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ABSTRACT BOOK

Sommario

26/09/2018 – CORSO – PRECONGRESSUALE

SEPSI: LA DIAGNOSTICA RAPIDA NELLA MICROBIOLOGIA DEL FUTURO

Diagnostic and prognostic role of biomarkers during clinical sepsis

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*Biomarkers are defined as characteristics that are objectively measured and evaluated as indicators. Following "*The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3)" *Sepsis is* as life-threatening organ dysfunction caused by a dysregulated host response to infection. Mortality rate observed in patients with sepsis may achieve 33.2%, while in patients with septic shock and other complications, mortality rate of 60% or higher is often estimated. Sepsis –associated health care expenses is constantly increasing. Thus biomarkers of sepsis which allow to establish an early diagnosis, an accurate prognosis and a very useful antibiotic stewardship are of paramount importance in the practice of intensive care units. Assessment scores (SOFA, APACHE and others) are excellent and often used tools to determine the extent of patient's organ dysfunction, however are not useful for early diagnosis and prognosis in septic patients. Single biomarkers (C-reactive protein, Procalcitonin and Presepsin, as well as pro-inflammatory and anti-inflammatory cytokines) are frequently elevated during the first hours of other critical non-infectious severe diseases such as systemic circulatory accidents (strokes, myocardial infarction and non-infectious complications of long and invasive surgery). Therefore up-to-date investigations on sepsis complex pathophysiology suggest the use of combination of at least 3 biomarkers (e.g. IL-6, PCT, and sTREM-1), in septic critical patients. Also precision medicine based on 3 sepsis endotypes (Inflammopathic, Adaptive and Coagulopathic) is a very recent approach. Such novel way to assess sepsis needs to evaluate a variable number (ranging from 4 to 100) of gene expression signatures, in order to include each septic patient in one of the sepsis subtypes, following laborious molecular data assessment by advanced computational methods. Cluster-based endotypes would suggest a personalized therapy for previously untyped sepsis patients, although diagnostic and prognostic aspects of endotypes warrant further investigations. In conclusion rapid phenotypic tests (e.g. IL-35, presepsin, [TIMP-2]·[IGFBP7] index) that meet the time-sensitive needs of patients with severe sepsis should be developed or further assessed, exploiting the wide experience and findings of the most recent sepsis research.

Execution, transport and preservation procedures of sampling for blood culture in cases of suspected sepsis: recommendations from a board of Italian experts

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Introduction. Bloodstream infection (BSI) is a major cause of death in developed countries and the diagnosis of BSI relies on the detection of pathogens, bacteria or fungi, in blood cultures (BC). BCs are still considered the gold standard for the diagnosis of BSI in order to achieve the pathogens identification and to assess their susceptibility profiles, although several rapid diagnostic tests have been recently developed. In the presence of a reasonable clinical suspicion of BSI, adopting proper methodological, technical, and organizational issues is strongly mandatory. The appropriate collection and quick transportation of BCs strongly influence the diagnostic yield of BCs. It is of paramount importance monitoring the entire pre-analytical process as recommended by the current guidelines.

Materials and Methods. A board of different specialists, sharing their clinical experience, elaborated a consensus national document on the whole BCs pre-analytical process, after a deep discussion of the available published guidelines in this field.

Results. Most of the existing guidelines are mainly focused on microbiology diagnosis and clinical management of BSI, whereas minor reviews are available that provide indications on the entire operational process of the pre-analytical step (from blood collection to the BC transportation to microbiology laboratory), and BC practices often deviate from there commendations. The available evidence suggests that the diagnostic yield of BCs is influenced by factors that can be classified in clinical aspects (such as correct ordering of BCs, timing of BC specimen collection, sampling strategies, and antibiotic treatment) and technical aspects (products for skin antisepsis, technical BS sampling methods, blood volume sampled, number of separate sets of BCs that should be collected, BCs storage, time to transportation, and safety of the operators).

Conclusions. The intent of this consensus national document is to provide a practical guide for Italian physicians and nurses on the best practice of the whole BCs pre-analytical process, including blood collection preparation, skin antisepsis, blood volume, technique and safety of the sampling, medium to be used, and time to transportation of BCs in cases of BSI.

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Elisabetta Nucleo

Klebsiella pneumoniae ed *Acinetobacter baumannii* Multi-Drug-Resistant (MDR) isolati dall'emocoltura di una paziente con storia di ospedalizzazione all'estero

Una donna di 62 anni viene operata d'urgenza in data 21/3/2016 in un Ospedale del Cairo, mentre era in vacanza a Sharm el Sheikh, per rottura di aneurisma ed emorragia cerebrale. Dopo il ricovero nel Reparto di Terapia Intensiva dell'Ospedale del Cairo, viene trasferita in Italia nel reparto Rianimazione dell'Azienda Sanitaria Ospedaliera "S. Croce e Carle" di Cuneo e successivamente in Neurochirurgia (20/4/16). Durante questa degenza, la paziente risulta positiva al tampone rettale per la ricerca di *Enterobacteriaceae* produttrici di carbapenemasi (CPE) e sviluppa una sepsi causata da *Klebsiella pneumoniae* ed *Acinetobacter baumannii* MDR. La paziente, in fase apparentemente di ripresa, in data 17/05/2016 viene trasferita nel reparto di Medicina Riabilitativa dell'Ospedale "S.S. Trinità" di Fossano. All'ingresso (17/05/2016) viene eseguito un tampone rettale per la ricerca di *K. pneumoniae* carbapenemasi produttrice (KPC). Il tampone viene seminato su piastre ChromID® Carba bioMérieux e le colonie positive confermate in biologia molecolare con il sistema GeneXpertCarbaR (Cepheid). Il tampone risulta positivo per un ceppo di *Klebsiella pneumoniae* OXA-48/NDM-1. In data 18/05/2016, in seguito al peggioramento della condizione clinica della paziente, vengono eseguiti urinocoltura e tre *set* di emocolture, due dei quali risultano positivi per *Enterobacter cloacae* OXA48 produttore. Viene iniziata la terapia antibiotica con imipenem e colistina (IMP S: 2 mg/L e COL S: <= 0.5 mg/L). L'urinocoltura risulta positiva per *E. coli* ES□L produttore. Nonostante le condizioni di salute migliorino, i tamponi rettali permangono positivi. In data 3/11/2016 la paziente viene dimessa e trasferita in una struttura di lungodegenza in provincia di Cuneo.

Appropriatezza delle indagini microbiologiche in paziente con peritonite secondaria

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STRATEGIA FARMACOLOGICA	*Terapia antibiotica specifica per batterio Gram negativo produttore di carbapenemasi e per batterio Gram positivi.*

Paziente uomo di anni 68, con fattori di rischio clinici per infezione da MDR, in shock settico da peritonite secondaria, post deiscenza di anastomosi per intervento di Myles effettuato 7 giorni prima. Rilevata colonizzazione da germe KPC produttore su tampone di sorveglianza.
Ammesso in terapia intensiva dopo re-intervento, inizio del processo diagnostico terapeutico che prende in considerazione i fattori di rischio con maggiore evidenza allo status di portatore da batterio produttore del gene KPC.
Rilevata co-infezione da *S. aureus* mediante indagini di biologia molecolare.
Inizio di terapia empirica di seconda linea e discussione sui report microbiologici e relativo antibiogramma per modulazione terapeutica.

Caratterizzazione molecolare di K.pneumoniae KPC e resistente a Ceftazidime/Avibactam

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SESSO	maschile
ETÀ	64
DESCRIZIONE E STORIA CLINICA	Leucemia mieloide cronica Insufficienza renale cronica Tamponamento cardiaco Batteriemia Diagnosi microbiologica: Batteriemia da *K.pneumoniae* KPC resistente a Ceftazidime/Avibactam
STRATEGIA FARMACOLOGICA	colistina 2.5 MUI x2/die meropenem 1gr x2/die ed ertapenem 1gr/die

Paziente maschio di 64 anni con leucemia mieloide cronica e insufficienza renale cronica, ricoverato presso l'U.O. di Malattie Infettive del P.O. "Annunziata" di Cosenza si presentava con tosse e dispnea. Dopo una settimana, la comparsa di dolore al fianco sx con sudorazione e astenia consigliava l'esecuzione di TC torace e addome, con esito di versamento pericardico. Trasferito presso l'U.O. di Cardiochirurgia del Policlinico Universitario Mater Domini di Catanzaro, veniva sottoposto ad intervento di sternotomia mediana con evacuazione della raccolta ematica pericardica. Durante il decorso post-operatorio veniva eseguita emocoltura da CVC con isolamento di *Klebsiella pneumoniae* KPC, con profilo fenotipico di resistenza a ceftazidime/avibactam e sensibilità a colistina.

Per studiare le basi molecolari della resistenza al ceftazidime/avibactam, in paziente naive a tale chemioterapico, è stata valutata la presenza dei geni KPC2 e KPC3 tramite PCR, ed è stata analizzata la sequenza nucleotidica ottenuta tramite tecnica di Sanger.

In conclusione, l'utilizzo di tecnologie molecolari incluso il sequenziamento ci è sembrato utile per un continuo e costante monitoraggio dell'insorgenza e diffusione di patogeni multiresist

27/09/2018 – VIRUS E TUMORI

Il virus dell'epatite B e carcinogenesi: meccanismi patogeni e prevenzione

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The hepatitis B virus (HBV) is responsible for about 50% of cases of hepatocellular carcinoma worldwide and this percentage can rise to 70% in the geographical areas where the infection is endemic. The risk of developing hepatocellular carcinoma is present not only in patients with chronic infection but also in patients with the so-called "serologically resolved" infection. Unlike other etiologies, 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. HBV pro-oncogenic potential can also be modulated by the viral protein known as HBx and by the production of aberrant forms of surface glycoproteins that accumulate in the hepatocyte and are able to induce oxidative stress and increased hepatocyte proliferation. Vaccination is an important strategy of primary prevention of hepatocellular carcinoma which has allowed to reduce the incidence of HBV-induced hepatocellular carcinoma cases. Despite this, some problems still remain to be resolved (suboptimal coverage, non-responders, circulation of immune-escape strains invisible to neutralizing antibodies) that hamper its total efficacy. As a secondary prevention strategy, antiviral treatment (based on inhibitors of HBV reverse transcriptase) can reduce the onset of liver cancer but cannot completely abrogate it. This is due to the fact that these drugs cannot inhibit the production of pro-oncogene proteins and integrated HBV-DNA, highlighting the importance of continuing monitoring for hepatocellular carcinoma even in patients who respond successfully to treatment.
Based on these assumptions, the availability of biomarkers that can predict the onset of liver cancer is crucial to identify patients at higher HCC-risk that may deserve intensive liver monitoring. This represents an unmet medical need, answering to the issue "assess host genetic and viral markers to determine prognosis and optimise patients' management" raised by European HBV Guidelines promoted by EASL (www.easl.eu).

Novel therapeutic target for high-risk HPVs

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Introduction: Identification of novel mechanistic insights into hrHPV immune evasion is critical for understanding how these viruses can persistently infect steadily unreactive cells and promote cancer. In a recent report, we demonstrated that downregulation of pattern recognition receptors (PRRs), mainly cGAS-STING pathway, in human papillomavirus (HPV)-18 harboring cells create an unreactive cellular milieu suitable for viral persistence, replication and tumorigenesis.

Materials, Methods and Results: We have showed that HPV18 persistence in keratinocytes leads to the inhibition of both type I and type III IFNs in response to DNA ligands, and this effect was mainly due to the suppression of cGAS-STING and RIG-I pathways by epigenetic modifications. We have identified SUV39H1 as the principal enzyme responsible for the accumulation of histone H3 containing a trimethyl group at its lysine 9 (H3K9me3) at the promoter regions of the aforementioned genes in NIKSmcHPV18 and HeLa cells. Using both pharmacological inhibitor (namely chaetocin) and siRNA technology against Suv39h1, we demonstrate 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 in Suv39h1-depleted-HeLa cells. In turn, Suv39h1 is targeted by the histone deacetylase SIRT1 and its activity is regulated by acetylation at lysine residue 266 in its catalytic SET domain. SIRT1 levels have been found increased in cells containing episomes of high-risk HPV types and in HeLa cells as well. Here, we show that also Sirt1 inhibition leads to changes in chromatin structure from activated to repress state at the promoter regions of the cGAS, RIG-I, and STING genes. Transient expression of HPV18 E6 and E7 oncoproteins in 293 cells allowed us to identify the E7 protein as the principle inducer of both Suv39h1 and Sirt1 activity. Gene silencing of E6 and E7 in HeLa cells corroborated this finding.

Conclusions: Consistent with recent data reporting frequent suppression of cGAS and STING in many types of human cancer, inhibition of this signaling pathway by HPV18 is consistent with a model whereby infected cells escape the attention of the immune surveillance system, acquire further genetic mutations, and eventually become transformed. The observed inhibition of cGAS–STING signaling may also help to clarify why cells harboring hrHPV infection do replicate, despite activation of the DNA damage response, which ordinarily arrests cellular replication. We also show that hrHPV-harboring cells treated with histone modifiers and PRR agonists regain the capability to produce IFNs. Overall, our findings provide new mechanistic understanding of virus-induced immune evasion that contributes to cancer progression.

EBV-associated lymphoproliferative disorders in immunocompromised patients

Fausto Baldanti, MD

EBV infection is associated with diseases both in its replicative and latent phases. In particular, is EBV latency that the virus fully expresses its oncogenic potential. EBV associated tumors range from poly-oligoclonal lymphoproliferations to a wide range of B and NK/T lymphomas to gastric carcinoma and leiomyosarcomas. In all cases, expression of EBV latency genes promote cell proliferation while inhibiting cell apoptosis. A crucial role is played by EBV-specific T-cell response in the control of the oncogenetic pathway. This control might be hampered in immunocompromised patients leading to a higher risk of EBV-associated lymphoproliferation, while virus-driven immunomodulation events may further increase the risk of limphomagenesis.

Inhibitors of cellular mechanisms essential for viral replication: an opportunity to treat viruses with no specific therapy

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Introduction. To replicate, viruses exploit the metabolic machinery, and proteins, lipids and other cellular compounds. Besides cellular molecules used by one or a few viruses, a large number of studies demonstrated that a large number of viruses, also of different families, usurp same cellular pathways, proteins and lipids. This finding and the demonstration that perturbation of one or more of these host-virus interactions reduce efficiency of viral replication, prompted experimental studies aimed to develop drugs specifically targeting cellular molecules that viruses use for their replication. If these host-targeted agents (HTAs) inhibit pathways used by many viruses, they may exhibit broad spectrum of activity, including viruses orphan of therapy. On the other side, by blocking a host molecule HTAs may also result toxic for cells themselves. For this reason and the difficulty of identifying efficient cellular targets, available HTAs are a few and with limited range of application.

Materials and Methods. New technologies and possibility to dissect molecular mechanisms with unprecedented precision and detail gave strong impulse to the quest for development HTAs, especially for novel and emerging viruses. By using one of such technologies and testing compounds specifically developed to inhibit a cellular enzyme, here we describe the path to the development of two novel classes of HTAs.

Results. Ablation of a cellular enzyme involved in a lepidic metabolism used by a wide array of viruses and at different steps of replication cycle, we have created cells deficient for this enzyme and that will be use as platform to analyze viral replication in a context of deranged lipid metabolism. Further, in a collaboration studies aimed to develop drugs blocking a cellular enzyme involved in synthesis of nucleic acids and many other documented activities, we are testing such drugs against Coxsackieviruses, viruses with no specific therapy largely diffused and causing a broad spectrum of clinical manifestations.

Discussion and Conclusions. New technologies and in-depth analyses of host-virus interplay are unveiling many cellular processes usurped by viruses. Specific and efficient HTAs blocking key point of interaction may therefore limit viral spread in the host and, ultimately, halt viral replication. On this ground, this talk illustrates recent progresses made on two novel targets that, being used by a large number of viruses, may provide rationale and room for developing broad spectrum HTAs.

27/09/2018 – INTERVENTO PREORDINATO SU MICROBIOLOGIA ALIMENTARE

Selection of indigenous yeasts for the improvement of Sicilian wine production

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1. Introduction

In wine production, yeasts transform grape sugars into alcohol, and produce key molecules for wine quality, as fermentation aromas typical of young wines, and glycerol, responsible for the smoothness and roundness in the mouth. Here we report the isolation and selection of new strains of *Saccharomyces* and non-*Saccharomyces* yeasts useful in winemaking.

2. Materials and Methods

We isolated yeasts from spontaneous fermentations carried out in old wineries of South-Eastern Sicily (for *Saccharomyces*), and from juice of grapes harvested in Western Sicily and Linosa Island (for non-*Saccharomyces*). We performed species and strains identification by molecular methods, phenotypic characterization by microbiological methods. Chemical analyses of wines produced in Sicilian wineries were carried out according to OIV official methods, identification of volatile organic compounds by Gas Chromatography-Mass Spectrometry.

3. Results

Starting from 930 *Saccharomyces* yeast colonies isolated from spontaneous fermentations, we identified 209 strains of *S. cerevisiae*, and demonstrated that many of them possessed good oenological characters. 14 strains were used in experimental winemaking with black grapes, three strains with white grapes and one strain in second fermentation in bottle: the selected strains were able to prevent the multiplication of unwanted spoilage microorganisms and to produce wines of excellent quality. The three strains giving the best results were used successfully as starter in industrial vinification of still and sparkling wines in four different Sicilian cellars.

Then we characterized 2575 non-*Saccharomyces* yeasts colonies from early stages fermentation musts and 14 *Starmerella bacillaris* (sin. *Candida zemplinina*) strains were isolated. Three were used during three vintage seasons for the production of experimental red wines with more glycerol (50% more on average) and less alcohol than those made with industrial *S. cerevisiae* strains. Afterwards four Sicilian wineries used one strain in red wine industrial production.

Finally, from 3939 isolates from the Linosa Island grapes, we identified 17 yeasts species. Among these, we used one *Kluyveromyces marxianus* strain for the production of more aromatic experimental white wines, thanks to the production of beta-glucosidase and esters.

4. Discussion and Conclusions

Industry is always looking for new yeast strains able to increase wine typicality and quality. In the European Union, use of genetically modified yeasts is not allowed and interest is strong for natural strains. After a patient work of selection, today we are able to provide wineries with yeast strains isolated in Sicily and able to produce high quality wines, including softer and rounder red wines and more aromatic white wines.

27/09/2018 – SESSIONE SPECIALE INFLUENZA:

PREVENZIONE, EFFICACIA VACCINALE E MECCANISMI PATOGENETICI

Dinamiche epidemiche dell'influenza stagionale e pandemica: trend storici e recenti

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Introduction. Influenza is a viral disease which is widespread worldwide. Human illness is due to to several virus types which are differentiated into a series of subtypes. Type A and B human influenza viruses are the most important causes of human disease, being characterized by typical epidemic dynamics. Inter-species passage of influenza virus strains from animals to humans may be the cause of severe pandemics.

Materials and Methods. To perform a review of the literature, to analyze Italian and international epidemiological and microbiological data, interpreting phylogenetic studies and epidemic dynamics.

