can also be induced by viral infection, thereby facilitating adhesion and invasion by pathogenic bacterial species. The progression of disease can reflect an airway environment increasingly suitable for the growth of such pathogens, leading to expansion of their populations. Moreover, with aging, changes in the regional conditions (mucus, nutrients, pH, clearance, and immunity) can lead to an unbalance in the equilibrium between migration and elimination thus favoring regional growth in the lower airways of typical or atypical pathogens.

A correlation between causing biallelic gene mutations and long-term evolution of lung function, as a general marker of respiratory health, is demonstrated in primary ciliary dyskinesia (PCD). Moreover, a significantly greater decline in lung function was observed in patients infected with *P. aeruginosa* compared with those without infections and patients with other bacterial infections. However, because PCD children who became infected with *P. aeruginosa* had significantly worse spirometry at enrolment, *P. aeruginosa* may be a marker for a patient already doing badly, rather than itself increasing disease severity. Finally, it is possible that cilia which are completely immotile favours the development of biofilms thus starting a vicious circle of chronic infection and continuous worsening of spirometry parameters.

## *Le micobatteriosi atipiche*

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Atypical mycobacterial infection has been described in the medical literature since the mid 1950s. Nontuberculous mycobacteria (NTM) are classified based on their growth rates: rapid and slow growing NTM. Atypical mycobacteria are obligate aerobes that can be found in the environment in soil, water, vegetables, and even in domestic animals and dairy products.

Because infections by NTM were not reportable in the past, few systematically collected data about their frequency and distribution are available. NTM infections began to be reported more frequently after the incidence of tuberculosis declined in the 1950s.

Distribution of atypical mycobacterial infection is worldwide.

NTM are transmitted via inhalation into the respiratory tract and ingestion into the GI tract. Disseminated NTM infection usually develops in patients with AIDS and/or lymphomas. The most important risk factor for NTM disease in patients without HIV infection is underlying lung disease, pulmonary infection is the most common manifestation. NTM infection is associated with pulmonary infection and bronchiectasis in elderly women without pre-existing lung disease. Deficiency of IFN-gamma and TNF-alpha production and absence or defects of IFN-gamma receptors are associated with infections with NTM. Other possible risk factors for NTM infections include gastroesophageal reflux disease (GERD), peptic acid suppression, and aspiration or microaspiration.

Untreated patients with significant lung disease may develop respiratory insufficiency or weight loss, severe disability or death may result from respiratory failure.

The clinical course of pulmonary MAC (Mycobacterium Avium Complex) infection is usually indolent. Treatment success rate in patients without HIV infection have ranged from 20-90% in various studies, with an average of 50-60% clinical success and 60-75% of sputum conversion rates. Fibrocavitary pulmonary disease, BMI less than 18.5 kg/m<sup>2</sup>, and anemia are negative prognostic factors for both all-cause and MAC-specific mortality in HIV-negative patients. Therefore treatment should not be delayed in these patients with positive MAC cultures.

Pulmonary NTM infection in immunocompetent hosts generally manifests as cough, sputum production, weight loss, fever, lethargy, and night sweats.

Diagnostic testing includes chest X ray and/or TC scan, acid-fast bacillus (AFB) staining and culture of sputum specimens.

If patient is unable to produce sputum, sputum induction may be helpful in obtaining respiratory tract sample. Procedures such as bronchoscopy with bronchoalveolar lavage (BAL) with or without biopsy may be necessary to obtain appropriate respiratory specimen.

## *Interferoni e malattie respiratorie*

Carolina Scagnolari

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Respiratory viral infections are a leading cause of disease and mortality. The severity of these illnesses can vary markedly from mild or asymptomatic upper airway infections to severe wheezing, bronchiolitis or pneumonia. Common respiratory viral pathogens include adenovirus, enterovirus, human coronavirus, human metapneumovirus, rhinovirus (RV), influenza, parainfluenza and respiratory syncytial virus (RSV). Moreover new genotypes/strains of respiratory viruses (e.g. RSV-ON1, enterovirus D68, adenovirus 14p1, RV-C) are emerging as important cause of hospitalization in the last decades. The mechanisms that determine why some individuals suffer from severe respiratory illness whilst others do not are not well understood. In this regard, it is known that lung airway epithelial cells and mucosal immune cells are the primary cells for respiratory viruses. Following viral infection, these cells generate a range of mediators, including type I (IFN- $\alpha$ , IFN- $\beta$ ) and III interferon (IFN- $\lambda$ 1, IFN- $\lambda$ 2, IFN- $\lambda$ 3), proinflammatory cytokines, and chemokines, which not only have pivotal roles in virus control but also determine the development of inflammation and disease. However, the molecular logic underlying innate immune responses to environmental cues is governed by a bow-tie architecture. In particular the bow-tie is an ordered control system, comprised of three crucial elements: (i) input signals (pattern recognition receptors); (ii) the core (adaptors proteins and transcriptional factors, IRFs and NF $\kappa$ B); and (iii) output signals (IFN, cytokines, chemokines, ROS, etc). Both input and output signals are highly variable, flexible, robust and diverse; by contrast, the core is specialized, efficient and rigid. This principle has been exploited by several respiratory viruses, which target core molecules to evade the antiviral innate immune response. However, it has been reported that severe respiratory viral infections caused by highly pathogenic strains of influenza virus or coronavirus are associated with a heightened innate immune response and an excessive production of inflammation mediators including cytokines and IFN (“cytokine storm”). One interpretation of these data is that although a robust innate immune response becomes activated, it is not able to control the virus, resulting in death due to direct viral damage to the airways. Directly related to this it has been shown using a SARS-coronavirus animal model that a rapid and robust virus replication associated with delayed IFN-I can lead to lung immunopathology, with fatal outcomes. In agreement it has been also reported that the relative timing of the IFN-I response and maximal virus replication is key in determining outcomes of MERS-COV infection. However, it must be considered that since their inception, IFNs have displayed ever increasing diversity and demonstrated activity far more refined than the original observation of the ability to ‘interfere’ with viral replication. There are several subtypes of IFN, such as IFN- $\alpha$ , IFN- $\beta$  and IFN- $\lambda$ , but it was not known how each subtype helps to combat respiratory viruses. Type I IFNs and type III IFNs share many properties, including induction by viral infection, activation of shared signaling pathways, and transcriptional programs. By contrast, type III IFNs have been shown to serve as a front-line defense that controls infection at epithelial barriers while minimizing damaging inflammatory responses, reserving the more potent type I IFN response for when local responses are insufficient. Thus far, IFN- $\lambda$  has been considered mainly as an epithelial cytokine, which restricts viral replication in epithelial cells and constitutes an added layer of protection at mucosal sites. Moreover, it is now increasingly recognized that IFNLR1 is expressed broadly, and that immune cells such as neutrophils and dendritic cells also respond to IFN- $\lambda$ . However, recent studies pointed out that a robust type III IFN activation can be detrimental for the host during severe RSV infection and HRV-wheezing episodes. In addition IFN- $\lambda$  induced by influenza virus can contribute, as previously shown for type I IFN, to nasal colonization and pneumonia due to *S. aureus* superinfection. Thus, additional studies are urgent required to understand the complexity of IFN system and caution should be exercised in the classification of IFN on the basis of their detrimental action during respiratory viral infections.

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## IL NUOVO CHE AVANZA IN MICOLOGIA

*Nuove prospettive nella diagnostica micologica: dalla ricerca alla real life*

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Invasive fungal infections (IFIs) represent a public health burden worldwide, especially in immunocompromised patients and their incidence has risen over the last decades, due to the increased use of immunosuppressive and cytotoxic therapies, new finer diagnostic techniques, greater awareness and clinical suspicion. A rapid and accurate diagnosis has been shown to reduce mortality and morbidity in these patients, but diagnosis is even now a challenge. Several biomarkers have been studied and international guidelines recommend the use of (1-3) - $\beta$ -D-Glucan (BG) in the diagnosis of IC or Galactomannan if IA was suspected. An overview on the latest available test and on more recent molecular test useful for the clinical laboratory and biomarkers will be presented. It will be introduced the use in real life of these test and its utility in the antimicrobial stewardship program activated in the university hospital of Verona.

## *Importance of biomarkers in medical mycology (Importanza dei biomarcatori in micologia medica)*

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Non-culture-based diagnostics are useful to help establish an early diagnosis of invasive fungal infections (IFIs), including aspergillosis, candidiasis, and *Pneumocystis jirovecii* pneumonia (PJP). Recent studies show that antigen-based tests can significantly affect the diagnosis of IFI in high-risk patients. *Aspergillus galactomannan* (GM) testing is an important adjunct to the diagnosis of invasive aspergillosis, particularly when used along with PCR testing, and a surrogate marker for outcome when used in serial testing. Beta-D-glucan (BDG) testing is non-specific for several fungal genera, including *Aspergillus* and *Candida*, and in high-risk patients may enhance the diagnosis of IFI. However, the low sensitivity and specificity in some clinical settings limit the usefulness of the BDG, which is not a commonly adopted practice by European microbiology laboratories. BDG can be detected in the serum of patients with PJP, and several studies have reported a good diagnostic performance in HIV-infected PJP patients. Overall, the BDG assay has good accuracy for distinguishing patients with proven or probable IFIs from patients without IFI, and in some cases is helpful to monitor the response to antifungal treatment. As intended as point-of-care testing (POCT) methods, lateral-flow immunoassays allow rapid testing of clinical samples for the detection of an extracellular glycoprotein (mannoprotein) antigen secreted by actively growing *Aspergillus* species. A similar method developed to detect cryptococcal antigen has demonstrated a performance comparable to those of ELISA and latex agglutination on both cerebrospinal fluid and serum. Its low cost, ease of use, high accuracy make the lateral-flow immunoassay well suited as a POCT diagnostic method for the screening of large numbers of patients in resource-limited settings.

## 20/09/2019 – LA DIAGNOSI RAPIDA DELLE INFEZIONI DA VIRUS INFLUENZALI

### *Influenza virus infection: state of the art*

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One hundred years have passed since the largest influenza virus (IV) pandemic on record, spread around the world in three consecutive waves (spring-autumn 1918 and winter 1918-19), infecting over one third of the world's population and killing an estimated 50 million people with unusually severe clinical manifestations in previously healthy young adults. IVs are segmented negative-strand RNA viruses belonging to the *Orthomyxoviridae* family. The high mutation rate of their genome makes these viruses incredibly successful, particularly antigenic drift allows for annual epidemics resulting in thousands of deaths and millions of hospitalizations; while the emergence of new strains through antigenic shift (e.g. swine-origin influenza A) can cause devastating global outbreaks of infection. In addition to virus genetic mutations, multiple host factors like age, sex, general health, metabolic and redox conditions, having important repercussions on different steps of the virus life-cycle as well as on the host response, may dictate the outcome and severity of infection. The aging process, for example, is accompanied by complex age-related changes affecting the cell redox homeostasis, the immunity and the host defenses, thus increasing the susceptibility of old individuals to infections and predisposing them to a reduced response to vaccination. Furthermore, sex disparities are reported in response to IV infection. Females mount a higher immune response than males and possess intrinsically higher antioxidant capacity that persists during infection and contributes in part to female resistance to IV infection. Finally, enzymes regulating glycolytic pathway, like glucose-6-phosphate dehydrogenase (G6PD), are down-modulated during IV infection. G6PD is also involved in the re-establishment of redox homeostasis, therefore its reduction during infection probably contributes to oxidative stress, a condition needed for the activation of redox-regulated pathways useful for viral replication.

Three families of inhibitors, acting directly on specific viral proteins, are currently available for fighting IV infection and halting its spread. However, their efficacy is limited due to toxicity, short therapeutic window of action and the emergence of drug resistance. Vaccination is a key component of defense strategies against influenza, although effective vaccines cannot be produced quickly enough to deal with emerging threats. Therefore, the developing of rapid diagnostic assay and the search of new therapeutic strategies, ideally *ad personam*, especially for people at high risk of infection including elderly, males, asthmatics or people with metabolic syndromes, like obesity and diabetes, are the future goals to control influenza epidemics and eventual pandemics.

## *Molecular diagnosis of Influenza virus infection: an update*

Alessandra Pierangeli

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Influenza virus infection is a major cause of morbidity and mortality among children and adults, especially the elderly. Vaccination is the best method for influenza control in order to either prevent the infection or reduce illness severity. However, several host factors may alter vaccine efficacy especially in aging population with more chronic conditions. Influenza is often misdiagnosed because of the low sensitivity of clinical diagnosis that is based on nonspecific signs and symptoms. In surveillance studies of respiratory infections in children and adults attending emergency departments in Rome during the first winter season of pandemic Influenza A H1N1 2009, we showed that clinical and epidemiological criteria defined as Influenza-like illness (ILI), cases that were attributed to rhinoviruses and respiratory syncytial virus infections using molecular diagnosis. On the other hand, patients not presenting a classical ILI, may attend hospital with pulmonary complications of influenza with or without bacterial co-infections.

Recent advances in molecular diagnostic techniques enable to detect multiple respiratory viruses and bacteria simultaneously but their clinical utility is still debated. Several studies have shown that respiratory pathogen testing offers a potential way forward combating antibiotic overuse, but others did not find any relevant impact on medical management. Moreover, the ever-increasing number of multiplex molecular assays can be disorienting and not of straightforward interpretation for clinical use. This could be overcome by using rapid molecular diagnostic tests thus providing a more appropriate patient management and therapy, if they prove to have adequate performance. Studies will be presented on molecular testing for Influenza virus during its epidemic season, comparing different approaches.

## 20/09/2019 – COMUNICAZIONI ORALI SUL TEMA MICROBIOMA INTESTINALE E NON SOLO

### *Gut Microbiota Genetic and Functional Markers for Non-Communicable Gastro-Intestinal Diseases*

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Non-Communicable Diseases (NCDs) represent major causes of morbidity, disability, health-related retirement, and premature death in the EU, accounting for most healthcare expenses. A number of NCDs, such as gastric cancer, colorectal cancer (CRC), diabetes (T1D and T2D), celiac disease (CD), liver diseases (NAFLD-Cirrhosis-Cancer), Pseudomembranous Colitis, Inflammatory Bowel Disease (IBD), and others, feature aberrations in the intestinal homeostasis and gut microbiota. Such changes are expected, in turn, to play major a role in the NCD etiopathogenetic process.

Hence, the expertise of medical microbiologists is now urged to contribute to the clinical profiling of such NCDs patients, at time of diagnosis, follow up and prognosis.

However, while gut microbiota markers are expected to serve as indexes of the host intestinal homeostasis, the dynamics of gut microbial ecological systems pose significant hurdles on the road toward their precise definition.

In this talk, the relationships between gut microbiota and few examples of NCDs will be discussed, with a focus on the current attempts to provide diagnostic/prognostic values to specific microbiota markers and eubiosis/dysbiosis indexes. The significance of genetic and functional bacterial features will be highlighted with respect to specific experimental designs aimed at identifying microbial markers of clinical value for CRC and CD.

## ***ID 183 - Fecal Microbiota Transplantation: Microbiologist opinion***

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### **Introduction**

Fecal Microbiota Transplantation (FMT) consists of a fecal solution prepared in laboratory to be infused by colonoscopy, nasal-duodenal tube or administered orally by encapsulation. To date, FMT is primarily applied in the treatment of recurrent *Clostridium difficile* infections (rCDI). However, being gut microbiota involved in systemic diseases such as metabolic or neurological syndromes, FMT is proposed to be used in these clinical conditions as well.

### **Materials and Methods**

Since 2013, the laboratory of Microbiology in the teaching hospital Fondazione Polclinico-“A. Gemelli” – IRCCS – in Rome prepared and infused 378 fecal solutions in patients affected by rCDI and Intestinal Bowel Disease (IBD). Currently clinical trials with this infusion therapy are conducted on Amyotrophic Lateral Sclerosis (ALS) patients.

### **Results**

Since 2013 we have been developing laboratory procedures and optimized workflows.

### **Discussion and conclusions**

We report our experience.

## **ID 133 - Changes of microbiome structure and circulating miRNAs in saliva of autistic children and their correlation with cognitive impairment**

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### **Introduction**

It is well known that several pathological conditions, including neuro-psychiatric diseases, may modify salivary molecules such as metabolites, proteins, RNAs and bacterial populations. Autism Spectrum Disorder (ASD), a complex neurodevelopmental disorders whose etiopathogenesis is still unclear, is believed to be the complex result of a combination of genetic, epigenetic and environmental factors. Immune dysregulation and gastrointestinal abnormalities are of particular interest in the light of several papers reporting ASD-associated disturbances. In fact, a gut microbiota dysbiosis in ASD patients could play a key role in the alterations of brain structure and function development because of interactions between the Central Nervous System (CNS) and the gut microbiota, the so-called gut-brain axis. The aim of this study was to combine the alterations of the salivary microbiome and miRNA expression profiles in ASD and healthy (NC) subjects and investigate their association with neuropsychological parameters to discover new biomarkers of ASD.

### **Material and methods**

In this experimental plan, were evaluated changes in the microbial composition of the salivary microbiome in 53 ASD and 27 healthy samples, which were sequenced using the Illumina MiSeq platform. To profile the circulating miRNA expression from saliva, NanoString Counter system assays were performed using the NanoString platform.

### **Results**

The microbial profile, obtained by 16S rRNA sequencing analysis of ASD patients and NC subjects, revealed statistically significant differences of abundance at the genus and species levels. In particular, *Rothia*, *Filifactor*, *Actinobacillus*, *Weeksellaceae*, *Ralstonia*, *Pasteurellaceae* and *Aggregatibacter* increased their abundance rates in the saliva of ASD patients, while *Tannerella*, *Moryella* and TM7-3 decreased. In addition, 5 salivary miRNAs were statistically altered in ASD patients compared to NCs. Variations of both miRNAs and microbes were statistically correlated to different neuropsychological scores related to anomalies in social interaction and communication. We also found a negative correlation between salivary miR-141-3p expression and *Tannerella* abundance.

### **Discussion and Conclusions**

Our results demonstrated that miRNA and microbiome dysregulations found in the saliva of ASD children are associated with cognitive impairment of the subjects and a potential cross-talking between circulating miRNAs and resident bacteria alterations could exist. Moreover, these findings could pave the way to new potential tools for molecular diagnosis of ASD.

## **ID 310 - Characterization of nasal microbiota in pediatric patients with adenoid hypertrophy and allergic rhinitis.**

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The human nasal cavity hosts a complex bacterial community that is mainly stable at the genus level but can vary between individuals and with the season. Several researchers have shown evidence for dysbiosis of the nasal microbiome in the context of allergic and inflammatory diseases of the airways. However, there are no studies regarding allergic rhinitis (AR) and adenoid hypertrophy (AH), that are common in children and are often associated with each other. AR is a nasal allergic disease characterized by nasal hyper responsiveness, immunoglobulin E (IgE) production, skewing of mucosal immune homeostasis toward a TH2-type response, and accumulation of eosinophils and mast cells. AH is the unusual enlargement of the adenoid tonsil, as a consequence of antigenic stimulation associated with chronic inflammation. Both of them may exist simultaneously and may cause similar clinical symptoms, mainly as nasal obstruction and snoring. This study was aimed to identify the community structure, composition, and functional potential of the commensal microbiota in the anterior nares of 75 pediatric subjects suffering of AR, AH or both, by 16s rDNA gene sequencing. Overall, our results highlight the dysbiosis as a characteristic condition of the nares ecosystem in pediatric patients suffering from allergic diseases. Although we did not profile viral or fungal components of nasal microbiota, we found significant differences at different taxonomic levels among diseased groups and controls. A total of 12 different phyla, 118 genera, and 269 bacterial species were found across all samples examined. The analysis of the relative abundance among the different groups revealed *Proteobacteria* as the most abundant phylum in all groups followed by *Actinobacteria* and *Firmicutes*. Regarding  $\beta$ -diversity, statistically, significant separations were determined across all different phenotypes. At the species level, *Moraxella nonliquefaciens*, *Corynebacterium pseudodiphthericum* and *Dolosigranulum pigrum* were significantly more abundant in the control group while *Acinetobacter guillouiae* had the highest mean relative abundance in all diseased groups followed by *Pseudomonas brenneri* and *P. fluorescens*. A highly conserved component of microbiota, as well as keystone taxa with promising diagnostic potential, have been identified. Furthermore, the analysis of correlations among taxa in health and diseases evidenced an inflammation driven loss of ecological interactions. Although our results contribute to a more comprehensive knowledge on nasal microbiota and allergic diseases, further studies are necessary to clarify if microbiota modification is the cause of the diseases or it is a consequence of the diseases themselves.

## *ID 141 - Mapping CSF virome in patients with suspect neuroinvasive infection.*

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**Introduction** The aetiology of most neuroinvasive infections remains undetermined. Next Generation Sequencing Metagenomics (mNGS) represents a valuable approach to characterise the microbial population, to estimate the viral diversity (virome), and to complement classical diagnostics. In this study, we analyzed the viruses present in cerebrospinal fluid (CSF) from patients with clinical suspect of infectious meningo-encephalitis, using mNGS. Sixty CSF samples from 58 patients that resulted negative to routine diagnostic procedures were analyzed.

**Material and Methods** After RNA extraction, cDNA was prepared by Sequence Independent Single Primer Amplification technique. We prepared NGS libraries by fragmentation, adapter ligation, and amplification. Sequencing was performed using Ion-Torrent S5 instrument. A median of 40.8 (range 23.0-53.9) million reads, 179 nt length (range 30-300 nt) per sample were obtained. Illumina platform was also used in a fraction of samples.

High-quality reads were filtered using Bowtie2 with high stringency parameters (90% of homology over 80% of read sequence), to subtract human-derived reads (99% of reads). Filtered reads were mapped to NCBI Nucleotide Database with an E-value cut-off of  $10^{-5}$ . All results were then merged and grouped following NCBI Taxonomy database classification with an home-made python program, collecting the overall reads mapped to every taxa. Finally, the presence of the identified viruses was confirmed by targeted PCR. Experimental protocol and pipeline were optimized using either samples spiked with known RNA and DNA viruses or clinical specimens with known viral content.

**Results** In the majority of samples, sequences from human-endogenous-retroviruses (n=32) and phages were detected (n=51). Environmental viral reads were retrieved in 26 samples. In addition, sequences of other human viruses were detected in 12 samples, including: HIV (3), Epstein Barr virus (1), Cytomegalovirus (1), Torque Teno virus (2), Circoviruses (3), HPV (2), Merkel cell polyomavirus (1), Human Parechovirus (1), and Rinovirus (1). Among these, the virus was represented by one single read in 4 cases (2 HIV, 1 EV, 1 HPV), whose relevance has to be established. In some samples, the presence of specific viral sequences was confirmed by targeted PCR.

**Discussion and Conclusion** mNGS represents a promising opportunity to investigate the potential role of viruses in the pathogenesis of nervous system disorders through an unbiased method able to identify known and novel pathogens. mNGS performed in combination with conventional diagnostic test may potentially improve diagnosis of neurological syndrome. However, extensive efforts are needed for discriminating between pathogenic organisms or bystanders (non-pathogenic) and contaminants.

## **ID 176 - Dietary treatment promotes gut microbial community changes in subjects affected by glycogenosis type 1**

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**Introduction-** The glycogen-storage diseases (GSDs) are caused by enzyme defects of glycogen degradation. These enzymes are mainly localized in the liver. Hepatomegaly and hypoglycemia are the principal abnormalities. The glycogen storage disease Ia and Ib are due to the defects of the glucose-6-phosphatase and glucose-6-phosphate translocase, respectively. The aim of dietary treatment is to prevent hypoglycemia and suppress secondary metabolic complications, by the intake of slow-release carbohydrates. Such a dietary intervention could affect the availability of substrates for microbial fermentation. In this study, we compared the gut microbiota composition and microbial metabolite production (i.e. short chain fatty acids -SCFAs-) of subjects with glycogenosis type 1 and healthy subjects, sex- and age-matched.

**Materials and Methods-** Nine GSD1 subjects (6 males, age range 4-38 years old) and 12 healthy controls (CTR) were enrolled. We assessed dietary intake and performed gut microbiota analysis by next-generation sequencing using V3–V4 hypervariable 16S rRNA genomic region. Fecal SCFAs were quantified by gas chromatography.

**Results-** Alpha-diversity analysis revealed a significant reduction in microbial richness and evenness in the GSD group compared with the CTR group (PD whole tree,  $p=0.03$ ; observed species,  $p=0.02$ ; Shannon,  $p=0.002$ ). Phylogenetic analysis highlighted a significant separation of gut microbiota according to both unweighted ( $p=0.004$ ) and weighted Unifrac distances ( $p=0.01$ ). In particular, GSD subjects were characterized by an increase in the relative abundance of *Enterobacteriaceae* ( $p=0.006$ ) and *Veillonellaceae* ( $p=0.01$ ), whereas the CTR group was enriched in *Ruminococcaceae* ( $p=0.001$ ). SCFAs quantification revealed an increase of fecal acetate and propionate in GSD subjects ( $p=0.03$  and  $p=0.04$ , respectively).

**Discussion-** Despite GSD diet is enriched in resistant starch, usually considered a good substrate for beneficial microbes, we found a dramatic increase in Proteobacteria. This phylum, and in particular *Enterobacteriaceae*, had been suggested to exert pro-inflammatory activity both locally, at the gastrointestinal mucosa, and systemically. Indeed, GSD subjects are at higher risk to develop chronic inflammatory bowel diseases.

Whether our findings represent an effect of the disease itself, or a consequence of the diet is still unclear.

## 20/09/2019 – COMUNICAZIONI ORALI SUL TEMA INFEZIONI VIRALI PERSISTENTI

### *Hepatitis B virus as a model of persistent infection induced by a DNA virus*

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After the entry into the nucleus of infected hepatocytes, HBV genome is converted into a highly stable DNA molecule, the so-called circular covalently closed DNA (cccDNA). cccDNA acts as a minichromosome, allowing HBV to establish persistent infections in form of chronic and also occult infection. In the setting of occult infection, our immune responses suppress the transcriptional activity of cccDNA thus favoring the entry into a latent or minimally-replicating status. Occult HBV infection is associated with a large variety of serological profiles including the so-called sero-negative occult HBV infection characterized by the negativity to all diagnostic markers of HBV infection. Occult HBV infection has been intensively investigated since it can give origin to HBV reactivation under conditions of immunosuppression. The risk of HBV reactivation is increasing due to the growing use of immune-suppressive therapies in several medical fields. Furthermore, if not properly prevented by an adequate antiviral prophylaxis, immune-suppression driven HBV reactivation can induce severe forms of hepatitis that can lead to patients' death. Interestingly, viral strains, circulating in patients with HBV reactivation, are peculiar since they are characterized by an enrichment of immune-escape mutations capable to hamper HBV recognition by endogenous antibodies and also by antibodies used by diagnostic assays. This can lead to HBsAg-negative results despite active viral replication, thus posing issues for the diagnosis of HBV reactivation if only HBsAg is used as marker of HBV replication.

Beyond occult HBV infection, HBV can establish chronic infection that in the long run can promote the onset of hepatocellular carcinoma. Unlike other aetiologies, HBV-induced hepatocellular carcinoma may also occur in the absence of cirrhosis in young adults, highlighting the existence of direct mechanisms that HBV can implement to promote hepatocyte transformation. In particular, HBV can integrate portions of its genome into the cellular genome, an event that can lead to genomic instability, loss of onco-suppressor genes, up-regulation of oncogenes and (as evidenced by recent studies) to the formation of chimeric transcripts (viral- human) able to activate intracellular pathways associated with increased cell proliferation. The risk of HBV integration increases by enhancing cccDNA transcriptional activity. HBV integration can occur since the early phases of chronic HBV infection supporting the importance to set up an early treatment in order to prevent the integration of HBV-DNA into hepatocytes' genome and in turn the risk of liver cancer. Overall findings highlight the role of HBV cccDNA in the pathogenesis of HBV infection and the need to develop innovative anti-HBV drugs aimed at achieving HBV cure.

*ID 299 - Preclinical studies on the role of endogenous human retroviruses as potential markers of disease and prognosis of chronic lymphocytic leukemia*

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**Introduction:** the transcriptional activity, protein and viral particle production of human endogenous retrovirus K (HERV-K) have been demonstrated in tissue, patient serum and cell lines isolated from different types of tumors, such as ovarian, breast, prostate, teratocarcinoma, lymphomas, leukemias, sarcomas and melanoma. The mechanisms underlying HERV-K oncogenic activity could depend on the expression of oncogenic viral proteins, on the induced immune escape mechanisms, on the regulation of gene expression mediated by the long terminal repeat sequences or by the ability of retro-transposition determining genomic instability and alteration of the expression of neighbouring genes. In the field of oncohematology some studies have identified alterations of HERVs messengers and proteins expression in human lymphoid leukemic cells and the presence of circulating antibodies to HERV-K. On these bases, the objective of the study was to evaluate the potential use of distinct HERVs families as biomarkers of disease and prognosis of chronic lymphocytic leukemia (CLL).

**Materials and methods:** 27 patients with CLL diagnosis and 30 healthy donors were recruited. Data on diagnosis, therapy, outcome, biochemical parameters and mutational status were collected. A sample of peripheral venous blood was collected in order to separate peripheral blood mononuclear cells (PBMCs) by density gradient centrifugation. Afterwards, the cells were cultured for 72 hours in RPMI with 10% FBS, in absence or presence of IL-2 and in a serum free stem cell medium. The transcriptional activity of HERV-K (HML-2) and HERV-H was analysed by RT-Real Time PCR. The non-parametric Mann-Whitney test and the calculation the Rho coefficient of Spearman were used for statistical analysis.

**Results:** the molecular analysis showed a significant high expression of HERV-K and HERV-H in the patients compared to healthy donors at the baseline level. Besides, PBMCs cultured in different conditions exhibited high transcriptional activity of HERVs. Moreover, a positive correlation among HERV-K and HERV-H expression and between HERV-K and the biochemical parameter beta-2-microglobulin in patients at baseline levels was demonstrated. Finally, based on the HERVs expression, we are able to discriminate the two distinct populations of healthy subject and patients.

**Discussion and conclusion:** the results suggest HERVs expression as distinctive marker of CLL and their involvement in etiopathogenesis of the disease. The ongoing study could provide an indication of the role of HERVs as markers associated with genetic instability and as prognostic factors, in order to identify subgroups of CLL patients who could benefit from targeted therapeutic approaches.

## *ID 027 - Longitudinal study on the origin and pattern of Human Polyomaviruses replication after kidney transplantation*

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**Introduction:** Human Polyomaviruses (HPyVs) infections are common, ranging from 60% to 100% in the general population, depending on the virus. After primary infection, which occurs asymptotically during childhood, HPyVs can establish a life-long latency in tubular kidney epithelial cells. Immunosuppression, typical of transplant recipients, is a risk factor for their reactivation and may lead to clinically HPyVs-induced diseases. HPyVs may be latent in the transplanted host and reactivate in case of decrease of the immune function or may be transmitted by the donor to the recipient through the graft. Origin, replication pattern and clinical significance of HPyVs infection in kidney transplantation (KTx) remain unclear.

**Material and Methods:** A total of 630 urine or kidney biopsies were collected from 58 KTx donor/recipient pairs, before KTx and, periodically, from 1 up to 900 days post KTx. Samples were tested for BK Polyomavirus (BKPyV), JC Polyomavirus (JCPyV), Merkel Cell Polyomavirus (MCPyV), Human Polyomavirus 7 and 9 (HPyV7, HPyV9) genome by virus-specific duplex TaqMan Real Time PCR. Viral strains were molecularly characterized by automatic sequencing. T-test, fisher's exact test and Kaplan-Meier curve analysis were used for analyzing the data.

**Results:** Patients' follow up was conducted for a mean time of 486 days (range: 119-900 days) post KTx. HPyVs viruria was present in 29/58 (50.0%) donors and 43/58 (74.1%) recipients. JCPyV DNA was detected in 26/58 (44.8%) donors, and in 23/58 (39.6%) recipients; 18/58 (31.0%) donor/recipient pairs showed identical JCPyV strains. BKPyV DNA was detected in 3/58 (5.17%) donors and 18/58 recipients (31.0%), whereas MCPyV genome was detected in 2/58 (3.5%) donors and 15/58 (25.9%) recipients. The median time of JCPyV, BKPyV and MCPyV reactivation was 1 (range 0-398), 81 (range 0-375) e 98 (range 0-544) days post KTx, respectively. HPyV replication in kidney biopsies confirmed viruria data. Based on the association results, JCPyV infection was protective against BKPyV, but not MCPyV infection ( $p < 0.05$ ). One patient developed nephropathy associated to BKPyV/JCPyV coinfection.

**Conclusions:** JCPyV reactivation occurred in the early KTx phase and was probably due to the strain transmitted from the donor. MCPyV and BKPyV replications occurred later post KTx and was likely due to reactivation of recipient strains or primary infection. JCPyV replication occurring during the first weeks post KTx might prevent BKPyV reactivation/infection. These data, taken together, are significant to define the implication of HPyVs in kidney transplantation. Screening for viral replication could be the most useful tool for the identification of patients at risk of post-transplant complications.

**ID 225 - RT-QuIC assay for the *in vivo* detection of PrP<sup>s</sup>CJD from the cerebrospinal fluid of CJD-suspected cases at early clinical stage of disease**

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**Introduction:** Detection and measurement of prions associated with various transmissible spongiform encephalopathies (TSE) is a key challenge for the management of these diseases. In the last years several methods have been developed to increase the sensitivity of prion detection with the aim of finding a reliable assay that could allow early diagnosis of prion diseases using a less invasive sampling.

The Real Time Quaking Induced Conversion (RT-QuIC) assay is a rapid and highly sensitive assay for prion seeding activity that has been successfully applied to detect scrapie isoform of the prion protein (PrP<sup>Sc</sup>) in several different types of TSEs including sporadic Creutzfeldt-Jakob Disease (sCJD). Recently, the European Center for Disease Prevention and Control (ECDC) has proposed that RT-QuIC analysis of cerebrospinal fluid (CSF) or other tissues could be used for early diagnosis of sCJD. In the present study we focused on the *in vivo* detection of CSF-derived PrP<sup>s</sup>CJD in a series of Sardinian patients with an early clinical stage of sCJD.

**Materials And Methods:** CSF samples from 30 suspected subjects of sCJD recruited by Neurological Units from Cagliari and other Sardinian cities were collected and stored to -80 °C before analysis for the detection of prion by RT-QuIC assay. In the reactions, PrP<sup>s</sup>CJD propagates *in vitro* by converting freshly added normal recombinant-PrP into the misfolded forms. Western blotting and ELISA test were used for the measurement of 14-3-3 and tau marker proteins.

**Results:** RT-QuIC analysis showed positive reactions in 15/15 patients with probable or definite sCJD and negative reactions in all other subjects without sCJD, in according with ECDC diagnostic criteria.

RT-QuIC assay achieved a sensitivity and specificity of 100%, compared with other tests performed on the cerebrospinal fluid (53% and 70% for 14-3-3; 86% and 82% for tau protein, respectively).

**Discussion And Conclusions:** Although the number of clinical samples analyzed in this study is small due to the low incidence of the disease, the results are in line with previous studies and demonstrate that RT-QuIC is a robust, specific and reliable assay for an early and *ante-mortem* diagnosis of sCJD and has the potential to be used routinely in authorized clinical laboratories.

***ID 275 - Gene Editing Efficiently Cuts Out The Integrated Human Immunodeficiency Virus Genome From The Host Cell Genome, But The Virus Has A Chance To Start Over***

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**Abstract**

Gene editing created great expectancies of eliminating human immunodeficiency virus type-1 (HIV-1) from host cells and eradicating the infection. Little is known however about the fate of excised proviruses. In this study, we treated cells bearing integrated HIV genome (provirus) with clustered regularly interspaced short palindromic repeats/Cas9 (CRISPR/Cas9) and examined what happened to the provirus once excised from the host cell genome.

To this aim, cells stably transduced with green fluorescent protein- or luciferase-labeled HIV molecular clones were treated with CRISPR/Cas9 targeting the HIV long terminal repeats (LTR). The excised provirus was monitored for persistence, rearrangements, and transcriptional activity.

The results showed that the HIV provirus is efficiently cut out from the host cell genome but also demonstrated that the provirus persists for several weeks and rearranges in inter-molecular concatemers with the aid of HIV Integrase. Furthermore, circularization restores functional LTR exhibiting modest transcriptional activity *per se* that is strongly enhanced in the presence of exogenous Tat and Rev as it may occur in a superinfection.

In conclusion, gene editing is indeed a promising tool to eradicate HIV infection, but the virus may still be lurking and seeking for a second chance. This work highlights therefore a potential issue that warrants further investigations before deploying CRISPR/Cas9 editing on a routine clinical basis.

## ***ID 284 - Analytical treatment interruption and rearrangement of HIV-1 drug resistance mutations in peripheral reservoir***

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**Introduction:** Achieving antiretroviral therapy-free HIV remission in infected individuals are under active investigation in HIV cure strategies. Little is known about the impact of short analytical treatment interruption (ATI) on archived major resistance mutations (MRMs).

**Materials and Methods:** 7 chronically HIV-1 infected patients (pts) with HIV-RNA <50cps/ml for  $\geq 10$  years, undergoing towards ATI (APACHE) were analysed for total HIV-DNA (cps/ $10^6$ CD4<sup>+</sup>T, by ddPCR) and pol sequences (HXB2 pol nt:170-1415, by Illumina MiSeq) before ATI (T1), at viral rebound (VR) during ATI (T2) and at achievement of undetectable HIV-RNA after ART resumption (T3). These data are also obtained at 3 similar time-points in 7 ART-treated pts with HIV-RNA <50cps/ml for  $\geq 1$  year enrolled in the MODAT study (control-group, all in triple-therapy). Intra-patient prevalence of MRMs (Stanford 2018) and of APOBEC3G-related mutations was assessed at each time point. Wilcoxon signed-rank and Mann-Whitney tests are used to test changes in MRM prevalence within and between APACHE and MODAT pts, respectively.

**Results:** APACHE pts experienced VR after ATI at a median (IQR) time of 4 (3-7) weeks and, after ART resumption, achieved HIV-RNA <50cps/ml in 24 (4-29) weeks. Median (IQR) HIV-DNA cps/ $10^6$ CD4<sup>+</sup>T was 982 (692-1286) at T1, 1892 (146-3798) at T2, and 992 (553-2183) at T3, with no significant change in total HIV-DNA overtime, despite a temporary expansion of peripheral HIV reservoir during ATI (P=0.368). Comparing APACHE and MODAT subjects, no differences were found on the first and third time for HIV-DNA (P= 0.620 and 0.530).

At baseline (BL), 5/7 APACHE and 3/7 MODAT carried MRMs with a median (IQR) intra-patient prevalence of 38.1 (6.2-99.6) and 99.0 (47.1-99.5), respectively.

In APACHE, MRMs persisted during ATI in 2/7 (28.6%, both with an intra-patient prevalence >99.0%) and in 4/7 (57.1%, with a median [IQR] intra-patient prevalence of 53.1 [2.6-99.7]) at plasma level and in PBMCs, respectively. Post-ATI, HIV-DNA MRMs were found in 3/7 (42.8%) individuals, with an intra-patient prevalence of 1.4%, 99.6%, and 99.8%. Comparing pre- and post-ATI HIV-DNA sequences, MRMs with a BL intra-patient prevalence <80% significantly decreased from pre- to post-ATI (intra-patient prevalence: 7.2 [1.8-29.8] vs. 0.0 [0.0-0.6], P=0.01). This decrease occurred mainly in APOBEC-3G related MRMs. MODAT pts did not change MRM prevalence between BL and follow-up, even considering MRMs with a BL intra-patient prevalence <80% (P=0.180).

**Discussion and Conclusion:** This proof of concept study confirms that ATI does not affect the amount of post-ATI peripheral HIV-DNA value, but suggests that ATI may be associated with rearrangement of peripheral archived MRMs, and in some cases with their complete reversal.

*ID 198 - A hyperfusogenic fusion machinery is observed in measles central nervous system infection from an Italian case.*

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**Introduction** Measles (MeV) infection can be severe in immunocompromised individuals and lead to complications including measles inclusion body encephalitis (MIBE). In some cases, MeV persistence and subacute sclerosing panencephalitis (SSPE) occur even in the face of an intact immune response. While relatively rare complications of MeV infection, MIBE and SSPE are lethal. MIBE occurs in immunocompromised people 1 to 9 months after the viral infection, while SSPE appears several years later, often in immunocompetent individuals. The pathogenesis of these diseases remains poorly understood. In 2017 a large measles outbreak in Italy caused 4885 reported cases and 4 deaths.

**Materials and methods** MeV sequences have been obtained from the brain of a patient who died of MIBE, during the Italian outbreak. The sequencing of the viral genome revealed alterations in several genes including the envelope proteins --the receptor binding protein (hemagglutinin; H) and the fusion protein (F). We characterized the biological effects of these mutations and compared them to the mutated envelope proteins identified in patients who died of MIBE or SSPE during a South African outbreak in 2009/2010. The fusion properties of the different F proteins have been assessed by the fusion assay. The conformational state of the Fs has been evaluated using monoclonal antibodies that recognize the pre-fusion or post-fusion conformation of the F protein.

**Results** In the more recent clinical MIBE case, like in the South African cases we characterized, the viral fusion machinery was deregulated. The Italian F protein had three amino acid alterations, and mediated cell fusion in the absence of known MeV cellular receptors -- in distinction to wt MeV F which requires receptor-engaged H in order to fuse -- and was unstable and more readily activated by heat. An alteration in H decreased its ability to promote F-mediated fusion.

**Discussion and conclusions** Analysis of singly mutated F proteins showed that all three altered residues contributed to the receptor independent fusion and the thermal instability. The F bearing all three mutations was activated most readily and was unstable even at 32° C. These results suggest that the destabilization of F protein and increased fusogenicity represent features shared by several CNS-associated MeV isolates.

## POSTER BATTERIOLOGIA

### *P 001 – ID 008 - HLA-G levels in sputum samples of COPD patients: correlation with disease manifestation and airway microbiological detection.*

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**Introduction:** Chronic obstructive pulmonary disease (COPD) is characterized by persistent respiratory symptoms and airflow limitation due to airway and/or alveolar abnormalities. COPD presents recurrent episodes of worsening of symptoms called exacerbations, where airway bacterial colonization is a common feature. The composition of the lung microbiome changes between healthy, smoking and COPD patients and also between acute phase or stable state of COPD. Understanding the inflammatory and immunological events that lead to infectious exacerbations and/or changes in the airway microbiome has the potential to provide novel pharmacological target for intervention in COPD. The Human leukocyte antigen(HLA)-G has functions of regulation of the immune response, is involved in various inflammatory pathologies and plays an immune-escape role for microbiological infections (Rizzo et al. Future Microbiol 2016). We aimed to evaluate the levels of soluble (s)HLA-G molecule in COPD at stable state and during exacerbations and to correlate the level of sHLA-G with sputum microbial detection.

**Materials and Methods:** This is a post-hoc analysis of a previous study aimed to evaluate the effect of adding inhaled corticosteroid (ICS) to long acting bronchodilator (LABA) on sputum bacterial load (Contoli et al. ERJ 2017) in COPD (n=60). Stable moderate COPD patients were recruited and randomized to receive either salmeterol/fluticasone 50/500 mcg bid (ICS/LABA) or SALM 50 mcg bid (LABA) for 12 months. After baseline assessment, patients were seen every 3 months. At each visit lung function was assessed and induced sputum collected to evaluate quantitative/qualitative bacteriology. sHLA-G levels was evaluated in sputum supernatants by commercially available ELISA tests.

**Results:** Forty paired-samples of sputum obtained from COPD patients at baseline and after 1 yr of treatment were included in the analysis. No demographic, functional or clinical parameters correlated with sHLA-G levels at baseline. At baseline the higher are the levels of sHLA-G, the higher are the levels of total bacterial load ( $p=0.05$ ;  $r=0.39$ ). In COPD patients treated with ICS/LABA, the increase in bacterial load was paralleled by an increase in sputum sHLA-G levels ( $p<0.05$  at 1-yr vs baseline). A significant increase in sputum sHLA-G levels was found at exacerbation ( $n=11$ ;  $p<0.001$ ) and in particular when a potential pathogen bacterium (PPB) was detected.

**Discussion and conclusions:** At stable state, sputum sHLA-G levels reflect modification of sputum microbiological composition in COPD patients. The highest levels are detected during exacerbation when a PPB is concomitantly detected, suggesting the role as an immune-escape mechanism. Targeting HLA-G can represent a pharmacological intervention able to modulate susceptibility to infections in COPD.

***P 002 – ID 010 - Therapeutic effect of the predatory bacterium *Bdellovibrio bacteriovorus* against the Adherent Invasive *Escherichia coli* (AIEC) strain, isolated from Crohn's Disease (CD) patients.***

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**Introduction:** Crohn's Disease (CD), included in inflammatory bowel disease, is characterized by continuous remissions and reactivations. CD patients show over-colonization of the intestinal mucosa, mainly due to species belonging to the family of Enterobacteriaceae, and particularly *Escherichia coli*. Adherent-invasive *Escherichia coli* (AIEC) strains, a new pathotype of *E.coli*, were isolated for the first time from CD patients intestinal mucosae. These strains are able to survive and replicate within epithelial cells and macrophages, without inducing apoptosis. The presence of AIEC destabilizes the intestinal barrier directly, thanks to its adhesive-invasive properties, or indirectly by activating resident macrophages to produce pro-inflammatory cytokines. Minimize CD symptoms, improve quality of life, avoid disease complications, reach and maintain a remission state in CD patients, represent the main therapies targets. Given the impact of AIEC on CD pathogenesis, many researchers are evaluating possible treatments aimed at controlling AIEC strains in intestinal mucosa. Strategies are all aimed to prevent AIEC replication or its adhesion and invasion of intestinal cells and macrophage. Nowadays, however, a truly effective strategy is not present, and further investigations are needed in this area.

**Aim:** The objective of the present study was to evaluate the predatory ability of *B. bacteriovorus* versus the AIEC pathotype (strain LF82), in order to assess its possible therapeutic use in CD patients.

**Materials and Methods:** Predation assays were carried out in planktonic co-cultures of predator and prey. The predatory activity of *B. bacteriovorus* was evaluated both as therapeutic agent, on preformed biofilms, and as biofilm formation prevention agent. The impact of *B. bacteriovorus* on AIEC adhesive and invasive abilities on intestinal cell lines was also assessed. Finally, the therapeutic capacity of the predator *B. bacteriovorus* was evaluated on the animal model *Galleria mellonella*.

**Results.** *B. bacteriovorus* showed a clear predatory activity against the AIEC strain both in planktonic growth form and on prevention and destruction of AIEC biofilm. Furthermore, the adhesive and invasive capacity of the AIEC strain on the Caco2 intestinal line decreased in presence of *B. bacteriovorus*. Preliminary results of the experiments with *Galleria mellonella* indicated the non-toxicity of the predator strain *B. bacteriovorus* towards *Galleria mellonella*.

**Discussion.** The results obtained indicate *B. bacteriovorus* as a good candidate, which could be useful for the control of AIEC strain at intestinal mucosa level of CD patients. The reduction of the AIEC strain will help reducing the damage to intestinal tissues, as well as lowering the level of inflammation.

## ***P 003 - ID 015 - New synthesized silver ultra nanoclusters (SUNc) activity against Helicobacter pylori***

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**Introduction:** *Helicobacter pylori* (*H. pylori*) infections is widely spread and still difficult to treat due to the significant increase of antibiotic resistance. Therefore, many efforts are required in order to find a new therapeutic approaches. It has been widely demonstrated that silver nanoparticles have a strong antimicrobial activity against Gram-positive and Gram-negative bacteria, as well as viruses and fungi. The aim of this study was to evaluate the antimicrobial activity of a new synthesized (EP-18181873) silver ultra-nanoclusters (SUNc) formulation against *H. pylori*, alone or in combination with metronidazole (MNZ).

**Materials and Methods:** Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of SUNc were evaluated *versus* eight clinical *H. pylori* isolates with a different antibiotic susceptibility pattern, and one reference strain *H. pylori* ATCC 43504, using the broth microdilution methodology. The effects of combination of SUNc and MNZ were determined by checkerboard assay, and evaluated using fractional inhibitory concentration index (FICI), against 2 strains with different susceptibility to MNZ. Furthermore, the toxicity of SUNc was determined on AGS cells by using the MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazoliumbromide) assay.

**Results:** The MICs and MBCs of SUNc ranged between 0.16 to 0.33 µg/ml. The checkerboard titration assay revealed that the combination SUNc-MNZ showed a synergistic effect confirmed by a increased susceptibility to MNZ. The citotoxicity test on AGS cells demonstrated that SUNc is not toxic until 1.30 µg/ml.

**Conclusion:** The preliminary data obtained show that SUNc are active against *Helicobacter pylori* and could represent, particularly in combination with MNZ, a novel strategy for the treatment of the infection associated with multidrug resistance strains.

## ***P 004 – ID 017 - Molecular assays detecting antibiotic resistance genes on respiratory samples: an unavoidable issue***

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**Introduction:** Bacterial respiratory tract infections are responsible for considerable morbidity and mortality, especially in patients with infections acquired in health care facilities. The worldwide spread of multidrug resistant bacteria has reduced the number of effective drugs and complicated the empirical treatment choice. Rapid microbiological diagnosis plays a crucial role in optimizing targeted therapies and reducing antibiotic resistance selection. Molecular assays have been successfully used for effective prediction of antimicrobial resistance on blood cultures but few data are available on respiratory samples. The aim of this study was to determine whether molecular assays would expedite the detection of carbapenemase- and extended-spectrum beta-lactamase (ESBL) encoding genes as well as the *S. aureus* resistance determinants *mecA* and *mecC* directly from respiratory samples.

**Materials and Methods:** The CRE and ESBL ELITE MGB<sup>®</sup> kits are two qualitative multiplex real-time PCR assays for the detection of the most prevalent carbapenemase and ESBL encoding genes in *Enterobacteriaceae* (EB), respectively. The MRSA/SA ELITE MGB<sup>®</sup> kit is a multiplexed assay designed to simultaneously detect a conserved sequence of the *S. aureus*, *mecA* gene and its homologous variant *mecA*<sub>ALGA251</sub> (*mecC*). The CRE ELITE MGB<sup>®</sup> Kit and ESBL ELITE MGB<sup>®</sup> Kit were assayed on bronchoalveolar lavage (BAL, n=134) positive for Gram-negative rods at microscope observation. The MRSA/SA ELITE MGB<sup>®</sup> Kit was tested on sputum (n=21), tracheal aspirate (n=18), bronchial aspirate (n=15) and BAL (n=37) positive for Gram-positive cocci at microscope observation. Molecular results obtained in less than 3 hours were then compared with those of conventional testing.

**Results:** Among the 134 BAL positive for Gram-negative rods, carbapenemase-producing and ESBL EB were isolated in 17 (12.7%) and in 13 (9.7%) specimens by standard culture methods, respectively. Among the 91 respiratory samples positive to Gram-positive cocci, *S. aureus* was isolated in 70 (76.9%) specimens. The presence of methicillin resistance tested by conventional method was found in 24 (34.3%) *S. aureus* isolates. Agreement results between molecular and conventional results were: 97.8%, 97.8%, and 91.2% for ESBL ELITE MGB<sup>®</sup>, CRE ELITE MGB<sup>®</sup>, and MRSA/SA ELITE MGB<sup>®</sup> kit, respectively.

**Discussion and Conclusions:** Excellent agreement results between genotypic and conventional phenotypic approaches were obtained. Molecular assays may constitute a valid approach for effective prediction of antimicrobial resistance on respiratory samples. Detection of resistance genes from respiratory samples and the implementation of antimicrobial stewardship programs can probably expedite infection control practices, thereby improving patient outcome.

## ***P 005 – ID 019 - Self-assembling temporin-derived peptides with improved antimicrobial activity***

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**Introduction:** Antimicrobial peptides (AMPs) represent a valid chance to overcome and control the antibiotic resistance. The recent identification of common characteristics shared by antibacterial and self-assembling peptides provides a paradigm shift towards the development of novel antibacterial agents. Temporins are AMPs with intrinsic self-assembling properties and a wide antimicrobial activity. We designed and synthesized Temporin L (TL) analogues by introducing fatty acids of variable length (tridecanoic, undecanoic, heptanoic, and valeric acid) as chemical motif (tag) in both N- and C-terminal of the reference peptide TL48, with known antimicrobial activity.

**Material and Methods:** The synthesis was performed *via* a solid-phase peptide strategy. Starting from TL-48 we developed a short library of lipidated analogues. The addition of fatty acids, both in N- and C-terminal regions of TL-48, was carried out using the coupling reagents (COMU/Oxyma) in the presence of a base such as DIEA, in DMF. The antimicrobial activity was evaluated by the broth microdilution assay to determine MIC on *S.aureus* ATCC 29213, *P.aeruginosa* ATCC 27853 and *K. pneumoniae* ATCC BAA-1705 (carbapenemase producer). The self-assembling of peptides was further determined by critical aggregation concentration, while their mechanism of action was investigated by fluorescence assays, using liposome-systems to mimic Gram-positive and Gram-negative membranes (Thioflavin T, Laurdan and Membrane Leakage assays).

**Results:** Compared to TL-48 (MIC 15.1 microM), only peptides, featuring heptanoic and valeric acid at N-terminal, respectively, showed a stronger activity on *S. aureus* (MIC 7.6 and 7.5 microM, respectively), but a slight activity against Gram-negative bacteria (MIC 30.4 microM). Starting from these peptides, we developed new analogues to explore the effects of the same modification to C-terminus. However, these compounds did not show a better activity. To evaluate if the positively charged residues are crucial for interaction with bacterial membrane, we synthesized a novel analogue with an alkyl chain (5C) in para position of the aromatic ring of Phe1 in TL-48. It showed a stronger activity against *S. aureus* (MIC of 7.11 microM), and an appreciable activity versus *P. aeruginosa* (14.3 microM) and *K. pneumoniae* (14.3 microM). Finally, we demonstrated that this peptide adsorbs and self-assembles into the membrane of both Gram-negative and Gram-positive, inducing an alteration of the membrane fluidity and promoting bacterial cell death by pore formation.

**Discussion and Conclusion:** We discovered a novel TL-48 analogue characterized by an improved antimicrobial activity against Gram-negative and Gram-positive bacteria, causing cell death by pore formation.

## **P 006 – ID 021 - Role of the environmental microbiome on preterm newborns colonization: a pilot study by NGS**

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**Introduction:** Extremely preterm infants, due to their characteristics, represent an extremely at-risk group of contracting healthcare-associated infections (HAIs). Late-onset sepsis is a major cause of morbidity and mortality in newborns admitted to neonatal intensive care units (NICUs). In this study we characterized the colonization by environmental microbiome on preterm infants, by analyzing nasal swabs from preterm infants at the time of birth and during the permanence in the NICU.

**Materials and Methods:** A total of 55 nasal swabs were collected from 30 newborns admitted to NICU, independently from their clinical conditions. The study time-course of samples collection included: 30 swabs at the time of birth (group N), 18 after 9 days (group S) and 7 after 13 days (group SA). At the same time points, samples from the environment have been collected including sinks, footboards of the beds, and floors. Microbiome analyses were simultaneously performed by NGS and by a custom real-time PCR (qPCR) microarray kit.

**Results:** In nasal swabs, the bacteria most frequently detected with the NGS method were: *Corynebacterium spp.* (N: 40%, S: 56%, SA: 57%), *Staphylococcus spp.* (N: 53%, S: 94%, SA: 100%), *Streptococcus spp.* (N: 53%, S: 61%, SA: 71%) and *Escherichia-Shigella spp.* (N: 33%, S: 61%, SA: 100%). Other bacteria detected frequently were *Acinetobacter spp.*, *Pseudomonas spp.*, *Klebsiella spp.*, *Enterobacter spp.*, *Cutibacterium spp.* and *Rothia spp.* The qPCR analysis allowed the identification of bacteria up to species level. To be noted, within the genus *Staphylococcus* the main species were *aureus* (N: 3%, S: 33%, SA: 43%) and *epidermidis* (N: 27%, S: 89%, SA: 100%). Instead, within the genus *Streptococcus* the most frequently detected species were *pneumoniae*, *infantis*, *oralis* and *salivarius*, with higher identification rates in the SA group. This method also allowed the identification of the fungus *Candida albicans* in group S (6%) and SA (43%). Environmental NGS analysis of the NICU showed the presence mainly of *Staphylococcus spp.*, *Streptococcus spp.*, *Corynebacterium spp.*, *Cutibacterium spp.*, *Acinetobacter spp.*, *Escherichia-Shigella spp.* and *Pseudomonas spp.* Interestingly, the similarity between the microbiome of nasal swabs and the environmental one increased with the stay of the newborns in the ward.

**Discussion and Conclusions:** Based on molecular analyses, our study highlights the importance of routine screening to assess the rate and type of colonization of fragile newborns by the environmental microbiome. The introduction of this new approach may be important for environmental monitoring and for consequent clinical management of newborns admitted to NICU ward.

## ***P 007 – ID 022 - Antimicrobial efficacy of essential oils against pathogens isolates from cystic fibrosis patients by using a machine learning analysis***

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**Introduction:** Cystic fibrosis (CF) is one of the most common lethal genetic disorders in Caucasian population. CF patients manifest a variety of multi-organ problems due to the alteration of sodium and chloride secretion across cell membranes and the subsequent luminal dehydration. The impairment of muco-ciliary clearance leads to the production of a thick and dehydrated mucus in the CF lung which promotes the airway chronic bacterial colonization. The microbiology of CF respiratory tract is peculiar. In the early stage of life, it is characterized by the prevalence of *S. aureus*. In early adolescence, the lung of CF patients typically becomes chronically infected with Gram-negative non-fermenting bacteria. Among these, *P. aeruginosa* is the most relevant and recurring. These bacterial species rapidly acquire multi-drug resistance, primarily due to cumulative exposure to antibiotics. Recently, several reports indicated in vitro efficacy of natural compounds as promising treatment to reduce the development of the CF associated infections. Among these, essential oils (EOs) seemed to be the most promising agents. Recently machine learning (ML) has been proved as tool to enable the deep investigation on EOs chemical components modulation role against both *P. aeruginosa* and *S. aureus*. In this study an extensive study on 61 commercial EOs against a panel of 40 bacterial strains isolated from CF patients is reported.

**Materials and Methods:** Clinical bacterial isolates were classified on the basis of phenotypic and genotypic features (descriptors). To speed-up the in vitro procedure, classification algorithms allowed the strains clusterization in to select representatives to be subjected to EOs antimicrobial evaluation.

**Results:** Some EOs, showing a strong efficacy to impair the growth of microorganisms, were promptly assayed against all the clinical isolates. Among them three EOs demonstrated their ability to inhibit all bacterial growths. The potent EOs were analyzed for by means of gas chromatography coupled with mass spectrometry to investigate on the likely chemical components mainly responsible for the antibacterial activity.

### **Discussion and Conclusions**

Investigation of the most important components by means of feature importance and partial dependence plots allowed us to indicate the chemical components mostly related to antimicrobial activity of three active EOs.

## ***P 008 – ID 023 - Ten-year retrospective analysis of Legionella diffusion in Hospital water systems and its serogroup seasonal variation***

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**Introduction:** *Legionella* spp. is a Gram-negative microorganism that can develop at a temperature between 25°C and 50°C, especially in collection systems. It can be isolated for example in hot and/or cold water systems, wet soil, air conditioning systems, cooling towers, spas, swimming pools and fountains. Legionellosis is normally acquired by direct inhalation of aerosols from an environmental source contaminated with *Legionella*. The aim of this study was to determine the prevalence of *L. pneumophila* in the water supply systems of some hospitals in southern Sardinia in order to assess the criticality of the water distribution network and strengthen preventive measures. The distribution of serogroup 1 (SG1) and serogroups 2-15 (SG2-15) was also evaluated in the autumn-winter and spring-summer periods.

**Materials and Methods:** From 2009 to 2018, 769 water samples were collected and divided according to seasonality and then analyzed according to the standard methods indicated in ISO 11731-2:2004 and in ISO 11731:2017 for *Legionella* detection.

**Results:** The samples were positive in 37.1% cases (n. 285). In the autumn-winter period SG 1 showed a positivity of 41.2% (n. 40) with a decrease in the spring-summer period with 9.6% (n. 18) of positivity. In contrast, SG 2-15 showed a positivity of 30.9% (n. 30) in autumn-winter, which tends to increase to 56.9% (n. 112) in spring-summer ( $p < 0.001$ ). SG 1 appears to be isolated with a significantly higher frequency in autumn-winter than in spring-summer. This result is the opposite if we consider SG 2-15 group which is more present in spring-summer than in autumn-winter.

**Discussion and Conclusions:** Legionellosis is one of the emerging public health issues. *Legionella*-related illnesses are increasing in number but are probably still underestimated because of a lack of awareness on the part of clinicians along with difficulties in diagnosis. Environmental microbiological surveillance and risk assessment should be performed more frequently to control the environmental spread of *Legionella* spp. Disinfection must be carried out, even in the presence of low levels of contamination, especially in health facilities where people are more susceptible to infections. Our findings suggest that different serogroups may have different ecological niches and that SG 1 needs further examination on account of its riskiness.

## ***P 009 – ID 024 - Characterization of the microbiological quality of well water in south Sardinia***

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**Introduction:** Well water requires a chemical, physical-chemical and microbiological analysis to ensure that the water used for irrigation or for human consumption presents no specific risks for health and the environment. Safe water is one of the fundamental prerequisites for healthy living, but water-borne diseases are still numerous and represent a major cause of death worldwide, particularly in developing countries. The aim of the present work was to determine the microbiological quality of samples taken from wells in central-southern Sardinia.

**Materials and Methods:** In this survey, 55 water samples were taken from private wells at a depth between 8 and 25 m, in the period from 2014 to 2018. The microbiological analysis was carried out by investigating the main contamination factors: *E. coli*, Enterococci, *Pseudomonas aeruginosa*, total Coliforms, total mesophilic counts at 22°C and 36°C. Quality was assessed through the detection of the parameters required by national law.

**Results:** The results showed that in the period between 2014 to 2018, 55 wells were examined and 69.1% samples (n. 38) were analyzed in the spring-summer period. The analysis of the data showed that only 36.4% of the samples (n. 20) complied with law limits for water for human consumption. As regards the individual parameters that were searched for, a positivity was found for *E. coli* in 5.6% of the samples (3 wells) with a median of 60 CFU (1-170), for Coliforms in 26.4% (n. 14) with a median of 23 CFU (9-70), for Enterococci in 14.0% of the samples (n. 7) with a median of 34 CFU (2-54) and for *P. aeruginosa* in 29.1% of the samples (n. 16) with a median of 15 CFU (1–220). As regards the counts at 22°C, a positivity of 22.9% was found with a median of 510 CFU (170-2600), while the counts at 36°C showed a positivity of 45.8% with a median of 98 (50-430). Finally, as regards the analysis of seasonality, no significant associations were found between the isolation frequencies of positive or negative samples and the two periods considered (autumn-winter and spring-summer).

**Discussion and Conclusions:** It can be noted that water destined for human consumption should be periodically subjected to analysis to ascertain its wholesomeness and suitability for use. The high concentration of *P. aeruginosa* and recorded coliforms suggests that the disinfection of the wells was insufficient or not performed properly if implemented.

The results obtained through this survey highlight the levels of contamination both of faecal and environmental origin. Such data suggest the need to pay more attention to the waters used not only for human consumption but also for irrigation, since the cultivated plants could become a vehicle for potentially pathogenic microorganisms.

## ***P 010 - ID 026 - Microbial biofilm correlates with an increased antibiotic tolerance and poor therapeutic outcome in infective endocarditis***

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**1. Introduction:** infective endocarditis (IE) is associated with high rates of mortality. Prolonged treatments with high-dose intravenous antibiotics often fail to eradicate the infection, frequently leading to high-risk surgical intervention. By providing a mechanism of antibiotic tolerance, which escapes conventional antibiotic susceptibility profiling, microbial biofilm represents a key diagnostic and therapeutic challenge for clinicians. This study aims at assessing a rapid biofilm identification assay and a targeted antimicrobial susceptibility profile of biofilm-growing bacteria in patients with IE, which were unresponsive to antibiotic therapy.

**2. Methods:** Patients with surgically-treated IE were enrolled in the study. The level of biofilm production was assessed by the clinical Biofilm Ring Test® (cBRT) and confocal microscopy. Microbial susceptibility profiles to conventional drugs were determined by the microdilution broth method for both planktonic and biofilm cells.

**3. Results:** *Staphylococcus aureus* was the most common isolate (50%), followed by *Enterococcus faecalis* (25%) and *Streptococcus gallolyticus* (25%). All microbial isolates were found capable to readily adhere and produce large and structured biofilms. As expected, antibiotic treatment either administered on the basis of antibiogram or chosen empirically among those considered first line antibiotics for IE, including ceftriaxone, daptomycin, tigecycline and vancomycin, were not effective at eradicating biofilm-growing bacteria. Conversely, antimicrobial susceptibility profile of biofilm-growing bacteria indicated that teicoplanin, oxacillin and fusidic acid were most effective against *S. aureus* biofilm, while ampicillin was the most active against *S. gallolyticus* and *E. faecalis* biofilm, respectively.

**4. Conclusions and discussion:** This study indicates that biofilm-producing bacteria, from surgically treated IE, display a high tolerance to antibiotics, which is undetected by conventional antibiograms. The rapid identification and antimicrobial tolerance profiling of biofilm-growing bacteria in IE can provide key information for both antimicrobial therapy and prevention strategies.

## ***P 011 - ID 032 - Investigating the metabolic potential of the rare actinomycete genus Actinospica.***

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**Introduction:** Natural products still remain an incomparable source of chemical novelty, especially when previously unexplored strains are evaluated. Our proprietary strain collection, including >40.000 actinomycetes, has been built following this rationale and is therefore enriched in strains belonging to uncommon lineages. In this work we analysed the metabolites produced by strains classified as *Actinospica*, an actinomycete genus for which only four strains had been previously described.

**Materials and Methods:** A total of 22 previously uncharacterized *Actinospica* strains from our library were evaluated by sequencing of the PCR-amplified 16S rRNA gene and cultured in two liquid media. Fermentation extracts were prepared from biomass by ethanol extraction and from spent medium by solid phase extraction. Metabolites were analysed by LC-MS followed by molecular networking through GNPS platform (Global Natural Product Social Molecular Networking). Antibiotic activity of extracts against *Staphylococcus aureus* and *Escherichia coli* was evaluated in agar diffusion tests and used for extracts prioritization. Active compounds characterization was based on LC/MS and NMR analyses.

**Results:** Phylogenetic analysis indicated that the strains represent different lineages within the genus *Actinospica*, including some not related to the three described species. Extracts from strains G8 and G23 were active against *S. aureus*. Molecular networking analysis indicated production of unique metabolites by three strains, including G8 and G23, which were differentiated by all others also by 16S phylogeny. Bioactive extracts from strains G8 and G23 were further characterized. Strain G23 produces a novel ionophoric polyether, while G8 produced novel ansamycin-like polyketides.

**Discussion and Conclusions:** We characterized two novel bioactive compounds from a limited number of strains belonging to an uncommon actinomycete genus, *Actinospica*, confirming that poorly characterized actinomycete group are promising candidates for the discovery of new metabolites. Identification of the most promising strains derived from the combination of taxonomic, bioactivity and molecular networking data, this approach is currently being applied to our screening projects for strain/extract prioritization. Our analysis indicates that only a portion of the available *Actinospica* genetic diversity has been accessed so far, therefore suggesting that more new metabolites could be discovered by deeper characterization of this lineage.

## ***P 012 – ID 034 - Molecular surveillance of MDR carrier state of patients hospitalized in an oncohaematology unit in Rome.***

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**Introduction:** The burst isolation of multidrug resistant microorganisms is linked to the high use of both antibiotics and invasive devices, particularly in high-risk patients such as onco-hematology patients. Nowadays, the measures to contain the spread of MDR microorganisms are fundamental and, in this perspective, active surveillance is of critical importance. This study performed in onco-hematology patients aimed to compare the culture-based method to detect rectal carriers of MDR to a multiplex Real Time (RT)-PCR molecular method.

**Materials and Methods:** A total of 193 rectal swabs were collected from onco-hematological patients hospitalized in Policlinico Umberto I of Rome, Italy. Each sample was cultured on selective and chromogenic media such as MacConkey II Agar, Brilliance™ ESBL Agar and Brilliance™ CRE Agar, to provide a presumptive identification of extended beta-lactamase (ESBL) producing and carbapenem-resistant *Enterobacteriaceae*; Brilliance™ VRE Agar was used for the detection of vancomycin resistant *Enterococci* (VRE). Rectal swabs were concurrently analyzed on Nimbus extraction platform (Hamilton Robotics, Bonaduz, Switzerland), followed by multiplex RT-PCR (Allplex™ Entero-DR Assay, Seegene, Seoul, Korea) that detects and identifies 8 antibiotic resistance genes simultaneously: CTX-M, KPC, NDM, IMP, VIM, OXA-48, VanA and VanB.

**Results:** Out of 193 swabs analyzed with both systems, 14 were invalidated by the molecular method due to factors related to improper sampling. Full agreement between the molecular method and phenotypic appearance on cultures was observed in 129 out of the 181 remaining samples. No agreement between the methods was observed in 49 samples. Indeed, RT-PCR detected a gene not revealed by the cultural phenotype in 43 samples, and 7 displayed VRE phenotype, but were negative at the molecular analysis. Therefore, the extraction platform combined with multiplex real time PCR recognized 11.9% positive samples that were not recognized by the culture method. The turnaround time of the molecular technique was of 3-3.5 hours for 40 samples, compared to that of 24/48 h of the cultural method.

**Discussion and Conclusions:** The routine cultural methods, relatively inexpensive, required for the report 24-48 hours, and failed to show evidences on the state of ESBL and/or carbapenemase carrier state in an apparently small, but significant, proportion of rectal swabs derived from oncohematological patients. The higher speed and accuracy should justify the relatively higher costs of the molecular method in the identification of resistance genes, allowing the clinician to a prompt implementation on the needed control measures in avoiding the spread of life-threatening infections in a cohort of high-risk patients.

***P 013 – ID 035 - Direct Beta-lactam inactivation Method (dBLIM): a novel low cost assay for the rapid detection of ESBL- and Carbapenemase-producing Enterobacteriaceae directly from positive blood culture bottles***

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**Introduction:** The rapid detection of ESBL-producing *Enterobacteriaceae* (Ep-EB) and carbapenemase-producing *Enterobacteriaceae* (Cp-EB) from severe infections, especially bloodstream infections (BSIs), is of paramount importance, since the early administration of effective antibiotic therapy may help to both improve patient outcomes and limit the spread of these highly resistant pathogens. In this study, we present and evaluate Direct Beta-Lactam inactivation Method (dBLIM), a novel method for the rapid detection of ESBL and carbapenemase activity directly from positive blood culture bottles. dBLIM originates from Carbapenem Inactivation Method (CIM), an easy, inexpensive, highly sensitive phenotypic method for carbapenemase activity detection in GN isolates. To date, no studies experimented potentiality of this diagnostic tool to detect other hydrolysing enzymes, such as ESBLs, and furthermore its direct application to clinical specimens, e.g. BC samples. dBLIM that we proposed here, is intended to simultaneously detect both ESBLs and carbapenemases production directly from positive BC bottles achieving results in less than 7 hours.

**Materials and methods:** dBLIM was performed on both aerobic and anaerobic BC bottles spiked with 422 characterized EB strains classifiable in 4 phenotypic group: extended-spectrum-cephalosporinases/carbapenemase-non-producing (nEp-nCp) EB (n= 116), ESBL-producing EB (n= 111), AmpC- $\beta$ -lactamase-producing EB (n=33) and carbapenemase-producing EB (n=162).

**Results:** No false positive results was obtained in nEp-nCp EB, in ESBL and in AmpC groups, proving an 100% overall specificity. No significant discrepancy was observed in the performance of dBLIM between aerobic and anaerobic BCs for all groups except for VIM-expressing EB. A sensitivity rate of 53.6% were obtained in anaerobic bottles vs 100% achieved in aerobic ones among BCs spiked with *bla*<sub>VIM</sub>-harbouring EB. This finding gives rise to the hypothesis that the metallo-enzymes may be downexpressed or rapidly degraded or inactivated in anaerobic bottles conditions. Conversely, in KPC, OXA-48, NDM and carbapenemases co-producers groups, an excellent performance was observed, ranging from 99% to 100% in both bottle types. About the detection of extended-spectrum-cephalosporinases activity, dBLIM showed a sensitivity of 100% and 84-87% for ESBL and AmpC-type, respectively.

**Discussion and conclusions:** The dBLIM test may be a cost-effective and highly robust phenotypic method for the reliable detection of extended-spectrum-cephalosporinases and carbapenemases directly from BCs in the same day of bottles positivity detection. As it requires no specialized equipment, reagents and trained personnel, it might be used in most clinical microbiology laboratories.

***P 014 – ID 037 - The role of biofilm production in a case of persistent bacteraemia by *Ralstonia mannitolilytica* and *R. pickettii*, complicated with *Candida parapsilosis* colonization of the central venous catheter.***

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**Introduction.** Members of the genus *Ralstonia* are emerging opportunistic pathogens that include *R. pickettii* and *R. mannitolilytica*, which have been shown resistant to many classes of antibiotics. We recently observed an unusual case of persistent and recurrent bacteraemia caused by *R. pickettii* and *R. mannitolilytica*, which successfully lasted in 80 days. During the infectious episode, the patient showed positive blood and central venous catheter (CVC) cultures for both *Ralstonia* species as well as for *Candida parapsilosis*. Clearance of candidemia was obtained after 4 days of antifungal therapy. Instead, both species of *Ralstonia* were difficult to eliminate by antibiotic therapy. Since both *Ralstonia* spp. and *C. parapsilosis* are recognized to be biofilm producer species, we wanted to verify the hypothesis that biofilm production could be involved in the development of the infection. Therefore, we measured in an *in vitro* model, the biofilm production capacity of both *Ralstonia* spp. and *C. parapsilosis* alone or in combination.

**Materials and Methods.** The bacterial and yeast strains were isolated from blood and CVC cultures and identified by the MALDI-TOF (Bruker, Germany) technique. The strains were then placed on 96 polystyrene microtiter plates and biofilm production was measured using either the XTT or Biotimer assays (BTA). Briefly, XTT method rely on the detection of the enzymatic activity of mitochondrial oxidoreductases by XTT (2,3-Bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide) reduction. The reaction is witnessed by the color change of the substrate, thus measuring the enzymatic activity of the biofilm-forming cells. On the other hand, BTA reveals the microbial metabolism in time as a function of the initial bacterial concentration, therefore allowing the determination of the bacterial concentration in the body of biofilm.

**Results.** BTA allowed the measurement of biofilm production by the two species of *Ralstonia* spp. and of *C. parapsilosis*, either individually or in combination. Instead, as expected, XTT assay witnessed the measurement of biofilm for *C. parapsilosis* only. Both isolates of *Ralstonia* spp. produced biofilm in a dose-dependent manner with bacterial concentrations as low as 75 live cells/mL, as measured by BTA. Both XTT method and BTA showed reduction of biofilm production by *C. parapsilosis*, when the yeast was co-cultured with both *Ralstonia* species.

**Discussion and Conclusions.** The reduction of the biofilm produced by *C. parapsilosis* when incubated with the two species of *Ralstonia*, seemed to justify the fast elimination of the yeast through the antifungal therapy, while the stable production of biofilm by *Ralstonia* spp. could have a crucial role in the persistence of the bacterial infection in the patient.

## ***P 015 – ID 041 - Colistin resistance in sequentially *A. baumannii* strains isolated after in vivo exposure: alterations in pmrCAB related to PmrB mutations.***

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**Introduction:** Main mechanism reported to confer colistin resistance is modification of lipid A associated with mutations in *pmrA/pmrB* genes. Herein, we investigated amino acid variations (AA) of PmrAB two-component regulatory systems and *pmrA*, *pmrB* and *pmrC* mRNA expression in three pairs of colistin-susceptible (ColS) and colistin resistant (ColR) *A. baumannii* strains.

**Materials and Methods:** ColS and ColR strains were sequentially isolated from three patients before and after colistin treatment, respectively. Patient#1 admitted for suspected acute coronary syndrome was diagnosed with VAP caused by XDR-*A. baumannii* susceptible only to colistin (1.5 MU every 24h, and aerosolized, 1 MU every 24h). Patient#2 admitted for trivalvular coronary disease. Clinical course was complicated by surgical site infection due to XDR isolate for which a combination therapy with colistin (3MU every 12h) plus tigeciclin was administered. Patient#3 admitted for bowel obstruction. Post-operative course was complicated by VAP caused by XDR strain, for which intravenous colistin (4.5MU every 12h) plus tigeciclin was administered. Minimum Inhibitory Concentrations (MICs) were determined by Vitek 2 system (bioMérieux, France) and confirmed by Microscan Walkaway system (Beckman Coulter, USA). Resistance was observed at day 9, 10 and 14 of colistin treatment for patient #1, #2 and #3, respectively. The isolates of each pair showed 100% similarity, resulting indistinguishable by PFGE. The *pmrA* (675 bp) and *pmrB* (1335 bp) genes were sequenced by Sanger method and compared with those of *A. baumannii* ATCC19606. Sequences were edited by Chromas and analyzed using BLAST and Mega6 multiple sequence alignment software. AA positions and their effect on protein structure were predicted by InterPro and PROVEAN tools. Expression of *pmrA*, *pmrB* and *pmrC* genes was assessed by *in house* real-time RT-PCR.