Results. In the tropics or in subtropical areas, such as Hong-Kong, influenza cases and outbreaks may occur all the year round, while in temperate areas influenza tends to reemerge every year causing epidemic waves during the cold season. In recent years, a co-circulation of A/H3N2, A/H1N1, and a couple of B virus strains has been reported. The spread of seasonal influenza viruses is favored by the high level of population susceptibility to new virus strains which emerge continuously due to high frequency of small mutations in the virus RNA (so-called virus "drift"). When major changes (so-called "shift") in the virus genome occur - i.e., due to rearrangement of human and animal flu viruses or to cross-species passage of viruses from birds or swine to humans - a novel virus may emerge in the human population, and major epidemics or even pandemics then occur. This happened three times in the last century (H1N1 in 1918, H2N2 in 1957, and H3N2 in 1968) and again in 2009 (a swine H1N1 variant). Differently from seasonal viruses, pandemic viruses do not respect seasonality patterns and are not predictable. In recent years, bird viruses, such as the A/H5N1 and H7N9, have caused a number human cases with limited human-to-human transmission The low R_0 of these viruses due to the current low efficiency of inter-human transmission explains the lack of sustained transmission in human communities.

Discussion and Conclusions. Seasonal influenza causes a high burden of disease and financial costs that may be reduced by vaccination. Unpredictably, pandemic viruses may emerge from animal reservoirs, representing a global public health threat. Monitoring the circulation of potential emerging strains is important to implement early and appropriate intervention.

Virological surveillance of influenza: An epidemiological tool for vaccine formulation

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Virological Surveillance is the basis for attempts to control influenza in order to identify genetic / antigenic changes in circulating strains, but also to select future vaccine strains through the verification of the "match" with the formulation of the current vaccine, identify new viruses with pandemic potential and, to establish the seasonality identifying the period of active surveillance and also the period of activity for the control of influenza.
The Global Influenza Surveillance and Response System (GISRS) is a technical collaboration, coordinated by WHO, of existing institutions and networks, which bring together human and technical resources for rapid identification, confirmation and response to epidemic episodes of international relevance. The network provides an operating framework for connecting experts and technicians to maintain a constant alert of the international community on the outbreaks of epidemic outbreaks.
A continuously updated activity on circulation, typing and seasonality of influenza viruses with particular attention to the differences that occur each year between the two northern and southern hemispheres of the globe. However, based on the identification of the WHO Sub Regions, each sub-region has its own network.
The European Monitoring Network is coordinated by the WHO Influenza Center in London and, through the ECDC, supports member states, in enhancing the efficiency of the surveillance network.
In Italy, the NATIONAL CENTER FOR INFLUENZA (NIC) is located at the Istituto Superiore di Sanità and coordinates a network of national centers, whose objectives are, coherently with the supranational networks, to prevent and control epidemic and pandemic events, studying the circulation of influenza viruses (among ILI cases) in the population, evaluate the antigenic homology between epidemic strains and vaccine strains, evaluate the pathogenicity profile of circulating viruses, evaluate the degree of sensitivity / resistance of viral isolates to influenza drugs, provide to the International Bodies of Reference (WHO, ECDC) data on the characteristics of viruses circulating in Italy.
Surveillance data are collected from the 46th week of the year until the 17th week of the following year with the contribution of "Sentinel Doctors" which are General Practitioners (GPs) and Family Pediatricians (PLS).
However, in addition to the contribution to the national network, the role of virological surveillance assumes an added value for the local clinical epidemiology in order to define the epidemic curve and the type of genotypes circulating during the seasonal epidemic, the comparison of the type of circulating genotypes during the seasonal epidemic with the previous seasons, the definition of the degree of vaccination mismatch of influenza B-type viruses on an annual basis, of the seasonal impact data by age and type of influenza virus.
Ultimately, all the World's Surveillance Centers contribute with strains locally isolated to the final definition of the vaccine composition of both seasonal and tetravalent vaccines, up to the recommendation for the composition of influenza vaccines for the coming season that in the case of the northern hemisphere, was issued by WHO in February 2018 for the season 18/19 giving the start to the production of commercial lots of flu vaccines.

Universal influenza vaccine development strategies

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Introduction

Influenza viruses cause both sporadic pandemics and seasonal epidemics. The latter, result in about three to five million cases of severe illness and about 290.000 to 650.000 deaths per year worldwide. Currently available antivirals are burdened by drug resistance. The main weapon for fighting epidemics relies on prevention through vaccines. Despite the great effort of vaccines in reducing seasonal influenza diseases, they are still burdened by several drawbacks such as their time-consuming preparation; possible mismatches between predicted vaccine strains and drifted seasonal influenza isolates and the lack of their ready availability in case of pandemics. Under this light, the availability of universal flu vaccines targeting highly divergent flu isolates, certainly represents a compelling medical need. For this reasons, the study of virus hemagglutinin (HA) and the characterisation of its protective epitopes largely conserved amongst flu viruses is certainly one of the most ambitious approaches described so far for developing such a vaccine.

Materials and Methods

Protective epitopes on virus HA can be characterised through different approaches. The most promising so far consists in characterising the human B-cell protective response, endowed with broad range activity against a plurality of influenza subtypes, for identifying and structurally defining the neutralising epitopes on virus HA shared amongst virus isolates. The availability of both HA crystal structures and the identification of three dimensional moieties on HA recognised by broad neutralising human monoclonal antibodies (Hu-mAbs) represent the starting point for designing and validating mimotopes or modified HAs able to elicit cross-protection in immunised subjects.

Results

Several attempts performed to date for identifying neutralising epitopes shared amongst virus isolates on the HA-stem region, allowed to define possible "universal" flu immunogens. Giving the low variability of HA-stem, the so called "*headless approach*" was first described. Notwithstanding the high potential of such approach, the structure of the headless-HA resulted heavily impaired in its stability. Therefore, other most promising approaches involving the engineering of HA followed by validation with heterosubtype Hu-mAbs were performed with more success.

Discussions and Conclusions

The path taken years ago for designing novel heterosubtype "universal" flu vaccines is not ended right now. However, thanks to the availability of antibodies of human origin able to neutralise influenza viruses belonging to both phylogenetic Group 1 and 2 and to protect animals from virus lethal challenge, the validation and development of novel immunogens to be used as effective vaccines is now much closer.

Complicanze associate alle infezioni causate da Influenza A e B

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Introduction

The 2017/18 influenza season has been characterized by the uncommon early high rate circulation of influenza B virus. In fact, based on epidemiological surveillance data of ECDC the majority of influenza viruses detected were type B (63%), representing a high level of circulation of influenza B viruses compared to recent seasons.

Material and Methods

A total of 764 influenza laboratory-confirmed cases in ICUs patients have reported in Italy during the last influenza season and 172 (22.5%) of them occurred in the Lombardy Region. The typing of Influenza positive samples (A and B strains) and sequencing of HA gene were performed as previously described.

Results

Eighty-three out of 172 (48.3%) cases were typed as A/H1N1pdm09, 71 (41.3%) as influenza B, 1 (0.6%) as influenza A/H3N2 and from 10 (20.2%) no typing results were obtained. HA sequencing was performed for 58/83 (69.9%) influenza A and 33/71 (46.5%) influenza B strains. Forty-two (72.4%) Influenza A/H1N1pdm09 strains were characterized by the T120A change as observed. Overall, mutations (G/N/A) at codon 222 were observed in 5/58 (8.6%) influenza A/H1N1pdm09 strains. All influenza B strains belonged to the B/Phuket/3073/2013 clade and were characterized by L172Q and M251V changes.

Discussion and Conclusions

An upsurge of influenza B cases in patients admitted to ICU with a severe respiratory infections has been observed during the last influenza season and a rare case of myocarditis related with influenza B infection was observed in one patient. No molecular signatures associated with increased severity were observed among Influenza B strains. On the contrary, Influenza A/H1N1pdm09 virus strains harbored, also in samples of URTI, the more aggressive 222G/N mutations.

27/09/2018 – GRUPPI DI LAVORO

INFEZIONI ASSOCIATE A BIOFILM

The anti-inflammatory drug 6-mercaptopurine inhibits biofilm formation, cell adhesion and virulence in adherent-invasive Escherichia coli (AIEC) associated with 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 onset of the chronic inflammation typical of the disease. However, the effects of anti-inflammatory drugs used for CD treatment on AIEC virulence have not yet been investigated.

Materials and methods: We have tested the effects of amino-6-mercaptopurine riboside (6-MP), the active metabolite of azathioprine, one of the most widely used anti-inflammatory drugs in CD, on the virulence factors of AIEC strain LF82**.**

Results: 6-MP impairs its ability to adhere to, and consequently to invade, human epithelial cells. Notably, phagocytosis of LF82 treated with 6-MP by human macrophages is also reduced, suggesting that 6-MP affects AIEC cell surface determinants involved both in interaction with epithelial cells and in uptake by macrophages. Consistent with these results, 6-MP affects expression of cell adhesion-related genes, such as the *csg* genes, encoding thin aggregative fimbriae (curli). To further investigate whether 6-MP can indeed inhibit c-di-GMP signalling in AIEC, we performed biofilm and motility assays and determination of extracellular polysaccharides. 6-MP clearly affected biofilm formation and cellulose production, but also, unexpectedly, reduced cell motility, itself an important virulence factor for AIEC.

Discussion and conclusions: Our results provide strong evidence that 6-MP can affect AIEC-host cell interaction by acting on the bacterial cell, thus strengthening the hypothesis that mercaptopurines might promote CD remission also by affecting gut microbiota composition and/or physiology, and suggesting that novel drugs targeting bacterial virulence and signalling might be effective in preventing chronic inflammation in CD.

Which technique for the evaluation of the antibiotic susceptibility of bacterial biofilm? State-of-the-art and future perspectives

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Biofilms are organized bacterial consortia embedded in an extracellular polymeric matrix, produced by the bacteria and/or derived from the host, and attached to living or abiotic surfaces. The reduced antimicrobial susceptibility of sessile microorganisms, due to different resistance and tolerance mechanisms, makes treatment of biofilm-related infections very difficult thus promoting their persistence. Chronic biofilm-based infections have been found in almost all tissues of the human body, especially affecting patients with chronic wound infections and those with chronic lung infections such as cystic fibrosis. The lack of correlation between classic susceptibility test results and prediction of therapeutic success in chronic infections is probably due to the use of planktonically growing instead of biofilm-growing bacteria. Thus, there is growing interest in the development of laboratory models to evaluate, under conditions that more appropriately mimic the in vivo biofilm, the antimicrobial activity on biofilms: microtitre plate-based assays, substratum suspending reactors, flow cell system, microfluidics, alginate bead model, etc. However, studies establishing the clinical validity of these models are still lacking. Likewise, new pharmacodynamic (PD) parameters, including minimal biofilm inhibitory concentration, minimal biofilm-eradication concentration, biofilm bactericidal concentration, and biofilm-prevention concentration, were defined in recent years to quantify antibiotic activity in biofilms. Here we discuss: i) the need to introduce new standardized methods for antibiotic susceptibility testing of microbial biofilms in clinical practice to guide decisions about treatment; ii) the advantages and limitations of laboratory models proposed to assess the antibiotic susceptibility of biofilms; iii) the significance of PD parameters recently defined for predicting the *in vivo* activity of antibiotics on mature biofilms; and iv) the need for standardization of the methods - along with parameters and breakpoints, and the interpretation of results - before they are implemented in clinical microbiology laboratories for obtaining data relevant to the clinical setting.

New insights into the multi-factorial anti-biofilm activity of Sb-1 bacteriophage against Staphylococcus aureus

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Introduction: Sb-1 is a therapeutically relevant bacteriophage targeting *Staphylococcus aureus* (*Sa*). Previous experiments have shown that Sb-1 has synergic eradicating activity against *Sa* biofilm when used in combination with antibiotics. In this study, we further investigated the capability of Sb-1 to degrade the biofilm matrix and target "persister" cells of a methicillin resistant *Sa*, to understand the basis of its antibiofilm activity.

Materials and Methods: Following a 24h treatment with increasing Sb-1 titers (10^2 to 10^7 PFU/ml), the viability of biofilm-embedded *Sa* ATCC43300 was analyzed by isothermal-microcalorimetry. The matrix of the phage-treated biofilm was observed by confocal laser scanning microscopy following staining with wheat germ agglutinin conjugate with Alexafluor488 (WGA_{488}). Persister cells were either isolated by exposing *Sa* biofilm to high ciprofloxacin concentrations or induced by exposing *Sa* stationary phase cells to 400 µg/ml carbonyl cyanide m-chlorophenylhydrazone. 10^6 CFU/ml persister cells were treated with 10^7 and 10^4 PFU/ml Sb-1 for 3h and plated for CFU counting. Alternatively, phage-treated bacteria were washed and incubated in fresh medium for a further 24h.

Results: Although treatment with 10^7 PFU/ml Sb-1 determined a reduction of viable sessile *Sa* cells, the phage alone could not completely eradicate the biofilm. Interestingly, Sb-1-treatment was shown to affect the exopolysaccharide component of the matrix in a dose-dependent manner. Indeed, exposure to 10^6 PFU/ml Sb-1 resulted in the disruption of the matrix, as attested by the lack of signal emitted from the specific staining, although bacterial viability was not impaired. Moreover, the incubation with 10^7 PFU/ml Sb-1 determined a strong reduction of persister CFU/ml (2-5 Log). Interestingly, although 10^4 PFU/ml Sb-1 did not kill *Sa* persister cells in PBS, those cells were completely killed when bacteria were reverted to an active growing phenotype by transfer to fresh media.

Discussion and Conclusions: We have demonstrated that sub-inhibitory concentrations of Sb-1 target *Sa* exopolysaccharide matrix, and that this phage displays killing activity against *Sa* persister cells, by inducing either immediate "cell lysis from without" at high titer or delayed "cell lysis from within" by means of a "*Trojan horse effect*" at lower titers. Results obtained suggest that Sb-1 could adsorb on metabolically quiescent cells, inject its nucleic acid, and then induce the viral lytic cycle as soon as the cells revert to an active-growing phenotype, thus triggering cell death. This study provides valuable data on the matrix-disrupting and persister-killing abilities of Sb-1, which make this phage an interesting tool for alternative therapy of biofilm-associated infections due to *Sa*.

Pentadecanal inspired synthetic molecules as new anti-biofilm agents against S. epidermidis

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1. Introduction

Staphylococcus spp, including *Staphylococcus epidermidis*, are the prevalent species involved in orthopaedic device-related infections. A key factor contributing to the virulence of *S. epidermidis* is its ability to form biofilm. In this field, the interest in the development of new approaches for the prevention and treatment of bacterial adhesion and biofilm formation has increased. In a recent paper we reported the purification and the identification of pentadecanal able to impair *S. epidermidis* biofilm formation. Interestingly, this is the first piece of evidence that a long-chain fatty aldehyde is endowed with anti-biofilm molecule.

2. Materials and Methods

Pentadecanal derivatives used in this work were: pentadecanoic acid purchased from Sigma-Aldrich; methyl ester and dimethyl acetal were obtained from chemical synthesis. Biofilm formation of *S. epidermidis* was assessed by crystal violet and CLSM. Biofilm biomass was determined by COMSTAT software. Biofilm cell viability was also determined by the LIVE/DEAD® Kit. Synergy test of these molecules in combination with vancomycin was also evaluated by using checkerboard assay. Finally, their toxicity on eukaryotic cells was investigated (biocompatibility assay).

3. Results

The synthetized derivatives resulted to have anti-biofilm activity against *S. epidermidis* strains with different capability. This effect was further investigated by a CLSM analysis. Furthermore, the viability of cells encapsulated in the biofilm in the presence of derivatives confirmed the absence of any bactericidal activity.

The pentadecanal and its synthetic derivatives use in combination with antibiotics on mature biofilm were also explored. Results obtained showed that pentadecanoic acid modulated the antimicrobial activity of the vancomycin. In particular, the MBIC (minimal biofilm inhibition concentration) and MBEC (minimal biofilm eradication concentration) values were reduced by 2–fold after combination with pentadecanoic acid. To explore the clinical applications of pentadecanal and of its derivatives, their biocompatibility was investigated on fibroblasts and keratinocytes. All molecules resulted to be fully biocompatible under 50µg/mL.

4. Discussion and Conclusions

As the pentadecanal is an aldehyde (a chemically reactive agent), it could easily undergo oxidation reactions. One of the aims of this work was the design of some pentadecanal derivatives to enrich the arsenal of weapons to fight biofilm development.

This work endorses the pentadecanal and its derivatives as key molecules for the development of innovative approaches for the prevention and, in case of the pentadecanoic acid, for the treatment of *S. epidermidis* biofilm-associated infections in combination with an opportune antibiotic therapy.

Anti-biofilm efficacy of a non-ionic surfactant, Poloxamer 338, adsorbed on silicone urinary catheters

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Introduction: Poloxamers are amphiphilic tri-block poly(ethylene oxide)-poly(propylene oxide)-poly(propylene oxide) (PEO-PPO-PEO) copolymers available in different molecular weights. They are used in pharmaceutical and cosmetic formulations as surfactants, emulsifying, solubilizing and dispersing agents. Lately, poloxamer 188 and poloxamer 407 have been investigated as anti-adhesive coatings for poly(methyl methacrylate) (PMMA), polystyrene (PE), and silicone surfaces. Poloxamer 188 at 0.1% on PE and PMMA provided conflicting results against *Escherichia coli* and *Staphylococcus epidermidis* biofilms, respectively, while Poloxamer 407 induced 1 log reduction (90%) of *S. epidermidis* both in static (0.1% on PE) and dynamic (0.05% on silicone rubber) conditions. In this study, the ability of Poloxamer 338, adsorbed on 100% silicone urinary catheter, to prevent *Escherichia coli* biofilm development both in static and in dynamic conditions was investigated.

Materials and Methods: *E. coli* EC5-FSL strain isolated from urine of catheterized patient of Fondazione Santa Lucia has been used. Growth curves have been performed in presence and absence of 5 mg/ml (0.5%) Poloxamer 338 solution. Colony-forming units have been calculated by detaching EC5-FSL cells grown as biofilm on 1 cm-long segments of 100% silicone urinary catheter, as it is or left in contact with a solution of 5 mg/ml Poloxamer 338 for 6h. Atomic force microscopy was used to determine Poloxamer layer thickness. The ability of the Poloxamer 338 to interfere with adhesion process in dynamic conditions has been investigated with Bioflux system, by layering Poloxamer 338 onto the microfluidic silicone channel that mimics a urinary catheter. As control, microfluidic channel as it is was used. EC5-FSL (OD_{600} 0.25) was inoculated into the system and, after 1h adhesion, fresh medium has been instilled for 15 h. A time-lapse recording monitored biofilm formation.

Results: Poloxamer 338 in solution did not significantly influence *E. coli* growth, while, when layered on silicone catheter (30-40 nm thickness), it drastically reduced the ability of the EC5-FSL strain to form biofilm, with 0.8 $\log_{10}$ reduction. In dynamic conditions, *E. coli* is totally unable to adhere on Poloxamer 338-coated silicone channel, also after 24h washing time.

Discussion and Conclusions: The stronger antifouling property of Poloxamer 338 coating against *E. coli*, compared to Poloxamer 188, together with the higher stability of the adsorption over the time with respect to Poloxamer 407 and the startling ability to completely avoid (100%) *E. coli* adhesion in dynamic conditions makes Poloxamer 338 a safe and more effective alternative to control catheter related-urinary tract infections.

Antifungal activity of Bacillus amyloliquefaciens supernatants against Candida albicans biofilm

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Introduction- Biofilm-formation and fungal filamentation are well-recognized virulence factors in *Candida* pathogenesis, helping the fungus to invade host tissues and promoting its persistence. Modern medicine, characterized by a wide use of biomedical devices, offers new niches for opportunistic fungi able to form biofilm communities. Conventional antifungal drugs have been demonstrated to be ineffective against biofilms, and alternative strategies and/or drug targets are needed to overcome biofilm-dependent resistance.

Recently, some *Bacillus amyloliquefaciens* strains were reported to have probiotic activity, to be effective for the biocontrol of multiple plant diseases caused by soil-borne fungal pathogens, and to possess mycotoxin degrading ability. In this study, we evaluated the activity of secondary metabolites produced by *B. amyloliquefaciens* on *Candida albicans* biofilm.

Materials and Methods – *C. albicans* SC5314, a strong biofilm producer, was used in this study. Bacterial supernatants were obtained by centrifuging 48- and 72h-old *B. amyloliquefaciens* (*Ba*), strain TITI, broth cultures. Biofilm formation was induced by sub-culturing *C. albicans* in a defined medium in 96-well plate, for total biomass measurement by crystal violet staining, and on 18-mm-diameter round coverslips, for confocal laser-scanning microscope (CLSM) analysis. Gene expression of hyphal wall protein 1 (HWP1) and agglutinin-like sequence 3 (ALS-3), known to be relevant for *C. albicans* filamentation and biofilm-formation, was investigated by real-time PCR.

Results - We observed a dramatic inhibition of *C. albicans* ability to adhere to polystyrene surface by both 48- and 72h-old *Ba* supernatants. Moreover, *Ba* supernatants applied to 24-h preformed biofilms resulted in the detachment of most cells from the wells.

CLSM analysis with calcofluor white staining, targeting fungal chitin, showed alterations in morphology of the growing yeast that resulted in stubby hyphae unable to express typical thigmotrophic features. HWP-1 and ALS-3 were downregulated in the presence of *Ba* supernatant.

Discussion and Conclusions - *Bacillus* species are good producers of antimicrobial substances such as peptide and lipopeptide antibiotics, and bacteriocins that have become a highly effective alternative to traditional fungicides against environmental molds. *Ba* cyclic lipopeptides seem to belong to the surfactin, iturin and fengycin families that act as biosurfactants, perturbating fungal membranes. Our data demonstrate that such compounds could be also effective against medically relevant yeasts and could have potential uses in biotechnology and biopharmaceutical applications.

27/09/2018 – FOCUS ON

PNCAR, ICA E ANTIMICROBIAL STEWARDSHIP

Il ruolo del microbiologo nelle ICA - The microbiologist role in HAIs

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The Healthcare Associated Infections (HAIs) are acquired infections that can occur in all areas of care, including acute care hospitals, day-hospital / day-surgery, long-term care, outpatient clinics, home care, and nursing homes. HAIs are the most frequent and serious health care complications.
The microbiologist, a specialist with diagnostic skills, supported by the knowledge of infectious agents and their pathogenic power, antibiotic-resistance mechanisms and epidemiological events, has a professional heritage that finds practical application in the control and surveillance of infections.
The organization of a computerized microbiological surveillance and of signalling systems that make it possible to quickly monitor / communicate to the Operative Unit and to the Hospital Infection Committee (HIC) the identification of "sentinel" microorganisms and / or the detection of epidemic clusters appears to be essential. This allows both the rapid adoption of pre-established *ad hoc* measures for the containment of the infection, and the beginning of microbiological investigations (e.g. molecular typing) aiming the identification of the source (screening activity on patients, staff, and environment).
The elaboration of periodic reports to share data concerning the prevalence of ICAs, the microorganisms involved, and the related antibiotic-resistance phenotypes, then represent important tools for the knowledge of local epidemiology and the implementation of appropriate control measures.
The microbiologist must guarantee his / her active participation in the work of HIC, for the drafting of guidelines on behaviors, preventive interventions and measures of isolation / control to be adopted, and on the rational use of antibiotics in both hospital and community. Finally, the microbiologist is involved in the organization of training programs on the main methods of prevention / containment of infections, both at the intra-hospital level and in long-term care facilities on the territory, as well as urged to adhere to regional projects of active monitoring of infection and antibiotic-resistance.

27/09/2018 – FOCUS ON

VIRUS E MALATTIE NEUROLOGICHE

PML in HIV and in the course of molecular target therapies: two sides of the same coin

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Progressive multifocal leukoencephalopathy (PML) is a degenerative pathology with a fatal course, characterized by the formation of demyelinating lesions on the white matter. PML was first identified in 1958 when typical neuropathological changes were observed in the brains of neurological patients who died during lymphoproliferative diseases. In 1971 it was shown that the etiologic agent of the disease is the human polyomavirus JC Virus (JCV), whose oligodendrocyte lithic infection causes brain damage.
From the time of its discovery until the beginning of the AIDS epidemic, PML was extremely rare and only 230 cases were reported worldwide. The disease instead became very frequent in patients with AIDS to the point of being one of the most frequent opportunistic infections with a lethality among these patients that could reach up to 7% depending on the various cases.
The drastic decrease in AIDS cases following HAART therapy seemed that even PML should return to being a rare disease and instead, starting from 2005 it became clear that JCV can be reactivated and cause PML with high frequency even in patients with oncologic or autoimmune diseases treated with biological drugs. In this aspect of particular importance is the Natalizumab, a monoclonal used in the therapy of Multiple Sclerosis (MS), which has induced the development of PML in a large number of patients with MS.
The dissemination of PML allowed researchers to refine diagnostic methods and to conduct important pathogenetic studies both regarding the characteristics of the different JCV strains involved, and the importance of the immune response and neuropathology aspects through sophisticated neuroimaging studies.
In the lecture will be discussed the current knowledge on PML, the virological and immunological aspects and as a disease once considered monophasic and lethal is in fact a group of disorders of various severity and with different clinical and pathological presentations.

Central nervous system viral infections transmitted by arthropods

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Vector-borne viral infections of the central nervous system (CNS) represent a major global public health concern. They are mainly caused by RNA viruses belonging to different families, incuding flaviviruses, such as Tick Born Encephalitis (TBEV), West Nile (WNV), Usutu (USUV) and Zika (ZIKV). The spectrum of associated central or peripheral nervous system disorders is broad, also depending on the host immune status.

TBEV was firstly identified in Italy in 1994 , today it is endemic in northern-eastern Italy. WNV has been reported in Europe since 1958 and is considered the most widespread flavivirus in the world; in Italy, after a lag of about 10 years it reappeared in the north-east of the country in 2008, and became endemic, with cases reported every year in almost 22 regions. USUV was first observed in Europe in 1996 in birds, and circulates in several countries including Italy. WNV and USUV differ substantially for rate of mortality in birds (high for USUV and low WNV), and public health impact (WNV is responsible of neuroinvasive clinical forms in humans, while these are rare for USUV). WNV and USUV are transmitted to humans by an infected mosquito feeding on an infected animal (main reservoir: birds). However human-to-human transmission may occur through blood transfusion and organ transplantation, with high incidence of neuroinvasive disease for WNV. The "Integrated National Surveillance and Response Plan for West Nile and Usutu Viruses" is issued annually by the Ministry of Health to addresses entomological, veterinary and human surveillance and reporting.

ZIKV jumped to the public health attention in the last years, due to extensive outbreaks occurring in Latin America, with several imported cases in Europe and Italy. Inter-human transmission is efficiently mediated by mosquito bite. ZIKV-associated illness is generally self-limiting, but severe clinical complications, including fetal/newborn microcephaly, autoimmune-neurological presentations including cranial nerve dysfunction, and Guillain-Barré Syndrome in adults emerged during the recent massive outbreak.

Overall, the diagnosis of these infections is challenged by the paucity or even lack of available commercial kits, and by the high cross-reactivity among viruses of the same family, requiring trained personnel and expert advice for correct interpretation. NAT screening for WNV of blood and organ donors, which is applied to asymptomatic persons, has problems linked to the sensitivity of the methods, with transient and low level viremia. In addition the available NAT methods do not distinguish between WNV and USUV, suggesting the need of improving diagnostic tools and highlighting the importance of an integrated approach, also taking into account serological results.

Recurrent Herpes Simplex Virus-1 infection induces molecular hallmarks of neurodegeneration and cognitive deficits in mice

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Introduction

Several literature reports suggested that herpes simplex virus type 1 (HSV-1) triggers neurodegenerative processes that are reminiscent of Alzheimer's disease (AD), a neurodegenerative disorder characterized by a progressive decline in cognitive function and the accumulation in the brain of amyloid-β peptides (Aβs), hyperphosphorylated forms of tau protein, high levels of oxidative stress markers and neuroinflammation. However, a cause-effect relationship between the multiple HSV-1 reactivations characterizing the human disease and the development of an AD-like phenotype has yet to be demonstrated. Hence, we set up a mouse model of recurrent HSV-1 infection and investigated the appearance and accumulation over time of biochemical and functional AD markers.

Methods

6-8 week-old BALB/c mice were infected by labial HSV-1 inoculation and subsequently subjected to thermal stress (TS) every 6-8 weeks over the next 10 months to induce repeated virus reactivations. Virus spreading and replication into the brain were assessed by PCR amplification of viral gene/mRNA, immunofluorescence detection of viral protein and infectious virus isolation from brain tissues. Immunohistochemistry, western blot (WB), ELISA assay as well as redox proteomic were used to reveal AD hallmarks in the brain following repeated TSs. Novel object Recognition (NOR) and Y-Maze behavioral test were used to assess cognitive dysfunction.

Results

We found that multiple HSV-1 reactivations triggered, in mouse brain, the accumulation of AD hallmarks, including Aβ, hyperphosphorylated tau and neuroinflammation. These AD hallmarks were paralleled by increased levels of 4-hydroxynonenal (HNE, marker of lipid. peroxidation), 3-nitrotyrosine (3NT, marker of protein nytrosylation) and protein carboxylation, indicating generalized conditions of oxidative stress. Specifically we found the alteration in the level of 13 HNE-modified proteins involved in energy metabolism, protein folding, cell structure, and signal transduction, cognitive dysfunction. Accordingly, behavioral tests evidenced cognitive deficits that became greater following several TSs, thus suggesting their correlation with the progressive accumulation of virus-dependent AD-like damages.

Conclusions

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.

28/09/2018 – INTERAZIONI TRANSKINGDOM

Sialic acids as molecular mediators of transkingdom interactions in the nasopharyngeal mucosa

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Introduction. Sialic acids are anionic nine-carbon sugars that in mammals are found as terminal sugars of secreted glycoproteins or glycolipids and glycoproteins on cell surface, and are involved in essential processes and regulation of immune system. The human nasopharyngeal mucosa is lined with pseudostratified columnar epithelium, and is covered with a mucus layer that contains antimicrobial compounds and sialylated mucins. This environment hosts a complex and diverse microbiota, and is the entry site of invasive bacterial pathogens and viruses, which often exploit sialic acids to modulate their interaction with the host. Some bacterial pathogens decorate their outer surface with sialic acid on LOS/LPS or capsule masking them from the immune system. Others, such as *Streptococcus pneumoniae*, while not producing sialic acid, are equipped, like some viruses, with neuraminidases that are key virulence factors.

Materials and Methods. By using molecular and cell biology approaches, cell infection and animal models the role of meningococcal surface-exposed sialic acids was investigated.

Results. Sialic acid capsule and LOS sialylation are of utmost importance for *Neisseria meningitidis* virulence. Most of the attention has been focused on the role of surface-exposed sialic acids in mediating resistance to both phagocytosis and complement-mediated killing resulting in enhanced meningococcal survival in the bloodstream and in the cerebrospinal fluid. Less is known about their role in colonization and invasion of nasopharyngeal mucosa, except that they play a negative role in the adhesion of meningococci to epithelial cells. It is also known that sialic acid capsules are less expressed when meningococci use lactic acid instead of glucose as main carbon source, and are subject to frequent, reversible on-off phase variation in mutator strains. Nevertheless, there is evidence that surface-exposed sialic acids play a positive role in mucosal invasion. We demonstrated that they are essential for meningococcal survival and growth within epithelial cells, protect bacteria from killing by cationic antimicrobial peptides, and, together with a two-partner secretion system, mediate the interaction between meningococci, host cell microtubules and motor proteins.

Discussion and Conclusions. The contrasting role of surface-exposed sialic acids during adhesion and invasion points out to the importance of their dynamic regulation in response to microenvironment changes and transkingdom interactions. How these interactions between meningococci and other bacteria and viruses may modulate the expression of surface-exposed sialic acids thereby affecting nasopharyngeal colonization and the outcome of meningococcal infection will be discussed.

Interazioni transkingdom nel microbiota vaginale

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Vaginal colonization by a *Lactobacillus* species is associated with healthy gynecologic and reproductive outcomes. Vaginal microbial communities play an essential role in maintaining homeostasis in the vaginal environment. When lattobacilli do not dominate the local microbioma, other microbioal taxa can proliferate leading to a "dysbiotic state". The most common curable sexually transmitted disease is the trichomoniasis: the etiogical agent of the infection, the flagellate protist *Trichomonas vaginalis*, is able to imbalance the microbial community structure. *T. vaginalis* infections can cause severe vaginitis, yet 85% of infections are asymptomatic and untreated. Trichomoniasis is associate to cervical and prostate cancer with increased rates of acquisition and transmission of HIV and other sexually transmitted infections, pelvic inflammatory disease, spontaneous abortions in the first trimester of pregnancy, preterm birth and other adverse outcomes of pregnancy.

Recent studies have demonstrated *T. vaginalis* acts in concert with bacteria and viruses in the vaginal microbiota and these interactions can modulate host immune response and associated pathology. Thus, most *T. vaginalis* isolates harbor intracellular *Mycoplasma* species (in particular *M.hominis* and *M.girerdii*), dsRNA virus, or a combination of these symbionts. Interestingly, recent studies have demonstrated direct effects of symbiosis on pathogenicity and host immune response, up-regulating the inflammation, contributing to infection sequelae. These data indicate that the T.vaginalis/mycoplasma/dsRNA virus consortia can be considered 'pathogroups', or units of pathogenicity. The study of trichomoniaisis pathogroups will elucidate mechanisms underlying the pathogenesis of the infection, leading to the development of effective novel therapeutic approaches to specifically target the negative health outcomes associated with trichomoniasis.

Trans-kingdom interactions in biofilms

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In their natural habitat, i.e. environment and human niches, microorganisms exist in close association with a plethora of species. Metagenomic and proteomic studies allowed for deepening our knowledge about such microbial communities and highlighted the co- existence of prokaryotes, eukaryotes and viruses. Both bacteria and fungi organize into biofilms, three-dimensional structures constituted of cell aggregates embedded in a self- produced extracellular matrix.

Polymicrobial biofilms represent an underestimated clinical problem and emerging evidence shows that molecular interactions between species, synergistic or antagonistic, can have important implications for human colonization and disease, including increased recalcitrance to both host defenses and antimicrobial therapies.

Among fungi, *Candida albicans* is the most common fungus isolated in the clinical setting and the fourth leading cause of bloodstream infections. *C. albicans* readily forms biofilm on abiotic and biotic surfaces, and almost all medical devices support its growth hence, possibly serving as portal entry to the blood. It is estimated that up to 27% of nosocomial *C. albicans* infections are polymicrobial, with *Staphylococcus aureus* one of the most commonly co- isolated organism. Polymicrobial biofilm-formation appears to be a key factor in worsening patient outcome.

For better characterizing this trans-kingdom interaction *in vivo*, we investigated the pathogenicity and the antimicrobial response of *C. albicans* and *S. aureus* infection using the *Galleria mellonella* model. Lower inoculum of S. aureus, in the presence of *C. albicans*, resulted in increased mortality and displayed reduced vancomycin response. Combination therapy with anidulafungin and vancomycin increased larval survival but less than expected, suggesting that polymicrobial biofilms are unique entities that behave differently from the originating species.

Unravelling the molecular mechanisms underlying these infections would be pivotal to implement *ad hoc* antimicrobial therapies.

Transkingdom control of viral pathogenesis

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Introduction. Additionally, to our own cells and genomes, humans harbour a vast array of microbes, including bacteria, viruses, and fungi which together constitute the microbiome. The microbiome is not neutral neighbour, rather it's an essential contributor to host physiology, acting in a symbiotic relationship and forming with the host a complex superorganism termed "holobiont". The holobiont concept has led to a re-evaluation of our understanding of different physiological processes and diseases. Currently, the focus of the studies in this field is to investigate causal relationships between microbes related to promoting or inhibiting each other's presence in the host. In particular, area of active investigation is the identification of which specific taxa and/or bacterial functions are responsible for control of viral pathogenesis.

Materials and Methods. The advent of new high throughput sequencing technologies, in combination with other molecular tools and novel imaging platforms, has made a fundamental contribution to microbiome characterization and to how we can now define many roles that bacteria play in influencing/regulating viral infections.

Results. Emerging data indicate that bacteria regulate, and are in turn regulated by, viruses through a series of processes termed "transkingdom interactions". It's now clear that either the individual bacterial species or bacterial ligands have a profound effect on the overall outcome of virus infections being able a) to directly enhance viral infectivity by stabilizing virions and promoting viral binding to the surface of host cells, b) to promote viral evasion by skewing the antiviral immune response, b) to facilitate or hinder viral replication and virally-induced disease, and c) to increase or decrease the ability of viral infection to promote carcinogenesis.

Discussion and Conclusions. We have only become to scratch the surface of transkingdom interactions in physiological and pathological human conditions, and future studies will undoubtedly seek to delineate their effects more in detail. However right now, it's tempting to speculate that composition and transkingdom interactions of an individual's bacterial microbiota can influence disease outcome during viral infection, making it a potential target for therapeutic intervention. Understanding these interactions will be crucial to design better treatments and preventions of virally-induced diseases.