**Results:** We found nonsynonymous substitutions such as P233T, E301G and L168K on *pmrB* of all ColR strains. The E301G and L168K represent novel mutations, not previously described. Relative expression of *pmrA*, *pmrB* and *pmrC* mRNA increased in all ColR strains.

**Discussion and Conclusions:** The *pmrB* substitutions were associated with *pmrC* over-expression and colistin resistance. These mutations maybe result in activation of PmrB in absence of physiological activating signals. In ColR isolates expression of *pmrA* was increased, suggesting that the changes determined a constitutive activation of PmrA and over expression of *pmrC*. Moreover, emergence of resistance could have been favored by a suboptimal use of colistin, according to the current guidelines and practice not available when these patients were treated. However, the exact role of these novel changes in resistance needs to be further investigated.

## ***P 016 – ID 042 - Titration of Igs contained in an Intravenous IgM-enriched preparation against selected MDR pathogens***

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**Introduction:** Pentaglobin<sup>®</sup> is an immunoglobulin preparation containing IgG, IgA, and enriched in IgM with a high antibacterial and endotoxic effect by polymorphonuclear leukocytes activation against a wide range of Gram-negative and Gram-positive bacteria. The goal of our work was to titer the IgG, IgM and IgA in Pentaglobin<sup>®</sup> targeting specific surface antigens of Gram-positive and Gram-negative bacteria commonly involved in sepsis, as well as a *C. albicans* strain.

**Materials and Methods:** Lipopolysaccharides from Gram-negative bacteria, peptidoglycan and lipoteichoic acid from the other microorganisms were extracted and used in several ELISA assays in order to determine the titer of Pentaglobin<sup>®</sup> immunoglobulins directed towards the aforementioned surface antigens.

**Results:** Our results showed an overall immunoglobulin titer of at least  $10^3$  in Pentaglobin<sup>®</sup> when tested with *S.aureus*, *Enterococcus* spp, *S. agalactiae*, *E. coli*, *A. baumannii* and *C. albicans*, with some exceptions only for the IgA titers. *K. pneumoniae* and *P. aeruginosa* Pentaglobin<sup>®</sup> IgG, IgM and IgA titers were  $\geq 10^2$ .

**Discussion and Conclusion:** According to these results, Pentaglobin<sup>®</sup> can be considered as a potential adjuvant for antimicrobial therapy.

## ***P 017 – ID 045 - Time-Kill Assay of Citrus bergamia distilled extract on multi-drug resistant clinical isolates***

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**Introduction:** In recent years a growing increase of multi-drug resistant (MDR) bacteria has been documented due to indiscriminate and often inappropriate use of antibiotics. Several studies have shown the antimicrobial properties of bergamot (*Citrus bergamia* Risso et Poiteau), especially of the distilled extract. The aim of this study is to evaluate the *in vitro* sensitivity of MDR microorganisms, isolated from clinical samples, to distilled bergamot extract and its bactericidal effects over time using the *time-kill* assay.

**Materials and Methods:** MDR bacteria were isolated from patients admitted to the Catanzaro University Hospital (Italy). Bacterial growth was monitored with the automated system Alfred 60AST (Alifax) until exponential growth was reached. For the determination of the Minimal Bactericidal Concentration (MBC), serial dilutions of the bergamot distilled extract were prepared on a microwell plate. Each dilution was tested with a microbial concentration of  $10^5$  CFU/ml. After 24 hours of incubation, 1  $\mu$ L of each well was inoculated on blood agar plates. Over 24 hours period, seven exposure times to the bergamot distillate were established for the evaluation of the killing kinetic curve. Colony counts were carried out following culture on blood agar plates.

**Results:** Our results show that the bergamot distilled extract has a slow bactericidal activity on Gram-positive bacteria, while the bactericidal effect of the distillate on Gram-negative bacteria was observed after 2 hours of exposure. Finally, on the *Candida* strains the fungicidal effect was confirmed after as short time as 30 minutes of exposure.

**Discussion and Conclusions:** Antimicrobial action of bergamot distillate on MDR clinical isolates was confirmed. The prompt fungicidal action on *Candida* strains can be accounted by the presence of sterols on the yeast cell wall, allowing a faster interaction with the lipophilic components of the bergamot distillate. The greater sensitivity of Gram-negatives compared to Gram-positives can be explained by the different structure pattern of mostly lipid layers found in Gram-negatives. Also, our findings may be explicated by the synergistic activity of the different components of the distillate.

## ***P 018 – ID 052 - T2 magnetic resonance assay allows rapid detection of both bacteria and fungi directly from whole blood: is there a clinical impact relative to standard blood culture?***

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**Introduction:** Fungal and bacterial bloodstream infections and associated clinical sepsis are one of the major cause of mortality among hospitalized patients. The T2Dx (Biosystem), an automated instrument platform, uses nonculture T2 magnetic resonance (T2MR) technology to detect nucleic acids and microbial cells directly within whole blood, reducing detection and reporting time. In this study we show data on the performance of T2MR, in parallel with the gold standard method, to provide accurate and timely diagnosis of sepsis and to optimize the antimicrobial stewardship.

**Materials and methods:** For the present study we enrolled 25 selected critically ill patients, admitted to the Catanzaro University Hospital (Italy), with suspected sepsis. On samples, we performed both whole blood detection by T2MR technology and blood cultures by Virtuo (bioMerieux ) plus Vitek2 System.

**Results:** A total of 30 samples from enrolled patients were processed. T2MR technology (T2Bacteria panel and Candida panel) showed a concordance of 100% for Candida species and of 76.5% for bacterial isolates in comparison with conventional method. In two patients, magnetic resonance was able to detect *K. pneumoniae* and *E.coli*, while conventional methods identified *E.coli* alone. *K. pneumoniae* nucleic acids detection, probably derived from alveolar bacterial DNA translocation in absence of alive bacteria, was useful to identify respiratory tract infections. These data was confirmed by culture methods later on, and allows clinicians to optimize the antimicrobial therapy. On 4/17 samples (tested by T2 bacteria panel) we found discordant results: in particular *S. capitis*, *S. epidermidis* and *E. cloacae complex*, which were not present in the T2bacteria panel, and *P.aeruginosa* detected by T2MR technology alone.

**Discussion and conclusions:** T2 Bacteria and Candida panels, using magnetic resonance, are a rapid and innovative assay which, compared to conventional methods, might support an earlier therapeutic management of bloodstream infection patients. Further studies with clinical outcomes are necessary to validate T2 as a useful tool to improve escalation strategies for antibiotic therapy.

## ***P 019 – ID 054 - Bioactive compounds derived from different fungi as innovative antimicrobial technologies against *Staphylococcus pseudintermedius****

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### **1. Introduction**

Filamentous fungi biosynthesize a wide range of chemically different secondary metabolites during processes of competition with other micro- and macro-organisms, symbiosis, parasitism or pathogenesis. Some of these natural compounds have antibiotic properties, which enable the microbe to inhibit and/or kill other microorganisms. The objective of this study was to evaluate the antimicrobial activity of bioactive compounds extracted from selected microbes belonging to *Trichoderma*, *Talaromyces*, *Clonostachys* and *Coniothyrium* genera against both methicillin-resistant (MRSP) and methicillin-susceptible (MSSP) *S. pseudintermedius* strains. The MRSP strain is an important reservoir of antibiotic resistance genes in addition to the *mecA* gene. The prudent antimicrobial use in veterinary medicine promotes the identification of new and appropriate technologies to reduce the impact of this pathogen.

### **2. Materials and Methods.**

Canine MRSP and MSSP strains were selected at the Microbiology Laboratory of Dept Veterinary Medicine and Animal Production, University of Naples “Federico II”. The colonies were isolated on Mannitol Salt Agar and identified by MALDI-TOF-MS. The antimicrobial susceptibility patterns were determined by disk diffusion method and the detection of *mecA* gene by PCR. The extracted natural compounds from the selected fungal strains were analyzed by LC-MS QTOF system and compared to a fungal metabolite database. The minimum inhibitory concentration (MIC) on MRSP and MSSP of the fungal extracts was determined by broth microdilution method. Anti-biofilm activity was also tested.

### **3. Results**

The antibiotic assays of the extracts showed no significant differences between MRSP and MSSP strains. Furthermore, these fungal metabolites demonstrated a good bacterial growth inhibition with MIC less than 64 µg ml<sup>-1</sup>. Biofilm inhibition activity was observed only for two strains at concentrations of 32 µg/ml and any significant activity was registered on biofilm disruption. LC-MS qTOF analysis revealed the presence of several secondary metabolites belonging to different classes of microbial metabolites (i.e. tetramic acid derivatives, diketopiperazines, macrolides, funicones, etc.).

### **4. Discussion and Conclusion.**

The antibiotic-resistant pathogens are a great problem with a growing global healthcare crisis. Our results show the antimicrobial activity of a large panel of bioactive extracts against MRSP and MSSP. In addition, two of these fungal exo-metabolites showed a significant activity against biofilm formation. Isolation and chemical characterization of fungal metabolites responsible of the antibiotic activity and the roles that these molecules may have to control the disease development, are being investigated.

## ***P 020 – ID 055 - Antibacterial and antimycotic activity of hydroxypyridinone-based iron-chelating co-polymer (DIBI) against pathogens carried by companion animals.***

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### **1. Introduction**

Antibiotic resistance is one of the most urgent threats to public's health and the increasingly limited therapeutic options both in human and veterinary medicine underline the need of new alternative therapeutic approaches, in order to limit the ever-increasing spread of multidrug-resistant strains among companion animals. Among the different aetiological agents, bacteria and yeasts represent the main pathogens associated with cutaneous diseases. Bacteria such as Staphylococci, in particular *Staphylococcus pseudintermedius*, *Streptococci*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Escherichia coli*, and yeasts like *Malassezia pachydermatis* are frequently isolated from diseased cats and dogs. DIBI, a novel water-soluble hydroxypyridinone-containing iron chelating polymer, developed by Chelation Partners Inc. (Canada), provides a potential new antibacterial treatment by denying pathogens of iron as needed for their growth. Herein, we tested DIBI against different strains isolated from dogs suffering from skin disorders.

### **2. Materials and Methods.**

Some strains of bacteria and mycetes belonging to the stored collection obtained from diagnostic routine at the Microbiology Laboratory of Department of Veterinary Medicine and Animal Production, University of Naples “Federico II” (Italy) were selected. Cutaneous specimens were inoculated onto different selective and differential agar plates to isolate gram-positive and gram-negative bacteria or yeasts. Isolates were identified by MALDI-TOF-MS and their antibiotic resistance profiles were evaluated by disk diffusion method. Then MIC susceptibilities to DIBI were evaluated using the broth microdilution method in 96-well round-bottomed plates against eighteen microbial strains.

### **3. Results**

DIBI activity was found to be strongly inhibitory to selected gram-positive bacteria (*Staphylococcus aureus* and *Staphylococcus pseudintermedius*,) and two *Malassezia pachydermatis* strains, while resulted to be moderately inhibitory to the selected gram-negative bacteria (*Pseudomonas aeruginosa* and *Proteus mirabilis*). Precisely, gram-positive bacteria and mycetes displayed a DIBI MIC range lower to 4 µg/mL. *Pseudomonas aeruginosa* and *Proteus mirabilis* showed a wider spectrum of sensitivity with values lower to 128 µg/mL.

### **4. Discussion and Conclusion.**

Widespread emergence of multidrug-resistant bacterial pathogens represents an important problem to animal health and an increasing therapeutic challenge in veterinary medicine. Thus, new alternative approaches are necessary, and this study demonstrated that DIBI represents a promising non-antibiotic alternative therapy against different microbial agents involving in cutaneous infectious diseases of companion animals.

## ***P 021 – ID 057 - Invasive meningococcal serogroup C disease in Italy, 2012-2018.***

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**Introduction:** In Italy, the introduction of the meningococcal C conjugated vaccine (MCC) in 2005 has led to a reduction in the cases of serogroup C disease, mostly among children for whom the vaccination is targeted. However, invasive meningococcal diseases (IMD) due to serogroup C strains (MenC) are still spreading through different countries with high morbidity and mortality. In Italy one outbreak in 2009 and an epidemic in Tuscany Region in 2015-2016 occurred due to the hyperinvasive MenC strains. The aim of this study was to describe the epidemiology of MenC in Italy during 2012-18. Moreover, the molecular features, including genetic relationships among MenC isolates, were analyzed.

**Materials and Methods:** Bacterial isolates and clinical samples (blood or cerebrospinal fluid) from invasive cases are collected and characterized at the National Reference Laboratory of Istituto Superiore di Sanità in Rome. Antimicrobial susceptibility was determined by MIC Test Strip Method (EUCAST breakpoints). Genotypic characteristics, including serogroup identification, multilocus sequence typing (MLST), finetype and antibiotic resistance genes were performed and analyzed using the PubMLST database (<http://pubmlst.org/neisseria/>). Moreover, whole genome sequencing (WGS) was performed on a subsample of MenC isolates and compared using cgMLST.

**Results:** A total of 346 laboratory confirmed cases due to MenC were reported within the National Surveillance System (<http://old.iss.it/mabi/>). The MenC IMD showed an average annual incidence of 0.08 per 100,000 population, with a peak of 0.1-0.13/100,000 in 2015-2016, respectively, determined by the outbreak occurred in Tuscany. The median age was 32 years. The main clinical pictures were sepsis (42%), meningitis and meningitis/sepsis. The case fatality rate was 27%. Two isolates were resistant to rifampicin (MIC 0.38 mg/L), whereas the 69% of isolates showed a decreased susceptibility to penicillin G. Thirteen different clonal complexes (cc) (cc11 was the predominant), and 33 genotypic formulas -the most frequent C:P1.5-1,10-8:F3-6:ST-11(cc11)- were identified. Phylogenetic analysis based on cgMLST clustered the genomes by ccs. In particular, MenC:cc11 splitted into two subgroups, C:P1.5-1,10-8:F3-6:ST-11(cc11) and C:P1.5,2:F3-3:ST-11(cc11).

**Discussions and Conclusions:** In Italy, MenC continues to be responsible of severe IMD cases and outbreaks. From 2012 to 2018, the C:P1.5-1,10-8:F3-6:ST-11(cc11) is the main strain identified and responsible of an epidemic in 2015-2016 with high case fatality rate. In the post vaccination era, the monitoring and the genomic analysis of MenC meningococci should be maintained in order to evaluate the relationship among IMD cases and the occurrence of hyperinvasive strains.

## ***P 022 – ID 059 - Novel silver-functionalized poly( $\epsilon$ -caprolactone)/hydroxyapatite biomaterials designed to counteract post-surgical infections.***

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**Introduction:** In surgery, device implantation establishes a non-negligible incidence of infections, representing one of the major causes of morbidity and mortality in this medical field. A foreign body presence is the triggering event for biomaterial-associated infections (BAIs), because biomaterial surface and roughness not only attract host eukaryotic cells involved in tissue regeneration, but also free-floating bacteria. Indeed, as soon as a contamination occurs, the “race to the surface” begins, determining the fate of infection development. These concerns led to the exploration of synthetic biodegradable polymers, such as poly ( $\epsilon$ -caprolactone) (PCL), a hydrophobic bioresorbable material with a great potential in biomedical applications, either as a porous scaffold in bone tissue reconstruction or as vehicle for controlled delivery of therapeutic molecules. Aim of the present research is the development of novel PCL-based biomaterials, modified with both hydroxyapatite (HA), to impart bioactive/bioresorbable properties, and silver, to supply antibacterial behavior. Therefore, the designed scaffold will be able to promote fast and physiological bone integration and avoid bacterial contamination.

**Materials and methods:** PCL and HA/PCL porous pellets, functionalized with silver ions ( $\text{Ag}^+$ ) were developed by salt-leaching methods, sieved in the range 125-355  $\mu\text{m}$ , as a pore former. Samples were further characterized from the chemical and morphological point of view. Antibacterial tests were performed by assaying *Staphylococcus aureus* adhesion on biomaterials through a sonication protocol to dislodge adherent microorganisms without altering their viability. The planktonic bacteria number was also determined.

**Results:** Field Emission Scanning Electron Microscopy showed that the samples were characterized by square-shaped macropores, whose average dimension was in agreement with that of the starting salt. X-Ray Diffraction analysis confirmed the presence of PCL and HA phases, while Energy Dispersive X-ray Diffraction confirmed the presence of Ag in the correct amount. The antibacterial tests revealed a significant ( $p < 0.001$ ) reduction of the adherent staphylococci on the Ag-functionalized surfaces, after 24 h of incubation, with values of about  $10^4/10^3$  CFU/ml respect to  $10^9$  CFU/ml for controls. Additionally, a similar significant ( $p < 0.001$ ) decrease in CFU/ml was also detected for planktonic bacteria, thus proving the Ag release from the enriched PCL-based samples.

**Discussion and Conclusions:** Due to the combined antimicrobial and biodegradable properties, the PCL-based scaffolds enriched with silver showed good potential for bone tissue engineering and offer a promising strategy, as an ideal microbial anti-adhesive tool, for the reduction in BAIs and antimicrobial molecules-targeted delivery.

## ***P 023 – ID 061 - Uncommon isolation of Streptococcus pneumoniae from male urethral samples***

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**Introduction** *Streptococcus pneumoniae* is not a common agent of urinary tract infections (UTIs); indeed, literature data about its isolation in genitourinary samples of both adults and children are limited. Here, we report 13 cases of *S. pneumoniae* isolated from male urethral samples.

**Materials and Methods** From June 2018 to May 2019, a total of 281 urethral samples, collected with transport swabs and transferred to the Microbiology Laboratory of the ‘Infermi Hospital’ (Rivoli, Turin), was examined for suspected urethritis. Specimens were analyzed for the presence of opportunistic bacteria, fungi, *Mycoplasma* spp and *Trichomonas* spp with cultural examination and for *Chlamydia trachomatis* and gonococci by strand displacement amplification. All the isolates were tested for antimicrobial susceptibilities to many widely used antibiotics. Resistant ones were confirmed by manual susceptibility testing.

**Results** One hundred sixty-one (57,3%) of 281 male patients were negative to all researched pathogens. The other 120 cases (42,7%) were microbiologically positive: in details, bacteria or fungi were isolated in pure culture in 105 samples, whereas the remaining 12,5% was characterised by coinfections. Remarkably, out of 120 positive subjects, *S. pneumoniae* was detected in 13 men (10,83%; age range: 20-70 years; median age: 43 years) with symptoms of urethritis. Examination of their urethral specimens revealed the presence of Gram-positive cocci, confirmed as *S. pneumoniae* based on colony morphology, alpha haemolysis, Gram stain and optochin susceptibility. In three patients microscopy and cultural analysis highlighted a polymicrobial infection caused by *S. pneumoniae* with *Staphylococcus haemolyticus*, or *Streptococcus agalactiae*, or *Ureaplasma urealyticum*, respectively. The majority of analyzed *S. pneumoniae* was resistant to erythromycin and clindamycin. In some isolates, further resistance was noted; in particular, one case presented multi-resistance to different classes of antibiotics.

**Discussion and Conclusions** *S. pneumoniae* is infrequently isolated from urethral specimens. However, our study described an unusual high number of urethritis cases caused by pneumococci in a relatively short period (11 months) in an enclosed area (ASL TO3, Turin). *S. pneumoniae* as etiologic agent of urethritis was confirmed by its isolation in pure culture in 10 cases out of 13. As *S. pneumoniae* is a commensal of the upper respiratory tract, orogenital sexual contact has been suggested to be responsible for direct inoculation of the microorganism in the male urethral mucosa. With the increase in sexually transmitted diseases and variable human sexual behaviors, the significance of *S. pneumoniae* as a sexually transmitted pathogen should be more considered and it would be useful to look for *S. pneumoniae* during routine screening for UTIs.

## ***P 024 – ID 062 - Synthesis of silver nanoparticles by Pichia pastoris and its efficacy against different bacteria.***

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**Introduction:** Day by day bacteria becoming resistance against available antibiotics. Therefore, to overcome this problem, there is a persistent need to develop antibacterial agents. Hence AgNPs may serve as better alternative antibacterial agent against different pathogens. The main aim of present study was to evaluate *in vitro* antibacterial activity of AgNPs synthesised from *Pichia pastoris* against different bacterial strains.

**Materials and Method:** The *Pichia pastoris* cell filtrate was treated with 1mM silver nitrate solution and colour changes from colourless to brown is due to the formation of silver nanoparticles and the excitation of surface plasmons. Different techniques have been used for characterize silver nanoparticle i.e. UV–VIS Spectroscopic Analysis, dynamic light scattering (DLS), Zeta potential Analysis, Fourier-transform infrared spectroscopy (FTIR) and transmission electron microscope (TEM).

**Results:** The AgNPs was characterized by visual observation followed by UV-Vis spectrophotometric analysis, which showed a peak at about 420 nm. Moreover, to find out the presence of proteins as capping agent AgNPs was analysed by Fourier Transform Infrared Spectroscopy (FTIR). Transmission Electron Microscopy (TEM) was carried out to detect the size and shape of Ag-NPs. To evaluate antibacterial activity of silver nanoparticles disc diffusion method against different bacterial strains was carried out.

**Discussion and Conclusion:** The cell filtrate of *Pichia pastoris* changes color from yellow to dark brown when treated with AgNO<sub>3</sub> and its UV-vis spectra showed at 420 nm and average size measured by DLS was found to be 40 nm. The zeta potential for silver nanoparticles was found to be -21 mV. The FTIR analysis showed presence of protein capping agent when AgNPs scanned 400-4000 cm<sup>-1</sup>. The synergistic effects of AgNPs were maximum activity against Gram positive than Gram negative bacteria. It is concluded that the environmentally benign silver nanoparticles synthesized from *Pichia pastoris* can be used against bacterial strains. The synthesized silver nanoparticles may have important advantage over conventional antibiotics to which the bacteria got resistance.

## ***P 025 – ID 063 - Antibacterial resistance trends of *Pseudomonas aeruginosa* from 2017 to 2018 at the University Hospital “Luigi Vanvitelli” of Naples***

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**Introduction:** *Pseudomonas aeruginosa* (PA) is one of the most common pathogens associated with nosocomial infections. PA is a gram-negative and non-fermentative bacteria, able to colonize a great variety of ecological niches, in particular humid environments. It is rarely found in the normal microbial population of healthy individuals, however it can colonize the skin, the gastrointestinal tract and the respiratory tract. The pathologies linked with this microorganism involve a wide range of disease, from skin superficial infections to fulminant sepsis and they include: ear or eye infections, urinary tract infections, osteomyelitis, pneumonia and bloodstream infections. Different epidemiological studies show that antibiotic resistance in PA is increasing in clinical isolates resulting in higher morbidity and mortality.

**Materials and Methods:** This study was conducted from January 2017 to December 2018. During this period 283 PA isolates were collected from urine, wounds, catheters, body fluids, blood, respiratory tract specimens. The selected samples were grown on routine MacConkey medium where they resulted lactose Non-fermenting pale colonies oxidase positive. The colonies were identified with MALDI-TOF (matrix-assisted laser desorption/ionization time-of-flight) and antibiotic susceptibility tests were performed by the disc-diffusion (Modified-Kirby Bauer disc diffusion method) according to CLSIs guidelines.

**Results:** Sex prevalence of clinical isolates showed that infections caused by PA are more common in males (56,5%) compared to female (43,5%). The majority of clinical isolates were isolated from the respiratory secretion followed by the bloodstream and urine. Resistance to antibiotic classes tested showed a rate of 55,5% Fluoroquinolones, 46,6% Carbapenems, 40,6% Extended-spectrum Cephalosporins, 39,9% Aminoglycosides, 27,2% Piperacillin. On the other hand, the prevalence of PA-MDR, defined as resistant to at least 3 classes of antibiotics, was 41% and the MDR strains were mainly isolated from respiratory secretion.

**Discussion and Conclusions:** This study shows that clinical isolates of PA are becoming resistant to commonly used antibiotics and gaining more and more resistance to newer antibiotics. The spread of the resistant bacteria is need to be implemented with strict antimicrobial policies, surveillance programs for multi-drug resistant organisms and infection control procedures. Meanwhile, the antibiotics susceptibility pattern should be continuously monitored and the results should be acknowledged by clinicians.

## ***P 026 – ID 064 - Alternative strategies to reduce the presence of potential pathogens by using negative and positive ions***

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**Introduction** In recent years, there has been renewed interest in the use of air ionizers to control the spread of airborne infections. Bacteria and viruses attached to air particles circling in a room can be charged by ions, so they fall out and are removed from the air. Furthermore, negative and positive ions have been shown to have microbicidal effects on various bacterial and fungal species. Thereby the use of ionizers may contribute to disinfecting the atmosphere and stopping the transmission of microorganisms. The aim of this study was to determine if the sensitivity of bacteria to ions could be influenced by variation in experimental parameters.

**Materials and methods** An ionizer producing positive and negative ions was used to determine potential effect on bacterial cells related to: 1) bacterial type; 2) bacterial load; 3) action area and 4) distance of the ion generator. Hence, ion effects on Gram positive (*Staphylococcus aureus*) and Gram negative (*Escherichia coli*) ATCC bacteria, at two different concentrations ( $10^4$  and  $10^7$  UFC/ml) have been studied. Bacteria were plated on Petri dishes of two different diameter (90 or 150 mm) and placed at two different distances from the ionizer (5 or 10 cm), for different incubation times (1, 3, 8, 12 hours) in aerobic sterile conditions at room temperature and constant relative humidity.

**Results** The average concentration of positive and negative ions emitted by the ionizer was 12 million ions/cm<sup>3</sup> at 5cm of distance and 2.6 million ions/cm<sup>3</sup> at 10cm of distance. The results evidenced a promising efficacy of ions on both the Gram positive or negative bacterium. A more significant ( $p < 0.05$ ) ion activity was observed on *S. aureus* both at  $10^4$  or  $10^7$  UFC/ml, independently from the distance from the ionizer and from the plate diameter, already starting from 3 hours of incubation and up to 12 hours.

**Discussion and Conclusions:** These results indicate that negative and positive ions are responsible for reducing bacterial survival in different experimental conditions, suggesting the ionizer use as a promising alternative treatment for a microbial load reduction in various fields, such as healthcare facilities, with the potential to reduce the amount of antimicrobials used.

***P 027 – ID 065 - Prevalence and antibiotic susceptibility patterns of methicillin-resistant Staphylococcus aureus strains among clinical isolates from “Luigi Vanvitelli” University Hospital of Naples, Italy.***

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**Introduction:** Healthcare-associated methicillin-resistant *Staphylococcus aureus* (MRSA) is a common pathogen worldwide and its multidrug resistance is a major threat. Indeed, in addition to beta-lactams, several MRSA strains have shown an increasing resistance to macrolide-lincosamide-streptogramin B (MLS<sub>B</sub>) agents, to glycopeptides, such as vancomycin, and to fluorochinolones, greatly reducing the number of available therapeutic options. Here we evaluated the clinical characteristics and antibiotic susceptibility profile of healthcare-associated MRSA with focus on resistance to vancomycin and MLS<sub>B</sub> agents.

**Materials and Methods:** A total of 417 *S. aureus* cases were isolated from several clinical specimens collected from hospitalized patients of “Luigi Vanvitelli” University Hospital in Naples, during January 2017 – December 2018. All *S. aureus* isolates were identified by matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS) and submitted to antibiotic susceptibility testing by Kirby-Bauer disc diffusion method. The inducible clindamycin resistance was determined by double-disk diffusion test (D-test).

**Results:** Out of 417 healthcare-associated *S. aureus* cases, 140 were MRSA (33,6%) and of these, 88% were respiratory infections. All of the MRSA and MSSA isolates were susceptible to linezolid and daptomycin, while the majority of MRSA isolates were resistant to ciprofloxacin (77,1%), clindamycin (57,9%), and erythromycin (65%). In comparison to MSSA isolates, MRSA isolates were significantly more resistant to ciprofloxacin, and trimethoprim/sulfamethoxazole (P<0,05). Two MRSA cases, isolated from two patients with bloodstream infections, exhibited resistance to vancomycin (1,4%) and one of these was of constitutive MLS<sub>B</sub> phenotype. Finally, out of 140 MRSA strains, 16 (11,4%) were constitutive MLS<sub>B</sub> phenotypes, 61 (43,6%) were inducible MLS<sub>B</sub> phenotypes and 12 (8,6%) were macrolide-streptogramin B phenotypes.

**Discussion and Conclusions:** In this study a high rate of healthcare-associated MRSA infections was observed. A greater number of MRSA cases are multidrug-resistant. Antibiotic resistance profiles can optimize the treatment of multi-drug resistant *S. aureus*. For this reason, a D-test should be performed before selecting clindamycin to treat cases of MRSA that appear susceptible to clindamycin but resistant to erythromycin in order to minimize the risk of treatment failure. Also the susceptibility to vancomycin should be proven before using this antibiotic for the treatment of the serious MRSA infections. As one, it is necessary to avoid the excessive and inappropriate use of antibiotics in order to stop the further increase of resistant strains and prevent the development of new ones.

## **P 028 – ID 067 - Antibacterial activity of N-Alkylimidazolium salts**

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**Introduction:** Prosthetic implants-associated infections represent one of the most common and severe post-surgical issues. Mainly, there is a combination of bacteria growth and biofilm production. Despite the antibiotic treatment, often the prosthetic implant removal is necessary. In this scenario, the possibility to use antibacterial and antibiofilm functionalized materials in implant surgery could represent the next future. On this basis, the study proposes two different N-Alkyl imidazolium salts (N-Alkyl IMS) as antimicrobial bionanocomposites. Imidazolium salts (IMS) derive from the imidazole rings through the alkylation of both nitrogen atoms in the heterocycle. They are found in different natural products and play essential functional and structural roles. The biological activity of the imidazole ring is linked to its ability to bind to a wide variety of functional groups. Based on the acquired group, IMS can be applied as an antimicrobial, antitumor, antioxidant and antifibrous agent. N-Alkyl IMS salts have been recognized as promising antimicrobial agents. In this study, the antibacterial and antibiofilm activity of C<sub>16</sub>IMS and C<sub>10</sub>(10)IMS against Gram-positive and Gram-negative bacteria was evaluated.

**Materials and Methods:** Susceptibility testing was performed following the broth micro-dilution method. The Gram-positive bacterium *Staphylococcus aureus* ATCC 6538 and the Gram-negative bacterium *Escherichia coli* ATCC 11219 were used as test organisms. The antibacterial action of C<sub>16</sub>IMS and C<sub>10</sub>(10)IMS was defined by determining their minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) ranging from 50 to 0.78 µg/mL. The drug's ability to degrade the mature biofilm of the tested strains was performed through the Crystal Violet assay ranging from 100 to 1.56 µg/mL.

**Results:** The results show that both N-Alkyl IMS are active against Gram-positive and Gram-negative bacteria. For *Staphylococcus aureus*, C<sub>16</sub>IMS and C<sub>10</sub>(10)IMS recorded MIC of 3.125 µg/mL and a MBC of 25 and 12.5 µg/mL, respectively. For *Escherichia coli*, C<sub>16</sub>IMS and C<sub>10</sub>(10)IMS show a MIC of 25 and 12.5 µg/mL, respectively and a MBC greater than 50 µg/mL. Both N-Alkyl IMS are capable to degrade about 40% of mature biofilm at 100 µg/mL.

**Discussion and Conclusions:** C<sub>16</sub>IMS and C<sub>10</sub>(10)IMS could represent potential antimicrobial bionanocomposites for overcoming prosthetic implants-associated infections.

## ***P 029 – ID 073 - Microbiological assessment of cricket and cricket flour: preliminary study***

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**1. Introduction:** Following the introduction of foods derived from the processing of edible insects on the European market, entomophagy has become an increasingly common practice. The food safety of these ‘novel foods’, such as cricket flour, is limited by the lack of knowledge of their microbiota. As a few studies have been conducted on crickets (*Acheta domesticum*) and its final processing product (flour), the aim of this research was to evaluate crickets’ microbiota and that one of the flour obtained after heat treatment applied on crickets reared under controlled and monitored housing conditions.

**2. Materials and Methods:** Different categories of crickets (male, female, young, adult), and related samples of flour (obtained after heat treatment at 80°C for 6 hours) were subjected to bacteriological and mycological investigations in aerobic and anaerobic conditions, and evaluated for the total bacterial (TBC) and fungal counts (TFC: cfu/g). Cultures of main pathogenic agents like *Salmonella* spp, *L. monocytogenes*, *V. cholerae*, *Campylobacter* spp., *Neisseria* spp., *Haemophilus* spp., *S. aureus*, *B. cereus*, *H. pylori*, *C. perfringens* and sulphite-reducing clostridia, *Candida* spp, were carried out. Phenotypic (Remel, ThermoFischer) and molecular identifications (PCR and sequencing) were performed.

**3. Results:** On raw crickets, the mean TBC and TFC resulted of  $7520 \times 10^6 \pm 4073 \times 10^6$  cfu/g and  $570 \times 10^6 \pm 40 \times 10^6$  cfu/g, respectively; while on flour samples a lower loads were observed ( $20750 \pm 2100$  cfu/g TBC, and  $250 \pm 40$  cfu/g ( $P < 0.05$ ) TFC;  $P < 0.05$ ). On crickets, *E. coli*, *E. faecalis*, *K. oxytoca*, *P. aeruginosa*, *S. paucimobilis*, and *Staphylococcus* Coagulase negative, *Streptococcus* spp., and *Bacillus* spp. were the bacteria more representative. After drying, only *Staphylococcus* Coagulase negative, *Streptococcus* spp., and *Bacillus* spp. were isolated. All samples resulted negative for pathogens. Yeast of the genus *Yarrowia lipolytica*, was identified both on cricket and flour samples.

**4. Discussion and conclusions:** The study contributes to fill the gap in the scientific knowledge of the microbiota of *Acheta domesticum*. *Yarrowia lipolytica* has been shown to be a yeast of high probiotic interest and its presence in the final product suggests its potential added value. The absence of *Staphylococcus* spp. coagulase positive could be a sign of a correct management in compliance with good manufacturing practices. Thanks to the breeding technique and the heat treatment applied, it was also possible to monitor the presence of pathogens relevant to public health, both in the crickets and in the derived flour. These results, together with the significant decrease in the total bacterial and fungal load in the cricket flour, permit to classify this innovative product as a safe novel food and feed.

## ***P 030 – ID 076 - Horizontal gene transfer in the gut of a single patient: bla<sub>VIM-1</sub> carbapenemase in E. coli, K. oxytoca, C. freundii and E. cloacae***

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**Introduction:** The rapid spread of Carbapenem Resistant Enterobacteriaceae (CRE) still is a current worldwide growing public health threat, and plasmid mediated horizontal transfer may account for dissemination of carbapenemase genes carried by CRE strains. The *bla<sub>VIM</sub>* carbapenemase gene was originally detected in Italy from *Pseudomonas aeruginosa* strains. Nowadays, in Italy VIM-producers are the cause of sporadic outbreak and limited regional spread, while KPC is the most diffused carbapenemase in CRE, since it is endemic and with a nation-wide distribution. In the present case we report about the *bla<sub>VIM-1</sub>* gene found in four different bacterial species in the gut of a single patient.

**Material and methods:** The complete genomes of the four strains were obtained and analysed for in silico identification of multilocus sequence types, resistance, plasmid, and virulence gene content of the different bacterial recipients. The IncA plasmid was completely assembled and compared with ten other plasmids of the same family isolated in Italy or in other countries for phylogenetic analysis.

**Results:** The *bla<sub>VIM-1</sub>* gene was found as an integron-borne gene cassette located on an IncA plasmid in three bacterial strains. These strains were identified as *Escherichia coli* ST349, *Citrobacter freundii* ST18 and *Klebsiella oxytoca* ST35 by multilocus sequence typing. The IncA plasmid harboured other resistance genes such as *bla<sub>SHV-12</sub>*, *sul1* and *strA*, *strB*, *sul2*, *mph(A)*, *aph(3')-XV* and *qnrS1*, conferring resistance to aminoglycosides, macrolides, cotrimoxazole quinolones. In *E. coli* an IncX1 and an IncFIB plasmids were also found. Differently, an *Enterobacter cloacae* ST93 also harboured *bla<sub>VIM-1</sub>* but it was located on its chromosome. This strain also carried IncHI2 and IncL plasmids, encoding the mobile colistin resistance *mcr-9* gene and the carbapenemase *bla<sub>OXA-48</sub>* gene, respectively.

**Discussion and conclusions:** IncA plasmids carrying *bla<sub>VIM-1</sub>* gene have been recently described in other Italian hospitals in Bologna and Naples in *Kluyvera cryocrescens* and *Klebsiella pneumoniae*, respectively.

Even though it is not possible to know exactly which has been the path followed by *bla<sub>VIM-1</sub>* the main hypothesis is that the spread of this resistance gene started from the chromosome of *E. cloacae* and from there it was acquired by a IncA plasmid within the gut of the patient and spread to the other *Enterobacteriaceae*. The IncA plasmid demonstrated a great conjugative ability in given the different species where it has been found, surely it can be defined as an exceptional broad-host range plasmid. This suggest that the novel association of *bla<sub>VIM-1</sub>*-IncA may support a wider spread of this carbapenemase gene in the next future in Italy.

## ***P 031 – ID 077 - Bacterial strains isolated from patients with Urinary Tract Infection: prevalence and antimicrobial resistance pattern.***

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**Introduction:** Urinary Tract Infections (UTI) is the most common and expensive health problem in the world. The treatment of UTIs is difficult due to the onset of bacterial strains resistant to antibiotics. The analysis of antimicrobial susceptibility profiles of the pathogenic strains will improve the choice of antibiotics for the treatment of UTIs. In this study, we estimated the frequency of UTI-associated pathogens and their antimicrobial susceptibility profiles.

**Materials and Methods:** This retrospective study was conducted on 1747 urine samples from patients of UOC University Hospital of Campania “Luigi Vanvitelli” in the Naples from January 2017 to December 2018. Bacterial identification and antibiotic susceptibility testing were performed using Matrix assisted laser desorption ionization - time of flight mass spectrometry (MALDI-TOF MS) and Phoenix BD.

**Results:** Among the 1747 studied patients, 543 (31,1%) were positive and 1204 (68,9%) negative for bacterial growth, respectively. Positive patients 325 (60,1%) are females while 216 (39,9%) are males. With regard to the pathogenic strains, 426 (78,1%) were Gram-negative and 108 (19,9%) were Gram-positive. The most isolated Gram-negative strain is *E. coli* (53,5%) of which 23,8% were strains producing extended-spectrum  $\beta$ -lactamases (ESBL). *K.pneumoniae*, *E.coli*, *P.aeruginosa* and *P.mirabilis*, were the most frequent gram negative isolates, that showed a resistance to most of tested antimicrobials except for Amikacin, Gentamicin, Imipenem, Meropenem, Trimethoprim/Sulfamethoxazole and Piperacillin/Tazobactam too. The highest resistant is the Ampicillin 315 (73,9%) with the increasing resistance of Ciprofloxacin and Levofloxacin 156 (36,6%), which are commonly used as empirical treatment in most of UTIs.

**Discussion and Conclusions.** The aim of the study is to determine the prevalence of the bacterial causative agent of urinary tract infection among different groups in UOC University Hospital of Campania “L.Vanvitelli”. The result, similar to other epidemiological study, show that a prevalence of isolates was belonged to Gram negative bacteria. *E. coli* were the most prevalent but also *K. pneumoniae* and *P. aeruginosa* were isolated. This finding was in agreement with the common knowledge about the causative agents of UTI and the relative profile of resistance. In this view for the treatment of urinary tract infections is preferable to use antimicrobial drug that demonstrated high sensitivity, with restriction to some of them adverse effects. Moreover, further studies in our UOC and also the knowledge of the antimicrobial stewardship program are imperative to highlight the emergence of multi-drug resistance among clinical bacterial species.

## ***P 032 – ID 078 - Molecular typing of ceftazidime-avibactam resistant Klebsiella pneumoniae strains isolated from hospitalized patients***

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**Introduction:** Carbapenem-resistant Enterobacteriaceae (CRE) have emerged as major pathogens worldwide. In Italy, production of *Klebsiella pneumoniae* carbapenemases (KPCs) is the predominant mechanism of carbapenem resistance, especially associated to *Klebsiella pneumoniae*. Since its approval in 2015, ceftazidime-avibactam (CAZ-AVI) has been successfully used for treating complicated KPC-*K. pneumoniae* infections, until increasing reports of resistance, due to mutations in the conserved omega loop of the KPC gene, began to emerge. The objective of the study was to investigate the resistance mechanism to CAZ-AVI of clinical *K. pneumoniae* isolates collected from different hospitals in Rome during a six-month survey.

**Materials and methods:** Between December 2018 and May 2019, 10 CAZ-AVI resistant KPC-*K. pneumoniae* strains were isolated from five different hospitals in Rome. Identification and antimicrobial susceptibility testing were performed using the MALDI-TOF MS (bioMérieux, France) and Phoenix system (Becton Dickinson Diagnostics, USA). MIC breakpoints were interpreted according to EUCAST recommendations.

Carbapenemase production was confirmed with agar diffusion assays with phenyl boronic acid (Liofilchem) followed by molecular screening by RT-PCR for KPC, VIM, NDM, and OXA-48 genes (Progenie Molecular). The sequence type (ST) of isolates was determined by traditional Multi Locus Sequence Typing of seven *housekeeping* genes; the KPC alleles were investigated by Sanger sequencing.

**Results:** Typing performed by MLST revealed that 8 *K. pneumoniae* isolates belonged to ST512, one to ST307 and one isolate to a new ST. Phenotypic characterization of the strains showed an MDR profile with resistance to CAZ-AVI with MICs ranging from 12 to 128 mg/L.

Molecular characterization of 5 strains showed a mutation in the omega loop of KPC enzymes; four of them did not produce carbapenemases, despite the presence of the gene and were susceptible to imipenem and meropenem. The remaining 5 CAZ-AVI resistant strains harboured a wild-type KPC-3 gene and were resistant to all carbapenems.

**Discussion and Conclusions:** The therapeutic options available for the treatment of serious infections sustained by KPC-*K. pneumoniae* are limited and resistance to CAZ-AVI represent today a global challenge. Taken together, our results support the observation that the omega loop of KPC enzymes plays a key role in the emergence resistance to CAZ-AVI. Further analyses on porin alterations (OmpK35 and OmpK36) are needed to identify possible mutations involved in CAZ-AVI resistance in the wild-type KPC-3 producing isolates.

## ***P 033 – ID 085 - Enterococcus spp. from wild mammals of Apuan Alps Regional Park (Tuscany, Italy): antibiotic susceptibility and virulence genes***

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Today, antibiotic resistance (AMR) represents one of the main concerns for public health. While the number of studies concerning farm animals is consistent, few researches has been carried out in wild animals. The aim of the study was to assess the antibiotic susceptibility and virulence factors of enterococci from wildlife ranging in the Apuan Alps Regional Park, Tuscany (Italy).

*Enterococcus* spp. isolates were obtained on Slanetz and Bartley agar and identified by MALDI-TOF mass spectrometry (MS). For the isolates with a log(score) value lower than 2,000, additional identification analysis were performed (16S rRNA and rpoA gene sequencing and analysis of 16S-23S rDNA regions). Isolates were then characterized for their antibiotic susceptibility (determination of MIC values), and the presence of antibiotic resistance and virulence factors genes by PCR. From the 36 fecal samples, most belonging to canids (wolves and foxes), 52 isolates were obtained and classified as *E. faecium* (46% of isolates), *E. hirae* (19%), *E. faecalis* (13%), *E. gallinarum* (8%), *E. casseliflavus* (6%), *E. durans* (4%), *E. mundtii* (2%), and *E. canintestini* (2%). The most frequent resistances were observed against tetracycline (36.5%), ciprofloxacin (36.5%) and erythromycin (25%). Three isolates showed high level of resistance to vancomycin and teikoplaine (MIC $\geq$ 1024 microg/ml), and 15% of the isolates demonstrated multidrug-resistance. Most of the enterococci showed a tiamulin MIC value  $\geq$ 32 microg/ml. Resistance to ampicillin, linezolid and streptomycin was not detected. Among resistance genes, *aac(6)-Ii* (50% isolates), *msrA/B* (48%), *msrC* (42%), and *tetM* (35%) were most frequently identified. The occurrence of the *tetL*, *ermB*, *cat*, *aph(3')-IIIa*, *aac(6')-Ie-aph(2'')-Ia*, *ant(4')-Ia*, and *ant(6)-Ia* genes was sporadic. *VanA*, *vanB*, *vanC1/C2* or *vanM* gene were not detected in glycopeptide-resistant isolates; 29% of the isolates contained the Tn916/Tn1545 transposon integrase gene. All *E. faecium* and *E. faecalis* isolates were positive for the *efaAfm* and *efaAfs* genes, respectively. Other virulence-associated genes, i.e. *gelE*, *cylA*, *asa1*, *esp*, and *ace* occurred mainly in *E. faecalis* isolates.

Obtained results indicated that wild mammal might act as reservoirs of resistance and virulence genetic determinants. Most of the analyzed samples belonged to canids, top predator carnivorous species, able to disperse microorganisms across long distances. Indeed, due to the reduction of natural prey, farm animals become an important nutritional source, pushing predators toward anthropized settlements, with public health implications. Additional analysis, such as MLST could be performed on representative *E. faecalis* and *E. faecium* in order to assess the diversity of the isolates and understand their origin.

## ***P 034 – ID 087 - “Green synthesis” of silver nanoparticles: characterization and in vitro antibacterial activity against P. aeruginosa and S. pseudintermedius isolates***

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**Introduction:** Over the last few years metal nanoparticles (NPs) have been studied as alternative methods to fight against infectious diseases. “Green synthesis” of silver nanoparticles (AgNPs) is a fast-growing area of nanoscience research. AgNPs can be used for multifunctional bio-application such as an antibacterial, antifungal and antiviral. The aims of this study were the production of AgNPs using green synthesis methods and the determination of their antibacterial and anti-biofilm ability against *P. aeruginosa* and *S. pseudintermedius* isolates.

**Materials and Methods:** AgNPs were biosynthesized using two eco-friendly methods: an infusion of *Curcuma longa* and the culture supernatant of *E. coli*. The reduction of  $\text{Ag}^+$  to  $\text{Ag}^0$  was monitored using UV-vis spectra analysis and the distribution of the nanoparticles was determined by TEM (Transmission Electron Microscopy). Ten isolates of *P. aeruginosa* and ten of multidrug resistant *S. pseudintermedius* were enrolled in this study to determine the antibacterial activity of AgNPs. Purified nanoparticles from *C. longa* (ClAgNPs) and *E. coli* (EcAgNPs) were used alone and in combination with carbenicillin and ampicillin in the Kirby-Bauer disk diffusion assay. The minimum inhibitory concentration (MIC) of both ClAgNPs and EcAgNPs was determined using microdilution method. Mature biofilm, grown in 96-wells plate, from both *Staphylococcus* and *Pseudomonas* strains was exposed to NPs.

**Results:** The ultraviolet-visible spectrum analysis revealed a maximum absorption peak around at 440 nm for both ClAgNPs and EcAgNPs confirming the synthesis of metal nanoparticles. TEM showed mean diameter and standard deviation of  $11.107 \pm 2.701$  nm and  $27.282 \pm 2.48$  nm for ClAgNPs and EcAgNPs respectively. All the *Pseudomonas* strains were resistant to carbenicillin, ClAgNPs and EcAgNPs alone showed a mean inhibition halo of 9.5 and 14.4 mm respectively resulting in statistically difference if compared to carbenicillin+ClAgNPs (14 mm) and carbenicillin+EcAgNPs (17.45 mm). Staphylococcal strains were resistant to ampicillin and significant differences were found between ClAgNPs and EcAgNPs alone (9.75 mm and 16.40 mm) and ampicillin+ClAgNPs (16.21 mm) and ampicillin EcAgNPs (21.38 mm) halos. The MIC of the ClAgNPs against *P. aeruginosa* and *S. pseudintermedius* was 71 nM and 140 nM respectively. Nanoparticles from *E. coli* showed lower MIC of 0.39 nM and 3.73 nM for *Pseudomonas* and *Staphylococcus* respectively. Anti-biofilm properties were displayed by both ClAgNPs and EcAgNPs only in *Pseudomonas* strains.

**Discussion and Conclusions:** These results confirmed the potential antibacterial ability of AgNPs used alone or in combination with antibiotics, suggesting their potential application for the treatment of infectious diseases caused by MDR bacteria too.

## ***P 035 – ID 091 - Quaternized chitosan derivatives as innovative antimicrobial strategy with antibacterial, antibiofilm and anti-virulence properties***

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**Introduction:** In the era of antimicrobial resistance, identification of new antimicrobials is a research priority at the global level. In this regard, the attention towards functional polymers, with biomedical/pharmaceutical grade, having anti-infective properties has recently grown. Their application may help against multidrug resistant infections, and serve as local delivery systems for active agents. Among those, chitosan (CS), a biopolymer of marine origin, has significant antimicrobial properties, is biocompatible and biodegradable. However, its properties are mostly limited to pH values below 6, due to its poor water solubility at physiological pHs. To overcome this limit, chitosan derivatives, in particular quaternary ammonium ones, have been synthetically obtained having water solubility irrespective of pH, muco-adhesive properties and wound healing promotion features. The aim of this study was to evaluate the antibacterial and antibiofilm activities of new quaternized CS derivatives against *P. aeruginosa* and *S. epidermidis* as well as their anti-virulence properties towards *P. aeruginosa*.

**Materials and Methods.** Low (QAL) and high (QAH) molecular weight quaternized chitosan derivatives were synthesized and their antibacterial activity evaluated as MIC and MBC. Ability of the derivatives to prevent biofilm formation was assessed by crystal violet staining. The antivirulence effects of CS derivatives were evaluated quantifying, pyoverdine, proteases and the staphylolysin LasA in supernatants of stationary-phase cultures of *P. aeruginosa* exposed to sub-inhibitory concentrations of the compounds.

**Results.** Both QAL and QAH derivatives exerted a bactericidal and/or inhibitory activity on the growth of *P. aeruginosa* and *S. epidermidis*. The same compounds also showed marked dose-dependent anti-biofilm activity. Furthermore, the high molecular weight (QAH) derivative was used to functionalize titanium plates. The successful functionalization, demonstrated by electron microscopy, was able to inhibit partially the adhesion of *S. epidermidis* at 6 hours of incubation. Interestingly, unlike CS, the CS-derivative QAL was also able to markedly reduce the production of relevant virulence factors (e.g. LasA and proteases) in a clinical isolate and in the reference strain ATCC 27853 of *P. aeruginosa*.

**Conclusions.** Antivirulence drugs are likely to have distinct properties from those of antibiotics, including reduced resistance induction and minimal perturbation of the healthy microbiota. The demonstrated ability of the CS-derivatives tested to both inhibit bacterial growth and suppress the production of relevant virulence factors reveals their potential as multifunctional molecules against multidrug resistant infections.

## **P 036 – ID 092 - Development of an *in vitro* gut microbiota model**

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**Introduction:** The use of *in vitro* models of the gut microbiota is deemed essential to study the dense network of interactions between microorganisms and analyze the impact of positive or negative perturbations on microbial balance. In this study, a 3D *in vitro* model of the gut microbiota was reproduced on electrospun gelatin structures.

**Materials and Methods:** *In vitro* bioengineered gut microbiota was developed by growing fecal microorganisms on 3D gelatin structures. These structures were created by electrospinning of gelatin, acetic acid, and (3-Glycidoxypropyl)trimethoxysilane with a Linari apparatus. The gut microbiota was recovered from a stool sample of a selected healthy donor treated according to the European guidelines for fecal microbiota transplantation. Scaffolds were incubated with an aliquot of filtered fecal suspension at 37°C in anaerobic atmosphere for up to 7 days. At different time intervals, samples were analyzed by confocal laser scanning microscopy (CLSM) of DAPI-stained preparations with and without rodamine, as contrast staining, and by the crystal violet 0.1% adhesion assay. Microbial communities grown on the gelatin structures were evaluated in composition and amount by 16S metagenomic sequencing and Real-Time quantitative PCR.

**Results:** Gut microbiota was able to adhere to the scaffolds, proliferate, and form long-lasting biofilms. Quantitative biofilm assays revealed a stable and increasing adhesion of the microbiota on the structures compared to the controls. CLSM showed the spatial disposition of microbial populations, both on surface and in the entire thickness of the structures. Microbial composition analysis demonstrated that most microbial communities are conserved in the bioengineered microbiota compared to the fecal sample, with the exception for a significant increase in the number of microorganisms belonging to the *phylum Proteobacteria*.

**Discussion and Conclusions:** Due of the stability of the microbial consortia grown on the scaffolds, electrospun gelatin structures can constitute a valid substrate to reproduce the 3D microbial gut environment. All microbial *phyla* of the fecal microbiota persist during incubation, despite small qualitative and quantitative fluctuations in genera and species can be observed. This model appears useful for studying microbial interactions in the gut and can represent a good platform for microbiota investigations under variable conditions in complex systems, like bioreactors.

## **P 037 – ID 096 - Activity of carvacrol against planktonic and biofilm growth of drug-resistant rapidly growing mycobacteria**

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**Introduction.** Rapidly growing mycobacteria (RGM) are a subset of non-tuberculous mycobacteria (NTM) divided into six major groups, including the *Mycobacterium chelonae*/*M. abscessus* complex, *M. fortuitum* group, *M. smegmatis* group, *M. mucogenicum* group, *M. mageritense*/*M. wolinskyi* group and the pigmented RGM. They are environmental bacteria found worldwide with a propensity to produce skin and soft-tissue infections. *Mycobacterium chelonae*, *M. abscessus* and *M. fortuitum* represent above 80% of clinical RGM isolates. The resistance of RGM to antibiotics and ability to form biofilm contribute considerably to the treatment failure. The goal of the present study was to evaluate the antimicrobial activity of carvacrol (CAR), either in liquid and vapour phase, against different species of drug-resistant RGM. Moreover, the effect of CAR against RGM species capable to form biofilm was also evaluated.

**Materials and Methods.** The susceptibility of RGM isolates [*M. abscessus* (n = 7), *M. chelonae* (n = 1), *M. fortuitum* (n = 1), *M. mucogenicum* (n = 1), *M. smegmatis* (n = 1)] to eight antibiotics and CAR was assessed by MIC/MBC evaluations using microdilution methods in according to Clinical and Laboratory Standards Institute guidelines. The activity of CAR vapours was evaluated with an invert Petri dishes method. The effect of CAR on biofilm formation and preformed biofilm at two different maturation stages (4- and 8-days old) was measured by evaluation of planktonic bacterial growth, biofilm biomass and biofilm metabolic activity.

**Results.** The results of susceptibility tests showed that RGM strains were resistant to most antibiotics assayed. MIC values of CAR were equal to 64 µg/mL for 7 out of 11 RGM isolates and ranged from 32 to 512 µg/mL for the remaining four *M. abscessus* isolates. The MBCs were 2-4 times higher than MICs and MICs of vapours were lower than those in liquid phase (16 µg/mL for 10 out of 11 RGM strains and 64 µg/mL for one *M. abscessus* strain). Regarding the biofilm, CAR at concentrations of  $1/2 \times \text{MIC}$  and  $1/4 \times \text{MIC}$  showed a strong inhibition of biofilm formation (61-77%) and at concentration above the MIC ( $2-8 \times \text{MIC}$ ) produced significant inhibition of 4- and 8-day preformed biofilms.

**Discussion and Conclusions.** CAR could have the potential for implementation of strategies for treating RGM strains also in a sessile lifestyle and offers interesting applicative prospects related to its volatility such as diffusion and penetration into inaccessible areas.

## ***P 038 – ID 098 - Insights into the periplasmic proteins of *Acinetobacter baumannii* AB5075 and the impact of imipenem exposure: a proteomic approach***

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1. Introduction Carbapenem-resistant *Acinetobacter baumannii* strains cause life-threatening infections due to the lack of therapeutic options. Since the periplasmic space contains carbapenemases as well as proteins with crucial cellular functions, we have studied the periplasmic proteome of the multidrug-resistant (MDR) *A. baumannii* AB5075 strain, grown in the absence and presence of imipenem (IMP).
2. Materials and Methods The periplasmic proteins identification was achieved by combining mono-dimensional protein gel electrophoresis with nano-High-resolution Liquid Chromatography - ElectroSpray Ionization-tandem mass spectrometry (nano-HPLC-ESI-MS/MS). Semi-quantitative analysis on common proteins was performed using exponentially modified protein abundance index (emPAI) values.
3. Results and Discussion We identified 65 unique periplasmic proteins common in both growth conditions and among them 8 were differentially expressed. These proteins belonged to protein fate, response to oxidative stress, antibiotic-resistance, energy metabolism and uncharacterized proteins. Among those involved in protein fate, two proteins presented the tetratricopeptide repeat motif, mediating protein-protein interactions. ABUW\_1746 gene product was significantly overexpressed in LB and downregulated in the presence of IMP, whereas ABUW\_2363 showed the opposite expression profile. This expression switch suggests the need for different protein interactions to respond to changed environmental conditions. Also the heat shock protein, encoded by ABUW\_2868, likely involved in response to oxidative stress, was significantly upregulated in bacteria cultured in the presence of IMP. The upregulation of ABUW\_2868 gene product as well as other protein(s) conferring protection against oxidative stress under IMP exposure was confirmed also by an antioxidant activity assay.
4. Conclusions Overall, this study provides the first insights about the composition of the periplasmic proteins of a MDR *A. baumannii* strain, its biological response to IMP and suggests possible new bacterial targets to develop alternative antibiotic drugs.

## ***P 039 – ID 103 - Microbiological analysis of port catheters explanted from patients and comparison of methods to recover microbial cells from infected catheters***

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**Introduction:** Implanted port catheters are important vascular access devices for patients with malignancies requiring long-term chemotherapy. They are considered to be safe and reliable, but may lead to major complications including infections and thrombosis. Laboratory criteria for the diagnosis of catheter-related blood stream infections (CRBSI) are precise, but differences in definitions and methodologies among various studies have made data difficult to compare. In the present study, we compared three different procedures to recover bacterial/fungal cells from infected catheters and to perform a microbiological analysis of port catheters explanted from patients to determine the type of microorganisms involved and the part of the device being the probable source of infection.

**Materials and Methods:** Anesthesiology and Pain Therapy Unit of Pisa University Hospital provided the port catheters removed from patients with suspected infection (n=18) or for end-of-use (n=7), after a period ranging from 26 days to 7 years. The port reservoir, the septum, and the catheter tip were separately subjected to different treatments for the recovery of adherent microorganisms: a) flushing of the catheter lumen, b) sonication and flushing, c) treatment with DTT and flushing. An *in vitro* model of catheter infection with *Staphylococcus epidermidis* was also developed to compare the 3 proposed methods.

**Results:** The results obtained demonstrated that the simple "flushing" of the catheter lumen allows the recovery of a number of microorganisms comparable to that of more complex methods such as sonication or treatment with DTT. More than half of the port catheters removed due to suspected infection (10/18) were culture-negative, despite the positivity of blood culture, suggesting that the blood stream infections were not port catheter related. Some catheters removed due to suspected infection gave positive results to culture of the septum and reservoir, but not of the catheter tip. This suggests that the reservoir was the probable source of infection. 3/7 catheters removed for end-of-use were infected (tip and/or reservoir) suggesting that colonization may occur without symptoms. The most frequently isolated microorganisms were *Staphylococcus spp.* and *Candida spp.*

**Discussion and conclusions:** When a venous access port catheter is removed because of suspected CRBSI, sending the port reservoir in addition to the catheter tip to the microbiology laboratory may increase the sensitivity of diagnostic procedures. The finding that 56% of the removed port catheters were culture-negative suggests to evaluate carefully the paired blood cultures from peripheral vein and port catheter prior to the decision to remove the catheter.

Funded by University of Pisa, Project PRA 2017\_18.

## ***P 040 – ID 106 - Bordetella pertussis antigenic diversity following the introduction of acellular pertussis vaccines, Italy.***

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**Introduction.** Antigenic divergence among *Bordetella pertussis* strains has been described in several countries where acellular pertussis vaccines are widely used, leading to the supposition that the bacterial population could adapt to substantial vaccine-induced immune pressure. In Italy, acellular pertussis vaccines replaced the whole-cell vaccine in the late '90s with high vaccine coverage. The aim of this study was to explore the antigenic repertoire of *B. pertussis* isolates collected in Italy before and after the introduction of acellular pertussis vaccines, with particular regard to the population at greatest risk of severe disease.

**Materials and Methods.** Fifty-eight Italian *B. pertussis* isolates, causing disease among babies less than 6 months of age, were included in the study; of these, 19 were collected in 1994 (pre-acellular pertussis vaccine era, wP) and 39 in 2013-2018 (post-acellular pertussis vaccine era, aP). Genotyping of the acellular vaccines antigens was performed using the Multilocus Antigen Sequence Typing (MAST). Pertactin deficiency was evaluated through indirect ELISA and/or whole *prn* gene sequencing.

**Results.** The wP group showed the *ptxP1/ptxA1/fim2-1/fim3-1* profile. No pertactin deficient strains were detected; three *prn* alleles were identified and equally distributed: *prn1* (32%), *prn2* (36%) and *prn3* (32%). The aP group showed the *ptxP3/ptxA1/fim2-1* profile and two different *fim3* alleles: *fim3-1* (85%) and *fim3-2* (15%). The proportion of pertactin-deficient isolates reached the value of 62% and *prn2* represented the unique allele among the remaining. The molecular mechanisms that affected pertactin expression included: the introduction of a premature stop codon (48%), the insertion of IS481 element (43%) and the disruption of the promoter region (9%).

**Discussion and Conclusions.** The genetic shift here described, in line with that observed internationally, emphasize the need to deeper investigate strain diversity to better understand the *B. pertussis* evolutionary patterns during the time.

## ***P 041 – ID 107 - Correlation between tcdB gene PCR cycle threshold and severe Clostridium difficile disease***

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**Introduction:** *Clostridioides difficile* is one of the most common causes of hospital acquired infections, accounting for 15-25% of all antibiotic associated diarrhea. The choice of the optimal test to diagnose CDI infection is still controversial. The European guidelines advise to include toxin EIA in the diagnostic algorithm, while the results obtained by different studies suggest an approach based on NAAT stand-alone test: as a low cycle threshold (C<sub>T</sub>) in real time PCR, corresponding to higher bacterial loads, correlates with the presence of free toxins. Since 2015, our center has been using NAAT (GeneXpert) assay as a single test for assessing CDI; our study proposes to correlate the C<sub>T</sub> values deriving from PCR amplification of the tcdB gene and the level of infection in order to optimize patient management.

**Materials and Methods:** The degree of disease severity was defined upon the combination of the recommendations of ESCMID and IDSA/SHEA: mild to moderate disease with leukocyte count <15000 cells / ml and creatinine <1.5 times the premorbid level; severe disease with WBC > 15000 cells / ml, serum creatinine > 1.5 times the premorbid level and serum albumin level <3 g / dl. Between February 2015 and August 2018, 4441 stool samples with Bristol scales between 5 and 7 were tested on arrival in the laboratory using the GeneXpert test.

**Results:** 421/4441 (9.4%) samples were positive for the tcdB gene and were included in the study. Among these, in 133 samples (31.5%) the gene encoding the binary toxin was detected. Among 421 patients, 222 and 199 were classified as having mild / moderate and severe disease, respectively. Analysis of the data showed that there is a statistically significant correlation between C<sub>T</sub> values of tcdB PCR (<25) and patients with severe disease (P = 0.0075), while higher C<sub>T</sub> values (> 25) are correlated with patients with mild / moderate disease (P = 0.0075). A significant difference was found between mean age and C<sub>T</sub> values <25 (79) and > 25 (74) (P = 0.004). The mean C<sub>T</sub> values of tcdB PCR in patients with mild / moderate disease were significantly higher (28.1) than in patients with severe disease (25.9) (P = 0.00001). We found a statistically significant correlation with leukocytosis (tau = -0.131; P <0.001), but not with the alteration of creatinine levels or serum albumin level.

**Discussion and Conclusions:** In conclusion, we suggest that a C<sub>T</sub> value of ≤26 could be used as a surrogate for fecal-free toxins and could be used, complemented by a picture of leukocytosis, to assess the severity of the disease and to guide the treatment of patients with CDI.

## ***P 043 – ID 111 - Identification of seven sexually transmitted infectious agents by the Anyplex II STI-7e multiplex real-time PCR in urogenital samples from patients attending Pisa University Hospital***

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**Introduction:** Annually there are around 376 million new cases of four curable sexually transmitted infections (STIs) worldwide among people aged 15-49 years: *Chlamydia trachomatis* (CT, 127 million), *Neisseria gonorrhoeae* (NG, 87 million), syphilis (6 million), or *Trichomonas vaginalis* (TV, 156 million). These epidemics have a profound impact on the health and lives of population and in particular CT and NG are important causes of infertility worldwide. The majority of STIs have no or only mild symptoms that may not be recognized. Moreover, some STI pathogens are difficult to culture and multiple STI are rather common. Thus, methods based on multiplex PCR which allow for simultaneous detection of multiple pathogens in the same sample were recently being increasingly in use for the diagnosis of STIs. We aimed to evaluate the prevalence of seven STI pathogens and coinfection rates in samples from in and out-patients of Pisa University hospital.

**Materials and Methods:** The presence of CT, NG, TV, *Mycoplasma hominis* (MH), *M. genitalium* (MG), *Ureaplasma urealyticum* (UU), and *U. parvum* (UP), were assessed in 3308 samples (52.4% from females, 47.6% from males) arrived at the Microbiology Unit of Pisa University hospital from June 2017 to June 2019. Anyplex II STI-7e multiplex real-time PCR assay based on TOCE technology was used for identification. Such assay makes it possible to detect seven pathogens and a cellularity control in a single fluorescence channel on real-time PCR instrument. Flocked swabs and a transport medium were used to collect the urogenital samples while the urine or sperm samples were collected in sterile containers.

**Results:** Around 30% of the total samples were positive for at least one pathogen and in almost 20% of the positive samples a coinfection with 2 or 3 pathogens was observed. Interestingly, female patients had significantly higher number of positive samples (38.7%) compared to males (17.9,  $P < 0.01$  Chi-Square). UP was the most frequent pathogen identified (20% of all patients, 31% of females and 8% of males), followed by UU and MH. CT was the 4<sup>th</sup> frequent STI pathogen (2.1% overall, 2.7% in males, 1.6% in females). MH&UP (females) and MH&UU (males) were the pathogens causing the most frequent coinfections. Endocervical swab represented the most positive sample type.

**Discussion and Conclusions:** Female patients had more than twice STI compared to male patients, which was mainly attributable to higher presence of urogenital mycoplasmas/ureaplasmas. This result may in part be due to presence of ureaplasma/mycoplasma in the female urogenital tract as commensals. The finding of 20% coinfection in positive samples encourage the use of multiplex assays to assess the presence of multiple STIs in one sample regardless of specific symptoms.

## ***P 044 - ID 113 - Anti-bacterial activity against methicillin-resistant and -susceptible *Staphylococcus aureus* strains of extracts from the kurdish medicinal plant *Onobrychis carduchorum****

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**1. Introduction.** Due to the increase of numerous drug resistant strains there is an urgent need to develop new antimicrobial agents. *Staphylococcus aureus* is one of the most important human pathogens, although it is part of the normal human microflora. Methicillin resistance development in *S. aureus* strains represents a huge problem worldwide. Both in developing and industrialized countries the use of medicinal plants is a valuable resource of alternative and natural compound with antibacterial activity. *Onobrychis carduchorum* Taumsend, belonging to the family of Fabaceae, is a widely employed plant in the Kurdish traditional medicine to cure inflammations and other skin diseases.

**2. Materials and Methods.** Forty four *S. aureus* strains isolated from different human sample were identified and characterized for their antibiotic resistance profile using the VITEK® 2 automated system. Secondary metabolites were isolated from the acetone extract of leaves and flowers of *O. carduchorum*. The inhibition efficacy of the produced extracts and Vancomycin was tested against *S. aureus* NCTC6571 and clinical isolates using paper disk diffusion assay.

**3. Results.** Among the forty four *S. aureus* strains, characterized for their antibiotic resistance profile, 41% were methicillin resistant. Ten different phenolic metabolites were isolated from the acetone extract and resulted belonging to three different classes: 1. iso-flavones, 2. flavanones and 3. dihydro-stilbenes. Some of the isolated compounds have a strong inhibition activity on the growth of *Staphylococcus aureus*. In particular, compound 1 shows a growth inhibitory activity that was comparable to that of vancomycin.

**4. Discussion and Conclusions.** The above compounds have been found to date, mostly in other Fabaceae, as *Glycyrrhiza glabra*. However, not much is known about their bioactivities. Here we report the antibacterial activities of these new compounds, even if with preliminary results. The basic idea is the use of these data for the development of new antibacterial formulation for the treatment of *S. aureus* infections. Clearly, it is necessary to implement the study to better understand the biological activity of these compounds and to characterize their safety.

## ***P 045 – ID 118 - Evaluation of the Sepsis Flow Chip kit for detection of multidrug-resistant bacteria***

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**Introduction:** The increased incidence of systemic infections caused by multidrug-resistant (MDR) bacteria is a global health worrisom. The implementation of new tools in the microbiological diagnosis with the ability to detect rapidly and efficiently these organisms is a necessity to prevent spreading of these in the community and among hospital patients. The aim of this study was to evaluate the reliability, accuracy, and usefulness of the commercial Sepsis Flow Chip kit (SFC) for the detection of MDR bacteria.

**Materials and Methods:** A collection of 60 clinical samples, in particular 27 oropharyngeal swabs (OS), 27 nasal swabs (NS), 6 rectal swabs (RS) from hospitalized patients (University Hospital Federico II), were analyzed with the Sepsis Flow Chip (SFC) kit. The SFC (Master Diagnostica, Spain) is a qualitative molecular diagnostic test based on DNA Flow Technology and consisting of the simultaneous detection of at least 36 bacteria species, fungi species, and 20 resistance markers by multiplex-PCR followed by automatic reverse dot blot hybridization to specific DNA probes immobilized onto a nylon membrane. The kit includes, in a single assay, the detection of the main resistance genes in gram positive bacteria: *mecA* for *S. aureus* and CoN-Staphylococci; *vanA-vanB* for *Enterococcus* spp., and those in gram negative bacteria: two extended spectrum beta-lactamases (ESBLs) and fifteen Carbapenemases (CPMs).

**Results:** After processing the 60 samples by SFC system, 24 out of 27 OS samples were identified as positive and 3 OS as negative. The most frequently identified microorganims were *Streptococcus* spp. (24/24 samples) followed by *Streptococcus pneumoniae* (1/24 samples). The same patient was positive for *S. pneumoniae* also in the nasal swab, the remaining NS were negative for all pathogens included in the SFC. Two out of 6 RS samples were identified as positive. The identified microorganims were *Escherichia coli* and *Pseudomonas aeruginosa*. Resistance gene, ESBL (CTX-M), was also detected in RS while no positive cases were detected for carbapenemase genes. SFC kit demonstrated a 100% concordance with the results obtained by standard clinical microbiological protocols.

**Conclusion:** SFC is a reliable, fast (<4 hours), robust, and automated single test for simultaneous identification of the most important human pathogens and their resistant forms in hospital acquired infections. Rapid pathogen detection by molecular diagnostic tools may facilitate the rapid diagnosis. Moreover, the simultaneous detection of multidrug-resistant (MDR) strains will lead to an earlier administration of efficient antibiotic therapy. Further retrospective and prospective clinical validation of this new assay is currently under process.

## **P 046 – ID 120 - Clinical sensitivity evaluation of the EuSepScreen Plus kit in the diagnosis of *Streptococcus pneumoniae* infection in subjects with otitis media**

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**Introduction:** Otitis media (OM) comprises a heterogeneous group of inflammatory disorders affecting the middle ear. With over 700 million cases annually, OM is the most common reason for pediatric emergency room visits worldwide. OM generally affects children and it is characterized by a sudden onset of symptoms, significant pain, and signs of inflammation with accumulation of purulent fluid behind the tympanic membrane. One of the most principal etiological agent of OM is *Streptococcus pneumoniae*. The aim of the present study was the validation of the clinical sensitivity of the EuSepScreen Plus kit (Eurospital) in the diagnosis of *S. pneumoniae* infection by DNA extracted from oropharyngeal swab and Real Time (RT)-PCR in subjects suffering OM.

**Materials and Methods:** Oropharyngeal swabs were collected according to standard procedures, subjects with an age ranging from 5 to 7 years were enrolled for the study. A total of 40 swabs were collected both from patients with OM (n=20) and from healthy controls (n=20), to determine the correct diagnostic and analytical sensitivity of the EuSepScreen Plus kit. The EZ1 DSP Virus handbook kit was used to extract the bacterial DNA from the collected biological samples. This method uses a technology of magnetic particles to absorb the bacterial DNA and release it in the final elution step. The RT-PCR procedure, provides the detection of multiple targets that include *Neisseria meningitidis*, *S. pneumoniae* and human Beta-globin gene as control. The RT-PCR reaction was set up according to the protocol provided by the kit. To each tube were added 37.5 µl of diluent buffer (Reagent B), and 62.5 µl of Reagent A containing the Master Mix for the RT-PCR. Subsequently, 20 µl of each mix was added to the wells of 96-Well Microtiter Plates with 5 µl of each extracted DNA.

**Results:** From the first results obtained it was possible to observe that for healthy controls only 1% was positive for the presence of *S. pneumoniae* DNA. While for the patients with OM, the 40% of subject were positive. The result obtained in this case is in perfect agreement with their clinical picture as it is much more invasive, with the presence of infections at the level of the mastoid and a slight complication of hearing ability.

**Discussion and Conclusion:** The present study, although conducted in a small cohort of patients, confirmed the efficacy of the EuSepScreen plus kit for the detection of *S. pneumoniae* DNA from a polymicrobial biological sample such as the oropharyngeal swab. It would be interesting in the future to analyze these samples through culture assay to verify the possible presence of antibiotic resistance in isolated strains.

## ***P 047 – ID 122 - Evaluation of the effect of HIV infection on Quantiferon-Plus results in patients with active tuberculosis and latent infection***

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**Introduction:** In 2017, WHO estimated that tuberculosis (TB) caused 300,000 deaths among HIV-infected people. Treatment for latent TB infection (LTBI) is strongly recommended in HIV-infected people. The QuantiFERON-TB-Plus (QFT-P), one of the IGRAs worldwide used to diagnose LTBI, measures IFN $\gamma$  after M. tuberculosis-stimulation in TB1 and TB2 tubes containing peptides specific to elicit a CD4- or a CD4- CD8 response respectively. Aim of this study is to evaluate the effect of HIV-infection on QFT-P test in a low TB endemic country.

**Material and Methods:** we prospectively enrolled 167 subjects with HIV-infection (32 with active TB: HIV-TB; 45 with LTBI: HIV-LTBI, 90 without TB infection) and 298 individuals without HIV-infection (170 with active TB disease, 110 with LTBI, 18 without TB infection). Each enrolled patient performed QFT-P.

**Results:** The sensitivity of QFT-P assay calculated in the active TB population was not significantly different in HIV-infected and -uninfected subjects (69% in HIV-TB- and 80% in e TB-patients). Comparing the levels of IFN $\gamma$  response to QFT-P antigens, we observed a lower IFN $\gamma$  level in HIV-LTBI compared to LTBI (TB1 stimulation:  $p=0.004$ ; TB2 stimulation:  $p=0.002$ ). Differently, the level on IFN $\gamma$  in HIV-TB patients was similar to TB patients without HIV infection. In active TB patients we found that the majority of IFN $\gamma$  values fell out of the uncertain range of 0.2-0.7 IU/mL (Nemes AJRCCM 2017) independently of HIV-status (HIV-TB: 82 %; TB: 85%,  $p>0.999$ ). Differently, in the HIV-LTBI group a higher proportion of results were in the uncertain range compared to HIV- uninfected subjects (HIV-LTBI: 24 %; LTBI: 11%,  $p=0.045$ ). Surprisingly, in HIV-TB patients we did not find any association between the CD4 count, ART status and distribution of IFN $\gamma$  results. Evaluating the ability to respond to TB1 and/or TB2, we found that HIV-LTBI has a higher risk to have an “only TB1 response” (odds ratio 5.27, CI: 1.07-25.89  $p=0.04$ ) and higher risk to have discordant results (TB1 positive and TB2 negative or the contrary) (odds ratio 2.79, CI: 1.19- 6.54,  $p=0.02$ ) compared to LTBI. Interestingly, the majority of discordant results had IFN $\gamma$  values falling in the uncertain range.

**Discussion and Conclusions:** HIV infection did not have an impact on the sensitivity of QFT-P in patients with active TB. The CD4 counts and ART status did not influence the distribution of IFN $\gamma$  values in response to QFT-P in HIV-infected patients with active TB. Notably, HIV-LTBI has a higher number of results falling in the uncertain zone compared to LTBI. More studies are necessary to understand if it is needed a different cut-off to detect LTBI in HIV-infected subjects.

## **P 048 – ID 128 - Antimicrobial photodynamic therapy against endodontic *Enterococcus faecalis*.**

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**Introduction.** Light Emitting Diodes (LEDs) are complex semiconductors that convert electrical current into incoherent narrow-spectrum light. D’Ercole et al. have shown that the concurrent treatment for 5 min with 1.0% sodium hypochlorite (NaOCl) and Near-Infrared (NIR)-LED is able to significantly decrease *Enterococcus faecalis* viability, *in vitro*. Some authors have claimed that from irradiated sites bacteria were able to regrowth and reestablish a new infection after only 24 h. So, it is fundamental to be sure of the stability of the antimicrobial activity of LEDs, in order to permit the long-term success of the treatment. The aim of this study was to monitor the effects of NIR-LED on the *E. faecalis*, along a relatively long time of 1 week.

**Materials and Methods.** *E. faecalis* ATCC 29212 bacterial suspensions were treated with 880 nm NIR-LED irradiation and NaOCl alone and combined to each other, according the following scheme: NaOCl treatment with 0.5%-NaOCl for 5 min; LED-5 with 880-nm NIR-LED irradiation for 5 min; LED+NaOCl contemporary with NIR-LED and 0.5%-NaOCl for 5 min. Treated samples were tested in respect to the controls for (i) colony forming units per milliliter (CFU/mL) for the quantification of cultivable cells and (ii) cells viability by LIVE/DEAD (L/D) analysis. The analysis were recorded at 5 min, 24 h, 48 h and 1 week. The data were submitted to ANOVA and LSD *post hoc* tests at a level of significance of 0.05.

**Results.** All treatments were able to decrease significantly CFU/ml respect to control. In particular, after 5 min, 24 h and 48 h the most effective reduction was measured for NaOCl and NaOCl+LED-5. After 48 h NIR-LED-5 reduced significantly CFU/ml respect to control, but it was higher respect the other groups. On the contrary, after 1 week the samples treated with NIR-LED were characterized by a decrement of CFU/ml. The L/D analysis showed that vitality of control remains constant at all time points. The more significant effect of NIR-LED-5 is visible after 1 week, with the evidence of an aggregate of dead cells. NaOCl shows an evident killing effect at all time points observed. The combination of NIR-LED-5+NaOCl results in a marked killing effect on the population of *E. faecalis* in all measurements made with a progressive aggregation of dead cells.

**Discussion and Conclusions.** The antibacterial effect of NIR-LED treatment is persistent after 1 week from irradiation and seems to increase over time, contrary to the effect of NaOCl. L/D staining shows that NIR-LED irradiation seems to potentiate the killing effect of NaOCl and a marked antibacterial activity is evident. However, NIR-LED-5 is characterized by an antibacterial effect, with a progressive aggregation of dead cells that reach the maximum after 1 week from the irradiation.

## **P 049 – ID 130 - Microbiota characterization of Agnano thermal water**

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**Introduction:** For years, thermal waters have been used to treat a different number of diseases. The benefits depend especially to their chemical and physical characteristics, but their anti-inflammatory and skin regeneration traits cannot be exclusively linked to their mineral composition. For this reason, the aim of our study was to identify non-pathogenic bacteria in the thermal waters, focusing on the presence of some species probably involved in skin regeneration with anti-inflammatory action.

**Materials and Methods:** Agnano thermal water was collected in January 2019. A portion of 100 ml of thermal water was filtered through seven 0.20 µm pore cellulose nitrate membranes. Each membrane was plated on differential and selective media (filtration procedure). Another portion of 10 ml of thermal water was transferred in 90 mL of Brain Heart Infusion (BHI) broth and incubated at 37°C with 220 rpm (enrichment procedure). Once an observable turbidity was obtained, the broth culture was harvested by centrifugation at 3500 rpm for 10' at room temperature and serial dilutions were plated on differential and selective media. The colonies obtained through the filtration and enrichment procedure were stored at -80 °C in BHI broth + 10% glycerol and subsequently analyzed by Matrix Assisted Laser Desorption/Ionization (MALDI) mass spectrometer.

**Results:** Microbiological analysis showed that the isolated bacterial species from Agnano thermal water belong mainly to three different phyla: Actinobacteria, Firmicutes and Proteobacteria. The filtration procedure allowed the isolation of seven bacterial species, while through the enrichment procedure twenty species were isolated, of which two bacterial isolates were also identified through filtration.

**Discussion and Conclusions:** The therapeutic effects of defined spring waters are currently shown to be related not only to their peculiar mineral composition, but also to the complex activity due to their resident non-pathogenic bacterial populations. Although the present study provides only preliminary data, some of non-pathogenic bacterial species isolated from Agnano spring water could be likely to produce molecular mediators involved in skin regeneration process that, thus far, remain unknown. In the future, we will focus our attention on the study of the molecules produced by selected bacteria and will analyze their potential therapeutic effect on *in vitro* cellular systems.

## ***P 050 – ID 135 - Evaluation of a chemiluminescence immunoassay for rapid detection of serological IgG and IgM antibodies of *Treponema pallidum****

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**Introduction:** Syphilis is one of the most important sexually transmitted infections (STIs), being a public health problem both because of the magnitude they reach globally and because of its complications and sequelae. In 2016, 29365 confirmed syphilis cases were reported in 28 European countries, giving a crude notification rate of 6.1 cases per 100 000 population (European Centre for Disease prevention and Control surveillance report 2016) and the male-to-female ratio in 2016 was 7.9:1, with rates of 10.8 cases per 100 000 population in men. For these reasons diagnosis of syphilis must be as accurate and fast as possible. The objective of this work is the evaluation of a new automated technique of indirect chemiluminescence immunoassay (VirClia® by Vircell, Spain), in the detection of separately IgG and IgM class antibodies of *Treponema pallidum* in a population of men who have sex with men.

**Material and methods:** We selected a total of 310 serum samples from men who have sex with men (82 of them HIV positive) with more than 10 different sexual partners/year. Within 24 hours from the collection all samples were processed by VirClia chemiluminescence immunoassay for the detection of IgM and IgG antibodies to *T. pallidum*, Rapid Plasma Reagin (Futura System Group, Italy) and treponemal hemagglutination assay TPHA (Futura System Group, Italy) and syphilis screen antibodies IgG+IgM (Diasorin, Italy). In presence of genital ulcers a swab for *Treponema pallidum* DNA Real Time PCR (Sacace biotechnologies, Italy) was collected.

**Results:** 175 samples were negative to all the tests performed, 130 were positive to IgG, screening and TPHA, 26 all tests positive, 54 had all tests positive except IgM. 4 samples had IgG value in the borderline area and were referred to patients with a diagnosis of syphilis more than 15 years ago. One patient had only a positive IgM value, but his clinical diagnosis of primary syphilis was confirmed by lesional Real time PCR.

**Discussion and Conclusion:** The diagnosis of syphilis in pluripartner men who have sex with men must take place quickly to allow a timely antibiotic therapy aimed to reduce the spread of the disease.

Introducing in luetic serological diagnosis also the dosage of IgG and IgM against *Treponema pallidum* is necessary for a correct diagnostic investigation of the pathology. The assay we tested showed excellent sensitivity and specificity and in one case allowed the serological diagnosis of syphilis in the absence of other positive tests.

## ***P 051 – ID 137 - High Taxonomic Resolution Analysis of Vibrio populations associated with Bivalve tissues by a new target enrichment Next-Generation Sequencing strategy***

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**Introduction:** *Vibrio* are emergent pathogens indigenous in the marine environment that can be transmitted to humans and animals through direct contact or ingestion of contaminated food and water. In particular, consumption of raw or insufficiently cooked filter-feeding bivalve molluscs may result in illness due to the presence of these microorganisms. Detection and enumeration of *Vibrio* species in environmental samples or bivalve tissues is not straightforward due to technical limitations of currently employed methods. In particular, conventional methods for *Vibrio* detection, such as cultivation in association with phenotypic tests or PCR based methods are limited by the time and labour involved in analyzing a large number of species/samples. Lack of phylogenetic value of marker genes (e.g. 16S rRNA) employed in amplicon sequencing analysis as well as difficulties in separation of host DNA from microbial DNA in shotgun metagenomic approaches make the application of NGS molecular techniques also challenging. This research aimed to develop and apply a new molecular approach to comprehensively study *Vibrio* population in complex environmental and animal samples.

**Material and Methods:** A target enrichment next generation sequencing protocol was applied for high taxonomic resolution analysis of the marine *Vibrio* community on a total of 12 *Crassostrea gigas* tissue samples collected at two marine farming sites in Europe. A mock community sample (positive control) and a nuclease-free water sample (negative control) were also included in the analysis. A sequence database containing 884 phylogenetic and virulence markers of the *Vibrio* and marine pathogen community was built and used to produce a total of 12,114 biotinylated 100-mer RNA baits for selective capturing of target DNA via hybridization using the MYbaits technology. The produced enriched libraries were pooled and sequenced on a MiSeq Illumina platform.

**Results:** By applying this new methodological approach several *Vibrio* species were identified in bivalve tissues including the human potential pathogenic species *V. alginolyticus* and *V. toranzoniae*. Other identified species included recognized potential bivalve pathogens (e.g. *V. splendidus*, *V. tasmaniensis* LGP32 strain, *V. crassostreae*), the coral pathogen *V. coralliilyticus* and other *Vibrio* species (e.g. *V. cyclitrophicus*, *V. lentus*, *V. cortegadensis*, *V. pomeroyi* and *V. mediterranei*)

**Discussion and conclusion:** The methodological approach developed in this study allowed, for the first time to our knowledge, high taxonomic resolution analysis (species/strain level) of *Vibrio* populations associated with bivalve tissues. Such approach could have wide application in studies investigating *Vibrio* species in complex environmental and animal samples

***P 052 – ID 142 - Microbiological characterisation of Group A streptococci (GAS) isolated from children with chronic tics and their first grade relatives in the European Multicentre Tics Study (EMTICS).***

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**Introduction:** Tic disorders during childhood have a detrimental effect on quality of life. Genetic and environmental risk factors, including exposure to GAS are poorly understood and were investigated in EMTICS.

**Materials and Methods:** EMTICS comprised two longitudinal cohort studies: COURSE, consisting of 715 tic-affected children, aged 3-16 years, followed for 16 months; ONSET, that included 260 high-risk children (having a first degree relative with a diagnosis of Tourette syndrome and no tics at study entry), aged 3-10 years, followed for three years. EMTICS children were throat swabbed both at enrolment and at planned visits. Swabs were processed following a common validated microbiological protocol for GAS detection and isolation. Microbiological characterisation included *emm* typing, screening of superantigen genes including their allelic variants and of surface protein R28 gene, clonality by MLVA and MLST. Susceptibility to erythromycin and clindamycin was also assessed.

**Results:** GAS positivity largely varied between participating centres ranging from 0% to 47.6%. In total, 296 GAS strains were collected. As a control population, 42 GAS strains from pharyngitis were received and typed. There were not differences in the serotype distribution, virulence and clonal relationships between GAS strains isolated from ONSET and COURSE. In both populations, *emm28* and *emm89* were the most diffuse serotypes. Noteworthy, these *emm* types don't produce capsule because of missense mutations (*emm28*) or absence (*emm89*) of the *hasA* gene. Further unidentified frameshift mutations have been identified in this study. Overall, the incidence of non-capsulated EMTICS strains was 50.7%, more frequent in ONSET than COURSE (55.3% vs 49.7%). The most diffuse serotypes in pharyngitis were the capsulated *emm1* and *emm3*; the proportion of non-capsulated strains was 30.9%. When GAS was isolated during exacerbation of tics symptoms, in severe tic symptoms or persistent throat colonisation, an enrichment of *emm12* strains was noted. The superantigen *speC* gene was abundant in both ONSET and COURSE (68.2% vs 58.9%) but *SpeC1* allele was significantly more present in COURSE (Fisher's exact test: 0.04). Surface adhesin R28 was associated to certain *emm* types (2,28,48,77). Resistance to erythromycin was 9.9% (3.5% and 12.5% in ONSET and COURSE, respectively).

**Discussion and Conclusions:** It is not evaluable how much the different incidences of GAS positive children between participating centres could have affected the microbiological analysis. *emm12*, *speC1* allele and erythromycin resistance were asymmetrically represented in GAS strains from ONSET and COURSE. The large proportion of non-capsulated GAS strains may suggest that carriage more than infection was relevant in EMTICS children.

## ***P 053 – ID 144 - A DNJ (deoxynojirimycin) derivative inhibits both biofilm formation and virulence-factors expression in Staphylococcus aureus***

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**Introduction:** *Staphylococcus aureus*, a major human pathogen associated with refractory biofilm-associated infections, can resist treatment due to the emergence of antibiotic-resistant strains and through biofilm formation. *S. aureus* is also capable of secreting several exotoxins associated with the pathogenesis of sepsis and pneumonia. Iminosugars, structural analogues of carbohydrates in which the oxygen atom is replaced by nitrogen, are currently under investigation as potential therapeutic agents in many human disorders. Very few studies have been dedicated to the effect of iminosugars on planktonic and biofilm cells. The aim of this study was to assess whether a small library of DNJ derivatives, characterized by differences in the total length of the alkyl chain, can prevent the growth of planktonic cells and on the formation *S. aureus* biofilm, and whether it may have synergistic effects with antibiotics *in vitro*. The molecular mechanism of DNJ derivatives activity was also studied.

**Materials and Methods:** A series of N-alkylated iminosugars in both enantiomeric form (compounds L-1 to L-5 and D-1 to D-5) were synthesized. The MIC values were calculated by broth microdilution method. The activity of iminosugars was tested against *S. aureus* biofilms at different stages of development by confocal laser scanning microscopy, crystal violet and tetrazolium salt reduction assay. The transcription of selected virulence-related genes was verified by qRT-PCR.

**Results:** The compound L-1 showed maximum antimicrobial and antibiofilm activities in a dose-dependent way. In addition, it exhibited a synergistic or additive antimicrobial activity with conventional antibiotics against *S. aureus*. Sub-MICs of L-1 exhibited no bactericidal activity against *S. aureus*, but affected *S. aureus* biofilm development in a dose dependent manner, inducing a strong reduction in biofilm biomass. Biofilm thickness and interference with cell-to-cell adhesion were also affected. Transcriptional analysis was used to elucidate the mechanisms responsible for the inhibition of *S. aureus* growth and biofilm formation. At sub-inhibitory concentration, compound L-1 repressed the expression of the several virulence factors of *S. aureus*, including toxins (*hla*, *hly*, *lukD*, *lukE*), and crucial global regulators, such as *RNAIII*, *sarA*, and *saeR*.

**Discussion and Conclusions:** The overall findings suggest that L-1 acts as a good antibacterial as well as antibiofilm agent and may represent a novel potential molecule for treatment of biofilm-related *S. aureus* infections. In addition, compound L-1 could attenuate *S. aureus* virulence by affecting virulence-associated genes.

## ***P 054 – ID 153 - Characterization of a *poxtA*- and *optrA*-co-carrying conjugative multiresistance plasmid from *Enterococcus faecium* of swine origin***

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**Introduction:** The non-mutational resistance mechanisms to oxazolidinones, reported in both animal and human strains, include: modification of the 23S rRNA by the Cfr methylases, resulting in co-resistance to phenicols, lincosamides, oxazolidinones, pleuromutilins, and streptogramins A, and ribosomal protection by ABC-F proteins *OptrA* and *PoxtA* leading to co-resistance to oxazolidinones (including tedizolid), phenicols and tetracyclines (only the *PoxtA* protein). The acquired oxazolidinone-resistance genes are associated with mobile genetic elements, mostly plasmids, which facilitate their dissemination among bacteria and enhance their epidemiological importance. In this work, we studied a porcine *Enterococcus faecium* strain, namely S157, harbouring *optrA* and *poxtA*.

**Materials and Methods:** Antimicrobial susceptibility testing was carried out by broth microdilution. S1-PFGE, southern blotting and hybridization with *optrA*- and *poxtA*-specific probes have been used to determine genes location. Transferability was assessed by conjugation experiments using *E. faecium* 64/3 as recipient. Genetic contexts were analyzed by WGS, Sanger sequencing and PCR mapping.

**Results:** *E. faecium* S157 showed resistance to florfenicol, linezolid, tedizolid and tetracycline, and harbored 4 plasmids ranging from ca. 30 to ca. 145 kb. *poxtA*-probe hybridized with a ca. 30 kb-plasmid, while *optrA*-probe with a ca. 50 kb-plasmid. Interestingly, both probes also hybridized with a plasmid of ca. 100 kb. The three plasmids were detected in all transconjugants. WGS revealed that the *poxtA*-carrying plasmid (31.2 kb) was a mosaic structure derived from the recombination between a portion of the plasmid pF120805 and a chromosomal multi-resistance region of *Erysipelothrix rhusiopathiae*; *poxtA* gene was flanked by two *IS1216* with the same orientation. The *optrA* gene was associated with *fexA* in the ca. 50-kb plasmid resulting from the recombination between two plasmid portions: (i) pW033-13 of *Staphylococcus sciuri*, and (ii) plasmid 5 of *E. faecium*. PCR mapping and sequencing experiments showed that both *poxtA* and *optrA* plasmids recombined to form the ca. 100 kb plasmid.

**Discussion and Conclusions:** In enterococci, antibiotic resistance is mainly linked to the acquisition of resistance genes localized on mobile genetic elements such as conjugative plasmids. It is already known that recombination events occurred between plasmids, especially in the *Enterococcus* spp. In our study, we characterized two conjugative plasmids carrying the oxazolidinones resistance genes *poxtA* and *optrA* that could have recombined to a more complex structure. To the best of our knowledge, this is the first report of the co-location of the oxazolidinone resistance genes *poxtA* and *optrA* on conjugative plasmids in *E. faecium*.

## ***P 055 – ID 156 - Preliminary investigation of Streptococcus salivarius-mediated interference against S. pneumoniae and S. pyogenes***

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**Introduction:** Adherence of *S. pneumoniae* and *S. pyogenes* strains to nasopharynx epithelia represents the first step for their colonization and is considered a prerequisite for infections such as acute otitis media (AOM). In this context, *S. salivarius* 24SMBc, isolated from the nasopharynx of a healthy child, represents an oral probiotic that is well known for its ability to inhibit AOM pathogens. The goal of this work was to investigate the capability of *S. salivarius* 24SMBc to interfere against a clinical isolate of *S. pneumoniae*, belonging to serogroup 19A (*S. pneumoniae* SpA1) and a *S. pyogenes* strain (*S. pyogenes* Spy1), belonging to the emm-type 1 group. The probiotic *S. salivarius* 24SMBc was tested for its ability to interfere with otopathogen adhesion and to co-aggregate with them.

**Material and methods:** The protocol used to investigate the effects of the probiotic *S. salivarius* on the adherence of *S. pneumoniae* and *S. pyogenes* strains included: i) preparation of epithelial cell monolayers and bacterial cells, ii) infection of epithelial cell monolayers with  $1.5 \times 10^9$  CFU/ml,  $1.5 \times 10^8$  CFU/ml and  $1.5 \times 10^7$  CFU/ml of *S. salivarius* 24SMBc versus  $1.5 \times 10^8$  CFU/ml of otopathogens iii) detection of adherent *S. pneumoniae* and *S. pyogenes* strains by viable counts. In addition, we assayed auto-aggregation and co-aggregation for our probiotic versus the same otopathogens.

**Results:** The *S. pneumoniae* and *S. pyogenes* strains assayed showed good adherence on HEp-2 cells (>10% of inocula adhering to epithelial cells after a 3 h incubation). *S. salivarius* 24SMBc reduced their adhesion of 1 log when it was inoculated in a 24-well plate at the concentration of  $10^9$  and  $10^8$  CFU/ml, while we observed a minor effect at the concentration of  $10^7$  CFU/ml. Moreover, *S. salivarius* 24SMBc had a high auto-aggregation value of 83.05% and co-aggregated with pathogens showing percentage value of 64% for *S. pneumoniae* SpA1 and 78% for *S. pyogenes* Spy1, being significant because it was higher than their auto-aggregation value, 40.8% and 70% respectively.

**Discussion and Conclusions:** Our preliminary results *in vitro* showed that *S. salivarius* 24SMBc plays a key role in the adhesion interference of *S. pneumoniae* SpA1 and *S. pyogenes* Spy1, moreover it was able to co-aggregate with them. Thus, our probiotic strain is able to reduce the adhesion of pathogens on the respiratory epithelium by competing for adhesion sites and forming co-aggregates. Our data can also provide information on preferential dosing strategies. In conclusion, *S. salivarius* 24SMBc administration probably impacted negatively on the colonization of *S. pneumoniae* and *S. pyogenes* strains in otitis-prone children modulating or restoring the normal nasopharynx flora.

## ***P 056 – ID 160 - Clinical, epidemiological, and genetic features of human Mycobacterium avium, Mycobacterium intracellulare and Mycobacterium chimaera strains isolated in Tuscany, Italy, from 2004 to 2019***

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**Introduction:** *Mycobacterium avium* complex (MAC) is a group of nontuberculous mycobacteria responsible for most of the human-associated infections. Recently, the high emergence of MAC human diseases has attracted increased attention worldwide. MAC predominantly consists of *Mycobacterium avium* and *Mycobacterium intracellulare* that are implicated in a wide spectrum of clinical manifestations, including respiratory tract infections, lymphadenitis in children and disseminated infections in severely immunocompromised patients. *Mycobacterium chimaera* has been recently involved in a global outbreak of infection related to heat-cooler unit contamination. The aim of the present study is to provide the clinical, epidemiological and genetic characterization of MAC clinical strains isolated in Tuscany, Italy, over the last 16 years.

**Materials and Methods:** A total of 171 MAC strains, identified by InnoLipa probes, were collected from 2004 to 2019 in the Laboratory of Clinical Mycobacteriology of the University Hospital of Pisa, Italy. The minimum inhibitory concentration (MIC), determined by broth microdilution assay, was used to evaluate the drug susceptibility against several drugs, including clarithromycin. Variable numbers of tandem repeats (VNTR) typing was performed to genotype *M. avium* and *M. intracellulare*.

**Results:** Of the 171 MAC isolates, 113 were *M. avium*, 50 were *M. intracellulare* and 8 were *M. chimaera*. MAC strains were prevalently isolated from subjects older than 65 years (51%), in whom the infection occurred primarily at the lung level. *M. intracellulare* and *M. chimaera* were isolated almost exclusively from respiratory samples (90%); of the *M. avium* isolates, 79% were from respiratory specimens, 10% from lymph nodes, 4,5% from blood and the remaining 6,5% from other clinical specimens. We found that most MAC isolates, including all the *M. chimaera* strains, were susceptible to clarithromycin; only 5 isolates of *M. avium* and 1 isolate of *M. intracellulare* were resistant to clarithromycin. The results obtained by the VNTR analysis showed that *M. avium* isolates displayed a high degree of genetic relatedness, whereas *M. intracellulare* isolates did not show a close genetic relationship.

**Discussion and conclusions:** The characterization of the MAC clinical isolates of the last sixteen years showed that *M. avium* was the most abundant organism, followed by *M. intracellulare*. *M. chimaera* strains were exclusively associated with pulmonary infections and not with endocarditis or disseminated infections after cardiac surgery. The findings from the molecular analysis indicated that strains of *M. avium*, but not *M. intracellulare*, exhibit a high phylogenetic proximity, suggesting that MAC strains may have different sources and route of transmission.

## ***P 057 – ID 162 - Biofilm related cutaneous infections: a comparative analysis of the performance of different diagnostic techniques***

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**Introduction.** Intact skin constitutes a physical barrier against bacterial infections. Any cutaneous lesion may behave as an ideal means by which microorganisms can invade tissues and actively grow inside the host. Microbial infection of the skin may shift from an initial acute phase to a long, difficult to treat, chronic illness. In most cases, the chronicization process is frequently associated to the presence of a bacterial biofilm in the lesion, which confers a certain degree of resistance to the most common drugs and less susceptibility of bacteria to host immune responses. The complex bacterial community constituting a biofilm is hard to be eradicated and contributes to hinder bacterial pathogens during microbiological diagnostic procedures. This study aimed at optimizing the procedures for isolation and identification of microorganisms responsible for biofilm-associated infections.

**Materials and methods.** Punch biopsies were obtained from patients with chronic ulcers attending the Dermatology Unit of the Pisa University Hospital. Skin fragments were weighted, suspended in sterile saline and treated as follows to detach microorganisms from skin tissues: i) homogenized by hand shaking (conventional microbiology procedure); ii) homogenized using silica beads and 2000 rpm shaking for two minutes. Appropriate dilutions of 100 µL aliquots were seeded on selective and discriminative media. Plates were incubated for 18-24 h at 37°C, CFUs counted and each isolate identified by Matrix Assisted Laser Desorption Ionization-Time Of Flight (MALDI-TOF) mass spectrometry.

**Results.** The prevalent species isolated by the conventional procedure was *Staphylococcus aureus* ( $2 \times 10^5$  CFUs/g), which is an opportunistic pathogen of the skin and a biofilm producer. Silica bead homogenization mainly led to the isolation of *Enterococcus faecalis* at high amounts (4000 CFUs/g). *Proteus mirabilis* and *Morganella morganii* were isolated at less amounts.

**Discussion and conclusions.** The overall data indicate that silica-bead homogenization lead to the isolation of a greater number of species, when compared to conventional methods. Such a procedure probably favors bacteria detachment from superficial and deep skin tissues and bacterial biofilms, without reducing bacterial vitality. Our data further support that the isolation of *E. faecalis* as responsible for cutaneous infections is quite common. Indeed, *Enterococcus* spp. are frequently responsible for wound and soft tissue infections, especially among trauma patients, most likely because of its antibiotic resistance and its ability to produce biofilms.

## ***P 058 – ID 165 - Isolation, identification and characterization of seven novel strains belonging to the genus *Bifidobacterium* and *Lactobacillus* from the faeces of breast-fed infants and honeybees' gut***

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**Introduction.** The genera *Bifidobacterium* and *Lactobacillus*, which have been frequently associated with health-promoting effects in both humans and animals, are the most commonly included as bioactive components in functional foods, dairy products, as well as in food supplements and pharma products. The aim of the study was to isolate and identify and characterize novel strains of lactic acid bacteria and bifidobacteria from faeces of breast-fed infants and honeybees' gut.

**Materials and Methods.** A total of 20 fresh faecal samples and 10 samples from worker honeybees' gut were analysed. The selection of donors (Algerian infants aged 3-6 months) and sampling was carried out as recommended by Ethics Committee. After homogenizing the samples were inoculated in a reducing medium containing Brain Heart Infusion (BHI) broth (Oxoid, Italy), 0.5% glucose, 0.5% yeast extract (Oxoid, Italy), 0.25% cysteine (Merck), 10 µg vitamin K1 L<sup>-1</sup> (Sigma Aldrich, Italy), and 0.02 g hemin L<sup>-1</sup> (Sigma Aldrich, Italy). Then, serial dilutions of each sample were plated in duplicate on MRS agar (De Man et al., 1960) supplemented with 0.25 % (w/v) L-cysteine hydrochloride (MRSc) and BSM agar (Bifidobacterium Selective Medium - BSM, Sigma Aldrich, Italy) and incubated at 37°C in anaerobic conditions using jars for 24-72 h. The distinction between *Lactobacillus* and *Bifidobacterium* was done by microscopic observation. Selected isolates were initially submitted to physiological and biochemical tests including: catalase test, oxidase test, spore formation, gelatin liquefaction, production of NH<sub>3</sub> from arginine, production of indole, production of CO<sub>2</sub> from glucose, hemolytic activity. Moreover, the isolates were tested for acid and bile tolerance. The isolates which showed the best properties were characterized at genus level using specific primers.

**Results.** A total of 4 isolate for 20 fresh fecal samples and 3 isolates from 10 worker honeybees' gut exhibited a phenotypical and metabolic profile compatible with the genus *Lactobacillus* and *Bifidobacterium*. Moreover, these seven isolates showed resistance to low pH, tolerance to high concentrations of bile salts. The preliminary genotypically characterization performed by sequencing of part of their 16S rRNA gene and following comparison of the sequences to those on the databases, the results identified the isolates from fecal samples as *Lactobacillus* spp. and the isolates from worker honeybees' gut as *Bifidobacterium* spp..

### **Discussion and Conclusions.**

In selecting potential bioactive strains for biotechnological application as bioactive components in functional foods and dairy products, the identification at species and at strain level and the evaluation of the safety and of physic-chemical and functional properties, are highly important. Further researches will be necessary to characterize the isolate at species and strain level and to evaluate their possible technological and toxicological activities.

## ***P 059 – ID 166 - Inhibition of Quorum Sensing-dependent biofilm formation and virulence factors in Pseudomonas aeruginosa by the boronic acid SM23***

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**Introduction:** Quorum sensing (QS) regulates the expression of virulence factors in *P. aeruginosa*. Inhibiting QS-controlled virulence factors without affecting the growth of *P. aeruginosa* may represent a promising strategy for overcoming its widespread and constantly increasing drug-resistance. In this study, we investigated the effects of SM23, a boronic acid, which was specifically designed as beta-lactamase inhibitor, on biofilm formation and virulence factor production by *P. aeruginosa*

**Material and Methods:** the bioluminescent *P. aeruginosa* strain P1242 was employed. The effect of the boronic acid SM23 on *P. aeruginosa* were assessed by evaluating a) the biofilm formation and its morphology by crystal violet staining/bioluminescence and confocal microscopy and b) the production in cell supernatant of the virulence factors, pyoverdines and elastase. The pyoverdine release was assessed by measuring the fluorescence emission with a multi-well fluorescence plate reader and mass spectrometry, while the elastase activity was determined by the Ohman's method, using the Elastin-Congo red as substrate. Finally a qRT-PCR was employed to study the SM23-induced changes in the expression of the QS genes *lasI* and *lasR*.

**Results:** the SM23 significantly inhibited the development of biofilm and the production of virulence factors, as pyoverdines and elastase, without affecting bacterial growth. Preincubation of bacteria with *P. aeruginosa*-conditioned (24 h) medium completely prevented the binding of SM23 to the cells. By investigating the transcriptional changes related to QS, we found that *Pseudomonas* exposure to SM23 caused a notable decrease in the levels of *lasI* and *lasR* gene expression. Finally, the SM23 significantly reduced *P. aeruginosa* biofilm and pyoverdine production on endotracheal tubes, an *in vitro* condition closely mimicking clinical settings.

**Discussion and Conclusions:** taken together, our results indicate that boronic acid SM23, besides inhibiting beta-lactamase, can also act as potent inhibitor of QS in *P. aeruginosa*, suggesting that it may have a potential application in the prevention and treatment of biofilm-associated *P. aeruginosa* infections.

## ***P 060 - ID 171 - Characterization of carbapenemes resistant Klebsiella pneumoniae isolates from blood and bronchoalveolar lavages in the Area Vasta 3***

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**Introduction:** Carbapenem-resistant *Klebsiella pneumoniae* (CRKP) is a global threat which has spread throughout the world. Data from the European Antimicrobial Resistance Surveillance Network (EARS-Net) for 2016 showed that Greece, Italy and Romania are among the countries reporting the highest prevalence of bloodstream infections by CRKP worldwide (67, 34 and 31%, respectively).

Aim of this work is the study of CRKP prevalence and the mechanism of resistance in the Macerata province.

**Materials and Methods:** Fifty-two non duplicated *Klebsiella pneumoniae* isolates were collected by the Microbiology lab of the Civitanova Marche hospital from blood or bronchoalveolar lavages in the period April - November 2016. Identification and antibiotic susceptibility were performed by BD Phoenix 100. Carbapenems resistance was confirmed by disc diffusion, the resistance phenotype was confirmed using the KPC/MBL/OXA-48 (Rosco diagnostica) and the genotype by PCR (*bla*<sub>KPC</sub>, *bla*<sub>NDM</sub>, *bla*<sub>VIM</sub>, *bla*<sub>OXA48-like</sub>, *bla*<sub>IMP</sub>). Clonal relatedness of the carbapenems resistant strains was assessed by PFGE analysis of *Xba*I macro-restricted gDNA. PFGE patterns were analysed using PyElph software (v. 1.3) and distance computed by UPGMA.

**Results:** Among the 52 KP, 29 were obtained from blood cultures and 23 from bronchoalveolar lavages. Fourteen isolates (27%) were meropenem and/or imipenem resistant (M.I.C. > 8mg/L and > 4mg/L, respectively). Most of them (11) came from blood samples (38% of strains were resistant). The disk diffusion synergy tests revealed that eleven isolates were KPC, 2 MBL and 1 AMPC. All the KPC strains harboured the *bla*<sub>KPC</sub> gene while the MBL resistance was due to the *bla*<sub>VIM</sub> gene.

PFGE analysis sorted two main clusters composed of closely related strains.

Discussion and Conclusions

This study showed that, in the Macerata province, prevalence of CR in invasive *Klebsiella pneumoniae* is high and that KPC-production was the most prevalent carbapenem-resistance mechanism in *K. pneumoniae*.

## ***P 061 – ID 178 - Diamines produced by vaginal bacteria in women undergoing in-vitro fertilization as biomarkers of dysbiosis.***

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**Introduction:** Hormonal alterations induce the onset of dismicrobisms that facilitate the establishment of real infections affecting the vaginal tract. It is known that alterations of the vaginal microbiota have a greater incidence in infertile women, although their influence on the result of Medically Assisted Procreation is still controversial. The aim of this work was to evaluate the effect of treatment with exogenous gonadotropins, on the vaginal microbiota of women undergoing controlled ovarian stimulation (COH), in the context of Medically Assisted Procreation procedures.

**Materials and Methods:** A cohort of 108 infertile patients, undergoing hormone therapy for ovarian stimulation for in vitro fertilization (IVF) treatment at the Center for Couple Infertility (University of Siena), were prospectively enrolled in this study. Two vaginal swabs from patients were collected: the first during the screening visit one month before hormone therapy, and the second was collected after the hormone therapy, before egg retrieval. Changes in the vaginal microflora were assessed using a new Vaginal Diamines Assay (VADA) for the quantification of diamines which can be used as biomarkers of the catabolic activity of bacteria involved in BV. We also have analyzed the samples with culture based techniques (Columbia Blood, Columbia CNA, Gardnerella, Mannitol Salt, Mac Conkey, Sabouraud, Rogosa and Sheadler agar plates).

**Results:** In this study, we showed a consistent and significant increase in diamines in vaginal fluids, with an average increase of 34.46  $\mu\text{M}$  following hormonal treatment. This flora shift was also confirmed by the culture analysis of vaginal swabs and the most represented species appear to be *Enterobacteriaceae* and *Streptococcus* spp, which increase after hormone treatment by 13.8% and 30.8% respectively. A highly significant association ( $p \leq 0.0001$ ) between reduced amounts of vaginal lactobacilli ( $< 10^4$  cfu/swab) and the presence of genital tract pathogens was found in the woman study population. Finally, the implantation rate resulted significantly decreased in patients with vaginal samples positive for diamine content and presence of microbial pathogens.

**Discussion and Conclusions:** This study shows that there is a significant shift in the vaginal microbiota of women after undergoing hormone treatment towards pathogenic bacteria which could create a condition like BV. The value of this observations may be significant and may support the presence of a link between the changes in the flora after hormone treatment and very low pregnancy rates in IVF.

## ***P 062 – ID 187 - Emergence and spread of carbapenemase-producing Serratia marcescens strains in a Nephrology Unit of a large Italian hospital***

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**Introduction:** The emergence of Gram-negative carbapenemase-producing bacteria is of concern worldwide due to limited treatment options. This problem has increased during last years especially due to KPC-producing *Klebsiella pneumoniae*. Due to an increase of carbapenem resistant *Serratia marcescens* isolated from urine samples between June and October 2018 in the Clinical Microbiology Laboratory of San Martino Hospital (Genoa, Italy), a molecular study was carried out to characterize such strains.

**Materials And Methods:** Ten non repetitive carbapenem resistant *S.marcescens* isolated from urines of patients hospitalized in Nephrology unit from June to October 2018 were studied. Vitek MS and Vitek AES 2 (BioMerieux, France) were used to identify the strains and to determine antimicrobial susceptibility.

Susceptibility results were interpreted according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST). The modified Hodge test to detect the production of carbapenemases and the phenotypic test for detection of metallo-beta-lactamase were performed. For molecular analysis PCR was used to identify the genes corresponding to the production of carbapenemases and Random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) was used for genotypic characterization of the *S.marcescens* isolates. The genotypes were characterized as identical (identical banding pattern), highly related (one mismatch in banding pattern), or unrelated.

**Results:** All isolates showed carbapenem resistance (minimum inhibitory concentrations of meropenem > 8mg /l). The modified Hodge test for carbapenemase production was positive and the presence of blaVIM gene, was detected for all strains. Lastly, 7 out of 10 strains showed identical banding pattern (Profile A), one strain had a different profile (Profile B) and the remaining two had an identical banding pattern related to profile A (Profile A1).

**Discussion And Conclusions:** These early data suggest that 7 out of 10 strains belong to a main clonal group and these results show that in the period from June to October in the Nephrology unit there was a small outbreak of carbapenem-resistant *S. marcescens*. This is the first case of an outbreak caused by carbapenem-resistant *S. marcescens* in the San Martino Hospital and highlights the importance of strengthening strict adherence to infection control procedures and the need for continuous surveillance of antibiotic-resistant pathogens in local hospitals.

## ***P 063 – ID 194 - Rapid detection and containment of a NDM-1 *Proteus mirabilis* clone in an Italian Hospital***

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**Introduction:** the acquisition of carbapenemases codifying genes in *Proteus mirabilis*, a species intrinsically resistant to tetracycline, tigecycline and colistin, is worryingly emerging. Aim of the study was to characterize eight *bla*NDM-positive *P. mirabilis* (Pm-NDM) strains collected from inpatients and environment of “S. Angelo Lodigiano” Hospital (LO), Northern Italy.

**Materials and Methods:** in April 2019, four *P. mirabilis* strains were selected on CHROMagar™ CPE (BD) at Lodi Hospital Clinical Microbiology Laboratory from two infected/colonized inpatients at the Sub-Acute Care Unit of the “S. Angelo Lodigiano” Hospital. Since the *bla*NDM-type determinants detection by XpertCarba-R System (Cepheid), further n=10 samples, of which n=8 from the patients’ room and n=2 from the patients’ devices, were obtained with containment purposes. Species identification and antimicrobial susceptibility testing were performed by Vitek-2 System (BioMérieux) and confirmed by AutoScan4 System (Beckman Coulter). Interpretation was done according to EUCAST (v8.0-2018, <http://www.eucast.org>). Check-MDR CT103XL (Checkpoints) microarray, PCR and sequencing were used for beta-lactamases, *ArmA*, *AphA6*, *rmtB*, *rmtC* genes screening and *bla*NDM-type identification, respectively. The transferability of *bla*NDM-type determinants was verified by conjugation experiments using *E. coli* J53 (Rif<sup>R</sup>) as a recipient. Plasmids and clones characterization was accomplished using PBRT Kit (Diatheva) and PFGE (*Sfi*I) (BioRad), respectively.

**Results:** a total of eight Pm-NDM strains, obtained from: urine (n=2), rectal/skin swabs (n=4), patients’ room (n=1) and patient’s device (n=1), were susceptible only to gentamicin, ciprofloxacin, levofloxacin and aztreonam. Microarray, PCR and sequencing assays confirmed the presence of a *bla*NDM-1 gene variant together with *AphA6* gene. The *bla*NDM-1 gene was transferable to *E. coli* J53 by conjugation. While the plasmid resulted un-typeable by PBRT Kit, all the isolates showed a well-defined, identical PFGE profile. The patients’ room disinfection by hydrogen peroxide nebulization, the patient’s cohorting and the infection control measures undertaken allowed the resolution of the Pm-NDM spread within one month.

**Discussion and Conclusions:** this is the first report on the detection and the rapid containment of a MDR Pm-NDM-1 clone with high spreading potential, in a Northern Italy Hospital. The findings highlight the importance of class B carbapenemases screening in *P. mirabilis* in Italian area, as such determinants are emerging in Sudan, Tunisia, Greece and Austria, with 18-67% infections mortality rate associated.

## ***P 064 – ID 208 - Rapid molecular tests for detection of antimicrobial resistance determinants in Gram-negative organisms from positive blood cultures: a systematic review and meta-analysis***

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**Introduction:** Timely detection of antimicrobial (cephalosporin/carbapenem) resistance (AMR) determinants is crucial to the clinical management of bloodstream infections caused by Gram-negative bacteria (GNB).

**Materials and methods:** The objective of our study was to review and meta-analyse the evidence for using commercially available molecular tests for the direct detection of AMR determinants in GNB-positive blood cultures (PBCs).

Data sources: PubMed, Scopus and ISI Web of Knowledge.

Study eligibility criteria: Clinical studies evaluating the performance of two major commercial systems, namely the Verigene<sup>®</sup> and FilmArray<sup>®</sup> systems, for rapid testing of GNB-PBCs, in comparison with the phenotypic or genotypic methods performed on GNB-PBC isolates.

Methods: Literature search according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses criteria and, for meta-analysis, bivariate random-effects model.

**Results:** Twenty studies were identified (3307 isolates) from 2006 to 2019. Nine studies were conducted in East Asia. In 15 studies using phenotypic comparators (1029 isolates), 1014 (52.6%) isolates were *Escherichia coli*, and 287 (14.9%) displayed AMR phenotypes. In 5 studies using genotypic comparators (1378 isolates), 585 (42.4%) were *E. coli*, and 100 (7.3%) displayed AMR genotypes. Pooled sensitivity and specificity estimates for detection of AMR determinants by the Verigene<sup>®</sup> (i.e. CTX-M, IMP, KPC, NDM, OXA and VIM) and/or FilmArray<sup>®</sup> (i.e. KPC) systems were 85.3% (95% CI 79.9%–89.4%) and 99.1% (95% CI 98.2%–99.5%), respectively, across the 15 studies, and 95.5% (95% CI 89.2%–98.2%) and 99.7% (95% CI 99.1%–99.9%), respectively, across the 5 studies.

**Discussion and conclusions:** Our findings show that the Verigene<sup>®</sup> and FilmArray<sup>®</sup> systems may be a valid adjunct to the conventional microbiology (phenotypic or genotypic) methods used to identify AMR in GNBs. While FilmArray<sup>®</sup> system currently detects only one AMR determinant, both the Verigene<sup>®</sup> and FilmArray<sup>®</sup> systems can miss important cephalosporin/carbapenem resistance phenotypes. Further studies will establish the prominence of such rapid diagnostics as standard of care in patients with bloodstream infections.

## **P 066 - ID 212 - Pre-medicated gauze with hydrogel and hydrolat of *Citrus aurantium* var *amara* (flowers) for the prevention of microbial infections of the damaged skin**

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**Introduction.** As known, one of the major problems linked to the wound care is the highest probability of infection. Until now, many studies have been developed to modify gauzes made with hydrogel, with the only function to make the wound moist, giving it also an antimicrobial action. However, articles published to date show gauzes modified with the addition of antibiotics or chemical compounds with antimicrobial action or at most essential oils like *Melaleuca alternifolia* (Tea Tree Oil). Our studies focused on the antimicrobial action of a hydrogel, like the gellan gel, when modified with the addition of hydrolates (Hys), natural waste products of the essential oils production method.

**Materials and Methods.** On seven fungal strains (4 clinical isolates and 3 ATCC strains) and 11 GRAM+ strains both sensible and resistant to antibiotics (6 clinical isolates and 5 ATCC strains), micro-broth dilution test, in line with EUCAST guidelines, was performed. We studied the cytotoxic action of 1 commercial Hy (*Citrus aurantium* var *amara* - CA), and 4 Hys produced at the DISTAL department of the University of Bologna (*Monarda citriodora*, *M. didyma*, *M. fistulosa*, *Lavandula vera*). The same test was performed to evaluate the antimicrobial action of preservatives present in the commercial Hy. The cytotoxic action of a prototype of hydrogel-impregnated gauze (HIG) modified with 50% v/v of the Hy of CA was evaluated. Ten microL of a suspension of each strain were adsorbed on HIG and incubated overnight at 37°C. Subsequently, the HIGs were printed on Muller Hilton agar medium that was further incubated at 37°C for 24h. To assess the *in vivo* efficacy, preliminary study of subcutaneous *S. aureus* infections were developed in 8 Wistar rats by inoculating 100 microL of 1x10<sup>7</sup> cfu/mL suspension and bandaging only the treated group (n=4) with HIG. Every day, for 5 days, the HIG was changed and the rat was monitored for the development of subcutaneous abscess.

**Results.** Among all the investigated Hys, CA was the most effective (MIC<sub>90</sub> ≤ 3.125% v/v). Tests carried out on preservatives alone did not show any cytotoxic action (MIC > 50% v/v); while, when associated with the Hy, they enhance the action of hydrolate alone (MIC<sub>90</sub> = 12.5% v/v). Tests performed on HIG showed a cytotoxic action on all tested strains. Preliminary *in vivo* data showed a reduction of about 70% of volume of abscess in treated rats compared to those in control group.

**Discussion and Conclusions.** Our data showed a cytotoxic action of commercial Hy of CA vs fungal and bacterial strains potential skin pathogens. Although further *in vivo* studies are needed to confirm this activity, our study points out to a possible use of the Hy of CA in pre-medicated gauze for the treatment of skin wounds.

## ***P 067 – ID 213 - Characterization of farm animal and human Clostridioides (Clostridium) difficile strains in Italy***

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**Introduction:** *Clostridioides (Clostridium) difficile* is one of the most important causes of healthcare-associated infections worldwide. A higher incidence and severity of *C. difficile* infection (CDI) in hospitals and increasing number of cases in the community have recently been reported. This epidemiological change has been associated with the emergence of highly virulent (HV) lineages, such as RT 027 and RT 078, able to infect various species and to be geographically widespread. Since farm animals are indicated as possible reservoirs of *C. difficile*, in this study isolates from humans, swine and cattle were characterized and compared in order to investigate a possible overlap of *C. difficile* types.

**Materials and Methods:** *C. difficile* were grown on selective culture plates and incubated in anaerobic atmosphere. Capillary PCR-ribotyping was used for molecular typing of strains. The presence of genes encoding for toxin A, B and binary toxin (CDT) was investigated by PCR assays. Susceptibility analysis for moxifloxacin (MXF), erythromycin (ERY), tetracycline (TET), amoxicillin (AMX), metronidazole (MTZ) and vancomycin (VAN) was performed by Etest.

**Results:** In total, 364 strains were analyzed, 267 from animals (250 swine and 17 cattle) and 97 from humans. The most frequent toxigenic profile observed in human strains was A + B + CDT- (79 strains), while the most common among animal strains was A + B + CDT + (252 strains). Twenty nine different RTs were detected among human strains and 11 among animal strains. Seven RTs (001, 005, 078, 085, 126, 620, 569) were detected in both human and animal isolates. The predominant type in human strains was RT 018 (39%), followed by RT 014 and RT 078 (both 8%). Conversely, RT 078-lineage was predominant in animals (97%). This lineage includes RT 033, RT 045, RT 066/2, RT 078, RT 126 and RT 620. In particular, RT 078 was the prevalent (86%) in this study. Susceptibility analysis showed that 98% of strains RT 078-lineage from animals were resistant to ERY and MXF, while strains RT 018 of human origin are known to be resistant to ERY, MXF and to other classes of antibiotics, including clindamycin and rifampicin.

**Discussion and Conclusions:** The results obtained in this study indicated a partial overlap of RTs detected from human and farm animal strains isolated in Italy. RT 078-lineage was confirmed as one of the most successful lineage in animals, especially swine, and a relevant cause of CDI in humans. Our data support animals as a potential source of highly virulent and resistant *C. difficile* strains and highlight the necessity of a close surveillance in this field in accordance with a One Health approach.

The research leading to these results has received funding from Ministry of Health RC IZSLER 07/15

**P 068 – ID 214 - Rapid and simultaneous detection by a multiplex real-time PCR assay of bacterial vaginosis (BV) among women admitted to Gynecology Unit of Sapienza University Hospital “Policlinico Umberto I”.**

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**Introduction:** Bacterial vaginosis (BV), a vaginal disorder which affects millions of women, is typically characterized from a large decrease in *Lactobacillus spp.* and their replacement with severe or facultative anaerobic bacteria. To date, BV is diagnosed using Amsel’s clinical criteria and microscopy based on Nugent scoring, considered the gold standard, although the accuracy is less than optimal. The use of quantitative Polymerase Chain Reaction (q-PCR) method could improve the diagnosis of BV. The aim of this study was to perform a diagnosis of BV among women admitted to Gynecology Unit of Sapienza University Hospital “Policlinico Umberto I” using a multiplex real time PCR, Allplex™ Bacterial Vaginosis Assay (Seegene, Korea). Moreover, in order to obtain a complete clinical framework of patients enrolled, also the potential presence of sexually transmitted pathogens was investigated by using the same platform. Finally, molecular data were compared with those obtained through the gynecologist’s microscopic observation of vaginal fluid.

**Materials and Methods:** A total of 274 genital swab, collected from Caucasian women sexually active and not pregnant, were analyzed by Allplex™ molecular method. Samples automatic process was performed on Nimbus IVD extraction platform (Hamilton Robotics, Switzerland), followed by a multiplex q-PCR (Allplex™ Bacterial Vaginosis Assay, Seegene, Korea) specific for the detection of bacteria associated with vaginosis: *Lactobacillus spp.* (*L. crispatus*, *L. gasseri* and *L. jensenii*), *Gardnerella vaginalis*, *Atopobium vaginae*, *Megasphaera* Type 1, *Bacteroides fragilis* and *Mobiluncus spp.* (*M. mulieris* and *M. curtisii*). Furthermore, from the same tube, the detection and the identification of 7 *Candida spp.* and of 7 pathogens, causing sexually transmitted infections (*Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Trichomonas vaginalis*, *Mycoplasma genitalium*, *Mycoplasma hominis*, *Ureaplasma urealyticum* and *Ureaplasma parvum*), was performed.

**Results:** Data obtained were analyzed by Seegene Viewer software and interpreted as positive or negative. Out of a total of 274 specimens, 33 samples were found positive for BV. Moreover, 4/33 BV positive samples were also positive for *Candida spp.* and 26/33 showed positivity for sexually transmitted pathogens. Comparison of molecular data with those obtained through the gynecologist’s microscopic observation, evidenced that 26/33 were in agreement to Nugent scoring and Amsel criteria whereas 7 resulted undiagnosed by microscopic observation.

**Conclusions:** Although Amsel and Nugent criteria remain the gold standard for BV diagnosis, the Allplex™ molecular method ensures a high sensitivity and allows the detection of more pathogens simultaneously with a Turn Around Time (TAT) of less than 5 hr.

## ***P 069 – ID 215 - Development of a novel Loop-Mediated Isothermal Amplification (LAMP)-based kit for the rapid detection of Legionella spp. in environmental samples***

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**Introduction.** *Legionella* spp. are found ubiquitously in freshwater and also in man-made water systems. Despite *Legionella pneumophila* accounts for the great majority of reported legionellosis cases, many more species have been associated with disease, causing infections above all in immunocompromised hosts. *Legionella* spp. may grow on BCYE culture media, but usually they are slow-growing and require even more than a week for their detection. Hence, environmental contaminations by *Legionella* spp. may often be underdiagnosed. To address these deficiencies, an innovative LAMP-based kit was developed allowing a rapid, specific and sensitive detection of *Legionella* spp.

**Materials and Methods.** Following filtration, a 10-minute DNA extraction from membranes occurs, using a specific ready-to-use extraction buffer. Reactions are prepared by adding 22 µl of LAMP mix and 30 µl of mineral oil, already supplied inside the kit, in the test tubes containing the lyophilized primers. Afterwards, genetic amplification is carried out in the dedicated device ICGENE mini (Enbiotech Cat.N. EBT 801). *Legionella* spp. kit performances were tested according to validation by AFNOR NF148 (2015). Specifically, sensitivity, inclusivity, exclusivity and method robustness were analysed. In addition, kit performances were compared by testing samples both with the reference cultural method and with the LAMP method.

**Results.** The method proved to be sensitive, with a detection limit of 32 Genomic Units/µl. Results of robustness testing showed a 100% performance for the different types of water, as all the samples contaminated with the *L. pneumophila* strain at various levels of contamination were amplified. Inclusivity and exclusivity criteria were met.

**Discussion and Conclusions.** Reference cultural method may take more than one week for a negative result, while the above-mentioned LAMP kit offers a rapid, cost-effective and sensitive option for the effective detection of *Legionella* spp. in various types of water samples. In this study, all the criteria and parameters requested by AFNOR validation were met, thus offering significant advantages for official controls, helping to prevent the spread of the disease.

## ***P 070 – ID 216 - Bordetella pertussis DNA in the Blood from a Child with Silver-Tube Tracheostomy Detected Prior to Seroconversion***

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**Introduction:** *Bordetella pertussis* is a fastidious aerobic, Gram-negative coccobacillus that causes the classical disease of whooping cough. We present a 3-year-old male, with a silver tube tracheostomy, landed from Ivory Coast to Rome, because of a programmed admission at the pediatric neuropsychiatry ward of our hospital. Few hours after his arrival, the patient had general malaise and shaking chills that forced him to present to the emergency department of the same hospital.

**Materials and methods:** A suite of tests were ordered, including blood culture (BC) and other microbiological exams. A patient's sample of whole blood in EDTA was collected for direct testing with an in-house multiplex real-time (qPCR)-based molecular assay, MicroScan. We have previously developed the assay, CE-IVD-marked, (Liotti FM et al., submitted for publication) for rapidly detecting a wide range of target microbe's densities, starting from automatically extracted and then dispensed microbial DNA into qPCR reagents-prefilled micro-well strips before their manual loading onto the BioRad CFX Thermal Cycler.

**Results:** The laboratory exams showed a high white blood cell count, C-reactive protein, and procalcitonin value. Molecular testing for the malaria parasite and influenza virus yielded negative results. In waiting for the results of BCs that will be reported as negative five days later, we found that the whole blood sample tested in MicroScan was positive for *B. pertussis* DNA. Based on these findings, patient's nasopharyngeal swabs were obtained for either molecular testing or cultures that will be negative for *B. pertussis*. On hospitalization day 4, serological exams were performed and showed positive IgM and negative IgG *B. pertussis* antibody titers that were suggestive of acute patient's infection. On hospitalization day 12, blood patient's sample serological testing showed a 4-fold increase of the IgG titer against *B. pertussis*, which indicated seroconversion.

**Discussion and conclusions:** To the best of our knowledge, the present is one of four *B. pertussis* infection (BPI) cases reporting the recovery of *B. pertussis* from patients' blood. In the present case, the cough was apparently absent because our patient had a tracheostomy. We cannot exclude that the stressful environment in our patient's trachea had limited the thriving of *B. pertussis* in the upper respiratory tract, which represents its preferred colonization site. However, only in our case, the classical serologic course in response to the infecting organism confirmed the diagnosis of BPI. Since *B. pertussis* DNAemia may be the first presenting sign in a child with tracheostomy, use of a PCR assay may aid to timely and effectively identify all critically ill patients with active infection.

## ***P 071 – ID 222 - Rapid diagnosis from positive blood culture: the experience of the Microbiology and Virology Unit of the Sapienza University Hospital “Policlinico Umberto I”.***

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**Introduction:** An accurate identification and characterization of pathogens is crucial in the management of the infected-patient, particularly in blood stream infections. Traditionally, microbiological diagnosis is based on conventional and culture-dependent approaches, which however requires at least 48 hours for the bacterial identification and antimicrobial susceptibility testing (AST). With the aim to provide a quick and, hopefully, inexpensive answer to clinicians, our group set up a procedure, based on the already described methods published in the literature, for a rapid microbial identification from positive blood cultures.

**Materials and Methods:** A total of 285 positive blood culture bottles were examined and subjected to Gram staining. The rapid method was applied for the identification of Gram-negative bacteria, *Streptococcus spp.* and *Enterococcus spp.* at first isolation and for the identification of *Staphylococcus spp.* found at least in three positive culture media bottles. An amount of 5 ml of blood culture sample was centrifuged in sterile tubes at 250 rcf for 5 min. In order to obtain a bacterial pellet, a washing of the supernatant was carried out with an equal volume of cold sterile water. The sample was centrifuged for a second time at 1500 rcf for 5 min. The resulting pellet was used to set up: 1) mass spectrometry identification by the MALDI-TOF system (Bruker Daltonics BD, Germany), 2) AST by the Vitek 2 semi-automated system and 3) RT-PCR assay by GeneXpert® (Cepheid, France) for carbapenemases detection. Moreover, a rapid antibiotic susceptibility testing (RAST) was carried out according to EUCAST guidelines.

**Results:** Out of 285 positive blood cultures, 100 were found positive for Gram-positive bacteria: among them, 71% were identified at the level of species, 13% at level of genus and 16% were not identified. For Gram-negative microorganisms, 185 were positive: among them, 87% were identified at the level of species, 8.6% at level of genus and 4.4% were not identified. The most represented species were *Klebsiella pneumoniae*, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Enterococcus faecalis* and *Enterococcus faecium*. Regarding carbapenemases detection, 64.7% of *K. pneumoniae* was *Klebsiella pneumoniae* carbapenemase (KPC). The concordance rate between the RAST and AST results was of 88.5% for the Gram-positive and 95% for the Gram-negative.

**Discussion and conclusions:** This inexpensive method, without any chemical reagents, may contribute to early effective identification and antibacterial treatment, by providing reliable results of RAST within 6 h. This procedure allowed an identification of 93% of Gram-negative bacilli within 1 hour. On the other hand, the power of this method is low in identifying *Staphylococcus spp.* and, as expected, it is not able to discriminate fungi infections and polymicrobial blood cultures.

## ***P 072 - ID 226 - Arcobacter spp. in environmental waters in Sicily: occurrence, antimicrobial resistance and relationship with bacteria indicators of fecal pollution***

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**Introduction:** *Arcobacter* spp. are emerging enteropathogens and potential zoonotic agents that can be transmitted by food and water. The species *A. butzleri* and *A. cryaerophilus* have been associated with bacteremia, gastroenteritis and diarrhea in humans and with abortion and diarrhea in animals: the water plays an important role in the transmission. Aims of the present study were to evaluate the presence of *Arcobacter* spp. from different water sources, to assess the possible correlation with the levels of fecal indicator bacteria and to determine the antimicrobial resistance of the isolates.

**Materials and methods:** A total of 100 samples including rivers, ponds, drinking water, spring water, well water, seawater and seaweeds, were examined for the presence of *Arcobacter* by bacteriological and molecular methods (multiplex-PCR and sequencing). Antimicrobial resistance testing of the isolates against 10 antibiotics was performed by the disk diffusion method. In the water samples, the MPN procedures for *E. coli* and for enterococci were used.

**Results:** *Arcobacter* were isolated from 27 (27%) of the samples examined: the highest prevalence rate was 81.8% in rivers followed by 62.5% in artificial ponds, 40% in streams, 14.2% in seawater, 10% in well water and 5.8% in springs water samples. The analysis of algae, taken together with sea waters, was positive with a value of 83.3%. All drinking water samples were negative. However, *Arcobacter* spp. was identified in a non-chlorinated source water sample (spring water) used to drink with 0 MPN/100 ml of *E. coli* and enterococci. *Arcobacter* has also been isolated in some water sources with low or no levels of fecal indicator bacteria. Based on the multiplex PCR targeting both 16S rRNA and 23S rRNA genes, the isolates were identified as belonging to the species *A. butzleri* (96.3%) and *A. cryaerophilus* (3.7%) and confirmed by the sequencing analysis. In one samples of artificial pond with aquatic birds, the two species were co-isolated. All the arcobacters were susceptible to antibiotics commonly used in therapy but two strains resulted resistant to ciprofloxacin. Multidrug resistance was observed in all isolates tested.

**Discussion and conclusions:** This work supports previous studies of arcobacters findings in environmental water samples and their presence is recognized as a potential risk for human health. Moreover, our results show that pathogen bacteria of the genus *Arcobacter* may be present in aquatic sources, even with low or no values of *E. coli* and enterococci. It is important to deepen these investigations mainly in the water, which can be used for water supply and irrigation, to better assess the dangers associated with human health. Furthermore, it is recognized that the waters represented a reservoir of antibiotic resistance genes.

## ***P 073 – ID 227 - Prevalence of sexually transmitted infections in a cohort of migrant women arriving in Palermo from sub-Saharan Africa***

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**Introduction:** The considerable increase in migratory flows, in some regions, such as Sicily, pushes to analyze the epidemiological change in the spread of sexually transmitted infections (STIs). Mobility itself creates fragility and susceptibility to the risk of contracting sexually transmitted infections. The main STIs therefore require continuous monitoring; in fact, their prevalence is an indicator of living conditions (eg poverty, housing, sexual habits or sexual abuse) and access to prevention. The purpose of this study was to evaluate the prevalence of different sexually transmitted infections (STIs) in migrant women arriving in Palermo and coming from the countries of sub-Saharan Africa.

**Methods:** 53 women with mean age 24 (range 12-45 years) were enrolled after having undergone a gynecological examination. To each, with the help of the cultural mediator, a questionnaire was given concerning the migratory path and the medical history information. Cervical swabs were collected for molecular detection of Human papillomavirus (HPV), *Chlamydia trachomatis* (CT), *Neisseria gonorrhoea* (NG), *Trichomonas vaginalis* (TV), *Mycoplasma spp* (M) and *Ureaplasma spp* (U).

**Results:** Of 53 cervical samples 27/53 (51%) were HPV positive. Oncogenic types were found in 23/27 (85.2%) positive samples. Multiple HPV type infections were shown in 15/53 (28,3%) of the samples of whom 5 (33.3%) had two genotypes, 5 (33.3%) three genotypes, 2 (13.3%) four genotypes, 1 (6.7%) five genotypes, 1 (6.7%) six genotypes and 1 (6.7%) nine genotypes. 100% of multiple infections; and 66.7% (8/12) of single infections contained at least one high-risk genotype (HR-HPV). By use of a multiplex PCR we found: CT in 2/53 sample (3,8%) TV in 3/53 (5,7%), M in 10/53 (18,9% as *M. hominis* or *M. genitalium*) and in 38/53 (71,7% as *U. urealyticum* or *U. parvum*). In no sample was found NG. In 14 samples (26,4%) we highlighted more than one microorganism.

**Discussion and conclusions:** Human papillomavirus (HPV) is the most common sexually transmitted disease. The prevalence of HPV infection found was relatively high (51%), in line with other published studies. Among the bacteria the positivity to *U. urealyticum* or *U. parvum* (71.7%) is significantly high compared to other pathogens such as *M. hominis* or *M. genitalium* (18.9%). Although there is a persistent controversy regarding to *Ureaplasma* as a pathogen in sexually transmitted infections, since it is not clear whether it is colonization or infection, several publications highlight its role as a risk factor for HPV infection. In conclusion, given the high prevalence of oncogenic genotypes, the implementation of vaccination programs in the case of migrants could therefore become an effective prevention approach, capable of producing substantial benefits.

## ***P 074 – ID 229 - Construction and characterization of Major Capsid Protein and Major Tail Protein deletion mutants of Streptococcus pyogenes phi1207.3 phage.***

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### **1. Introduction**

Phi1207.3 is a prophage of *Streptococcus pyogenes* carrying the macrolide efflux resistance genes *mef(A)/msr(D)*. The phage was previously transferred to a *Streptococcus pneumoniae* strain, devoid of prophages or ICEs and not naturally competent for transformation, where it is able to produce complete phage particles with a Siphoviridae morphology. The mechanism of transfer of phi1207.3 still needs to be elucidated, as it is able to move among streptococcal species by a mechanism which likely depends on the cell-to-cell contact rather than on the interaction of free-floating phage particles with bacterial cells.

Here, we constructed Major Capsid Protein (MCP) and Major Tail Protein (MTP) deletion mutants to better understand the phi1207.3 phage interaction with the bacterial cells and its lysogenic transfer.

### **2. Materials and Methods**

Mutagenic constructs were obtained by PCR Gene Splicing by Overlap Extension (SOEing) and transformed in *S. pneumoniae* competent cells. Genotype of recombinants was determined by nanopore sequencing on the GridION. Mutant phage preparations, after Mitomycin C induction, were obtained by ultracentrifugation of culture supernatants and characterized by (i) Real Time-PCR analysis, using divergent primer pairs directed at the ends of the phage genome, (ii) transmission electron microscopy (TEM) imaging after negative staining, and (iii) lysogenic transfer on a solid substrate.

### **3. Results**

Real Time-PCR analysis showed that MTP mutant can produce phage circular genomes, suggesting that MTP absence does not prevent circular and concatemeric genomes packaging, while MCP mutant is able to produce circular genomes with a lower value compared to the wild type (wt), showing that MCP deletion affects the genome packaging.

TEM imaging of MTP mutant showed the presence of phage particles with an icosahedral capsid, demonstrating that the absence of the Major Tail Protein does not affect phage capsid assembly.

Even when induced with Mitomycin C, both wt and mutated phi1207.3 produce a limited phage burst without evident cell lysis. Only the wt phi1207.3 was capable of lysogenic transfer, whereas for MCP and MTP mutants transfer could not be achieved, indicating that both structural proteins are essential for an efficient phage transfer.

### **4. Discussion and Conclusions**

Upon deletion of the Major Tail Protein, genome packaging and phage capsid assembly are not impaired, even though the lysogenic transfer does not occur.

Deletion of the Major Capsid Protein, instead, influences both genome packaging and lysogenic transfer.

These results suggest that the transfer mechanism of phi1207.3 phage is still not clear, although it is likely to be dependent on the cell-to-cell contact and to involve both the Major Capsid and Major Tail proteins.

## ***P 075 – ID 232 - Extended spectrum beta-lactamase-producing Escherichia coli from human and animal sources in Italy: a “One Health” approach.***

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**Introduction.** *Escherichia coli* producing extended spectrum beta-lactamase (ESBL) is a serious public health concern. Food-producing animals have been suggested as a potential source for ESBL-producing *E. coli* affecting humans, thus surveillance requires a One Health approach. This study aimed to compare ESBL-producing *E. coli* isolates from humans and food-producing animals with respect to ESBL gene, phylogenetic group and sequence type (ST).

**Materials and Methods.** Overall, 919 ESBL-producing *E. coli* isolates from humans (n. 474) and food-producing animals (n. 445) were collected in six Italian Regions (2016-2017). Human clinical isolates were collected from urine (n. 375) or blood (n. 99) while indicator isolates from food-producing animals were recovered from the intestinal content or faecal samples of bovines (n. 131) swine (n. 120) and poultry (n. 194). Phenotypically confirmed ESBL-producing *E. coli* isolates were screened for the presence of the *bla*<sub>CTX-M</sub>, *bla*<sub>SHV</sub> and *bla*<sub>CMY-2-like</sub> genes by PCR and sequencing and classified according to phylogenetic typing and MLST genotyping.

**Results.** CTX-M was the most frequent ESBL type in both human and animal isolates with CTX-M-15 predominant in humans (74.8%) and bovine (51.1%) but not in poultry (36.6%) and swine (31.7%). CTX-M-1 was common (58%) in swine. SHV type and CMY-2-like were found mainly in animal isolates, especially in poultry (28.9% for CMY-2-like and 17.0% for SHV12). Human ESBL-producing *E. coli* isolates mostly (76.8%) belonged to phylogroup B2, while animal isolates were distributed among groups A (35.7%), B1 (26.1%) and C (12.4%). Only a few animal isolates (almost all recovered from poultry) were classified into group B2 (4.3%). Most human isolates (81.6%) belonged to the pandemic ST131 clone and frequently carried CTX-M-15 (66.3%). Among animal isolates, ST131 was rarely detected (n. 3 isolates from poultry) and never carried CTX-M-15. Other than ST131 isolates were disseminated among several different STs. Twelve STs

were shared by human and animal isolates with ST10, ST410 and ST38 more frequently detected. Of note, ST410 isolates of both human and animal origin shared the same ESBL profiles including either CTX-M-15 or CTX-M-1.

**Discussion and Conclusions.** Different subgroups of ESBL-producing *E. coli* isolates from both human and animal source may share: i) ESBL genes but carried by different ST clones, ii) ST clones but containing distinct ESBL genes, iii) both ESBL genes and ST clones. According to our results the potential exchange of ESBL genes through plasmids or isolates from animal to humans and vice versa is feasible, underlying the need for a strict monitoring based on an “One Health” approach.

## **P 076 – ID 233 - *Kocuria* species from eyelid margin of patients undergoing cataract surgery**

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**Introduction:** Organisms in the genus *Kocuria* are gram-positive coccoid bacteria of the family *Micrococcaceae*. Though they are environmental bacteria, as well as human skin and oropharynx mucosa commensals, they can cause infections like peritonitis, endocarditis, cholecystitis, pneumonitis, and urinary catheter-related infections. *Kocuria* spp. are also unusual causative organisms in ocular infectious diseases. This study aimed to evaluate the antibiotics sensitivity and biofilm formation of *Kocuria* spp. isolated from eyelid margin of patients undergoing cataract surgery.

**Materials and Methods:** Forty eyes of 20 patients undergoing serial unilateral cataract surgery, between May and June 2019, were randomised. All volunteers had to sign informed consent forms. Ocular swabs were taken from the eyelid margin of the eye selected for surgery (cataract surgery group) and from that of the other eye (control group) before, during and after cataract-surgery. These swabs were used to inoculate blood agar and chocolate agar plates for culturing. After growth of the bacterial flora, the antibiotics sensitivity standard method using netilmicin (NET), tobramycin (TOB), levofloxacin (LEV), vancomycin (VA), cefuroxime (CXM) and imipenem-cilastatin (IMC), was applied. The biofilm-forming capability of selected strains was evaluated by using a microplate assay. Results. Bacterial isolates were identified by API System, namely, *Kocuria varians* (n=2), *K. rosea* (n=1), *K. kristinae* (n=1); coagulase-negative *staphylococcus* (CoNS) such as *S. epidermidis* (n=10), *S. lugdunensis* (n=4), *S. warneri* (n=4), *S. hominis* (n=2), *S. xylosus* (n=4), *S. capitis* (n=2); *S. aureus* (n=4); *Streptococcus* spp. (n=2) and *Bacillus* spp. (n=2). The *K. varians* strains were isolated before and after cataract-surgery in both groups, whereas *K. rosea* and *K. kristinae* after cataract-surgery in control-group.

**Results:** of antibiotic sensitivity test showed that all *Kocuria* spp. were resistant to IMC. *K. varians* was also resistant to TOB (50%) and CXM (25%), and *K. rosea* to TOB (25%). Biofilm production in *Kocuria* species was highest in *K. kristinae* (Optical Density = 1.208±0.47) which exhibited a lower incidence of antimicrobial resistance.

**Discussion and Conclusions:** Post-cataract surgery endophthalmitis remains the greatest nightmare in cataract surgery. *Kocuria* endophthalmitis is a relatively rare but emerging cause of endophthalmitis and is often misdiagnosed as CoNS endophthalmitis.

## ***P 077 – ID 238 - Detection of ESBLs and carbapenemases-producing Enterobacterales from surface waters in Pavia area, Northern Italy***

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**Introduction:** Multi-drug resistant microorganisms (MDRO), originating from anthropogenic activity, could reach the water compartment via urban waterways with serious risks for human health. The aim of the work was to screen for the presence of broad-spectrum cephalosporins and/or carbapenems resistant *Enterobacterales* in both different surface waters and a wastewater treatment plant (WWTP) in Pavia area (Northern Italy).

**Materials and Methods:** A total of 18 water samples were collected during May 2019 the 13<sup>th</sup>-14<sup>th</sup>, from four streams, two ponds, four spring waters, one sewer and one treatment plant, located in Pavia. The chosen sampling sites were located up- and downstream of the urban center. A water volume of 1 ml was passed through 0.45 µm-pore size membranes by filtration method. Filter membrane were placed on Plate Count Agar (PCA), MacConkey (MCA) and selective MCA containing 0,25 µg/ml and 4 µg/ml meropenem (MP) and 1 and 2 µg/ml of cefotaxime (CTX), respectively. The bacterial count was estimated after 18-24h incubation, at 37° C. was identified at species level. Species identification and susceptibility testing were performed on each colony grown on selective MCA and distinguishable for the unique morphotype, using the semi-automated system MicroScan autoSCAN-4 (Beckman Coulter). Susceptibilities profiles were interpreted according to both EUCAST 2019 epidemiological and clinical breakpoints. CT103XL microarray (Check-points) and PCR were used for resistance genes investigation.

**Results:** the size of the enterobacterial population identified on PCA ranged from 61 CFU ml<sup>-1</sup> (from a pond spring water located downstream the urban center) to 1,000 CFU ml<sup>-1</sup> (from a sewer, at the edge of the city). Twenty-seven species of enterobacteria grew on selective MCA: 66.6% (n=18/27) *E. coli*, 14.8% (n=4/27) *Klebsiella spp.* (n=3 *K. pneumoniae* and n=1 *K. oxytoca*), 7.4% (n=2/27) *Citrobacter freundii*, 7.4% (n=2/27) *Kluyvera intermedia* and 3.7% (n=1/27) *Enterobacter aerogenes*. The 74% (20/27) of the above isolates (n=17 *E. coli* and n=3 *K. pneumoniae*) were ESbetaLs-positive by MicroScan autoSCAN-4.

The 72.2%, 66.6%, 38.8%, 27.7%, of the ESbetaL-producing *E. coli* isolates resulted ciprofloxacin, levofloxacin, trimethoprim-sulfamethoxazole (TMS) and gentamycin resistant, respectively. The *bla*TEM/SHV- and *bla*KPC-type genes co-existed in a MDR *K. pneumoniae* strain collected from the Vernavola stream (northern Pavia area), by Microarray and PCR. The KPC-*K. pneumoniae* strain retained susceptibility only to colistin and TMS.

**Discussion and Conclusions:** The occurrence of MDRO and ESbetaL/KPC-producing *Enterobacterales* in hydric compartment poses potential risks for human health, highlighting the importance to improve remediation actions on surface waters.

## ***P 078 – ID 243 - Antibiotic activity of antimicrobial peptides from various sources.***

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**Introduction:** The growing demand of new antibiotics active against multiresistant bacteria has encouraged the research of antimicrobial agents from natural sources. Peptides are the most common weapon that all organisms produce to prevent invasion by external pathogens. Antimicrobial peptides (AMPs) are short peptides consisting of 10-100 amino acids produced by all organisms that, unlike common antibiotics, do not trigger resistance and are specific toward bacterial cells. Although several studies dealing with AMPs have been performed, little is known about their mechanism of action. Here we report the investigation of the antimicrobial activities of several AMPs different in size, charge and polarity against a wide range of pathogenic, non pathogenic and resistant bacterial strains to define possible relationships between AMPs structure and bacterial characteristics.

**Materials and Methods:** The effect of different AMPs on the EUCAST panel of reference strains for susceptibility testing was determined by measuring the minimal inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC). Morphological changes on bacterial cells induced by AMPs treatment were monitored by Transmission Electron Microscopy (TEM). Finally, cytotoxicity of AMPs was determined through an hemolytic test.

**Results:** A collection of reference bacterial strains was screened with both natural occurring and *in silico* designed AMPs to test the effect of antimicrobial peptides characterised by a broad spectrum of different structural features. Preliminary results demonstrated that peptides have different effects on pathogenic, non pathogenic and resistant bacterial strains. MIC and the MBC measurements performed to estimate the antimicrobial and the antibacterial activity of the AMPs indicated a wide range of different behavior. Upon the AMPs tested, Temporin L (TL) showed a strong antimicrobial activity against both Gram-negative and Gram-positive strains. However, morphological investigation by TEM analyses demonstrated a largely different effect of TL on *E. coli* and *S. aureus*. In particular, TEM images clearly indicated that TL impaired cell division in *E. coli*, while in *S. aureus* the TL treatment originated the formation of membrane vesicles on the bacterial surface.

### **Discussion and Conclusions**

Different AMPs showed different activities on various bacterial strains. TL exhibited a potent antimicrobial activity against both Gram-negative and Gram-positive strains. However, the peptide displayed different mechanisms of action on *E. coli* and *S. aureus*.

## ***P 079 – ID 244 - Molecular typing of Legionella pneumophila by SBT from BAL of two clinical cases***

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**Introduction:** "Legionnaires' disease", is a lung infection caused by a ubiquitous bacterium that spreads also through the water systems of buildings. Although several of the more than 60 species described in the genus *Legionella* may cause disease, *L. pneumophila* is the major agent, responsible for nearly 90% of all cases worldwide. Transmission of bacteria from the environment to humans occurs via inhalation or aspiration of Legionella-containing aerosols. Cooling towers, hot springs, and potable water systems in large facilities, hotels, hospitals, and public baths that are contaminated with *Legionella* spp. are the implicated sources of outbreaks and sporadic cases of Legionnaires' disease. The current gold-standard *L. pneumophila* genotyping assay for epidemiological investigations is sequence-based typing (SBT), developed as a variant of multilocus sequence typing. We described the typing of *L. pneumophila* isolated from the clinical samples, the cases of Legionellosis of a woman and a man in a Sicily hospital.