28/09/2018 – NUOVE STRATEGIE ANTIMICROBICHE

Essential oils as antimicrobial agents: future perspectives

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The increasing spread of multi-drug resistance encourages the search of new antimicrobial agents. A potential new approach is offered by the essential oils (EOs), complex mixtures of plant secondary metabolites consisting of terpene hydrocarbons, aromatic compounds and terpenoids. Recently, the interest in the EOs and closely related components has considerably increased due to their antimicrobial activity against a wide spectrum of microorganisms including pathogenic and spoilage strains, either in the planktonic or the biofilm phase. Their activity against multidrug-resistant strains such as *Acinetobacter baumannii*, *Escherichia coli*, methicillin-resistant *Staphylococcus aureus*, *Mycobacterium tuberculosis*, *Pseudomonas aeruginosa* and *Candida albicans*, also in synergy with conventional drugs, suggests a possible role as new antimicrobial agents. An important characteristic of EOs is hydrophobicity, which enables them to partition with the lipids present in the cell membrane of bacteria and mitochondria, rendering them more permeable by disturbing the cell structures. Despite the promising results given by *in vitro* studies so far, the direct application of EOs still encounters limitations due to the poor solubility and high volatility. New technologies aimed to their incorporation into polymeric materials or encapsulation in solid particles/films provide novel tools to overcome these limitations. In this context, the chemico-physical properties of EOs allow them to dissolve, diffuse, and permeate into the most common polymers. Several studies demonstrate the efficacy of EOs incorporated into synthetic or natural matrices (polypropylene, polyethylene, ethylene vinyl alcohol, polylactic acid, polycaprolactone, chitosan, alginate, starch) by different processing such as melt mixing, extrusion, solvent casting and electrospinning. Accordingly, this presentation provides a brief overview of relevant literature on antimicrobial EOs and describes the most recent advances made on the preparation, biocidal action and possible application of new polymeric systems. Specifically, the effectiveness of carvacrol, a monoterpene present in many EOs, incorporated into ethylene-vinyl-acetate films or polylactic acid nanofibers against *Escherichia coli*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Candida albicans* is discussed. The EOs and their components incorporated into polymeric materials open up new horizons in the development of effective antimicrobial systems in biomedical area for drug delivery, devices coating or wound healing and in food industry for the next generation of packaging materials.

Fighting multidrug resistant infections with bioactive lipids: towards the development of a novel liposome-based immunotherapeutic platform to enhance antimicrobial immune response

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Introduction. Phagocytosis is a key mechanism of innate immunity, which is favored by the timely and coordinated intervention of host lipid second messengers and whose metabolism may be altered during infections, as intracellular survival strategy of the pathogen, or in the immunocompromised host. On these grounds, a possible immunotherapeutic approach aimed at increasing the concentration of selected bioactive lipids within immature phagosomes to promote (auto)phagosome maturation and intracellular pathogen clearance may represent a promising strategy to enhance antimicrobial response.

Materials and Methods. We have generated asymmetric apoptotic body-like liposomes (ABL) to deliver bioactive lipids within target macrophages. These liposomes were characterized by the presence of i) phosphatidylserine (PS) at the outer surface of the liposome membrane, to resemble apoptotic bodies and to allow liposome internalization by macrophages or dendritic cells, and ii) different bioactive lipids, such as phosphatidic acid (PA), phosphatydilinositol 3-phosphate (PI3P) and phosphatydilinositol 5-phosphate (PI5P), at the inner surface of liposome membrane. The immunotherapeutic value of ABLs was assessed i) in a model of pathogen-inhibited antimicrobial response, by using human macrophages infected with different mycobacterial strains, and ii) in a model of host-impaired antimicrobial response, by using primary macrophages expressing a pharmacologically-inhibited or a naturally mutated cystic fibrosis transmembrane conductance regulator (CFTR) and infected with a wide range of multidrug resistant clinical isolates of *P. aeruginosa*.

Results. The delivery of PA, PI3P or PI5P by ABL promoted phagosome maturation, reactive oxygen species generation and intracellular mycobacterial killing by human macrophages during *in vitro* infection with BCG or with mycobacterial clinical isolates belonging to the ancient EAI and modern CAS, but not Beijing, lineage. Moreover, the delivery of PA or PI5P by ABLs improved antimicrobial response in macrophages expressing a pharmacologically inhibited or a naturally mutated CFTR, by enhancing i) phagocytosis, ii) phagosome acidification and iii) intracellular killing of a wide range of multidrug resistant clinical isolates of *P. aeruginosa*.

Discussion and conclusions. Altogether, these results show that the delivery of bioactive lipids by ABL enhances antimicrobial response of human macrophages and may represent an exploitable host-directed strategy to potentiate all phases of phagocytosis process (from pathogen internalization to phagolysosome maturation), irrespectively of its impairment by a bacterial pathogen (e.g. MTB) or by a genetic defect (e.g. Cystic Fibrosis).

Nanoceramics with immunomodulatory and antibacterial properties for restoration of middle ear damage

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Introduction: The tympanic membrane (TM) is a thin, circular layer of tissue that separates the outer and middle ear. It consists of three main layers: an outer epithelium, a middle fibrous lamina propria, and an inner mucosal layer. These periodic inner structures become deformed or aperiodic in various clinical conditions, such as in Acute and Chronic Otitis Media (AOM and COM) and the associated inflammation. In particular, in COM a complex biological processes and changes are involved, including the later development of middle-ear biofilms that serve as reservoirs for antibiotic-resistant bacteria, resulting in a prolonged and often recurring bacterial infection with TM perforation and otorrhoea, condition named Chronic Suppurative Otitis Media (CSOM). The bacterial spectrum most often identified in the CSOM-affected middle ear is dominated by *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Enterobacteriaceae* such as *Proteus* spp. and *Klebsiella pneumoniae*. Nanomaterials, both ceramics and polymers can be produced in the submicrometric-to-nano size, which may elicit unpredictable reactions in the biosystems. The aim of this study was to investigate the cytocompatibility, anti-inflammatory and immunomodulatory properties of some piezoelectric nanomaterials, barium titanate nanospheres, lithium niobate nanoparticles and polyvinylidene fluoride (PVDF) ultrafine fibers in perspective of their application in restoration of damaged TM.

Methods: In our experimental system, Hacat cells were treated with stable suspensions of nano-objects obtained by using glycol chitosan at different dilutions and time of exposure. The expression levels of pro- and anti-inflammatory cytokines and antimicrobial peptide human beta-defensin-2 (HBD-2) were evaluated at 6 and 24 h of using LightCycler software.

Results: The results obtained, showed that piezoelectric materials possess relevant immunomodulatory activity and were also able to stimulate a significant increase in the production of HBD-2, which indicated the presence of an indirect antibacterial activity proper of the innate immune response. These findings suggested that these piezoelectric nanoparticles and ultrafine fibers can possess unique properties for interfacing with tissues, including but not limited to piezoelectricity. Areas of applications may involve delicate microenvironments such as the cochlear organ.

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New biomaterial functionalized with natural antimicrobials to counteract microbial implant infections

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Introduction. Mentha Piperita essential oil (EO) is a complex mixture of different natural biomolecules with proven antibacterial activity against Gram-positive and Gram-negative bacteria, fungi and viruses. Despite its increasing use in phytotherapy, application of Mentha Piperita EO in medicine is still quite scarce and almost no research work considers its application in combination with biomaterials. On the other hand, the possibility to employ the antibacterial properties of this EO together with its low resistance development risk and low toxicity is a challenging approach for the development of smart biomaterials for prosthetic applications. This work aimed to combine the antibacterial properties of Mentha Piperita EO with those of bioactive titanium alloy (Ti6Al4V) for medical applications in which good osteointegrability and antimicrobial effects are required.

Materials and methods: Different concentrations of Mentha Piperita EO in ethanol were considered for the treatment of Ti6Al4V, made bioactive with a patented chemical treatment. The samples were characterized by means of X-ray photoelectron spectroscopy (XPS), Fourier transform infrared spectroscopy (FTIR), wettability and z potential analysis. Antibacterial tests were performed by assaying the *Staphylococcus aureus* adhesion on biomaterials through a sonication protocol, that allows to dislodge bacteria without altering their viability.

Results: XPS analyses evidenced a significant increase in the carbon content for the modified Ti6Al4V alloy; FTIR analyses highlighted, on the modified samples, the presence of three peaks attributable to chelate compounds characteristic of the organic elements in EO. The wettability tests showed a reduction of the wettability after the modification with the EO and the z potential highlighted a shift of the isoelectric point to a less acidic value after the surface functionalization process. The antibacterial tests showed a significant ($p<0.001$) reduction of the adherent staphylococci on the modified surfaces after 24 h of incubation, with values of 1.71×10^8 CFU/ml for control samples and 6.80×10^7 CFU/ml for samples enriched with Mentha Piperita EO.

Discussion and Conclusions: A multi-functional biomaterial for prosthetic implants that could enhance osteogenesis, and simultaneously prevent post-surgical infection and inflammation, is urgently needed. This research work shows the great potential of Mentha Piperita EO use for biomaterial surface functionalization with enhanced antibacterial properties. Furthermore, the use of Mentha Piperita oil of Pancalieri is consistent with the European policy of exploitation of the local regional natural resources (Piedmont).

Antimicrobial and antibiofilm effect of graphene oxide against chronic wound microorganisms

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Introduction. Chronic wounds are difficult to heal and represent an increasing problem worldwide, becoming an important challenge for the healthcare system.
Microbial infections in chronic wound together with the presence of biofilm and multidrug resistance strains may contribute in the prolongation of the inflammatory phase interfering seriously with healing processes. Graphene oxide (GO) has been reported to exhibit strong antibacterial activity towards both Gram-positive and Gram-negative bacteria. The aim of this work was to investigate the *in vitro* antimicrobial and antibiofilm efficacy of GO against chronic wound pathogens also in a *in vitro* wound model.

Materials and Methods. *Staphylococcus aureus* PECHA 10, *Pseudomonas aeruginosa* PECHA 4 and *Candida albicans* X3 clinical isolates were incubated with 50 mg/L of GO for 2h and 24 h to evaluate the antimicrobial immediate and tardive effect. Optical and Atomic force microscopy images were performed to visualize the GO effect on microbial cells. The antibiofilm effect of GO was tested on *S. aureus, P. aeruginosa* and *C. albicans* biofilms both in formation and mature.
Moreover, an innovative GO hydrocellulose film was synthetized and used to treat the *S. aureus* and *P. aeruginosa* dual-biofilm grown in an innovative *in vitro* wound model (Lubbock model).

Results. When compared to the respective time controls, GO significantly reduced the *S. aureus* growth both at 2 and 24 h and displayed a bacteriostatic effect in respect to the GO t=0; an immediate (after 2 h) slowdown of bacterial growth was detected for *P. aeruginosa* whereas a tardive effect (after 24 h) was recorded for *C. albicans*. Atomic force microscopy images showed the complete wrapping of *S. aureus* and *C. albicans* with GO sheets that explains its antimicrobial activity. Moreover, significant inhibition of biofilm formation and a reduction of mature biofilm were recorded for each detected microorganism.
Preliminary results about Lubbock model showed interesting antibacterial effect of GO hydrocellulose film on *S. aureus* and *P. aeruginosa* dual-biofilm.

Discussion and Conclusions. The antibacterial and antibiofilm properties of GO, also delivered by hydrocellulose film, against chronic wound microrganisms make it an interesting candidate to incorporate into wound bandages to treat and/or prevent microbial infections.

28/09/2018 – FOCUS ON

INFEZIONI SESSUALMENTE TRASMESSE

STI epidemiology

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Sexually transmitted infections (STI) are widely spread worldwide, affecting millions of people every year. In Italy, two sentinel surveillance systems collect data on a number of STI. The first one, which started in 1991, is based on a network of 12 large STI clinics and collects data on patients with a symptomatic STI. The second one, which started in 2009, is based on a network of 13 large laboratories and collects data on people tested for at least one of the following STI (regardless of the presence of symptoms): *Chlamydia trachomatis* infection, *Trichomonas vaginalis* infection, gonorrhoea.

Data reported by these surveillance systems shows that the number of people with a confirmed STI has increased between 2010 and 2016. In recent years, a relevant increase in the number of cases has been observed for *C. trachomatis* infections, genital warts and syphilis.

The proportion of HIV-positive individuals in this population is very high and has been growing since 2008.

STIs: syndromic diagnostic approach

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Microbiological diagnosis of syndromes that can be caused by multiple pathogens, such as sexually transmitted infections (STIs) may be challenging. Therefore, methods able to detect multiple microorganisms in a clinical specimen at the same time are essential.
Several molecular diagnostic assays, flexible and easy to use, have recently been commercialized to assist the syndromic diagnosis of STIs, allow to overcome some limitations of conventional diagnostic assays, such as microscopy, culture and antigen detection assays.
Furthermore, the detection of many different pathogens from a variety of clinical specimens in a single reaction represents an important advantage offered by these new molecular syndromic methods for the differential diagnosis of many common syndromes, such as urethritis and cervicitis.
In addition, microorganisms usually not detected by conventional diagnostic methods (i.e. *Mycoplasma genitalium*) can be identified by these assays, thus reducing the cases in which a microbiological diagnosis cannot be achieved for a given STI.
Finally, multi-sample option and multiplex detection ability of these tests is strongly recommended for the detection of multi-etiological syndromes such as STIs, even in asymptomatic carriers and low prevalence populations.

28/09/2018 – FOCUS ON

DIAGNOSTICA MOLECOLARE

Semplicità e potenza nei test sindromici

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A syndrome is a set of symptoms and signs that are correlated with each other and, often, with a specific disease. Key syndromes include respiratory and gastrointestinal Infections, meningitis, encephalitis, sepsis, bone and joint infections and sexually transmitted diseases, all of them having multiple pathogens as causative agents.

Diagnostic laboratories can make use of a variety of methodologies and technologies to detect and recover the causative organism of an infection, but the single pathogen detection approach, or the use of bacterial culture often lead to pathogen misidentification and application of inefficient broad targeted therapies, resulting in therapy failures, longer recovery times, higher costs and eventually patient mortality.

Platforms for high multiplexed fast syndromic testing are now helping healthcare providers to deliver efficient and timely patient-centred care. They greatly reduce diagnostic uncertainty, and help overcome mistakes and inefficiencies that commonly result from testing one or few organisms at a time.

A platform for syndromic testing shall be flexible, scalable and closer to the patient, with minimal contamination risk and transparency on result calculations to better understand and target co-infections which are commonly found in syndromes and more frequently in children

Testing multiple pathogens in a single test gives answers that are more accurate, comprehensive, and actionable for real-life decisions in critical care, bringing invaluable benefits to the patient and health care providers.

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Recenti acquisizioni negli approcci di genomica per la diagnosi e caratterizzazione di Neisseria meningitidis e la gestione di outbreak

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Introduction. The Whole Genome Sequencing (WGS) provides a further detailed characterization of Invasive Meningococcal Disease (IMD) for surveillance purposes and for outbreak investigations to identify links between cases and groups at higher risk to which address targeted immunization programs. Hereby, the use of WGS in two recent outbreaks is described: one occurred in Sardinia island in 2018 and one in Tuscany region, 2015-2017.

Materials and Methods. For each isolate, 1 ng of DNA was used for sequencing libraries with Nextera XT DNA protocol in Illumina MiSeq platform (kit v3, 600 cycles). The final assembly ranged from 201 to 506 (median = 247) contigs/sample covering the ~2.2 Mb of *N. meningitidis* genome. Draft genomes were uploaded to PubMLST.org database to perform core genome MLST (cgMLST).

Results. WGS is currently in place as routine at NRL of ISS within the National Surveillance System of IMD. The outbreak of five cases in individuals aged 19-21 years, two of them died, occurred in Sardinia in 2018 due to the hypervirulent MenB:cc11 strain. cgMLST confirmed both the close similarity among the five outbreak isolates and to MenC:cc11 strains circulating in Italy in the same period. The close similarity among the genomes of B and C capsular serogroups, strongly suggests capsular switching from C to B meningococci and identified specific molecular traits. These results supported the immunization strategies against meningococcus B to control the outbreak. Moreover, the outbreak in Tuscany in more than 70 IMD cases, mostly in adults, was completely characterized by WGS helping to understand the contact tracing and the main molecular traits of the strain. This outbreak appears to be due to a series of interconnected clusters determined by C:P1.5-1,10-8:F:3-6:cc11 strain, leading then to a general picture of raised incidence and characterized by a high fatality rate.

Discussion and Conclusions. Here it was described the added value given by WGS over conventional sequence-based methods in outbreak investigations. Detailed molecular information is key to understand epidemic and hyper-endemic dynamics and re-modulate vaccination strategies, as recently occurred in Italy to manage the two IMD outbreaks. In conclusion, WGS facilitates the rapid and accurate identification of the pathogen. It allows to confirm and define the true size of the outbreak and supports towards a more focused investigation.

Recenti sviluppi delle piattaforme molecolari e loro implicazioni nella gestione clinica delle patologie virali: il caso dell'HIV

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The management of HIV infection and the search for a cure has highlighted the need for increasingly
sensitive and precise assays to measure HIV-1 RNA viral load (VL). Recently, Hologic Inc. released the fully automated Aptima HIV-1 Quant Dx assay (AQ) for blood plasma VL quantitation, based on the dual target approach to detect and quantify *pol* and *5'LTR* regions of HIV in the same sample. Though both signals are generated, most of quantitative final results are obtained by *pol* amplification. However, in some patients, only 5'LTR signal is suitable to measure viremia. The clinical meaning of VL data obtained with dual-target method and the impact on management of HIV+ infection is at moment controversial and deserves investigations.

Valutazione di una nuova piattaforma molecolare per la diagnosi di gastroenteriti virali

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Introduction: Diarrhea has been the second leading cause of death among children under the age of five. The rapid and accurate pathogen diagnosis in patients with diarrhea is crucial for reducing morbidity and mortality. Viruses are major etiological agents of childhood gastroenteritis. In recent years, several molecular platforms for the detection of viral enteric pathogens have become available. Two newly developed one-step multiplex real-time RT-PCR assays were evaluated for ability to detect Norovirus GI and GII (Norovirus ELITe panel MGB® test), and Rotavirus, Adenovirus and Astrovirus (Gastrointestinal viral ELITe panel MGB® test) in stool samples, using a fully automated cassette based sample-to-results solution (ELITe InGenius).

Material and Methods: The performance of the ELITe InGenius assays was compared with consolidated real-time RT-PCR for Norovirus and Rotavirus, conventional RT-PCR for Astrovirus and commercial ICT assay (Vikia Rota-Adeno, BioMeieux SA, Lyon, France) for Adenovirus using 284 stool samples from children with acute gastroenteritis.

Results: The overall sensitivity and specificity for the ELITe InGenius assays were for 100% and 95.7% for Norovirus, 98% and 90.35% for Rotavirus, 97% and 99.2% for Adenovirus, 100% and 96.6% for Astrovirus, respectively. Substantial agreement rates between the two commercial tests and the reference methods (k: 0.83-0.96) were observed. A wide panel of different types of Norovirus (one GI and six GII genotypes), Rotavirus (six genotypes) and Astrovirus (two genotypes) were detected. Overall, the Ct values obtained by InGenius assays were significantly lower than those produced by the reference methods.

Discussion and conclusions: The InGenius Norovirus and Gastrointestinal virus ELITe assays showed a high concordance with the reference methods coupled with an elevate sensitivity and specificity and can be recommended as a valuable methods for accurate diagnosis of epidemic and sporadic gastroenteritis. Studies of considerable sample size are required to determine robust Ct cut-off values to effectively distinguish disease-causing infection for individual patients. Performing simplified straightforward molecular assays may help solve the dilemma of correlating viral loads to severity of disease.