**Materials and method:** The clinical samples were obtained from two patients admitted with signs of infection of the lower respiratory tract. The woman and man were respectively 63 and 70 years old. The respiratory samples were cultured on buffered charcoal yeast extract agar (BCYE). Suspected colonies isolated from two patients' BAL were confirmed by Latex agglutination. Using SBT, typing method recommended by EWGLI (European Working Group for Legionella Infections) it was possible to typify the strains. The strains were discriminated using SBT analyzer.

**Results:** Suspicious colonies were identified as *L. pneumophila* serogroup 1 by Latex testing. In the first case, from woman's clinical sample, the molecular typing by SBT has highlighted a new ST, with the following profile: 1, 4, 3, 1, 31, 1, 1. This profile entered in the EWGLI database, has been associated with ST 2689. In the second case, from man's clinical sample, we obtained the following profile: 2, 3, 9, 10, 2, 1, 6. This profile coincide with ST 23.

**Discussion and Conclusions:** In accordance with our experience to avoid epidemics or cases of legionellosis it is recommended to monitor the water systems of public and private structures. We also emphasize the importance of cultural investigation from clinical samples as it allows us to evaluate the presence of other serogroups than serogroup 1, detected only by the urinary antigen. In the present study we confirmed the utility of SBT for performing molecular epidemiological studies. Moreover, thanks to molecular methods, we have found a new type of ST.

## ***P 080 – ID 247 - Effect of edible fungi extracts *Pleorotus eryngii* var. *elaeoselini* and *Grifola frondosa* against strains of *Staphylococcus aureus* methicillin-resistance producers of biofilm***

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**Introduction:** *Staphylococcus aureus* is a Gram positive bacterium that can be found on the skin and in the nasal mucosa. It can generate pathologies in immunocompromised individuals and represents one of the most important causes of nosocomial infections, above all because it is easily transmissible, for example through contact with infected individuals. It is a bacterium increasingly resistant to drugs. The resistance is made more effective also thanks to the ability to form biofilm. For this reason, we are constantly looking for drugs that eliminate these pathogens. Lately, we focused on the study of some natural fungi extracts that have an inhibiting action on bacteria. In particular, we have used wild edible Basidiomycota extracts derived from *Pleorotus eryngii* var. *elaeoselini* and *Grifola frondosa*.

**Materials and Methods:** The bacterial strain ATCC 43300 of *S. aureus* and clinical strains of *S. aureus* methicillin-resistant (MRSA), isolated at the Policlinico P. Giaccone of Palermo, were seeded in TSA (Tryptone Soy Agar) media and incubated at 37° C for 24 h. The bacterial suspensions (0.5 McFarland in Broth Tryptose) were dispensed into 96-well sterile polystyrene plates. For whites, only the BT medium (broth tryptose) was dispensed. The plate was incubated at 37° C for 24 h. We proceeded using the extracts of the mushrooms at a concentration of 25% (*Pleorotus eryngii* var. *elaeoselini* and *Grifola frondosa*) and diluting in BT. In the whites and the two wells for each strain, the BT was dispensed again. The plate was incubated at 37° C for 24 h. The biofilm was colored with crystal violet (0.5%), dissolved with ethanol. The optical density reading was performed at 540 nm.

**Results:** The bacterial strains of *S. aureus* are good biofilm producers. In most samples, both fungi extracts have led to a decrease in growth and there is a reduction of the ability to form biofilm that is evidenced by a decrement of optical density.

**Discussion and Conclusions:** The results show a slowdown in the biofilm production of *S.aureus* methicillin-resistant strains and it is hoped that increasing their concentration, the inhibitory effect on biofilm will be more positive. Moreover, considering the total edibility of the mushrooms from which the extracts of *P.eryngii* var. *elaeoselini* and *G. frondosa* were obtained, the aim could be to be able to use these natural components to treat patients who are infected with these microorganisms.

## ***P 081 – ID 248 - A Fast method for Pseudomonas aeruginosa detection in water samples***

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**Introduction-** *Pseudomonas aeruginosa* is a relevant opportunistic pathogen characterized by multidrug resistance, thus representing a health risk in hospitals with various types of infections. *P. aeruginosa* may be detected in spring water, underground and surface water, water for human consumption and pool water. Therefore, it is important to establish a rapid, specific and sensitive method for its detection. The reference cultural method determines enumeration of *P. aeruginosa* after incubation for 48 hours. Loop-mediated isothermal amplification assay (LAMP) is a novel nucleic acid amplification method for rapid and sensitive target detection.

**Materials and Methods-** Tap autoclaved water samples were inoculated with a *P. aeruginosa* ATCC 27853 suspension to obtain 3 contamination levels: 5 CFU/100 ml, 50 CFU/100 ml, 100 CFU/100 ml. After filtration of the inoculated samples, DNA extraction was performed by placing the filter in a ready-to-use extraction buffer for 10 min at 85°C. The LAMP assay was performed, detecting *P. aeruginosa* in water samples with 100% specificity. In order to improve the sensitivity of the method a short enrichment was introduced and 6 differently formulated broths were tested. These broths were evaluated through optical density (in McFarland units) at T<sub>0</sub>, T<sub>1</sub> (3h) and T<sub>2</sub> (4h) after inoculation and the most performing broth (B2) was selected. Subsequently, B2 was tested with water spiked at the above-mentioned contamination levels, incubating the related filters with 3 ml of B2 for 3 and 4 hours. Following incubation, broths were centrifuged at 8000 rpm for 5 minutes. DNA extraction was performed adding the extraction buffer to the pellet and incubating for 10 minutes at 85°C. Afterwards, the LAMP assay was carried out.

**Results-** The selected broth (B2) showed a higher McFarland value, both at T<sub>1</sub> (0,17 McF) and T<sub>2</sub> (0,31 McF) in comparison to other broths, which showed similar values at T<sub>1</sub>, but values under 0,25 at T<sub>2</sub>. Following filtration of the spiked water samples at the 3 contamination levels and DNA extraction, LAMP assay showed no amplification at 3 hours for the 5 CFU/100ml spiked sample, while at 4 hours amplification occurred also for 5cfu/100ml. Therefore, the 4-hour incubation time showed to improve considerably sensitivity.

**Discussion and Conclusions-** This approach, which combines the enrichment with the LAMP assay, can detect a concentration of *P. aeruginosa* ≤5 CFU/100ml in just over 5 hours. Hence, it is more rapid than reference method and shows a higher sensitivity than other molecular methods. This new assay can help providing timely and accurate risk assessment to prevent *P. aeruginosa* exposure from water, resulting in reduced disease burden, especially among immunocompromised and susceptible individuals.

## ***P 082 – ID 252 - Occurrence of *Pseudomonas aeruginosa* in several sources of drinking water***

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**Introduction:** *Pseudomonas aeruginosa* is an environmental ubiquitous bacterium that may cause opportunistic waterborne infections in immunocompromised host. It can be isolated in several aquatic habitat where it shows the ability to survive in a quiescent metabolic state under stressful conditions and to revert to a metabolically active status when environmental conditions allow. In domestic environment it is not rare to find it associated with the water piping system, producing a thick layer of biofilm. Many strains that are biofilm producers express virulence factors and resistances to several antibiotic classes. This study aims to isolate and characterize *P. aeruginosa* and other Gram-negative bacteria in a range of types of drinking water.

**Materials and Methods:** For each water source, 1 liter sample was aseptically collected in sterile flasks containing sodium thiosulfate to neutralize chlorine. The samples were kept at +4 °C and analyzed within 24 hours. Isolation and enumeration of *P. aeruginosa* was carried out by membrane filtration technique according to the UNI EN ISO 16266: 2008. The isolated strains were identified and tested for antibiotic resistance by Vitek 2 (Biomérieux). All *Pseudomonas* isolates were tested for biofilm production by violet crystal staining in multiwell 96 plates and their growth was compared to those of strongly producing biofilm reference strains. All strains were stored at -80 °C for further studies.

**Results:** A total of one hundred water samples from different sources was analyzed: tap water, domestic deionizer water (from home filtration devices), water dispensers, ground water and mineral water springs. In most of the samples directly from the drinking water system a microbial load < 10 CFU/L has been detected. In 1/3 of the samples till now analyzed, Gram-negative species, resistant to the chlorine, have been identified.

A higher percentage of samples from domestic deionizer and dispenser waters resulted positive for the presence of *P. aeruginosa* than ground and tap water samples.

**Discussion and Conclusions:** Our preliminary data highlight a critical issue related to the home filtration devices installed in the terminal part of taps. Although the water suppliers constantly check the ensuring compliance with the microbial load parameters, the last part of the private pipes frequently hosts microorganisms organized in biofilms mono or multi species able to alter the microbiological quality of water.

Deionized water samples have been resulted a large reservoir of microbial diversity. Indeed, the great filtering surface provides perfect substrate to sessile forms triggering the biofilm production and enhancing the growth of bacteria routinely living in drinking water.

Further sampling and analysis are necessary to drive reliable conclusions.

## ***P 083 – ID 256 - Antimicrobial and antibiofilm properties of graphene oxide on root dentine: A pilot study***

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**Introduction:** Graphene is a single atomic sheet of sp<sup>2</sup>-bonded carbon atoms arranged in a honeycomb pattern with extremely high mechanical strength and modulus of elasticity. Graphene has many derivatives, such as graphene oxide (GO), reduced graphene oxide (rGO) and Graphene nanoplatelets (GNP). They differ from each other in terms of surface properties, number of layers, and size. Graphene and graphene oxide are recognized as promising nanomaterial to be applied in biologic and medical fields, such as dentistry. Based on few articles, it is known that graphene and GO can inhibit the growth of some dental pathogens as *E. coli*, *S. aureus* and *B. subtilis*. *E. faecalis*, that is known for its ability to form intra- and extraradicular biofilms, can be resistant to endodontic therapy procedures and may serve an important role in contributing to endodontic treatment failure. The aim of this study is to determine the antimicrobial and antibiofilm properties of GO on root dentin surfaces.

**Materials and Methods:** Twelve single-rooted teeth (maxillary incisors, maxillary canines and maxillary premolars) were selected for this study. After root canal preparation, the teeth were sliced using a water-cooled diamond saw and 30 disks (3-3,5 mm thick) were prepared. Root-Disks were attached to 24-Well Plate microtiter and each specimen was sterilized with plasma sterilization (Steris V-PRO® maX Low Temperature Sterilization System Group, Bordeaux, France). Fifteen samples were treated with GO (particles size < 10 µm) (Graphenea - Graphene Oxide Water Dispersion, 0.4 wt% concentration, San Sebastián, Spain) using the Drop Casting Method (Go Group) and fifteen samples were not treated (NT Group). Then, the specimens inside 24-Well Plate, were inoculated with 1 ml of suspension of *E. faecalis* (ATCC 29212). After 48 hours, bacteria were spread on ESA-plates. After overnight incubation at 37°C, colonies on the plates were counted to determine the number of CFU. Moreover, the specimens were observe under an electronic scanning microscope (SEM) and the presence of clumps were evaluated using a semi-qualitative evaluation. The data were analyzed using Kruskal-Wallis test (p<0.05).

**Results:** The CFU of GO Group and NT Group showed a statistical significant differences (p<0.05). The SEM results (clumps) showed a statistical significant difference between groups (p<0.05)

**Discussion and Conclusion:** Several studies have shown the antimicrobial efficacy of GO. Indeed, GO can interact and have effects on bacterial cells. In this study, the GO on root dentin showed an antimicrobial efficacy. Moreover SEM analyses highlighted GO inhibited bacterial aggregation and biofilm formation. Further studies are necessary to assess the interaction between GO and root dentin.

## ***P 084 – ID 261 - Antimicrobial properties of *Satureja montana* L. essential oils and nanoemulsions***

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**Introduction** *Satureja montana* (Family of *Lamiaceae*) Essential Oil (SEO) presents a wide range of biological activities due to the high content of active phytochemicals. The composition of four different SEOs (SEO1-3, from wild plants, and SEOT, from commercial source) has been analyzed. Subsequently, the antimicrobial properties against different Gram-negative and Gram-positive bacterial strains have been assessed determining the minimum inhibitory (MIC) and the minimum bactericidal (MBC) concentration. In addition, antibiofilm activity was tested. In order to improve the EO properties Oil in Water Nanoemulsions (NEs), composed by SEO and Tween20 or Tween80 were prepared.

**Materials and Methods** The composition of the complex metabolic mixtures, differing for the biological sample harvesting and distillation time, have been determined by means of high-resolution Fourier-transform ion cyclotron resonance, mass spectrometry, coupled to electrospray ionization and atmospheric pressure chemical ionization sources. The morphology of bacterial cell treated with the SEOs was evaluated by scanning electron microscopy (SEM), in order to visualize possible damages. Quantification of biofilm production was assessed by crystal violet staining. NEs were produced in different ratio of SEO and Tween20 or Tween80. All formulations were analyzed in terms of hydrodynamic diameter,  $\zeta$ -potential and polydispersity index (PDI) by using the Dynamic Light Scattering. The MIC and MBC in brain heart infusion broth of the different SEOs and NEs were also determined against different bacterial strains.

**Results** Terpenes, terpenoids, fatty acids, polyketides, amino acids and polyphenols were found as the main components of the mixtures. All the SEOs samples have shown, at different extent, antibacterial activity. The droplet size of the NEs was found in the range of 55-120 nm and the dimensions seem to be correlated with the surfactant amount. In particular, increasing surfactant content, the dimensions decrease. The PDI value lower than 0.3 indicates that all formulations are characterized by homogeneous population. Sub-MIC values of SEOs and NEs showed a significant reduction of biofilm formation; SEOT in addition exerted an effect, albeit weak, on the established biofilm mass. SEM confirmed that the main SEO targets are the cell wall and the membrane.

**Discussion and Conclusions** This work confirms how the knowledge of different chemical profiles of SEOs can be a crucial point to understand the antimicrobial activity. MIC and MBC values fluctuated slightly depending on variations in their chemical profiles. In addition, the results confirmed the formation of stable NEs characterized by homogeneous dimensions that could be applied as innovative drug delivery system.

## ***P 085 - ID 264 - Biofilm formation and antibiotic resistance in Stenotrophomonas maltophilia: a prospective multicentre study.***

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**Introduction:** *Stenotrophomonas maltophilia* is an environmental multidrug-resistant opportunistic pathogen causing serious infections. Although its ability to grow as a biofilm both onto abiotic and biotic surfaces recently moved into the focus of research, the evidence that this lifestyle contributes to diseases caused by *S. maltophilia* is lacking. Herein, a large collection of clinically relevant isolates was evaluated *in vitro* for biofilm formation. Further, the relationship between biofilm formation and antibiotic resistance was investigated.

**Materials and methods:** Between February and April 2019, 109 *S. maltophilia* isolates were collected at five international medical centres (Prague, Belgrade, Freiburg, Las Palmas de Gran Canaria, Rome). The clinical relevance of each isolate was defined (“definite”, “probable”, “possible”, “non pathogen”) according to the CDC guidelines. Biofilm formation, assessed by crystal violet microtiter plate assay, was cross-referenced with microbiological data and the *in vitro* activity of seven antibiotics tested by disk diffusion method. Further, susceptibility of preformed biofilm to cotrimoxazole and levofloxacin was evaluated by measuring the Minimal Biofilm Inhibitory (MBIC) and Eradication (MBEC) Concentrations.

**Results:** Biofilm formation was highly preserved in *S. maltophilia* population studied, regardless of etiological relevance (93.9% vs. 90.8%, respectively for nonpathogen and pathogenic isolates). However, “strong-producer” isolates were more prevalent among “definite” pathogens than “probable”, and “possible” ones (73.9% vs. 42.2% and 25%, respectively;  $p < 0.05$ ). The “strong-producer” isolates were also more prevalent among isolates from blood (78.3%), wound (64.3%) and non-cystic fibrosis patient respiratory tract (54.5%). A trend for higher biofilm formation was observed among isolates causing hospital- vs. community-acquired infections. Pathogenic isolates susceptible to colistin, ceftazidime, levofloxacin, and ticarcillin-clavulanic acid produced significantly more biofilm biomass than the resistant counterparts, whereas no differences were found among non-pathogenic isolates. A negative relationship was found between biofilm amount and the number of resistances to antimicrobials tested. *In vitro* data showed that planktonic-to-biofilm transition significantly increased antibiotic resistance both to cotrimoxazole (MBIC values up to 256xMIC; MBEC values up to >64xMBC) and levofloxacin (MBIC values up to >128xMIC; MBEC values up to 128xMBC).

**Discussion and conclusions:** The relationship seen in *S. maltophilia* between biofilm formation and antimicrobial resistance highlights the importance of the sessile phenotype as a potential virulence factor that might contribute to therapeutic failure and chronicization of infection.

## ***P 086 – ID 265 - Polyclonal circulation of Klebsiella pneumoniae isolated from blood infections in Sicily***

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**Introduction:** Carbapenem-resistant *Klebsiella pneumoniae* has emerged as one of the major multidrug-resistant bacterial pathogens worldwide. This strain is highly transmissible and able to cause severe infections in both the hospital and the community. The resistance of this pathogen is conferred on it by numerous genes of resistance to antibiotics encoded by chromosome and plasmids (ARG). The international spread of this pathogen has been linked to a sequence type (ST) 258 predominant in North America and Europe and also to ST11, primary carbapenem-resistant *K. pneumoniae* clone in eastern Asia, especially in China. In recent years, *K. pneumoniae* ST11 strains have been increasingly identified in North America. In Italy, exists a surveillance system that reports all cases of blood flow infection to Carbapenem-resistant *Klebsiella pneumoniae* (CRKP). In this study we report the analysis of 602 CRKPs isolated from bloodstream infection of hospitalized patients and collected by the Regional Reference Laboratory for the Surveillance of infection by CPE.

**Materials and Methods:** From January 2017 until October 2018, the isolates from blood with antimicrobial MICs  $\geq 8$   $\mu\text{g/ml}$  for meropenem and/or imipenem-resistance, according to EUCAST clinical breakpoints, are submitted to a molecular typing. Detection of ST-258/512 clone was performed by a multiplex PCR assay to detect three genes that are *pilV-l*, *is-66* and *prp*. To determine the isolates ST-258/512 clone negative, we use an ERIC PCR and a multiplex PCR assay to detect two genes *kphp* and *pilL* that are considerate the markers for ST11. *pilV* was selected as a marker for ST258 and differentiates two different sequence type. While, we detect two ST11 associated capsules, K locus type 47 (KL47) and KL64. And also, we analyzed for ST11 four virulence genes *rmpA*, *rmpA2*, *iroN* and *iutA*. Among them, *rmpA/rmpA2* are the mucoid phenotype regulator genes, whereas *iroN* and *iutA* are two genes linked to the siderophore-mediated iron-acquisition operon.

**Results:** Our results showed the 114/602 CRKP belonged to the clonal complex 258 (CC-258) with prevalence of 19%. Furthermore, 10/602 CRKP isolates belong to the clone ST11 with prevalence of 1,6%. All 10 isolates were positive for *iutA*, and *rmpA/rmpA2*, while they result negative for *iroN* and *KL47/KL64*. Genotyping of *K. pneumoniae* revealed 31 different ERIC types showed that there is no specific ERIC profile.

**Discussion and Conclusion:** Infections with CRKP are an emerging clinical threat especially in patients with blood infections. The low prevalence of CC-258 suggests a change of the epidemiology of CRKP population towards a more complex polyclonal spread in Sicilian hospitals.

## ***P 087 - ID 267 - Antimicrobial and antibiofilm properties of Temporin-L on Pseudomonas fluorescens, in static and dynamic conditions.***

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**Introduction:** Biofilms consist of an extracellular matrix composed of proteins and polysaccharides that constitute the principal cause of the bacteria resistance against a large number of antibiotics. Therefore, research is focused on finding alternative antimicrobial substances able to either hamper biofilm formation or to prevent bacterial growth. Recently, we showed that the antimicrobial peptide Temporin L impairs *E.coli* growth by inhibiting cell division. Here we investigate the effect of Temporin-L (TL) on biofilm formation in *Pseudomonas fluorescens* (*P. fluorescens*) both in static and dynamic conditions, demonstrating both antimicrobial and antibiofilm properties.

**Materials and methods:** Biofilm formation in static conditions was performed on coverslips and analyzed by the Crystal Violet assay while biofilm morphology was assessed using imaging techniques. In dynamic conditions, biofilms were grown in a flow chamber using a microfluidic system and images were recorded by confocal microscopy.

**Results:** The *P.fluorescens* cells were either grown in the presence of TL or incubated with the antimicrobial peptide following biofilm formation both in static and dynamic conditions using different concentrations of the peptide. When TL was added during cell growth, the peptide affected biofilm formation at 25  $\mu$ M. Confocal microscopy demonstrated that at this concentration *P.fluorescens* cells were still alive while antimicrobial activity was clearly evident at 50  $\mu$ M. When *P.fluorescens* cells were treated with TL following biofilm formation, confocal images demonstrated that the peptide exerted a strong antimicrobial activity leading to cell death but leaving the biofilm architecture unaltered.

### **Discussion and Conclusions**

Investigation of TL effect on *P. fluorescens* confirmed the antimicrobial activity of this peptide already observed on *E.coli* cells when TL was added during *P. fluorescens* growth. In these conditions, at lower concentration the peptide showed also antibiofilm properties impairing biofilm formation both in static and dynamic conditions. However, confocal microscopy experiments demonstrated the absence in *P. fluorescens* of the long necklace-like structure observed in *E.coli* indicating that a different molecular mechanism of cell death had taken place. Furthermore, when TL was added to *P. fluorescens* biofilm, static and dynamic experiments demonstrated that a strong antimicrobial activity was exerted by the peptide suggesting that TL might penetrate biofilm architecture with a still unknown mechanism leading to *P. fluorescens* cell death.

## ***P 088 – ID 268 - Carbapenem-resistant Enterobacteriaceae resistance (CRE): nine months of surveillance in Sicily***

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**Introduction:** Carbapenems are considered to be one of the few drugs that are useful for the treatment of infections caused by Multi-resistant Gram-negative bacteria. The emergence of carbapenem-resistant *Enterobacteriaceae* is a serious public health due to the large spectrum of resistant genes and to the lack of therapeutic options. Carbapenem-hydrolyzing  $\beta$ -lactamases belonging to molecular class A (e.g., KPC, GES, IMI, SME), class B (e.g. IMP, VIM, NDM, GIM) and class D (e.g. OXA-23 and OXA-48), are the main source of antibiotic resistance in *Enterobacteriaceae*. Since the risk of nosocomial infections related to Carbapenem-resistant *Enterobacteriaceae* is very high. The aims of this study was to activate an surveillance system in the following departments: surgery ICU, hematology, emergency room, neonatology and infectious diseases.

**Materials and methods:** From September 2018 until June 2019, 349 patients were enrolled. The colonization due CRE was evaluated by phenotypic methods. While the GeneXpert CARBA-R was used to evaluate the genes correlated to the resistance.

**Results:** Our results show that 21.4% (75/349) of patients were colonized by CRE. Particularly 52% (39/75) of the strains were positive for *bla*<sub>KPC</sub>. While 1,3% (1/75) were positive for *bla*<sub>OXA-48</sub>, *bla*<sub>VIM</sub> and *bla*<sub>NDM</sub> respectively. In addition 6.6% (5/75) of the strains were positive for two different genes for *bla*<sub>KPC</sub>-*bla*<sub>OXA-48</sub> and 1,3% (1/75) for *bla*<sub>KPC</sub>/*bla*<sub>VIM</sub>. However 36% of the strains resistant to carbapenems did not show any genes analyzed.

**Discussion and Conclusion:** The surveillance system proposed that one of the main risk factors in the rapid spread of carbapenem-resistant bacteria is represented by nosocomial infection. Data collected reveal that hospitalization time exposes patients to high probability of colonization. In conclusion, a comprehensive infection surveillance strategy against CRE infections, based on the screening of rectal swabs reduces both the incidence of colonization by enterobacteriaceae resistant but also health care costs for patient treatment.

## ***P 089 – ID 272 - First detection and characterization of Hospital-Associated Vancomycin Resistant *Enterococcus faecium* isolates in the ICU of the AOU hospital of Sassari, Italy.***

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### **1. Introduction**

Hospital-Associated Vancomycin Resistant *Enterococcus faecium* (VRE) has emerged worldwide becoming an important nosocomial pathogen causing serious infections leaving scarce therapeutic options. In clinical settings, vancomycin resistance is mainly due to the acquisition of vanA and vanB transposons contributing to the dissemination of resistance. Phenotypic and genotypic characterisation of isolates is essential for the prevention and effective management of enterococcal infections. Here we describe the prevalence of *E. faecium* from different wards and the characterization of the first two VRE strains isolated at the University Hospital of AOU Sassari in Italy.

### **2. Materials and Methods**

Epidemiological data of *E. faecium* were retrospectively analyzed from 2013 to 2018. Two VRE strains isolated in January 2018 were analyzed by WGS for resistance and virulence determinants, MLST and clonal relationship, additional eight VSE isolates from 2013, 2014 and 2017 were included in the study.

### **3. Results**

During the study period 396 clinical isolates of *E. faecium* were isolated mostly from Surgery, intensive unit care (ICU) and Medicine wards, mainly from urine (26%) and blood (11%) samples. For the first time two multidrug resistant VRE strains (VAN MIC $\geq$ 8) were isolated from the same patient admitted at ICU. WGS analysis revealed a new Tn1549-VanB<sub>2</sub> with mutations in vanS, vanW, and vanB genes, the IS16 marker and *esp* gene. VRE strains were nosocomial of ST117 of Clonal Complex 17. They were closely related but not clonal, differing by 23 cgSNPs. VSE strains were nosocomial of ST117, ST780, ST80, ST78 and the new ST916 (SLV of ST17). One isolate from ICU was vancomycin variable (VVE) (VAN MIC $\leq$  0.5) of ST117 carrying a similar vanB<sub>2</sub> operon to VRE displaying common mutations in vanB and vanH and different in vanW genes.

### **4. Discussion and Conclusions**

Vancomycin resistance appeared in 2018 in ICU ward associated to a new variant of Tn1549-vanB<sub>2</sub>. VREf differed by 23 SNPs in the core genome suggesting its evolution within patient. Phylogenetic analysis revealed a different origin of VRE and VVE from the endemic ST117 VSEf clones circulating in the hospital at least from 2013. Interestingly, WGS revealed a VVE clone characterized by a stealthy VanB operon. This type of strains may revert to a vancomycin-resistant phenotype on exposure to vancomycin through additional mutations in van operon. Because of their susceptibility to vancomycin these clones escape the traditional phenotypic method having considerable potential to spread silently and cause treatment failures, posing thus new challenge to the current detection of VRE.

## ***P 090 – ID 279 - Longitudinal genomic analysis of Pseudomonas aeruginosa as a tool for the definition of persistence/reinfection in the airways of Cystic Fibrosis patients.***

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**Introduction:** In cystic fibrosis (CF) patients chronic *Pseudomonas aeruginosa* (*Pa*) infection is associated with lung damage, a more rapid decline in lung function, and is an important prognostic factor of morbidity and mortality. *Pa* earlier acquisition shortens life expectancy, therefore, attempts to eradicate initial *Pa* acquisition and delay chronic airway infection is crucial for patient care. Aim of this study was to analyse the whole genome sequences (WGS) of *Pa* isolates obtained from a child over a 4 years period in order to define if she was subjected to uncommonly frequent reinfections or if she has acquired an early chronic *Pa* infection.

**Materials And Methods:** *Pa* isolates (n = 32) were subjected to genomic DNA extraction. Nucleic acid was quantified by Quantus fluorimetric system (Promega) and evaluation of its purity was carried out by Nanodrop (Thermo Scientific). Genomic libraries were prepared using the Nextera XT Flex DNA kit and were run using the Miseq system (Illumina) for the generation of paired-end 2x250bp reads. Raw reads were evaluated on the basis of sequence quality criteria using FastQC. Filtered reads (Trimmomatic software) have been *de novo* assembled through the SPAdes v3.9.0 software. Assembled products were used as input for genetic characterization by comparison with specific databases such as virulence gene databases (Virulent Factors Database, VFDB) and antibiotic resistance (Resfinder). Phylogenetic relationship of the isolates was evaluated using a SNP-based approaches (CFSAN, FDA or kSNP3). SNPs matrix was used as input for phylogenetic analyses performed with the RaxML software that uses Maximum Likelihood (ML) algorithms to determine the relationships between genomic sequences of *Pa* isolates obtained from this study and the ones that are present in databases.

**Results:** WGS analysis carried out with CFSAN pipeline using PAO1 as reference genome of *Pa* highlighted the presence of two clusters whose isolates differ in about 1000 pairwise SNPs. Within the same cluster, *Pa* isolates had a maximum of 6 SNPs difference confirming the clonality of different isolates. The main cluster comprises all the *Pa* strains isolated in the period 2015-2017, when the child had two >6-months period of *Pa*-free cultures and some strains isolated in 2018/2019 (cluster I), whereas the cluster II contains only recent strains (years 2018-2019).

**Conclusions:** Results have shown that, starting the first *Pa* isolation, the child suffered from a chronic infection and that a superinfection occurred some years later. Evaluation of *Pa* clonality by WGS may support studies aimed to determine efficacy of eradication therapies and may help to manage patients for obtaining a better clinical outcomes.

## ***P 091 – ID 288 - Staphylococcus aureus strains isolated from osteomyelitis in Italy: molecular typing and virulence profile***

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**Introduction:** *Staphylococcus aureus* is the most common causative organism of osteomyelitis (OM). To evaluate the phenotypic and genotypic characteristics and the virulence profiles of the *S. aureus* strains causing OM in Italy, a collection of *S. aureus* isolated from OM patients was analysed.

**Materials and Methods:** Twenty-six *S. aureus* strains were collected from orthopedic patients suffering from OM. The identification of the isolates and the antimicrobial susceptibility pattern were obtained by MALDI-TOF and broth microdilution, respectively. Genes encoding for adhesins of the MSCRAMMs family, such as ClfA, ClfB, FnbA, FnBb, Cna and Bbp, and the *icaA* gene for biofilm formation were searched by PCR. In addition, the presence of PVL-encoding gene was also assayed. All strains were *spa* typed and the Sequence types (STs) were deduced by the Ridom SpaServer.

**Results:** Most of strains (21/26, 80.7%), were obtained from patients with local risk factors as orthopedic implants or orthopedic surgery. Methicillin-resistant *Staphylococcus aureus* (MRSA) accounted for 30.7% (8/26) of strains. The *spa* typing showed 21 different *spa* types distributed among 10 clones. The most prevalent clone among MRSA was ST22 (4/8, 50%) detected only among MRSA strains. ST30 was present only among methicillin-susceptible *Staphylococcus aureus* (MSSA), also representing the most common type among MSSA (3/18, 16.6%). PVL was detected in only 2 MSSA strains belonging to ST30 (7.7%). Genes encoding for ClfA, ClfB, and FnbA were present in almost all strains (25/26; 96.2%) while genes encoding for Cna and Bbp were detected in 30.8% (8/26) and 26.9% (7/26) of the strains, respectively. The *icaA* gene was present in all isolates.

**Discussion and Conclusions:** The molecular epidemiology of *S. aureus* responsible for OM in Italy is quite scarce. Despite the limited number of strains examined, this preliminary study shows the diversity of *S. aureus* strains causing OM in Italy, and highlights differences among MSSA and MRSA strains. All isolates are well equipped with virulence factors important for adhesion to the bone and for establishing the infection. Some virulence factors were present in almost all strains, while Cna was found only in strains belonging to ST30 and ST22. An in-depth analysis of the genotypic characteristics of these strains could help understanding the importance of the virulence factors for the establishment of the OM. Future approaches, such as whole genome sequencing and *in vitro* models of infection and biofilm formation could contribute to gain further insight into the role of these virulence traits.

## ***P 092 – ID 290 - Analysis and Biological Characterization of the Outer Membrane Vesicles produced by Helicobacter pylori Planktonic and Biofilm Phenotypes***

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**Introduction:** *Helicobacter pylori* causes gastro-duodenal diseases, which may be mediated in part by the outer membrane vesicles (OMVs) constitutively shed by the pathogen. Outer Membrane Vesicles (OMVs) deliver virulence factors, proteins and nucleic acids and are involved in cell-cell interactions during biofilm formation. The aim of the present study was to analyze and characterize the OMVs isolated by the planktonic (pOMVs) and biofilm (bOMVs) phenotypes at 2, 6 and 10 days of incubation to clarify how these structures are associated with *H. pylori* pathogenesis.

**Materials and Methods:** *H. pylori* ATCC 43629 biofilm formation was evaluated at 2, 6 and 10 days of incubation using live/dead staining and confocal laser scanning microscopy (CLSM) followed by COMSTAT image analysis. The pOMVs and bOMVs isolated by ultracentrifugation were treated for: (1) Proteomic analysis by nano LC-MS/MS; (2) Extracellular DNA (eDNA) concentration by using QuantiT<sup>TM</sup>PicoGreendsDNA assay; (3) Enumeration and eDNA detection by PKH26 and PicoGreen staining followed by flow cytometry.

**Results:** COMSTAT analysis showed no significant changes in thickness, biomass and roughness over time. The biofilm had a well-developed 3D-structure with an equal amount of live and dead cells. The PicoGreen assay showed that the content of the eDNA associated with OMVs is in the range of 29-54 pg/μl for the pOMVs and 0,6-2 pg/μl for the bOMVs. Flow cytometry data confirmed that most of the detected vesicles contained eDNA (eDNA-OMVs), in particular the eDNA-OMVs represented the 60-99 % of the total vesicles detected. Proteomic analysis revealed a time and phenotype dependent modulation of many virulence related proteins, such as the Vacuolating cytotoxin transporter which resulted 5 times more abundant in the bOMVs. Furthermore, proteins related to the pathogen survival such as the Alkyl hydroperoxide reductase C, was only detected in the biofilm phenotype at each time point.

**Discussion and Conclusions:** *H. pylori* biofilm reaches maturity after 2 days of incubation as shown by COMSTAT analysis. The most of OMVs are associated with eDNA, suggesting a possible key role in horizontal gene transfer as well as in the biofilm development. The proteomic analysis of the OMVs suggests a phenotype dependent role, which may be associated with *H. pylori* colonization and pathogenesis.

## ***P 093 – ID 291 - Potential activity of Vitamin E in reducing the biofilm formation by human pathogens on the surface of medical devices.***

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**Introduction:** The use of medical devices for the healthcare of patients is often associated with the onset of healthcare-associated infections (HCAIs). These undesired infections are caused by development on the surface of such devices of bacterial biofilm assemblages, which confer to the pathogen's resistance against the antibiotic treatment. Due to the clinical and economic relevance of this topic, new strategies for the treatment of infections caused by the biofilm proliferation are unceasingly searched by scientists. The present study speculated the potential use of vitamin E as pretreatment of surfaces to avoid/reduce the biofilm formation by a large panel of human pathogens, including *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Pseudomonas putida*.

**Materials and Methods:** Vitamin E was applied as an interface between the bacterial cultures and the polystyrene walls of a 96 well plate at different concentrations of glucose and its influence on *in vitro* biofilm production was evaluated. Moreover, for *Staphylococcus* genus a further investigation was conducted verifying the potentiality of an anti-biofilm pretreatment with vitamin E on the silicone surface of urinary catheters lumen. The vitamin E was spread on the lumen of the catheters and then inocula of stains were performed. The ability of these strains to colonize the pretreated surfaces was finally evaluated.

**Results:** Vitamin E has been able to interfere with bacterial adhesion and prevent *in vitro* biofilm formation. Furthermore, the ability of *S. aureus* and *S. epidermidis* to colonize the catheter surface resulted in being decreased by vitamin E application.

**Discussion and Conclusions:** In the present study, we evaluated the *in vitro* ability of vitamin E to interfere with bacterial colonization of plastic and of medical devices surfaces by a large panel of human pathogens implicated in the HCAIs onset. Results related to biofilm formation on such materials have shown that the vitamin concentration used can reduce the biofilm production of all tested strains, with a variable efficacy between strains and without any correlation to the gram-negative or positive group. Furthermore, for *S. aureus* and *S. epidermidis*, resulted more susceptible to the action of vitamin E, a potential role of vitamin for the prevention of biofilm formation on the surface of medical devices was also demonstrated. Although further studies are needed to better clarify the mechanisms the spectrum of vitamin E activity and the influence on that of glucose adding, our findings together suggest the promising use of vitamin E as coating molecule to prevent implant-associated infections and also improve the post-operative course.

***P 094 – ID 292 - Seizing the Staphylococcus aureus catabolites can constitute an innovative strategy for ameliorating Atopic Dermatitis symptoms. Preliminary data in vivo on Galleria mellonella preclinical model.***

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**Introduction.** Atopic Dermatitis (AD) is a common chronic inflammatory skin disease, highly disabling, characterised by dry, itchy, red, swollen, and cracked skin. Although the etiopathogenesis up today is still unclear and debated, it is well recognized that the cutaneous dysbiosis contributes to the pathophysiology of this disease. During an eczema flare, the diversity of the normal microflora is diminished, allowing *Staphylococcus aureus* to proliferate. *Staphylococcus aureus* expresses several molecules that contribute to the intensity of symptoms of this pathology, including delta-toxin, alpha-toxin, Phenol-soluble modules, protein A, superantigens which trigger B cell expansion and cytokine release and proinflammatory lipoproteins. LimpiAD is a new dermatological formula developed by Aileens pharma pointing to improve the symptoms of AD. LimpiAD, through its active component (a fragment of a bacterial cell wall with a carrier mucopolysaccharide matrix), could act on the pathogens responsible for the main clinical signs of the disease.

**Materials and Methods:** *Staphylococcus aureus* ATCC BAA1680 and *Staphylococcus aureus* ATCC 29213 were grown in their specific medium, and the overnight cultures were centrifuged and diluted in saline solution. The diluted supernatants were mixed with three different formulations of LimpiAD: LimpiAD A (base formula, without active component), LimpiAD B (with 2,5% active component) and active component alone. After 1h and 4h incubation with the formulations, the supernatants were used to inoculate *G. mellonella* larvae. Larvae treated injected with supernatants (without formulation) and culture medium alone (Brain Heart Infusion broth) in a 1:2 ratio were used as controls. After injection, larvae for each condition were incubated in a Petri dishes at 35°C and survival was observed in the following 96 hours.

**Results.** For the *S. aureus* ATCC BAA1680 strain, the formula B produced greater survival increasing. Regarding the strain *S. aureus* ATCC 29213, the active component produced the greater increasing of larvae survival both in 1h and in 4h pre-treatments. For both strains, the lower activity was confirmed for the base formula A.

**Discussion and Conclusion:** Our results suggested that the active components could interfere with the pathologic mechanisms associated to the catabolites produced by *S. aureus*, in particular, the intensity of interference resulted higher as more prolonged is the putative interaction with the catabolites in supernatants. This study, it makes us confident to hypothesize a promising potential of LimpiAD in seizing of catabolites and setting the stage for an innovative and original approach to the treatment of AD.

## ***P 095 – ID 295 - Evaluation of biofilm formation in Klebsiella pneumoniae Multidrug-Resistant isolated from urine and blood samples***

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**Introduction:** Strains of *Klebsiella pneumoniae* are often involved in urinary tract infections and blood infection associated also with sepsis in compromised individuals. It is the most frequently recovered agent from nosocomial infections. The bacterium is able to form a layer of biofilm which contributes to make the microorganism resistant to several antibiotics. The antibiotic resistance correlated to the biofilm formation have contributed to the global spread of *K.pneumoniae* infections. The aim of this study was to analyze the biofilm formation ability of clinical *K. pneumoniae* strains isolated from urine and blood samples.

**Materials and Methods:** Using fresh colonies the *K.pneumoniae* strains were diluted in 3 ml of Brain-Heart Infusion (BHI) and quantified to 0.5 McFarland. 200 µl of each culture broth was added into three wells of microtiter plate and a negative and positive control were also included. The biofilm assay was performed in triplicate. It was analyzed according to G.Donelli et al.

**Results:** Our results showed that 88 % of strains isolated from urine samples and 80 % isolated from blood culture were producers of biofilm. Particular *K.pneumoniae* of urine samples have formed a more adherent biofilm. In addition 16% of strains isolated from urine samples and 8% from blood cultures belong to clonal complex CC-258.

**Discussion and Conclusion:** In our study we evidence the capacity of Multidrug-Resistant *K. pneumoniae* to produce biofilm, particular as nosocomial infection in patients with catheter. The results obtained revealed that the ability of urine isolates to produce biofilm is higher than that of blood isolates. So the biofilm formation probably is correlated with the site of infection and it is also one of the determining factors the antibiotic resistance.

***P 096 – ID 296 - Bactericidal activity of 0.6% povidone iodine eye drops formulation in preparation of patients for cataract surgery: preliminary results of a multicentre, prospective, clinical study***

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**Introduction:** Endophthalmitis is one of the most serious complications of cataract surgery. Application of 5% PVP-I before cataract surgery is recommended but dilute povidone-iodine (PVP-I) in humans is not enough investigated. The greater availability of diatomic free iodine in diluted PVP-I solution kills bacteria faster than at more conventional concentrations (5-10%). The objective of this study was to assess the bacterial load variation of the normal conjunctival flora following repetitive applications of PVP-I containing eye drops prior preoperative cataract surgery for endophthalmitis prophylaxis. IODIM™ (Medivis SRL, Italy), was administered as repetitive applications for 3 days in the eye of patients undergoing cataract surgery.

**Materials and methods:** In this study on medical device lasting 4 days, 120 patients, candidates for cataract extraction, in groups of 20 from 6 centers, were enrolled to assess the effectiveness of 0.6% PVP-I solution in the eye subjected to cataract surgery. Four conjunctival swabs were collected using the Copan ESwab™ collection device, (a tube with 1 ml liquid Amies medium and a FLOQSwab®), from the eyes of each patient in two different time-points. Aliquots of 100 ul of each conjunctival ESwab™ samples was plate on three different agar plates to assess microbial growth and total bacterial count. Bacterial identification was performed by MALDI-TOF mass spectroscopy and, for some species, antibiotic-resistance related phenotype was performed. The untreated contralateral eye was used as control.

**Results:** Overall more than 70 different bacterial species were identified from conjunctival ESwab™ samples with a total bacterial count/eye ranging from 1x10<sup>1</sup> to 8x10<sup>3</sup> UFC/mL with *S. epidermidis* as the most representative specie. More than 85% of patients showed a significant reduction (2-3 log units) in resident microbial load up to the total eradication of bacteria in 46% of patients. The comparison with the untreated eye confirmed the action of IODIM™ in decreasing the load, while the untreated eye showed an extreme variability of bacterial load between the two times.

**Discussion and conclusions:** IODIM™ has been shown to significantly lower the bacterial load of the ocular surface in patients undergoing cataract surgery. ESwab™ demonstrated to optimally collect and transport live cells till 36h after from conjunctival eyes samples.

***P 097 – ID 298 - Akkermansia muciniphila as a therapeutic target in inflammatory bowel diseases treatment: an in vivo study.***

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**Introduction:** *Akkermansia muciniphila* (*A. muciniphila*) have been recently pointing out among the obligate anaerobic gut commensal bacteria because that could exert beneficial effects on human health. Indeed the presence of *A. muciniphila* in the human intestine is associated with homeostasis, an inversely proportional relationship between the presence of *A. muciniphila* and intestinal inflammations such as appendicitis, Crohn's disease, ulcerative colitis and inflammatory bowel disease (IBD) have been demonstrated. This study aimed to evaluate the potential role of *A. muciniphila* for treatment of inflammatory bowel diseases (IBD).

**Material and Methods:** *A. muciniphila* DSM 22959 (DSMZ, German Collection of Microorganisms and Cell cultures) and *A. muciniphila* BAA835 (ATCC, American Type Culture Collection) were tested on C57BL/6 mice animal model of intestinal inflammation induced by Sodium Dextran Sulfate (SDS). The course of the disease was monitored through three parameters: body weight, colon weight and DAI (disease activity index). Immediately after euthanasia, the animals were laparotomized, and then the colon was isolated and measured. Colon sections were subjected to histological analysis by hematoxylin and eosin (H&E) and periodic acid Schiff (PAS) staining. Moreover, colon serum levels of pro-inflammatory cytokines and cytokines (interleukin-6, TNF- $\alpha$  and INF- $\gamma$ ) were monitored. Mice faeces were collected and analysed by PCR to verify the effective presence of *A. muciniphila*.

**Results:** The body weight of all C57BL/6 mice decreased drastically when colitis was induced, but *A. muciniphila* was able to protect animals from the heavy weight loss of those not treated. IL-6 serum levels of rats treated with SDS only, are much higher than those pre-treated with *A. muciniphila*. Also the expression analysis of TNF- $\alpha$  and INF- $\gamma$  demonstrated how *A. muciniphila* can protect against inflammation induced by experimental colitis.

**Discussion and Conclusions:** This promising result allows us to hope that *A. muciniphila* can be exploited, in the future, as an excellent therapeutic target in inflammatory bowel diseases treatment.

## ***P 099 – ID 306 - Development of a novel fast-break carbapenem-inactivation method for the low cost detection of carbapenemase-producing Gram-negative bacteria***

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**1. Introduction.** Infections mediated by carbapenemase-producing Gram-negative pathogens are increasingly reported worldwide and represent a threat for the healthcare system. Due to the scarcity of therapeutic options, a prompt diagnosis of infections mediated by carbapenemase-producing *Enterobacterales*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, which are among the most common causes of infections worldwide and are endemic in Italy, is of particular importance. Different techniques have been developed for the detection of carbapenemase-producing Gram-negative pathogens, including different molecular, colorimetric and enzymatic inactivation methods. In this study a novel fast and cheap variant of the carbapenem-inactivation method is described.

**2. Materials and Methods.** 30 clinical strains, including 8 *Klebsiella pneumoniae*, 6 *Escherichia coli*, 2 *Enterobacter cloacae* complex, 2 *Proteus mirabilis*, 8 *Pseudomonas aeruginosa* and 4 *Acinetobacter baumannii*, characterized for their carbapenemase content (producing KPC, VIM, NDM, IMP, FIM, NMC-A/IMI-2, GES-5, OXA-48, OXA-23, OXA-24 and OXA-58) or producing other beta-lactamases (CTX-M-15, PER, VEB, CMY-16 and FOX-7) were included. A 10 µl loopful of bacterial suspension was inserted in 200 µl of a commercial lysis buffer (Promega) for 20 minutes at room temperature with a 10 µg imipenem disk. Simultaneously, a 3 McFarland suspension of *E. coli* ATCC 25922 was streaked with a sterile swab on a Mueller Hinton Agar plate. After incubation, the antibiotic disk was placed in the plate, and incubated at 36±1°C and read after 3 and 18 hours.

**3. Results.** All 25 carbapenemase-producing Gram-negatives, except one OXA-23-producing *A. baumannii* and one OXA-232-producing *E. coli*, showed a significative reduction of the imipenem inhibition zone visible after three hours (confirmed by reading at 18 hours), while the 5 non-carbapenemase producers showed comparable results with the disk incubated with *E. coli* ATCC 25922.

**4. Discussion and Conclusions.** This novel and low-cost test reduced the turn around time for the detection of carbapenemase production compared to other currently used carbapenem-inactivation techniques. Further experiments will be required to explain the few discrepant results. The future implementation of this test directly to clinical specimens may represent a valid diagnostic option.

## ***P 100 - ID 309 - The epidemiology of Tuberculosis (TB) in Sicily***

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**Introduction:** Since 2013 a Tuberculosis (TB) surveillance program has been activated in Sicily by the Regional Health Department to develop and integrate conventional and molecular investigation tools in a region where acute migration waves, older migrant subgroups resulting by previous migrations and demographic evolution of the native population make up a very complex and rapidly evolving epidemiological landscape.

**Materials and Methods:** Mycobacterium tuberculosis complex (MTBC) isolates were collected from different patients hospitalized for TB different hospitals in Sicily, since 2014. MTBC isolates were cultured, identified and submitted to anti-TB drug susceptibility testing by the clinical microbiology laboratories of the Sicilian hospitals and periodically sent for molecular typing to the regional laboratory for TB surveillance and control in Palermo.

**Results:** The notification rate of TB is increased from 38.5% in 2010 to 67.3% in 2017 with a wide range among the 9 Sicilian Provincial Health Authorities (ASP). The increase is sustained by the increase in cases of TB in foreign-born individuals (from 24.7% to 63.0%). We collected in a period of five years (2014-2018) 487 strains, among which 26 were found Multi-Drug Resistant (MDR) strains and 2 Extremely-Drug Resistant (XDR) strains. Molecular characterization by spoligotyping and 24-MIRU-VNTR typing showed a wide heterogeneity of the main lineages, with 42 clonal complexes among which, in 2017, a cluster of MDR strains.

**Discussion and Conclusions:** TB epidemiology in Sicily is complex and is rapidly changing. The most striking features are the increasing proportion of cases in the foreign-born population and the wide heterogeneity of MTBC isolates. Strain typing is a useful tool in understanding TB distribution and has a potential in formulating appropriate strategies for the disease control in a particular region. The emergence and potential spread of MDR and XDR strains require constant monitoring of drug sensitivity.

## POSTER MICOLOGIA, PARASSITOLOGIA

### *P 065 – ID 209 - Discrimination of Candida species from primary cultures using different selective mycological media*

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**Introduction:** Beside the traditional Sabouraud dextrose agar (SDA), *Candida* chromogenic medium (CHROM) is the most widely used method for easily discriminating *Candida* species from primary cultures based on different colony morphologies. *Candida* bromcresol green (BCG) agar medium allows to reveal colour-based differences among yeast colonies following pH changes induced by their differential growth. The objective of this study was to compare BCG, CHROM and SDA media for the primary isolation and presumptive identification of *Candida* species.

**Materials and Methods:** The study was performed at the clinical microbiology laboratory of the Fondazione Policlinico Universitario A. Gemelli IRCCS (Rome, Italy). We used 20 clinical isolates of *Candida* species at a 10<sup>3</sup> CFU/ml concentration to prepare suspensions of either single or randomly mixed species. We plated each suspension in parallel on the three BCG agar, CHROM agar (Brilliance Chromogenic Agar [Oxoid]) and SDA media and incubated aerobically at 37°C. Three independent readers, blinded to number and type of inoculated species, and with a high (operator #1, operator #2) to medium (operator #3) skill in plate readings, observed each set of plates daily up to 3 days (final endpoint) and recorded the number of morphologies found on each plate. We calculated the percentages of correct, overestimated or underestimated colony detections.

**Results:** Sixty monomicrobial and 570 polymicrobial plates were prepared overall, which were equally distributed across BCG, CHROM and SDA plates. Correct identification rates for BCG were almost always superior to those of CHROM and SDA, irrespectively of the operator skills. The rate averages (1- to 3-day readings) were 70.3%, 78.0%, 75.8% (BCG), 41.6%, 60.3%, 55.0% (CHROM), and 61.5%, 80.8%, 67.7% (SDA) for operators #1, #2, and #3, respectively. The underestimated detection rate averages were higher for CHROM (40.2%) than for BCG (15.3%) and SDA (22.3%) plates. The overestimated detection rates were low at all reading days (<10%), but were higher for BCG than CHROM and SDA plates and this effect was particularly evident at 2-day (4.5%, 2.3% and 2.2%) and 3-day (4.4%, 2.0% and 2.1%) readings.

**Discussion and Conclusions:** BCG agar medium can be effectively used for *Candida* species discrimination from primary cultures even in the absence of high skilled mycologists.

## ***P 101 - ID 043 - Antifungal and anti-biofilm activity of the first cryptic antimicrobial peptide from an archaeal protein against Candida spp. clinical isolates***

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**Introduction:** *Candida* species are the most prevalent opportunistic fungal pathogens worldwide, with a high mortality rate especially in immunocompromised patients. *Candida* bloodstream infection is often associated with the presence of implanted medical devices, on which *Candida* grows as a resilient biofilm. The emergence of resistance of *Candida* spp. to the most common drugs, also due to biofilm formation, requires the development of alternative antifungal agents. VLL-28 is a cryptic antimicrobial peptide derived from a transcriptional factor of *Sulfolobus islandicus*. Here we investigated the antifungal activity of VLL-28 towards pathogenic *albicans* and *non-albicans Candida* spp. isolated from blood infections.

**Materials And Methods:** we studied the *in vitro* ability of VLL-28 to (1) inhibit yeast cells growth in a planktonic state using a standardized broth microdilution method (CLSI document M27-A2), (2) prevent cell adhesion and (3) eradicate established biofilms using the XTT-reduction assay, a tool for the quantitative measurement of bacterial and fungal metabolic activity and response to antimicrobial treatments. Confocal laser scanning microscopy (CLSM) was used to illustrate the effect of peptide on the viability and architecture of biofilms of *Candida* species using the LIVE/DEAD FungaLight Yeast Viability Kit. CLSM was also used to study the intracellular target of the peptides by double staining of the yeast cells with FITC-labelled peptides and MitoTracker Orange.

**Results:** VLL-28 showed comparable antifungal activity against tested isolates of *Candida* spp. It effectively prevents biofilm formation by reducing the cells' adhesion to the abiotic surfaces of all the strains tested, except for *C. krusei*. CLSM analyses performed on mature biofilms revealed that VLL-28 is differentially active on tested strains, with *C. albicans* and *C. parapsilosis* being the most sensitive ones. The fluoresceinated peptide has bound to the surface of planktonic cells and no internalization was observed.

**Discussion And Conclusions:** VLL-28 exhibited fungicidal activity against almost all the planktonic *Candida* spp. tested with a reduced activity only towards *C. glabrata*. The poor susceptibility of *C. glabrata* may reflect the unique and distinctive features of its cell wall. Interestingly, VLL-28 also exhibited strong fungicidal activity towards the planktonic cells of *C. krusei*, which is intrinsically resistant to fluconazole and regarded as a potentially multidrug-resistant pathogen. The fluorescein-labelled peptide suggested that VLL-28 could exert its antifungal activity by damaging the cell wall. Notably, VLL-28 is the first example of an archaeal antimicrobial peptide that is active towards planktonic cells and mature biofilm of clinical isolates of *Candida* spp.

## ***P 102 – ID 046 - Transplacental transit of parasite antigens during maternal schistosomiasis.***

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**Introduction:** Urogenital schistosomiasis is a major public health concern in endemic countries of sub-Saharan Africa, where 40 million women of childbearing age are presently infected by *Schistosoma* spp. However, few studies are dealing with morbidities inflicted on pregnant women and their offspring.

**Materials and Methods:** We report on a case of a pregnant woman from Mali, who was affected by urogenital schistosomiasis based on urine parasitology and serological exams. We assessed some aspects of the innate and acquired immunity of the baby born to a mother with long-lasting *Schistosoma haematobium* infection. A 20 years old woman, arrived to Italy as migrant 4 months before the presentation to our University Hospital, at the 25th week of gestation. She reported occasional abdominal and pelvic pains during urination, starting about 6 years earlier. The suspect of schistosomiasis prompted us to carry out: i) a microscopic examination of the urine sediment; ii) urine *Schistosoma*-CCA assay by immunocromatographic card (NADAL CCA, Bilharzia Test); iii) anti-*Schistosoma* antibody level was evaluated by ELISA kit (NovaLisa, NovaTec Immunodiagnostica GMBH, Dietzenbach/Frankfurt, Germany). Although the lack of pregnancy-related disorders, one month before delivery, an amniocentesis was performed due to the previous parasitic diagnosis. An umbilical cord blood sample and a placenta aliquot were taken at the birth and we evaluated the newborn IgG4 values through nefelometry (BN II System, Siemens Healthcare, Italy). Moreover we tested Interleukin 35 (IL-35) by Elisa Kit, Cusabio Biotech Co., LTD and Interleukin 10 (IL-10) by Biochip ImmunoArray RANDOX.

**Results:** At delivery the newborn was positive for anti-*Schistosoma* IgG. By histological examination of the placental tissue, several eggs of *S. haematobium* were found. The CCA card revealed antigen of *S. haematobium* in both amniotic fluid and umbilical cord plasma. The newborn pathological values were: IgG4 0.985 g/l (n.v. 0.04-0.55), IL-35 223.63 pg/ml (n. v. 27.1 pg/ml), IL-10 0.44 pg/ml (n. v. 6.8 pg/ml).

**Discussion and Conclusions:** Our present data directly demonstrated the fetomaternal transmission of *S. haematobium* antigens. Both the immunosuppressive subclasses of IgG and the most specific Breg cytokine (IL-35) were increased in newborn serum samples, while Treg cytokine (IL-10) was decreased, suggesting a differential behavior of the Breg/Treg cascade in presence of *Schistosoma* antigens in newborn.

## P 103 – ID 056 - Synthetic 1,2-Dioxanes as potential anti-leishmanial drugs

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**Introduction:** Human leishmaniasis is a phlebotomine-transmitted infection caused by protozoan belonging to the genus *Leishmania*. Infections can manifest as tegumentary or visceral leishmaniasis, the latter being fatal if untreated. All currently used drugs are based on few compounds that are highly toxic, expensive and have a high rate of treatment failure. Thus, the development of new treatments for leishmaniasis is a priority. A number of recent studies on new drugs focuses on natural or semi-synthetic compounds. Among them, the endoperoxide artemisinin, extracted from *Artemisia annua*, and some of its derivatives have shown leishmanicidal activity. In the present work, a series of structurally simple, fully synthetic 1,2-dioxanes were tested to assess the *in vitro* anti-leishmanial potential evaluating their ability to affect parasite growth.

**Materials and methods:** The synthesis of 1,2-dioxanes was achieved through an efficient and low cost approach developed in our lab. The optimized standard protocol consists of five simple steps. Drug susceptibility assays were tested on promastigotes from reference strains of *L. donovani*, *L. tropica*, *L. major* and *L. infantum*, and against *L. donovani* amastigotes. Cytotoxicity of the dioxanes was analyzed on mammalian cells (Vero cell line).

**Results:** Screening of 22 newly synthesized dioxanes on *L. donovani* promastigotes revealed that 11 compounds out of 22 had a good inhibitory activity *in vitro* against the parasite, in the low micromolar range (IC<sub>50</sub> range between 16.4 μM and 4 μM). Moreover, 6 out of 11 compounds had low cytotoxicity, thus exhibiting a good selectivity index. The six most promising compounds in terms of activity and selectivity were further investigated for their antileishmanial activity on the promastigote forms of *L. tropica*, *L. major* and *L. infantum* and against *L. donovani* amastigotes. These six hits showed a good performance in terms of both potency and selectivity

**Discussion and conclusion:** These results indicate that six compounds proved to be significantly active against *Leishmania* promastigotes and amastigotes, with low cytotoxic effects towards mammalian cells. Based on the structure-activity relationships we identified the crucial role of: i) the side-chain at C4-position, being the aminopropyl imidazole and the triazolyl propyl phosphonium salt the two best substituents, ii) the lipophilic requirements at C6-position, iii) the short chain at C3-position to minimize the cytotoxicity. The excellent selective activity and the simple synthesis of endoperoxides suggested that they worth to be optimized and further evaluated.

## **P 104 – ID 058 - *Saccharomyces cerevisiae* as a new therapeutic agent against oropharyngeal candidiasis**

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**Introduction:** Oropharyngeal candidiasis is a common opportunistic mucosal infection of the oral cavity, mainly caused by an overgrowth of *Candida albicans* (*C. albicans*). This infection can inhibit nutritional intakes and strongly affect quality of life. To date, standard therapeutic strategies involving the administration of antifungal drugs can bring several side-effects, not least the emergence of drug-resistant strains. The purpose of this study is to investigate the effectiveness of *Saccharomyces cerevisiae* based probiotic (*S.-cerevisiae*) against oropharyngeal candidiasis.

**Materials and methods.** C57BL/6J mice were immunosuppressed with subcutaneously injection of cortisone acetate. The mice were infected with bioluminescent (BLI) *C. albicans* and then treated sublingually with *S. cerevisiae*. In these mice fungal burden was imaged and quantified in the IVIS Lumina XRMS Imaging system. Gene expression of *C. albicans* virulence factors such as ALS3, SAP2 and SAP6 was examined. Histopathologic lesions of tongue were also evaluated.

**Results:** Our results show that administration of *S. cerevisiae* in the oral cavity of C57BL/6J mice resulted in a protective effect against oropharyngeal candidiasis. This was related to: i) a decrease of *C. albicans* load in the oral cavity, esophagus, stomach and duodenum; ii) an early resolution of inflammatory process in the tongue; iii) a marked reduction of *C. albicans* virulence factors

**Discussion and Conclusions:** These findings suggest that probiotic *S. cerevisiae* is able to positively reverse/attenuate the course of OPC infection.

## P 105 – ID 082 - Virulence factors in *Malassezia* species

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**1.Introduction:** *Malassezia* genus includes a group of lipophilic and mostly lipid-dependent yeasts that are recognized as members of the normal skin mycobiome of both human and other homoeothermic organisms. However, *Malassezia* species are natural inhabitants of the healthy skin, under certain conditions, they may cause or exacerbate several skin diseases as pityriasis versicolor (PV) seborrheic dermatitis (SD), and atopic dermatitis (AD). Furthermore, *Malassezia* species have been associated with systemic infections. Due to its ability to produce superficial and systemic infections in both immunocompromised and immunocompetent hosts, *Malassezia* is considered an important emerging pathogen. To allow a better knowledge of its pathogenicity, it is important to investigate the expression of some virulence factors. In this study, we evaluated the cellular surface hydrophobicity (CSH) levels, adherence capacity and biofilm formation of 4 *Malassezia* species frequently involved in various dermatological processes.

**2. Materials and methods:** Twenty-six clinical *Malassezia* isolates from PV, SD, AD, and health subjects (HS) were studied; 4 *M. furfur* from lesions and 1 HS, 13 *M. sympodialis* 8 from lesions and 5 HS, 4 *M. globosa* 3 from lesions and 1 HS, 2 *M. slooffiae* from lesions. The adherence capacity was determined polystyrene surface and CSH levels using two-phase system. A semiquantitative measurement of biofilm formation was made by tetrazolium salt (XTT) reduction assay and by Scanning Electron Microscopy (SEM).

**3. Results.** All isolates were hydrophobic and adherent. CSH showed values in a range of  $9.8 \pm 2.1\%$  to  $87.7 \pm 15.2\%$ , about 50% of the strains were highly hydrophobic. The adherence was between  $15 \pm 2.8\%$  to  $98.7 \pm 3.2\%$ . *M. globosa* and *M. slooffiae* showed mayor adherence than *M. sympodialis*. The average of XTT values were, for *M. furfur*  $0.60 \pm 0.3$ , *M. sympodialis*  $0.464 \pm 0.17$ , *M. globosa*  $0.60 \pm 0.05$  and for *M. slooffiae*  $0.161 \pm 0.13$ . The averages values obtained for the three virulence factors were high when the strains were isolated in HS respect to isolates in lesions. SEM observations confirmed the biofilms formations characterized by abundant extracellular matrix covering the yeast cells.

**4.Discussion and conclusions.** Hydrophobicity is an important factor to adherence and to biofilm formation on abiotic surface, this characteristic was present in all isolates studied with different capacity. These important virulence factors can influence the capacity of *M. furfur*, *M. sympodialis*, *M. globosa*, and *M. slooffiae* to change from commensal to pathogen status. The observed capacity to form biofilms is related to the capacity to colonize catheters and consequently to produce fungemia.

## ***P 106 – ID 100 - Antifungal effects of Melaleuca alternifolia essential oil (TTO) alone and in combination with itraconazole or ketoconazole against Trichophyton rubrum.***

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**Introduction:** *Trichophyton rubrum* is the major cause of chronic or recurrent human dermatophytosis included among the fungal diseases that most affect the worldwide population. Many azoles, such as itraconazole (ITC), often resulting in complete clearance of the lesions. *T. rubrum* can develop resistance to azoles after prolonged exposure to subinhibitory concentrations of these drugs leading to therapeutic failures and to persistence and chronicity of the infections. This problem has stimulated the search for therapeutic alternatives, including essential oils recognized for their potential antimicrobial role. The essential oil of *Melaleuca alternifolia* (Myrtaceae; Tea Tree Oil, TTO) has recently received much attention for its antifungal properties: due to its lipophilic nature, which facilitates skin penetration it is used as local formulation for dermatological disorders. This study aims to analyze the effects induced by TTO on *T. rubrum* and its potential synergism when used in combination with conventional drugs such as ITC or ketoconazole (KCZ).

**Material and Methods:** *In vitro* susceptibility testing assays of TTO or azoles on 4 *T. rubrum* clinical isolates were performed using the CLSI M38-A2 broth microdilution method, except for inoculum suspensions (Granade & Artis, 1980). MICs were defined as the lowest concentration that induces 80% of mould growth inhibition. The synergic action of TTO with ITC was studied with the checkerboard method. The nature of interaction was defined by means of fractional inhibitory concentrations (FICs) calculated as the MIC of the combination of TTO with drug, divided by the MIC of TTO or drug alone. FIC index (FICI) was obtained by adding both FICs. FICI:  $\leq 0.5$ , synergistic; 0.5–4.0, no interaction;  $> 4.0$ , antagonistic.

**Results:** Confirmation of the antimicrobial activity of the TTO and azoles was undertaken against the 4 test moulds. The MICs did not differ significantly with regard to tested fungal strains: the mean MIC value obtained for TTO was 1.08  $\mu\text{g/ml}$ , while for ITC or KCZ was 0.5  $\mu\text{g/ml}$  and 0.25  $\mu\text{g/ml}$ , respectively. TTO was most effective in combination therapy, showing a strong synergy with azoles (FICI values 0.37 and 0.245, respectively) and reducing the MIC both of them up to 8-fold. Moreover, for each one of these interactions, the MIC for TTO alone was below the lowest testing concentration.

**Discussion and Conclusions:** The results obtained have demonstrated both the effects of TTO on *T. rubrum* and the synergism when it was used in combination with azoles. Synergy might permit lower doses of the individual drugs to be used more effectively and safely, offering a promise for combination topical treatment regimens for superficial mycoses. Therefore, we can hypothesize the use of this combination within future pharmaceutical preparation.

## ***P 107 - ID 108 - Performance of a new whole-blood test for detecting reactivation of echinococcal cysts***

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**Introduction:** Human cystic echinococcosis (CE) is diagnosed by imaging and serology, but the diagnostic performance of the latter is poor, especially in the post-treatment follow-up. We set up a whole-blood immune test based on Interleukin (IL)-4 detection by an enzyme-linked immunosorbent assay in response to the native Antigen B (AgB) of *Echinococcus granulosus sensu lato*. The aim of this prospective pilot study was to evaluate the performance of the whole-blood test in discriminating cyst viability and in detecting cyst reactivation.

**Materials and Methods:** The test was performed at baseline (T0) in 67 patients with liver CE: 30 had CE3b cysts (viable, active cysts); 37 had spontaneously inactivated CE4 cysts (non-viable, inactive cysts). After enrolment, 6 patients with CE3b cysts received albendazole, resulting in cyst solidification (CE4) in 4 of them. However, it is known that CE3b cysts treated with albendazole almost invariably reactivate over time. The whole-blood test could be repeated after 1 year (T1) in 15 patients per group.

**Results:** The IL-4 levels at baseline were significantly increased in patients with CE3b cysts compared to patients with CE4 cysts ( $p=0.006$ ). Although significant area under curve results were obtained ( $p=0.007$ ) upon ROC analysis, the cut-off maximizing sensitivity for CE3b cysts diagnosis predicted CE3b with a 60% sensitivity and 76% specificity, whereas the cut-off chosen to maximize specificity predicted CE3b with 33% sensitivity and 95% specificity. As expected, we observed no change in IL-4 levels in the CE4 group at T1 with the exception of 4 patients, 3 of whom had a decrease and 1 an increase in IL-4 levels. Regarding the CE3b group, among the 4/15 subjects whose cysts evolved to inactive stage at T1, 2 (50%) showed an IL-4 decline compared to the baseline levels.

**Discussion and Conclusions:** Levels of IL-4 produced in response to AgB are increased in samples from patients with active compared to inactive CE. Interpretation of trends in variations of IL-4 levels during the follow-up would require additional follow-up time points (ongoing).

***P 108 – ID 112 - Molecular characterization of Trichomonas vaginalis virus (TVV) and associations with Mycoplasma hominis symbiosis and metronidazole susceptibility in Trichomonas vaginalis isolated in Italy***

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**Introduction:** The flagellated protozoon *Trichomonas vaginalis* can establish endosymbiotic relationship with *Mycoplasma hominis* and can harbour double-stranded RNA (dsRNA) viruses, known as *T.vaginalis* viruses, recently classified as Trichomonasvirus genus, within the family of *Totiviridae* (TVVs). Four TVVs species have been identified by phylogenetic analyses and comparisons of genomic sequences, demonstrating that their presence strongly influences protozoan virulence. In this study, we investigated the prevalence of the four TVVs species among *T.vaginalis* isolates from women affected by trichomoniasis, also reporting the pattern association between TVVs and *M.hominis*, and the correlation of viral infection and metronidazole resistance. This study is the first report on the prevalence of TVVs in Italy and the second one in Europe.

**Material and Methods:** Real time PCRs were performed by testing 48 *T. vaginalis* strains, in order to determine the protozoan association with the four TVV species and *M.hominis*. The intracellular location of TVVs was also confirmed by immunofluorescence experiments. *In vitro* metronidazole susceptibility assays were performed by testing 19 representative *T. vaginalis* isolates. Protozoan cells were incubated, under microaerophilic atmosphere, with increasing concentration of metronidazole to determine the minimum lethal concentration (MLC).

**Results:** The prevalence rate of TVV-positive *T.vaginalis* isolates was 50%, with a prevalence of TVV2 infection (79,17%). Interestingly, 8,33% of *T.vaginalis* strains were infected by TVV4, showing presence of this virus species for the first time in Europe. Finally, a total of 37,5% of isolates showed a mixed TVVs species infection. *M.hominis* prevalence was found in 81,25% of *T.vaginalis* isolated: our results have shown any statistical correlation between *M.hominis* and TVV species. Finally, our data demonstrate the lack of correlation between metronidazole resistance and presence of TVVs in *T. vaginalis* isolated

**Discussion and Conclusion:** Our data demonstrated a high prevalence of trichomonad strains infected with both TVVs and *M. hominis*, confirming the capability of *T.vaginalis* to establish endosymbiotic relationship with several microorganisms. We also reported the lack of correlation between metronidazole resistance and presence of TVVs or *M.hominis* in our *T.vaginalis* isolated. Since the interaction among TVVs, *M. hominis* and *T. vaginalis* may modify the host response to *T.vaginalis* infection and influence the virulence of protozoa, the study of the relationships between *T.vaginalis* and its symbiotic microorganisms represents a new exciting field of research.

## ***P 109 – ID 114 - Rhizosphere metaproteome: a tool to evaluate biodiversity and fungi-plant interaction***

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**1. Introduction.** Rhizospheric bacterial composition is very important to improve plant growth and health, especially in agricultural conditions. Metaproteomics, has the potential to transform the study of microbial community functioning in soil: this is a very promising tool to study microbial activity in environmental samples such as soil, thus obtaining a deeper understanding of plant-microbe interactions. The aim of this work was to characterize fungal community associated with *Vitis vinifera* cv. Pinot Noir roots using a metaproteome approach.

**2. Materials and Methods.** The bulk and the rhizospheric soil of the grapevine were sampled before fruiting. Soil proteins were extracted using NoviPure® Soil Protein Extraction Kit, optimized for LC-MS/MS analysis. Protein samples were trypsin digested, purified and peptides were analyzed by two-dimensional liquid chromatography/tandem mass spectrometry (2D-LC-MS/MS). The MASCOT Search engine was used for protein database searches.

**3. Results.** The high diversity of identified proteins revealed differences in the community composition at the genus level among the considered soils. Among filamentous fungi, the most active genera were *Aspergillus*, *Colletotrichum*, *Fusarium* and *Saccharomyces*. The biological activity related to the activity of filamentous fungi in the rhizosphere involved mainly cellular nitrogen compound and macromolecule metabolic process.

**4. Discussion and Conclusions.** To our knowledge, few papers have described the rhizosphere metaproteome of agricultural plants and no data are available in the literature regarding the rhizosphere proteome of *V. vinifera*, especially describing filamentous fungi activity in the rhizosphere. Our interest in characterizing grapevines rhizosphere is justified by both economic and historical reasons. In fact, in 2016 the International Organization of Vine and Wine declared that in Italy, 690,000 ha of agricultural land are cultivated with grapevine resulting in 7.9 millions of tons of fruits and 50.9 millions of hl of wine production and in Piedmont, 43,500 ha of agricultural land are cultivated with grapevine leading to the production of 2.5 millions of hl of high quality wine. Moreover, since in 2014 the hills of the Piedmont (Italy) area covering the Langhe, Roero and Monferrato were included in the “Unesco World Heritage” (<http://whc.unesco.org/en/list/1390>).

## ***P 110 – ID 115 – Trichomonas vaginalis transports and transmit virulent Mycoplasma hominis to human cells after metronidazole treatment: a potential role in bacterial invasion of fetal membranes and amniotic fluid***

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**Introduction:** *Mycoplasma hominis* is an opportunistic pathogen able to colonize the lower urogenital tract and is associated with severe pregnancy and postpartum complications. Molecular mechanisms of pathogenicity and virulence effectors remain poorly characterized. Several studies have demonstrated that *M.hominis* can establish an endosymbiotic relationship with *Trichomonas vaginalis*, urogenital parasitic protozoon, also linked to preterm birth that can infect the vagina, without reaching the uterus and placental membranes. Recently, two bacterial genes (*alr* and *goiB*) associated with amniotic cavity invasion and a single gene (*goiC*) associated with intra-amniotic infections and high risk of preterm delivery, have been identified in *M.hominis* isolated from a group of pregnant patients. The first objective of this work was the identification of three genes in *M.hominis* symbiotically associated with *T.vaginalis* clinical isolates, representing an additional potential risk factor for adverse maternal outcomes during trichomoniasis. Subsequently, we set up an *in vitro* model to assess the transmissibility of virulent *M.hominis* released from metronidazole-killed *T.vaginalis* to human derived cells.

**Materials and Methods:** The presence of *M.hominis* in 34 *T.vaginalis* strains was evaluated by PCR. Then, the presence of *alr*, *goiB*, and *goiC* genes in *T.vaginalis*-associated *M.hominis* was assessed by specific PCR. Moreover, we set up *in vitro* experiments coculturing human amnion-derived cells (WISH) and *T.vaginalis* infected with *M.hominis* positive for all virulence genes with and without metronidazole treatment, in order to study the transmissibility of intracellular *M.hominis* from *T.vaginalis* to WISH.

**Results:** We demonstrate that a high number of *M.hominis* intracellularly associated with *T.vaginalis* have *goiC* gene, in association with *alr* and *goiB*. In addition, our data showed that metronidazole treatment of *M.hominis*-infected *T.vaginalis* allows delivering viable intracellular *goiC* positive *M.hominis* from antibiotic-killed protozoa and that free *M.hominis* can infect human cell cultures.

**Discussion and Conclusion:** Our data reveal that *M.hominis* symbiotically associated with *T.vaginalis* can possess the three genes associated with amniotic membranes colonization and adverse pregnancy outcomes. Moreover, our results suggest that intracellular *M.hominis* are massively delivered from metronidazole-killed *T.vaginalis*, leading the subsequent infection of genital tissues and the direct bacterial invasion of placental membranes and amniotic fluid. The intracellular localization of bacteria in *T.vaginalis* cells can explain the paradoxical results reporting the failure of metronidazole treatment of subclinical trichomoniasis to prevent preterm delivery in pregnant women.

## ***P 111 – ID 146 - Citrinin overproduction under different growth conditions by a new strain of *Penicillium citrinum* showing non-canonical phenotypic features***

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**Introduction:** *Penicillium spp* are moulds among the most widespread in both indoor and outdoor environments. They are responsible of spoilage of food and related products (e.g. coffee beans, cassava, spices, maize derived products, grains or wheat, peppercorns, cheese, and fermented meat), where they can release different mycotoxins. Their spores are associated with allergic reactions. Species of the genus may also be responsible of infectious diseases in humans. We accidentally isolated from the environment a non-readily classifiable *Penicillium* strain producing a bright red liquid exudate. The aims of this work were its identification, characterization, and seek for possible biotechnological applications.

**Materials and Methods:** The mould was grown on Czapek Yeast Autolysate and Sabouraud Dextrose. Size, color and other morphological features of colonies and mycelium were recorded and compared with available data in manuals and atlas. Internal Transcribers Regions (ITS) sequencing was performed following standard protocols. The exudate was withdrawn from the surface of colonies; its composition analyzed by HPLC-MS, and tested for antibacterial activity against different Gram-positive and Gram-negative representative species as per an adapted EUCAST protocol. The mycotoxin production was quantified by a spectrophotometric method.

**Results:** Albeit growth behaviour and morphology were atypical, the ITS sequencing and the identification of the main mycotoxin produced assigned the isolated mould to the species *Penicillium citrinum* (strain designation: PcL1). The main component of the exudates was the polyketide mycotoxin citrinin. The exudates showed a narrow spectrum of antibacterial activity, limited to Gram-positive cocci. However, this activity was maintained against methicillin- and macrolide-resistant *Staphylococcus aureus* strains. Efforts were done to increase/maximise citrinin production by modulating different growth parameters such as temperature, exposure to light, basic medium composition, carbon source type and concentration. The yield of citrinin production was increased from about 0.9 g/L to 4.5 g/L of batch culture.

**Discussion and Conclusions:** The described strain is an interesting phenotypic variant of *P. citrinum*. It may represent a useful reference for the cost-effective production of citrinin on a laboratory scale and also for industrial applications, upon genetic modification and selection. Additionally, *P. citrinum* PcL1 may be used as a reference strain to test in vitro the antifungal and anti-mycotoxin producing activity of new compounds.

## ***P 112 – ID 149 - Use of real-time multiplex PCR for the diagnosis of dermatophytes onychomycosis***

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**Introduction:** Onychomycosis represents one of the most frequent mycoses in the world with a incidence progressively increases with age up to a prevalence of 20% between 40 and 60 years and exceeds 40% in the elderly. The main etiological agents are dermatophytes but yeasts and nondermatophyte moulds can also be involved. The laboratory diagnosis of dermatophytosis is currently based on microscopy and culture assays however, molecular methods are becoming increasingly popular in this field. The aim of the present study was to evaluate the real-time multiplex PCR for detecting and identifying dermatophytes in the nail plate compared with microscopy and culture methods.

**Materials and methods:** Over period between January and May 2019, 96 patients were observed. Nail material was taken through scraping from clinically abnormal nails. The nail scrapings was divided into three parts: one for microscopy in 15% potassium hydroxide, one for culture in Sabouraud's dextrose agar medium supplemented with 0.5% cycloheximide and 1% chloramphenicol and the remaining part for DNA extraction and PCR assay. The DermaGenius® Nail real-time multiplex PCR (PathoNostics, The Netherlands) was use for the detection the most clinical prevalent dermatophytes species. The DNA was extracted by using the PathoNostics Extraction Kit following the manufacturer's instructions. Data were analyzed using the MedCalc Statistical Software.

**Results:** A total of 61 (63.5%) cases of onychomycosis were confirmed. The microscopy was positive in 52/96 samples (54.2%) among which 37 specimens had a fungal culture positive for dermatophytes (25), yeasts (2), or nondermatophyte moulds (10). The fungal cultures were positive in 46/96 samples (48%) and in particular 29 were dermatophytes, 3 were yeasts and 14 were positive for a non-dermatophyte mould. The PCR was positive in 51/96 nail samples (53.1%), among which 38 where microscopy positive (38/51, 74.5%) and 29 where culture positive for dermatophytes (29/51, 56.9%). The PCR assay was positive in 9 samples that showed microscopy and culture negative and in 13 samples with negative culture for a dermatophyte but with a positive microscopy showing its ability to identify non growing fungal agents.

**Discussion and conclusions:** This study makes a further contribution to the clinical evaluation of DermaGenius® Nail real-time multiplex PCR, a commercial multiplex PCR assay for the diagnosis of onychomycosis. The data obtained show that the PCR assay is a reliable that shortens the time to diagnosis and can unmask the presence of non growing fungal pathogens in nails.

## ***P 113 – ID 158 - Comparative analysis of commensal and pathogenic vulvovaginal clinical isolates of *Candida albicans*: hyphal morphology, molecular genetics and phylogenetic relationships***

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**Introduction.** Vulvovaginal candidiasis (VVC) is a common disorder affecting approximately 75% of women of childbearing age at least once during their life and it is caused primarily by *Candida albicans*. However, *C. albicans* is also a normal harmless commensal of the vaginal mucosa, hence a long-standing question is how the fungus switches from commensal state to a virulent pathogen. Studies performed in a murine vaginitis model suggest that host inflammatory processes drive the onset of symptomatic infection. Accordingly, our recent work on clinical samples from colonized and symptomatic women revealed different propensity to form hyphae. In addition,  $\beta$ -glucan, a fungal cell wall pro-inflammatory polysaccharide, was largely masked from immune recognition during vaginitis and enhanced  $\beta$ -glucan availability was only found in hyphae from symptomatic patients with a concomitant massive neutrophil infiltration.

**Materials and Methods.** To further investigate any association between fungal virulence traits and VVC outcome, the following parameters were analyzed on commensal and clinically relevant isolates: MLST analysis, sequencing of the gene encoding the candidalysin toxin and percentage of hyphal fragments.

**Results.** The results obtained so far suggest that none of these fungus-related parameters allow to discriminate between commensal and clinically relevant isolates.

**Discussion and Conclusions.** Taken together, our preliminary data indicate that host-intrinsic mechanisms, rather than the fungus' intrinsic virulence traits, may play a key role in the occurrence of VVC.

## ***P 114 – ID 161 - Antifungal activity of the human lactoferricin derived peptide hLF1-11 in combination with caspofungin against Candida species***

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**Introduction:** *Candida species are the main fungal opportunistic pathogens causing mucosal and systemic infections often associated with drug resistance. The difficulty to find new antifungal drugs has led to an increased interest in the use of combination therapies. The present study was aimed at investigating the possible synergistic activity of a synthetic N-terminal peptide of human lactoferricin, named hLF1–11, with caspofungin against Candida albicans and Candida parapsilosis.*

**Materials and Methods:** The interaction between hLF1–11 and caspofungin was evaluated by the checkerboard assay using 96-well round bottom polystyrene microtiter plates. Yeast cells ( $10^3$  CFU/mL) were incubated with the antifungal compounds, alone or in combination, for 24 h at 37°C, following the CLSI guidelines. The synergistic activity was evaluated for *C. albicans* on a reference strain (SC5314) and a fluconazole resistant (MIC: 64 µg/mL) clinical isolate (CA688), and for *C. parapsilosis* on a reference strain (ATCC 22019) and a clinical isolate (CP7) selected for its strong ability to produce biofilm. An in vitro killing assay was performed to evaluate the possible fungicidal activity of synergistic concentrations of hLF1-11 and caspofungin against the *C. albicans* reference strain. The number of CFU/mL was assessed by plating serial dilutions of cultures after 2 and 24 h incubation with the compounds. A fractional inhibitory concentration (FIC) index  $\leq 0.5$  was interpreted as synergy.

**Results:** A strong synergistic effect was observed against all the tested *C. albicans* and *C. parapsilosis* strains. For *C. albicans* strains, the FIC index ranged from 0.07 to 0.09, and for *C. parapsilosis* from 0.18 to 0.28. In the killing assay, synergy was defined as a decrease in CFU/ml of  $\geq 2$  Log by the combination of hLF1–11 and caspofungin in comparison with the most active constituent. After a 2 h incubation, no synergistic activity was detected, whereas after 24 h incubation synergy was found with various combinations of concentrations.

**Discussion and Conclusions:** The synergistic effect observed between caspofungin and hLF1-11 against *C. albicans* and *C. parapsilosis* is of potential clinical relevance, representing a possible novel approach to target drug resistant fungal infections. Future studies will help to elucidate the mechanisms of action of the synergistic effect between hLF1-11 and caspofungin, and the potential efficacy of such a combination against *Candida* species showing different caspofungin susceptibility.

Acknowledgement: Supported by a research grant awarded under the Investigator Initiated Study Program of Merck & Co.

## ***P 115 - ID 170 - Liquid-, Solid- and vapor-phase antifungal activities of thirteen essential oils: susceptibility of selected *Aspergillus* spp. strains.***

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**Introduction:** Treatment options for aspergillosis include amphotericin B (AMB) and azole compounds, such as voriconazole and itraconazole (ITZ). However, there have been severe side effects associated with these antifungal agents, and antifungal resistance continues to increase. Essential oils (EOs), a mixture of volatile compounds with well-known antifungal activity, have been used to treat human infections and other maladies for centuries. In this study, we present the antifungal screening of 13 EOs against *A. flavus*, *A. fumigatus*, and *A. niger*. AMB and ITZ were used as comparator antifungal agents.

**Materials and Methods:** A collection of commercially-available EOs: bitter orange (*Citrus aurantium*), citronella (*Cymbopogon nardus*), clove (*Syzygium aromaticum*), eucalyptus (*Eucalyptus globulus*), geranium (*Pelargonium graveolens*), lavender (*Lavandula angustifolia*), lemon (*Citrus limon*), neem (*Azadirachta indica*), oregano (*Origanum vulgare*), rosemary (*Rosmarinus officinalis*), tea tree (*Melaleuca alternifolia*), thyme red (*Thymus vulgaris*), true lavender (*Lavandula angustifolia* XL *latifolia*) has been screened for antifungal activity against different clinical fungi AMB-resistant and ITZ-resistant strains: 1A. *flavus*, 7A. *fumigatus*, and 5A. *niger*.

EOs were obtained from Flora (Italy). AMB and ITZ were purchased from Sigma-Aldrich (Italy). The chemical composition of the EOs was analysed by Gas Chromatography–Mass Spectrometry (GC/MS). The EOs antifungal activity was determined by a microdilution method, a vapor contact assay, and a disk diffusion assay. We evaluated the Minimum Inhibitory Concentration (MIC) and the Minimum Fungicidal Concentration (MFC) of EOs and drugs according to the CLSI M38-A2 with some modifications for EOs. The final concentrations ranged from 1% to 0.0078% (v/v) for EOs. AMB and ITZ were used as positive controls.

**Results.** Geranium, citronella, and clove were found to be the EOs with the better activity against all fungi tested. In fact, the MICs value of geranium and citronella EOs were 0.06% against *A. fumigatus* and *A. niger* (MFC=0.5%). The MIC value of clove EO was 0.125% against *A. flavus*, *A. fumigatus* and *A. niger* (MFC=0.5%). The volatile vapor of geranium, citronella, and clove EOs at 0.075% concentration completely inhibited the growth of all *Aspergillus* tested. Disk diffusion assay confirm antifungal activity of these 3 EOs, showing mycelial growth inhibition.

**Discussion and Conclusions.** These data encourage adequately controlled and randomized clinical investigations. Moreover, the use of OEs could be a valid support to limit the spread of resistance (including recovery of resistant isolates from the environment), likely related to the use of fungicides in numerous agricultural practices and commercial products.

***P 116 – ID 174 - First application of Fourier-transform infrared spectroscopy (FT-IR) technology in the subtyping of the yeast *Saprochaete clavata*.***

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**Introduction:** Invasive fungal infections due to the yeast *S. clavata* are an under-estimated healthcare problem especially in hematological wards. Recent improvements in DNA based technologies, in particular the whole-genome sequencing (WGS), allow nowadays to infer clonal relationship between isolates. Besides the high discriminatory power, the high costs and expertise required, can limit WGS feasibility for nosocomial outbreak analysis. FT-IR analyzes absorption bands due to the stretching vibrations of specific functional groups, resulting in different spectroscopic fingerprints depending on the differences existing in the biochemical composition of microorganisms. The aim of the present study was to evaluate the potentiality of FT-IR technology in the subtyping of eleven isolates of the yeast *S. clavata*, collected from single patient's episodes of candidemia, and compared with the cluster analysis obtained by MALDI-TOF protein fingerprint.

**Materials and methods:** For all isolates, replicates' profiles were obtained on an IR Biotyper system running the IR Biotyper software and analyzed in the range between 800 and 1300  $\text{cm}^{-1}$  wavelength. FT-IR spectra distance was evaluated using the Euclidean average linkage method and the dendrogram obtained was compared with the hierarchical cluster analysis resulting from the MALDI-TOF mass spectra profiles.

**Results:** In the dendrogram obtained from the FT-IR spectra four out of eleven *S. clavata* isolates clustered together and the clustering derived from MALDI-TOF protein profiles was highly similar.

**Discussion and conclusions:** The comparison with WGS analysis would add further value to FT-IR technology subtyping results; this new rapid and easy to perform approach could represent an alternative to molecular methods.

## ***P 117 – ID 175 - Vaccinium macrocarpon urine metabolites identification and quantification and evaluation of their effect on Candida albicans adhesion***

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**Introduction-** *Candida albicans* urinary tract infections (UTI) are increasingly common in hospital settings due to its high propensity to form biofilms on mucosal surface and plastic surface of indwelling devices.

*Vaccinium macrocarpon* (cranberry) has been widely used for decades in the prevention of UTIs in the general population. Proanthocyanidins (PACs), in particular the A-type, are the main responsible for the *in vitro* activity. Nevertheless, there are controversial results on their presence in human urine after cranberry oral intake. The reason of such different results can be the use of different dosages as well as non-standardized cranberry products. The aim of the work was *i*) to identify and quantify cranberry components and metabolites in human urine after the oral intake of a highly-standardized cranberry extract (Anthocran<sup>TM</sup>, Indena S.p.A.), *ii*) to evaluate the urine *ex vivo* effect on *C. albicans* adhesion and biofilm formation and *iii*) to test the *in vitro* activity of a mixture of metabolites identified in the active fractions.

**Materials and Methods-** Ten young healthy female volunteers took 2 capsules Anthocran<sup>TM</sup>/day for 7 days. Urine samples were collected before starting supplementation and at the following time-points after the last dose: 1, 2, 4, 6, 10, 12, 24 hours. An HPLC-MS/MS method was set-up using a LTQ-XL-Orbitrap working in data dependent scan mode to perform the analyses. A targeted and an untargeted approach was used to identify known metabolites and compounds hereto unreported in the literature. Urine fractions were tested *in vitro* against the reference strain *C. albicans* SC5314 and eight clinical isolates from UTIs.

**Results-** Urine fractions collected after 1 and 12 hours were found to significantly reduce the adhesion. The *ex vivo* effect of cranberry metabolites was then confirmed by evaluating the significant inhibitory effect of a reconstituted mixture of metabolites on *C. albicans* adhesion and biofilm formation.

**Conclusions-** The data reported in the present work demonstrate that *i*) PACs are metabolized after cranberry oral intake, *ii*) urines collected following one week of cranberry treatment are able to significantly reduce *C. albicans* adhesion and biofilm formation, *iii*) the activity can be due to a synergistic effect of identified cranberry metabolites including PACs metabolites.

## ***P 118 – ID 177 - Unexpected activity of bromiphen, a new quaternary ammonium compound, against Candida auris***

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**Introduction-** *Candida auris* has the ability to persistently colonize hospital environments, with the propensity for transmission in health care settings, and to survive for weeks on plastics, fabrics, and nonporous surfaces. Cationic agents are an important class of anti-infectives widely used for environmental infection control. In particular, quaternary ammonium compounds (QACs) are largely recognized as antiseptics and disinfectants. Nevertheless, commonly used QACs seem to be ineffective against *C. auris*. The aim of the present study was to assess the efficacy of the new QAC bromiphen, a derivative of domiphen, in inhibiting *C. auris* adhesion and growth.

**Materials and Methods-** We assessed the antimicrobial activity of both domiphen (D) and bromiphen (*p*-bromo-domiphen, Br-D, patents numbers WO2019053626 and PCT/IB2018/057007) against several *Candida* species, including *C. auris*. Benzalkonium chloride was used as reference QAC. Minimal inhibitory concentrations (MICs) were evaluated using EUCAST protocol for yeasts. Cytotoxicity was performed on mammalian cells (HEp-2 ATCC CCL-23) by MTT assay, and in the invertebrate model *Galleria mellonella* by directly injecting various drug concentrations into the haemocoel.

**Results-** Compared to the parental compound domiphen bromide, bromiphen, namely *p*-bromo-domiphen bromide, resulted more active in all the performed tests. In particular, planktonic MIC<sub>90</sub> for *C. auris* were 8 mg/L and 2 mg/L for D and Br-D, respectively. Concerning the cytotoxicity, the IC<sub>50</sub> (a concentration that causes a reduction by half of the activity of mitochondrial dehydrogenase) were 10 mg/L for Br-D and 7 mg/L for D. Selectivity index (SI) values were 0.8 for D and 5 for Br-D. Doses up to 8 mg/L of both compounds were nontoxic to *G. mellonella* larvae.

**Discussion-** The environment of patients colonized or infected with *C. auris* can become heavily contaminated. Our preliminary data suggest that bromiphen is a promising QAC for environmental and possible skin decontamination against *C. auris* persistence.

## ***P 119 – ID 234 - Epigenetic modulators as a new strategies to reduce Aspergillus pathogenicity***

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*Aspergillus* species are the most important pathogenic filamentous fungus in humans causing a spectrum of diseases including allergic bronchopulmonary aspergillosis, chronic pulmonary aspergillosis and invasive aspergillosis. The exoproteins of *Aspergillus* such as gliotoxin, hydrolases (alkaline protease, amylase, lipase, and catalase) have a significant role in causing pathogenicity to the host. The aim of this work has been to evaluate the activity of selective epigenetic inhibitor compounds on mycelial growth and on expression of virulence-related exoproteins produced by *Aspergillus*. Moreover, the antifungal activity of these compounds was assessed alone and in combination with some antifungal drugs. In particular, JUMONJY inhibitor, bromodomain extra terminal protein inhibitor, DNA methyltransferase-1 inhibitor, enhancer of zeste homolog 2 inhibitor, histone deacetylase inhibitor, lysine-specific demethylase 1 inhibitor and acetyltransferases inhibitors, have been tested against *Aspergillus*. The results have showed that epigenetic modulators significantly modulated extracellular proteins and that deacetylase inhibitors reduced mycelial growth. Further studies will be conducted to identify the mechanisms involved, with the aim of being able to use active molecules to reduce *Aspergillus* virulence.

## **P 120 – ID 239 - Clarification of the mechanism of action of ozonized oil eye drops in liposomes against *Candida* spp.**

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**Introduction:** Ozone is commonly used as sanitiser for several and different purposes in a number of fields. The antimicrobial, antifungal and antiviral action is well known, as its ability to prevent the development of antimicrobial resistance and allergic reaction, since the product of reaction is molecular oxygen. The use of ozonized oils to treat external urogenital or wound infections is encouraged specially where the infection is sustained by MDR strains. Keratitis are often associated with fungal infections induced by filamentous fungi (*Aspergillus*, *Fusarium*) or yeast-like fungi (*Candida albicans* and other *Candida* species). Specifically, they are more dangerous and devastating than bacterial keratitis and their diagnostic path and treatment is challenging. Moreover, *Candida albicans* keratitis are frequently associate with systemic illness or complicated chronic ocular diseases. The aim of this study was investigated the action mechanism of ozonized oil eye drops in liposomes (**ed-OZO**) against four clinical strains of *Candida* species: *C. albicans* (CA), *C. glabrata* (CG), *C. krusei* (CK) and *C. orthopsilosis* (CO).

**Material and Methods:** *Candida* spp. isolates were obtained from S.Salvatore Hospital in L'Aquila. The antifungal activity of ed-OZO versus *Candida* isolates was evaluated using microdilution broth method for the calculation of MICs (EUCAST-AFST) Edef7.2. Half maximal growth inhibitory concentrations (IC<sub>50</sub>) for all strains were calculated by non-linear fitting using the software Origin 2018. CA membrane depolarization was investigated by fluorimetric assay using 3,3'-dipropylthiadicarbocyanine iodide DisC3(3) in a range of ed-OZO from 1/128×MIC to 1×MIC.

**Results:** *Candida* spp. isolates are susceptible to ed-OZO at the following concentrations (vol/vol): CA, 3.125%; CG, 0.195%; CK, 0.78%; CO, 6.25%. CA membrane depolarization occurs instantaneously after exposure to ed-OZO at concentrations ranging from 1/32×MIC to 1×MIC. In this range, dose dependent membrane depolarization is observed (form 3.76% to 32% with respect to a positive control). Increasing in ROS is observed at the highest ed-OZO concentrations, as well as membrane lipid peroxidation.

**Discussion and Conclusion:** This study was addressed to the evaluation of the antimycotic activity and investigation of the mechanism of action of ozonized oil eye drop stabilised in liposomes. It shows good antimycotic activity at dilution ranging from 32- to 512-fold against all candida species. In *Candida albicans*, used as model for mechanism investigation, eye drop acts inducing membrane permeabilization as demonstrated by membrane depolarization. The increase in ROS species and subsequent membrane lipid peroxidation might explain the strong antimycotic activity.

## ***P 121 – ID 249 - Transfusion-transmitted malaria of P.malariae in Palermo, Sicily***

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**Introduction:** Transfusion-transmitted malaria (TTM) is a rare occurrence with serious consequences for the recipient. It is an accidental Plasmodium infection caused by whole blood or a blood component transfusion from a malaria infected donor to a recipient. The incidence of transfusion-transmitted malaria is very low in non-endemic countries due to strict donor selection but it may cause morbidity and mortality especially in non-endemic areas because the individuals have no premunition to malaria. *Plasmodium falciparum*, *P. vivax* and *P. malariae* are the species most frequently detected in TTM. A clinical case of transfusion-transmitted malaria, caused by *Plasmodium malariae*, is presented in a patient with acute hemorrhagic gastropathy.

**Materials and Methods:** In April 2019, a 70-year-old Italian man with recurrent spiking fever for four days, thrombocytopenia (131,000 to 48,000), anemia (10.5 to 8.2 mg / dl), acute renal failure (azotemia 185 mg / dl, creatinine 4.73 mg / dl uricemia 9.3 mg / dl), was diagnosed with a *Plasmodium malariae* infection confirmed by microscopy and Real-time PCR. The patient had never been abroad, but about two months before, he had received red blood cell transfusion for anaemia. Going back to the donor, we discovered that it was a missionary priest who often went to tropical regions. *Plasmodium* PCR was also used on donor blood to confirm the causal link.

**Discussion and Conclusions:** The main problem related to transfusion-transmitted malaria is related to the presence of asymptomatic blood donors who are predominantly "semi-immune" with very low parasitic loads. Different surveillance strategies can be used to reduce cases of transfusion-transmitted malaria. These include: pre-donation questionnaires, laboratory screening or their combination of them. The laboratory, with serological and molecular tests, could play an important role in the prevention of transfusion-transmitted malaria. This case suggests that *P. malariae* infections in asymptomatic semi-immune donors are a threat to transfusion safety. Microscopy, which is still the gold standard for the diagnosis of malaria, has a limited sensitivity to detect parasitemias so low. Serological tests, combined with the donor's questionnaire, are the screening method used today in non-endemic countries, although not ideal with regard to sensitivity and specificity. In the literature it is recommended to adopt a unique national serological test, and the development of screening algorithms based on multiple laboratory tests, including molecular tests.

## ***P 122 - ID 293 - A rare case of onychomycosis due to *Knufia epidermidis****

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**Introduction:** Onychomycosis is a common clinical finding, caused by fungal infection of the nail, characterized by alteration of color, thickening and onycholysis of the nail. In fungal infection are rare reported infection due to black yeast like or melanized fungi. We present a case of black yeast-like, *Knufia epidermidis*.

**Materials and Methods:** we did nail scraping for coltural examination and identification in sabouraud agar plate with chloramphenicol implementation and gene sequencing analysis of the Internal Transcribed Spacer (ITS) of 18S rDNA in the rRNA and the sequence data were analyzed using the National Center for Biotechnology Information BLASTsystem.

**Results:** The sequences analyses has led to identification of species *Knufia epidermidis*.

**Discussion and Conclusions:** The intensive use of nail polish, especially if they come from Countries not subjected to strictly quality controls, can be too aggressive for the nail and the basis for various nail diseases. New trends like fake nails, nail art, nail ring and others, in some cases have led to excessive stress on the nails; a wrong and unharmonious application of the nail polish, like when air bubbles are created, could lead to a hood effect, in which a micro environment is created that can favor the growth of yeast, fungi and moulds.

## ***P 123 – ID 301 - Effect of Myrtus communis essential oil on Trichomonas vaginalis sensitive and resistant to metronidazole***

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**Introduction:** *Trichomonas vaginalis* is a protozoan parasite responsible for trichomoniasis, the most common non viral sexually transmitted disease. Metronidazole is the drug of choice for *T. vaginalis* infections. The increase in metronidazole resistant parasites and undesirable side effects of this drug makes the search for an alternative a priority for the management of trichomoniasis. *Myrtus communis* oil is known for its effects on several microorganism, but has never been tested on *T.vaginalis* so far. The present study was carried out to investigate the in vitro effects of *Myrtus communis* essential oil on strains *T. vaginalis* of different geographical origin, both sensitive and resistant to metronidazole.

**Material and methods:** The effects of different concentrations of *Myrtus communis* essential oil ranging from 2 mg/ml to 120 µg/ml was evaluated on exponentially growing trichomonad cells at 24 and 48 hours of incubation at 37% °C. Several protozoan strains of different geographical origin, both sensitive and resistant to metronidazole, were tested. Minimal lethal concentration (MLC) was determined by both microscopy and MTT assay.

**Results:** A prompt effect of the essential oil of *Myrtus communis* was demonstrated, with an average MLC of 250 µg/ml at 24 h and of 125 µg/ml at 48 hours on both metronidazole sensitive and resistant trichomonad strains.

**Discussion and conclusions:** A high antitrichomonad activity was observed on both metronidazole sensitive and resistant strains, suggesting that *Myrtus communis* essential oil may be a promising candidate for the development of antitrichomonad treatment alternative to metronidazole.

## POSTER RAPPORTI MICRORGANISMO-OSPITE

### *P 124 - ID 001 - Pathogens In Bile Microbiota: The Fallen In Patients With Biliary And Pancreatic Tract Disease*

*Paola Di Carlo (1) - Teresa Fasciana (1) - Nicola Serra (2) - Consolato Maria Sergi (3) - Francesco D'arpa (4) - Emanuele Battaglia (4) - Anna Giammanco (1)*

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3. *University of Alberta, Laboratory Medicine and Pathology, Canada*
4. *Di.Chir.On.S. Department, University of Palermo, Italy*

**Background:** The pancreatic biliary tract is the site of acute, chronic inflammatory processes and also of tumor etiology. The studies we have conducted so far have demonstrated the existence of microorganisms in the bile samples of subjects with acute, chronic cholecystitis, pancreatic head and gallbladder cancer. Although bile is notoriously defined as a sterile liquid, our studies have shown the presence of pathogens in bile specimens, so much so that today we talk about bile microbiome. Studies conducted by our research group have shown that gram-negative MDR pathogens are the most isolated in the bile of patients with chronic inflammatory and tumor processes of the pancreatic biliary tract

**Aim:** We evaluated the effect of bile microbiota on survival in patients with pancreas and biliary tract (PBD).

**Patients And Methods:** We investigated 152 Italian patients with cholelithiasis (CHL), cholangitis (CHA), cholangiocarcinoma (CCA), gallbladder carcinoma (GBC), pancreas head carcinoma (PHC), ampullary carcinoma (ACA), and chronic pancreatitis (CHP).

The patients were hospitalized at the Department of General and Emergency Surgery, University Hospital of Palermo, Italy, between June 2010 and June 2014, with follow-up until December 2016. The study population consisted of patients with positive culture of bile samples collected during endoscopic retrograde cholangiopancreatography (ERCP) from patients harboring hepatobiliary disease at an external quality assurance-certified General Surgery and Emergency Academic Unit of Policlinic University Paolo Giaccone, Palermo, Italy . Microbiota identification with antimicrobial susceptibility testing was performed at the Department of Microbiology and Virology Unit, Paolao Giaccone University Hospital of Palermo, IT. Demographics, bile cultures, therapy, and survival rates were analyzed in cohorts (T1 death <6 months; T2 death <12 months; T3 death <18 months, T3S alive at 18 months).

**Results:** the most 10 common bacteria in T1 were *E. coli*, *K. pneumoniae*, and *P. aeruginosa*. In T2, the most common bacteria were *E. coli* and *P. aeruginosa*. In T3, there were no significant bacteria isolated, while in T3S the most common bacteria were like those found in T1. *E. coli* and *K. pneumoniae* were positive predictors of survival for PHC and ACA, respectively. *E. coli*, *K. pneumoniae*, and *P. aeruginosa* showed a high percentage of resistant to 3CGS, amino- 15 glycosides class, and quinolone group especially at T1 and T2 in cancer patients. An unprecedented increase of *E. coli* in bile leads to a decrease in survival.

**Conclusions:** We suggest that some strains isolated in bile samples may be considered within the group of risk factors in carcinogenesis and/or progression of hepato-biliary malignancy. A better understanding of bile microbiota in patients with PBD should lead to a multifaceted approach to rapidly detect and treat pathogens before patients enter the surgical setting in tandem with the implementation of the infection control policy.

## ***P 125 - ID 002 - A leopard can change its spots: how HCMV genetic variability impacts viral fitness and NK ligands immunomodulation***

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**Introduction:** Human cytomegalovirus (HCMV) is the leading cause of congenital infection resulting in severe morbidity and mortality among infected newborns worldwide; however, mechanisms and virulence factors contributing to HCMV pathogenesis and particular clinical outcomes remain unclear. To successfully establish a persistent infection, the virus must have evolved multiple mechanisms to avoid host immune recognition. For example, HCMV infected cells exhibit remarkable resistance to natural killer (NK) cell-mediated cytotoxicity via encoding a large set of immunomodulatory proteins. Furthermore, HCMV demonstrates an exceptionally high degree of variability. Against this background, our aim was to determine whether and to what extent the differences in genetic composition affects viral fitness, its ability to modulate NK response, and clinical outcome.

**Materials and Methods:** For this purpose, we enrolled a cohort of 21 pediatric patients with confirmed HCMV congenital infection. We evaluated the degree of genetic polymorphism of HCMV clinical strains by next generation sequencing (NGS), primarily focusing on viral genes known to encode proteins with potent NK immunomodulatory functions. In parallel, we ran an extensive *in vitro* analysis of all clinical isolates to characterize viral growth properties in different cellular models.

**Results:** Here we report extraordinary genetic and phenotypic diversity of the clinical isolates, reflected in both viral growth properties and ability to modulate NK cells. Growth analysis of HCMV clinical isolates revealed different patterns of replication and dissemination. Although we observed no difference in cell tropism, the fast-replicative isolates form a unique morphological pattern. The analysis of NK cell activating ligands at both RNA and protein level, demonstrated that HCMV clinical strains affect NK ligands to different degrees. For instance, all viruses downmodulate ULBP2/5/6 ligand, with the strongest effect observed with the phenotypically more aggressive isolates. Moreover, there was a strong up-regulation of PVR observed with fast-replicative isolates. Conversely, other NK ligands, such as MICA, MICB, ULBP3 and B7-H6, were not modulated by any of the HCMV isolates. Finally, to assess whether HCMV isolates affect NK recognition, we analyzed IFN-gamma production. Likewise, we observed a great variation between clinical strains, indicating that the genetic variability actually reflects variability in functionality.

**Discussion and Conclusions:** Overall, our study contributes to understanding the impact of viral genetic variability on viral fitness and immune system modulation, with the ultimate goal to identify valuable markers for the management of congenitally infected newborns.

**Keywords:** Human cytomegalovirus (HCMV), congenital infection, clinical isolates, genetic variability, viral phenotypes, immunomodulation, NK ligands.

## ***P 126 – ID 003 - Metabolic games: IFI16-driven regulation of cellular metabolism upon HCMV infection***

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**Introduction:** Human cytomegalovirus (HCMV) is associated with acute and chronic disease in both healthy and immunocompromised populations. A critical feature of HCMV is that it modulates cellular metabolism to promote viral replication. Indeed, HCMV infection has been associated with the increased lipogenesis in infected cells that is likely required for the envelopment of the newly formed virions. However, the fundamental mechanisms responsible for HCMV-induced activation of lipid synthesis remain poorly understood. We have previously reported that interferon-gamma-inducible protein 16 (IFI16) is a restriction factor for HCMV and it is delocalized from the nucleus to the cytoplasm as viral escape mechanism.

**Methods:** To understand if IFI16 interferes with one or more metabolic effects during HCMV infection we generated knockout (KO) gene variants in human foreskin fibroblasts (HFFs) through CRISPR-Cas 9 technology.

**Results:** Here, we demonstrate that IFI16 reduces the expression of the glucose transporter GLUT4, resulting in decreased glucose import and translocation of the carbohydrate-response element binding protein (ChREBP) to the nucleus. Consequently, reduced transcription of the genes encoding lipogenic enzymes leads to decreased lipid synthesis and enhanced generation of the enveloped viral particles in infected cells.

**Discussion and Conclusions:** These data may shed light on the potential impact of IFI16 on regulation of glucose and lipid metabolism upon HCMV replication suggesting new promising targets for antiviral therapy.

## ***P 128 – ID 009 - The interplay between the vaginal microbiome and local host immune response influences the susceptibility to the oncogenic viral infections***

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**Introduction:** Oncogenic viruses are generally present in a large part of the population without causing disease. In the female genital tract, oncogenic viruses can enter and asymptotically persist as a consequence of the vaginal mucosal barrier failure and inflammation deriving from the local microbial dysbiosis. Among these viruses, members of *Papillomaviridae*, *Polyomaviridae* and *Herpesviridae* families have been studied for their oncogenic potential. Though, studies addressing their dynamic interaction with the resident vaginal bacteria and host immune response are scarce.

**Materials and Methods:** We evaluated the relationship between oncoviruses and disruptions in vaginal Community State Types (CSTs) and host immune response in 90 Caucasian immunocompetent women of reproductive age. Precisely, we performed the V3-16S rRNA gene sequencing, we dosed 48 immune soluble factors and we used a multiplex type-specific PCR for 44 oncoviruses.

**Results:** *Polyomaviridae* and *Papillomaviridae* DNA was detected in samples from each CST with a different incidence while *Herpesviridae* were absent. The presence of viral DNA was significantly associated with changes in the resident bacteria of CST I and IV ( $p < 0.05$ ). Precisely, *Lactobacillus crispatus* increased in samples from CST I infected with *Polyomaviruses* while *Prevotella timonensis* and *Sneathia sanguinegens* increased in samples from CST IV infected with *Papillomaviruses*. Conversely, CST II and III showed a significant alteration of the host response leading to reduced antiviral efficacy. CST II infected samples showed a decrease of Eotaxin, MCP-1, and IL-7 while CST III infected samples showed a decrease of IL-9 and IL-15 ( $p < 0.05$ ). In addition, an efficient viral clearance was registered only in women from CST I group.

**Discussion and Conclusions:** Persistent infections caused by *Papillomaviruses* and *Polyomaviruses* specifically benefit from the different local immune-bacterial crosstalk observed in each CST, in which a key role in vaginal defence is played by *Lactobacillus* species. Indeed, *L. crispatus* (CST I) correlated with the highest rate of viral clearance while *Lactobacillus gasseri* (CST II) and *Lactobacillus iners* (CST III) exerted a lower antiviral role. Newly, we detected the co-occurrence of *Human Papillomavirus* and *Merkel Cell Polyomavirus* mainly in samples with molecular signatures of mucosal inflammation suggesting a similarity in the pathogenic pathway. In conclusion, our *in vivo* study begins to address the knowledge gap with respect to the role of vaginal bacteria and immune response in the susceptibility to oncoviral infections.

## ***P 129 - ID 012 - Atopobium vaginae and Porphyromonas somerae induce proinflammatory cytokines expression in endometrial cells: a possible implication for endometrial cancer?***

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**Introduction:** The female lower vaginal tract has long been known to have an active microbiota, but a uterine microbiota was recognized only recently, with unique characteristics differing from that of the vagina. Although the exact role of uterine microbiota is unclear, recent studies suggest that it could affect important conditions including endometriosis and cancer. In particular, *Atopobium vaginae* and *Porphyromonas somerae* species resulted particularly significant in endometrial cancer, but there is currently uncertainty about the mechanisms by which such bacteria might influence endometrial microenvironment. Our study was thus aimed to analyze *in vitro* the effect of *A. vaginae* and *P. somerae* on the expression of pro-inflammatory cytokines by endometrial cells, as they are implied in the establishment of an inflammatory environment potentially favoring the onset/progression of endometrial cancer.

**Materials and Methods:** The human HEC-1A endometrial cells were cultured in the presence or absence of *A. vaginae*, *P. somerae* or *Lactobacillus vaginalis* (as a control). Cells and cell supernatant were collected at 24, 48 and 72 hours post infection (h.p.i.), and analyzed for proinflammatory cytokine expression and release by ELISA and transcription analysis.

**Results:** *A. vaginae* and *P. somerae* induced an evident release of proinflammatory cytokines by endometrial cells, with different patterns for *A. vaginae/P. somerae* (IL1alpha, IL1beta, IL17alpha, and TNFalpha) compared to *L. vaginalis* (IL-8). Cytokine mRNA expression analysis, performed by microarray real-time PCR, showed that all the cytokines detected by ELISA were activated at the transcriptional level. In addition, other chemokines resulted significantly altered by co-culture with *Atopobium* and *Porphyromonas*, including CCL8, CXCL2, IL22 and IL9, all of them previously reported to be associated with cancer progression. In particular, CCL8 has been associated with tumor progression and dissemination, CXCL2 is induced by TNFalpha and induces chemoresistance, IL22 promotes progression in breast cancer, and IL9 prevents apoptosis, promoting proliferation and metastasis.

**Discussion and Conclusions:** Our *in vitro* observations show for the first time that certain bacteria have the ability to induce expression of proinflammatory cytokines and chemokines by endometrial cells, and suggest that their presence in the uterine environment might be associated with the establishment of conditions promoting inflammation, providing a starting point for future researches focusing on the impact of uterine microbiota on uterine physiology and pathologies, and hopefully potentially useful tools for diagnosis and effective clinical interventions.

## ***P 130 – ID 025 - Assessment of biofilm production and drug resistance profile for therapeutic management of infected chronic ulcers***

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1. **Introduction:** Bacterial biofilm is a key factor contributing to delayed wound healing and associated with increased antimicrobial drug resistance. The necrotic tissue and debris promote bacterial attachment, and Chronic ulcers (CU) are susceptible to infection due to impaired host immune response. Therefore, establishing the role of pathogenic biofilms in CU will help to guide a more efficient diagnosis and targeted therapeutic intervention. In this study we aimed at developing a novel clinical protocol for the rapid microbiological assessment of biofilm production and antibiotic tolerance and at exploring their role in sustaining bacterial invasion and persistence in patients with CU.

2. **Materials and Methods:** 150 patients were enrolled in the study. Microbial identification was performed by MALDI-TOF and drug susceptibility was determined by broth microdilution tests. Biofilm production of was assessed by the clinical BioFilm Ring Test (cBRT). The antibiotic susceptibility profile on biofilm-growing bacteria was further obtained by the Anti-Biofilm Test (ABT).

3. **Results:** A total 246 bacterial strains were identified. Gram-negative bacteria were the most represented microorganisms (61%). Nevertheless, considering individual bacterial species, *Staphylococcus aureus* was the most common pathogen (35.8%), followed by *Pseudomonas aeruginosa* (16.7%). 78.7% of the strains were moderate/high biofilm producers, while most of the weak biofilm producer bacteria were found in polymicrobial colonization in association with strong biofilm producer strains. The level of biofilm production correlated with a consistent reduction of the effective antibiotic options. Aminoglycosides were the most active antibiotics against biofilm-growing Gram-negative bacteria, whereas oxacillin and fusidic acid showed a good penetration in *Staphylococcus aureus* biofilm.

4. **Discussion and Conclusions.** Moderate/high biofilm producers were isolated in most CU thus promoting bacterial persistence and antimicrobial tolerance. The assessment of biofilm strength combined with the antibiotic profiling of biofilm growing bacteria may help direct a targeted therapeutic intervention in CU, improving the clinical outcome of these difficult infections.

## ***P 131 – ID 031 - Female infertility: impact of the vaginal and endometrial microbiota on women's reproductive health***

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**Introduction:** The existence of a uterine microbiota has been recently demonstrated, and a qualitatively and quantitatively different microbiota, between the vaginal and uterine sites, has been showed. Additionally several studies showed differences in the vaginal microbiota, among pregnant women and infertile women. Few studies showed the impact of the uterine microbiota on the success of sterility treatments/assisted fertilization.

**Aim:** Characterize the vaginal and endometrial microbiota in female infertility.

**Methods:** 34 women, aged between 20-45 years, experiencing cycles of Medically Assisted Procreation (PMA) for at least a year, were recruited. After informed consent, during PMA, post-hormonal stimulation and immediately before the oocytes collection, two types of sample were collected from the enrolled woman: vaginal fluid in the posterior fornix, and biopsy of the endometrium. Total DNA was extracted from samples using dedicate kits (Qiagen). V3 and V4 regions of 16S rRNA were sequenced using Illumina Miseq platform. DNA sequences obtained (FASTQ) were analyzed for quality control and filtered (for sequencing errors, and chimeras), grouped into operational taxonomic units (OTUs), and analyzed. Alpha diversity, OTUs index and the Shannon index were calculated.

**Results:** The group with a positive pregnancy outcome showed a vaginal microbiota dominated by two species: *Lactobacillus iners* and *Lactobacillus crispatus*, respect to the group with negative outcome, showing a greater heterogeneity in the composition. In the endometrial microbiota the absence of Lactobacilli and a predominance of Lachnospiraceae and Enterobacteriaceae, were showed in the group with positive pregnancy outcome.

**Discussion and conclusions:** Results indicate two distinct microbial environments between vagina and uterus, the first dominated by Lactobacilli, the second characterized by a polymicrobial composition in which there are species that have never been detected before in the human genital tract, such as Kocuria. Furthermore a difference in microbiota composition was observed, in both considered sites, among the women with positive pregnancy outcome and the one with negative outcome. The results indicate that both, vaginal and uterine microbiota composition, play an important role that strongly influences the outcome of PMA. We could hypothesize that combined use of hormonal stimulation, and therapies aimed at re-establishing the correct vaginal and endometrial microenvironment, could be helpful for PMA success.

## ***P 132 – ID 036 - Induction of apoptosis and inhibition of DNA repair mechanisms on triple negative breast cancer cells by U94 of human herpes virus 6***

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**Introduction:** Triple-negative breast cancer (TNBC) prognosis is still poor with no effective specific targeted therapy available. U94, the latency gene of human herpes virus 6 (HHV-6), is able to interfere with proliferation and crucial steps of the metastatic cascade in TNBC models. U94 exerts its activity upon its arrival to the nucleus. Based on these evidences, we characterized the molecular targets of the viral protein.

**Materials and Methods:** TNBCs (MDA-MB 231, MDA-MB 468 and BT-549) were transduced for twenty-four hours with amplicon vectors expressing U94 or enhanced green fluorescent protein (EGFP). The Affimetrix array was performed and its results were confirmed by real-time PCR. Apoptosis was assessed by flow cytometry, western blot and TUNNEL assay. Chemotherapeutic drugs cytotoxic rate was evaluated by counting cells using the COULTER COUNTER® Analyzer.

**Results:** The Affimetrix array analysis demonstrated that U94 is able to inhibit TNBCs proliferation and DNA repair mechanisms. Flow cytometric and TUNNEL analysis revealed that following U94 expression, TNBCs undergo apoptosis as demonstrated by morphological changes in cell membranes and by DNA fragmentation. Western blot analysis showed that U94 induces apoptosis through down-modulation of the anti-apoptotic protein Bcl-2 and activation of the pro-apoptotic proteins Bax and BAD. Moreover, U94 increased the expression of cleaved poly (ADP-ribose) polymerase and caspase-3. Here we show that, U94 induces apoptosis via the intrinsic pathway as attested by the expression of active caspase-9. Based on this evidence, we tested whether U94 could act as chemo-sensitizer in TNBCs, we exposed transduced cells to cisplatin and doxorubicin, DNA-damaging drugs, and to taxol, a microtubule inhibitor. Interestingly, U94 transduction of TNBCs enhanced cisplatin and doxorubicin cytotoxicity whereas no differences were detected after taxol treatment.

**Discussion and Conclusions:** U94 can be considered as a potential selective chemotherapy sensitizer for the killing of cancer cells which lack DNA repair. In this scenario, U94 is a hopeful therapeutic TNBC treatment as a single agent or in combination.

**P 133 – ID 040 - Vaginal Epithelial Cells discriminate between yeast and hyphae of *C. albicans* in women who are colonized with or have vaginal candidiasis**

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**Introduction:** Vaginal candidiasis is common disease affecting women; however, how *C. albicans* shift from commensalism towards a pathogenic status remains poorly understood. The present study investigated the vaginal epithelial cell (EC) response dynamics under various conditions.

**Materials and Methods:** Healthy women, asymptomatic *C. albicans* carriers, and symptomatic patients with vaginal candidiasis were enrolled in this study. ECs in the vaginal swabs were analyzed with cytofluorimetric analysis for pattern recognition receptors and intracellular signals, with LDH performed for cell damage and an ELISA for cytokine expression.

**Results:** The level of TLR4, TLR2, and EphA2 expression was significantly higher in ECs from asymptomatic and symptomatic subjects compared to healthy donors. Activation of the transcription factors NF- $\kappa$ B and c-Fos-p-38 was observed in ECs from symptomatic and asymptomatic pseudo-hyphae/hyphae carriers, but not from the asymptomatic yeast carriers.

**Discussion and Conclusions:** The presence of pseudo hyphae/hyphae is required to determine vaginal candidiasis; however, it may be not sufficient to induce the pathological process associated with neutrophil recruitment and EC damage.

## **P 134 – ID 051 - Inhibition of Transglutaminase 2 as a potential host-directed therapy against *Mycobacterium tuberculosis***

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**Introduction:** Host-directed therapies (HDTs) are emerging as a potential valid support in the treatment of drug-resistant tuberculosis (TB). Following our recent report indicating that genetic and pharmacological inhibition of transglutaminase 2 (TG2) restricts *Mycobacterium tuberculosis* (*Mtb*) replication in macrophages, we aimed to investigate the potentials of the TG2-inhibitors cystamine and cysteamine as HDTs against TB.

**Materials and Methods:** To investigate whether cysteamine and cystamine had an anti-microbial activity against *Mtb* in macrophages, THP-1 monocyte-derived macrophages and in primary human monocyte-derived macrophages (hMDM), were infected with *Mtb* H37Rv and then treated with cystamine and cysteamine at concentrations compatible to those that achieved *in vivo*. We previously showed that genetic inactivation of TG2 in murine macrophages results in the impairment of the LC3/autophagy homeostasis, which nevertheless correlates with the restriction of *Mtb* intracellular replication. To further investigate the impact of the two TG2 inhibitors cystamine and cysteamine on autophagy, we quantitatively evaluated the autophagic flux by confocal pH-imaging of the autophagic intermediates on THP-1 cells transfected with mRFP-GFP-LC3B. . To analyze the activity of these drugs as HDTs against TB, we infected human peripheral blood monocyte cells (PBMCs) with *Mtb*, evaluating the effect on granuloma-like structures (GLS).

**Results:** We showed that both cysteamine and cystamine restricted *Mtb* replication in infected macrophages when provided at equimolar concentrations and did not exert any antibacterial activity when administered directly on *Mtb* cultures. Interestingly, infection of monocyte-derived THP-1 mRFP-GFP-LC3B cells followed by the determination of the autophagic intermediates pH distribution (AIPD) showed that cystamine inhibited the autophagic flux while restricting *Mtb* replication. Moreover, both cystamine and cysteamine had a similar antimicrobial activity in primary macrophages infected with a panel of *Mtb* clinical strains belonging to different phylogeographic lineages. Evaluation of cysteamine and cystamine activity in the human *ex vivo* model of granuloma-like structures (GLS) further confirmed the ability of these drugs to restrict *Mtb* replication and to reduce the size of GLS.

**Discussion and Conclusions:** The antimicrobial activity of the TG2-inhibitors synergized with a second-line anti-TB drug as amikacin in human monocyte-derived macrophages and in the GLSs model. Overall, the results of this study support the potential usefulness of the TG2-inhibitors cysteamine and cystamine as HDTs against TB.

## ***P 135 - ID 053 - Bovine herpesvirus 1 induces autophagy in neuro-2A***

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### **1. Introduction**

Bovine herpesvirus 1 (BoHV-1), a member of the alpha-herpesvirinae sub-family, initiates acute infection in mucosal surfaces and provokes serious clinical signs in the upper respiratory tract of cattle in addition to a transient immune-suppression. BoHV-1 establishes latency in sensory neurons within trigeminal ganglia (TG), but periodically may reactivate from latency, and this ability is critical for virus transmission. Recent studies suggest that mouse neuroblastoma (neuro-2A) cell line might be a good model to examine cellular mechanisms which control BoHV-1 productive infection as well as cytotoxicity in neuronal cells.

### **2. Materials and methods**

We investigated the effects of BoHV-1 (Cooper strain) infection in neuro-2A cells.

### **3. Results**

Following the infection for 48 h, a slow time-dependent decrease in cell viability was detected. Cell morphology investigation showed chromatin condensation, fragmentation, expanded cytoplasm, pyknotic nuclei and an high degree of vacuolization. Biochemical analysis revealed a late weak activation of caspase 3 and early signs of a strong autophagy, due to an increase in the conversion of LC3 from LC3-I to LC3-II and to enhanced levels of autophagy related proteins. Finally, a decrease in virus replication, from 24 to 48 h post infection, was detected by virus titer, assayed through TICD<sub>50</sub> in MDBK cells.

### **4. Discussion and Conclusions**

Our results suggest that intriguingly BoHV-1 in neuro-2a cells may exert its potential neurotoxicity through a small rate of necrosis and/or apoptosis, but a predominant stimulation of autophagy seems counteract this phenomenon. Thus, autophagy might be seen as an important mechanism of neuroprotection. Indeed, autophagy is an essential vacuolar process of the cell which is important in maintaining homeostasis. Several herpesviruses have developed strategies to escape this degradation, by expression of specific anti-autophagic proteins. However, during latency, autophagy can also be activated by latent proteins encoded by different herpesviruses to promote cell survival and achieve long term viral persistence *in vivo*. The lifelong latency of BoHV-1 requires that significant numbers of infected sensory neurons survive infection and maintain normal functions. Sensory neurons in TG of calves latently infected with BoHV-1 express latency-related gene products, together with the protein ORF2 that interacts with beta-catenin, a signaling pathway differentially regulated during the latency-reactivation cycle, which increasing facilitates survival of latently infected neurons, probably due to an activation of autophagy.

Future studies about viral proteins could help to clarify a potential support with neuronal factors to maintain latency.

## ***P 136 – ID 066 - Virulence potential of Klebsiella pneumoniae outer membrane vesicles***

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**Introduction:** Outer membrane vesicles (OMVs) are naturally secreted by gram negative bacteria. They are spherical nanostructures (20-250 nm), which carry nucleic acids, lipopolysaccharide (LPS), phospholipids, peptidoglycan, outer membrane proteins, periplasmic, cytoplasmic and membrane proteins. OMVs play a crucial role in the interaction among bacteria. Moreover, it is nowadays clear the pivotal role of OMVs in the bacteria-host interaction. OMVs play fundamental process in many protective functions for the bacterium itself and offensive towards the host. Since *Klebsiella pneumoniae* has emerged as an important opportunistic pathogen, mostly causing nosocomial infections, we focused on the isolation, characterization and evaluation of the virulence potential of its OMVs.

**Material and Methods:** Bacteria strain ATCC 10031 was cultured in LB broth and OMVs were harvested from culture supernatants. To characterize them we performed Transmission Electron Microscopy (TEM), Dynamic Light Scattering (DLS) and 10% sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). The proteins in the gel slices were digested with trypsin and the derived peptide mixtures analyzed using LC-MS/MS techniques. Protein identification occurred through the MASCOT database. The inflammatory activity of OMVs was tested by treating BEAS-2B cells with 0.5, 1, 5 µg/ml of OMVs from *K. pneumoniae* for 24 h. The expression of pro-inflammatory cytokine genes (IL-8, IL-6, IL-1β and TNF-α) was determined by quantitative PCR (qPCR) whereas the amount of inflammatory cytokines was investigated by enzyme-linked immunosorbent assay (ELISA).

**Results:** TEM identified spherical vesicles of a generally uniform size, without any contamination. DLS measured a Z-average of 86nm and proved a homogeneous size distribution with a polydispersion index of 0.236. SDS-PAGE revealed two major bands in *K. pneumoniae* OMVs, with a clear difference compared to the protein profile of the whole-cell lysate. Proteomic analyses identified 101 proteins in the *K. pneumoniae* OMVs. As concerns qPCR, expression of all tested cytokine genes was significantly increased in BEAS-2B cells treated with 5 µg/ml of OMVs compared to that in untreated control and to that treated with LPS at 10 µg/ml. BEAS-2B cells responded to 5 µg/ml of OMVs with a significant increase of IL-8 and IL-6 secretion, while no significant amount of IL-1β and TNF-α was observed by ELISA.

**Conclusions:** Our study confirmed that *K. pneumoniae* produces and secretes OMVs into the extracellular milieu during in vitro culture and suggested the pathogenicity of *K. pneumoniae* OMVs observing a strong proinflammatory response. Since OMVs are clearly involved in the pathogenesis of this bacterium, they could be future targets for novel therapy and potential vaccine against *K. pneumoniae*.

## ***P 137 – ID 070 - Sample preparation methods for the metaproteogenomic characterization of the oral microbiota***

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**Introduction:** Variations in the structure and functionality of the oral microbiota can be indicators of the health/disease status, not only regarding the oral cavity, but also at a global scale. While metagenomics has established as a standard mean to determine the microbiota composition, metaproteomics deserves growing attention for its ability to identify protein functions from both the microbiota and the host, that might serve as clinical biomarkers. Here, we aimed to critically evaluate different sample preparation methods for the metaproteogenomic analysis of the oral microbiota.

**Materials and Methods:** Saliva and oral mucosa samples were collected in parallel from healthy volunteers, making use, respectively, of Salivette<sup>®</sup> collection systems and buccal swabs. DNA and protein extraction were performed according to different methods, comprising both commercial kits and in-house protocols. The V4 region of the 16S rRNA gene was amplified by subjecting the microbial DNA to direct PCR amplification or to a nested PCR approach. On the other hand, proteins were processed according to a modified filter-aided sample preparation procedure. Finally, DNA amplicons were sequenced with an Illumina MiSeq sequencer, while the peptide mixtures were analyzed by ultra-performance liquid chromatography coupled with high-resolution mass spectrometry. Metagenomic and metaproteomic data were finally parsed using dedicated bioinformatic tools, such as QIIME and Unipept.

**Results:** Different data concerning the microbiota composition were obtained when comparing saliva and buccal swab samples, as well as when comparing different sample preparation methods, according to both metagenomic and metaproteomic results. Relative identification yields, the ratio between microbial and host protein information, the method reproducibility and the impact of sample storage were also comparatively evaluated.

**Discussion and Conclusions:** This pilot study supports the use of metaproteogenomics for the characterization of the oral microbiota, providing several methodological indications on how to maximize microbial information and to optimize the sample preparation steps in order to increase identification yields. Oral metaproteogenomic methods hold promise to identify novel biomarkers associated to infectious and oncological diseases affecting the oral cavity as well as other organs.

## ***P 138 – ID 071 - Isolation of bacterial RNA from infected host tissues: application for transcriptomic studies of group B streptococci***

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**Introduction:** Group B Streptococcus (GBS), an encapsulated, Gram-positive pathogen, is an important cause of perinatal systemic infections and of sepsis in the elderly. Understanding the mechanisms by which GBS regulate virulence factor expression *in vivo* is crucial to develop alternative strategies to control these infections. We have recently identified a gene encoding for the plasminogen binding protein PbsP, which is expressed *in vivo* at extremely high levels signal transduction by the SaeR/S two-component system. Since purification of microbial RNA from infected organs is technically challenging, we developed a new method for maximizing the yield of bacterial RNA, with the ultimate objective to study SaeR/S-mediated, genome-wide transcription regulation.

**Materials and Methods:** Different strains of mice were infected i.v. and i.p. with  $1 \times 10^8$  CFU of the BM110 strain (clonal complex 17) and sacrificed at 48 h after infection to collect organs and peritoneal lavage fluids. Bacterial RNA was extracted from the organs of infected mice, retro-transcribed and analyzed by RT-PCR.

**Results:** We optimized bacterial RNA extraction from infected tissues by combining different approaches including: 1) selecting a specific strain of immune-deficient mice (Myd88 KO) to obtain high bacterial loads, particularly in GBS target organs, such as the brain; 2) selective lysis of host cells using guanidium-based methods that preserve bacterial RNA integrity; 3) using beads of specific size and homogenization cycle numbers in order to disrupt GBS. The RNA quality and quantity was evaluated by gel electrophoresis and qRT-PCR analysis. Results indicate that the method is adequate for organ-specific transcriptomic studies.

**Discussion and Conclusions:** Our newly developed protocol results in considerably improved recovery of bacterial cells from infected organs and in higher yields of high-quality RNA, overcoming a major limitation in the study of organ-specific bacterial transcriptomic analysis. The method allowed us to obtain novel information on virulence and colonization factors of GBS that rely on the SaeR/S two-component regulation system.

## **P 139 – ID 072 - Antimicrobial activity of two food-intake regulatory neuropeptides: Ghrelin and Orexin B**

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**Introduction:** Abuse and animal husbandry use of antibiotics have contributed to the emergence of resistant bacteria, this is a global health threat so the discover of new antimicrobial agents is an absolut priority. In this scenario endogenous peptides are resulting as emerging as novel potential candidates. Ghrelin is a 28-amino acid peptide expressed mainly in the stomach, pancreas and also present in plasma and saliva. It binds to the growth hormone receptor (GHS-R1a) in pituitary cells, inducing the secretion of growth hormone and an appetite-stimulating effect. Orexins are hypothalamic neuropeptides that have been implicated in the regulation of feeding. They are cleaved from a common precursor molecule, prepro-orexin, forming orexin A (33 amino-acids) and orexin B (28 amino-acids). Interestingly, the chemical properties of orexin B and ghrelin are very similar to those of vasoactive intestinal polypeptide (VIP) reported to have antimicrobial activities. Since antimicrobial action of a peptide can be predicted by its chemical properties, we hypothesized that the two neuropeptides may function as anti-infective molecules. In the present work we have analysed their antibacterial activity against Gram-negative (*Escherichia coli* and *Salmonella Typhimurium*) and Gram-positive (*Staphylococcus aureus*) ATCC bacteria and we have evaluated the antiviral proprieties of the same peptides against herpes simplex virus 1 (HSV-1). **Materials and methods:** Susceptibility testing was performed following the broth micro-dilution method. In particular we have defined the antibacterial action of peptides by determining their minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) used a range of concentrations between 100 and 0.78  $\mu$ M. Moreover, we have also evaluated the cytotoxicity of the two neuropeptides. To evaluate the effect of the peptides on inhibition of HSV-1 infectivity, a co-treatment experiment was performed. Vero cells (ATCC CCL-81) were incubated with scalar concentrations of the peptides, from 50 to 0,781  $\mu$ g/ml of substance with the virus ( $10^3$  PFU / ml) and the infection was monitored by plaque assay. **Results:** Ghrelin and orexin B have a strong antibacterial activity against Gram-negative bacteria with a minimal inhibitory concentration at 25  $\mu$ g/ml, while the effects against Gram-positive *S. aureus* are absent. They don't show toxicity effects until highest concentrations tested. However, orexin B shows a high antiviral activity at the concentration of 50  $\mu$ g/ml. **Discussion and conclusions:** We demonstrated that ghrelin and orexin B possess similar antibacterial activity against Gram-negative, while only orexin B shows a high antiviral effect in a co-treatment assay. The pivotal mechanism of action have to be investigated.

## ***P 140 – ID 074 - Plasminogen binding and immunoprotective properties of the Mk-rich domain of PbsP, a cell wall protein from Streptococcus agalactiae***

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**Introduction:** *Streptococcus agalactiae* (Group B *Streptococcus* or GBS) is a frequent cause of sepsis and meningitis in neonates and in patients with underlying chronic diseases. Therefore, the development of effective measures to control GBS disease represents a public health priority, for which further knowledge of pathogenic mechanisms is required. We have recently described PbsP, a plasminogen-binding cell wall-anchored protein expressed by all GBS strains. PbsP is required for breaching of the blood-brain barrier by GBS and can induce immune protection against brain invasion. This protein is highly expressed *in vivo* and contains two 150-aa SSURE domains and a methionine and lysine-rich (MK-rich) region. Our previous studies demonstrated that the Mk-rich and the SSURE domains mediate GBS binding to plasminogen (Plg) and vitronectin, respectively. Therefore, PbsP or its isolated domains are attractive vaccine candidates.

**Materials and Methods:** We studied the mechanisms by which the Mk-rich domain binds Plg and induces protective immunity. **1.** We generated recombinant fragments of the Mk-rich domain to identify the minimal region that still maintains the Plg binding ability. **2.** We determined the role of lysine binding sites in the Plg molecule for its ability to interact with the Mk rich domain. To this end, we replaced lysine residues with alanine in the MK-rich domain. **3** We tested the ability of wild-type and mutated recombinant MK-rich fragments to prevent brain invasion in a mouse model of GBS meningitis.

**Results:** We found that, similarly to the entire PbsP protein, the Mk-rich domain binds the Kringle 4 domain of human Plg. Moreover, the Plg binding activity of the Mk-rich domain is confined to its C-terminal region (S<sub>444</sub> to K<sub>429</sub>). L-lysine and its analog epsilon-aminocaproic acid were able to inhibit the binding between Plg and Mk-rich domain, suggesting an involvement of Plg lysine binding sites (LBS) in the Plg molecule. Surprisingly, however, an Mk-rich construct in which all lysine residues were mutated to alanine still fully retained the ability to bind Plg. Moreover, this mutated fragment was highly protective in a in mouse model of GBS meningitis.

**Discussion and Conclusion:** We have identified here highly immunogenic recombinant fragments derived from the MK-rich domain of PbsP that retain the ability of the entire protein to bind Plg. These fragments could be included in a universal anti-GBS vaccine as isolated components or as carriers after conjugation with capsular polysaccharides.

## ***P 141 – ID 075 - Vaginal microbiota evaluation in HPV infection women***

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**Introduction.** Human papilloma virus (HPV) is a small, non-enveloped and icosahedral DNA virus. According to the Centers for Disease Control and Prevention (CDC), HPV is the main cause of sexually transmitted infections. There are more than 100 types of HPV, among them two serotypes (16 and 18) are related to 70% of cervical cancers and pre-cancerous cervical lesions. In 2018, 570000 new cases have been estimated and about the half part of patients are died. The vaginal microbiota has a considerable role in the HPV cervical cancers. It is well known that bacteria are strongly associated with vaginal inflammation and oncogenic mutations in the human cells. Our aim is to investigate if HPV infection could influence the bacterial microbiota composition in the uterine cervix.

**Materials and Methods.** A total of 48 women were enrolled in the study. DNA samples from the uterine cervix were included: the vaginal swabs had been collected as mucosal scrapings with a brush. The methods were performed according to the guidelines and regulations. Subsequently, HPV DNA was extracted by QIAamp DNA Microbiome (QIAGEN, code 51704). Isolated DNA concentrations were measured using a Qubit 2.0 Fluorometer (Life Technology) and normalized to 1 ng/μL. The V3-V4-V6 region of 16S rDNA gene was amplified by Polymerase Chain Reaction (PCR) followed by sequencing via Next Generation Sequencing (NGS) (ARROWforNGS, code AD-002.024).

**Results.** The data obtained show that *Lactobacillus* is the main bacterial species in women with a negative HPV test. Meanwhile, there is a significative decrease in *Lactobacillus* population in the most women. The results are supported by the prevalence of other bacterial populations, such as the genera *Gardenerella*, *Prevotella*, *Atopobium*.

**Discussion and Conclusions.** The vaginal microbiota, together with vaginitis and HPV infection, may modulate the development or prevention of cervical cancers. Further studies are required to determine whether the regulation of the vaginal microbiota via probiotics could be used as a strategy in the prevention of cervical cancers.

## ***P 142 – ID 080 - Airborne microbial flora in Buffalo farms in a Mediterranean climate.***

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**Introduction:** Mediterranean buffaloes are an important part of the animal industry. Indeed, they are important for the production of meat, skins, milk and derivatives. The last few decades have seen an increase in intensive buffalo farms worldwide. Microorganisms are easily cumulated and aerosolized in stables with high animal population density. Inhalation of airborne microorganisms involves risks for environment, human and animal health. For this reason, in this study we focused on gathering microbiological air sampling in Southern Italian buffalo farms.

**Materials and Methods:** Ten buffalo farms have been selected in Campania, homogeneous in size and organization. Each growing house has the capacity to hold 400 buffaloes. The bioaerosol samples were collected with a Thermo-Scientific six-stage Andersen Cascade Impactor, following the method described by Andersen et al. Furthermore, for each farm, three samples taken from five sites were tiled on different culture mediums. After sampling, the plates were immediately incubated at 30°C for 24 to 36 h for mesophilic bacteria, and at 37°C for 24 h to 36 h for coliforms. For yeasts and moulds the cultures were incubated at room temperature (23 ± 3°C). The plates were inspected after 4 days and periodically up to 14 days after primary exposure.

**Results:** The results showed the concentrations of cultivable microorganisms in 10 farms in Southern Italy during the year 2018. The minimum and maximum values of the bacterial concentration found, ranging from 7.9 x 10<sup>4</sup> to 2.4 x 10<sup>6</sup> CFU/m<sup>3</sup> for the summer, 6.5 x 10<sup>4</sup> to 1.1 x 10<sup>6</sup> CFU/m<sup>3</sup> for the spring, 6.2 x 10<sup>4</sup> to 9.8 x 10<sup>5</sup> CFU/m<sup>3</sup> for the fall and 6.3 x 10<sup>3</sup> to 3.2 x 10<sup>5</sup> CFU/m<sup>3</sup> for the winter. Likewise, the values for fungi are: 9.1 x 10<sup>2</sup> to 4.1 x 10<sup>3</sup> CFU/m<sup>3</sup> for the summer, 6.6 x 10<sup>2</sup> CFU/m<sup>3</sup> to 3.3 x 10<sup>3</sup> for the spring, 7.1 x 10<sup>2</sup> to 2.9 x 10<sup>3</sup> CFU/m<sup>3</sup> for the fall and 3.3 x 10<sup>2</sup> to 1.7 x 10<sup>3</sup> CFU/m<sup>3</sup> for the winter. A great variety of bacterial and fungal species or genera were found in buffalo farms. Among these, the most numerous belonged to the following species: *Enterobacteriaceae*, *Campylobacteriaceae* and *Pseudomonadaceae*. *Candida*, *Saccharomyces*, *Aspergillus* and *Cladosporium* were the most representative of the identified fungi in all stables.

**Conclusions:** This study was designed to obtain information about the airborne microorganism present in buffalo farms of Southern Italy. Bacterial concentrations were greater than fungal concentrations. Nevertheless, the results show that in stables where animals and workers are in good health, no airborne aerosol particles containing pathogenic microorganisms are present.

## ***P 143 – ID 081 - IFI16 protein binds to LPS with high affinity and boosts inflammatory cytokine production***

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**Introduction:** Lipopolysaccharide (LPS) is the main cause of gram-negative bacterial sepsis. When introduced into the bloodstream, LPS is recognized by LPS binding protein (LBP), transferred to CD14 and finally to the TLR4-MD2 receptor. Other proteins are known for their ability to bind to and amplify the LPS-mediated pro-inflammatory process. The interferon- $\gamma$ -inducible protein 16 (IFI16) is a nuclear phosphoprotein acting as DNA sensor and antiviral restriction factor. In addition, IFI16 can be released in the extracellular space upon a variety of stimuli and found in the sera of patients with autoimmune diseases. IFI16 protein per se or upon binding to LPS from *E. coli* O111:B4 (EB-LPS) acts like DAMP (damage-associated molecular pattern) propagating danger signals and amplifying the secretion of IL-6 and IL-8.

**Methods:** GST-pull down assays and saturation binding experiments with recombinant IFI16 were used to characterize IFI16 binding activity to LPS of different origin, including high and weak TLR4 agonists. Then, the human monocytic cell line THP-1 and the renal carcinoma cell line 786-O were used to characterize the biological activity of IFI16 alone or pre-complexed with the different LPS variants. Finally, co-immunoprecipitation (co-IP) and proximity ligation (PLA) assays were used to investigate IFI16 direct interaction with TLR4.

**Results:** GST pull down assays and saturation binding experiments were used to demonstrate that the IFI16 HIN-B domain binds to the lipid A moiety of both high and weak TLR4 agonist LPS variants, regardless their number of acyl chains or phosphate groups. Interestingly, the binding also occurred with LPS from *P.gingivalis* (Pg-LPS) and *R.sphaeroides* (Rs-LPS), weak TLR4 agonists. Moreover, treatment of THP-1 cells or 786-O cells with IFI16 alone led to increased production of proinflammatory cytokines, which was further amplified upon treatment with pre-complexed LPS-IFI16. Finally, through co-IP experiments we showed that IFI16 interacts directly with TLR4. This was also corroborated by PLA experiments and by the observation that TLR4 silencing through specific siRNAs completely abolished the proinflammatory activity of LPS-IFI16 complexes in both THP-1 and 786-O cell lines.

**Conclusion:** Collectively, our data provide the first evidence that IFI16 is an alarmin that acts propagating inflammation. Moreover, its activity is strongly enhanced upon binding to the lipid A moiety of the complete LPS derived from *E. coli* O111:B4 and F583 strains, which are regarded as full TLR4 activators. Finally, the interaction with the weak TLR4 agonists, namely Pg-LPS, Rs-LPS and MPLA, and with TLR4 deserve further analysis, and might pave the way to new strategies targeting IFI16 in the course of systemic or organ-specific inflammatory diseases, such as autoimmunity, sepsis or LPS-driven acute kidney injury.

***P 144 – ID 086 - Beta-defensins and analoges in Helicobacter pylori infections: mRNA expression levels, DNA methylation and antibacterial activity.***

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**Introduction:** antimicrobial peptides can defend mucosal gastric from bacteria, but *Helicobacter pylori* can equally colonise the gastric apparatus. To know the function of beta-defensins are in *H. pylori*-associated chronic gastritis, we investigated susceptibility, mRNA expression levels of human beta-defensins and DNA methylation changes in their promoter in gastric mucosa with or without *H. pylori* infection.

**Materials and methods:** we evaluated, by bisulfite genomic sequencing, the methylation levels of promoter CGIs beta-defensin genes (HBD2-4) in gastric biopsy specimens in 15 controls and 10 patients colonised with *H. pylori*. We also tested, by Real-Time PCR, the pattern of induction of HBD2-4 by *H. pylori* and its susceptibility to the treatments with synthetic analogs of beta-defensins, by antimicrobial assay.

**Results:** our findings demonstrate that infection with *H. pylori* is related to a gastric enhancement of the inducible HBD2, whereas the levels of HBD3 and HBD4 inducible expression remained unchanged. The methylation levels of HBD2 gene was largely incremented in samples *H. pylori*-negative and relatively demethylated in *H. pylori*-positive samples.

Moreover, we assessed the antimicrobial susceptibility by growth on blood agar. *H. pylori* strain *Tox+* was susceptible to all defensins tested and their analogs (3N, 3NI).

**Discussion and Conclusions:** our study provides, for the first time, a comprehensive analysis for transcript and DNA methylation levels of one of the most prominent human beta-defensins in patients infected by *H. pylori* with a bacterial effect. A finer insight of the mechanisms regarding resistance and susceptibility of *H. pylori* against other antimicrobial peptides might favor the detection of possible targets for new eradication therapeutics.

## ***P 145 – ID 090 - The assessing of the infectious risk with disease-modifying therapies***

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**Introduction:** Ocrelizumab, recently approved for the treatment of primary progressive and relapsing multiple sclerosis (MS), targets B cells expressing the CD20 receptor and increases the risk of infections. The aim of the study: “take a picture” of the infectious status and define the infectious risk related to ocrelizumab treatment in MS patients.

**Materials and methods:** At the Neuroinfectious Unit of Policlinico Umberto I (Rome), an infectious screening (“Carta Infettivologica”) for MS patients candidate for ocrelizumab treatment was carried out. Two levels were established: the first one common for all patients, to highlight any positivity to HBV, HAV, HCV, HSV-1/2, VZV, EBV, rubella and measles viruses, CMV, toxoplasma, anti-JCV antibodies, TB, HIV and HPV; the second one, for those patients who resulted positive in the first-level surveys.

**Results:** Fifty-four MS patients (19 females, 37 males) with a median age [interquartile range (IQR)] of 52.5 (43.3-58), median years of disease (IQR) of 11(4.3-18.8) and median EDSS (IQR) of 5.5 (3.6-6), were enrolled. At baseline, the “Carta Infettivologica” showed 10 MS patients with antibodies against HBV surface and core antigens (HBsAb and HBcAb, respectively), while HBV surface and e antigens (HBsAg and HBeAg, respectively) and antibodies against HBV e antigen (HBeAb) were negative. For all 10 patients, pre-ocrelizumab HBV-DNA was undetectable (<10 IU/ml), liver enzymes were within normal ranges and ocrelizumab treatment was started. A pre-emptive approach for HBV reactivation was adopted with monthly assessment of liver enzymes and HBV-DNA. Among these HBsAb+, HBsAg- and HBcAb+ patients, one showed HBV reactivation after three months from ocrelizumab first administration. The patient was still asymptomatic, liver enzymes remained within normal ranges and prompt treatment with specific nucleoside analogues was started. After a month HBV-DNA was undetectable and no further positivity detection was found. In another HBsAb+, HBsAg- and HBcAb+ patient (asymptomatic and with liver enzymes within normal ranges) HBV-DNA passed from “undetectable” to “detectable <10 IU/ml”, after 13 months of ocrelizumab first administration.

**Discussion and conclusion:** During ocrelizumab treatment, HBV infection can reactivate. In HBsAb+, HBsAg- and HBcAb+ MS patients, a pre-emptive approach with HBV-DNA monthly assessment seems a valid and safe approach to promptly detect HBV reactivation. Prompt treatment with specific nucleoside analogues can contrast HBV reactivation and allows to continue ocrelizumab treatment. By the “Carta Infettivologica” the infectious risk can be reduced, caused by the acquisition of new pathogens or by the reactivation of those already latently present in the host.

**P 146 – ID 097 - Fatal attraction: *Acinetobacter baumannii* exploits carcinoembryonic antigen-related cell adhesion molecules (CEACAMs) for cellular adherence**

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**1. Introduction:** Bacterial cell adherence is an essential first step in establishing colonization and, eventually, infection. *Acinetobacter baumannii* is a life-threatening bacterium mainly responsible for ventilator-associated pneumonia. It adheres and invades host cells by the interaction between the bacterial phosphorilcholine-containing outer membrane protein and the host platelet-activating factor receptor. Receptor engagement triggers a cascade of pathways that leads to bacterial internalization. Interestingly, several respiratory human pathogens engage carcinoembryonic antigen-related cell adhesion molecules (CEACAMs) for host adhesion and invasion. CEACAMs are immunoglobulin-related glycoproteins that trigger cell–cell communication, inflammation and cancer progression. CEACAM1, CEACAM5, and CEACAM6 are found on various epithelial cell types, while others such as CEACAM8 are expressed sole on granulocytes. In this study, we have hypothesized that *A. baumannii* could exploit CEACAMs to increase adhesion to epithelia.

**2. Materials and methods:** Pull-down and fluorescence immunostaining approaches with specific antibodies as well as adhesion experiments using stably transfected lung epithelial A549 cells individually expressing CEACAM1, CEACAM5, CEACAM6 and CEACAM8 were performed

**3. Results:** We demonstrated that *A. baumannii* strain AB5075 associated with purified CEACAM1, CEACAM5 and CEACAM6 receptor proteins, but not with CEACAM8. Deletion of the N-terminal IgV-like domain of recombinant CEACAM1 protein abrogated the interaction, highlighting the importance of this domain for interaction. The adhesion rates of strain AB5075 were significantly increased in A549 cells individually expressing CEACAM1, CEACAM5 and CEACAM6 in comparison to A549 transfected with the empty vector or CEACAM8. Pull-down experiments performed with proteolytically digested *A. baumannii* cells abolished CEACAM specific interaction, indicating the involvement of bacterial proteinaceous element(s). Incubation of CEACAM transfected A549 cells with an inhibitor of N-glycosylation (i.e. tunicamycin) showed no decrease in the adhesion rates of strain AB5075, suggesting that bacterial-CEACAM-interaction does not relies on CEACAM major surface-associated saccharides.

**4. Conclusions:** Since increased epithelial cell adhesion creates the potential for invasion, binding to CEACAMs on the cell surface could exert a critical role in increasing the invasiveness of *A. baumannii*.

## ***P 147 – ID 099 - Anti-inflammatory strategies to tackle *Pseudomonas aeruginosa* lung infections: exploring the potential of the antimicrobial peptide lin-SB056-1 and its dendrimeric derivative***

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**Introduction:** Chronic lung infections sustained by *Pseudomonas aeruginosa* are commonly associated with a persistent inflammatory state, which represents the major process leading to substantial airway damage and reduced life expectancy. Antimicrobial peptides (AMPs) have been shown to profoundly modulate the host immune response, thus gaining increasing attention as novel therapeutic solutions to tackle uncontrolled inflammation during chronic infections. In the present study, we examined the anti-inflammatory potential of the semi-synthetic peptide lin-SB056-1 and its dendrimeric derivative (lin-SB056-1)<sub>2</sub>-K, which have previously demonstrated a significant antibacterial and antibiofilm activity in *in vivo*-like models of *P. aeruginosa* lung infections.