28/09/2018 – GRUPPI DI LAVORO

INFEZIONI VIRALI PERSISTENTI

Recent advances on human papilloma virus carcinogenesis and the role of host immunity factors

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Persistent infection with high-risk human papilloma viruses (HR-HPV) is the necessary, but not sufficient cause of several types of carcinomas. The key factors that determine the outcome of HR-HPV infections remain poorly understood due to paucity of model systems to study HPV natural history. In fact, fundamental properties of HPV viral proteins can be assessed in specialized cell culture systems but the complexity of the host responses would require preclinical surrogate models. This lecture will report on developments in understanding the first steps of HPV carcinogenesis and the role of host immunity factors. HR-HPV interfere with initial host immune response recognition, down-regulating the expression of Toll-like receptors (TLR) and other Pattern Recognition Receptor, and their signaling pathways. In cervical infections, HR-HPV genotypes activate TLR signaling at lower levels than the LR genotypes, consistently with the longer median time from infection to clearance estimated for HR as opposed to LR HPV. On the other hand, TLR overexpression (particularly TLR4 and TLR9) has been correlated with persistent infections and malignancy. Recent research has also been focused on how to HR-HPV escape immune surveillance mediated by the interferon (IFN) and cytokine responses. *In vitro* studies have shown that HR-HPV efficiently inhibits type I IFN response in the presence of elevated expression levels of the viral oncogenes E6 and E7. It is also known that type I IFN treatment can inhibit the growth of HPV-immortalized cervical epithelial cells and suppress the transcription of viral E6 and E7 oncogenes with different effects depending on the IFN subtype and concentration. The more recently described type III IFN, a key component of the mucosal innate immune response, is also target by HR-HPVs in a biological/clinical relevant way, since lower levels of expression of IFN lambda1 were found in patients with HR-HPV infection and in patients with SIL. Accordingly, type III IFN may have a protective role in the mucosal immune response to HPV and its use in the treatment of HPV-associated lesions should be evaluated. Undoubtedly, HR-HPV disturb the expression of genes (e.g. NF-κB) in keratinocytes, thus altering chemoattraction and a proper induction of adaptive response. As an example, IL-10, an anti-inflammatory cytokine, interacts with HPV E6/E7 proteins , favoring immunosuppression. Recent evidences indicate that C-C chemokine ligand CCL20/C-C chemokine receptor 6 axis is subjected to a complex modulation during different phases of HPV infections. Novel data highlighted how the host immune response may be strengthened to benefit patients through various pathways, especially in therapy of persistent HPV-infections and pre-cancerous lesions.

Novel N-Glycosylation sites in HBV surface antigen determine false HBsAg negativity despite immunosuppression-driven HBV reactivation by altering HBsAg antigenicity

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Introduction: To investigate N-linked-glycosylation sites (N-Glyc) patterns of HBsAg in immunosuppression-driven HBV-reactivation (HBV-R) in vivo and to evaluate their impact on HBsAg antigenicity and HBV replicative-transcriptional potential in vitro.

Materials and Methods: mutations associated with N-Glyc were identified by analysing HBsAg sequences of 55 patients with HBV-R. N-Glyc impact on pgRNA, core-particle-associated HBV-DNA and extracellular HBsAg were assessed by transfecting Huh7 cells with plasmids encoding WT or mutated HBV genotype-D full-genome. N-Glyc impact on HBsAg antigenicity was analyzed transfecting Huh7 cells with plasmids encoding for WT and mutated HBsAg linked to Streptavidin-tag (Strep-Tag-HBsAg). The Strep-Tag-HBsAg amount in supernatants was quantified through home-made ELISA targeting Streptavidin-Tag and also by two different commercial assays targeting HBsAg. Tunicamycin treatment (N-Glyc inhibitor) on Strep-Tag-HBsAg transfected cells confirms N-Glyc role in HBsAg antigenicity.

Results: At HBV-R, median[IQR] serum HBV-DNA was 6.7[5.3-8.0]logIU/mL while ALT 146[42-630]U/L. Notably, 7/55(12.7%) patients remained HBsAg-negative despite HBV-R(HBV-DNA:2.9-7.6logIU/mL). ≥1 additional N-Gly in HBsAg was detected in 5/7 HBsAg-negative patients.

In vitro, N-Glyc strongly reduced HBsAg titer without affecting viral replication. Specifically, S113N+T131N+M133T, ins114N, T115N and T123N caused a reduction of 80%,68%,62%,32% in HBsAg titer, respectively. Similarly, N-Glyc decreased Strep-Tag-HBsAg titre by commercial assays, but not in home-made ELISA, suggesting that N-glyc sites hamper HBsAg-recognition by antibodies without affecting HBsAg-release. Tunicamycin treatment confirms N-Glyc role in hampering HBsAg recognition by antibodies.

Discussion and Conclusions: Additional N-Glycosylation sites in HBsAg correlate with HBsAg-negativity in HBV-reactivation and alter HBsAg antigenicity in vitro without affecting viral replication, supporting their role in immune-escape and highlighting the importance of HBV-DNA for a proper diagnosis of HBV-reactivation.

Early post-transplant Torquetenovirus viremia predicts cytomegalovirus reactivations in solid organ transplant recipients

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Introduction. To date, there is no universal biomarker for measuring the overall level of immunosuppression conferred on a transplant patient treated with standard immunosuppressive regimen, and for assessing the overall integrity of the immune system. The currently used biomarkers have poor sensitivity, they don't measure slight changes in the immune system, and none of them are able to evaluate the capability of the immune system to control infections. Imbalance of the immune system in transplant recipients has a significant impact on replication of Torquetenovirus (TTV), the most representative and abundant virus of the human virome.

Materials and Methods. 280 adult patients who received kidney or liver transplant and who had completed a follow-up of at least one year were enrolled in the study. Peripheral blood serum samples were obtained from patients just before transplantation and every 10 days (± 5 days) within the first 3 months and then at 4, 5, 6, and 12 months (± 10 days) post-transplant. TTV kinetics were studied by a single step TaqMan-PCR assay designed on a highly conserved segment of untranslated region of TTV genome.

Results. 258 patients (92%) were plasma TTV positive before transplantation with mean TTV levels of 3.9 and 4.2 log DNA copies/ml of plasma in patients receiving kidney or liver transplant, respectively. Plasma TTV levels remained stable or changed slightly relative to baseline up to day 20. The first significant increase (about 1 log) of mean plasma TTV load occurred at day 30 and 40 for kidney and liver transplantation, respectively. Then, TTV viremia progressively increased to a maximum of 6.9 log at day 90 and of 6.5 log at day 80 in kidney and liver transplant patients, respectively. Subsequently, TTV stabilized at levels of approximately 2-3 logs higher than those at the baseline for the remaining 9 months of observation. Maintenance immunosuppression regimen based on cyclosporine A was associated with statistically significant higher median TTV loads compared to tacrolimus. TTV kinetics in patients who developed cytomegalovirus (CMV) reactivation within the first 4 months post-transplant differed from that observed in patients who did not experience CMV complications. Importantly, plasma TTV load measured between day 0 and 10 post-transplant was significantly higher in CMV DNA positive than in CMV DNA negative patients. TTV viremia above 3.45 log DNA copies/ml within the first 10 days post-transplant correlated with higher propensity to CMV reactivation following transplantation.

Discussion and Conclusions. This study provides evidence for the potential use of early post-transplant TTV viremia as innovative, simple and rapid biomarker to predict CMV reactivation in solid organ transplantations recipients.

Human endogenous retroviruses are overexpressed and demethylated in right colon cancer tissue, and packaged in plasma extracellular vesicles of colon cancer patients

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Introdcution

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 colorectal cancer were enrolled in this study. The expressions of HERV-H, -K, -R, and -P *env* gene, HERVs LTRs and Alu, LINE-1 methylation levels were investigated in the tumor and normal adjacent tissues and, when possible, in the blood. HERV expression was evaluated in the plasmatic extracellular vesicles (EVs). We further evaluated the associations among clinical characteristics and HERV expression and methylation levels.

Results

No differences were observed in HERV expression levels among the tumor, normal adjacent tissues and blood. Alu, LINE-1, HERV-H and -K LTRs were demethylated in the tumor compared to the normal adjacent tissues ($p<0.05$). The *env* gene was expressed in the EVs at low levels of 54% (-H), 38% (-K), 31% (-R), and 4% (-P) patients. Associations were found between HERV expression and right tumor colon location and between HERV methylation and vascular invasion.

Conclusions

Our findings demonstrate specificity of the changes in DNA methylation of retroelements in colorectal cancer, without reactivation of HERV expression, suggesting liberation of the LTRs from epigenetic control. The HERV sequences packaged in the EVs might be transferred from one cell to another, favoring cellular transformation. Investigations regarding the use of HERV expression/methylation as markers of prognosis are warranted.

Herpesvirus sub-clinical persistent infections in neurological diseases: the role of Natural Killer/B cells interactions

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Introduction: We have reported that Killer Ig-like (KIR)2DL2 inhibitory receptor expression on the surface of CD56bright Natural killer (NK) cells impairs the response to Human Herpesvirus (HHV) infections in a subgroup of Multiple Sclerosis patients. CD56bright NK cells may affect anti-viral response directly or by promoting B cell activation, particularly in MS, stimulating B-cell responses in part through CD40/CD40L activating signal. We aimed to analyze anti-HHV specific antibody synthesis and the expression of CD40/CD40L molecules in the subgroup of CD56brightKIR2DL2+ MS patients.
Materials and Methods: We enrolled 40 MS patients with a stable RRMS, 40 healthy controls and 40 patients with neuropsychiatric systemic lupus erythematosus (NLES). We analyzed herpesvirus (EBV, VZV, HHV-6, HSV-1, HSV-2) serology (IgM/IgG) in peripheral serum and immune-phenotyped NK cells for CD56, KIR2DL2, CD69, CD154 expression and B cells for CD19, CD40 and CD27 expression.
Results: We found a higher frequency of the number of simultaneously-increased specific anti-herpesvirus IgG titers in the blood of MS and NLES patients in comparison with controls (N: 5 vs 2; $p<0.01$). In particular, we found higher IgG titers towards EBV (EBNA1+VCA+) and HHV-6 in MS patients and towards EBV in NLES patients. Moreover, MS patients positive for KIR2DL2 expression on CD56bright NK cells demonstrated significantly increased IgG titers towards EBV and HHV-6, while no differences were observed in controls and NLES ($p<0.01$). No modifications in IgM titers were observed. No differences in CD40 expression on B lymphocyte among the three cohorts were observed. A subset comparison of CD56brightKIR2DL2+ patients and controls demonstrated a significant decrease in CD40 expression on B lymphocytes of MS patients ($p <0.0001$), while an increase in NLES ($p<0.001$). Conversely, CD56brightKIR2DL2+ NK cells from both MS and NLES patients showed an increased expression of CD40L (CD154) ($p<0.02$), while no differences were observed in controls.
Conclusions: These results suggest that the expression of KIR2DL2 receptor on CD56bright NK cells and the presence of sub-clinical persistent herpesvirus infections might affect not only NK cells functions but also B cells production of anti-herpesvirus antibodies in MS patients, possibly via CD40L up-modulation. Moreover, since soluble CD40L is reported to disrupt the blood-brain barrier, the increase of its expression in KIR2DL2 positive MS and NLES patients could have a broad effect on neurological disease etiopathogenesis.

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29/09/2018 – FOCUS ON

INFEZIONI ENTERICHE

Food and water-borne diseases: New epidemiological cycles

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Food and water-borne infections account for a substantial burden of disease worldwide. The impact of the large distribution of food commodities results in a large number of both sporadic cases and outbreaks, often with a trans-national dimension, particularly in the high-income countries.

The complexity of the international food trade networks, from the production to the point of exposure, makes it often difficult to trace back the sources of infections and demand for the involvement of task forces including actors from both the medical and veterinary fields with a range of skills including epidemiology, microbiology and molecular biology to face the investigation of outbreaks.

The introduction of pathogens into the food chain through the complex pathways of the food trade are well represented by a few examples of large food crises occurred recently in the EU and the rest of the world. The large Shiga toxin (Stx)-producing *E. coli* (STEC) O104:H4 outbreak occurred in 2011 in Germany and France is iconic of the introduction of an unusual type of STEC in the EU through the import of a food commodity from a third country. As a matter of fact, the event was caused by the consumption of fenugreek seeds contaminated with a Stx-producing Enteroaggregative *E. coli* imported from North Africa and used for producing sprouts eventually consumed raw. Additional examples of this global scenario are represented by the large outbreak of Hepatitis A linked to the consumption of frozen berries and affecting Italy and a large number of other countries in the EU and by the worst outbreak ever reported of Listeriosis occurred in South Africa between 2017 and 2018.

Beside the global food trade, other routes of dissemination of food- and water-borne pathogens are represented by the cycles triggered by the re-use of wastes, particularly the reclaimed water and the biomass-based fertilizers, in the agricultural settings. Water recovered at the wastewater treatment plants and the related sludges used, respectively, to irrigate and fertilize soils intended for crops and pastures indeed represent a risk of introducing pathogens in the food chain.

In order to cope with the many critical points in the food production chain introduced by the globalization of the production processes and the circular economy it is necessary to integrate the efforts involving multidisciplinary groups and to implement a “One Health” approach.

Virus enterici: minacce emergenti e strategie vaccinali

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Introduction: Enteric viruses are an heterogeneous group of viruses essentially sharing the fecal-oral transmission route and usually contaminating food or water, whose distinctive features are: low viral infective dose, high number of viral particles excreted with feces, and resistance in the environment. They are responsible for a wide panel of illnesses spanning from gastroenteritis (Adenovirus, Astrovirus, Caliciviridae in particular Norovirus, and Rotavirus), to hepatitis with fecal-oral transmission (Hepatitis A and E viruses), and other systemic infections of diverse severity like in the case of Enteroviruses. Due to their impact on human health, vaccines have been developed for Rotavirus and for polioviruses and for the latter, thanks to vaccination, an eradication effort is now close to its goal, but novel emerging or re-emerging threats have been presented by enteric viruses in recent years.

Materials and Methods: In the latest years the Italian Study Group for Enteric Viruses (ISGEV; https://isgev.net/) has carried on national surveillance of the circulation of enteric viruses, collecting stool samples from children hospitalized with acute gastroenteritis. In Sicily, syndromic surveillance has been integrated with environmental surveillance on urban sewages, collected from wastewater treatment plants.

Results: Surveillance of hospitalized diarrheic children has allowed monitoring the protective effects of Rotavirus vaccination, confirming the reduction of hospital admissions for Rotavirus gastroenteritis. However, in the same period Norovirus acquired an increasing role in acute pediatric enteric syndromes and food/water related outbreaks, possibly due to the emergence of novel antigenic variants. The combined syndromic/environmental surveillance allowed to exclude the risk of reintroduction in Italy of Poliovirus neurovirulent strains from endemic countries, but concomitantly demonstrated the widespread circulation of Hepatitis A virus in Sicily, probably linked to person-to-person transmission, including MSMs sexual contact, as recently reported in other European countries.

Discussion and Conclusions: Enteric viruses still represent a major threat to human health but vaccination has demonstrated its effectiveness, almost eradicating polioviruses worldwide and reducing hospitalizations for Rotavirus gastroenteritis. New vaccines would be much welcome for the control of Noroviruses and Hepatitis E virus, while extended recommendation for Hepatitis A vaccine should be considered. Surveillance activities will play an essential role in guiding prevention strategies and revealing emerging threats.

Tradizione e modernità nell'approccio diagnostico alle parassitosi intestinali

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At CTD – Negrar we evaluated if a change of our diagnostic routine for intestinal protozoa was justified. In detail, we intended to assess if a shorter routine, based on the analysis of a single fecal sample instead of the "classical" three, was sufficiently sensitive. The new approach to be evaluated included a classical coproparasitological exam plus a home-designed multiplex Rt-PCR, both on the same, unique sample. The data obtained showed that the sensitivity of the new proposed approach was higher than that of the examination of three distinct fecal samples, with microscopy only, with the advantage of reducing costs and saving time, both for patients and for the laboratory. However, molecular biology cannot replace microscopy completely, and the combination of both, though on a single specimen, appears to be the best compromise for the diagnosis of intestinal protozoa.

29/09/2018 – FOCUS ON

HIGHLIGHTS IN PARASSITOLOGIA

Giardia e giardiasi, un update!

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Introduction: *Giardia duodenalis* is a zoonotic enteric protozoan parasite that infects the small intestine and colonize the lumen and epithelial surface of human (and other mammals). It affects 200 million individuals worldwide and contributes to the global burden of diarrhoeal disease. Human infections are usually self-limited, but can be severe in some subjects. Clinical signs range from asymptomatic to severe disease, being also responsible of long-term sequelae. Children <5 year represent the risk category and impaired child growth and cognitive development, have been associated with recurrent infections. An effective control is hampered by the multiple infection routes (water, food or fecal-oral) and varied symptomatology. Drug treatment options are limited and efforts in this direction are limited- The mechanisms that contribute to giardiasis outcomes involve both parasite and host factors but are still only partly understood due to the lack of efficient *in vitro* and *in vivo* model. Genetic makeup likely accounts for biological differences among assemblages and isolates and it could potentially be significant for clinical outcomes of infection and epidemiology.
Materials and Methods: A review of the most recent literature is presented with the aim to highlights the advances on the understanding of the host-parasite relationship, pathogenic mechanisms and defining drug-resistance traits.
Results: Implemented *in vivo* infection model and *in vitro* assays suggest that a systemic dysbiosis of host gut microbial community is induced upon Giardia infection likely contributing to the establishment of the infection, induction of pathology, and/or eventual parasite clearance. Giardia-enterocyte co-culture proved that the parasite downregulates inflammatory response by cysteine proteases-mediated degradation of chemokines and cytokines, downregulation of proinflammatory transcriptional factors and degradation of cytokines transcripts in host enterocytes. Cysteine proteases also disrupt the epithelial barrier. Cases of untreatable giardiasis are emerging especially in patients returning from Asia but genetic resistance markers have not yet been identified. Extensive "omics" studies in experimental drug resistant laboratory lines identified broad and variable adaptive responses (post-transcriptional and post-translational alterations and metabolic adaptations), raising the question if resistance in Giardia is indeed an adaptive/tolerance mechanism.
Discussion and Conclusions: Future research in the field of Giardia and giardiasis will clearly attempt to further dissect the complex crosstalk between the host, the microbiota, and the parasite and to define resistance traits in clinical Giardia isolates.

Screening of candidate blood donors for malaria and Chagas disease

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Introduction

Transfusion with blood infected with *Plasmodium* spp. and *Trypanosoma cruzi* parasites represents a risk for malaria and Chagas disease transmission, respectively. Several countries attempt to avoid such risk by testing candidate blood donors who resided in endemic countries, in order to identify subjects carrying asymptomatic infection. In Italy, recent legislation introduced the use of serological tests for the detection of anti-*Plasmodium* and anti-*T. cruzi* antibodies, and the exclusion from donation for 2 years in case of a positive result. The Parasitology laboratory at Pisa University Hospital (PUH) is responsible for the screening of candidate blood donors referring to trasfusional centres of North-West Tuscany, and here we report the results obtained in the past two years.