**Materials and methods:** Human lung epithelial cells (A549) were exposed to *P. aeruginosa* lipopolysaccharide (LPS; 800 ng/mL) to induce an inflammatory response. The anti-inflammatory activity of lin-SB056-1 and (lin-SB056-1)<sub>2</sub>-K was evaluated by monitoring the production of pro-inflammatory cytokines (IL-8 and IL-6) through a bead-based flow cytometric assay. Lactate dehydrogenase assay and propidium iodide exclusion assay was performed to determine peptide cytotoxicity. LPS-neutralizing activity of the peptides was analysed by the Limulus amoebocyte lysate (LAL) assay. *Galleria mellonella* was exploited to assess the *in vivo* protective effect of the peptides against *P. aeruginosa* infection.

**Results:** Exposure of LPS-stimulated A549 cells to non-toxic concentrations of both lin-SB056-1 and (lin-SB056-1)<sub>2</sub>-K resulted in a dose-dependent decrease in cytokine release. The dendrimeric derivative demonstrated a stronger anti-inflammatory activity than its monomeric counterpart, reducing the levels of IL-8 by more than 3 times at 9.6 µM. Interestingly, the enhanced anti-inflammatory effect of (lin-SB056-1)<sub>2</sub>-K correlated with its higher ability to neutralize endotoxin as compared to lin-SB056-1. In particular, it showed almost 90% LPS-binding ability at the concentration of 9.6 µM. Although the dendrimeric peptide exerted a considerable antimicrobial and anti-inflammatory activity *in vitro*, it resulted to be poorly effective in protecting *G. mellonella* larvae from a lethal challenge with *P. aeruginosa*.

**Discussion and conclusions:** In addition to the remarkable antibiofilm properties stated in previous studies, the anti-inflammatory potential of (lin-SB056-1)<sub>2</sub>-K might have a critical role in improving the clinical outcome of *P. aeruginosa* chronic lung infection. Despite these promising results, further studies aimed at enhancing peptide stability and bioavailability are warranted to solve the lack of protective effect observed in the *G. mellonella* systemic infection model.

## ***P 148 – ID 101 - Fecal metaproteomic analysis reveals unique changes of the gut microbiome functions after consumption of sourdough Carasau bread***

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**1. Introduction:** Sourdough-leavened bread (SB) is acknowledged for its great variety of valuable effects on consumer's metabolism and health, including a low glycemic index and a reduced content of the possible carcinogen acrylamide. Here, we aimed to investigate how these effects influence the gut microbiota composition and functions.

**2. Materials and Methods:** Therefore, we subjected rats to a diet supplemented with SB, baker's yeast leavened bread (BB), or unsupplemented diet (chow), and, after 4 weeks of treatment, their gut microbiota was analyzed using a metaproteogenomic approach.

**3. Results:** Diet supplementation with SB led to a reduction of specific members of the intestinal microbiota previously associated to low protein diets, namely *Alistipes* and *Mucispirillum*, or known as intestinal pathobionts, i.e. *Mycoplasma*. Concerning functions, asparaginases expressed by *Bacteroides* were observed as more abundant in SB-fed rats, leading to hypothesize that in their colonic microbiota the enzyme substrate, asparagine, was available in higher amounts than in BB- and chow-fed rats. Another group of protein families, expressed by *Clostridium*, was detected as more abundant in animal fed SB-supplemented diet. Of these, manganese catalase, small acid-soluble proteins (SASP), Ser/Thr kinase PrkA, and V-ATPase proteolipid subunit have been all reported to take part in *Clostridium* sporulation, strongly suggesting that the diet supplementation with SB might promote environmental conditions inducing metabolic dormancy of *Clostridium* spp. within the gut microbiota.

**4. Discussion and conclusions:** Our data provide a picture of the effects of SB consumption on the intestinal microbiota taxonomy and functions in rats. Moreover, our results suggest that a metaproteogenomic approach can provide evidence of the interplay between metabolites deriving from bread digestion and microbial metabolism.

**P 149 – ID 102 - Celiac disease-associated *Neisseria flavescens* induces metabolic imbalance which is restored by *Lactobacillus paracasei* CBA L74.**

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**Introduction.** We previously identified a *Neisseria flavescens* strain in the duodenum of celiac disease (CD) patients that induced immune inflammation in *ex vivo* duodenal mucosal explants and in CaCo-2 cells. We also found that vesicular trafficking was delayed after the CD-immunogenic P31-43 gliadin peptide-entered CaCo-2 cells and that *Lactobacillus paracasei* CBA L74 (*L. paracasei*-CBA) supernatant reduced the peptide entry. In this study, we evaluated if metabolism and trafficking was altered in CD *N. flavescens*-infected CaCo-2 cells and if any alteration could be mitigated by pretreating cells with *L. paracasei*-CBA supernatant, despite the presence of P31-43.

**Materials and Methods.** We measured, before and after exposure to various treatment, CaCo-2 bioenergetics by an extracellular flux analyser. We evaluated *N. flavescens* and P31-43 intracellular trafficking by immunofluorescence, and ATP content in CaCo-2 cells by bioluminescence. We also verified bacteria viability by evaluating viable bacteria counts in CaCo-2 *N. flavescens* infected-cells.

**Results.** We found that CD-*N. flavescens* colocalized more than control *N. flavescens* with early endocytic vesicles and more escaped autophagy thereby surviving longer in infected cells. P31-43 increased colocalization of *N. flavescens* with early vesicles. Mitochondrial respiration was lower ( $P < 0.05$ ) in CD-*N. flavescens*-infected cells versus not-treated CaCo-2 cells, whereas pretreatment with *L. paracasei*-CBA did not significantly affect the entry of bacterium into the cells but reduced CD-*N. flavescens* viability and improved cell bioenergetics and trafficking.

**Discussion and Conclusions.** In conclusion, CD-*N. flavescens* induces metabolic imbalance in CaCo-2 cells, and the pretreatment with *L. paracasei*-CBA probiotic supernatant could be used to restore CD-associated dysbiosis.

## ***P 150 – ID 116 - Virulence analyses of a serogroup C meningococcus and defective mutant in surface-exposed sialic acid in a murine model of meningitis***

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**Introduction:** In serogroup C *Neisseria meningitidis*, the *cssA* gene encodes for an UDP-N-acetylglucosamine 2-epimerase that catalyzes the conversion of UDP-N-acetyl-D-glucosamine into N-acetyl-D-mannosamine and UDP in the first step of sialic acid biosynthesis. This enzyme is required for the biosynthesis of the (alpha2-9)-linked poly-sialic acid capsule and for lipooligosaccharide sialylation. In this study, we have used a reference serogroup C meningococcal strain and an isogenic *cssA* knockout mutant to investigate the pathogenetic role of surface-exposed sialic acids in a model of meningitis based on intracisternal inoculation of adult mice.

**Materials and Methods:** Before the development of the *in vivo* model we proceeded with the construction of a serogroup C *cssA*-defective isogenic mutant and with its characterization under *in vitro* conditions. Afterwards to investigate the infectious dynamics of the meningococcal disease in the mouse model, survival and microbiological analyses of the brain and peripheral organs of infected mice were performed. Subsequently, to analyze the histopathological features of the disease, histological evaluation, cerebral bleeding analysis, and localization of bacteria in brain structures were carried out.

**Results:** The mutant exhibited a growth rate comparable to the wild type and a relative fitness of 108% in *in vitro* conditions. Our data confirmed the key role of surface-exposed sialic acids in meningococcal pathogenesis. The LD<sub>50</sub> of the wild-type strain 93/4286 was about four orders of magnitude lower than that of the *cssA* mutant. Compared to the wild-type strain, the ability of mutant to replicate in brain and spread systemically was severely impaired. Evaluation of brain damage evidenced a significant reduction in cerebral hemorrhages in mice infected with the mutant in comparison with the levels in those challenged with the wild-type strain. Histological analysis of mice infected with wild type strain showed the typical features of bacterial meningitis. Noticeably, 80% of mice infected with the wild-type strain presented massive bacterial localization and inflammatory infiltrate in the *corpus callosum*, indicating a specific involvement of the *corpus callosum* in the mouse model of meningococcal meningitis.

**Discussion and Conclusions:** In the present study, we first aimed at validating the meningococcal meningitis mouse model by using a reference serogroup C strain and its attenuated isogenic *cssA* mutant. Then, comparison of the virulence levels of the two strains was also instrumental to further explore the pathogenesis of meningococcal disease and subsequent cerebral damage by analyzing possible interactions between meningococcal surface-exposed sialic acids and brain structures.

***P 151 - ID 119 - "Friendly fire": Pseudomonas aeruginosa-responsive Th1/17 cells are strongly enriched in the lung of Cystic Fibrosis patients and can contribute to promoting tissue damage and decline of lung function***

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**Introduction:** Cystic Fibrosis (CF) is a genetic disease characterized by recurrent and chronic lung infection, mostly caused by *Pseudomonas aeruginosa*, that trigger an exaggerated activation of immune system. In CF, the chronic lung inflammation is characterized by strong activation of neutrophils and macrophages, which, however, fail to get rid of *P. aeruginosa* infections, ultimately leading to lung failure. Two pro-inflammatory cytokines (IL-17 and IFN- $\gamma$ ) seem to play a critical role in this circuit of inflammation and tissue damage: IL-17 further induces neutrophil recruitment and activation, while IFN- $\gamma$  is directly implicated in epithelial disruption. Since Th1/17 lymphocytes are the major producers of IFN- $\gamma$ /IL-17, it is likely that these cells play a crucial role in lung decline of CF patients. However, the role of Th1/17 cell subsets in CF pathogenesis has not yet been characterized.

**Materials and methods:** Lung biopsies from CF and non-CF patients were processed and the levels of Th1/17 cell subsets, in lung mucosa as well as in the intraepithelial compartments, were quantified by cytofluorimetric analysis on the base of different surface markers expression. In addition, Th1/17 cell subsets were isolated by FACS sorting from lung of CF patients and analyzed with the antigen-specificity assay to analyze their activation in response to *P. aeruginosa* strains.

**Results:** Our preliminary results show that two specific Th1/17 cell subsets (called Th1/17EM and Th1/17\*) were significantly increased in the lung mucosa of CF patients chronically infected with *P. aeruginosa* compared to non-CF patients, while only Th1/17\* cells are significantly higher in the intraepithelial compartment. Interestingly, functional immunoassays showed that the *P. aeruginosa* laboratory PAO1 strain only elicited a weak proliferation of two Th1/17 subsets, while a clinical *P. aeruginosa* strain- isolated after years of persistence within CF lungs- triggered a strong proliferation of the Th1/17EM subset.

**Discussion and conclusions:** Our results demonstrated significantly higher levels of two Th1/17 subsets in CF lungs and a strong activation of Th1/17 cells in response to *P. aeruginosa* antigens. Interestingly, clinical strains overly stimulate Th1/17 cells compared to a *P. aeruginosa* laboratory strain, suggesting that mutations have occurred in the bacterium during adaptation to the CF lung result in a higher activation of Th1/17 cells. Taken together, our results confirm that in CF Th1/17 cells probably exert a pathogenic role, and strongly suggest that this process is mediated by their proliferation and production of high levels of IFN- $\gamma$ /IL-17 in response to *P. aeruginosa* clinical strains, thus resulting in chronic inflammation and consequent lung tissue damage.

## **P 152 – ID 125 - Role of non-typeable *Haemophilus influenzae* in the pathogenesis of Multiple Sclerosis: a novel triggering mechanism**

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**Introduction.** Multiple sclerosis (MS) is an autoimmune disease of the central nervous system in which autoreactive immune cells recognizing myelin antigens lead to demyelination and axonal injury. Mechanisms inducing and controlling the pathogenesis of MS have not been fully elucidated. Epidemiologic data suggest that exposure to infectious agents may be associated with increased MS risk and progression may be linked to exogenous bacterially-derived antigenic molecules, mimicking mammalian cell surface glycoconjugates triggering autoimmune responses. In particular, non-typeable *Haemophilus influenzae* (NTHi) expresses a N-glycosylated adhesin HMW1A, that could stimulate autoreactive MS immune cells to trigger an antibody response through a molecular mimicry mechanism. Furthermore previously, HMW1A specific antibodies have been identified in sera from a subpopulation of MS patients and have shown a cross-reaction with myelin in spinal cord tissue in an experimental animal model of MS. To better understand the role of NTHi in the pathogenesis of MS, we have characterized the oropharyngeal and rinopharyngeal microbiota of MS patients compared to a control group by analyzing the distribution of *Hemophilus spp.* **Materials and Methods.** Oropharyngeal and rinopharyngeal swabs were spread on: Chocolate agar supplemented with bacitracin and Selective chocolate agar plates containing bacitracin, vancomycin and clindamycin. All the plates were incubated at 37°C in humidified atmosphere with 5%CO<sub>2</sub> for 24-48h. Microbial growth was recorded and each pure culture was obtained by serial passage on chocolate enriched agar. For each isolated strain confirmatory test for X e V factor requirements was performed. The identification of all bacterial isolates was performed by Mass Spectrometry using the Matrix Assisted Laser Desorption/Ionization (MALDI) mass spectrometer. MALDI-TOF was used to differentiate *H. influenzae* from other *Hemophilus spp.* NTHi was confirmed by X- and V-factor dependence and capsule absence by *H. influenzae* Antiserum Poly (contains Types a, b, c, d, e, f). **Results.** Microbiological analysis of oropharyngeal and nasopharyngeal swabs for the research of *Hemophilus spp.* carried out on MS patients (n=17) and control subjects (n=10) showed that NTHi was found in 3 MS patients of 17 patients analyzed, while it was isolated only in 1 of 10 control patients. **Discussion and Conclusions.** Our preliminary data, although conducted on a small cohort of patients, could establish a connection between NTHi infection and MS. We are currently increasing the microbiological samples and for the same cohort of patients we are performing the antibody titration against HMW1A in order to better understand and characterize the biological role of NTHi.



## ***P 153 – ID 126 - Different AIEC-virulence determinants are involved in intracellular persistence and promotion of chronic inflammation in Crohn's disease***

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**Introduction:** Adherent-invasive *Escherichia coli* (AIEC) strains are overrepresented in the dysbiotic microbiota of Crohn's disease (CD) patients, and contribute to the exaggerated activation of adaptive immune system, in particular pathogenic Th1/17 cells, typical of the disease. However, AIEC determinants directly involved in this process, as well as the molecular mechanisms that trigger differentiation and activation of Th1/17 cells have not yet been investigated.

**Materials and methods:** We have generated a transposon insertion library of AIEC mutant strains, and tested them for their ability to reduce or even inhibit the secretion of Th1/17 polarizing cytokines and chemokines in human dendritic cells (hDC) and intestinal epithelial cells (IEC) compared to the parental AIEC-LF82 strain. Mutant strains with a lacking capacity to induce Th1/17 polarizing cytokines were tested both at the phenotypic level, for the expression of the main virulence factors described in AIEC, and at the genotypic level to identify the putative pathogenic-antigens expressed in the replaced DNA sequence.

**Results:** Independent immune-functional assays on hDC and IEC suggest that this library contains several mutants with an impaired ability to induce secretion of Th-polarizing cytokines such as IL-23, IL-12 and IL-1beta from hDC, or chemokine such as IL-8 and CCL20 from IEC, compared to the parental AIEC-LF82 strain. Notably, intracellular survival of these mutants within hDC or IEC did not always overlap with cytokine secretion patterns, suggesting that interaction with epithelial cells and/or uptake by dendritic cells of AIEC, and induction of proinflammatory cytokines are not mutually dependent and rely upon different determinants. Consistent with these results, these AIEC mutants displayed very different properties in terms of biofilm formation and cellulose production, thin aggregative fimbriae (curli) production, and sensitivity to oxidative stress in an acid and nutrient-poor medium that partly mimic the phagocytic-vacuole content.

**Discussion and conclusions:** Creation of an AIEC insertion mutant library, followed by a screening based on immune-functional and phenotypic assays, has already allowed us to select for mutants affected in the interaction with hDC and IEC, and it will be instrumental to identify the molecular mechanism behind differentiation and activation of pathogenic Th1/17 cells by AIEC. Our preliminary results provide strong evidence that several and distinct AIEC determinants are involved in the interaction with epithelial cells, uptake by hDC and induction of proinflammatory cytokines, suggesting that targeting only AIEC-determinants involved in intracellular survival or replication within hDC or IEC is not enough to prevent chronic inflammation in CD.

## ***P 154 – ID 127 - Insect diet modulates gut microbiota, SCFAs production and satiety-related hormone in laying hens.***

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**Introduction:** Insects represent a potential nutritional sources both for humans and animals. *Hermetia illucens*, with good amount of chitin and proteins, could be a suitable diet replacement for laying hens. In this study we investigated the effect of *H. illucens* larvae meal administration on cecal microbiota, short chain fatty acids (SCFAs) production, feed intake and glucagon like peptide-1 (GLP-1) gene expression in laying hens.

**Materials and Methods:** Two groups of Lohmann Brown Classic laying hens were fed ad libitum with isoproteic and isoenergetic diets: a Soybean meal (SD) group was fed a corn-soybean-based diet, and a completely replaced by *H. illucens* larvae meal (ID) group. 16S rDNA sequencing of cecal samples was performed on MiSeq platform; sequencing data were analyze with QIIME in order to pick Operational taxonomic units (OTUs) and their taxonomic classification, to describe alpha and beta diversity and to compare groups across different taxonomic levels. PICRUST analysis was applied to investigate KEGG functions involved in bacterial chitin degradation and SCFAs production. SCFAs level were analyzed, from cecal digesta samples, by gas chromatography; feed intake was measured weighing the amount of feed distributed and that of residual and scattered feed and was expressed as individual feed intake by day (grams/day/hen); finally brain GLP-1 mRNA expression was evaluated by using RT-qPCR.

**Results:** Type and relative abundance of caecal microbial species showed strong differences between SD and ID groups. *Alkaliphilus transvaalensis*, *Christensenella minuta*, and *Flavonifractor plautii* represented the principal contributors (FDR p-values < 0.05). of this shift and have the potential to degrade the chitin's insect meal and correlated with the observed high levels of gut SCFAs, in particular butyrate and propionate, in ID group. This group showed also a statistically significant reduction of feed intake and an increased brain expression of GLP-1.

**Discussion and Conclusions:** *H. illucens* may be a new potential prebiotic by well feeding gut microbiota in laying hens. Particularly the prebiotic activities of *H. illucens* derived from bacterial fermentation of chitin that promoted an ingreasing of SCFAs production. This latter may directly influence brain neurochemistry by increasing satiety-related hormone, GLP-1 and, consequently, reducing the feed intake behaviour. Insect-based food for animals and humans need to be evaluate, considering the worldwide growing interest, both as an alternative nutritional source and for its potential contribution on modulation of microbiota–gut–brain axis signalling, currently considered a main focus in both the aetiology and treatment of several metabolic disorders.

## ***P 155 – ID 132 - Selected Immunological Mediators and Cervical Microbial Signatures in Women with Chlamydia trachomatis Infection.***

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**Introduction.** In the female genital ecosystem, the complex interplay between the host immune system and the resident microflora protects against urogenital pathogens, like *Chlamydia trachomatis*. *C. trachomatis* is responsible for urethritis, cervicitis and salpingitis; however, most chlamydial infections are asymptomatic and, thus, not treated, potentially leading to severe reproductive sequelae. Here we investigated the interaction between the levels of selected immune mediators and the community state types of the cervical microbiota in *C. trachomatis*-infected women.

**Materials and Methods.** Cervical samples from 42 *C. trachomatis*-positive women and 103 matched healthy controls were analyzed through the metagenomic analysis of the hypervariable region v4 of the 16S rRNA gene, via Illumina MiSeq sequencing. Sequence data were analyzed by the Shannon diversity index, the UniFrac analysis, the linear discriminant analysis with effect size measurement (LEfSe), the analysis of composition of microbiome (ANCOM) and the sparse correlations for compositional data (SparCC). The determination of lactoferrin, interleukin 1 $\alpha$  (IL-1 $\alpha$ ), IL-6, alpha interferon (IFN- $\alpha$ ), IFN- $\beta$ , and IFN- $\gamma$  was performed in the cervical fluid by ELISA.

**Results.** Overall, *C. trachomatis* infection was significantly associated with a microbiota dominated by anaerobic bacteria ( $P = 0.000002$ ). In addition, a network of *Gardnerella vaginalis*, *Prevotella amnii*, *Prevotella buccalis*, *Prevotella timonensis*, *Aerococcus christensenii*, and *Variovorax guangxiensis* has been identified as a potential biomarker of *C. trachomatis* infection through a combination of the above described statistical approaches. Again, chlamydial infection was significantly correlated with an increased production of lactoferrin, IL-6, IL-1 $\alpha$ , IFN- $\alpha$ , and IFN- $\beta$  ( $P < 0.05$ ), whereas very low levels of IFN- $\gamma$  were observed in *C. trachomatis*-infected women, levels similar to those detected in healthy women.

**Discussion and Conclusions.** Our findings show a distinctive signature of *C. trachomatis* genital infection, characterized by a specific bacterial network and by increased levels of lactoferrin and proinflammatory cytokines (IL-1 $\alpha$ , IL-6, IFN- $\alpha$ , and IFN- $\beta$ ), accompanied by low levels of IFN- $\gamma$ . This complex picture may have physiopathological relevance, since it might be responsible for the incomplete clearance of *C. trachomatis*, leading to a persistent infection and, hence, to the development of chronic reproductive sequelae.

## ***P 156 – ID 134 - Chlamydia trachomatis suppresses the innate immune response in primary human Sertoli cells***

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**Introduction:** *C. trachomatis*, leading cause of bacterial sexually transmitted diseases worldwide, is able to disseminate and localize to the upper genital tract impairing the reproductive function. Specifically, ascending *C. trachomatis* genital infection has been demonstrated to cause epididymitis or epididymo-orchitis, well-known risk factors for male infertility. As previously demonstrated, *C. trachomatis* possesses the ability to infect primary human Sertoli cells, key elements for the spermatogenetic process in the testis. Therefore, herein, we investigated, for the first time, the immune response elicited in these cells following *C. trachomatis* infection, by evaluating the activation of toll-like receptors and the related downstream intracellular signaling pathways as well as the levels of inflammatory mediators.

**Materials and Methods:** The expression of TLR-2, TLR-3 and TLR-4, as well as of the transcription factors nuclear factor kappa-light-chain-enhancer of activated B cells (NF- $\kappa$ B) and interferon regulatory factor (IRF) 3 were investigated in *C. trachomatis*-infected human Sertoli cells at 5, 24 and 36 hours post infection, by western blot assay. The levels of interleukin (IL)-1 $\alpha$ , IL-6, interferon (IFN)- $\alpha$ , IFN- $\beta$  and IFN- $\gamma$  were determined via ELISA assay.

**Results:** *C. trachomatis* induced TLR-3 expression in human Sertoli cells whereas both TLR-2 and TLR-4 were not expressed. Nonetheless, *C. trachomatis* was able to evade the innate immune response in human Sertoli cells by disrupting the TLR-3 dependent signaling pathways, as evidenced by the marked down-regulation of NF- $\kappa$ B, IRF3 and the downstream inflammatory mediators.

**Discussion and Conclusions:** *C. trachomatis* elicited TLR-3 expression, usually involved in anti-viral response, but halted, at the same time, the activation of downstream signaling pathways, allowing its survival within human Sertoli cells. Overall, our findings suggest that *C. trachomatis* can suppress the innate immune response in Sertoli cells and, hence, evade the intracellular killing, potentially giving rise to a long-term infection that may exert negative effect on the male reproductive system.

## ***P 157 - ID 145 - Porphyromonas gingivalis adhesion on titanium implants is affected by Chlorhexidine gel***

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**Introduction.** Dental implants are used by dentists for oral rehabilitation in partially and completely edentulous patients. The settling and keeping of healthy soft tissues around implant abutments are considered to be important for the long-term storage of the oral implants. Oral microbiota and their capability to adhere and form biofilm on implant surface can be considered fundamental triggers of peri-implant inflammations, bone and implant loss. *Porphyromonas gingivalis* is the "keystone pathogen" most frequently found in peri-implantitis sites and appears to be strongly associated with progression of disease. In this study, we analysed the efficacy of 0.2% Chlorhexidine (CHX) gel treatment, used during all clinical procedures, to reduce *P. gingivalis* adhesion on titanium implants together with the marginal-bone-loss (MBL) in respect to a placebo gel group in a human, randomized, double blind clinical trial study.

**Materials and Methods.** Thirty two patients (16 placebo group; 16 CHX group) were enrolled and received a single implant. During each surgical and prosthetic stage, a gel containing 0.2% CHX or a placebo gel was used. After 8 weeks, the healing abutment was placed, after 2 weeks the first screw was replaced with a new one and used for microbiology analysis. In order to quantify the load of adhering *P. gingivalis*, TaqMan-PCR was performed and the clinical data (MBL, prosthetic failure, peri-implant and gingival index) were recorded.

**Results.** The quantification of *P. gingivalis* with TaqMan PCR shows its presence in all titanium implants except for three samples belonging to the 0.2% CHX group. The concentration of *P. gingivalis* in placebo group ranged from  $1.5 \times 10^3$  CFU/ml to  $3.5 \times 10^5$  CFU/ml and for 0.2% CHX group the values ranged from 0 to  $6.3 \times 10^4$  CFU/ml. Overall, the differences in terms of CFU between two groups were statistically significant ( $P < 0.01$ ). All implants showed a very low plaque and bleeding scores, statistical analysis showed a significant difference in terms of MBL between two groups.

**Discussion and Conclusion.** Several studies demonstrated the efficacy of CHX in the reducing post-surgical bacterial load to limit peri-implantitis. However, its benefits are limited because of its short-term application. In this randomized double blinded human study, we used 0.2% CHX gel inside the implants and during all surgical and prosthetic phases. Our results demonstrated that the use of 0.2% CHX gel reduced significantly the adhesion of *P. gingivalis* on implants and it induced a reduction of inflammatory response in terms of marginal-bone-loss. A rigid disinfection protocol based on 0.2% CHX in all phases from implant insertion to crown delivery is strongly recommended to reduce bacterial load on and around implants and peri-implant marginal bone loss.

## P 158 – ID 147 - MRSA invasive isolates: does the genetic background interfere with the ability to invade and persist into human osteoblasts?

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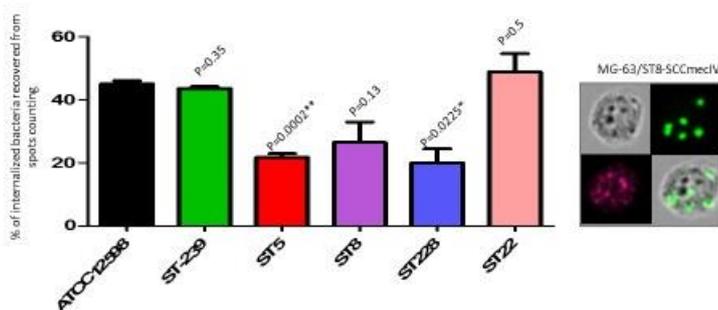
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**Introduction:** The host-pathogen interaction is dynamic and requires several changes to promote bacterial survival. All infection and post-invasion effects are dependent on the interplay between different factors. This study comparatively analyzed the interaction and persistence of different well-characterized *Staphylococcus aureus* clinical strains belonging to the main ST-MRSA-SCCmec clones within human MG-63 osteoblasts, and preliminarily the modulation of the expression of virulence factors.

**Materials/methods:** To overcome the limitations of the *ex-vivo* model, the internalization frequency was evaluated at a multiplicity of infection of 100, for 16 MRSA isolates and ATCC12598, by Flowcytometric assay (Amnis FlowSight® Imaging) after lysostaphin treatment and vancomycin-Bodipy staining, to determine the copy number and persistence of intracellular bacteria, 24h after infection. The graph shows the correlation between internalization and the statistical significance (GraphPad Prism,  $p < 0.05$ ). The MG-63 viability was evaluated by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium-bromide) reduction assay, at 2 and 24h post-infection. The *agr*, *hla*, *sigB*, *sarA* and *fnbA/B* expression levels were evaluated by RealTime-PCR (LC480 System, Roche), in intracellular condition model.

**Results:** The internalized bacteria were calculated counting the fluorescence spots inside MG-63 cells considering 10000 events. A statistical difference of internalization was found in ST5-SCCmecII and ST228-SCCmecI; ST239/241-SCCmecIII, ST8-SCCmecIV and ST22-SCCmecIVh showed the same ability to internalized of the ATCC12598. Cell viability during the infection period showed a growth slowdown in ST5-SCCmecII strains at 24h and in a ST8-SCCmecIV at 2h. Preliminary data expressed as the mean of CT values compared with *gmk* showed a relative increase in gene expression of *agrA* and *sigB*, compared with housekeeping gene (*gmk*), and a relative decrease of *fnbA/B*.

**Conclusions:** Using Flowcytometric assay, we obtained greater reproducibility rate of internalization and number/spots of intracellular bacteria, using live cells and lower time/cost consuming. The passage from the extracellular to the intracellular behavior showed changes in the fibronectin-binding protein expression, important for host/cell invasion but heavy for intracellular persistence. The combination of these techniques allowed us to understand how the interaction between host and clinical strain varies among the diverse genetic backgrounds.



## ***P 159 – ID 151 - Bartonella henselae invades human mesenchymal stromal cells inducing a neovascularization process***

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**Introduction.** *B. henselae* is the etiologic agent of Cat-scratch disease (CSD), a self-limiting chronic lymphadenopathy, in immunocompetent humans, mainly related to a cat scratch or bite. Instead, in immunocompromised patients *B. henselae* can cause bacillary peliosis hepatis angiomatosis. Endothelial cell (ECs) infection represents an important step in the pathogenesis of cat scratch disease and bacillary angiomatosis. In addition to ECs, bartonella also has a tropism for other cell types including cells of mononuclear phagocytes lineages. The cell types in which bartonella defend themselves from immune system have been considered as niches that could periodically seed bacteria into the bloodstream. This could explain the dissemination of *B. henselae* in the complication of CSD and the bacteraemic relapses that characterize the infection. However many aspects related to intracellular persistence of bartonella are still unclear and the presence of yet unrecognized niche has been hypothesized. Mesenchymal stem cells (MSCs) are multipotent adult stem cells that are present in multiple tissues, including bone marrow and adipose tissue. MSCs can differentiate into various cell lineages and have a proven ability to augment the neovascularization processes. The purpose of this study was to characterize the interaction of *B. henselae* with MSCs.

**Materials and methods.** Human adipocyte-derived mesenchymal stromal cells (Ad-MSCs) were stimulated with MOI 100 of *B. henselae* for 48-96 hr and 7 days. Different cytokines and chemokines upon *B. henselae* infection were measured by ELISA kits. The internalization of bacteria in Ad-MSCs was demonstrated by immunohistochemical and immunofluorescence analysis and by gentamicin protection assay. Cells were also evaluated by RT-PCR and FACS analysis for the expression of TLR-2/TLR-4 after *B. henselae* stimulation. Moreover, through preparation of conditioned medium from *B. henselae*-infected MSCs, we evaluated the capacity of MSCs to modulate angiogenesis.

**Results.** Infection of Ad-MSCs with *B. henselae* resulted in an increase of VEGF, CXCL8, IL-6, IP-10, CCL5 and PDGF-D secretion in a time-dependent manner. *B. henselae* can augment TLR-2 expression as demonstrated by RT-PCR and FACS analysis. Bacteria can enter the intracellular space of MSCs and can be detected as solitary bacteria in the perinuclear area and in clusters inside vacuolic compartments after 96 hr of infection. Moreover bacteria persist at 7 days post infection, as demonstrated by immunofluorescence. Finally the conditioned medium of infected MSCs can efficiently promote endothelial cells angiogenesis.

**Discussion and Conclusions.** We demonstrate that *B. henselae* invades and persist in MSCs. MSC infection triggers the production of pro-angiogenic factors that promote endothelial cells proliferation and capillary tube formation indicating that MSCs could represent a potential habitat of *B. henselae* and create a proangiogenic microenvironment typical of this bacterial infection.

## ***P 160 – ID 159 - Phagocytosis of *P. falciparum* gametocytes by macrophages***

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**Introduction:** Malaria is a parasitic vector-borne disease still causing millions of cases every year. The disease is transmitted by gametocytes (GCT), the sexual intraerythrocytic stage of the parasite, which develop in five stages (I-V). Stages I-IV are predominantly sequestered and differentiate in the extravascular compartment of the bone marrow, while stage V are released in the circulation from where they are ingested by a mosquito during a blood meal, causing malaria transmission. The role of the innate immunity against GCT is largely unknown. The aim of this work was: i) to set up a model to study the macrophages ability to phagocytose *P. falciparum* (*Pf*) GCT ii) to study macrophage activation by GCT.

**Materials And Methods:** GCT were obtained from the *Pf* transgenic strain 3D7elo1-pfs16-CBG99 expressing the luciferase CBG99 under the control of the GCT-specific promoter pfs16. Immortalized murine bone marrow derived macrophages (BMDM) or human monocytes (THP-1) differentiated to macrophages by phorbol esters (PMA) were incubated with immature (II-III) or mature (V) GCT for 2 hours in the presence or not of Cytochalasin D. Non-internalized parasites were removed by a lysis step. The cell permeable substrate luciferin was added and phagocytosis was measured by reading the luminescence using a Synergy4 (Biotek) reader. GCT phagocytosis was confirmed by Giemsa staining and confocal microscopy. The activation of macrophages was evaluated by measuring the production of nitric oxide (Griess assay).

**Results:** Both immature and mature GCT were phagocytized by BMDM. Upon pre-incubation with Cytochalasin D, an inhibitor of cell phagocytosis, the luminescent signal disappeared, indicating that the method is useful for quantifying phagocytosis of GCT. This was confirmed by Giemsa staining and confocal microscopy, that showed the presence of parasites inside macrophages. The method was then used to measure GCT phagocytosis by human PMA-differentiated THP-1. When nitric oxide was tested in the culture supernatants, it was found that only mature GCT but not immature ones induced macrophage activation.

**Discussion And Conclusions:** This method is suitable to measure phagocytosis of GCT by macrophages from different origin. An active interplay occurs between *Pf* GCT and macrophages suggesting an important, yet unexplored role of the innate immunity against the transmission stages of malaria.

## ***P 161 – ID 167 - Markers of B lymphocyte activation and their relationship to the infectious risk in multiple sclerosis patients treated with anti-CD20***

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**Introduction:** B cell activating factor (BAFF) and proliferation-inducing ligand (APRIL) are important cytokines involved in B cell survival and activation. Moreover, CD40L is a costimulatory molecule involved in B-cell activation, antibody production, and isotype class switching. Recently, ocrelizumab (an anti-CD20 monoclonal antibody) has been approved for the treatment of primary progressive and relapsing-remitting multiple sclerosis (MS). The aim of the study was to evaluate BAFF, APRIL and CD40L modifications during ocrelizumab treatment and their relation to the risk of infections.

**Materials and methods:** At the Neuroinfectious Unit of Policlinico Umberto I (Rome), 22 MS patients (6 females, 16 males) with a median age (interquartile range [IQR]) of 57 (51-60), median years of disease (IQR) of 9.5 (2.8-15) and median EDSS (IQR) of 6 (5-6.9) were enrolled. Plasma BAFF, APRIL and CD40L levels were longitudinally evaluated before, 6 and 12 months after ocrelizumab first administration (T0, T6 and T12, respectively).

**Results:** At T0, a positive correlation between plasma BAFF levels and EDSS value was identified ( $p=0.007$ ,  $r=0.55$ ). The longitudinal evaluation showed an increased in plasma BAFF levels ( $p=0.03$ ) and a trend toward the reduction in plasma APRIL and CD40L levels between T0 and T6 ( $p=0.06$ ). Given the close interconnection between BAFF and APRIL, the role of the BAFF/APRIL ratio was evaluated longitudinally, showing an increase from T0 to T6 ( $p=0.04$ ). Stratifying MS patients according to the occurrence of infectious complications, such as upper respiratory tract infections and urinary tract infections, during the first six months of treatment, at T0 the group with infectious complications showed higher BAFF values than the group without infections ( $p=0.04$ ). For a subgroup of nine MS patients, the longitudinal evaluation of plasma BAFF, APRIL and CD40L from T0 to T12, showed a reduction of plasma APRIL levels ( $p=0.03$ ) and a trend toward an increase of plasma BAFF and CD40L levels ( $p=0.06$  and  $p=0.009$ , respectively). The longitudinal evaluation of BAFF/APRIL ratio showed an increase from T0 to T12 ( $p=0.05$ ).

**Discussion and conclusions:** Although on a limited number of subjects, this study is the first to analyze the effect of ocrelizumab on BAFF, APRIL and CD40L plasma levels, and their relationship to the infectious risk. Noteworthy, the levels of BAFF were positively correlated with the degree of disability, and BAFF seemed to be a potential marker for disease monitoring and infectious complication risk stratification.

## ***P 162 - ID 168 - Innate immune response during Clostridium difficile infection: role of CD64 and CD11b as biomarkers of infection and treatment efficacy***

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**Introduction:** CD64, one of the Fc receptors for IgG, is constitutively present on macrophages, monocytes and eosinophils, and only to a small extent on resting neutrophils. However, neutrophil CD64 expression rapidly increases in the presence of microbial wall components. CD11b, a member of the beta-integrin family of adhesion proteins, is expressed at very low level on the surface of unstimulated neutrophils. While the expression of these biomarkers has been studied during neonatal sepsis, little is known regarding *Clostridium difficile* infection (CDI). The aim of the study was to investigate the dynamic changes of CD64 and CD11b expression before and after antibiotic treatment in patients with CDI.

**Material and methods:** Whole blood samples were collected before (T0) and at the end (T1) of CDI-specific antibiotic treatment in patients with CDI. Neutrophils were identified by electronic gating based forward and side scatter. Whole monocytes and their subsets were evaluated using the following monoclonal antibodies: HLA-DR, CD14 and CD16. The expression of CD64 and CD11b on peripheral blood neutrophils and monocytes was evaluated by flow cytometry.

**Results:** A total of 12 patients [median age 78 years (IQR 74.5-79)] with CDI were included in the study. Among them, 4/12 (30%) had a severe CDI. As for the 60-days outcome, 5/12 (41.6%) had a recurrence and 1/12 (8.3%) developed a nosocomial bloodstream infection. As a control group, 7 healthy donors (HD), matched for age and sex, were enrolled.

At T0, the neutrophils and intermediate monocyte (CD16+CD14+) percentages were significantly higher compared to HD ( $p=0.007$  and  $p=0.01$ , respectively). The CD64 median fluorescence intensity (MFI) on neutrophils and on total of monocytes was significantly higher compared to HD ( $p=0.04$  and  $p=0.004$ , respectively).

The longitudinal evaluation of CD64 and CD11b MFI showed a reduction on neutrophils and on monocytes, although not statistically significant. However, CD64 MFI results significantly reduced on intermediate monocyte (CD16+CD14+) ( $p=0.04$ ). Of note, at T1 values of CD64 on monocytes remained significantly higher than that observed in HD ( $p=0.002$ ).

Stratifying patients according to the development of recurrence or not (R+ and R- groups, respectively), the longitudinal evaluation showed a significantly reduction of CD64 and CD11b MFI on PMN and on monocytes in R- group whereas in R+ these biomarkers were persistently high ( $p=0.03$  and  $p=0.03$ , respectively).

**Discussion and conclusions:** CDI is associated with a high level of innate immune response which lowers at the end of CDI-specific therapy, however without reaching values comparable to HD. Overall, these findings highlight that the expression of CD64 on neutrophils and monocytes might be a specific indicator of bacterial infections while CD11b might represent a promising marker for the antibiotic treatment efficacy.

## ***P 163 – ID 169 - Leishmania infantum promastigotes stimulate CXCL8 production and neutrophils recruitment by endothelial cells***

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**Introduction:** Leishmaniasis is a protozoan disease caused by parasites belonging to *Leishmania* genus. More than 20 species of *Leishmania* cause symptomatic leishmaniasis, with disease severity ranging from cutaneous ulcerations to fatal visceral infections. *Leishmania* promastigotes are deposited into the human host's skin by the bite of infected phlebotomine sandflies and internalized by phagocytic cells where they develop into amastigotes and multiply. Neutrophils are the first cells recruited to the site of infection. They phagocytize *Leishmania* promastigotes, which are able to survive and infect other phagocytic cells. Endothelial cells are one of the first cells type encountered by *Leishmania* promastigotes. Their role in the establishment of the infection is unknown. Here, we investigate the *in vitro* effect of *Leishmania* promastigotes on the production of chemokines and neutrophil recruitment by endothelial cells.

**Materials And Methods:** Human microvascular endothelial cells (HMEC-1) were treated with *L. infantum* promastigotes at different cell/parasite ratio. Morphology of the parasites was observed by Giemsa stain and light microscopy. CXCL-8 production by HMEC-1 treated with *Leishmania* promastigotes was measured by ELISA. Conditioned medium from HMEC-1 cultured in the presence of *L. infantum* promastigotes was used as chemo-attractant in transwell systems. The number of migrated cells and the percentage of the different cells population were then determined.

**Results:** Morphological changes of promastigotes were detected after 4 and 24 hours of co-incubation with endothelial cells. Promastigotes of *L. infantum* stimulated the production of CXCL-8 by HMEC-1 in a dose and time-dependent manner. Conditioned medium from HMEC-1 stimulated by *L. infantum* promastigotes induced a 4-fold increase in cells migration respect to conditioned medium from control cells. This increase was comparable to that obtained using conditioned medium from HMEC-1 stimulated with TNF-alpha or LPS. After 2 and 24 hours of chemotaxis, more than 90% of migrated cells toward *L. infantum* conditioned medium were neutrophils.

**Discussion And Conclusions:** These data indicate that endothelial cells stimulated by *L. infantum* promastigotes produce CXCL-8, a chemokine involved in neutrophils recruitment, important for establishing the infection.

## ***P 164 - ID 172 - Bioactive liposomes as a novel host-directed therapeutic tool against opportunistic multidrug-resistant infections in Cystic Fibrosis patients***

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**Introduction.** Antimicrobial-resistant respiratory tract infections are increasing, presenting important management challenges worldwide, in particular in immunocompromised patients. Cystic Fibrosis (CF) is a genetic disease characterized by mutations at the Transmembrane Conductance Regulator channel (CFTR) gene and by the development of chronic infections by opportunistic pathogens, such as MDR *Pseudomonas aeruginosa* (MDR-PA) and *Mycobacterium abscessus* (MA). In this study, we have evaluated the efficacy of apoptotic body like liposomes loaded with phosphatidylinositol 5-phosphate (ABL/PI5P), a bioactive lipid involved in the activation of phagocytosis mechanisms, in *in vitro* and *in vivo* models of MA and MDR-PA infections.

**Materials and Methods.** Differentiated THP-1 cells (dTHP-1) and human primary macrophages, differentiated by peripheral monocytes coming from healthy donors (HD) or CF patients, infected or not with MDR-PA or MA, were *in vitro* stimulated with ABL/PI5P. ABL efficacy was evaluated in terms of bacterial phagocytosis and killing, phagosome maturation and cytokine production. Moreover, in several experiments, control macrophages were treated or not with INH172, used as a CFTR inhibitor, in order to get a CF phenotype. The efficacy of treatment with ABL/PI5P was also evaluated *in vivo* in a model of acute infection with MDR PA or chronic infection with MA, in terms of pulmonary leukocyte recruitment or pulmonary bacterial burden, respectively.

**Results.** Results concerning MDR-PA infection show that *in vitro* stimulation with ABL/PI5P of primary macrophages, with naturally mutated or pharmacologically inhibited CFTR and with impaired bactericidal activity, promotes bacterial internalization and phagosome acidification mediated- and ROS production- dependent intracellular killing. Moreover, the ABL/PI5P treatment induced a decrease in TNF-alpha, IL-1beta, and IL-6 and an increase of IL-10 production. The anti-inflammatory properties of ABL/PI5P were also confirmed *in vivo* in the murine model of acute MDR-PA infection. Results concerning MA infection show that ABL/PI5P promotes *in vitro* internalization and intracellular MA killing in human macrophages, which was associated with a significant ROS production and phagosome acidification. Finally, the intranasal treatment with ABL/PI5P in the murine model of chronic MA infection induces a mean reduction of about 50 fold of pulmonary mycobacterial burden.

**Discussion and Conclusions.** Altogether, these results show that ABL/PI5P treatment may represent a novel immunotherapeutic tool to be exploited to control opportunistic (myco)bacterial infections, that have acquired or are expected to acquire antibiotic resistance, and to reduce immunopathologic inflammatory reactions in CF patients.

## ***P 165 – ID 181 - Within-macrophages replication dynamics of Klebsiella pneumoniae***

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**Introduction:** *Klebsiella pneumoniae* (*Kp*) is a Gram-negative human pathogen. Hypervirulent lineages of *Kp* (predominantly capsule type K1/K2) are unique in their clinical presentation as they cause bacteraemic hepatic and splenic abscesses, occurring also in immunocompetent subjects. In addition, K2 ST25 strains are responsible for invasive disease in pigs. We have recently shown that intracellular replication of *Streptococcus pneumoniae* precedes the onset of bacteraemia. We aimed to test the hypothesis that *Kp* abscess formation starts after within macrophage replication.

**Materials and Methods:** In this work, eleven strains representative of both hypervirulent and multidrug resistant lineages were tested in an experimental infection in mice and a K2 ST25 strain in a porcine *ex vivo* spleen and liver perfusion model. Organs were analysed by CFU counting and confocal microscopy.

**Results:** Mucoïd, hypervirulent (K1 and K2) and MDR (KL17 and KL107) *Kp* were all detected in liver and spleens of mice. Bacteria localised in prevalently to CD169 positive macrophages, which in the case of the liver correspond to the Kupfer cells. Hypervirulent strains showed evidence for rapid replication in macrophages soon after challenge, while only single bacteria of the MDR lineages were detected intracellularly. In the porcine *ex vivo* spleen and liver perfusion system we could observe localisation to CD169+ macrophages in both organs with evidence also in this species for replication of the bacteria within macrophages.

**Discussion and Conclusions:** Our data indicate that the efficient replication of hypermucoïd isolates within CD169+ macrophages is the most likely mechanism for the origin of *K. pneumoniae* hepatic and splenic abscesses which characterise human disease.

***P 166 - ID 186 - Bovine lactoferrin pre-treatment induces intracellular killing of AIEC LF82 and reduces bacteria-induced DNA damage in differentiated human enterocytes***

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**Introduction:** AIEC reference strain LF82 is able to adhere to, invade and survival into human intestinal epithelial cells (IECs) without causing host cell death, usually leading to chronic infection states. LF82 induces also the production of pro-inflammatory cytokines, including IFN- $\gamma$  which, in turn, up-regulates CEACAM-6 receptor, the main surface molecule used by this bacterium to adhere to IECs. Moreover, LF82 is able to induce host DNA damage, impairing host defenses. Lactoferrin (Lf), a natural glycoprotein of innate immunity, is able to inhibit the bacterial adhesion/invasion to host cells, and it is able to enter into the cell nucleus where it binds to specific sequences of DNA thus regulating pro-inflammatory cytokine genes transcription. Here, the effect of Lf pre-treatment of Caco-2 cells on both LF82 invasiveness and survival as well as on cell DNA damage protection was tested.

**Materials and methods:** Lf was checked for purity, integrity, iron saturation rate, iron binding ability and lipopolysaccharide contamination. *E. coli* LF82, a prototype of AIEC strains isolated from a chronic ileal lesion of a Crohn's disease patient, and human epithelial colorectal adenocarcinoma cell-line (Caco-2) were used. Caco-2 cells, after 15 days of differentiation, were treated or not with IFN- $\gamma$  (50ng/ml) for 48h and/or with Lf (100 $\mu$ g/ml) for 12h before infection. After treatments, cells were infected immediately (T0) or after 3 (T3) or 10h (T10) with LF82 (MOI 1:10). The invasion and survival assays were carried out with gentamicin to kill extracellular bacteria. Synthesis of CEACAM-6 by Western blot as well as induction of apoptosis and Lf subcellular localization by immunofluorescence assay were carried out. Moreover, LF82-related DNA damage was determined by Comet assay and by the evaluation of phosphorylated ( $\gamma$ -H2A.X histone expression through Western blot.

**Results:** Our results showed that Lf pre-treatment is able to reduce the invasion and survival of LF82 in Caco-2 cells independently from its sub-cellular localization, induction of apoptosis and modulation of CEACAM-6 receptor expression. The genotoxicity induced by LF82, detected by Comet assay and histone  $\gamma$ -H2A.X expression, suggested that Lf protects Caco-2 cells from bacterial-induced DNA damage by triggering an intracellular pathway that remains active over time.

**Conclusion:** In conclusion, the data obtained in this work show that Lf is able to reduce the invasion and survival of AIEC LF82 in human intestinal epithelial cells, suggesting that the glycoprotein is able to trigger an intracellular pathway that remains active over time, as well as to counteract the bacterium-mediated genotoxicity thus efficiently protecting the host cells.

## ***P 167 - ID 191 - ZnO-loaded fiber meshes with anti-inflammatory and antibacterial properties for tissue regenerative applications***

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**Introduction:** New research fields are emerging based on the exploration of the nanoscale properties of fiber membranes to make them a real option for different tissue regenerative applications, like esophagus and lung. In this study, piezoelectric poly(vinylidene fluoride-co-trifluoroethylene) P(VDF-TrFE) fiber meshes containing Zinc Oxide (ZnO) nanoceramics were fabricated through electrospinning. P(VDF-TrFE) was chosen for its chemical resistance and piezoelectricity, and ZnO was chosen for its antibacterial properties. The fibers thus obtained were tested to evaluate their immunomodulatory and indirect antibacterial properties on pulmonary epithelial cells, and their ability to inhibit biofilms formation, adhesion and invasion of *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

**Methods:** ZnO nanopowder (20 w%) were sonicated in methyl ethyl ketone, then P(VDF-TrFE) was added and the final solution 20 (w/v%) and was electrospun into fibers using a collector velocity of 500 and 4000 rpm. Fiber morphology was analyzed via SEM. A549 cells were treated with fibers for 6 and 24 hours, then the expression levels of pro- and anti-inflammatory cytokines and antimicrobial peptide HBD-2 were evaluated using LightCycler software. For adhesiveness assays, semiconfluent monolayers were infected, in the presence or absence of fibers, with exponentially-growing *P. aeruginosa* or *S. aureus* at a multiplicity of infection of 100 bacteria/cell for 3h and (for invasiveness assay) further incubated for other 2 h in medium supplemented with gentamicin sulphate (250 µg ml<sup>-1</sup>). Total number (CFU/ml) of cell-associated or internalized bacteria was determined by lysing the monolayers with 0.1% Triton X-100 and by spreading serial dilutions on agar plates incubated at 37°C overnight. In Biofilm formation assay, the diluted bacterial suspension were placed into 96-well sterile polystyrene microplates in presence or absence of fibers and incubated overnight at 37 °C. The wells were then stained with 1% aqueous crystal violet solution and the OD<sub>570/655</sub> absorbance was measured on a microplate reader.

**Results:** SEM results demonstrated that ZnO nanoparticles distributed uniformly inside the fibers. The results obtained on A549 cells show that fibers possess relevant anti-inflammatory activity and were also able to stimulate a significant increase in the production of HBD-2; in addition, fibers have been shown to have significant anti-biofilm activity and to effectively inhibit the adhesion and invasion of both *P. aeruginosa* and *S. aureus* in pulmonary epithelial cells.

**Discussion and Conclusions:** the combination of antibacterial activity of ZnO, chemical inertia, and good mechanical properties, make these composite fiber meshes a good candidate for different applications.

## ***P 168 – ID 193 - Characterization of the B cell response upon vaccination with the chimeric H56 antigen of Mycobacterium tuberculosis***

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**Introduction:** Humoral immunity has been overlooked in the context of *Mycobacterium tuberculosis* vaccine design due to the perceived reduced relevance of antibody response for intracellular pathogen protection. On the contrary, emerging data have shown a role of antibodies in driving anti-microbial mechanisms, such as innate cellular cytotoxicity, in the context of *M. tuberculosis* infection. The generation of the B cell response upon vaccination is characterized by the induction of different functional and phenotypic subpopulations and is strongly dependent on the vaccine formulation, including the adjuvant used.

**Materials And Methods:** Here, we have assessed the humoral response and profiled the different B cell subsets elicited by vaccination of mice with the vaccine candidate H56, a chimeric tuberculosis vaccine antigen, combined with the liposome-based adjuvant CAF01. Immunizations were performed at weeks 0, 4 and 42 in order to investigate the persistence of the vaccine immune response. The induction of H56-specific IgG was assessed after primary and booster immunizations in sera at 2-5 weeks intervals, while the activation and differentiation of B cells was analysed in both draining lymph nodes and bone marrow upon booster immunization by multiparametric flow cytometry. The analysis of high-dimensional flow cytometric data was performed using a machine learning method based on clustering (FlowSOM) approach.

**Results:** A significant serum antibody response was elicited already after priming, and boosted by the secondary immunization, eliciting titers higher than 40000 that were persistently maintained for 9 months. A balanced production of IgG1, IgG2c and IgG2b subclasses was induced. Plasmablasts, plasma cells and germinal center B cells and many transient subsets, characterized on the basis of multiple markers coexpression, were detected into draining lymph nodes by automated flow cytometric analysis. Memory cells and long-lived plasmacells were also identified into the bone marrow of immunized mice, supporting the persistence of antibody titres.

**Discussion And Conclusion:** The H56 plus CAF01 vaccine formulation elicited a significant immune response against the tuberculosis vaccine antigen H56, that was characterized both in terms of antibodies and B cells. The computational analysis of our dataset allowed to identify different cellular phenotypes providing a signature of B cell recall response upon immunization with this vaccine formulation. The combined measure of humoral immunity and B cell phenotypes represents a powerful tool for the complex study of the B cell response upon vaccination.

## ***P 169 – ID 195 - Effects of a vegetal smallRNAs rich diet for gut microbiota modulation in pre-obese mice model***

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**Introduction:** The World Health Organization define obesity as a chronic and progressive disease, resulting from multiple environmental and genetic factors. It is associated with inflammation and alteration in microbiota composition and a fundamental role is played by food itself. Recently, the scientific community has focused on plant small RNA (p-sRNAs), in particular miRNAs, molecules able to modulate various biological processes by regulating human mRNAs expression (Cross-Kingdom interactions). The aim of the study was the evaluation of the effects of p-sRNAs introduced with the diet, on gut microbiome composition, in a murine model of obesity.

**Materials and Methods:** miRNAs in small RNA pool of *M. oleifera* seeds (*mol*-sR pool) were characterized by qRT-PCR. 12 C57BL/6J mice were randomly divided into 4 groups fed with a normal diet (ND), and a high fat diet (HFD) with/without *mol*-sR pool. After 5 weeks of treatment, weight and biochemical parameters were analysed. For microbiota analysis, 16S rDNA has been extracted, amplified, and deep sequenced from mice stools (Illumina MiSeq). High quality sequences were used to determine the diversity and richness of bacterial communities and the relative abundance based on operational taxonomic unit (OTU) using SortMeRNA and SUMACLUST. Kruskal- Wallis test was used to define significant differences in relative abundance among groups ( $p < 0.05$ ).

**Results:** In mice fed with HFD there is lipid accumulation both in adipose and liver's tissues respect to ND mice and HFD+*mol*-sR. Moreover, there is a loss of gut microbiota variability, typical phenomenon of dysbiosis (Shannon Index: 4.5 in HFD vs 4.8 in ND vs 5.0 in ND+*mol*-sR,  $p < 0.05$ ). It is appreciable an increase in Proteobacteria phylum in HFD respect to other groups (relative abundance, mean:  $1231 \pm 381$  in HFD,  $244 \pm 251$  in ND,  $414 \pm 262$  in HFD+*mol*-sR and  $523 \pm 310$  in ND+*mol*-sR,  $p < 0.05$ ) specifically of Enterobacteriaceae and Desulfovibrionaceae families, of genus *Parasutterella*, all related to states of inflammation. In HFD+*mol*-sR pool mice no lipid accumulation was observed in critical organs. Furthermore, Firmicutes/Bacteroidetes ratio is increased respect to HFD group. Likewise, there is a decrease in bacterial genera involved in intestinal inflammatory processes, such as *Akkermansia*, known for its role in inflammatory regulation (relative abundance, mean:  $547 \pm 148$  in HFD, 0 in HFD+*mol*-sR and  $0.13 \pm 0.35$  ND+*mol*-sR,  $p < 0.05$ ).

**Discussion and Conclusions:** This proof-of concept study shows that *mol*-sR pool could play a role in fat diet regimen decreasing fat accumulation and inflammation and inducing a balance of the main phyla involved in inflammation processes. The *mol*-sR pool is a biocomponent with beneficial properties making it a good candidate for obesity treatment.

## P 170 – ID 199 - HIV infection, plant small-RNAs and African traditional medicine

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**Introduction:** In Africa, up to 90% of the population depend on traditional medicine to help meet their health care needs. Traditional medicine is mainly chosen, because it is more affordable, more closely corresponds to the patient's cultural heritage, and allows greater public access to health information. In Africa, herbal medicines are used as primary treatment for HIV/AIDS and many studies have investigated the effects of natural products on HIV infection. Plants play an important role in providing indispensable micro-nutrients; among them, specific small RNAs are able to regulate human gene expression in a cross-kingdom manner. The aim of the study was to evaluate the effects of small RNAs from plants (p-sRNAs) on cell viability, immune response and HIV infection in PBMCs from HIV+ patients. **Material and Methods:** Thirty-five chronic HIV-infected patients were enrolled before antiretroviral treatment (cART-naïve) and their PBMCs have been transfected with a pool of p-sRNAs from *Moringa oleifera* (mol-sRs). This plant has been chosen, because is one of the most used in African traditional medicine. The presence of miRNAs belonging to 18 conserved families of plant miRNAs was detected at high concentration by qRT-PCR. Through bioinformatics analysis, several genes involved in cell cycle, apoptosis and immune response were identified as target of specific plant small RNAs. The effects of the p-sRNAs transfected into HIV-positive PBMCs were analysed by flow cytometry. **Results:** The p-sRNAs introduction determines a decrease of viability associated to an increase of apoptosis. An increase of T helper cells expressing Fas (CD4+CD95+) and a decrease of intracellular Bcl2 protein expression has been observed in PBMCs from HIV+ patients, while no effects are detected in PBMCs from Healthy donors. In CD4+ T cells the treatment significantly reduced cell activation (CD25+CD4+) and modified the T cell differentiation decreasing both central and effector memory cells (CCR7+CD45RA- and CCR7-CD45RA- respectively) and increasing effector memory RA cells (CCR7-CD45RA+). The p-sRNAs transfection induces a reduction of intracellular HIV p24 protein and of the viral DNA integration. All these effects are evident only in HIV+ patients who show CD4>200/mm<sup>3</sup> but not in patients with CD4 T cells <200/mm<sup>3</sup>, in which the transfection does not cause any significant change. Among the identified p-sRNAs, the miR858 targets VAV1, a protein involved in the regulation of HIV-pathogenesis. The transfection of HIV+ PBMCs with a synthetic plant miR858 determines a reduction of VAV1 protein expression and the decrease of HIV p24 protein expression. **Conclusions:** Altogether, these data may define a mechanism underlying the effectiveness of traditional medicine in HIV patients.

## **P 171 – ID 203 - Vaginal microbiota in HPV persistence: results of an observational, prospective study in a CIN2/CIN3 cohort of patients.**

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**Introduction.** The vaginal microbiota is recognized to have an important role in preventing or favoring vaginal diseases. Based on NGS-based studies, 5 main community-state-types (CSTs) can be observed, most of which are dominated by *Lactobacillus* spp., whereas the CST-4 is characterized by scarce *Lactobacilli* and increased diversity of anaerobic bacteria. Such condition is often associated with bacterial vaginosis, that increases host susceptibility to different kinds of disease, including HPV infection and consequent development of associated cancer, where the inflammatory environment generated by vaginal dysbiosis has a favoring role. However, the impact of vaginal microbiota on HPV persistence after tumor excision is not clear, thus our study was aimed to clarify this point in a cohort of patients with CIN-2/CIN-3 histological diagnosis, treated with surgical excision (LEEP).

**Materials and Methods.** Eighty patients were enrolled after clinical diagnosed and characterization for HPV presence. Clinical specimens included vaginal swabs and washings, collected before surgical treatment, and at 6-month follow-up. Swab samples were analyzed by real-time PCR (qPCR) microarray, allowing simultaneous identification and quantitation of 90 bacterial and fungal species of the vaginal microbiota. Vaginal washings were in parallel used for the evaluation of pro-inflammatory cytokines, by ELISA test.

**Results.** Compared with the healthy population (10%), in the enrolled CIN2/3 cohort a high prevalence of CST-4 group was observed (33%), which also correlated with high-grade lesions, as 75% of patients within this group were CIN3 patients. Interestingly, differences were observed in the microbiota of patients showing HPV clearance or persistence after surgery, being *L. crispatus* increased in the HPV-cleared but not in the HPV-recurrent patients (where instead *L. iners* was most represented, together with *A. vaginae* and *U. parvum*). At least five different pro-inflammatory cytokines were detected in patients at enrollment (IL-1alpha, IL-1beta, IL-6, IL-8, TNF-alpha), whereas they were profoundly decreased in the HPV-cleared group at follow up.

**Discussion and Conclusion.** Collected results suggest a predisposing role of CST-4 for HPV infection, as well as its association with malignant progression of cervical cancer and HPV persistence. Comparison between HPV cleared and persistent patients evidenced different type of vaginal microbiota, with negative or positive role in HPV persistence, also associated with different patterns of pro-inflammatory cytokine profile, highlighting the important role of vaginal microenvironment in tumor outcome.

**P 172 – ID 204 - Vitamin D and Palmitoylethanolamide modulate in synergic action the inflammatory response by *Chlamydia pneumoniae* infection in vitro**

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**Introduction.** *Chlamydia pneumoniae* (*Cp*), obligate intracellular bacterium, is a common respiratory pathogen with a tendency to chronicize. Recent data have suggested that has been associated with several disorders of the central nervous system (CNS). Microglial cells are the resident immune cells responsible for maintaining homeostasis in the CNS and when stimulated react and produce inflammatory mediators able to mediate multiple aspects of neuroinflammation. Vitamin D3 has been suggested to have an immunomodulatory effects and it was correlated with a decreased risk for developing some neurological disease. Palmitoylethanolamide (PEA) belongs to the class of long chain fatty acid ethanolamides that have been shown to have cytoprotective and anti-inflammatory activity. PEA is produced by neurons and glial cells in the CNS and is involved in the endogenous neuroprotective mechanisms that are activated following tissue damage or inflammation. The activation of microglia is part of an early defense mechanism following injury or disease. In pathological conditions, these cells assume an activated state characterized by morphological rearrangement, proliferation, chemotaxis towards the site of damage, and release of mediators. The majority of pathogens that reach the brain are physiologically eliminated or sequestered in a latent form by microglia. In the present study, we analyze effect of PEA and vitamin D on *Cp* infection of microglial cells.

**Materials and Methods.** We used a model of microglial cells (MG), isolated from the brains of CD-1 mice (P3-5) and infected with *Cp* at a MOI of 4 inclusion-forming units/cell. Microglial cells were infected with *Cp* to determine the influence of vitamin D and PEA, alone or in combination on *Cp* adhesion and on immunomodulatory response. In particular, we analyzed IL-8 and human- $\beta$ -defensin 3 (hBD-3) using Real time PCR and ELISA assay. For *Cp* adhesion, MG cells were infected for 1, 2 and 3h. Cell viability was evaluated by MTT.

**Results.** The results obtained demonstrate that infection with *Cp* is associated with increased levels of IL-8 and hBD-3. PEA (10 and 100nM) and vitamin D (10 and 20nM) used alone on our model showed a modulation of IL-8 and hBD-3. The combined use of PEA and vitamin D reduces the release of IL-8 cytokine and enhances the hBD-3 gene expression at both concentration tested.

**Conclusions.** Our results highlighted the ability of combined use of PEA and vitamin D to reinforce, synergistically, immunomodulatory response and pave the way for possible use in therapeutic applications in *Cp* chronic infection.

***P 173 – ID 218 - Identification of multifunctional CD4<sup>+</sup> T cells specific for the chimeric tuberculosis vaccine antigen H56, by combining MHC-II tetramer and intracellular cytokine staining technology***

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**Introduction.** Analysis of multifunctional CD4<sup>+</sup> T cells is fundamental for characterizing the immune responses to vaccination or infection. Peptide-MHC tetrameric complexes represent a powerful technology to detect antigen-specific T cells by the specific binding to their T cell receptor, and their combination with functional assays is fundamental for characterizing the antigen-specific immune response. Here we optimized a protocol for the detection of multiple intracellular cytokines within Mtb-specific CD4<sup>+</sup> T cells identified by the MCH class II tetramer technology.

**Material and Methods.** The detection of intracellular cytokine production within the activated epitope-specific CD4<sup>+</sup> T cells, was evaluated by different strategies that combined cellular restimulation (with the vaccine antigen or tetramers), tetramer staining (extracellular or intracellular) and intracellular cytokine labelling. The different procedures were tested in splenocytes from mice immunized with the chimeric tuberculosis vaccine antigen H56 mixed with the adjuvant CAF01.

**Results.** The protocol that better identified epitope-specific CD4<sup>+</sup> T cells and their effector function was based on the simultaneous intracellular staining with both MHC tetramers and cytokine-specific antibodies upon *in vitro* restimulation of cells with the H56 antigen.

**Discussion and Conclusions.** In this study we optimized a flow cytometric protocol for identifying at the single-cell level multifunctional epitope-specific CD4<sup>+</sup> T cells, elicited by immunization with the chimeric tuberculosis vaccine antigen H56. Demonstrating pros and cons of different protocols, we showed that the optimal procedure for the simultaneous detection of epitope-specific CD4<sup>+</sup> T cells and their effector function is based on the antigenic stimulation of cells combined with a single step of cytokine and tetramer staining in permeabilized cells. Analysis of multifunctional CD4<sup>+</sup> T cells is of critical importance for in depth characterization of cellular immune responses to tuberculosis vaccines. This protocol allows to better understand the complex functional profile of T cell responses upon vaccination or natural infection.

***P 174 – ID 230 - Study on the HPV-specific innate immune response in cervical cells of women, affected or not by bacterial vaginosis and other sexually transmitted infections.***

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**Introduction:** Human papilloma virus (HPV)-infected epithelial cells activate an Interferon (IFN) response following Toll-like receptors (TLR) signaling and produce chemokines to support chemotaxis of immune cells, thus promoting infection clearance. A healthy vaginal flora aids to calibrate the response to HPV whereas other sexually transmitted infections (STI) or bacterial vaginosis (BV) could deregulate it, thus contributing to HPV persistence. However, in this respect, the *in vivo* immune response to HPV was not extensively studied. We have previously reported that TLRs and type III Interferons (IFNs) were important determinants in the anti-HPV cervical innate immune response. The aim of this study is to appreciate whether patients' cervical cells may express different levels of innate immunity genes in relation to the HPV status in the presence or absence of other STI and BV.

**Material and Methods:** From women, 16-40 years old, attending gynecological clinics of Sapienza University Hospital, Rome, tested for HPV DNA, STI and BV during routine visits, residual cervical samples were tested in this study. The mRNA copy content of the TLRs 9 and 7, the IFN Lambda receptor chain, IFNLR1, the type III IFN L1-3, the IFN stimulated genes (ISG) ISG15 and UBP43, and of the chemokines CCL20 and CCL5 was measured by real-time RT-PCR and expressed relatively to a cellular invariant gene.

### **Results**

HPV DNA was detected in about 50% of the women; STI and BV rates were high and did not differ between HPV-positive and HPV-negative samples. Most women had a negative Pap test result. Despite a high inter-patients' variability, levels of expression of TLRs, and of the type III IFN genes were positively correlated to each other suggesting a biological relevance of these measurements. TLR7, IFNL2, and CCL20 expression in women positive for HPV were significantly higher with respect to the HPV negative. Differently, the expression of IFNL1 and TLR7, but not of CCL20, was higher in women positive to either BV or STI respect to the negative.

### **Discussion and Conclusions**

These preliminary data indicate that HPV-infected women, differentially expressed type III IFN subtypes and the CCL20 chemokine, with respect to women with other cervicovaginal infections. In particular, CCL20 has been shown to recruit Langerhans cells that may drive cell-mediated immune response but may also promote stromal inflammation. Thus, we plan to further investigate these women in follow-up visits to monitor whether HPV clearance is driven by CCL20 activation and influenced by the presence or not of bacterial co-infections.

## ***P 175 – ID 231 - Role of IL-18 in the etiopathogenesis of infections caused by Streptococcus agalactiae***

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**Introduction.** *Streptococcus agalactiae* or Group B *Streptococcus* (GBS), is a gram-positive pathogen that is the most common cause of life-threatening infections in newborns and pregnant women. Bacteria recognition is achieved by membrane-bound (such as TLRs) and cytosolic PRRs that signal through adaptor molecules to activate kinases which, in turns, activate IFN regulatory factors and NF- $\kappa$ B transcription factors. Inflammasomes are multiprotein complexes whose assembly cleave pro-caspase-1 into its mature form caspase-1, which promotes the maturation of proinflammatory cytokines IL-1 $\beta$  and IL-18. Since few studies have been conducted to investigate the role played by these cytokines during experimental GBS infections, in this work we have studied IL-18 involvement in the etiopathogenesis of streptococcal infections.

**Materials and Methods.** To examine the role played by IL-18, we have applied a systemic model of experimental GBS infection in adult and newborn mice. To this end, we employed IL-18 genetically defective mice and C57BL/6 wild-type (WT) as controls. The adult and neonatal mice were infected, respectively, intraperitoneally and subcutaneously with a sublethal dose of GBS COH-1 strain and lethality was monitored for different times. Furthermore, blood was taken to measure cytokine and CFU levels. To analyze the mechanisms underlying the increased susceptibility of IL-18 KO mice, we took the cells contained in the peritoneal lavage at different times after the ip infection with GBS.

**Results.** Adult and neonates IL-18 KO mice showed a most elevated susceptibility to GBS infection accompanied from CFU elevated number in the blood and kidneys at 24 and 48 h following COH-1 infection. To determine whether the increased mortality of IL-18 KO mice could be accounted for by alterations in the TNF-alpha and IFN-gamma production, two cytokines that are known to be fundamental in improving anti-GBS defenses, we measured at 6h and 18 h after i.v. inoculation with live GBS the levels of both cytokines in the kidney homogenates of WT and IL-18 KO mice. IFN-gamma concentrations were significantly lower in IL-18<sup>-/-</sup> mice than in WT, while TNF-alpha levels were similar in both groups. Ex vivo experiments indicated that at 3h and 6h after i.p. GBS inoculation was evident a significant increase in neutrophils numbers in IL-18 KO mice peritoneal cavity.

**Discussion and Conclusion.** Results showed that IL-18 KO mice were extremely susceptible to GBS infection and displayed an impaired ability to control bacterial growth. Furthermore, our data indicate that the protective role of endogenous IL-18 to GBS are mediated by IFN-gamma-, but not TNF-alpha-, dependent mechanisms. Finally, this study shows the IL-18 involvement in promoting neutrophil influx in PFL after GBS inoculation.

## ***P 176 – ID 235 - Cancer cells expressing HERVs and embryonic factors as target of antiretroviral drugs in the cancer therapy***

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**Introduction:** Human endogenous retroviruses (HERVs) are retrotransposable elements originated from retroviral infections of the germ cell line million years ago. One of the main features of HERVs is their responsiveness to factors present in the cell microenvironment, such as nutrients, hormones and cytokines, leading to their transcriptional reactivation. An altered expression of HERVs have been associated to the onset, progression and acquisition of aggressiveness features in tumour cells. Cancer stem cells (CSCs) are essential for tumor growth and spread and have been characterized by the presence of different markers, among which the surface marker CD133 and the embryonic factors OCT4, NANOG and SOX2. We previously demonstrated that, in microenvironmental stress conditions, HERV-K activation is required to maintain human melanoma cell plasticity and stemness features. Herein, we widen the assessment of the response to microenvironmental changes of cell lines from liver and lung cancer in relation to HERVs expression. Furthermore, with the aim to identify novel strategy to target aggressive cancer cells, we analysed the response to antiretroviral drugs treatment *in vitro*. **Materials and methods:** TVM-A12, HepG2 and A549 cell lines were cultured in standard or stem cell media. For treatment were used antiretroviral drugs zidovudine and efavirenz. The surface expression of CD133 and the apoptosis analysis were assessed by flow cytometry. The relative expression of HERV-K (HML-2), HERV-H, CD133 and embryonic genes (OCT4, NANOG, SOX2) were evaluated by RT-Real time PCR. Clonogenic activity and cell growth analysis was performed. **Results:** depending on microenvironmental changes, cell lines showed modifications in cellular morphology with the generation of sphere-like aggregates, associated to increase of HERV-K, HERV-H, CD133 and embryonic genes transcriptional activity. Treatment with antiretroviral drugs affected HERVs transcriptional activity and induced decrease of CD133 and embryonic factors gene expression, clonogenic activity and cell growth inhibition, accompanied by apoptosis induction. **Discussion and conclusion:** the HERVs could represent a linkage between microenvironmental changes and cancer cells phenotype switching, contributing to the acquisition of stemness features. Together with the use of standard cancer treatments, including chemotherapy, radiotherapy and surgery, new combination approaches are needed. In this view, we consider HERVs as target of the antiretroviral drugs as an alternative approach to improve response to therapy.

## ***P 177 – ID 245 - NF-κB-dependent regulation of apoptosis during HSV-1 infection of monocytic cells: possible role of miRNA.***

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**Introduction.** We have demonstrated that NF-κB activation in monocytic cells infected by HSV-1 limits the damage of an excessive apoptotic response to virus infection. However, molecular mechanisms involved in this phenomenon have not been elucidated. MicroRNAs (miRNAs) play an important role in the modulation of several, key cellular processes, including apoptosis regulatory pathways. Therefore, in this study, in order to get information on NF-κB-dependent restriction of virus-induced apoptosis, we aimed to identify differentially expressed miRNAs in U937 cells with functional NF-κB (U937-pcDNA) and in U937 cells in which NF-κB activation was inhibited by stably expressing a DN form of IκBa (U937-IκBa-DN), infected or mock-infected with HSV-1.

**Materials and methods.** Three independent samples of HSV-1-infected or mock-infected cells were collected from U937-pcDNA and U937-IκBa-DN cells, respectively. To determine the miRNA expression profiles of these two cell lines, the miRNA microarray platform, miRCURY LNA™ microRNA array, was used. Differentially expressed miRNAs were then analysed by DIANA-miRPath v3.0 software. Experimentally supported microRNA targets were selected by TarBase v6.0. Validated target genes were chosen taking into consideration those that could be involved in common, apoptosis regulating, pathways.

**Results.** Analysis of the raw data from differently expressed miRNA by database led to the identification of three predicted target genes that are primarily involved in different steps of the same apoptosis regulatory pathway, i.e. the PI3K/AKT/mTOR intracellular signalling pathway. The three miRNAs that were differentially expressed between U937-pcDNA and U937-IκBa-DN were has-miR-99a, has-miR-124-3p, and has-miR-494, that target mTOR, AKT, and PTEN genes, respectively.

**Discussion and conclusions.** This study refers to the first systematic analysis of the differences of miRNAs expression between monocytic cells with intact NF-κB activation and the same cells with abrogated NF-κB activation, following HSV-1 infection. In particular, in this study we focused our attention on miRNAs that could be possibly involved in the dramatically different apoptotic response to HSV-1 infection in monocytic cells, depending on their capacity or not to activate NF-κB. Target gene predictions indicated that differentially expressed miRNAs could play an important role in the apoptotic response to infection by affecting the PI3K/AKT/mTOR intracellular signalling pathway. However, further study on these miRNAs and their functions in the apoptotic response to HSV-1 infection is necessary. This finding may contribute to clarify mechanisms controlling HSV-1 infection in monocytic cells.

## ***P 178 – ID 255 - Long term immunogenicity and protection of a GMMA-based vaccine against Salmonella enterica serovar Typhimurium***

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**Introduction.** *Salmonella enterica* serovar Typhimurium (STm) represents the most prevalent cause of invasive nontyphoidal *Salmonellosis* (iNTS). Vaccination against iNTS has the potential for a major global health impact, but currently no licensed vaccine is available. In ongoing efforts to identify protective antigens, the O-antigen portion of STm (O:4,5) LPS has been recognized as an important vaccine target. Genetically modified STm exosomes, with GMMA (Generalized Modules for Membrane Antigens) technology, are being developed as one component of an iNTS vaccine. In this work, using a murine model, we characterized the long term immunogenicity and protection from challenge conferred by a GMMA-based vaccine against STm.

**Materials and Methods.** C57BL/6 mice were immunized subcutaneously at weeks 0 and 10 with Alhydrogel formulated STmGMMA as delivery system for O:4,5. Systemic and mucosal O:4,5-specific antibody response induced by STmGMMA/Alhydrogel vaccine were monitored for 38 weeks in serum and fecal samples by ELISA. The vaccine induced B cell response was analysed at sacrifice by multiparametric flow cytometric assay. Groups of immunized animals were also challenged intravenously (IV) with virulent STm and bacterial load in blood, spleen and liver were evaluated as indication of protection.