Materials and Methods

Sera samples were collected from candidate blood donors at transfusional centers in Livorno, Lucca, Massa-Carrara, Pisa, and Viareggio, and tested at PUH between february 2016 and august 2018. For Chagas disease, samples (N=1877) were tested by immunocromatography (ICT, Cypress Diagnostics) as screening assay and chemiluminescence (ChL, Abbot) as confirmatory assay. For malaria, samples (N=1415) were tested by ELISA (DRG). Candidate donors with a positive ELISA result were offered direct diagnosis on whole blood by Loop AMPlification method (LAMP, Meridian). Both for Chagas diseases and malaria, in absence of gold standard methods for serology, we compared results of different assays on samples from disease patients, in collaboration with the Centre for Tropical Diseases (CTD, Negrar, Verona; many thanks to F. Perandin, A. Angheben, Z. Bisoffi).

Results

For malaria, 93 candidate blood donors (6.6%) had a positive ELISA result. None of the 7 ELISA positive donors tested by LAMP were diagnosed with *Plasmodium* spp. infection. Testing malaria patients samples with five different ELISA kits showed low sensitivity of all kits compared to IFAT, and a highly variable agreement between ELISA results. For Chagas disease, 7 candidate blood donors (0.4%) had a positive ICT result. However, all 6 subjects had a negative ChL result. Testing Chagas disease patients samples with different assays showed a lower sensitivity of ICT compared to ChL, ELISA and Immunoblot.

Discussion and Conclusions

Our results show that the seroprevalence of malaria in the blood donors population of North-West Tuscany is considerable, while that of Chagas disease is very low. For malaria, an ELISA assay with better sensitivity should be developed and adopted for screening at national level. For Chagas disease, ELISA should substitute ICT as a screening assay, and a third test should be introduced when results of the screening and confirmatory tests are discordant. For both diseases, a direct molecular diagnostic method could be useful to rule out infection in seropositive individuals. These observations could guide the revision of current legislation regulating blood safety in Italy and other non-endemic countries.

Waterborne pathogens contamination in dental unit waterline of hospitals and dental clinics

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Introduction
Dental procedures generate aerosols containing microorganisms that proliferate within dental unit waterlines (DUWs). DUWs contamination is associated with environmental organisms such as Legionella spp., Pseudomonas aeruginosa, free living amoebae (FLA), which can increase the infection risk especially in immuno-compromised patients. We studied the microbial contamination and the potential health hazards for patients and dental staff in dental clinics where disinfection of DUWs was applied with different strategies.

Materials and methods
Dental clinics were investigated with 23 dental units in 4 teaching hospitals and 10 dental units in 7 dental premises. Tap water and DUWs systems (inlet, spittoon and handpiece) were sampled before and
after the disinfection treatment, where applied, to determine Total Viable Counts (TVCs) (ISO6222,2001), Legionella spp. (ISO11731-2,2004), P. aeruginosa (ISO16266,2006) and coliform bacteria (ISO9308-1,2004). Water samples were subjected to FLA detection by cultural method and PCR amplification of 18SrDNA region. To assign the FLA isolates to the species level, a comparison with available sequences in GenBank was performed by BLAST. Disinfection treatment was applied in 14/33 of dental units: 3% or 6% hydrogen peroxide (HP) (with and without surfactants) applied in DUWs for 1 hour and followed by water flushing was the prevalent shock disinfection practice followed by 0.2% peracetic acid or 2.5% quaternary ammonium compounds in continuous treatment.

Results
Microbiological quality of water varied between dental hospitals and smaller premises: Legionella pneumophila sg 2-14 was isolated in 30% (3/10) of DUWs housed in dental premises and 26% (6/23) of those in teaching hospitals. High concentrations (102-104 CFU/L) of Legionella spp. were detected in hospital tap water, handpieces (102-105 CFU/L) and spittoons (102- 103 CFU/L). P. aeruginosa was frequently associated with high TVCs and it was detected in 58% (19/33) of all DUWs, mostly in handpiece devices. Brevundimonas vesicularis was detected in 6% (2/33) of dental units. FLA were recovered from 18% (6/33) of hospital DUWs. Among all cells microscopically positive to the culture examination, all PCR positive isolates belonged to Vermoamoeba vermiformis (identity of 99%). Shock disinfection with 3% HP resulted effective against Legionella spp. and it was never detected after 10 days from treatment. However high TVCs were still observed and P. aeruginosa was isolated from handpiece and spittoon in 79% (11/14) of hospital dental units. Pseudomonas was subsequently eradicated with 6% HP and surfactants shock treatment. Our data suggest the presence of a large contamination and biofilm persistence in DUWs, as a source of hazards specific for patients and dental staff. The presence of amoebae and biofilm justify the inefficacy of low-level disinfectants. This occurrence requires a water risk management and an effective choice of DUWs disinfectant to obtain an hazards control during dental practices.

More than 100 years after the discovery of Toxoplasma gondii what's new

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A century after the discovery of *Toxoplasma gondii* many issues have been clarified but many others remain a matter of debate despite the extensive inbestigation of this apicomplexa parasite, Nowadays it is clear that the origin of the parasite is South America where the highest variability of the strain (with the most virulent type) is recorded . It is well known that toxoplasmosis is not just a foodborne disease but also a waterborne disease and recently the safety of different foods with regard to Toxoplasma have been examined as an important component of for the alimentary prophylaxis for non-immune pregnant women ad immunocompromised patients. Although screening for toxoplasma infection is mandatory only in a few countries, only many data support the prenatal screening also in countries with intermediate seroprevalence. However, the decrease in prevalence in many European nations could make the screening too expensive. One of the reasons in support for the screening, was that correct therapy could reduce the transmission and the severity of congenital infection. The introduction of automated tests for IgG and IgM detection and for IgG avidity allowed a more accurate and reproducible analysis in the mother and also real time PCR improved our diagnostic efficiency in congenital infection of the fetus. The availability of very sensitive Western Blot and Interferon Gamma Release Assay (IGRA) for newborns at risk allows diagnosis of congenital infection in the first 3 months of life, thus avoiding unnecessary therapy. In many countries the screening of graft donor and recipient nd prophylaxis have reduced the severity and mortality of toxoplasmosis, but this problem still exists. In transplanted patients the categories at risk are different according to the graft type: for solid organs transplanted patients those at risk are the seronegative recipients with seropositive donors , for hematopoietic stem cells transplanted patients those at risk are seropositives that became negative after the graft with seronegative donor. Also in these cases prophylaxis and diagnosis with sensitive and specific serological test , IGRA , real time PCR and prompt therapy can avoid disseminated, life-threatening infections. However, the interconnections between host and parasite during acute and chronic infection are still incompletely understood. A better knowledge of infection mechanism and its influence on neurobiochemical and neuropathological pathways of infection could help us explain the correlation between toxoplasmosis and neurological disorders.

POSTER BATTERIOLOGIA

P001 - Phycocyanin aqueous extracts from the cyanophyta Aphanizomenon flos-aquae with prebiotic beneficial effect

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Introduction: *Aphanizomenon flos-aquae* (AFA) is a freshwater unicellular blue-green alga consumed as a nutrient-dense food source and for its health-enhancing properties. One particular interesting feature in marine algae is their richness in natural pigments, including chlorophylls, carotenoids and phycobiliproteins (such as phycocyanins), that besides photosynthetic and pigmentation effects have showed health benefits, attracting much attention in food and pharmacology fields. The aim of this research was to investigate the prebiotic effect of two phycocyanin aqueous extracts derived from AFA (Blue Extract and Pure-PC) on viability and antimicrobial activity of different Lactic Acid Bacteria (LAB), such as *Lactobacillus rhamnosus* ATCC 53103, *Lactobacillus acidophilus* ATCC 4356 and *Bifidumbacterium bifidum* ATCC 29521.

Materials and Methods: Blue Extract and Pure-PC were added (2%) to standard broth culture medium (MRS broth) to verify the prolongation of viability of the examined LAB strains. Then, the antimicrobial activity of cell-free culture supernatants (CFCSs), obtained from LABs grown with or without the extracts, was determined against *Escherichia coli* O157:H7 ATCC 35150, *Staphylococcus aureus* ATCC 43387 and *Candida albicans* ATCC 14053 by time-kill studies. CFCS were selected on the basis of the data obtained from each LAB strains after their growth in MRS broth with and without Blue Extract or Pure-PC at different time of incubation (24, 48 and 120 hours).

Results: Media supplemented with Blue Extract and Pure-PC stimulated the growth of each LAB strain compared to that obtained in the standard medium, up to 346 h in the case of *B. bifidum*. The presence of Blue Extract and Pure-PC in culture medium also enhanced the antimicrobial activity of CFCSs from each LAB, resulting in a remarkable reduction of the selected human pathogens viability in time-kill experiments after 8h of incubation. This effect was probably related to the prolongation of viability of LAB strains in MRS broth supplemented with Blue Extracts and Pure-PC, particularly evident for *L. rhamnosus* ATCC 53103 and *B. bifidum* ATCC 29521. It can be also observed that the antimicrobial effect of CFCSs was higher when LAB strains were grown in presence of Pure-PC rather than with Blue Extract, because Pure-PC contains phycocyanin in high quantity and quality.

Discussion and Conclusion: Our data suggest that the examined phycocyanin extracts could be used as potential prebiotic substrata able to stimulate the growth of different LAB strains and to enhance their antimicrobial activity. For this, AFA extracts can be considered as suitable prebiotic substratum with beneficial effect on human microbiota and therefore on human health. The results are currently being patented.

P002 - Isolation and characterization of antibiotic-producing bacteria from marine invertebrates

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Introduction: The marine environment, due to its enormous biodiversity, is considered a rich but largely unexplored source of antimicrobial agents. As already known, both marine invertebrates and their associated microbiota can produce a large variety of secondary metabolites. Considering the need for new antibacterial agents active on antibiotic-resistant bacteria, we screened for antibiotic-producing bacteria among isolates recovered from Mediterranean marine sponge samples.

Materials and Methods: Marine invertebrates were collected in the Ligurian Sea (Mediterranean Sea, Italy). Samples were homogenized in artificial sea water and the cultivable microbial fraction of the marine sponge grown on several culture media specific for marine microorganisms (e. g. Marine Agar, Actinomycete Isolation Seawater Agar, Starch-Yeast Peptone and Starch Casein Agar). Bacterial quantification was determined after at least one week of incubation at 25 °C. Antibiotic-producing organisms were identified using the overlay method with *E. coli* CCUG^{T}, *S. epidermidis* ATCC 14990 and *B. subtilis* ATCC 6633 as reporter microorganisms. The cross-streak method was used to confirm antibiotic production in selected organisms. Preliminary bacterial identification was carried out using 16S rDNA amplification and sequencing. In selected organisms, growth conditions for antibiotic production were optimized.

Results: A total of nineteen antibiotic-producing bacterial isolates were found starting from six sponge homogenates. Preliminary characterization of organisms forming morphologically distinct colonies revealed that most isolates belong to the heterogeneous Bacillales order. One of the isolates, named AD1-26, could be easily grown in liquid medium, and allowed us to optimize the culture conditions (SB medium supplemented with 0.25 % glucose, 30 °C) most suitable for antibiotic production. The supernatant of AD1-26 cultures was used to purify the bioactive compound using adsorption on Amberlite XAD-4 resin followed by methanol extraction and HPLC purification. The purified extract showed activity on both *E. coli* CCUG^{T} and *B. subtilis* ATCC 6633.

Discussion and conclusion: Marine invertebrates, together with their bacterial symbionts represent one of the richest sources of biologically active secondary metabolites, some of which exhibit antibacterial properties. In this study, we isolated and characterized bacterial symbionts recovered from several samples of marine invertebrates from the Mediterranean Sea. One *Paenibacillus* spp. isolate, AD1-26, allowed the production and purification of an antibacterial compound showing activity on both Gram-negative and Gram-positive bacteria.

P003 - Antimicrobial activity of ircinin-2 isolated from a marine Mediterranean sponge Ircinia dendroides

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Introduction: Increasing levels of antibiotic resistance among clinically relevant bacterial pathogens, with the paucity of new drugs approved for the treatment of life-threatening infections, highlight the urgent need of new effective therapies. The marine environment, thanks to its biodiversity and the consequent chemical diversity represents a very promising source of new bioactive compounds. In this work, we report the isolation and characterization of an antibacterial compound isolated from the marine sponge *Ircinia dendroides* collected from the Mediterranean Sea.

Materials and Methods: A sample of *I. dendroides* was collected at 60 m depth in the Ligurian Sea (Italy). Sample extraction was carried out using a modified Kupchan's method. The antibacterial activity of organic extracts was evaluated by the agar diffusion method on a panel of both Gram-negative and Gram-positive bacteria. The chloroform extract was further purified by C18 reversed-phase column and NMR analysis was performed for structural determination. Determination of MIC and MBC were carried out using standard methods (CLSI) using both type strains or antibiotic-resistant clinical isolates. Time-kill studies were carried out with *Staphylococcus aureus* ATCC 25923 in the presence of 2x, 4x and 10x MIC of the purified compound for up to 24 hours at 37 °C.

Results: Although all obtained organic extracts showed, in agar diffusion tests, antibacterial activity against at least one of the tested bacterial species, the chloroform extract showed activity on 5 different organisms. HPLC purification of the extract allowed a good separation of the original mixture, with only 1 peak showing antibacterial activity. NMR analysis revealed the presence of two isomeric compounds in the purified extracts, which prevalently consisted of ircinin-2 and contained a marginal quantity of ircinin-1. The purified extracts showed antibacterial activity essentially against Gram-positive bacteria (MIC range, 2 – 8 microg/ml). In addition, it was also active on antibiotic-resistant clinical isolates of staphylococci, with MIC values ranging 4-8 microg/ml. Time-kill curves carried out with *S. aureus* ATCC 25923 confirmed the bactericidal activity of ircinin extracts, which showed a 4 log10 reduction of the bacterial load within 6 h.

Discussion and conclusions: Marine sponges, including of the genus *Ircinia*, are well known as a rich source of biologically active natural products with analgesic, anti-inflammatory and antitumor activities. In this work, we report the antibacterial properties of ircinin-2, a bioactive compound previously isolated from the marine sponge *Ircinia oros*. Ircinin-2 showed a bactericidal activity on *S. aureus* and was active on various antibiotic-resistant Gram-positive clinical isolates.

P004 - Bdellovibrio bacteriovorus to treat gut dysbiosis and pulmonary infections characterized by Gram-negative overgrowth

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Introduction. *Bdellovibrio bacteriovorus* is a Gram-negative predator bacterium, able to destroy bacterial biofilms that has already been proposed for treating bacterial infections. Our previous studies revealed the presence of *B. bacteriovorus* in healthy human intestinal mucosae and its significant decrease in intestinal mucosae of CD, UC and Celiac patients. Furthermore we showed *B. bacteriovorus* predatory activity *versus* the biofilm of the gram-positive *Staphylococcus aureus.* The aim of the present study was to evaluate *B. bacteriovorus* therapeutic potentiality for the treatment of the Aderent Invasive *Escherichia coli* (AIEC) and *Achromobacter xylosoxidans* overgrowth.

Materials and Methods. Strains: AIEC strain, a new *E. coli* patho-type isolated from intestinal mucosae of active CD patients, and the emergent pathogens *Achromobacter xylosoxidans* in Cystic Fibrosis (CF) lung infections (isolated from salivary samples of CF patients) were employed in the study. The predatory activity of *B. bacteriovorus* will be evaluated *versus* the selected strains in sessile and planktonic growth. The study also contemplates the use of intestinal epithelial cell line CaCo-2 and pulmonary cell line, A549 to evaluate the impact of *B. bacteriovorus* suspension and culture supernatant on, AIEC and *A. xylosoxidans*, adhesion and invasion ability.

Results. Preliminary results indicate a significant reduction of AIEC and *A. xylosoxidans* biofilms, when treated with *B. bacteriovorus* suspension or *B. bacteriovorus* culture supernatant.

Discussion and Conclusion. *B. bacteriovorus* by its predatory activity seems to be a good candidate to treat infections in vivo. Its presence in the intestinal mucosae of healthy subjects, and its absence in the mucosae of CD patients (where the overgrowth of gram negative, especially *E. coli*, occurs), clearly indicates its role in maintaining a balance among bacterial species, inside a microbial ecosystem. Our results will give us more information on its potential use against AIEC overgrowth in CD patients, and *A. xylosoxidans* pulmonary infections in CF patients.

P005 - Investigation of the mechanism of action of antimicrobial peptides: interactions with bacterial membrane proteins

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Introduction
The development of new drugs able to counteract pathogenic microbes is crucial in many application fields due to the large increase of drug-resistant bacterial strains. Antimicrobial peptides (AMPs) might constitute an alternative to conventional antibiotics; however, the possible development of AMPs as new drugs is strictly dependent on the definition of their molecular mechanism of action. We focused our attention on the elucidation of the molecular mechanism of Temporin-L by isolation and identification of the *E. coli* membrane proteins specifically binding the peptide. We proved that Temporin-L interacts with some essential proteins belonging to the elongasome and the divisome complexes and inhibits bacteria cell division by blocking the FtsZ protein crucial for the formation of the Z-ring at the beginning of the division process.

Materials and Methods
Data were obtained by functional proteomics approaches and confirmed by both optical and molecular spectroscopic assays.

Results
Biotinylated Temporin L was incubated with membrane proteins from *E.coli* cells. Putative protein interactors were eluted, digested *in situ* with trypsin and analysed by LC/MS-MS methodologies. Mass data were used for database search leading to protein identification. The results showed that Temporin-L interacts with many proteins belonging to the divisome and elongasome complexes. Optical microscopy investigation demonstrated that Temporin L inhibits bacterial cell division leading to the formation of long "necklace-like" cell structures. These data were confirmed by both Dynamic Light Scattering (DLS) and Small Angle Neutron Scattering **(**SANS) measurements that showed a rapid increase in the dimension of the "necklace-like" *E.coli* cell structures following Temporin L treatment. Sequence alignment of Temporin L with the CRAMP peptide suggested a possible interaction of the peptide with FtsZ protein crucial for Z-ring formation. A model of the complex was obtained by docking technique confirming the interaction of Temporin L with the T-loop of FtsZ.

Conclusions
Investigation of Temporin L mechanism proved that the AMP affects cell division by interacting with FtsZ and other proteins belonging to the divisome complex thus inhibiting the crucial step of Z-ring formation at the beginning of the division process.

P006 - Effect of Satureja montana L. (Lamiaceae) essential oil nanoemulsions against avian Escherichia coli strains

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Introduction
Massive use of antibiotics in breeding farm has contributed to the spread of multidrug resistant bacteria and poultry has been suggested as a *reservoir* for multidrug-resistant E*scherichia coli* strains. Many plant derived essential oils (EOs) exhibit biological activity mainly due to typical features of their components such as hydrophobicity, that allows partitioning with the lipids of the bacterial cell membrane interfering with the cell permeability and resulting in a bactericidal/bacteriostatic effect. Nanoemulsions (NEs) are colloidal dispersion in which main components are oil, emulsifying agents and aqueous phases. NEs were investigated to improving the delivery of active pharmaceutical or natural ingredients. In order to test the inhibiting activity on bacterial growth and biofilm production, NEs obtained from *S. montana* essential oil has been tested against *E. coli* isolates from healthy poultry fecal samples characterized for antibiotic resistance and metal tolerance.