**Results.** A single immunization with STmGMMA/Alhydrogel elicited O:4,5-specific serum IgG and IgM antibodies. The homologous boosting after 10 weeks induced a significant increase of antibody titers that were maintained for 38 weeks after immunization. Analysis of mucosal antibodies in fecal samples showed significantly higher O:4,5-specific IgG after STmGMMA/Alhydrogel boosting compared to control group, and levels persisted up to 38 weeks. B cell differentiation analysis showed the induction of B memory subpopulations following immunization with STmGMMA. Interestingly, the vaccine induced a significant decrease of bacterial load in blood, spleen and liver compared to unvaccinated mice upon STm challenge.

**Discussion and Conclusions.** These results demonstrate that immunization with a promising iNTS vaccine candidate induced long term persistence of O:4,5-specific IgG and IgM in serum, and O:4,5-specific IgG in fecal samples. Antibody persistence was associated with the presence of memory B cells. Additionally, STmGMMA/Alhydrogel reduced bacterial load following STm challenge, indicative of vaccine induced protection. These data support the development of a GMMA-based iNTS vaccine.

## ***P 179 – Id 257 - What Is The Correlation Of P53 Mutation, Dna Methylation And Microsatellite Instability In Patients With Helicobacter Pylori And Epstein Barr Virus Coinfection?***

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**Introduction:** *Helicobacter pylori* and Epstein-Barr virus (EBV) infection has recently been shown to be associated with gastric diseases. The aim of this study was to evaluate the relationship between *H. pylori* and EBV infection correlated to p53 mutations, DNA methylation and microsatellite instability. The investigation was considered interesting because Sicily, for geographic localization constitute one of the most complex mixtures of different ethnic elements in Europe.

**Methods:** Gastric biopsy samples of 100 patients with different gastric diseases were used. *H. pylori* and EBV infection was diagnosed by the detection of the *ureaseA* and BAMHI-W respectively. The virulence factors and resistance to clarithromycin of *H. pylori* were detected by polymerase chain reaction. Microsatellite instability (MSI) analysis was performed using the BAT26 primer set. Single-stranded conformational polymorphism (SSCP) was used for p53 mutation.

**Materials and Methods:** Our result showed that the *H. pylori* and EBV infection is present respectively in 44% and in 37% of the population analyzed while coinfection was found in 26% of patients.

About the analysis of different virulence factors of *H. pylori*, a close correlation was found between the various of the *oipA*, *vacA* and *cagA* genes.

COX2 gene was more frequently methylated in infected patients in contrast to non-infected patients, while microsatellite stability was more frequent in non-infected patients in contrast to infected patients. Microsatellite instability was more frequent in infected patients in contrast to non-infected patients.

Finally p53 mutation was observed in 26% of the cases, particularly no mutation was observed in patients with normal gastric mucosa, and our results showed that the exon 8 was mutated more frequently in patients with infection (*H. pylori* only, EBV only or both) in comparison to patients without infection, In addition the exon 8 was more frequently mutated in patients with co-infected.

**Discussion and Conclusions:** According to our results also in Sicilian population the gastric disease has different pathways.

## ***P 180 – ID 263 - Murine model of infection with Salmonella enterica serovar Typhimurium***

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**Introduction.** Invasive Nontyphoidal *Salmonella* (iNTS) infection is a major cause of death in sub-Saharan Africa, but currently no licensed vaccine against iNTS is available. Improved animal models that closely mimic natural infection are needed to study *in vivo* iNTS pathogenesis as well as host responses and vaccine induced protection. In the present study, a murine model of *Salmonella enterica* serovar Typhimurium infection was set up using intravenous (IV) and intragastric (IG) inocula, and animal survival, bacteremia, leukocyte presence and cytokine/chemokine levels in blood were investigated.

**Materials and Methods.** C57BL/6 mice were infected with different doses of *Salmonella* Typhimurium by the IV and IG routes. Survival of mice was monitored up to 20 days post infection, with a daily recording of clinical score and body weight. Bacterial counts in blood, liver and spleen were observed at different time points after IV infection and also in mesenteric lymph nodes and Peyer's Patches after IG infection. Leukocyte presence was assessed in blood by multiparametric flow cytometry, and cytokines/chemokines levels in serum were evaluated using Luminex immunoassay.

**Results.** For IV infection, the doses of  $10^3$ ,  $10^4$  and  $10^5$  colony forming units (CFU)/mouse were tested. The dose of  $10^4$  CFU, with survival rates of about 65%, 20%, and 0% three, four and five days post infection respectively, was selected as optimal infection dose. Bacterial loads one day post infection were about  $5 \times 10^3$  CFU/ml of blood,  $5 \times 10^5$  CFU/spleen and  $10^6$  CFU/liver and counts increased 3 days post infection. The analysis of leukocytes in blood indicated a significant dose-dependent presence of neutrophils. An increase of IFN-gamma and cytokines involved in granulocyte and monocyte recruitment, including G-CSF and MCP-1, was also observed. For IG infection, the doses of  $10^6$ ,  $10^7$  and  $10^8$  CFU/mouse were used. The dose of  $10^7$  CFU, with survival rates of about 65% and 30% six and eight days post infection respectively, that decreased up to day 15, was selected for further experiments. Bacterial dissemination in blood, spleen, liver, mesenteric lymph nodes and Peyer's Patches was evaluated.

**Discussion and Conclusions.** In the present work, we have set up a murine model of *Salmonella enterica* serovar Typhimurium infection by IG inoculum, that mimics the natural oro-fecal route of infection, and by the IV route that provides a specific window on the systemic infection. Bacterial dissemination, disease progression and the cellular immune response were characterised. Taken together, these data provide an important model to study host/pathogen interaction and protection conferred by *Salmonella* Typhimurium vaccines.

***P 181 – ID 270 - Computational analysis of multiparametric flow cytometric data to dissect the B cell response upon immunization with a Mycobacterium tuberculosis chimeric antigen***

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**Introduction:** Flow cytometry is a powerful technology widely used for studying the characteristics of cells upon interaction with antigens, such as vaccine antigens or microbial components. The progress of the technology has led to the development of instruments capable of measuring up to 50 parameters on large number of cells, promoting the necessity of developing advanced mathematical approaches for their analysis, capable of clustering cells on the base of their simultaneous marker expression in an unbiased way. Here, we have profiled the different B cell subsets elicited upon vaccination with a tuberculosis vaccine formulation, using two approaches to explore and visualize such multidimensional data, that are based on dimensionality reduction and unsupervised clustering.

**Materials and Methods:** The B cell response elicited by the vaccine candidate H56, a chimeric tuberculosis vaccine antigen, combined with the liposome system CAF01 was characterized using two automated methods based on clustering (FlowSOM) and dimensional reduction (t-SNE) approaches on R, a language and environment for statistical computing. The B cell signature identified with the adjuvanted-vaccine formulation was compared with the one induced by immunization with the H56 antigen alone.

**Results:** The clustering method identified the induction of different B cell populations, that included plasmablasts, plasma cells and germinal center B cells and their subsets characterized on the basis of multiple markers coexpression, while this profiling was more difficult with t-SNE. When undefined phenotypes were detected, their characterization could be improved by integrating the t-SNE spatial visualization of cells with the FlowSOM clusters. The frequency of some cellular subsets was significantly higher in lymph nodes of mice primed with the adjuvanted-formulation compared to antigen alone.

**Discussion And Conclusion:** Thanks to this automatic data analysis it was possible to identify, in an unbiased way, different B cell populations and also intermediate stages of cell differentiation elicited by immunization, thus providing a signature of B cell recall response that can be hardly obtained with the classical bi-dimensional gating analysis.

## ***P 182 – ID 271 - Role of host's immune response in infections caused by Streptococcus pneumoniae***

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**Introduction.** *S. pneumoniae* is a gram-positive bacterium which represent the most common cause of community-acquired bacterial pneumonia. Pneumococcus can cause lethal diseases in adult and young human and vaccines currently on the market do not give protection against all existing serotypes and so there is a need for alternative immune-based strategies. The fundamental role played by the host's immune system towards this microorganism is now known. In particular, previous studies has shown the role exercised by endosomal TLRs in the field of these infections. Infact in these studies, 3D mice, lacking a protein "chaperone" called UNC93B1 that regulate the "trafficking" of endosomal TLRs from endoplasmic reticulum to endosome, were particularly susceptible to pneumococcal infection. In this work we have studied host's immune sistem response in the experimental pneumococcal infection.

**Materials and Methods.** We set up a intraperitoneal (ip) experimental murine model of pneumococcal infection. To this end, we used genetically defective mice for 3D and C57BL/6 wild-type (WT) as controls. The mice were infected ip with  $2 \times 10^7$  CFU of *S. pneumoniae* D39 strain and at 3 hours after infection peritoneal liquid was harvested to analyze cell population and cytokine. Furthermore, as for *in vitro* experiments, we isolated neutrophil and bone marrow cells from WT and 3D mice, which were then differentiated *in vitro*, respectively, in dendritic and macrophage cells. These cells were infected with different infection multiplicities of D39 and supernatants tested for cytokines.

**Results.** In the peritoneal fluid of 3D mice, cytofluorimetric analysis showed a significant increase in the neutrophilic cell population. Furthermore, the levels of MIP-2 and KC were significantly reduced in mice lacking endosomal receptors while a production similar to the control mice was found for TNF-alpha and interleukin beta. In "*in vitro*" studies, neutrophil and differentiated macrophages and dendritic cells produced significantly smaller amounts of MIP-2 and KC in 3D than the C57BL/6J.

**Discussion and Conclusion.** Results showed that endosomal TLR absence caused a neutrophil recruitment in the cavity of mice infected with *S. pneumoniae* and a minor MIP-2 and KC production suggesting that signal transduction cascade from endosomal TLR activation is essential for the transcriptional activation of CXCL-1 and CXCL-2 genes. Furthermore, similar data were obtained *in vitro* experiments that used neutrophils and bone marrow cells from WT and 3D mice.

## ***P 183 – ID 289 - Blood biomarkers of pneumococcal infection of the murine lower respiratory tract***

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**Introduction:** *Streptococcus pneumoniae* is a major human pathogen causing life-threatening diseases. In order to develop alternative vaccination strategies or treatment candidates it is essential to have a reliable animal model that closely resembles the human disease. Biomarkers of infection are therefore necessary to evaluate the effectiveness of new vaccines and antibiotics. We developed a murine model of sublethal pneumonia and identified biomarkers of infection in the blood.

**Materials and Methods:** C57BL/6 mice were infected intranasally (i.n.) with  $10^7$  CFU of *S. pneumoniae* strain TIGR4 and blood was collected at 6h, 12h, 24h, 96h and 7 days post-infection (P.I.) to enumerate, by flow cytometry analysis, neutrophils, lymphocytes, monocytes, macrophages and natural killer cells.

I.n. infection with  $10^7$  CFU of TIGR4 was also performed (i) in mice vaccinated with two doses of pneumococcal conjugate vaccine (Pnevnar13) and (ii) in mice treated with 3 doses of ceftriaxone (20 mg/kg) at 12h and 36h and 84h after infection. Blood cells collected at 12h and 96 h P.I. were then evaluated with flow cytometry analysis.

**Results:** Neutrophils had a significant increase at 6h P.I. and remained elevated in the blood until day 7 after infection. Monocytes instead had only a slight and delayed increase. Macrophages, NK, T and B lymphocytes decreased significantly in the blood during the first 12h P.I. Leukocytes kinetics were also tested as biomarkers of infection on vaccinated and subsequently infected mice or in mice that were infected and received an antibiotic treatment. Infected mice had higher percentages of neutrophils and monocytes in the blood at 96h P.I. compared to vaccinated or treated mice. Vaccinated mice also did not show the early (6-12h P.I.) drop in macrophage counts compared to infected mice.

**Discussion and Conclusions:** In this work, we detected cellular biomarkers of infection in the blood upon induction of a sublethal respiratory infection with *S. pneumoniae*. In particular, neutrophils and monocytes can be considered positive biomarkers of infection since they were elevated in infected mice but not in mice previously vaccinated with the pneumococcal conjugate vaccine or treated with beta lactam antibiotics. Macrophages can be considered negative biomarkers of infection since they decreased in the blood of infected mice, while in vaccinated and treated mice values were similar to that of uninfected control mice. Therefore the kinetics of neutrophils, monocytes and macrophages in the blood were recognized as biomarkers of pneumococcal lung infection and represent valid tools to evaluate the effectiveness of new prophylactic and therapeutic candidates.

**P 184 – ID 294 - Effect of *Akkermansia muciniphila*, candidate probiotic species, on the response of *Galleria mellonella* larvae against *Escherichia coli* infections.**

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**Introduction:** *Akkermansia muciniphila* is a gram-negative a mucin-degrading member of the intestinal microbiota present in infants, adults, and the elderly both human and other mammalian species. *Akkermansia muciniphila* is recently proposed bacteria as new functional microbes with probiotic properties. The screening for new probiotic potentials is usually conducted using mammalian models, over the last years alternative models such as insects have become popular substitutes, considering the ethical implications and the high costs and time-consuming to obtain authorization for mammalian studies. *Galleria mellonella* is the organism widely proposed to study the pathogenesis, virulence and immune response mechanisms, also in the field of probiotics research. The use of this mini-host offer economic and ethical advantages compared to mammals; their short lifespan makes them suitable for high-throughput studies. In the present study, to assess the probiotic potential of akkermansia, we study the response of *Galleria mellonella* against *Escherichia coli* infection.

**Materials and Methods:** The bacterial strains used in this study are *Akkermansia muciniphila* DSMZ 22959, *Escherichia coli* ATCC 11775 and *Escherichia coli* ATCC 25922. The *G. mellonella* larvae were inoculated with 10 µL of *A. muciniphila* at the concentration of  $1 \times 10^5$  CFU/mL (not pathogenic dose for larvae). After two hours the first one inoculum, the larvae were infected by injection with 10 µL of *E. coli* suspension at the concentration of  $1 \times 10^5$  CFU/mL. After infection, the larvae were incubated in a Petri dish at 37°C and Health Index Scoring System was determined in the following 24 hours. Haemolymph was extracted at t = 0 and t = 24 hours p.i. Trypan blue (0.02% [vol/vol] in PBS) was added to the cells in the hemolymph and incubated at room temperature for 10 min. Viable cells were enumerated using a hemocytometer. Also, The hemolymph of four larvae per group was collected, and the serial dilutions in PBS were seeded to quantified the *E. coli* CFU/larvae count.

**Results:** These data show that *G. mellonella* larvae previously treated with  $10^3$  cells/larvae of *A. muciniphila* and then infected with  $10^3$  cells/larvae of *E. coli* have a higher health Index Scoring System than the control group. *A. muciniphila* infection it was able to stimulate the immune system of larvae by an increase in the number of hematocytes. A reduction in the number of *E.coli*/ larvae was also observed in the group pretreated with *A.muciniphila*.

**Discussion and Conclusions:** The *G. mellonella* larvae have proved a powerful model in the investigation of the probiotic properties. This finding demonstrated the potential protective action of *A. muciniphila* against *E. coli* infection.

## ***P 185 – ID 302 - Arginine rich C-terminal domain of the PE\_PGRS3 of Mycobacterium tuberculosis promotes adhesion and specifically binds phosphatidylinositols***

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PE\_PGRSs of *Mycobacterium tuberculosis* (Mtb) represent a family of complex and peculiar proteins, unique to MTB complex and few other pathogenic mycobacteria, whose role and function remain elusive.

We investigated PE\_PGRS3 expression in *Mycobacterium smegmatis* (Ms) and in Mtb, observing that this protein is specifically expressed in phosphate starvation growth condition. Heterologous expression of the full-length PE\_PGRS3 in Ms determined changing in both bacterial cell length and net surface charge. Furthermore, PE\_PGRS3 enhanced adhesion of Ms to macrophages and epithelial cells, and improved bacterial persistence in spleen tissue of intraperitoneally infected mice. We demonstrated that the arginine rich C-terminus (CT) was the key domain to exert PE\_PGRS3 biological function.

To further characterize the last 80 amino acids of the PE\_PGRS3 (rCT), this domain was purified and used in co-infection assays. Remarkably, addition of the rCT significantly increased adhesion of the Ms<sup>GFP</sup>PE\_PGRS3 $\Delta$ CT<sup>HA</sup> and Ms<sup>GFP</sup>. Furthermore, confocal microscopy analysis of infected macrophages and epithelial cells showed that Ms expressing full length PE\_PGRS3 developed distinctive and organized aggregates (Ms<sup>GFP</sup>PE\_PGRS3<sup>HA</sup>), compared to Ms expressing its mutant lacking CT (Ms<sup>GFP</sup>PE\_PGRS3 $\Delta$ CT<sup>HA</sup>) and Ms<sup>GFP</sup>.

These results suggest that the arginine rich CT may promote interactions to other mycobacteria and to host cells.

Finally, rCT was probed on a nitrocellulose membrane where diverse phospholipids were adsorbed, showing a specific binding to phosphatidylinositols (PtdIns). In addition, the surface charge of the Ms<sup>GFP</sup>PE\_PGRS3<sup>HA</sup> incubated with PtdIns was measured. Interestingly, PE\_PGRS3 was demonstrated to capture PtdIns restoring the negative surface charge up to standard values probably due to electrostatic attractions between positive charged arginine residues and negative charged phosphate groups.

Our results point to a critical role for the PE\_PGRS3 in tuberculosis pathogenesis, especially for the  $\approx$  80 amino acids long arginine-rich C-terminal domain, even though further studies will be needed to better elucidate its activity during infection.

## ***P 186 – ID 307 - Analysis of microbiota profiles in WAG/Rij rats and effect of fecal microbiota transplant on absence epilepsy phenotype***

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**Introduction.** A growing number of studies have found that the gut microbiota (GM) is closely connected with brain health, and dysbiosis has been reported to be associated to different neurological diseases. In particular, whether microbiome could play a role in the pathogenesis of epilepsy is becoming a question to be addressed given that: pivotal studies indicate changes in the GM of children with drug-resistant epilepsy and ketogenic diet significantly improves the GM composition with a decrease in seizure frequency in epileptic children. In order to probe a possible role of GM in epileptic disorders, WAG/Rij rats, a well validated animal model of absence epilepsy was used in the presented work to evaluate: 1) the ecological features variations of GM at baseline and during development with respect to age and sex-matched control Wistar rats 2) the effect of fecal microbiota transplant (FMT) on absence epilepsy phenotype.

**Material & Methods:** 16S rDNA fecal microbiota profiling was performed both in WAG/Rij and Wistar rats (1,4 and 8 months of age; n=6/group) and in recipient WAG/Rij and Wistar rats after FMT (n=6/group). Technology used for library preparation and sequencing was Illumina MiSeq platform, followed by statistical data analyses. SPecies IdentificatiON of metaGenOmic amplicons program was used to perform species classification on a representative sequence of each OTU of key genera discriminating the groups.

**Results.** WAG/Rij rats harbored an altered bacterial GM at baseline and during development with a markedly different abundance in Bacteroidetes and Firmicutes phyla with respect to control rats. Specific bacterial phylotypes defined the WAG/Rij rats microbiome being members of *Clostridiaceae* and *Lachnospiraceae* more abundant and *Lactobacillus* and *Phascolarctobacterium* prominently less abundant in WAG/Rij rats. Very interestingly, upon FMT from the control Wistar rats donors to WAG/Rij rats together with prominent remodeling of microbiota structure, EEG recordings showed a significant decrease in the the number and duration of spontaneous spike-wave disch SWDs in comparison to the age-matched control group.

**Conclusion.** Our findings indicate possible implication of microbiota-gut-brain axis in epilepsy and open to the hypothesis that manipulation of GM may be a novel therapeutic method for epilepsy.

## POSTER VIROLOGIA

### *P 188 – ID 007 - CXCR4 and CCR5 led to a different patterns of HIV-1 replication in macrophages*

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**Introduction:** To enter the target cell, HIV-1 binds not only CD4 but also a co-receptor mainly the  $\beta$ -chemokine receptor 5 (CCR5) or the  $\alpha$  chemokine receptor 4 (CXCR4). Limited information is available on the impact of co-receptor usage on HIV-1 replication in human primary monocyte-derived macrophages (MDM) and on the homeostasis of this crucial cellular reservoir.

**Materials and Methods:** MDM were obtained from PBMCs of healthy seronegative donors and infected in vitro with several CXCR4-tropic or CCR5-tropic HIV-1 strains. HIV-1 p24 viral Ag production was monitored at different time-points. DNA fragmentation was detected by propidium iodide incorporation measured by flow cytometric analysis. Gene activation was detected through microarray, and MAPK activation by western blotting assays.

**Results:** Replication of the CCR5-tropic 81A strain increased up to 10 days post-infection and then reached a plateau. Conversely, the replication of the CXCR4-tropic NL4.3 strain (after an initial increase up to day 7) underwent a drastic decrease becoming almost undetectable after 10 days post-infection. The ability of CCR5-tropic and CXCR4-tropic strains to induce cell death in MDM was then evaluated. While for CCR5-tropic 81A the rate of apoptosis in MDM was comparable to uninfected MDM, the infect of CXCR4-tropic NL4.3 in MDM was associated with a rate of 14.3% of apoptotic cells at day 6 reaching a peak of 43.5% at day 10 post-infection. This suggests that the decrease in CXCR4-tropic strains replication in MDM can be due to their ability to induce cell death in MDM. The increase in apoptosis was paralleled with a 2 fold increase in the phosphorylated form of p38 compared to WT. Furthermore microarray analysis showed modulation of proapoptotic and cancer-related genes induced by CXCR4-tropic strains starting from 24 hours after infection, whereas CCR5 viruses modulated the expression of genes not correlated with apoptotic-pathways.

**Discussion and Conclusions:** In conclusion, CXCR4-tropic strains can induce a remarkable depletion of MDM. Conversely, MDM can represent an important cellular reservoir for CCR5-tropic strains supporting the role of CCR5-usage in HIV-1 pathogenesis and as pharmacological target to contribute to HIV-1 cure. This work was supported by PRIN (Progetti di Rilevante Interesse Nazionale) Grant 2015W729WH\_007 and 2017M8R7N9\_004 from the MIUR, Italy and by GAIN (Agencia Gallega de Innovación) Grant IN606B-2016/012 from the Consellería de Cultura, Educación e Ordenación Universitaria e a Consellería de Economía, Emprego e Industria (Xunta de Galicia), Spain.

## ***P 189 – ID 016 - Peptidyl arginine deiminases: a novel antiviral target against human Cytomegalovirus***

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**Introduction.** Post-translational modifications (PTMs) represent a key virulence strategy to subvert host-cell immune control. Citrullination is an arginine PTM, catalyzed by peptidyl arginine deiminases (PADs); its dysregulation has been correlated with inflammation-dependent processes, including autoimmune diseases (ADs), cancer and neurodegenerative disorders. Strikingly, citrullination has never been associated with viral infections. Human Cytomegalovirus (HCMV) is a widespread beta-Herpesvirus carried by 70% up to 90% of the human population. Following primary infection, HCMV establishes a lifelong latency in cells of the myeloid lineage, where reactivation is often driven by inflammation. Against this background, the goal of this project is to characterize PTMs such as citrullination during infection with Herpesviruses, in particular with HCMV.

**Materials and Methods.** For this purpose, we determined the overall pattern of citrullination during HCMV infection in Human Foreskin Fibroblasts (HFF) using a citrulline-specific rhodamine phenylglyoxal (RhPG)-based probe. We then measured the PAD activity in infected HFFs using an *in vitro* antibody-based assay and we evaluated PADs expression both at mRNA and protein levels. Viral replication rate of the HCMV was assessed in the presence of Cl-amidine, a specific pan-PAD inhibitor, by standard plaque assay and quantitative PCR. Consistently, HCMV replication rate was assessed in cell lines KO for each PAD isoform by CRISPR-cas9 technology. Last, we generated the HCMV-induced citrulinome by mass-spectrometry-based analysis (LC-MS/MS) (in collaboration with Paul R. Thompson, UMass, USA).

**Results.** Here, for the first time we show that human Cytomegalovirus infection upregulates the overall pattern of citrullination in primary human fibroblasts. More interestingly, knock-out or pharmacological inhibition of specific peptidyl arginine deiminases isoforms strongly impairs the viral replication rate, indicating that citrullination is required for HCMV replication. Finally, by interrogating protein citrullination, we identify several interferon-inducible proteins as major target of citrullination, suggesting that citrullination can antagonize innate immune response to HCMV.

**Discussion and Conclusions.** Altogether, this study proposes that PAD-mediated citrullination is a novel mechanism of host viral adaptation in HCMV-infected cells that, even more interestingly, might represent an alternative strategy for efficient inhibition of HCMV viral replication and may shed light on the role of HCMV in the pathogenesis of HCMV-related diseases.

**Key words:** Human Cytomegalovirus, infection, citrullination, post-translational modification, peptidylarginine deiminase, immune system

**P 190 – ID 018 - Focus on antibody coverage IgG measles, mumps, rubella, and chickenpox virus in healthcare workers**

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**Introduction**

The issue of vaccination in recent years has been widely discussed .In Italy, in fact, there was a continuous and progressive decline in vaccinations that had brought the measles vaccine to insufficient coverage in 2015. The consequence was a serious epidemic in 2017 with about 5000 cases and several deaths. Following the decree (Decreto 1697/2018 Regione Lombardia) that imposed on health facilities for staff in contact with frail patients to be immunized for: measles, parotitis, chickenpox, rubella and whooping cough, in our Institute we have verified our healthcare workers. The aim of the study is evaluate antibody coverage for staff in contact with potentially immunocompromised patients.

**Materials and Methods**

The Institute's choice included all medical, fellow and nursing staff working in emergency room, coronary unit and intensive care unit for a total of 198 subjects (see table below). Pertussis control has been excluded, as there are no subjects under the age of 14 in our inpatients.

	N°	Male/female	age
nursing	63	37/26	27-65
fellow	41	53/21	32-66
physiician	94	21/20	27-36

Two different working methods were used: antibody titration of rubella and mumps viruses was performed with the VIDAS 3 (bioMérieux, SA F-69280 Marcy l'Etoile, France). VIDAS system uses the ELFA principle, combining the ELISA method with a final fluorescent reading; this technology ensures excellent sensitivity and specificity. Antibody titration of varicella and measles was performed with the ROCHE COBAS 8000 (Hoffmann-La Roche, Basilea CH); electrochemiluminescence (ECL) immunoassays.

	neg	doubt	pos
Rubella IgG Ab	<10I U/mL	10-15IU/mL	>=15IU/mL
Mumps IgG Ab	<0.35	0.35-0.50	>=0.50
Chickenpox IgG Ab	<0.6	0.6-0.9	>0.9
Measles IgG Ab	<13.5 AU/mL	13.5-16.5 AU/mL	>16.5 AU/mL

**Results**

In general, our methods have well discriminated against the subjects in the study having only 1 or 2 cases of doubt about all the dosages made. The coverage for chickenpox was very high, more disappointing results for measles and mumps.

	neg	duobt	pos			neg	doubt	pos

Measles					Mumps			
nursing	7		56			10		53
physician	5		88			9	1	84
fellow	13	1	27			7		34
tot	25	1	171			26	1	171

### Discussion and conclusions

The WHO states that vaccination coverage of more than 95% is necessary to prevent disease outbreaks. In our study we evaluated not the vaccination coverage but the immunization of a selected population such as the health care personnel of our institute. From the data we obtained, it can be seen that only 88% of our population has antibody coverage against measles. Data that are also confirmed in the case of mumps (88%), rubella (90%) less in the case of chickenpox (95%). Very important is that the younger population, most likely less covered by natural immunity is the most exposed. In fact, 18 out of 22 (85%) of the measles negatives are under 35 years old.

## ***P 191 – ID 020 - Strigolactones as antiviral drugs to inhibit Herpesvirus replication***

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**Introduction:** The human cytomegalovirus (HCMV) is the most frequent cause of congenital malformations in developed countries. Although several nucleoside analogues have been employed successfully against HCMV infection, their use in children is hampered by the occurrence of serious side-effects, such as neutropenia and thrombocytopenia. Thus, there is an urgent and unmet clinical need for less-toxic, but highly effective, antiviral drugs that could be safely administered against HCMV during pregnancy or in the neonatal period. Strigolactones (SLs) are a novel class of plant hormones with multifaceted roles. While the activity of SLs in plant-related fields is well characterized, their effects on human cells and their application in medicine are still emerging. The main data reported so far refer to the effect of SLs on cancer cells. However, the antiviral activity of SLs has never been demonstrated so far. In the present study, a panel of SL derivatives, named TH-EGO, EDOT, EGO-10, and GR24, has been evaluated for their antiviral activity against Herpesvirus infections, focusing on HCMV.

**Material And Methods:** Viral yield, attachment, and entry assays were performed on Human Foreskin Fibroblasts (HFFs) treated with a non-toxic concentration of the selected compounds and infected with HCMV. Western blot, flow cytometry, and computational analyses were performed to identify the molecular mechanism beyond SLs activity.

**Results:** we demonstrated that TH-EGO and EDOT markedly inhibit *in vitro* HCMV replication. We observed an antiviral effect also for other members of the Herpesvirus family, such as Herpes simplex virus type 1 and 2 (HSV-1 and HSV-2). Interestingly, SLs do not affect the first steps of HCMV replication (i.e. attachment and entry), but exert their role on the late phases, as indicated by a reduction of late protein expression, such as the HCMV tegument protein pUL99 (pp28). Finally, we pointed to the SL-dependent induction of apoptosis of HCMV infected cells, during the late stages of infection, as a contributing mechanism to their antiviral properties.

**Discussion And Conclusion:** Overall, our results indicate that SLs could provide an alternative to nucleoside analogues in the treatment of herpetic infections.

## P 192 – ID 028 - Antimicrobial activity of *Juglans regia* L. pellicle extract

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**Introduction:** The use of plant extracts for the treatment of a large variety of human diseases is well established since ancient time. Currently, there are growing interests on the natural extracts as a prominent source of chemical compounds with different biological activity. *Juglans regia* L. (common walnut) is a deciduous tree belonging to Juglandaceae family. Walnut was widely used in traditional medicine for its antidiabetic, antioxidant, antimicrobial, anti-atherogenic and anti-inflammatory effects. The aim of the present study was to evaluate the antibacterial and antiviral activities of walnuts pellicle extract.

**Materials and Methods:** Antibacterial activity of the extract was tested against thirty-two clinical isolates and eight ATCC strains by the microdilution method. Antiviral activity was evaluated against some DNA and RNA viruses, including *Herpes simplex* virus type 1 (HSV-1) and 2 (HSV-2) in VERO cells, *Echovirus* 9 (Hill strain) in LLCMK2 cells, *Poliovirus* 1 (Sabin strain), *Coxsackievirus* B1 and *Adenovirus* 2 in HEp2 cells. Total phenol content was determined spectrophotometrically, using the Folin-Ciocalteu method. Total flavonoid content was measured using a colorimetric assay. Ultra-Performance Liquid Chromatography coupled to Mass Spectrometry (UPLC-Ms/Ms) was performed to identify phytochemical compounds.

**Results:** Gram-positive strains were more sensitive, with MIC values ranging from 8.59 to >275.00 µg/ml. Gram-negative strains were less susceptible, with MIC values ranging from 275 to >275.00 µg/ml. Antibacterial activity of the extract was compared to that of reference antibiotic ciprofloxacin. *J. regia* L. pellicle extract inhibited HSV-1 and HSV-2 replication at doses below the cytotoxic dose. No virucidal effect was observed. The compound was ineffective against Polio 1, Adeno 2, ECHO 9, Coxsackie B1, viruses. Low flavonoid contents were found in the extract. These results are consistent with other studies showing that *J. regia* phenolics are mainly non-flavonoid type. UPLC-Ms/Ms confirmed that walnut pellicle extract is an important source of phenolic compounds.

**Discussion and Conclusions:** In antibacterial assay, Gram-negative strains were more resistant than Gram-positive. This could be explained by a different composition of their cell wall, formed by a thin layer of mucopeptides and a thick layer of lipoproteins and lipo-polysaccharides that make it not permeable to external agents. Interestingly, the extract showed also antiviral activity against HSV-1 and HSV-2. Taken together these data demonstrate a protective role of walnut pellicle extract against microbial infections. However, further studies will be need to investigate the possible mechanism of action of the biologically active chemical compound.

## ***P 193 – ID 029 - A recombinant minigenome of Parvovirus B19 for the overexpression of NS1 protein***

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**Introduction:** The non-structural protein 1 (NS1) of Parvovirus B19 (B19V) is the central protein involved in viral replication. Its molecular structure and cellular partners are still unknown, and a better characterisation of this protein and the cellular context in which it operates might be useful in order to develop new antiviral strategies against B19V.

**Materials and Methods:** Based on a model genetic system of B19V developed in our laboratory, we further introduced a deletion that generates a new RNA cleavage and polyadenylation site and removes almost completely the VP and 11-kDa genes, with the aim of overexpressing the only remaining gene, NS1. Then, we studied the expression of NS protein via transfection of UT7/EpoS1 cells and subsequent RT-PCR, IIF and flow cytometry analysis.

**Results:** A modified B19V genomic clone was generated, containing only the NS1 gene and lacking replication competence due to partial truncation of ITRs. After transfection of UT7/EpoS1 cells, this clone was able to increase the NS1 expression, compared to the unmodified control, in a simplified genetic context. The generation of a modified clone including complete ITRs failed, probably due to genetic instability induced by close secondary structure forming regions.

**Discussion and Conclusions:** Our study provides a useful genetic system to deepen the knowledge of NS1 protein. The system is appropriate to overexpress NS1 protein in order to I) purify the native protein for a better characterisation; II) identify cellular partners of the protein to clarify its mechanism of action and its interaction with cellular factors; III) have a simple genetic system useful for *in vitro* screening of putative antiviral molecules active on NS1 protein.

## ***P 194 – Id 038 - Presence Of Polyomavirus Jc Archetype Associated To Exosomes Enriched Vesicles In Circulation Can Be A New Way To Delivered Virus To Central Nervous System***

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**Background:** Polyomavirus JC (JCPyV) infects people indefinitely establishing a fine and successful interaction with the host causing progressive multifocal leukoencephalopathy in immunocompromised subjects. Increasing evidence have pointed out that JCPyV as other viruses exploit extracellular vesicles thereby helping viral persistence in the host. Here, the presence of JCPyV archetype form in extracellular vesicles circulating in cell supernatant and in human plasma was investigated.

**Materials and Methods:** JCPyV DNA status was studied in exosomes enriched vesicles (EEVs) obtained from cells supernatant after Cos-7 cells infection with JCPyV archetype molecular clone and in plasma-derived EEVs from 170 samples collected from 120 HIV positive patients and 50 healthy donors. EEVs were extracted from supernatant and plasma and characterized by Nanoparticle tracking analysis, by western blot for presence of tetraspanin CD63, CD81, annexin II, cytochrome C and Vp1 capsid protein and, finally, by immunoelectron microscopy (IEM). Presence and quantitation of JCPyV DNA were assessed with Multiplex real-time TaqMan PCR assay.

**Results:** The Extracellular vesicles extracted from Cos-7 cell supernatant after infection of JCPyV archetype exhibited a main size of 112 nm, had concentration of  $5.2 \times 10^8$ /ml, and scored positive for tetraspanin CD63, CD81 and annexin II, typical characteristic of the exosomes vesicles, and also for Vp1 capsid protein. The EEVs showed to contain JCPyV DNA  $1.6 \times 10^5$  copies per ml (2.14% of the total viral load). Important, JCPyV yield changed significantly following detergents and DNase treatment of supernatant before EEVs extraction. Moreover, EEVs purified from Cos-7 cell treated with GW4869 exosomes pathway inhibitor exhibited larger vesicles size and harbored a reduced JCPyV yield. Finally, JCPyV associated EEVs Cos-7 cells infection was proved and showed to be marginally neutralized by anti-Vp1 antibody but positively inhibited by anti-CD63 and anti-CD81 antibody. To explore the potential relevance in humans, investigation of well characterized plasma EEVs from 170 subjects (120 HIV-positive, and 50 healthy) reported JCPyV DNA detection in 15 (42%) of the viremic samples (14 were from HIV patients and 1 from healthy people) at a mean level of 23.5 copies/ml. The examination of EEVs selected samples reported the percentage of JCPyV DNA in EEVs of 5.4% of the total viral load. Moreover, IEM confirmed the presence of JCPyV-like particles associated to EEVs.

**Conclusions:** Collectively, these observations confirm the potential role of the JCPyV particles associated to EEVs and open new avenues and mechanistic insights on the molecular strategies adopted by this polyomavirus to persist in the host and spread to the central nervous system.

## ***P 195 – ID 039 - Evolution of HCV types in a teaching hospital, Calabria Region, Southern Italy over a decade (2008-2018).***

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**Introduction:** In the last years, therapy for Hepatitis C virus (HCV) improved with availability of several direct-acting antiviral (DAA) drugs, which allowed to avoid the use of standard of care treatment. Despite high rate of virological response with DAAs, efficacy of combination therapy appears to be influenced by HCV genotypes/subtypes, carrying resistance associated substitutions on target regions. Since determination of infecting genotype in patients cannot be avoided, we reported ten years' challenge about HCV types prevalence in our teaching Hospital.

**Materials and Methods:** During the 2008–2018 period, serum samples from 1,525 new consecutive HCV positive patients were collected. Cobas AmpliPrep/Cobas TaqMan HCV v.2.0 (Roche) test has been used for extraction and quantitation of RNA. Genotyping was performed by Versant HCV genotype 2.0 assay (LiPA, Siemens). The  $\chi^2$  analysis was used to evaluate the association between HCV types and demographic parameters ( $p < 0.05$  was considered significant).

**Results:** HCV1b was the most prevalent subtype (47.2%) followed by HCV2a/2c (20.2%). HCV1b decreased starting from 2010 (43.6%) to 2013 (39.5%), then it has been reported a constant prevalence until 2018 (48.8%), while HCV 2a/2c did not vary significantly over the time. HCV3 showed a peak of 13.3% during 2014, and a progressive increase from 2015 (7.0%) to 2018 (10.2%). HCV4 increased between 2010 and 2013 reaching the peak in 2013 (9.3%). No significant gender-related variation was detected in the distribution of both subtypes. HCV3 was the third most frequent (9.6%) and was significantly more common in male patients (86.9% vs 13.0% female,  $p < 0.05$ ). HCV4 showed a rate of 6.1%, with significant gender differences (62.3% male vs 37.6% female,  $p < 0.05$ ) followed by HCV1, HCV1a, HCV2 and HCV1a/1b. In particular, patients infected with HCV1b and HCV2a/2c were older (born before 1959) than those infected by HCV3 and HCV1a (born before 1977). Overall most of infected patients was born before 1949. HIV/HCV and HCV/HBV coinfection was diagnosed in 21 and 7 patients born before 1968, respectively. HIV positive patients were mainly intravenous drug users (IDU) and co-infected by HCV3 (10/21).

**Discussion and Conclusions:** The trend observed in this study showed an overall prevalence increase of HCV3. HCV genotype shift seems to be due to modified risk factors and different genotype susceptibility to antiviral drugs. HCV3 has a greater predisposition to fibrosis/liver cirrhosis and it is possibly associated with a lower sustained therapeutic response. This genotype, maintaining a high prevalence, could create a difficult to treat infection reservoir in risk categories (such as IDU, and inmates) causing an epidemiological increase.

## ***P 196 – ID 044 - Polyphenol-rich extracts derived from raw pistachios (*Pistacia vera*, L.) exhibited antiviral activity against HSV-1***

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### **1. Introduction**

The aim of the present research was to determine the effect of polyphenol-rich extracts derived from raw pistachios (*Pistacia vera* L.) on herpes simplex virus 1 (HSV-1) replication. HSV-1 is a common highly contagious infection usually asymptomatic, but under particular conditions it can cause more serious aggravation such as encephalitis, keratitis or gingivostomatitis. Drug-resistant strains of HSV frequently develop following therapeutic treatment, mostly in immune-compromised patients. Therefore, there is an increasing need to find novel anti-HSV drugs.

### **2. Materials and Methods**

The cell proliferation index (%) was calculated on Vero cells treated for 72h with raw pistachios extracts (0.4, 0.5, 0.6, 0.8, 1, 1.2, 1.4 mg/mL) on the basis of ATP levels using the ViaLight plus cell proliferation and cytotoxicity bioassay kit (Lonza Group Ltd., Basel, Switzerland). Plaque reduction assay was performed on Vero cells infected with HSV-1 (F) for 1 h at 37 °C and overlaid with DMEM medium containing 0.8% methylcellulose in the presence of pistachios extracts at different nontoxic-concentrations (0.1, 0.2, 0.4, 0.6, 0.8 mg/ml) for three days. The plaques were visualized and counted by staining cells with crystal violet. The DMSO was used in the HSV-1 control. Equal quantities of proteins from Vero cells infected with HSV-1 and treated with the raw pistachio extracts (0.4, 0.6, 0.8 mg/ml), were separated by electrophoresis on polyacrylamide gels, transferred onto a nitrocellulose membrane and probed with specific antibodies for ICP8, UL42, US11 to analyse the expression of viral antigens.

### **3. Results**

The results of viability assay indicate that the  $CC_{50}$  for raw extract was 1.2 mg/ml. The results of standard plaque reduction assay showed a significant decrease in the viral titer and plaque size after incubation with 0.4, 0.6, 0.8 mg/ml of raw extracts. Particularly, at concentrations of 0.4 and 0.6mg/ml a reduction of the viral titre greater than 50% was observed together with a reduced morphology of the plaque diameter (micro-plaques), while at the concentration of 0.8 mg/ml was reported a total absence of plaques. The  $EC_{50}$  was 0.4mg/ml. No reduction was observed after treatment with 0.2 and 0.1 mg/ml. In addition, a reduction in the expression of viral protein ICP8, UL42, US11 was also reported at 0.4, 0.6, 0.8 mg/ml of raw extracts and was depending on the increased concentration.

### **4. Discussion and Conclusions**

These results therefore suggest the existence of a direct association between the increasing concentration of the raw extracts and the inhibition of viral replication.

Natural products, such as pistachios, represent a good source of antiviral agents and could provide a novel treatment against HSV-1 infections.

## ***P 197 - ID 047 - Human endogenous retroviruses (HERV) methylation and expression in colorectal cancer patients***

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**Introduction:** Human endogenous retroviruses (HERV) are remnants of exogenous retroviral infections, representing 8% of the human genome. Their regulation is based on the DNA methylation of promoters, the long terminal repeats (LTRs). Transcripts from HERV have been associated with cancers, but reports concerning HERV expression in colorectal cancer remain sporadic.

**Materials and Methods:** Sixty-three patients with advanced stages of colorectal cancer were enrolled in this study. HERV-H, -K, -R and -P, LTRs, and Alu, and LINE-1 methylation levels, and the expressions of HERV *env* gene were investigated by RT qPCR in the tumor, normal adjacent tissues, and, when possible, blood and plasmatic extracellular vesicles (EVs). The expression of the HERV-K Pol protein was also evaluated by Western Blot.

**Results:** Alu, LINE-1, HERV-H and -K LTRs were demethylated in the tumor compared to the normal adjacent tissues ( $p < 0.05$ ), while no differences were observed in HERV *env* gene expression levels among the clinical specimens. The *env* gene was expressed in the EVs ( $p < 0.01$ ) of 54% (-H), 38% (-K), 31% (-R) patients. HERV K Pol protein was more expressed ( $p = 0.0013$ ) in the adjacent normal tissues compared to the tumor tissues.

**Discussion and Conclusions:** The changes in DNA methylation of retroelements is specific in colorectal cancer but does not correlate with viral overexpression. The Pol protein expression in the normal cells may induce the retrotranscription and the subsequent transfer of HERV sequences into other cells, possibly through EVs. HERV genome insertion might cause cells transformation.

## ***P 198 – ID 048 - Longitudinal analysis of HCMV-specific T-cell response in kidney transplant recipients (KTRs) during the first year after transplant***

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**Introduction:** Human Cytomegalovirus (HCMV) still represent a crucial issue in transplanted patients. The aim of the study was to evaluate the role of immunological markers in monitoring kidney transplant recipients (KTRs) at risk for HCMV infections.

**Material and Methods:** Eighty-six KTRs were prospectively enrolled and monitored for at least six months post-transplant. Pre-transplant HCMV serostatus was assessed, HCMV DNAemia and HCMV-specific T-cell response were monitored in the post-transplant. *Ex-vivo* ELISpot assay was performed using peptide pools representative of pp65, IE1 and IE2 proteins, for evaluation of HCMV-specific T-cell response. Interferon-gamma (IFN-gamma) producing T cells were quantified. HCMV DNAemia was measured in blood samples according to diagnostic protocols.

**Results:** Among 86 enrolled patients, 7 (8,1%) patients were D-/R- and they did not showed episodes of HCMV infection. Fourteen patients of 86 (16,3%) were D+/R- and 11 of them (78,6%) were treated for clinically relevant HCMV infection. HCMV-specific T-cell response was undetectable during the first three months post-transplant. A significantly increase in pp65- and IE2-specific T-cell responses was observed after 180 days post-transplant, followed by IE1-specific T-cell response.

Sixty-five patients of 86 (75,6%) were R+ at transplant and 22 of them were treated for clinically relevant HCMV reactivations (33,8%). Interestingly, 7/65 patients (10,8%) did not showed any episode of HCMV reactivation during the follow-up period. In this latter group of patients IE1-specific T-cell response was significantly higher at pre-transplant than respect to other R+ patients ( $p=0.0177$ ). Deficient IE1-T cell response was observed in treated patients.

**Discussion and Conclusions:** Deficiency in IE1-specific T-cell response should be considered as immunological marker for the risk of clinically relevant HCMV infections. However, further analysis are required on larger population for definition of clinical cut-off.

## ***P 199 – ID 068 - Identification of compounds with antiviral activity against Parvovirus B19***

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### **Introduction**

Parvovirus B19 (B19V) is a human ssDNA virus, responsible for a wide range of clinical manifestations, still lacking for a specific antiviral therapy. Alternative approaches, including testing of compounds with broad-spectrum antiviral activity, drug repositioning of existing approved drugs for a new clinical use, and small chemical libraries screening, led our group to the first identification of compounds with inhibitory activity against B19V with promising perspectives for a specific antiviral therapy.

### **Materials and Methods**

Experiments were carried out in erythroid progenitor cells (EPCs) and UT7/EpoS1 cells, infected with B19V and cultured in the presence of tested compounds. The dynamics of viral replication was evaluated by a qPCR assay, and the extent of inhibition of viral replication exerted by the compounds determined, along with the extent of inhibition on cell viability and proliferation.

### **Results**

Hydroxyurea (HU), a drug approved for treatment of sickle cell disease (SCD), inhibits B19V replication with EC<sub>50</sub> values of 96.2  $\mu$ M and 147.1  $\mu$ M in UT7/EpoS1 and EPCs, respectively. The antiviral activity occurs *in vitro* at concentrations lower than those affecting cellular DNA replication and viability and at levels measured in SCD patients undergoing HU therapy. The nucleoside phosphonate Cidofovir (CDV) and its lipid derivative Brincidofovir (BCV), with known antiviral activity against dsDNA viruses, also exert an inhibitory effect against B19V, with an enhanced activity of BCV compared to CDV. For BCV, calculated EC<sub>50</sub> values were in the range 6.6-14.3  $\mu$ M in EPCs and 0.22-0.63  $\mu$ M in UT7/EpoS1 cells, compared to EC<sub>50</sub> values for CDV >300  $\mu$ M in EPCs and 16.1  $\mu$ M in UT7/EpoS1 cells. Screening of a small chemical library indicated some coumarin derivatives as scaffold molecules with promising activity against B19V, further yielding derived compounds with partial but selective activity in inhibiting B19V replication, with EC<sub>50</sub> values in the range 6.5-6.7  $\mu$ M.

### **Discussion and Conclusions**

Efforts have been made in order to fill the gap in the development of a specific antiviral therapy against B19V. The demonstrated efficacy of HU is of relevance especially for the treatment of B19V transient aplastic crisis occurring mainly in children with SCD. Results on CDV and BCV extend their broad spectrum including a ssDNA virus, and the safety profile of BCV and its improved activity open the possibility of a specific treatment for B19V infection. Further progress in the knowledge of the biological characteristics of B19V and of its interaction with the cellular environment will lead to identifying potential viral and/or cellular targets for a specific antiviral strategy.

## ***P 200 – ID 069 - Synergic use of hormonal modulators and oncolytic viruses in the therapy of hormone-stimulated tumours***

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**Introduction:** Oncolytic viruses (OVs) are genetically modified viruses able to infect, replicate and lyse several malignant tumour cells. Their mechanism of action involves lysis of tumour cells and activation of the immune response, so that the recruited immune cells recognize the tumour and lead to its destruction. The aim is to research a new experimental combinatorial strategy between the oncolytic Herpes Simplex Virus-1 (oHSV-1) and different epigenetic and hormonal modulators in breast and prostate cancer cell lines. oHSV-1 is an engineered virus with deleted copies of ICP34.5 and disrupted ICP6 reading frame.

**Materials and methods:** oHSV-1 was propagated on VERO cells and used to infect both positive and negative breast cancer cell lines (MCF7; T47D and MDA) for the estrogen receptor (ER). A viral plaque assay was performed to determine the viral concentration by counting the plaque-forming units (PFU). After assessing the sub-cytotoxic concentration of the epigenetic substances to be used, synergic assays were carried out between oncolytic virus, pre-treated with epigenetic substances, and hormonal modulators, such as tamoxifen. A cell cycle analysis was performed by flow cytometry.

**Results:** The combination between virotherapy and epigenetic drugs improve the viral replication and the cell death. Furthermore, estrogen treatment also enhances the oHSV-1 infection and causes the reduction of apoptosis rates in ER+ breast cancer cells.

**Conclusions:** The use of oncolytic viruses associated with epigenetic modulators may represent an innovative and effective therapy against a large number of malignant tumours. The anticancer efficacy of oncolytic viruses depends on their ability to infect and propagate within the tumour and on the defence mechanisms of host cells that limit viral replication. These results allow the use of virotherapy in patients with hormone receptor positive breast cancer, more than the conventional therapies like chemotherapy and radiation.

## ***P 201 – ID 079 - The impairment of IFN response and dendritic cells maturation is similar during lineage 1 and 2 WNV infection***

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**Introduction:** Dendritic cells (DCs) are an important cell type during infection by multiple mosquito-borne flaviviruses including West Nile virus (WNV). Despite this, the interplay between different WNV lineages (L) and DCs remains poorly defined. To gain new insights into the ability of these viruses to induce the innate immune response, we evaluated whether WNVL1 and L2 varied in their type I/III interferon (IFN) and DCs antagonistic properties.

**Materials and Methods:** the single-growth curve replication of WNVs and mRNA levels of type I/III IFN subtypes were determined in DCs at different times post infection (p.i.) (MOI 0.1) using real-time RT-PCR. The antiviral activity of type I/III IFNs was also evaluated. Maturation of DCs after the infection with WNL1, L2 was assessed by flow cytometry. USUTU virus (USUV) was used as control IFN-inducing virus.

**Results:** Results showed that both WNVL1 and L2 titers peaked between 48 and 72 h p.i. in DCs. IFN-alpha subtypes (n=12), IFN-beta and IFN-lambdas1-2 levels in infected DCs followed a bimodal expression (maxima at 8 and 48 hrs), regardless of the WNV lineage (L1/L2). A similar type I IFN signature was recorded in WNVs and USUV infected DCs at 8 and 48 hrs p.i. Pretreatment of A549 cells (adenocarcinomic human alveolar basal epithelial cells) and SK-N-SH cells (human caucasian neuroblastoma cells) with IFN-alpha/beta/lambdas preparations, significantly reduced WNV L1/L2 replication. By contrast USUV replication was more sensitive and elicited a strong IFN response compared to both WNVs. Furthermore, both WNVs infected DCs were potentially less capable to induce a proper adaptive immune response compared to USUV-infected DCs, which instead showed a good expression of maturation markers (CD83, CD80, CD86 and CD54 ).

**Discussion and Conclusions:** In conclusion, IFN response appears to be impaired in DCs infected with WNVL1/L2 compared to USUV; WNVL2 could interact with the IFN and DCs response in the same way as WNVL1.

## ***P 202 – ID 083 - HIV-1 acute infection: back and forth between HIV-1 and host during the early phases of infection.***

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### **HIV-1 acute infection: back and forth between HIV-1 and host during the early phases of infection.**

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**Introduction:** Restriction Factors (RFs) are cellular proteins that target different essential steps of the HIV-1 life cycle, thereby providing an early line of defense against the virus. We studied the expression levels of some RFs to characterize the well-structured interplay between virus and host.

**Materials and methods:** PBMCs derived from 5 blood samples of healthy donors were infected with two different HIV-1 strains (3 infections were performed by using the HIV-1 3B strain, a CXCR4 tropic virus, and 2 with HIV-1 Bal, a CCR5 tropic strain of HIV-1). Infected PBMCs, collected at different time points post infection (pi) (2-12-24-48 hours (hpi) and 4-7 days pi (dpi), were collected to a) to analyze the RFs' expression (APOBEC3G, SAMHD1, SERINC3, MX2 and IFI16) b) to detect viral Pol RNAs c) to study the amount of proviral DNA (Pol and 2-LTR). Expression levels of viral and cellular elements were assessed using Real Time PCR. The fold increase were calculated compared to negative control.

**Results:** APOBEC3G and the cellular sensor IFI16 were both significantly overexpressed from 12hpi onwards [24h p.i. ( $p < 0.05$ ), 48hpi ( $p < 0.01$ ) and 4dpi ( $p < 0.05$ )]. On the contrary, no significant upregulation for the other RFs was observed in all experimental time points. Our data might suggest a pivotal role of APOBEC3G and IFI16 during the early phases of viral lifecycle. In particular, in our experimental conditions, a productive HIV infection detectable at 7dpi, as assessed by HIV p24, seem to be concomitant with a significant amount increment of Pol in RNA and DNA from 24hpi onwards, while 2-LTR showed a significant increase later (between 4 and 7dpi). Finally, our data did not show any correlation between the amount of viral infection and the increase of each RFs at our time points, but a correlation between the fold change of IFI16 and the other RFs, mainly for SAMHD1 ( $p = 0,0002$ ,  $R^2 = 0,978$ ), APOBEC3G ( $p = 0,05$ ), MX2 ( $p = 0,0011$ ,  $R^2 = 0,945$ ) e SERINC3 ( $p = 0,03$ ).

**Conclusion:** The early lines of defense are one of the many aspects of the complex host-pathogen interplay that characterizes HIV-1 infection. Our preliminary data suggest that during the early stages of viral replication, cells turn on several strategies to prevent viral replication and spread. Probably the absence of an upregulation of all RFs at each time point could depend from the activity of some viral accessory proteins, such as Nef, Vif, Vpu e Vpr, able to inhibit the RFs activity by counteracting the proteasomal complex for protein degradation.

## ***P 203 – ID 084 - Hepatitis C virus epidemiology during the Interferon-free Era in the metropolitan area of Naples, Italy***

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**Introduction:** Hepatitis C Virus (HCV) infection is a global health problem that affects approximately 3% of the global population. Indeed, the World Health Organization (WHO) estimates that 130–170 million people are infected with hepatitis C virus worldwide and that close to 400,000 people die each year of HCV-related liver diseases. HCV presents high level of variability, which translates into the existence of 8 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 evaluate the distribution of HCV genotypes and subtypes from 2010 to 2015 from patients living in the metropolitan area of Naples, Italy.

**Material and Method:** The study was performed on 1,520 anti-HCV-positive plasma samples collected at Azienda Ospedaliera Universitaria – Università della Campania “Luigi Vanvitelli” from April 2010 to December 2015. Of the 1,221 enrolled, 862 were naïve for antiviral therapy; the remaining 359 had no response to previous IFN-based treatment.

**Results:** Of the 1,520 blood samples collected during the study period, 1,240 (82.1%) were found to be positive for HCV- RNA and 280 (17.9%) negative. The blood samples were taken from 633 males (51.9%) and 588 females (48.1%), with a mean age of  $60 \pm 13$  (SD) 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.

**Discussion and Conclusion:** 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 6 years. Genotype 1b, historically the most prevalent in this area and in the whole of Italy, remains the most common (55.3%), followed by genotype 2 (23.7%), 1a and 3. 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. Thus, although HCV 1b remains the prevalent genotype, new subtypes may “emerge” to modify the future epidemiology.

## ***P 204 – ID 089 - Pluripotent stem cell derived human motor neurons as authentic model for assessing central nervous system measles infection***

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**Introduction:** Viral infections of the central nervous system (CNS) are one of the major causes of human morbidity and mortality worldwide. Among neurotropic viruses, Measles virus (MeV) infection presents in a variety of serious complications, including Measles Inclusion Body Encephalitis (MIBE) and Subacute Sclerosing Pan-Encephalitis (SSPE). Measles infection of the CNS is especially dangerous due to the lack of specific treatments. Neurons are one of the main cellular target in CNS for measles infection. Here, we report the use of human motor neurons as an *ex vivo* model to study virus-host interaction and to assess efficacy of antiviral intervention. Our findings highlight the potential application of fusion inhibitory peptides against MeV in human motor neurons.

**Materials and Methods:** We used a 14-day protocol for differentiation of iPSC (induced pluripotent stem cells) into spinal human motor neurons. Mature motor neurons were infected with eGFP recombinant MeV bearing a MIBE derived F (i.e., CNS adapted virus). Motor neurons were treated 72 h post infection with fusion-inhibitory peptides derived from the heptad repeat HRC region of the MeV fusion protein. Antiviral potency was monitored every 7 days post infection (up to 45 days post-infection) using high content fluorescent microscopy.

**Results:** The addition of MeV F derived HRC peptides, even 72 hours post infection, effectively blocked the CNS adapted MeV variant for up to 45 days. These results highlight the efficacy of blocking the MeV fusion machinery in preventing viral spread in authentic tissue.

**Discussion and Conclusions:** For accurate antiviral evaluation, cells or tissue relevant for natural infection are integral to recapitulate host conditions, not only to elucidate pathogenesis mechanisms but also to assess antiviral effects. These studies were conducted with human motor neurons that represent one of the natural MeV target in CNS infection. The results show that fusion blockage can prevent neuron-to-neuron viral spread.

## ***P 205 – ID 093 - Molecular epidemiology of Rhinovirus/Enterovirus and their role on cause severe and prolonged infections in hospitalized patients***

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**Introduction:** Rhinovirus (HRV) and Enterovirus (EV) are two of the most common respiratory viruses, causing from mild to severe respiratory syndromes (e.g. pneumonia). Here we report the molecular characterization of HRV/EV strains detected in a hospitalized-based population.

**Materials and Method:** From September 2017 to December 2018, respiratory samples collected from upper (FLOQSwabs™ collected in UTM) or lower (bronchoalveolar lavages) respiratory tract were tested for respiratory viruses using a set of laboratory developed multiplex real-time assays. All samples positive for HRV/EV were typed by sequencing the VP4-VP2 region.

**Results:** A total of 201 (8.0% of the total) patients were positive for a HRV infection. Median age of HRV-positive patients was 9 years (range 10 days-96 yrs). In 41 cases (20.4%) HRV was detected in co-infection with other respiratory viruses. Peak of cases were observed in the Sep-Nov period of 2017 and 2018. HRV-A was detected in 118/201 (58.7%) patients, HRV B in 18 (9.0%), HRV-C in 39 (19.4%) and EV in 13 (6.5%), while no typing results was obtained for 13 (6.5%) cases. A total of 10 patients were hospitalized in intensive care unit and in 12 immunocompromised patients a prolonged infection (>30 days) was observed (median duration 62 days, range 17-316). Based on typing and temporal analysis, 3 HRV outbreaks were identified, mainly in neonatal and oncoematology wards, caused by HRV-A89, A49, C43.

**Discussion and Conclusions:** Clinical impact of HRV/EV infections is not limited only to a common cold but these viruses should be considered as significant respiratory pathogens.

## ***P 206 – ID 094 - High prevalence of Anelloviridae in patients with periodontal disease***

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**Introduction.** The Anelloviridae are an emerging family of infectious agents. Their genome consists of small, single-stranded, circular, negative-sense DNA with a size of 30 nm and their virions lack envelopes. Three genera of the family Anelloviridae are identified in humans: Alphatorquevirus (Torque Teno Virus, TTV), Betatorquevirus (Torque Teno Mini Virus, TTMV), Gammatorquevirus (Torque Teno Midi Virus, TTMDV). Human Anelloviruses, especially TTV, are characterized by extremely high prevalence in the general population, with relatively uniform distribution worldwide and an apparent pan-tropism at the host level. They have been associated with various diseases, including hepatitis, cancer, respiratory diseases, hematological and autoimmune disorders, and periodontitis. Aim of this study was to investigate the evidence of the association between Anelloviridae and periodontitis.

**Materials and Methods.** Literature search through PubMed, Scopus, Web of Science, GOOGLE Scholar was performed, using [“(Anellovirus” OR “Torque Teno”) AND “periodont\*”] as search terms. Observational studies reporting prevalence of any type of Anelloviridae, assessed with any sampling method, in periodontitis and healthy patients were considered. Prevalence ratio (PR) with 95% confidence interval (95CI) were extracted/assessed from each selected study and the pooled PR was calculated. Sensitivity analysis to virus type also was performed.

**Results.** Five studies were identified, one of them (Priyanka et al. J Indian Soc Periodontol 2017) was excluded because there was not a control group. Of the included studies, two investigated two specific TTMV species (TTMV-222, Zhang et al. Sci Rep 2016 and TTMV-204, (Zhang et al. Virus Genes 2017), one investigated all the three genera (Spandole-Dinu et al. BMC Infect Dis 2018), one investigated TTV (Rotundo et al. J Periodontol 2004). In three studies the sampling methods was gingival biopsy, while Spandole-Dinu et al. performed serum samples. The pooled PRs assessed with the fixed-effects model, chosen because within-study heterogeneity was low, were: Anelloviridae 1.31 (95CI, 1.20-1.43, 4 studies); TTV 1.28 (95CI, 1.17-1.60, 2 studies); TTMV 1.37 (95CI, 1.18-1.60, 3 studies); TTMDV 1.27 (95CI, 1.13-1.42, 1 study).

**Discussion and Conclusions.** This analysis was based on few studies, thus making it impossible the investigation of result robustness. The strength of the evidence is, consequently, limited. Nevertheless, periodontitis patients resulted 30% more likely to be infected with any type of Anelloviridae than healthy individuals, with few differences between genera. The present data suggest that Anelloviridae could be important periodontitis markers, or even causally associated to this disease.

***P 207 – ID 095 - Increased innate antiviral response and IL-17 and/or IFN- $\gamma$  producing T-cell subsets levels in gut mucosa of long-term-treated HIV-1-infected women***

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**Introduction:** The influence of sex on gut mucosal innate antiviral response and T-cell response in HIV-1 infection is still substantially unknown. We explored whether gut mucosal Toll-Like Receptor (TLRs), Interferon I (IFN-I) subtypes gene expression levels and the frequencies of IFN-gamma and/or Interleukin 17 (IL-17) producing CD4 and CD8 T subsets cells and those expressing CD38 and/or HLA-DR differed between *post menopause* female and male HIV-1-infected patients.

**Methods:** Thirty long-term-treated HIV-1-infected individuals and six age and gender matched healthy controls were enrolled. Gut mucosal gene expression levels were examined by RT/Taq Man assays; the frequencies of naive, T central memory and T effector memory CD4 and CD8 T cell subsets producing IFN-gamma and/or IL-17 and expressing CD38 and/or HLA-DR were evaluated by multiparametric flow cytometry.

**Results:** HIV-1 infected patients expressed higher levels of gut mucosal TLRs and IFN-I mRNAs compared to healthy individuals ( $p < 0.001$  for all genes). A sex-specific differential expression of TLRs/IFN-I components was recorded among HIV-1 positive patients, with females showing a significantly higher mRNA expression of TLR3, TLR4, TLR8, TLR9, IFN alpha2, IFN alpha14, IFN beta, and IFN epsilon than the men counterparts. Males and females also differed in their gut T-cell response, with women being characterized by higher Th1, Th17, Tc1, Tc17, and Th1/Th17 cells subset levels than men. By contrast, the percentage of gut mucosal CD4 or CD8 T cell subsets expressing CD38 and/or HLA-DR were comparable between HIV-1 infected women and men.

**Conclusions:** Sex-based differences observed in the gut innate immunity and T-cell response of HIV-1-infected patients might contribute to the disease dimorphism.

## ***P 208 – ID 104 - Evaluation of Human herpes virus 8 and Malaria coinfection in Uganda***

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**Introduction.** *Human herpes virus 8 (HHV-8)*, etiological agent of Kaposi's Sarcoma (KS), is an important disease in public health in Africa sub-Saharan countries, where also *Plasmodium falciparum* malaria is most prevalent. The malaria infection could have an impact on HHV-8 reactivation and this may influence the transmission of Kaposi Sarcoma Associated Herpes Virus (KSHV) in endemic areas. As known HHV-8 establishes a long life persistent infection as result of a delicate equilibrium between viral replication and the host immune responses, that may be influenced by other pathogens such as *Plasmodium falciparum*.

**Materials and Methods.** In this surveys were enrolled children from two different zones of Uganda: Kampala suburbs (Central-Southern Uganda) and in rural sites of Karamoja region (North-Eastern Uganda). Fingerpick blood samples and saliva samples were spotted on Whatman grade 1 filter papers at the time of the field survey and then air-dried before being separately stored in sealed plastic containers. From each sample, the presence of *P. falciparum* DNA was investigated by nested PCR and the presence of HHV-8 DNA was detected by Real Time PCR. Statistical analysis was performed with the application of descriptive methods (means, SD, and percentage) and 95% confidence interval.

**Results.** We analyzed a sample of 259 children with mean age of 7.1 (1<13) years. *P. falciparum* DNA was detected in 36.7% (95% C. I. 31.0 – 42.7) of samples, while HHV-8-DNA in 5.8 % (95% C.I. 9.8 – 24.4). The coinfection was detected in 8.3%.

**Discussion and Conclusions.** These data show that a decrease in host immune response due to coinfection, in this case malaria, could represent a possible risk factor for infection or reactivation of latent HHV-8. Further studies are needed investigating other Africa sub-Saharan countries.

## ***P 209 – ID 105 - Functional analysis of a SSPE virus indicate that hyperfusogenic fusion protein contributes to measles virus infection in the brain***

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**1. Introduction.** Measles virus (MV) infection at infant age remains the main risk factor for neurological related complications such as measles inclusion body encephalitis (MIBE) and subacute sclerosing panencephalitis (SSPE). Although mechanisms have yet to be established, fact is that MV may enter, replicate and disseminate in the central nervous system (CNS). Recently, during a MV epidemic in South Africa, MIBE was diagnosed in several HIV-infected patients and, years later, SSPE as well.

**2. Materials and Methods.** To investigate the genetic and functional underpinnings of MV neuronal infection, we analyzed viral sequences and proteins function of viruses isolated from brain tissue samples of individuals with MIBE or SSPE infected during the same epidemic, after the onset of neurological disease and compared with wild type B3 virus sequences from the original outbreak.

**3. Results.** We found that both the SSPE and the MIBE viruses had amino acid substitutions in the ectodomain of the F protein that confer hyperfusogenic phenotypes. Moreover, functional analysis of the fusion complexes confirmed that both MIBE and SSPE F protein mutations promoted fusion with less dependence on interaction by the viral receptor-binding protein with known MV receptors. While the SSPE F required the presence of a homotypic attachment protein MV H, in order to fuse, MIBE F did not. Both F proteins had decreased thermal stability compared to that of the corresponding wild-type F protein. Finally, functional analysis of recombinant viruses expressing MIBE or SSPE fusion complexes showed that F can promote fusion independently of H binding to known MV receptors.

**4. Discussion and Conclusions.** Our results suggest that alterations to the MV fusion complex that promote fusion and cell-to-cell spread in the absence of known MV receptors may be a key property for brain infection. We also demonstrate that hyperfusogenic F proteins permit MV to enter cells and spread without the need to engage nectin-4 or CD150, known receptors for MV that are not present on neural cells a key change that is likely responsible for neuroinvasion. In conclusion, our findings indicate that the emergence of hyperfusogenic MV F proteins is associated with infection of the brain, though the existence of an alternative MV receptor present notably on neurons is also suspected.

## ***P 210 – ID 121 - Performance of rapid tests in the management of dengue fever imported cases: retrospective analysis from a Regional Reference Laboratory in Lazio, Italy 2014-2019***

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**Introduction:** Dengue virus (DENV) is the most common cause of febrile illness among people seeking for medical care after travel to Latin America or Asia; in Europe, it is second only to malaria as the febrile illness causing most hospitalizations after return from abroad. Early diagnosis of dengue fever (DF) is important to differentiate infections with similar symptoms and for appropriate patient management. Rapid diagnostic tests (RDTs) may represent an effective tool to guide and shorten medical treatment decisions, but the performance of these assays may vary according to different epidemiological and laboratory background.

**Material And Methods:** We reviewed laboratory records of two dengue RDTs adopted in different periods at the Lazio Regional Reference Laboratory (SD Bioline Dengue Duo RDT, Colorimetric RDT and SD Biosensor STANDARD F Dengue RDT, Fluorimetric RDT), to retrospectively establish their performance. Diagnostic accuracy, sensitivity, specificity, positive and negative predictive values for dengue acute infection were calculated using as reference the results of molecular (RT-PCR) and serological (immunofluorescence, IFA) confirmatory tests. Overall performance, calculated considering the final case definition, was also included in the accuracy assessment of RDTs.

**Results:** Based on case definition, sensitivity and specificity were 87.2% and 98.8% for Colorimetric RDT, 94.7% and 95.9% for Fluorimetric RDT. IgM detection by either RDTs was highly concordant with IFA results.

Colorimetric RDT specificity was further calculated considering 24 cases of non-dengue arboviroses (i.e. Zika, yellow fever and chikungunya virus): NS1 and IgG were negative in all cases, while IgM resulted positive in two Zika cases.

**Discussion And Conclusions:** RDTs are of undeniable help in the identification of dengue infections, but confirmatory tests are needed to rule in or rule out dengue fever diagnosis. Our analysis supports dengue RDTs utility as first line tools for prompt case identification, clinical management, surveillance activities, and vector control strategies. The set-up of control measures being crucial to arrest infection spread and establishment in non-endemic countries where competent vectors are present.

## ***P 211 – ID 123 - Inhibition Of Human Rhinovirus By Endogenous Oxysterols In Nasal And Bronchial Histocultures From Cystic Fibrosis Patients.***

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**Introduction:** Rhinovirus (RV) is the most frequent etiologic agent of upper respiratory tract infections, causing 50-70% of common colds worldwide, and the life-threatening exacerbation and recurrent worsening of chronic lung diseases, including asthma, chronic obstructive pulmonary disease, and cystic fibrosis. Both the healthcare and socio-economic impact of RV substantiate the need for intensive antiviral research of highly active and wide-spectrum antivirals. Nevertheless, in the face of this remarkable burden, the genetic diversity and high mutation rate of RVs hampered the development of both prophylactic and therapeutic strategies. To overcome these limitations, we focused our research on host-targeting antivirals as potential candidates for the development of a wide-spectrum anti-RV drug. Enzymatic oxysterols represent an important family of physiologic molecules endogenously originated from cholesterol, acting as host-targeting, sterol-lipid effectors of innate immunity against viral infections. In this study, we investigated the anti-RV efficacy of two enzymatic oxysterol, namely 25-hydroxycholesterol (25HC) and 27-hydroxycholesterol (27HC), along with their mechanism of action.

**Materials and methods:** We tested the antiviral efficacy of 25HC and 27HC by focus reduction assay. The toxicity profile of both oxysterols, along with their 50% cytotoxic concentration (CC<sub>50</sub>), was assessed by cell MTS assays and LDH assays. The antiviral activity was further confirmed on 3D in vitro fully reconstituted human nasal and bronchial epithelia starting from samples of respiratory mucosa of cystic fibrosis (CF) patients. The step of viral replication inhibited by oxysterols, along with their ability to select oxysterol-resistant RV strains, was investigated by in vitro assays.

**Results:** 25HC and 27HC can block the infectivity of two different RV strains, belonging to group A and B, at 50% effective concentrations (EC<sub>50</sub>) in the low micromolar range, and are characterized by selectivity indexes (SIs=CC<sub>50</sub>/EC<sub>50</sub>) above 100. Interestingly, both oxysterols can block RV replication at two different stages of viral replicative cycle, respectively before and after RV release from late endosomes. More importantly, unlike direct-acting anti-RV molecules such as Pleconaril or Rupintrivir, neither 25HC nor 27HC select drug-resistant strains.

**Discussion and Conclusions:** These findings suggest that oxysterols of endogenous origin might be a primary host strategy to counteract RV infection. In particular, given their anti-RV activity, and their low potential to select resistant strain, 25HC and 27HC could be considered for the development of new therapeutic strategies against RV.

## ***P 212 – ID 131 - Lack of Marseillevirus DNA in immunocompetent and immunocompromised Italian subjects***

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### **1. Introduction**

Nucleocytoplasmatic large DNA viruses (NCLDVs) belong to Megavirales, a proposed new viral order divided in seven viral families: *Ascoviridae*, *Asfarviridae*, *Iridoviridae*, *Mimiviridae*, *Phycodnaviridae*, *Poxviridae* and *Marseilleviridae*. These viruses are unique both in size and genetic makeup, and frequently include genes of bacterial, viral and cellular origin. Marseillevirus is the sixth largest known double-stranded DNA virus boasting a 250-nm icosahedral capsid enclosing a viral genome of about 400.000 bases. Although firstly isolated in amoebae, recent studies reported the presence of Marseillevirus also in humans and suggest that T lymphocytes sustain its replication. Single-centre studies worldwide have estimated a low prevalence of Marseillevirus both in symptomatic patients and in healthy donors but, to date, no informations are available on the prevalence of this giant virus in Italy.

### **2. Materials and Methods**

A total of 575 selected samples [482 sera, 63 respiratory specimens, and 30 cerebrospinal fluids (CSF)] from subjects living in central Italy were studied. All samples were collected from December 2017 to November 2018. Of the 482 sera, 285 were from healthy donors and 197 from immunosuppressed patients. After viral DNA extraction, all samples were tested for the presence of Marseillevirus DNA by a conventional PCR assay targeting the highly conserved ORF152 region. A positive control ( $10^3$  copies of extracted DNA from supernatant of Marseillevirus positive cell cultures) was run in each PCR.

### **3. Results**

None of the samples resulted positive for Marseillevirus DNA. Technical failures due to the presence of PCR inhibitors were excluded by testing all the extracted samples for the ubiquitous Torquetenovirus (TTV) DNA: all of them scored TTV DNA positive.

### **4. Discussion and Conclusions**

Although we cannot rule out the possibility that Marseillevirus circulates in Italy at a very low level or be so genetically different from the primers used to pass unnoticed, our results speak for a very low prevalence of this virus in Italian population and contribute at maintaining doubts about the real circulation of this virus in humans.

## ***P 213 – ID 136 - Rapid determination of antiviral drug susceptibility of Chikungunya virus by RT-real time PCR***

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### **1. Introduction**

Chikungunya virus (CHIKV) is the etiological agent of a (re)emerging arbovirus infection and represents a serious health problem worldwide. CHIKV has no specific antivirals. The global situation of diseases transmitted by arthropod-borne viruses such as CHIKV, Yellow Fever (YFV), Dengue (DENV), and Zika (ZIKV) viruses is alarming and treatment of human infection by these arboviruses faces various challenges. The recent discovery of broad-spectrum antiviral molecules, able to inactivate different groups of viruses, is an interesting approach. Among them, we are focusing on drugs able to inhibit the human RNA helicase DDX3, which is involved in both cellular specific pathways and replication of several viruses. By blocking DDX3, these drugs could therefore reduce the efficiency of the viral replication.

### **2. Materials and Methods**

Antiviral activities of DDX3 inhibitors against a commercial strain of CHIKV were determined in HuH7 cell monolayers. CHICK infected cells were treated with different concentrations of antiviral molecules. After three-five days of cultivation, drug susceptibility of CHIKV was determined by measuring CHIKV RNA levels in culture supernatants using an in-house RT-real time PCR. This assay uses primers designed on the conserved nsp1 region of virus genome. Before viral RNA extraction, cell supernatants were treated with RNases to eliminate non-encapsidated viral molecules. Plaque inhibition assay was used as reference method to compare results and PCR performance.

### **3. Results**

Some DDX3 inhibitors significantly reduced CHIKV replication. The assay with RT-real time PCR readout permitted rapid, objective, and reproducible determination of CHIKV drug susceptibilities with no need for stringent control of initial multiplicity of infection. Furthermore, the quantitative assay results showed good correlation with conventional plaque reduction assay.

### **4. Discussion and Conclusions**

DDX3 inhibitors could represent an innovative therapeutic approach able to bypass drug-resistance typical of virus-specific antiviral molecules. The PCR assay described proved a suitable quantitative method for measuring susceptibility of CHIKV to antiviral drugs.