Materials and Methods
For determining the qualitative and quantitative features of *S. montana* essential oil from Italy, analysis GC-MS were performed. Nanoemulsions are produced in a ratio of 1:1 with essential oil and Tween (20 and 80), and characterized for size and stability. Avian *E. coli* strains were analyzed for antibiotic resistance and heavy metal tolerance. Minimal inhibitory concentration and Minimal bactericidal concentration of NEs were determined by NEs and Mueller Hinton Broth in a ratio 1:4 in different volumes. Quantification of biofilm production in the presence of NEs was assessed by crystal violet staining.

Results
GC-MS analysis of the *S. montana* EO points to the presence of different classes of volatile phytochemicals, including terpenes and terpenoids. The most abundant components are carvacrol (25%), linalool (17%), g-terpinene (16%) and thymol (15%). The mass percentage of the thymol and carvacrol isomers correspond to 45% of the total. Increasing surfactant content, the nanodroplet dimensions decrease. Furthermore, all samples show negative zeta-potential values, useful to prevent nanodroplet aggregation. Microbiological results show that NEs have inhibiting properties both on biofilm production and growth of Gram negative bacteria.

Discussion and Conclusions
The results of this work confirm the antimicrobial activity of *S. montana* essential oil, as well as of newly designed nanoemulsions. Addition of *S. montana* NEs to the feed could represent a tool for improving delivery and intracellular concentration of natural antimicrobial compounds in antibiotic resistant poultry in chicken farms.

P007 - A promising potentiator to restore the effectiveness of Levofloxacin in multi-drugs resistant Helicobacter pylori: Pistacia vera L. oleoresin

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Introduction: *Helicobacter pylori* is a important gastrointestinal pathogen that it plays an important role in the pathogenesis of chronic gastritis, peptic ulcer, gastric adenocarcinoma, and gastric MALT lymphoma. *H. pylori* infection is difficult to eradicate and it is necessary to combine some different antibiotics: clarithromicyn, metronidazole, amoxicillin, levofloxacin and proton pump inhibitor. Increasing antimicrobial resistance and failing eradication rates underline the importance of the updated guidelines for the management of the *H. pylori* infection.
Oleoresin (ORS) of *P. vera* L., extracted from incisions made in the tree trunk is, up to now, less studied for its potential pharmacologic uses probably due for the lack of information about the traditional uses and because of the high economic value of the fruits that have left behind the potential exploitation of secondary products of its cultivation.
The aim of this study was to evaluate the *in vitro* antimicrobial and antibiofilm activities of *P. vera* L. ORS alone and combined to levofloxacin (LEVO) against multi-drugs resistant strains of *H. pylori*. Wax month *Galleria mellonella* was used as *in vivo* model to confirm the ORS antibacterial action.

Materials and methods: Five clinical strains of *H. pylori* and the reference strain were used for the study. The *in vitro* susceptibility to *P. vera* L. ORS, LEVO and their combinations were performed by the microdilution and checkerboard assay. The antibiofilm properties of ORS alone and associated with LEVO were also evaluated on biofilm formation by biomass measurement and the cellular viability by BacLight Live/Dead Kit. The *in vivo* ORS antibacterial action was determined as survival percentage of *G. mellonella* larvae.

Results: The MIC values of ORS ranged from 780 to 3120 mg/L and the MICs of LEVO ranged from 0.12 to 1.00 mg/L. In all strains a synergistic combination was recorded and in these cases the ORS was able to restore the effectiveness of LEVO reducing the MICs values. A significant percentage reduction was detected after treatment with ORS and LEVO in respect to the untreated samples. Synergistic combinations of ORS and LEVO induced a general reduction in microbial aggregates and substantial loss in cell viability. *In vivo*, ORS and LEVO displayed a protective effect against *H. pylori* infection with a survival percentage of *G. mellonella* larvae of 65% and 90%, respectively.

Discussion and conclusions: ORS is a compound capable of restoring the effectiveness of LEVO through the reduction of its MICs in *H. pylori* multi-drugs resistant strains. The strong effect of ORS and LEVO on biofilm and the *in vivo* protective effect suggest that ORS combined with antibiotics could provide a novel strategy to tackle the antibiotic resistance in *H. pylori*.

P008 - Biochemical characterization of clinical strains of Staphylococcus sp. and their sensitivity against polyphenols-rich extracts from pistachio (Pistacia vera L.)

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1. Introduction

Staphylococcus aureus and methicillin-resistant *S. aureus* (MRSA) pose a serious treat to public health and represent the most significant Gram-positive human pathogens. The aim of this work was to characterize clinical isolates of *Staphylococcus* sp. (methicillin-resistance, presence of coagulase, lipase and biochemical analysis with API system) and test their sensitivity against pistachio extracts.

2. Materials and Methods

The following strains, obtained from the University of Messina's in-house culture collection (Messina, Italy), were used for antimicrobial testing: *Staphylococcus aureus* ATCC 6538P, 31 clinical isolates of S. *sp.* obtained from swabs of patients with an orthopaedic infection. Out of the 31 clinical isolates, 16 were obtained from a knee prosthesis or surgical wound, 7 from hip prosthesis and 8 from other orthopaedic sites. MIC determinations were performed according to CLSI.

3. Results

Out of the 31 clinical isolates, 23 were coagulase positive and identified as *S. aureus*, of which 21 were MRSA. Polyphenols rich extracts from natural raw and roasted pistachios were active against *S. aureus* 6538P and the clinical isolates, roasted pistachios being the most active (MIC values ranging between 31.25 and 2000 μg/ml).

4. Conclusions

The antimicrobial effect of pistachios could be used to identify novel treatments for antibiotic resistance as well as topical agents for *S. aureus* skin infections.

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P009 - Comparative evaluation of the inhibitory effect of some essential oils against Multidrug Resistant Pseudomonas aeruginosa

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Introduction. The opportunistic pathogen *Pseudomonas aeruginosa*, like all members of the genus Pseudomonas, has the capacity to thrive in very different environments, such as water, plant roots, animals, including humans in whom it can cause severe infections especially in immunocompromised patients. Infections by *P.aeruginosa* are notoriously difficult to treat due to its intrinsic ability to resist many classes of antibiotics as well as its ability to acquire resistance.
Given the severity of *P.aeruginosa* infections and the limited antimicrobial arsenal with which to treat them, finding alternative prevention and treatment strategies is an urgent priority.
Hence, the objective of our study was to investigate the *in vitro* antibacterial activity of some essential oils (EOs) and their main components against *P.aeruginosa* strains. Amikacin (AMK) was used as reference drug.

Materials and Methods. A clinical multidrug resistant *P.aeruginosa* (MDR-PA) strain and *P.aeruginosa* ATCC 27853 were used. The EOs of *Mentha X piperita* and *Thymus vulgaris* and their main components (menthol and thymol) were tested for *in vitro* antibacterial activity. The EOs and components were purchased from Erbe Aromatiche Essenzialmenta (Pancalieri, Italy) and Primavera/Flora (Pisa, Italy). AMK was commercially purchased (Sigma-Aldrich, Milano, Italy). The chemical composition of the EOs was analysed by Gas Chromatography–Mass Spectometry (GC/MS). The Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal Concentration (MBC) of EOs, components and AMK were evaluated by means of the twofold serial microdilution assay according to the CLSI M100-S25 (2015) with some modifications for EOs. The final concentrations ranged from 4% to 0.0078% (v/v).

Results. In this study, the results showed that all tested oils were ineffective on MDR-PA and *P.aeruginosa* ATCC 27853 strains. In fact, the MIC and MBC values ranged from 4% to >4% (v/v).
Conversely, the menthol and thymol displayed an interesting activity: the MIC value of menthol was 0.25% v/v against MDR-PA (MBC=0.25% v/v) and *P.aeruginosa* ATCC 27853 (MBC=>2% v/v). The MIC value of thymol was 0.125% v/v against MDR-PA (MBC=0.25% v/v) and *P.aeruginosa* ATCC 27853 (MBC=0.5% v/v).

Discussion and Conclusions. Several studies have reported that *P. aeruginosa* appear to be least sensitive to the action of EOs. In the present study, the main components demonstrated promising antimicrobial activities against this bacterium. These results provide significant information on the activity of these components, suggesting their potential use. These encouraging results require further confirmation, to better clarify these data.

P010 - Attenuation of Listeria monocytogenes virulence by Cannabis sativa L. essential oil

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Introduction. In recent years, the use of plant products as alternative/adjunct antimicrobial agents to control pathogenic microorganisms has been attracting mounting interest. A major group of plant antimicrobial compounds is represented by essential oils (EOs), complex mixtures of volatile secondary metabolites belonging to different chemical families. *Cannabis sativa* L. has been grown for thousands of years for a multiplicity of purposes; in recent years, some genotypes containing low cannabinoid concentrations have been selected and used for research purposes. *Listeria monocytogenes* is a facultative intracellular food pathogen able to persist within biofilms and to develop resistance to sanitizers. In humans, the treatment of listeriosis is hampered by the intracellular location of listeriae and the poor intracellular penetration of some antibiotics. The purpose of this study was to investigate the *in vitro* and *in vivo* antibacterial and anti-virulence properties of an EO extracted from a legal *C. sativa* L. variety against *L. monocytogenes* isolates collected from patients with invasive listeriosis.

Materials and Methods. Eleven *L. monocytogenes* strains isolated in Italy in 2014-2016 were studied. The antibacterial and anti-virulence activity of the EO, extracted from *C. sativa* Futura 75 by steam-distillation, was determined by susceptibility tests, biofilm formation assays, Caco-2 invasion assays, motility assays, optical and scanning electron microscopy, Real-time RT-PCR experiments, and *Galleria mellonella* survival assays.

Results. A moderate bactericidal activity of the *C. sativa* EO was detected (Minimum Bactericidal Concentration >2048 mg/L). In presence of EO at sub-lethal concentrations, the ability to form biofilm and to invade Caco-2 cells was significantly reduced (up to 15% and 73%, respectively). Motility tests demonstrated that, listeriae became non-motile i.e. they didn't show the typical umbrella-like growth. Real-time RT-PCR assay demonstrated a significant downregulation of motility genes (*flaA*, *motA*, and *motB*) in *L. monocytogenes* exposed to the EO. In survival experiments with *Galleria mellonella*, larvae infected with *L. monocytogenes* grown in presence of EO showed much higher (93%) survival rates compared with controls (50%).

Discussion and Conclusions. Anti-virulence strategies are being explored among plant products as a novel approach to combat bacterial pathogens. The significant anti-virulence properties of *C. sativa* EO against *L. monocytogenes* suggests that it could be employed as an alternative agent to control *L. monocytogenes* infection.

P011 - Effect of pomegranate juice and peel extracts on cariogenic bacteria: an in vitro study

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Introduction: Even though the prevalence of dental caries has decreased through the use of preventive systems it still remains one of the most common chronic diseases that affect children. The etiology of tooth decay is multi-factorial and it's induced by three main factors: host, environment and bacteria. Today it's known that different bacterial species cause the strongest effect on the incidence of dental caries, including *Streptococcus mutans*. Many studies have revealed that *S. mutans* represents about the 20–40% of the cultivable flora in biofilms removed from carious lesion. *Rothia* spp., in particular *R. dentocariosa*, are common inhabitants of the oral cavity. Recent reports suggest that this species could be opportunistic pathogen, causing a number of diseases in addition to dental and periodontal pathologies. The control of microorganisms responsible for the pathogenesis of dental caries is still a challenge today as these germs have developed tolerance and resistance to many antimicrobial agents routinely used in the clinical practice. In this experimental context, numerous extracts of medicinal plants have shown antimicrobial activity; however, only a few natural products have found therapeutic applications. Recent studies have shown a powerful antioxidant activity of pomegranate (*Punica granatum L.*) for its high content of polyphenols, including ellagitannins and anthocyanins and an effective antimicrobial activity. The aim of the present study was to evaluate the antimicrobial activity of hydroalcoholic extracts of pomegranate peel and juice, against the microorganisms considered the main etiologic agents of dental caries.

Materials and methods: the values of the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of the extracts were determined against *S. mutans* Clarke ATCC 25175 strain and *R. dentocariosa* clinical isolate.

Results: peel extracts inhibit effectively the growth and survival of *S. mutans* ATCC 25175 strain and *R. dentocariosa* clinical isolate with MIC and MBC values of 10 μg/μl and 15μg/μl, respectively. Furthermore, the pomegranate juice extract showed high inhibitory activity against *S. mutans* ATCC 25175 strain with a MIC value of 25 μg/μl and a MBC of 40μg/μl, whereas, against *R. dentocariosa*, it has displayed a moderate inhibitory activity, with MIC and MBC values of 20 μg/μl and 140μg/μl, respectively.

Discussion and Conclusions: *in vitro* microbiological tests demonstrate that the hydroalcoholic extracts of pomegranate juice and peel are able to counteract the main cariogenic bacteria involved in tooth decay. Although being preliminary data, our results suggest that pomegranate polyphenolic compounds could represent a good adjuvant for the prevention and treatment of dental caries.

P012 - Antimicrobial and Antibiofilm Activities of the Italian Hemp (Cannabis sativa L.) Essential Oil Against Staphylococcus aureus

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Introduction: The renewed interest for industrial Hemp (*Cannabis sativa* L.) products has focused attention on its ecological, economic and social impact. The production, extraction and transformation of Hemp crops are often studied for the process of optimization, while the new potential applications, which are not related to the traditional uses, have not been fully investigated. In this context, the Essential Oil (EO) extraction could be an innovative application in pharmaceutical, cosmetic or food industries, therefore, the aim of the study was to evaluate the antimicrobial and antibiofilm properties of Hemp EO *versus Staphylococcus aureus* (*Sa*).

Materials and Methods: The essential oil was obtained by hydro-distillation of fresh aerial parts from industrial Hemp cv. Futura75 cultivated in Abruzzo region, Italy. The antimicrobial and antibiofilm activity of Hemp EO were evaluated on *Sa* ATCC 29213 and three clinical strains of *Sa* 101, *Sa* 104, *Sa* 105, isolated by different sources and characterized for the antimicrobial susceptibility pattern. The determination of the Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal Concentration (MBC) was carried out by using the broth microdilution method according to the NCCLS guidelines and the alamarBlue® (AB) viability assay in the range of 0.5-16 mg/mL. The antibiofilm effect was determined by the evaluation of Minimum Biofilm Eradication Concentration (MBEC) by using the broth micro-dilution method in the range of 16-32 mg/mL and the AB assay. The MBEC was confirmed by Live/Dead staining and fluorescent microscopy analysis. However, Colony Forming Unit (CFU) enumeration was performed in the biofilm and planktonic phenotypes. Moreover, the phenolic profile of the EO was determined by using a validated HPLC-PDA method in order to correlate the biological activities with the chemical profile on selected compounds.

Results: The Hemp EO showed a MIC corresponding to 8 mg/mL and a MBC at 16 mg/mL *versus* all *S. aureus* strains. Furthermore, the Hemp EO showed the capability to eradicate a mature biofilm developed by *S. aureus* with a MBEC at 24 mg/mL towards all the strains of *S. aureus* except for *S. aureus* 105 that showed a MBEC corresponding to 16 mg/mL. The effect of the Hemp EO in the eradication of *S. aureus* mature biofilms was confirmed by the significant reduction of the CFU count as well as by the Live/Dead staining and fluorescent microscopy that showed a multitude of dead cells in the biofilms treated with 24 mg/mL of Hemp EO.

Discussion and Conclusions: The antibacterial and antibiofilm activities of Hemp EO suggest that it could be a possible candidate in the treatment of infections associated with *S. aureus*.

P013 - Helix aspersa muller mucus (Helixcomplex ®) p41 protein prevents Pseudomonas aeruginosa growth and promotes mammalian bronchial epithelial cell proliferation

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Introduction: Snail mucus is a mixture of active substances commonly thought to have healthy properties for the treatment of skin disorders. Although snail mucus is an ingredient of various cosmetic and para-pharmaceutic products, the chemical composition and the biological effects are still unknown.

Materials and Methods: Crude extract from *Helix aspersa muller* mucus (HelixComplex®) was obtained (Patent N: 102017000117547) and, after chemical and molecular characterization, tested on *in vitro* experimental models of microbial infections and bronchial epithelial cell proliferation.

Results: Differently from what expected, HelixComplex® was characterized by the presence of small amounts of glycolic acid and allantoin. In size separation experiments, we observed a peculiar protein band at 30-40 kDa. Protein band was extracted and analyzed by ESI-Q-TOF-HPLC-MS instrument: the protein band in the range of 40-30kDa was composed by a single protein of 41kDa (p41). The product obtained from size exclusion centrifugation at 41 kDa showed anti-bacterial activities. In particular, it presents a strong bactericidal effect on *P. aeruginosa*, with an IC50 dose of 16ug/ul, as early as 15 minutes after treatment, on both laboratory strain PAO1 and *P. aeruginosa* clinical strains. This bactericidal effect was evident on both planktonic and biofilm-forming cultures of clinical *P. aeuruginosa* strains. By using different *in vitro* assays on epithelial bronchial cell cultures, we found that HelixComplex® lacked of cytotoxicity, and, importantly, that it was able to significantly induce cell proliferation.

Conclusions: These results identify the *H. aspersa* snail mucus p41 as a suitable antimicrobial compound for the treatment of *P. aeruginosa* associated infections, where we need to: i) block *P. aeruginosa* proliferation and biofilm formation; ii) ensure a regenerative efficacy on bronchial epithelial cells. Interestingly, we obtained efficacy results only in the presence of a protein complex purified by size exclusion centrifugation. This procedure guarantees the presence of the p41, but also of other actives present in the *H. aspersa* mucus, confirming the power and uniqueness of the actives present inside this natural product.

P014 - Epigenetic modulator UVI5008 inhibits MRSA by interfering with bacterial gyrase

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Introduction: The impact of multi-drug resistant bacterial strains on human health is reaching worrisome levels. The re-purposing of existing drugs, developed for other application, as antimicrobial agents is nowadays an interesting approach. To this end, we screened a panel of drugs previously characterized to be epigenetic drugs. Among them we selected UVI5008 that alter Gram-positive growth.

Materials and Methods: Susceptibility testing was performed following the broth micro-dilution method against four pathogenic bacterial strains including Gram-negative bacteria (*Escherichia coli* and *Klebsiella pneumonia*) and Gram-positive (*Staphylococcus aureus* and *Enterococcus faecalis*). The antibacterial action of UVI5008 was defined by determining its minimum inhibitory concentration (MIC) on the multi-sensitive standard strain *Staphylococcus aureus* ATCC and its four corresponding clinical isolates. The ability of the drug to form and degrade the biofilm was performed through the 2,3,5-triphenyl-tetrazolium chloride reduction assay.The key structural motifs was identified by testing its 18 structural modifications. The mechanism of UVI5008 action was evaluated through DNA gyrase-specific inhibition and DNA gyrase ATPase linked assay. Its effect on the morphology of the bacterium was assessed by scanning electron microscopy.