## ***P 214 – ID 140 - First large-scale epidemiological study on Torquetenovirus (TTV) DNA prevalence and loads in healthy donors***

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### **1. Introduction**

TTV is emerging as a marker of functional immune competence with potential to predict transplant-related adverse events and finally customize dosage of maintenance immunosuppression. In order to better standardize measurements and improve predictive powers, large-scale epidemiological studies are needed to understand how basal TTV prevalence and loads vary in immunocompetent healthy donors according to age.

### **2. Materials and Methods**

Plasma samples from 1014 healthy subjects aged 18-69, which were stratified according to age, sex, ethnicity, and previously confirmed or unknown fitness, were tested for TTV DNA by using a commercial quantitative real-time PCR assay. In order to assess relationship with previous immune system activation, a subcohort of 384 subjects was also tested for serum anti-CMV antibodies. In order to assess the best biological matrix for TTV viremia quantification, a subcohort of 100 subjects was also tested on a paired whole blood sample.

### **3. Results**

Overall TTV prevalence was 64% but highly related to age increasing by 10% every 5 years of age increase. In the virus-positive subjects, TTV viremia was in mean of 904 copies/ml or 2.3 Log, and weakly correlated with age ( $r = 0.135$ ,  $p < 0.001$ ) but not with anti-CMV serum IgG level. Anti-CMV antibodies significantly correlated with age ( $r = 0.310$ ,  $P < 0.0001$ ). Linear regression analysis with ANOVA using Log TTV viremia as dependent variable and anti-CMV IgG level, age and sex as predictors showed a predominant effect of age. Ten of the 34 healthy donors with TTV-negative plasma turned TTV-positive when whole blood was tested, while the 66 donors with TTV-positive plasma showed a mean 50-fold increase in copy numbers when whole blood was tested.

### **4. Discussion and Conclusions**

The study establishes prevalence and mean value of TTV viremia in a large cohort of healthy subjects and suggests that healthy ageing causes only minimal increases in TTV viremia. Since whole blood shows increased prevalence and titer of TTV, this matrix should be preferred in epidemiological studies.

## ***P 215 – ID 148 - Glia-neurons crosstalk during HSV-1 infection and its role in neuronal damage***

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**Introduction.** Herpes simplex virus type 1 (HSV-1) establishes a lifelong infection in the peripheral ganglia and, following periodic reactivation, may reach the Central Nervous System (CNS) where its replication has been associated to the induction of neurodegenerative processes typical of Alzheimer's disease (AD). Previous data showed that in vitro HSV-1 infection enhances neuronal appearance of AD hallmarks: production/accumulation of neurotoxic fragments of Amyloid Precursor Proteins (APP) and hyperphosphorylation of Tau protein; these data were recently confirmed in our in vivo recurrent HSV-1 infection model. It is known that abnormal activation of glial component of CNS (astrocytes and microglia) are involved in AD pathophysiology. Thus, it is reasonable to hypothesize that HSV-1-activated glial cells may concur to virus-mediated neuronal damage.

**Materials and Methods.** Primary cultures of neuronal and glial cells were obtained by E17 rat or mouse embryo brains. Confluent cellular monolayers were infected with HSV-1 (strain F) at a m.o.i. of 1 for 18 hours and analyzed by Western Blot to detect tau phosphorylation and APP fragmentation. HSV-1 titers were measured in conditioned media by standard plaque assay. Cytokines and chemokines production were evaluated by ELISA. Amyloid beta peptides production was investigated by confocal Microscopy assay.

**Results.** To investigate the glia-neurons crosstalk during HSV-1 infection and its role in neuronal damage, we used different HSV-1-infected neurons–glia co-culture models, and analyzed the occurrence of glia activation in terms of hyperproduction of reactive oxygen species (ROS) and proinflammatory cytokines. We carried out a time-course analysis of ROS production, and detected an increase with respect to uninfected single cultures, mostly during the first hours of HSV-1 infection. At the same time, we observed the onset of endoplasmic reticulum stress, along with NF- $\kappa$ B activation and consequent increased expression of pro-inflammatory cytokines. Furthermore, we also investigated the effects of virus-activated glia on HSV-1-induced Tau phosphorylation and APP amyloidogenic processing. We found that the presence of glial cells influenced Tau phosphorylation (particularly at Thr 205 residue) and A $\beta$  oligomer formation in HSV-1 infected neurons–glia co-cultures compared to infection of single cultures.

**Discussion and Conclusions.** Our data suggest that HSV-1 infection concurs to neurodegeneration also through the molecular crosstalk between glia and neurons.

## ***P 216 - ID 155 - Molecular typing of Adenovirus in pediatric patients***

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**Introduction:** Adenovirus (HAdV) infection usually occurs in children younger than 5 years of age with a mild and self-limiting course. In immunocompetent individuals the infection may cause acute febrile illness with respiratory and/or gastrointestinal symptoms and in rare case a number of clinical manifestations including keratoconjunctivitis, cystitis, hepatitis, myocarditis and meningoencephalitis. Differently, in immunocompromised patients, Adenovirus infection is often associated with high morbidity and mortality. Over 80 serotypes of HAdV have been identified and grouped, according to the symptomatology, in 7 subgroups (HAdV A-G). The main serotypes associated with human disease are C1, C2, C5, B3, B21, E4 and F41. The objective of our study was to evaluate the circulation of HAdV in the Bambino Gesù Children's Hospital in Rome (Italy), in relation to patient characteristics.

**Materials and methods:** From September 2017 to September 2018, 78 sequencing analysis of the hypervariable region of the HAdV exon gene was conducted on HAdV DNA extracted from different samples (blood, stools, urine, broncho-alveolar lavage, rhino-pharyngeal wash and swab), collected from 72 pediatric patients. According to the immunological status, patients were classified as 43/72 immunocompetent and 29/72 immunocompromised.

**Results:** We detected 10 genotypes: HAdV-A12 (4%); HAdV-A31 (8%); HAdV-B3 (4%); HAdV-B11 (1.5%); HAdV-B21 (1.5%); HAdV-C1 (21%); HAdV-C2 (47%); HAdV-C5 (7%); HAdV-C6 (3%) and HAdV-F41 (3%). Genotypes mostly associated with immunocompromised patients were A31 (21%) and C2 (45%) with immunocompetent patients were genotypes C1 (26%) and C2 (49%). Patients analyzed were between 0 and 18 years of age, the majority under 3 years (54%). Of these 82% showed HAdV-C infections, related to respiratory diseases. For 6 patients was possible to analyze samples collected from two different body district (stool versus blood or versus respiratory specimen), confirming the presence of one genotype in each patient.

**Discussion and Conclusions:** HAdV-C2 resulted the genotype more frequently detected in our population, being revealed in 21/43 immunocompetent patients and in 13/29 immunocompromised ones. It is noteworthy that genotype A31, that is not often described in bone marrow transplant patients, is very represented in our population. According to literature, in immunocompetent patients primary infection interested one district, while in immunocompromised patients there are a large number of systemic infections. We are going on in collecting data to evaluate the correlation between genotype and severity of the disease/immunological response. Moreover, Adenovirus typing is a useful tool for epidemiological evaluation of nosocomial outbreak.

## ***P 217 – ID 164 - Increasing severity of bronchiolitis during the last epidemic seasons associated to novel variants of respiratory syncytial virus A ON1***

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**Introduction:** Respiratory syncytial virus (RSV), the most common cause of infant hospitalization for bronchiolitis, is more variable than previously thought; genetic variability has an impact on its clinical severity. Since 2012, the novel genotype ON1 is the only RSV-A circulating at present; we demonstrated that infants hospitalized with ON1 bronchiolitis, overall experienced a clinical course milder than an older RSV-A genotype. However, we observed an increase in the hospitalization rate and in bronchiolitis severity of in the 16/17 and 17/18 epidemic seasons, with respect to the previous ones. The aim of this study is to characterize ON1 variability to understand virulence factors.

**Material and Methods:** Previously healthy infants hospitalized for bronchiolitis in the Paediatric Emergency Department, "Sapienza" University of Rome, from 2012-2013 through 2017-2018 were prospectively tested for 14 respiratory viruses using molecular techniques. About 100 RSV-positive bronchiolitis cases were sequenced from patients' nasopharyngeal aspirates in the attachment glycoprotein G gene including the conserved domain and the second hypervariable region. ON1 sequences were aligned with BioEdit and a phylogenetic analysis was performed with unique patients' and reference strains, with MEGA 6. Clinical and patient data obtained with a structured questionnaire were analyzed in correlation with genomic differences, using SPSS.

**Results:** A stratified analysis showed differences in bronchiolitis cases caused by RSV-A ON1 along the various epidemic seasons. Severity scores and hospitalization length, were significantly different among groups, indicating higher clinical severity in infants hospitalized in 16-17 and 17-18 with respect to the other epidemic seasons. The phylogenetic analysis showed several genomic variants appearing since 2012; some of them were not maintained later on but others evolved forming distinct clades with highly significant bootstrap values. Most strains sequenced during 16–17 and 17-18 set are characterized by several amino acid changes, some of which, never observed before, are located in escape mutant sites. Interestingly, the most divergent 17/18 cluster acquired a novel N-linked glycosylation site and strains belonging to the ON1 divergent lineage ON1-1.2 acquired around ten potential O-linked glycosylation sites with respect to the first ON1-1.1 strains.

**Discussion and Conclusions:** The ON1 strains causing more severe clinical course were characterized by both several amino acid substitutions and new glycosylation patterns, which may result in differential pathogenesis and immune escape. Considering that RSV strains are constantly evolving, a continued surveillance is important for clinical and immunoprophylactic strategies.

## ***P 218 – ID 180 - Temporin G, an amphibian antimicrobial peptide, effective against influenza and parainfluenza viruses***

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**Introduction:** Temporins are mildly cationic short peptides, synthesized by frog dermal glands, stored within granules and released by a holocrine-type mechanism upon stress or physical injury. While bactericidal activity of temporins has been widely described, very little is known about their antiviral efficacy. We have recently demonstrated the anti-herpetic effect of Temporin B, particularly its virucidal activity in *in vitro* model of infection. In this study, we investigated the potential antiviral activity of different Temporins against two respiratory viruses, influenza and parainfluenza.

**Materials and Methods:** Epithelial lung cells (A549) were infected with influenza A/Puerto Rico 8/H1N1 (PR8) or with parainfluenza type 1 (Sendai) viruses and treated with different concentrations of Temporins A, B, and G. The cytotoxicity was evaluated by Trypan blue exclusion and by MTT assay. Viral production was evaluated at 24 and 48 h post infection (p.i.), by means of haemagglutination assay, 50% tissue culture infectious dose and in cell western assay. Viral copies were quantified by RT PCR.

**Results:** Since TA was toxic at low concentrations it was excluded for the following experiments. TB and TG were not toxic until the concentration of 50  $\mu$ g/ml therefore both peptides were added at different times from viral infection (before, during or p.i.). TB was not effective at any concentration used, while TG significantly inhibited influenza virus replication (about 50% inhibition respect to untreated cells) when added during (ADS) or during and after (ADS+POST) viral adsorption. A strong reduction (about 70%) was also found against parainfluenza virus, especially when added after (POST) infection, thus suggesting a different mechanism of TG in blocking replication of both viruses. This trend was confirmed by analysis in western blotting of viral proteins only for PR8 infection. In fact, treatment with TG inhibited all proteins of about 50% respect to untreated cells. Unexpectedly, TG was not able to reduce Sendai protein expression, suggesting that it inhibited parainfluenza virus replication at post-translational level.

To evaluate an eventual direct effect of TG on the viral particles, the peptide was pre-incubated with each virus for 1 h at 37°C and the mixtures were used to infect A549 cells for 24 h. The results showed no inhibition either when TG was pre-incubated with the viruses or if added directly to cell monolayers for 2 h before viral infection, thus excluding its effect before infection.

**Discussion and conclusions:** Our results indicate that only TG was effective against both respiratory viruses, however since the peptide acts at different steps of virus life-cycle, further studies are needed to deepen the mechanism underlying its inhibitory effect.

## **P 219 – ID 185 - Environmental surveillance of Poliovirus circulation in Sicily**

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**Introduction:** Global Eradication of Poliomyelitis is promoted since 1988 by the World Health Organization (WHO) through vaccination campaigns that have obtained a massive reduction of the disease, which nowadays is limited only to Nigeria, Pakistan and Afghanistan. Since 1996, the Sub-National Reference Laboratory (SNRL) at PROMISE University Department in Palermo participates to the global polio eradication acute flaccid paralysis (AFP) active surveillance network, coordinated by the Italian Ministry of Health and National Institute of Health (NIH). Although Europe was declared "polio-free" in 2002, Italy is considered at risk of re-introduction of Poliovirus (PV) due to migratory flows. In accordance with the WHO guidelines, since 2013 environmental sampling campaigns have been performed in Sicily at Reception Centers for Immigrants and wastewater treatment plants, for the surveillance of the reintroduction of wild or vaccine-derived PV (VDPV).

**Materials and methods:** Wastewater samples have been collected for virological investigations according to standard protocols approved by the WHO, including: clarification and concentration of samples (centrifugation and sedimentation in dextran/PEG gradient); isolation of PV and non-polio enteroviruses on susceptible cell lines (L20B and RD); and characterization of positive samples (cytopathic effect, CPE) by molecular techniques (at NIH for confirmation).

**Results:** A novel environmental sampling campaign started in May 2019 providing for the collection of wastewater samples for virological analysis twice a month, and is planned to end in October 2020 after a total of more than 300 samplings. The Sicilian environmental surveillance network includes 5 Wastewater Treatment Plants: Piana degli Albanesi (PA), Pantano d'Archi (CT), Mili (ME), Syracuse and Trapani; 2 Hotspots: Rosolini (SR) and Pozzallo (RG); 2 Reception Centers for Asylum Seekers (CARA): Milo (TP) and Pian del Lago (CL). A dedicated website (<https://www.unipa.it/dipartimenti/promise/terza-missione/laboratori-sorveglianza>) has been created in order to share the guidelines for environmental surveillance, optimize the communication network between the sampling teams and the SNRL and publish surveillance updates.

**Discussions and conclusions:** A wide environmental surveillance network allows to better define, prepare and coordinate the actions to be taken in the event of a PV outbreak (involving both wild or VDPV). A higher frequency of sampling will improve the sensitivity of surveillance at demonstrating the absence of PV circulation. Constantly negative results for >12 months would consent to exclude PV reintroduction in the local population, but surveillance should be continued until the achievement of global polio eradication.

## ***P 220 – ID 196 - Human Herpesvirus 8 infection contributes to ROS production in diabetes type 2 patients***

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**1. Introduction.** The Human Herpesvirus 8 (HHV8), the causative agent of Kaposi's sarcoma, establishes a latent-persistent infection for the host's lifespan and causes intense and long-lasting alterations in the physiology of infected cells. HHV8 induces reactive oxygen species (ROS) at an extremely early stage of infection. Recently, HHV8 has also been associated to some widely diffused chronic diseases such as cardiovascular disease and diabetes mellitus type 2 (DM2). It is noteworthy that ROS, leading to oxidative stress, may have a key role in metabolic modifications induced by DM2. Interestingly, agents that increase or generate ROS result in stimulation of basal insulin secretion. Thus, we focused on the role of HHV8-infection in the alteration of the plasma redox status of a DM2 cohort.

**2. Materials and Methods.** Blood samples collected from DM2 and control subjects were screened for serological positivity to antibodies against HHV8. As biomarkers of oxidative stress, we determined the malondialdehyde (MDA), the more stable products of radical damage on the plasma lipid fraction, and more sensible and precise markers of the lipid peroxidation process such as fatty acid hydroperoxides (HP), 7-ketocholesterol, oxidation products of unsaturated fatty acids (UFA) and cholesterol, respectively. The level of plasma lipophilic antioxidant  $\alpha$ -tocopherol was also measured.

**3. Results.** Relevant differences were observed in the redox status in plasma from DM2 and either HHV8-positive or –negative control subjects. The level of the antioxidant  $\alpha$ -tocopherol significantly decreased in both DM2 and HHV8-positive subjects. Levels of the main lipid oxidation products (MDA, HP and 7-ketocholesterol) were much higher in HHV8-positive and DM2 subjects, indicating that plasma oxidative stress is a common feature in both DM2 and HHV8-infection. In addition, 7-ketocholesterol was further increased in HHV8-positive DM2 patients. No significant differences were found for cholesterol and unsaturated fatty acids.

**4. Discussion and Conclusions.** Our results support the assumption that DM2 metabolic alterations and endothelial dysfunction are strictly correlated to plasma oxidative stress and highlight a similar condition in HHV8-positive patients. Therefore, we hypothesize that the HHV8-infection may contribute to the production of ROS, and hence to the oxidative stress closely related to the pathogenesis and development of DM2. These results support the idea of HHV8 as an additional risk factor for DM2.

## ***P 221 – ID 200 - A bioinformatic tool for in-silico evaluation of the most suitable molecular tests to detect CCHF infection taking into account patient's travel history and geographic distribution of CCHFV strains***

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**Introduction:** Crimean-Congo hemorrhagic fever virus (CCHFV) is considered a major emerging infectious threat according to the WHO R&D blueprint. Reverse-transcriptase PCR (RT-PCR) is the method of choice for rapid laboratory diagnosis during acute phase of infection. A wide range of CCHFV molecular assays have been developed, employing varied primer/probe combinations. The high genetic variability of CCHFV often hampers the efficacy of available molecular tests and can affect their diagnostic potential. Recently, more and more complete CCHFV genomic sequences are becoming available, allowing to better appreciate the genomic evolution of this virus. We summarized the current knowledge on RT-PCR methods and we developed a new bioinformatic tool (CCHFV Primer Checker) to evaluate existing molecular assays in detecting different CCHFV strains.

**Materials and Methods:** Molecular methods have been obtained by a web-search in PubMed, with a deliberately relaxed query, in order to capture a large set of articles. All CCHFV genomes available by 31 December 2018 were retrieved from GenBank. All S segments including the complete coding region and with available data about host, collection country and collection date were selected. Phylogenetic analysis was performed with RAxM software. CCHFV sequences were clustered according to their geographic origin and relative genetic distance. An home-made program has been developed in python to evaluate molecular assay, and to investigate detection efficacy of assay combinations.

**Results:** Twenty-two RT-PCR methods were screened from 206 publications, and 181 complete S segment sequences were selected from 2,729 CCHFV records. CCHFV sequences were divided into nine phylogenetic groups, accordingly to previous works. Primers and probes of each assay showed a wide range of nucleotide mismatches within CCHFV strains (from zero up to 28 mismatches per assay). Only few cases of perfect match with all strains among a CCHFV group were found. Moreover, using combinations of up to three RT-PCR methods markedly increase the number of perfect matches, for most of CCHFV phylogenetic groups.

**Discussion and Conclusions:** In-silico PCR analysis confirms that assay sensitivity is strongly correlated to geographic area of virus origin and that no test available seems to be able to detect all known CCHFV strains. This assessment further supports the good practice for CCHFV detection of performing more than one assay, aimed to different sequence targets, to avoid exclusive reliance on a single assay and on a single target sequence. The choice of the most appropriate tests must take into account patient's travel history and geographic distribution of CCHFV strains.

## ***P 222 – ID 201 - Specific polymorphisms of the NS5A protein of hepatitis C virus genotype 1b are associated with hepatocellular carcinoma in cirrhotic patients***

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**Introduction:** Hepatocellular Carcinoma (HCC) generally arises after a stage of advanced liver cirrhosis with an annual incidence of 2-8%. The HCV protein, NS5A, is known to modulate viral fitness and to interact with different cellular proteins, including P53 and PI3K, thus inducing intracellular signaling pathways associated with cell proliferation and apoptosis. In this light, we examined the genetic variations in NS5A associated with HCC.

**Materials and Methods:** This study comprises 188 patients (pts) chronically infected with HCV-1b, all cirrhotic and DAA-naïve: 34 diagnosed with HCC and 154 controls without HCC. NS5A domain-1 (aa:1-183) sequences were obtained for all pts by Sanger method from plasma samples. Association of mutations with HCC was assessed by Fisher Exact test. Shannon Entropy (SE) was used to identify residues with significantly higher ( $P<0.05$ ) variability ( $SE>0.2$ ) in HCC compared to No-HCC.

**Results:** HCC pts had a comparable median (IQR) log serum HCV-RNA [5.6(5.3-6.1) vs 5.8(5.3-6.1)IU/ml], ALT[65(37-86) vs 71(50-112)U/l], and significantly higher liver stiffness [28(20-33) vs 19(15-26)KPa,  $P<0.001$ ] compared to No-HCC pts. By mutational analysis, four specific NS5A mutations significantly correlated with HCC: S3T(8.8 vs 1.3%,  $P=0.01$ ), T122M(8.8% vs 0.0%,  $P<0.001$ ), M133I(20.6 vs 3.9%,  $P<0.001$ ), and Q181E(11.8 vs 0.6%,  $P<0.001$ ). Multivariate analysis confirmed that the presence of  $\geq 1$  of the identified mutation is independently associated with HCC occurrence (adjusted OR 12.93, 95% CI 4.27-39.15;  $P<0.001$ ). Of note, HCC pts with  $\geq 1$  of these mutations tend to have higher HCV-RNA levels respect to HCC pts without mutation [median (IQR): 5.7(5.4-6.2) vs 5.3(4.4-5.6) log IU/ml,  $P=0.02$ ], suggesting their role in enhancing viral replications. By SE, other three residues were more variable in HCC: C13R/S( $SE=0.264$ ;  $P=0.03$ , located in highly conserved N-terminus NS5A-D1), F127L/S( $SE=0.225$ ;  $P=0.03$ ), and N137D/K( $SE=0.264$ ;  $P<0.001$ ). Moreover, an enrichment of additional mutations is observed at residue 181(Q181E/G/H/P,  $SE=0.410$ ;  $P=0.01$ ). Notably, all the above-mentioned residues are localized in regions of NS5A-D1 known to interact with cellular proteins as P53(aa:1-149), involved in the apoptosis regulation, and/or with p85-PI3K(aa:1-112), involved in Wnt/Beta-Catenin signaling pathway regulating the cell growth.

**Conclusions:** The association of specific NS5A polymorphisms with HCC provides a focus for further investigations aimed at elucidating the molecular basis of HCV-mediated oncogenesis. These viral signatures, if confirmed in a larger population, could play a critical role as prognostic markers of HCC, especially in cirrhotic-HCV pts, helping to identify pts at higher HCC-risk, deserving more intense liver evaluation and/or early treatment.

## ***P 223 – ID 202 - Surveillance of Norovirus and Rotavirus gastrointestinal infections associated with health care in Palermo***

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**Introduction:** Viral Acute Gastroenteritis (AGE) is an important cause of morbidity and mortality mainly due to Rotavirus A (RVA), in infant age, and Norovirus (NoV), across all ages. Enteric viruses can be easily transmitted in healthcare facilities, due to low infectious dose, environmental persistence, and increased person to-person contact. The aim of this research was to evaluate the role of NoV and RVA in the onset of healthcare-associated infections (HAI) and nosocomial infections (NI) in paediatric and geriatric patients in Palermo.

**Materials and methods:** Faecal samples and data were collected from patients admitted with AGE or developing AGE during hospitalization at the Paediatric Hospital “G. Di Cristina” or at the Geriatrics Department of the “P. Giaccone” University Hospital of Palermo. NI criteria were established based on admission, discharge, onset or recurrence of AGE symptoms. Faecal specimens were screened for RVA and NoV by RT-RealTime-PCR and positive samples were genotyped.

**Results:** From March 2018 to February 2019, faecal samples were collected from 413 paediatric and 20 geriatric patients with AGE. For 223 of these subjects (208 paediatric and 15 geriatric) anamnestic, clinical and epidemiological data were collected and 5 paediatric patients (2.4%) had a clinical and epidemiological history that fulfilled the criteria for NI. Of these, 3 were positive for NoV and 2 for RVA. When representative stool samples (131/433; 30.3%) were screened for NoV and RVA regardless of nosocomial origin of the infection, 20 (15.3%) were positive for RVA and 17 (13%) for NoV. In 2 samples (1.5%) RVA/NoV co-infection was found. Most of the RVA positive samples (16/22, 72.7%) were genotyped and the prevailing genotype was G3/P[8] (9/16, 56.3%), followed by G1/P[8] (3/16, 18.8%), G2/P[4], G3/nt, G9/P[4] and G9/nt (1/16, 6.3% each). Among the NoV-positive samples genotyped (17/19; 89.5%), GII.P16/GII.4 Sydney2012 (10/17, 58.8%) was prevalent, followed by GII.P21/GII.13 (2/17, 11.8%), GII.P16/GII.4, GII.P16/GII.2, GII.P7/GII.6, nt/GII.4 Sydney2012 and nt/GII.4 (1/17, 5.9% each).

**Discussion and conclusions:** Despite its relevance, the diagnosis of viral AGE is carried out in a limited number of hospital laboratories, and only a few specialized centres carry out their molecular characterization. In Italy, few data are available on viral enteric NIs acquired during and following health treatments. The information gathered in this study will provide a clearer epidemiological-virological picture on viral AGEs, allowing to evaluate the real burden of HAIs and identify subjects at risk and also helping to define guidelines and protocols for diagnostic purposes and for control interventions.

## ***P 224 – ID 205 - The integration of Hepatitis B virus is a frequent event in the setting of HBeAg negative chronic infection: implications for an altered cell metabolism and disease progression.***

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**Introduction:** HBV integration in human genome was mainly described in HCC patients (pts), proving its role in oncogenesis. Few data are available on HBV integration in HBeAg-negative pts with limited disease progression. Here, we aim to analyze HBV intrahepatic reservoir and integration in HBeAg-negative pts with no/mild liver fibrosis.

**M&M:** We analysed liver tissues from 40 HBeAg-negative pts classified as: group-1 (HBV-DNA<2,000IU/ml; n=8), group-2 (HBV-DNA 2,000-20,000IU/ml; n=14), group-3 (HBV-DNA>20,000IU/ml; n=18). cccDNA, intrahepatic total (it)-HBV-DNA were quantified by RT-PCR and pgRNA by digital-PCR. Exome Sequencing [Illumina, median(IQR) coverage: 115x (90x-140x)] was performed for 40/40 pts. HBV integration was recognized as chimeric HBV-human sequences applying a bioinformatic pipeline (Virus-Clip). The role of genes involved in HBV integration was analysed by GeneCards.

**Results:** Group-1 and -2 show a comparable intrahepatic HBV reservoir. Conversely, compared to group-2, group-3 is characterized by higher median[IQR] cccDNA (2.6[2.3-2.7] vs 2.0[0.9-2.3]log copies/1000cells, P=0.01), it-HBV-DNA (3.9[3.5-4.4] vs 3.1[2.2-3.9]log copies/1000cells, p=0.005) and pgRNA (190[7-770] vs 3.3[1.5-12] copies/1000cells, p=0.02).

HBV-DNA integration is found in all groups of pts with the highest prevalence in group 3 (55.6%, 10/18). Notably, HBV-DNA integration is evident in 14.3% (2/14) and 25% (2/8) of pts in group 2 and 1, respectively, despite lower viremia and a more limited HBV reservoir. Integration events are detected in 12 chromosomes without evidence of preferential chromosomal hotspots. Most HBV-DNA integration events (68.7%, 11/16) occurs within exon-flanking intronic regions, close to RNA splicing-site, regions critical for a proper mRNAs production. Notably, by gene ontology, HBV-DNA integration involves human genes regulating cell proliferation (NUP85, COL18A1, AGBL5, ANKRD52) and, thus, specifically involved in hepatocarcinogenesis and it also occurs in genes regulating lipid metabolism (NR3C1, CYP2UI, LMF-1) or antiviral/inflammatory responses (ISG, IFITM-1). Finally, a higher amount of serum HBsAg is the only factor correlated with HBV integration (p<0.001): HBsAg>5,000IU/ml identifies HBV integration with the best diagnostic-accuracy (83.5%), 92% sensitivity and 73% specificity.

### **Conclusions**

HBV integration occurs across all types of HBeAg-negative pts, despite low-viremia and limited liver disease. The localization of HBV integration suggests that this event is not restricted to carcinogenesis but may also be involved in mechanisms regulating hepatocyte metabolism and inflammation. Overall, our findings underline the complexity of HBeAg-negative infection and are a timely reminder that also these pts are at risk of disease progression.

***P 225 – ID 206 - Merkel cell Polyomavirus DNA detection in respiratory samples: study of a cohort of patients affected by cystic fibrosis.***

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**Introduction:** The role of Merkel cell Polyomavirus (MCPyV) as a respiratory pathogen is controversial and it is still unclear in patients with cystic fibrosis (CF). The aim of the study was to define the MCPyV prevalence and epidemiology in CF patients, to gain new insights into the association between MCPyV infection and respiratory diseases.

**Materials and Methods:** A 1-year-long study was conducted testing oropharyngeal aspirate samples from 249 and 124 CF and non-CF patients respectively. The detection of MCPyV was carried out by nested polymerase chain reaction (PCR). Moreover, a sequence alignment to examine viral capsid protein 1 (VP1) and a phylogenetic analysis were performed.

**Results:** MCPyV DNA was detected in 65 out of 249 samples analyzed CF (26%), a percentage that is higher than that recorded in non-CF patients (0.8%). There were no statistically significant differences in MCPyV prevalence according to gender, while there is a correlation between MCPyV detection and age. Interestingly, an association between the presence of MCPyV and the concurrent isolation of *Staphylococcus aureus* was found. Sequence analysis of MCPyV VP1 and phylogenetic analysis revealed a 99% homology with the published sequences of these viruses in GenBank.

**Conclusions:** Detection of MCPyV in CF patients' specimens pointed out a possible interaction between the virus and CF. Further studies are necessary to fully understand the involvement of MCPyV in the pathogenesis of respiratory disorders.

## ***P 226 – ID 210 - Nitazoxanide is an effective inhibitor of human rhinovirus replication in vitro***

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**Introduction:** Human rhinoviruses (HRVs) are ssRNA-nonenveloped viruses belonging to the *Picornaviridae* family. HRVs are responsible for more than 50% of upper airway infections, and are the leading cause of the common cold. HRVs are also associated with lower respiratory tract infections in immunocompromised patients, chronic obstructive pulmonary disease and asthma exacerbations. To date there is no effective antiviral treatment for HRV infections. Nitazoxanide (NTZ) inhibits the replication of a broad range of respiratory viruses in cell culture, including influenza and parainfluenza viruses. Herein we investigated the effect of NTZ and its bioactive metabolite tizoxanide (TIZ) on human rhinovirus infection *in vitro*.

**Materials and Methods:** HeLa R19 cells were infected with human rhinoviruses serotype 2 and 16 (HRV-A2 and HRV-A16) for 1 hour at 33°C under single-step (3 PFU/cell) or multi-step (0.01 PFU/cell) growth conditions. Cells were maintained at 33°C in DMEM culture medium containing 2% FCS. NTZ and TIZ, dissolved in DMSO, were diluted in culture medium, added to cells at different times before or after virus adsorption and kept in the medium for the duration of the experiment. Controls received equal amounts of DMSO. Virus yield was determined by TCID<sub>50</sub> assay or plaque assay, and cell viability was determined by MTT assay. Viral protein synthesis was evaluated by SDS/PAGE-autoradiography after [<sup>35</sup>S]methionine/cysteine-labeling, Western-blot and immunofluorescence analysis, and viral RNA levels were determined by qPCR.

**Results:** Nitazoxanide was found to inhibit the replication of HRV-A2 and HRV-A16 at non-cytotoxic doses, with EC<sub>50</sub>s ranging between 0.1 and 1.5 µg/ml, depending on the multiplicity of infection and the virus serotype. NTZ did not affect virus adsorption, but acted on an early event of the virus replication cycle, causing a block of both HRV RNA and protein synthesis. NTZ was also found to be cytoprotective in infected cells up to 48 hours after infection. Tizoxanide was similarly effective against HRV infection.

**Discussion and Conclusions:** Nitazoxanide is used in the clinic as a safe and effective antiprotozoal/antimicrobial drug and its antiviral activity was shown in patients infected with hepatitis-C virus, rotavirus and influenza viruses. The present results indicate that NTZ is effective also against HRV infection *in vitro*. Based on these observations, NTZ is undergoing Phase III clinical development for treatment of colds due to rhinovirus infection.

***P 227 – ID 211 - Polyomaviruses stool shedding in patients with hematological disorders: detection analysis and study of the non-coding control region genetic variability.***

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**Introduction:** Fragmented data are available on the human polyomavirus (HPyVs) prevalence in the gastrointestinal tract. Rearrangements in the non-coding control region (NCCR) of JCPyV and BKPyV have been extensively studied and correlated to clinical outcome; instead, little information is available for KIPyV, WUPyV and MCPyV NCCRs. To get insights into the role of HPyVs in the gastrointestinal tract, we investigated JCPyV, BKPyV, KIPyV, WUPyV and MCPyV distribution among hematological patients in concomitance with gastrointestinal symptoms. In addition, NCCRs and VP1 sequences were examined to characterize the strains circulating among the enrolled patients.

**Materials and Methods:** DNA was extracted from 62 stool samples and Q-PCR was carried out to detect and quantify JCPyV, BKPyV, KIPyV, WUPyV, MCPyV genomes. Positive samples were subsequently amplified and sequenced for NCCR and VP1 regions. A phylogenetic tree was constructed aligning the obtained VP1 sequences to a set of reference sequences.

**Results:** Q-PCR revealed low viral loads for all HPyVs searched. Mono and co-infections were detected. A significant correlation was found between gastrointestinal complications and KIPyV infection. Archetype-like NCCRs were found for JCPyV and BKPyV, and a high degree of NCCRs stability was observed for KIPyV, WUPyV and MCPyV. Analysis of the VP1 sequences revealed a 99% identity with the VP1 reference sequences.

**Conclusions:** The study adds important information on HPyVs prevalence and persistence in the gastrointestinal tract. Gastrointestinal signs were correlated with the presence of KIPyV, although definitive conclusions cannot be drawn. HPyVs NCCRs showed a high degree of sequence stability, suggesting that sequence rearrangements are rare in this anatomical site.

***P 228 – ID 217 - Effects of combination treatment with an inhibitor of I-kappa-B-alpha phosphorylation and azidothymidine on regulated cell death of HTLV-1 infected cells.***

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**Introduction:** We have previously demonstrated that azidothymidine (AZT) is endowed with an intrinsic pro-apoptotic potential towards PBMC from healthy donors as well as tumor cell lines, but that this cytotoxic potential cannot be fully achieved unless I-kappa-B-alpha (IkBa) phosphorylation is inhibited. Since constitutive activation of NF-kappa-B appears a common biological basis shared among untransformed or transformed HTLV-1-infected cells, we have investigated whether a pharmacological inhibition of IkBa phosphorylation could be a potential strategy for potentiating the efficacy of AZT to induce regulated cell death (RCD) in HTLV-1 infected cells.

**Materials and Methods:** As HTLV-1 infected cells, the chronically HTLV-1-infected human cell lines MT-2, C91/PL, C5/MJ, and the IL-2 dependent HTLV-1 infected cell lines, BM7 (CD4+CD8+, 53 weeks post infection) and BM24 (CD4+, 12 weeks post infection) immortalized in our laboratory, were utilized. For comparison, peripheral blood mononuclear cells (PBMC) from healthy adult donors, stimulated with and kept in IL-2 for 3 and 6 days, were also utilized. After preliminary experiments, 1 µM Bay 11-7085, as IkBa phosphorylation inhibitor, and 128 µM AZT, were utilized for the combination treatment. Apoptotic RCD was assessed by flow cytometry analysis following detergent treatment and propidium iodide (PI) staining. RNA isolation was performed using a NucleoSpin RNA kit, and RNase-free DNase. NF-kB activation was detected by non-radioactive EMSA. For quantization of the NF-kB protein p65, p50 and p52 binding, a commercial enzyme-linked immunosorbent assay was utilized. The transcriptional profile was assessed by SuperArray and subsequent specific confirmation by Real-time quantitative reverse transcription PCR.

**Results:** HTLV-1 infected cells of different types, as well as uninfected PBMCs, were low susceptible to apoptosis induced by single treatments with Bay 11-7085 or AZT, while the combination treatment induced increased levels of apoptosis with respect to single treatments, and, importantly, levels of RCD were considerably higher in HTLV-1 infected cells in comparison with the uninfected ones. Inhibition of NF-kB activation was confirmed by the different assays and the transcriptional analysis revealed that the addition of Bay 11-7085 to AZT treatment in HTLV-1-infected cells induced the upregulation of some pro-apoptotic genes together with the down-regulation of some anti-apoptotic genes with respect to single treatments.

**Discussion and Conclusions:** Our data suggest that addition of adequate concentrations of IkBa phosphorylation inhibitor to therapeutic regimens including AZT could be a promising strategy in HTLV-1 related diseases.



## ***P 229 – ID 228 - Age and genotype related Rhinovirus infection rate in Cystic Fibrosis patients attending a Regional Reference Center***

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**Introduction:** The bacterial colonization in Cystic Fibrosis (CF) patients airways is enhanced by the alteration of the normal mucociliary clearance and the dysregulation of the epithelial defense activity resulting from CF Transmembrane Regulator dysfunction. The role of respiratory viruses in the pathologic state of CF patients and related diagnostics is still poorly considered with respect to the importance given to bacteria. Among the acute viral respiratory CF infections, Rhinovirus (RV) is the most common cause of exacerbation and is associated with significant morbidity.

**Material and Methods:** Respiratory samples (n=515) were collected from the upper respiratory tract (oropharyngeal aspirate and sputum) of CF patients attending the Regional Reference Center for Cystic Fibrosis, Policlinico Umberto I, for routine visit during 2018. In addition to the usual microbiological investigation, RV was tested by RT-PCR in all samples. Participants were divided into 3 age groups according to the literature on microbiology setting and disease evolution: the children cohort (0-10 years; 131), adolescents/young adults (11-24y; 170) and the older cohort ( $\geq 25y$ ; 214). The genotype characterization resulted in homozygotes deltaF508, heterozygotes deltaF508/other and other mutations. RV infection was evaluated in relation to microbiological and patients data collected by clinicians, using SPSS.

**Results:** RV positivity was found in 80 out of 515 patients (15.5%) with a similar rate in all groups: 23/131 (17.5%) in the children cohort, 33/170 (19.4%) in the adolescent/young adults cohort and 24/214 (11.2%) in the older cohort. RV infection cases peaked in January in all groups, in contrast to seasonal prevalence in spring and fall in the general population. The RV infection in all age groups was not related to sex, body mass index and to the presence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*, alone or in coinfection. Interestingly, in the children cohort, RV infection occurred far more frequently ( $p=0.004$ ) in the deltaF508/deltaF508 (17/57; 29.8%) with respect to the other genotypes (2/39; 5.1% - 4/35; 11.4%); no correlation was found in the other groups. Moreover, no difference was found between groups in relation to microbiology, in each age cohort.

**Discussion and Conclusion:** In this preliminary study, we found an higher rate of RV infection in young CF patients with deltaF508/deltaF508 mutations. Since this genotype is associated with a more severe phenotype and that RV infections are likely one of the factors initiating the onset of CF lung disease, our results point to the importance to diagnose RV infections at least in winter months, and to possibly prevent them in an effort to reduce the overall burden of respiratory illness in CF patients.

## ***P 230 – ID 241 - Recent out-of-Africa migration of human herpes simplex viruses***

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**Introduction:** Herpes simplex viruses types 1 and 2 (HSV-1 and HSV-2) are ubiquitous human pathogens. Both viruses evolved from simplexviruses infecting African primates. It is commonly thought that HSV-1 and HSV-2 originated in Africa and migrated from this continent with their human hosts. Although this hypothesis is in line with the notion that herpesvirus lineages frequently originate through co-speciation events, it still awaits formal testing.

**Materials and Methods:** We applied different approaches on HSV-1 (n=140) and HSV-2 (n=141) circulating strains to test the African origin hypothesis and to analyze the time-frame of their worldwide dispersal. Population structure and geographic clustering were analyzed using STRUCTURE; we estimated nucleotide diversity ( $\theta_w$  and  $\pi$ ); geographic origin was inferred using the discrete model implemented in the BEAST software and the the BBM method; to account for the time-dependent rate phenomenon, dating was achieved by the application of a power law rate decay model to convert units of branch lengths from substitutions/site to time.

**Results:** Results indicated that HSV-1 populations have limited geographic structure and the most evident clustering by geography is likely due to recent bottlenecks. For HSV-2, the only level of population structure is accounted for by the so-called “worldwide” and “African” lineages. Analysis of ancestry components and nucleotide diversity, however, did not support the view that the worldwide lineage followed early humans during out-of-Africa dispersal. Although phylogeographic analysis confirmed an African origin for both viruses, molecular dating with a method that corrects for the time-dependent rate phenomenon indicated that HSV-1 and HSV-2 migrated from Africa in relatively recent times. In particular, we estimated that the HSV-2 worldwide lineage left the continent in the 18<sup>th</sup> Century, which corresponds to the height of the transatlantic slave trade, possibly explaining the high prevalence of HSV-2 in the Americas (second highest after Africa). The limited geographic clustering of HSV-1 makes it difficult to date its exit from Africa. The split between the basal clade containing mostly African sequences and all other strains was dated at ~5,000 years ago.

**Discussion and Conclusions:** In this study, we confirm the African origin of circulating HSV-1 and HSV-2 strains. The African origin does not necessarily imply that these viruses left the continent and dispersed worldwide during the major migratory events of early human populations. Our data do not exclude the possibility that herpes simplex viruses infected early humans, but show that the worldwide distribution of circulating strains is the result of relatively recent events.

## ***P 231 – ID 250 - Ancient and recent adaptive evolution of HCMV***

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**Introduction:** HCMV possesses the largest genome among human-infecting viruses and displays high genetic diversity. Cytomegaloviruses closely related to HCMV infect non-human primates (NHPs) with high species-specificity, indicating long-term virus-host coevolution. We performed a two-tier evolutionary analysis by searching for positively selected mutations that arose during HCMV adaptation to its human host and HCMV variants that are positively selected in circulating HCMV clinical isolates.

**Methods:** Molecular evolution analyses were performed to search for episodic positive selection on the HCMV branch (branch-site test, PAML models) or ongoing selection in HCMV populations (phylogenetic-population genetics approach, gammaMap). The functional effect of positively selected sites was tested by using a reverse-genetic approach (i.e., BAC mutants) or by expressing variant proteins in human cells.

**Results:** We found that 34 genes were positively selected during HCMV adaptation to the human host. Core genes were more frequently targeted by positive selection than non-core genes. Selection drove the evolution of three capsid proteins and of numerous tegument proteins that participate to an interaction network. Glycoproteins were also targets of positive selection, as well as core viral enzymes (e.g., UL70, the primase). Two UL70 selected sites were tested for their phenotypic effect in vitro using a BAC-based system in HHF and ARPE-19 cells. In both cell types, increased viral replication was observed for UL70 mutants compared to the wild-type, suggesting that these variants arose as a temperance strategy. Analysis of ongoing selection in HCMV clinical isolates found 32 positively selected genes. Isolates from urine and blood/plasma had more abundant and more divergent signals of positive selection compared to those from amniotic fluid. Positively selected sites were significantly enriched in protein regions exposed on the virion surface and in the extracellular domains of viral proteins located at the plasma membrane, indicating a major selective pressure by the host immune system. Positively sites were also significantly enriched within signal peptide regions. As a proof of concept, we tested the effect of positively selected sites in the UL144 signal peptide using time-course immunofluorescence. Results showed that amino acid variation at the selected sites alters UL144 intracellular dynamics by modulating its timing of association with the translocon.

**Discussion and Conclusions:** We show *that* the evolutionary processes that drove host adaptation do not necessarily parallel those that occur at the intra-species level. We provide a catalog of positively selected sites that are expected to modulate viral phenotypes, as the UL70 and UL144 experiments testify.

## *P 232 – ID 251 - Shift of HCV genotypes distribution in Sicily*

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**Introduction:** The HCV genotypes distribution show regional variations, as result of to the prevalent route of transmission and age of infection. A good understanding of HCV epidemiology should be required to develop strategies to eradicate HCV

**Materials and Methods:** Between 1/1997 and 12/2018, serum from 5692 consecutive patients with HCV infection were genotyping by a reverse hybridization of a line probe assay

**Results:** 41.8% patients were female and 58.11% male. Mean age was  $54 \pm 14.08$  (range 1–89 years). The distribution of HCV genotypes showed that HCV/1b, decreased from 81.2% in 1997 to 64.9% in 2018, with a parallel increase of the other genotypes: 1a (4.7% to 10.7%), 3a (5.9% to 8.7%), 2 (5.9% to 9.0%), 4 (2.4% to 5.2%). Genotypes 1b, 2 and 4 distribution do not show significant differences between men and women, while genotypes 1a and 3a have a greater prevalence in men. The distribution of HCV genotypes was compared in the periods 1997-2007 and 2008-2018. Genotype 1b was observed more frequently in the female in both periods and 1a and 3a genotypes were more frequent in males, with a statistically significant difference only for genotype 3a. Genotype 2 was more frequent in both periods in females, reaching statistical significance only in the second period. Genotype 4 was more represented in males in both periods, but without statistical significance

The prevalence of each genotype assessed by splitting patients into age groups: <25, 26-35, 36-45, 46-55, 56-65, 66-75 and > 75 years has shown that HCV/1b is predominant in all age groups (> 45%) and particularly in the age group 66-75 (78.9%); while genotype 3a shows a prevalence of about 20% in patients <25 years and 36-45 years, 10% in the 46-55 age group and 1.5-% in subjects > 56 years. Genotype 1a reaches 14% prevalence in subjects between 26 and 45 years. Genotype 2 show a prevalence >10% in subjects >46 years and 19.0% in patients >75 years. The prevalence of genotype 4 remains <5% in all age groups.

**Discussion and Conclusions:** The genotype 1b still remains the most widespread, followed by the genotype 2, especially in the elderly and with an evident cohort effect. The spread of these genotypes is linked to their almost exclusive presence in the general population several decades ago, when there were numerous infections with new iatrogenic transmissions. The cohort effect is also evident for genotypes 1a and 3a although involving the younger age groups. Despite the proximity and the notable migratory flows coming from the countries of North Africa where the genotype 4 is endemic, in Sicily this genotype shows a low prevalence. The surveillance of uncommon and low therapy responding HCV genotypes spread could provide very useful data for assess global HCV eradication goals.

## ***P 233 – ID 260 - Temporin G and B as new potent antiviral agents against HSV-1***

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**Introduction:** One of the most widespread human pathogens is the herpes simplex virus type 1 (HSV-1), an enveloped double-stranded DNA virus. More than 60% of people under age 50 has been found HSV-1 infected worldwide. Another common human pathogen is JC, a small and naked virus with double-stranded DNA genome enclosed by an icosahedral capsid. JC virus has an almost ubiquitous distribution with a worldwide seroprevalence of about 80%. HSV-1 infections are usually treated with antiviral agents such as acyclovir and its derivatives, but immunocompromised patients are unable to control HSV-1 infection and generally need long-term anti-herpetic therapy that could induce drug resistance. On the contrary, to date, drug therapies for JC infections are not available. Thus, in this context it is essential to develop novel anti-viral agents. We have recently demonstrated the virucidal anti-HSV-1 properties of temporin B (TB), a short and cationic peptide derived from frog dermal glands. Here we investigated the potential antiviral effect of another peptide (temporin G, TG), with similar length (13 aa), but different aminoacid composition, respect to TB.

**Methods:** To this aim, confluent monolayers of African green monkey (Vero) cells were infected with HSV-1 at a multiplicity of infection of 1 for 20 hours, while COS-7, simian kidney cells expressing SV40 Tag, were infected with supernatant containing JC virions corresponding to  $3 \times 10^5$  genome equivalents per milliliter (gEq/ml). Infected cells were subjected to time-of-addition assay and virucidal test with TG (50 µg/ml) or TB (20 µg/ml). Viral titers were quantified by standard assays.

**Results:** Our results demonstrated that TG treatment was HSV-1 virucidal like TB and, surprisingly, it was also able to almost completely inhibit the adsorption phase of HSV-1 life-cycle (more than 3 log inhibition respect to TG-not treated), as confirmed by adsorption and entry assays. Moreover, TG didn't alter the binding of the virus to the host cell by interfering with some receptors on the cell surface, as demonstrated by pre-treatment assay, suggesting that the peptide could bind one or more viral glycoproteins needed for viral attach to host cells. On the contrary, both TG and TB treatments didn't inhibit JC life-cycle or exert a virucidal effect, probably due to the absence of the viral envelope.

**Conclusions:** Overall, our data showed that both peptides interfered with the virus envelope, in particular while TB acted as virucidal agent on the envelope of HSV-1, TG inhibited also the adsorption of the virus, thus suggesting this latter as a more potent antiviral agent, that could both disrupt the HSV-1 envelope (like TB) and bind viral glycoproteins with a key role in virus attachment to host cells.

***P 234 – ID 269 - Influence of Sexually Transmitted Infections on seminal HIV levels among patients on ART: preliminary data of a case-control study.***

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**Introduction:** In the context of an undetectable viremia, seminal HIV-RNA and DNA can be detected after 6-months of ART, but no risk of HIV transmission is even reported. However, sexually transmitted infections (STI) are known to increase the HIV shedding in semen of ART naïve patients, and their role in influencing the HIV seminal compartment despite peripheral undetectable HIV-RNA is still unclear.

**Materials and methods:** This preliminary study includes 19 HIV-1 patients (pts) with undetectable viremia (<20cps/ml) for at least 1 year. At the enrolment, 9 are STI positive (cases: 7 syphilis, 2 Mycoplasma spp urethritis, 1 C. trachomatis), while 10 (controls) are STI negative. Pts are analysed for total HIV-DNA (detection limit 32cps/106CD4+cells) and residual viremia (detection limit 2 cps/ml) in both blood and seminal compartments by home-made protocols using ddPCR. Blood and semen specimens from cases are retrieved at baseline and after STI treatment.

**Results:** Pts are mainly MSM (89.5%) and HIV-1 infected by B subtype (73.7%), with a median (IQR) age of 35 (29-44) years. Median (IQR) CD4+ and CD8+ cell counts are 772 (576-1042) and 704 (626-927) cells/mm<sup>3</sup> respectively. All pts are on successful NRTI-based regimen (median [IQR] time of undetectability: 172 [104-270] weeks; 3rd drug: 10 INSTI; 4 NNRTI; 5 PI). No differences are found for these parameters between case and control groups. Peripheral total HIV-DNA is detectable in 17 pts (89.5%), with a median (IQR) value of 1157 (305-3433) cps/106CD4+cells. Differently, seminal total HIV-DNA is detectable only in 2 pts (10.5%, 1 case and 1 control), in both cases with a quantification <32cps/106CD4+cells. Peripheral and seminal HIV-RNA are detectable in 11 (57.9%) and 9 (47.4%) pts respectively. The HIV-RNA quantification in both compartments never exceeds the 20cp/ml (median [IQR]: 2.7 [<2.0-3.5] in peripheral plasma vs. 5.5 [3.5-8.5] in seminal plasma). Again no differences are found when HIV-DNA and RNA values in peripheral and seminal compartments are compared between cases and controls (P>0.50). However, 5 out of 19 pts (26.3%) show a seminal HIV-RNA positivity despite the peripheral HIV-RNA negativity. This discordance is more frequently observed in STI pts (4/9, 44.4%) respect to controls (1/10, 10.0%) (P=0.14). Six STI cases are analyzed at enrolment and after antibiotic treatment. Among these 2 (33.3%) maintain undetectable seminal HIV-RNA, 3 (50.0%) show a reduction (-3.4, -2.3, and -12.2) and only one (16.7%) experiences a seminal HIV-RNA increase to 12.1cps/ml.

**Discussion and Conclusions:** These preliminary data show that successful combined antiretroviral treatment avoids the presence of HIV-DNA in the seminal cells in the majority of pts, maintaining HIV-RNA in seminal compartment at non-relevant levels, despite STI.

## ***P 235 – ID 274 - Transcription profile of DNA damage response genes in naïve and cART-treated HIV-1 positive patients.***

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**Introduction:** The understanding of molecular mechanisms that support human immunodeficiency virus replication allowed to develop therapeutic approaches that effectively control viral replication and dramatically improving the survival of HIV-infected patients. Nevertheless, many aspects of pathogenesis and persistence of infection are still unknown. During the last years, significant progresses have been made in the delineation of mechanisms underlying HIV-induced cell death. Among these, viral integration plays an important role together with pre- and post-integration processes. The induction of cell-killing triggered during viral integration involved the activation of DNA-dependent protein kinase (DNA-PK), a central integrator of the DNA damage response (DDR), which caused phosphorylation of p53 and histone H2AX. The aim of this study was to evaluate whether virological suppression could affect mRNA expression profile of genes involved in DDR, by comparing cART-treated individuals with treatment-naïve patients.

**Material And Methods:** Eighty PBMC samples were obtained from HIV+ patients (40 treatment naïve and 40 cART-treated) and 30 sample from healthy donors (HD). All HIV-infected patients on cART had viral load (VL) <50 cps/mL. mRNA levels of FasR, XRCC1, LIG III $\alpha$ , Parp-1, DNA PKc were determined by qPCR (Brilliant II Syber Green, Agilent Technologies) and normalized on  $\beta$ -actin; results were expressed as fold-change that was calculated by  $2^{-\Delta\Delta Ct}$  method. Differences between the different groups were analyzed for statistical significance using Mann-Whitney U and Kruskal-Wallis H tests.

**Results:** A significantly higher expression of mRNA levels of Parp-1, DNA PKc, LIG III $\alpha$  and FasR was detected in HIV infected individuals than in HD [Parp-1 1.8 (p=0.001), DNA PKc 16.7 (p<0.001), LIG III $\alpha$  3.1 (p=0.006), FasR 3.7 (p=0.001)]. Furthermore, splitting the infected population into two groups based on the viral load (naïve and cART-treated), a significant overexpression of DNA PKc and FasR mRNA levels was confirmed in both groups [naïve: DNA PKc 32 (p<0.001), FasR 17 (p<0.001); cART-treated: DNA PKc 13 (p<0.001), FasR 2.9 (p=0.013)] while an higher expression of Parp-1 and LIG III $\alpha$  was maintained only in cART-treated individuals [Parp-1 1.9 (p<0.001); LIG III $\alpha$  2.8 (p=0.004)].

**Conclusion:** Collectively, these results showed that the expression levels of some genes involved in the DNA damage response (Parp-1, DNA PKc, LIG III $\alpha$  and FasR) are higher in HIV+ patients than in healthy donors. Surprisingly, no significant differences between naïve and treated patients were observed. Although many mechanisms of post-transcriptional regulation have to be considered, these data suggest that a cellular damage persists despite suppression of viral replication.

## ***P 236 – ID 276 - Comparison of antiviral activity of human DEAD-Box 3 helicase inhibitors by inhibition of plaque formation and reduction in virus yield assays***

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**Introduction:** DDX3 helicase is a 70 kDa DEAD-box protein, an ATP-dependent RNA helicase ubiquitously expressed in human tissues and is involved in a wide variety of cellular processes. DDX3 is envisioned as target for antiviral therapy because it required by several viruses (West Nile, Japanese encephalitis virus, HCV and others) to replicate. Most of these viruses are translated immediately after entry by IRES (Internal Ribosome Entry Site)-dependent translation. However, there are no reported cases in the literature where DDX3 is investigated during negative sense ssRNA viruses. To compare antiviral activity of anti DDX-3 drugs against different viruses we performed plaque inhibition and virus yield reduction assays against vesicular stomatitis virus (VSV), measles virus (MeV) and Coxsackie virus serotype 5 (CV-B5).

**Materials and Methods:** Viral inhibition assays were conducted both with approved drugs (Ribavirin and Aciclovir), and with two DDX3 inhibitors that had proved efficient in previous studies. Inhibitory concentration 50% (IC<sub>50</sub>) of drugs on viral replication was obtained by comparing both the reduction of plaques and viral yield reduction. The plaque assay was used to evaluate the antiviral activity of the compounds in a qualitative way: for Coxsackie B5, VSV and HSV-2, which are lytic viruses, crystal violet coloring was used, while for MeV, which does not allow the formation of plaques, an immunocytochemical staining method was developed. In order to confirm and quantify the effect of drugs on viral growth, titration assays were finally performed for viral yield in the presence of drugs.

**Results:** The results demonstrate that virus yield reduction assays are more accurate and reliable in determining IC<sub>50</sub> values because they allow to quantify effects that cannot be quantified in plaque reduction assays. Indeed, in plaque reduction assays do not allow to quantify those antiviral effects that only reduce plaque size but leave the number of plaques unaltered.

**Discussion and conclusion:** The data obtained strongly suggests that the virus yield reduction assay is a more appropriate method to evaluate antiviral drugs than the plaque reduction assay.

## ***P 237 – ID 281 - Microbiological Diagnosis Of Cap In Patients Attending An Emergency Department During The Influenza Season***

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**Introduction:** Community acquired pneumonia (CAP) is a major cause of morbidity and mortality among adults, especially the elderly. *Streptococcus pneumoniae* and *Haemophilus influenzae* are considered the leading causes of pneumonia, but several factors may have altered this paradigm such as the introduction of pneumococcal conjugate vaccines, and aging population with more chronic conditions.

Recent studies have shown a change in CAP epidemiology accompanied by advances in molecular diagnostic techniques that enabled to detect multiple viruses and bacteria simultaneously. So, respiratory viral testing offers a potential way forward combating antibiotic overuse, a major driver of antimicrobial resistance. On the other hand, the ever-increasing number of multiplex molecular assays can be disorienting and not of immediate use in clinical practice.

In this study, viral and bacterial agents were tested using molecular methods in patients with a clinical diagnosis of CAP, and the etiology was analyzed with respect to the clinical course, to evaluate the utility of syndromic diagnostic panels.

**Materials and Methods:** Consecutive adult patients attending Policlinico Umberto I University hospital emergency department (ED) from 15/1 to 22/2/2019 during the influenza peak, were enrolled using as the inclusion criteria a clinical diagnosis of CAP. Nasopharyngeal swabs (NPS) were tested for respiratory pathogens using a Real Time Multiplex Assay (FTD Respiratory pathogens 21 plus) for: influenza A/B; rhinovirus; coronaviruses NL63, 229E, OC43, HKU1; parainfluenza 1-4; metapneumovirus; bocavirus; respiratory syncytial virus (RSV); adenovirus; enterovirus; parechovirus; *Mycoplasma pneumoniae*; *Chlamydia pneumoniae*; *Staphylococcus aureus*; *S. pneumoniae*; *H. influenzae*.

**Results:** Seventy-five patients were enrolled: 32 men (median age 70 years) and 43 women (median age 80 years). Bacterial agents were detected in 19/75 NPS (25.3%), viral infections in 17/75 (22.7%), viral-bacterial coinfection in 8/75 (10.7%) whereas 33/75 (44%) tested negative. Influenza A was the virus more frequently detected (11/25 of which four cases were in coinfection with *S. pneumoniae*), followed by rhinovirus and RSV. Viral CAP presented a more severe cumulative clinical index (CURB-65, median: 3 vs 2; p=0.011) and more comorbidities (CCI, median: 5 vs 4; p=0.015) than bacterial CAP.

**Discussion and Conclusions:** In this study in CAP, negative results, rates of viruses, bacteria and coinfections were similar to those of recent papers. The use of a sensitive syndromic molecular panel did not lower much the rate of CAP with no detected agents. Nonetheless, these results showed a great burden of viral CAP, during circulation of Influenza, that could be also diagnosed with Point of Care tests.

***P 238 – ID 283 - A three-years-long congenital CMV universal screening: results from a single center experience.***

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**Introduction:** Worldwide, Cytomegalovirus (CMV) is a prominent cause of foetal infections (congenital CMV, cCMV). Most newborns who have acquired infection in utero have no evident clinical signs but up to 15% of them will develop important symptoms during their early life (sensorineural hearing loss, neurological delay, cognitive and visual impairment). Therefore, in absence of universal neonatal screening, asymptomatic infections are under-diagnosed. Aim of this study was to better understand how many cCMV infections are not recognized at birth and if a universal postnatal CMV screening could result in more and earlier diagnoses and, consequently, in better clinical outcomes.

**Materials And Methods:** Saliva samples were obtained within 48 hours from birth by placing a flocked swab on the inside of infant cheek. Swabs were immediately eluted in 500 µl of transport medium and stored at -20°C at the laboratory site till their evaluation. CMV screening was performed using an in-house rt-PCR: a 2-µl aliquot of the eluate was amplified without undergoing nucleic acid extraction. Positive saliva samples were eventually subjected to DNA extraction and re-assayed using a commercial test. cCMV infection was definitively confirmed by quantitation of DNA load in urine.

**Results:** Between October 2015 and September 2018 saliva samples were collected from 9429 newborns. Thirty-eight infants (0.4%) were found CMV-positive both at screening and confirmation tests and among them, 19 neonates (50%) were unexpected, having no history of CMV infection/reactivation during their mother pregnancy. Following cCMV diagnosis, infants were subjected to second level examinations (physical and neurological examinations, laboratory exams, neuroimaging, hearing test, visual examination, abdomen ultrasound) to identify the symptomatic ones. Interestingly, as much as 7/19 unexpected babies (36%) showed abnormalities and started antiviral therapy. Clinical follow-up is still ongoing.

**Conclusion:** Universal post-natal CMV screening in saliva is a feasible method to identify unexpected symptomatic cCMV infants who might benefit from early clinical care and antiviral treatment.

## ***P 239 – ID 285 - Dynamics of TT virus infection in a cohort of HCV-infected patients achieving sustained virologic response after treatment with direct-acting antivirals***

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**Introduction:** HCV infection is a major public health problem due to the association with increased global morbidity and mortality. Hence the need to treat patients with hepatitis C to achieve sustained virologic response (SVR), which from numerous studies has highlighted a decrease in mortality, a reduction in risk of hepatocellular carcinoma and finally the reduction of recourse to transplantation. The new second generation drugs with direct antiviral activity (DAA) represented a real revolution in the treatment of HCV hepatitis: a 12 to 24 week-regimen allows obtaining an SVR ranging from 90 to 97% for patients with severe fibrosis, and from 87 to 95% of patients with non-decompensated cirrhosis. However, there is still little data in the long-term follow-up of patients treated with DAAs.

The study aims to evaluate the pre- and post-treatment dynamics of TTV viremia, considered an effective indirect marker of immunocompetence and immunological remodeling, in patients with advanced fibrosis or compensated liver cirrhosis treated with DAAs regimens with and without ribavirin and with SVR 12 weeks after the end of therapy.

**Materials And Methods:** In the study, 250 patients who achieved an RVS after 12-24 weeks of treatment with different DDA drug combinations were evaluated. The TTV viremia was determined before and after the end of the treatment with quantitative method and related to a series of clinical, instrumental and laboratory parameters to evaluate any significant correlations able to predict the improvement of the clinical picture, the reduction of relapse and the onset of hepatocellular carcinoma.

**Results:** The overall prevalence of TTV positivity was >80%. Significant differences were found between the naive patients and those previously treated with interferon plus ribavirin, in co-infected with HIV and in the male sex. Post-treatment different parameters, such as the degree of fibrosis and cirrhosis, steatosis, the absence of diabetes and hypertension, the previous alcohol abuse, the combination with two DAA drugs, the modification of inflammation parameters and angiogenesis correlated with a significant change in plasma TTV levels.

**Discussion And Conclusions:** It is necessary to understand whether the patients who responded to the new anti-HCV treatment are experiencing a real benefit from the clinical point of view, the regression of liver disease and the reduction of the risk of developing hepatocellular carcinoma. The results demonstrate that the dynamics of TTV viremia can be useful in the prospective evaluation of the residual risk of evolutionary liver disease as an indirect parameter of immunological remodeling. Indeed, this is preliminary evidence to be confirmed in the long-term follow-up.

## ***P 240 – ID 286 - Impact of recurrent Herpes Simplex Virus type 1 infection on brain aging: a focus on epigenetic markers***

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**Introduction:** In the last decades, Herpes Simplex Virus 1 (HSV-1) infection has been proposed as a risk co-factor for Alzheimer diseases (AD), the most common form of dementia in the elderly. Aging is the leading risk factor for AD and, in the brain, this process is characterized by a slow change in multiple physiological functions and cognitive decline. Genome instability driven by epigenetic imbalance is one of the main features of neuronal aging. Following previous results of our group, we hypothesized that recurrent HSV-1 infection may affect the epigenetic mechanisms, thus accelerating the normal brain aging. To verify this hypothesis, we evaluated the levels of specific aging epigenetic hallmarks, such as histone 3 acetylation (H3K56ac) and the histone chaperone HIRA levels, in mouse experimental models of acute and recurrent virus infection. We also analysed the expression of two key epigenetic regulators, such as Sin3/HDAC1 complex, both involved in the regulation of HSV-1-host interaction.

**Materials and Methods:** Entorhinal cortex homogenates from HSV-1- and Mock-infected BALB/c female mice (HSV1-M and CTRL-M, respectively) were analysed in western blot for H3K56ac, and HIRA and Sin3/HDAC1 expression. A group of mice was analysed 4 days post primary infection (dpi), whereas the others were subjected to multiple thermal stress (TSs), to induce repeated virus reactivations and sacrificed after the 3<sup>rd</sup> and the 7<sup>th</sup> TS. A group of 4 mice were sacrificed just before the 7<sup>th</sup> TS (during latent infection). The virus presence in the brain was assessed by molecular analysis of viral gene/protein expression as well as by virological methods.

**Results:** We found that TS-induced virus replication caused HSV1-M a significant decrease in H3K56ac as compared to those observed in CTRL-M. We also found a significant increase in Sin3/HDAC1 protein expression levels with respect to CTRL-M, starting from 4 dpi, and a similar increase was observed following 7 TSs, that may be associated with the H3K56 acetylation decrease. These results, together with a significant increase in HIRA protein levels, were also found in mice sacrificed before the 7<sup>th</sup> TS, during the virus latency, showing the presence of aging epigenetic markers and a potential maintenance of these effects.

**Discussion and Conclusions:** Recent data from our group showed that recurrent HSV-1 infection in mice induces a time-dependent accumulation of AD hallmarks, including amyloid-beta and cognitive deficits. In this model of recurrent infection, we investigated an additional mechanism by which recurrent HSV-1 infection could affect the host normal aging. Overall these data suggest that recurrent HSV-1 infection may induce specific aging epigenetic hallmarks and accelerate brain aging, persisting over time.

## ***P 241 – ID 287 - Role of extracellular vesicles in HSV-1-driven brain damage***

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**Introduction:** Extracellular vesicles (EVs) are involved in cell-to-cell communication, spreading of intracellular proteins and miRNA. They are also used by viruses to pass from a cell to another one. A growing body of evidence supports the role of EVs in transferring neurotoxic proteins such as Abeta and Tau, key players in Alzheimer's disease (AD). Several data support the role of Herpes Simplex Virus-1 (HSV-1) infection in AD pathogenesis. We recently demonstrated the effect of multiple viral reactivations on the accumulation of Tau-related brain damages in an *in vivo* mouse model. Interestingly, brain slices from HSV-1 infected animals showed a higher number of phosphorylated Tau (pTau) positive cells with respect to virus positive ones. Recent studies reported that aggregated forms of Tau can be transmitted among neurons and that exogenous aggregates of Tau could enter inside cells acting as seeds for the aggregation of the endogenous protein, thus propagating Tau-dependent damages in the brain. Here, we investigated whether HSV-1 infection in the brain could promote virus diffusion and Tau spreading among neurons via EVs.

**Materials And Methods:** EVs were isolated from supernatants of human neuroblastoma and primary cultures of rat neurons following 24-48h of HSV-1- or Mock-infection, analysed in western blot (WB) or treated with Acyclovir (ACV) and anti-HSV-1 neutralizing antibody, and incubated on uninfected neurons for 24h. Cell lysates were then analysed in WB for pTau content and compared to untreated cells.

**Results:** Results showed that EVs derived from HSV-1-infected cells contained both viral proteins, and increased levels of pTau, suggesting that they are exploited for viral spreading, as well as for pTau propagation among neurons. Accordingly, cells incubated with EVs isolated from HSV-1-infected cells showed the occurrence of HSV-1 productive infection, as visualized by the use of fluorescent recombinant HSV-1 virus, which is maintained after the use of anti HSV-1 neutralizing antibodies. Moreover, we found higher levels of pTau in cells pre-treated with ACV and incubated with EVs isolated from HSV-1-infected cells, with respect to those detected in cells incubated with EVs isolated from control cells, indicating that exosomes derived from infected cells can promote Tau phosphorylation and likely its aggregation.

**Conclusion:** Overall, these data indicate that the virus can promote pTau propagation among neurons via EVs, as well as virus spreading, and support the hypothesis that repeated HSV-1 reactivations into the brain may concur to neurodegeneration.

## ***P 242 – ID 297 - HEV seroprevalence in HIV-1 infected patients in a Roman Hospital is associated with other viral hepatitis coinfection history***

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**Background:** An increasing number of hepatitis E virus (HEV) infection is being reported in Italy. HEV is a public health issue mainly in immunocompromised people. Few clinical data are published about HEV-infection in patients living with HIV in Italy.

**Materials & Methods:** Within a Roman-hospital, a prospective enrolment of HIV-1 infected patients for studying HEV-seroprevalence is on-going. Sera are tested for HEV-IgG/IgM by ELISA and HEV-RNA by real-time PCR.

**Results:** Up to date, we enrolled 372 HIV-1 infected individuals and analysed 289 with clinical information and serological/virological results. The median (IQR) age of the analysed population was 47 (38-57) years; 75% were male and 82% were Italians; the most frequent HIV risk-factor was heterosexual (49%), followed by homosexual (35%). The median (IQR CD4<sup>+</sup> count was 591 (427-828) cells/mm<sup>3</sup>; and 80% had a plasma HIV-RNA <50 copies/mL. The prevalence of other viral-hepatitis was: anti-HAV (93/175, 53.1%), anti-HCV (36/262, 13.7%), HCV-RNA<sup>+</sup> (8/262, 3.1%), HBcAb (90/264, 34.1%), HBsAg (8/266, 3.0%), and anti-HDV (1/210, 0.5%). 35 out of 289 patients (12.1%) were HEV-IgG<sup>+</sup>, none of them showed HEV-IgM<sup>+</sup> and HEV-RNA<sup>+</sup>. Of these, 80% were Italians and 77% were males. Among the HEV-IgG<sup>+</sup> individuals, the most frequent HIV risk-factor was heterosexual (57%), followed by homosexual (23%). Among 142 patients with complete viral hepatitis serology markers, HEV<sup>+</sup> patients were older compared to HEV<sup>-</sup> [(53 (45-61) vs 45 (36-55), respectively, p=0.055)]. Drug-abusers showed a trend of higher proportion in HEV<sup>+</sup> (18.8%) compared to anti-HEV<sup>-</sup>(9.5%, p=0.258). HEV<sup>+</sup> patients were associated with a history of other viral hepatitis. Overall, HEV<sup>+</sup> patients showed a higher proportion of at least one positive viral hepatitis marker (13/16, 81.2%) compared to those HEV<sup>-</sup> (69/126, 54.8%, p=0.043). In particular, HEV<sup>+</sup> patients showed a significantly higher proportion of HAV<sup>+</sup> (75.0%) compared to HEV<sup>-</sup> (47.6%, p=0.039) and HEV<sup>+</sup> patients showed a significantly higher proportion of HBcAb<sup>+</sup>(56.2%) vs HEV<sup>-</sup> (24.6%, p=0.015). Moreover, HEV<sup>+</sup> patients showed a trend of higher proportion of HBsAg<sup>+</sup>(12.5%) vs HEV<sup>-</sup>(1.6%, p=0.063). Concerning previous HDV exposure, HEV<sup>+</sup> patients showed a trend of higher proportion of HDV<sup>+</sup>(6.2%) compared to those HEV<sup>-</sup>(0.0%, p=0.113).

**Discussion and conclusions:** In this ongoing study, we observed a similar HEV-seroprevalence in HIV-1 infected patients (12.1%) to what observed in a recent study of blood donors from Lazio (12.3%) (Spada *et al.*, 2018). The finding of HEV-seroprevalence association with other viral hepatitis coinfection history in HIV-1 infected individuals deserves further investigation.

## ***P 243 – ID 300 - Identification of Human Papillomavirus (HPV) in fresh and paraffin biopsies.***

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**Introduction:** Human papillomaviruses (HPVs) are sexually transmitted viruses. Some HPV genotypes (high risk-HPV, HR-HPV) are responsible for the development of cervical cancer, anal cancer, head and throat cancers. They exhibit a strict tropism for cutaneous and mucosal epithelium. Therefore, the samples of choice for the investigation of HPV are cervical, vulvar, and vaginal swabs in women, genital and urethral swabs in men, anal swabs and oral rinse in both of them. The investigation of HPV in biopsies is less frequent than the type of samples shown above, because of the invasivity of the collection procedure. The aim of this study was to identify HPV in female and male biopsies from ano-genital and oral districts.

**Materials and methods:** 86 biopsy samples had examined: 56 fresh and 30 paraffin biopsies received by the Virology laboratory of the UOC of Microbiology, Virology and Parasitology, AOU Policlinico “P. Giaccone” between February 2017 and May 2019, with HPV test request. In particular, we analyzed: 13 biopsies of female genital district (cervical, vaginal and vulvar), 9 biopsies of male genital district (penis, scrotum, foreskin, glans), 13 anal biopsies of women and men and 51 biopsy samples from the oral district (tongue, gums, palate, epiglottis, salivary glands, tonsils, larynx, pharynx, trachea) of women and men.

**Results:** Of 86 biopsies analyzed, 30/86 (35%) revealed the presence of HPV, in particular: 8/13 (61%) female samples from genital area, 5/9 (56%) male genital samples, 11/13 (85%) anal samples and 6/51 (12%) oral samples were HPV positive. Among these, we have been found that 1/13 (8%) in female genital samples, 1/9 (11%) in male genital samples, 2/13 (15%) in anal samples had multiple infections. All multiple infections contained at least one HR-HPV. No HPV multiple infections were found in oral samples. Single HPV infections were mostly low risk-HPV genotype (LR-HPV) except in 3/13 (23%) anal samples and in 4/51 (8%) oral samples where we found HR-HPV.

**Discussion and conclusions:** HPV is a well-established causative agent of malignancy of the cervix and a common sexually transmitted infection. Our study shows that all the samples with a history of low or high-grade lesions were HPV-positive. The presence of HR-HPV found is higher in the anal district (38%) than the others (8% in female and 15% in male samples, 8% in oral samples). Similar to cervical cancer, most anal cancers (89–100%) are induced by persistent infections with HR-HPV. To date there are no screening programs for anal, oral and male genital cancer. For this reason it would be important to analyze the presence of HR-HPV when precancerous lesions are removed. This could be a good starting point in preventing the onset of high-grade HPV-related injuries.

## ***P 244 – ID 305 - Combining innate immune and epigenetic modulators to reactivate latent HIV-1 reservoirs***

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**1. Introduction:** The presence of T cell reservoirs in which HIV establishes latency by integrating into the host genome, represents a major obstacle to a cure and has prompted the development of different strategies aimed at eradication of virus from latently infected cells. The “Shock and kill” strategy is one of the most pursued approaches directed towards the clearance of infection; although several Latency-Reversing Agents (LRAs) have shown promising reactivation activity, they have failed to reduce the cellular reservoir. Here, we evaluated a novel immune-mediated approach to clear latent HIV infection, based on the combination of innate immunity stimulation and epigenetic reprogramming.

**2. Materials and Methods:** The latency reversal activity of the cyclic di-nucleotide cGAMP and the FDA-approved Histone Deacetylase Inhibitor Resminostat was assessed in the J-Lat 10.6 and ACH-2 models of latency *in vitro*; Jurkat E6.1 and CEM A301 cells were used as uninfected control to evaluate the ability of these compounds to induce selective killing of infected cells. The experimental conditions tested *in vitro* were first replicated in a primary model of HIV latency, the CD4<sup>+</sup> T central memory (T<sub>CM</sub>) cells, obtained from healthy donors and infected with HIV-1 luciferase reporter virus, and finally in PBMCs obtained from HIV-positive patients on antiretroviral therapy (ART) from Policlinico Umberto I University Hospital.

**3. Results:** We found that the STING agonist cGAMP was able to induce latent HIV-1 reactivation by activating the NF-κB pathway. In addition, the combination of cGAMP and Resminostat resulted in a significant increase in HIV proviral reactivation and specific induction of apoptosis in HIV-infected cells *in vitro*. A reduction in HIV-harboring cells was also observed in CD4<sup>+</sup> T central memory (T<sub>CM</sub>) cells, a primary model of latency, upon stimulation with Resminostat and cGAMP, which induced high levels of selective cell death following reactivation. Finally, significant levels of cellular-associated HIV-RNA were found in PBMCs obtained from individuals on suppressive ART treated with Resminostat or cGAMP, although no synergistic effect was detected with the combination.

**4. Discussion and Conclusions:** Despite the ability of several LRAs to reverse HIV-1 latency, no functional cure has been achieved so far. Here we showed that a combination of two LRAs potentiates the latency reversal activity and induces apoptotic cell death in HIV-harboring cells. Collectively, these results represent a promising step towards HIV eradication by demonstrating the potential to exploit the immune system to reactivate latent HIV and induce specific killing of HIV-infected cells.