Results: The results show that UVI5008 is active against Gram-positive bacteria with a MIC of 8 and 25μM for *Staphylococcus aureus* ATCC and its four corresponding clinical isolates, respectively. The clinical isolates exhibit greater biofilm production than the standard strain, limiting the spread of the drug.UVI5008 degraded 50% of the mature biofilm at 50μM.The key structural motifs of compound are bromine in the para position, the specular portion of the molecule and the disulfide bridge. It reduced the enzymatic activity by about 80% with an IC50 of 20.4 μM, not showing direct activity on the B subunit. UVI5008 induced damage on the bacterial surface.

Conclusions: UVI5008 could represent an alternative approach to fight the methicillin-resistant *Staphylococcus aureus* strains diffusion.

P015 - Synergistic effect of abietic acid with oxacillin against methicillin-resistant Staphylococcus pseudintermedius

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1. Introduction

Resin acids are known to have antibacterial activity and are valued in traditional medicine for their antiseptic properties. Among these, abietic acid has been reported to be active against methicillin-resistant *Staphylococcus aureus* (MRSA) strains. In veterinary healthcare the methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) strain is an important reservoirs of antibiotic resistance genes including *mecA* gene, which encodes a lower-affinity penicillin binding protein PBP2′ which is absent in methicillin sensitive strains. The incidence of MRSP has been increasing, and treatment options in veterinary medicine are partial. Here, we evaluated the antibacterial activity of abietic acid, alone and in combinations with methicillin, against MRSP.

2. Materials and Methods.

One strain of MRSA and one of methicillin-susceptible *S. pseudintermedius* (MSSP) were selected at the Microbiology Laboratory of Department of Veterinary Medicine and Animal Production, University of Naples "Federico II", from canine auricular swabs. Coagulase-positive Staphylococcus cultures were identified by MALDI-TOF-MS. The antimicrobial susceptibility patterns were determined by disk diffusion test. Molecular profiling, using species-specific thermonuclease nuc gene, was also performed to confirm the phenotypical identification. The minimum inhibitory concentration (MIC) of the abietic acid against MRSP and MSSP isolates was determined by broth microdilution method. The interactions between abietic acid and methicillin against MRSP were evaluated by the checkerboard assay.

3. Results

Abietic acid showed a significant MIC_{90} value at 32 μg/mL (MRSP) and 8 μg/mL (MSSP), while the minimum bactericidal concentration (MBC) values were 64 μg/mL (2X MIC) and 32 μg/mL (4X MIC) for MRSP and MSSP, respectively. Furthermore, the checkerboard method showed a synergistic effect of abietic acid with oxacillin against MRSP, with a value of Fractional Inhibitory Concentration (FIC) index of 0,375. The highest synergistic interaction against MRSP was obtained in the wells with the best combination values of 4 μg/mL abietic acid (1/8 MIC) and 2,5 μg/mL oxacillin (1/4 MIC).

4. Discussion and Conclusion.

The extensive use of antibiotics has led to the emergence of multidrug resistant bacteria and the need to develop new antimicrobials. Our results demonstrate the antimicrobial activity of abietic acid against MRSP and MSSP. In addition to having a good antibacterial activity, abietic acid displays an important synergistic effect when used in combination with oxacillin. We suggest that abietic acid might represent a potential therapeutic candidate especially when used in combination therapy against MRSP.

P016 - Erythromycin-loaded nanodroplets as adjuvant therapeutics for infected chronic wounds caused by Streptococcus pyogenes

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Introduction: Chronic wounds are typically characterized by persistent tissue hypoxia, exacerbated inflammation, and impaired matrix remodeling. Moreover, chronic wounds are often worsened by microbial infections, with antimicrobial therapies being hindered by emerging resistant strains. *Streptococcus pyogenes* infections vary from superficial infection of the pharynx to serious skin and soft tissue ones that can lead to lethal invasive disease, despite antibiotic treatment. The use of nanoparticles has advantages in treating many dermatological conditions. Nanoparticle applications facilitate the body's response to foreign pathogens by improving innate and adaptive immune responses, increasing the effectiveness and reducing the adverse effects of antimicrobials and other therapeutic agents. The aim of this project is to assess the potential antimicrobial effect of oxygen free nanodroplets (OFNDs) and oxygen loaded nanodroplets (OLNDs) alone or coupled with erythromycin (Ery) in skin and soft tissue infections sustained by *S. pyogenes*, along with toxicity on human keratinocytes.

Materials and methods: Low molecular weight chitosan-shelled and 2H,3H-decafluoropentane-cored NDs, both OFNDs and OLNDs, and Ery-loaded NDs (Ery-LNDs) were prepared and characterized for physico-chemical parameters and drug release kinetics. Subsequently all NDs were tested for biocompatibility with human skin cells. Complementary analysis by confocal microscopy was also performed to study the physical interaction between NDs and *S. pyogenes* cell wall/membranes. Finally, bacterial growth was monitored upon incubation with or without NDs for increasing times (4, 6, 24 hours).

Results: All NDs were not toxic to human keratinocytes. A prolonged release kinetics of Ery from the NDs was *in vitro* demonstrated without initial burst effect. Analysis by confocal microscopy showed OFND and OLND internalization by *S. pyogenes* already after 3 h of incubation. According to cell counts, OFNDs and OLNDs displayed long term antimicrobial efficacy against *S. pyogenes* significantly inhibiting bacterial growth up to 24 h, thank to known chitosan antimicrobial properties. Intriguingly, Ery-LNDs were generally more effective, than erythromycin alone, in counteract streptococcal growth, probably due to Ery-LND internalization by bacteria.

Discussion and Conclusions: Taken together, these results support the hypothesis that NDs are capable antibacterial/skin-friendly devices and proper Ery-NDs might be a promising strategy for the topical treatment of streptococcal skin infections.

P017 - In vitro activity of Vancomycin-loaded nanodroplets against Enterococcus spp

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Introduction: Chronic wounds (diabetic, venous and pressure ulcers) represent an increasingly growing emergency among the elderly in Western countries. Persistent hypoxia is one of the main clinical features of chronic wounds and infections can cause either a delay in wound healing or worsening of the wound.
Adjunct therapies to facilitate chronic wounds to heal or to prevent their infections are urgently needed. In this context, oxygen-loaded nanodroplets (OLNDs) and vancomycin-loaded nanodroplets (VLNDs) have been proposed to counteract hypoxia effects and to deliver the antibiotic to bacteria, respectively. Nanodroplets (NDs) also display per se antimicrobial properties due to chitosan's presence in the outer shell. Skin infections are caused by different microbial agents including *Enterococcus spp.* In the present work, the activities of VLNDs and OLNDs were evaluated against *Eterococcus spp* in normoxia and hypoxia.

Materials and Methods: Eight strains of the *E. faecalis* and *E. faecium* isolated from infected wounds were cultivated in normoxic or hypoxic conditions. The antibacterial activity of free vancomycin, VLNDs or OLNDs was defined as the minimum inhibitory concentration (MIC), and determined through the microdilution broth method. The minimal bactericidal concentration (MBC) was also evaluated.

Results: Six out of the eight isolated strains exhibited sensitivity to vancomycin (MICs 0.5-1μg/ml), whereas two of them were resistant (MIC>256μg/ml). A slight decrease of the growth of 4 strains was observed in hypoxic condition. When OLNDs were added to the broth, increased growth of bacteria in hypoxic condition was observed. The MIC and MBC values for vancomycin were comparable in normoxic and hypoxic conditions. VLNDs showed better activity than free vancomycin against the *Enterococcus spp* and, at the effective concentrations on bacteria, no toxicity on the human cells was seen.

Conclusions: VLNDs were more effective than free vancomycin against *E. faecalis* and *E. faecium* and this may be due to the prolonged drug release profile. These data indicate that VLNDs can be promising tools for the topical treatment of infected and chronic ulcers.

P018 - Green synthesis and evaluation of antibacterial activity of silver nanoparticles prepared by using ulvan as novel reducing and stabilizing agent from renewable algal biomasses

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Introduction: Due to their strong antimicrobial properties, silver nanoparticles (AgNPs) have been intensively investigated in a wide range of industrial applications. However, the use of synthetic reducing and stabilizing agents for AgNPs preparation is increasingly hindered by costs and secondary toxicity, resulting unsuitable for being safely applied in biomedical applications. In this study, ulvan, a sulfated polysaccharide extracted from green algae belonging to *Ulva armoricana sp.*, was for the first time investigated and identified as reducing and stabilizing agent for AgNPs synthesis by using simple and eco-friendly methods. The antibacterial properties of the obtained AgNPs were investigated against clinically relevant bacterial species.

Materials and Methods: The heating method was selected as model strategy for the synthesis of ulvan-based AgNPs (Ulv-AgNPs). The antibacterial activity of Ulv-AgNPs was evaluated in standard bactericidal assays against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Staphylococcus epidermidis*. The bactericidal activity of Ulv-AgNPs was compared to that exerted by Citrate-AgNPs and free ulvan.

Results: Ulv-AgNPs exerted a marked bactericidal activity against both Gram+ and Gram- bacteria resulting in the complete eradication of the initial bacterial inoculum within 24 h of incubation. A dose-dependent reduction in the number of viable bacteria was observed for Gram+ bacteria, whereas the eradicating effect occurred at a threshold dose of Ulv-AgNPs in Gram- bacteria. A more rapid bactericidal effect was observed against Gram- than against Gram+ bacteria. Moreover, Ulv-AgNPs exerted a stronger antibacterial effect towards all tested bacterial species compared to free ulvan and Citrate-AgNPs, used as reference system of AgNPs.

Discussion and Conclusions: Ulvan was identified as active compound in the fabrication and stabilization of AgNPs by means of eco-friendly processes. Ulv-AgNPs exerted a strong and fast antibacterial activity against clinically relevant bacterial species. The dose-dependence and the killing kinetics differed slightly between Gram+ and Gram- bacteria suggesting that differences in structural features and/or surface composition between these two groups of bacteria may have an impact on the mechanism of antibacterial action of Ulv-AgNPs. The use of ulvan as natural compound for the development and stabilization of silver nanoparticles could represent an advance in the field of antimicrobial compounds since it represents a biocompatible polymeric material with unique biological and physicochemical properties which can be easily obtained from abundant waste biomasses through sustainable processes.

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P019 - Hydroxypyridinone-based iron-chelating co-polymer (DIBI) has antibacterial activity against Staphylococcus pseudintermedius strains isolated from canine otitis externa

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1. Introduction

Staphylococcus pseudintermedius (SP), an emerging zoonotic agent of canine origin, is an important opportunistic pathogen causing various infections in dogs and presents an increasing therapeutic challenge. 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 of *S. pseudintermedius* isolated from dogs suffering with otitis externa.

2. Materials and Methods

From dogs presented for screening at the Veterinary Teaching Hospital, we obtained auricular swabs which were processed at the Microbiology Laboratory of the Department of Veterinary Medicine and Animal Production, University of Naples Federico II (Italy). The swabs were inoculated onto Mannitol Salt Agar (MSA) and the isolates suspected to be SP were identified by matrix assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF-MS). Molecular profiling, using species-specific *nuc* gene, was also performed to confirm the phenotypical identification. The antibiotic resistance profiles were evaluated by a disk diffusion method on Mueller Hinton agar plates, according to the Clinical and Laboratory Standards Institute guidelines. Genetic profiles of methicillin resistance were carried out by PCR to assess the detection of *mecA* gene. The bacterial strains were routinely cultured from glycerol frozen stocks (-80 °C) and maintained on Trypticase Soy Agar (TSA). To test DIBI activity, liquid cultures were grown in Roswell Park Memorial Institute Medium 1640 (RPMI) supplemented with 2% (w/v) glucose, buffered with 0.165 M 3-(N-morpholino)-propanesulfonic acid (MOPS) for 18-24 h at 37 °C with shaking. DIBI stocks were prepared in RPMI (200 mg/mL) and all stock solutions were filter-sterilized (0.2 µm filter) before use.

3. Results

We isolated by phenotypic reaction profile, confirmed by PCR profiling and presence of *mecA* gene, one methicillin-resistant *S. pseudintermedius* (MRSP) strain and four methicillin-susceptible *S. pseudintermedius* (MSSP) strains. These strains showed resistance profiles to antibiotics that are frequently prescribed to dogs for MRSP or MSSP infections. All isolates were susceptible to DIBI with a MIC of 2µg/ml at 24 h. Furthermore, DIBI sensitivity was not linked to antibiotic sensitivity or resistance of the strains.

4. Discussion and Conclusions

Antibiotic resistance is one of the most urgent threats to the public's health and the increasingly limiting therapeutic options both in human and veterinary medicine underline the need of alternative treatments. DIBI has potential as a non-antibiotic alternative a therapeutic.

P020 - In vitro antimicrobial activity of vaginal lactobacilli cell-free supernatants against uro-pathogens by time-killing curves analysis

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Introduction: Healthy vaginal microbiota is dominated by *Lactobacillus spp*., which confers a critical line of defence against pathogens. This study aims to assess the *in vitro* antimicrobial activity of cell-free supernatants (CFSs) of three selected vaginal lactobacilli against a group of uro-genital pathogens by time-killing curves analysis.

Materials and Methods: The CFSs of *L. gasseri* 1A-TV, *L. fermentum* 18A-TV and *L. crispatus* 35A-TV (pH=4) obtained from a 48 h MRS broth cultures, after centrifugation and filtration. The *in vitro* antagonistic activity of these CFSs carried out by broth microdiluition against the indicator strains: *Streptococcus agalactiae*, *Enterococcus faecalis*, *Enterococcus faecium*, *Escherichia coli*, *Klebsiella pneumoniae*, *Candida albicans*, *Candida glabrata* and *Staphylococcus aureus*. The Lactobacilli CFSs antimicrobial effect on indicators was quantified by time-killing curves analysis at 6 and 24 hours. The viable microbial cell counts (CFU/ml) of each indicator strain, were recorded as $\log_{10}$ reduction from the starting inoculum. The effect of temperature and pH on the antibacterial activity of the three CFSs were also evaluated as described above, after heating at 121° C for 15 min and NaOH neutralization to pH 6.5.

Results: 1 A-TV CSF showed a bactericidal activity at T_6 on *S. agalactiae* and *K. pneumoniae*, while at T_{24} also on *E. coli* and *S. aureus.* 18A-TV and 35A-TV CFSs exhibited a bacteriostatic effect on *S. agalactiae*, *E. coli*, and *S. aureus* both at T_6 and T_{24}, while only on *K. pneumoniae* a bactericidal effect at T_{24}. All three CFSs displayed a bacteriostatic effect *versus E. faecium* and *E. faecalis* both at T_6 and $T_{24,}$ while no activity were observed *versus* both *Candida spp*. (Figure 1). The inhibitory activity of all three CFSs was totally affected by pH neutralization, restoring the normal growth of indicator strains. After heating, the CFSs inhibitory activity on *S. agalactiae* , *K. pneumoniae*, *E. coli* and *S. aureus* was mildly altered, still maintaining a bactericidal effect respect to control growth, while no effect was observed on *Enterococci* and *Candida spp.* The inhibitory activity of 18 A-TV CFS was not altered by heat, showing the same effect respect to untreated CFS (Figure 2).

Discussion and Conclusions: The *in vitro* assays show a promising antagonistic activity of the three selected lactobacilli CFSs. against some uro-pathogens. Our preliminary data suggested that different substances released by the three lactobacilli work in a synergic way to exert an antimicrobial activity. Further investigation will provide data on the real effect of these cell-free supernatants and their role on human vaginal health.

P021 - Effects of Cupral® on the formation and persistence of microbial biofilms in vitro

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Introduction: endodontic biofilm is a microbial community, enclosed in a polymeric matrix of polysaccharide origin where are frequently found pathogenic microorganisms, such as Gram+, Gram- and opportunistic fungi, belonging to *Candida* spp, responsible for several endodontic pathologies. As clinical importance is the fact that biofilm is extremely resistant to common intra-canal irrigants, antimicrobial drugs and host immune defenses. The aim of this *in vitro* study was to evaluate the efficacy of Cupral® on planktonic forms of some pathogens, as well as to assess its ability to prevent and affect the formation/persistence of microbial biofilms.

Materials and Methods: ATCC strains of *S. aureus*, *P. aeruginosa* and *C. albicans* were exposed to various concentrations of Cupral® (an antiseptic compound based on calcium and copper hydroxide, used in endodoncy) to investigate its antimicrobial efficacy. This activity has been evaluated in terms of microbial growth and cellular doubling time (optical density, colony forming units and doubling time assays), inhibition/persistence (crystal violet staining), viability of microbial cells embedded in the biofilms (live/dead stain) and pyoverdine production (fluorimetric assay). Finally, the morphology of Cupral®-treated biofilms was investigated by optical/confocal microscopy analysis.

Results: the addition of Cupral® to microbial cultures, influences, in a significantly and dose-dependent manner, the doubling time and growth of microbial cultures. Cupral® antimicrobial activity was also assessed on biofilms formation and persistence with meaningful decreases of residual biomass (observed reductions of 47-94% for *S. aureus*, 28-95% for *P. aeruginosa* and 27-75 % for *C. albicans*). Cupral®-treated biofilms analyzed by optical and confocal microscopy revealed loss of typical sessile structure, with few scattered microbial cells and a reduced thickness. Finally, the addition of Cupral® reduced both the number of embedded alive cells in the biofilms and the levels of pyoverdine in the culture supernatants.

Discussion and Conclusions: this pilot *in vitro* study provided the first evidences on Cupral® efficacy against microbial biofilms. The wide range of action (vs Gram+, Gram- and fungi) of Cupral® strongly suggests its use as compound in the prevention and treatment of main oral biofilm-associated infections.

P022 - *Biofilm production and rapid discrimination of L. pneumophila by matrix assisted laser desorption ionization time-of-flight*

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Introduction

Legionella pneumophila, Gram-negative bacterium, is responsible for the majority of legionellosis cases and is a significant contributor of community acquired, and hospital acquired pneumonia. *L. pneumophila* biofilm can contribute to the resistance of microorganism to various chemical and physical treatments. To typing of *L. pneumophila* different methods can be used, but in this study is taken into consideration pulsed-field gel electrophoresis (PFGE) and matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS). In recent years, MALDI-TOF-MS has become a valuable for the identification and typing of *L. pneumophila* isolates.

In this study, we report the ability to produce biofilm and the use of MALDI-TOF-MS for typing of 40 *L. pneumophila* strains.

Materials and methods

Thirty-eight strains of *L. pneumophila* were isolated from environmental samples and two from clinical samples. The isolates were divided as follows: 15 of nosocomial and 25 of community origin (19 coming from hotels and 6 coming from private homes). Instead, two clinical cases coming from: one isolated from the pleural fluid of an 11-year-old patient admitted to thoracic surgery and the other from a patient admitted to the hematology department of two separate hospitals. This samples have been analyzed with MALDI-TOF-MS. Instead, we have studied biofilm according to G. Donelli et al.

Results

In our study we found that the 45% is a maker of biofilm, and it is divided in strains moderate maker (15%) and weak maker (30%).

While clusterizzation by MALDI-TOF-MS we found that the strains belong to three different groups, particularly two clusterizzation together while one is independent.

Discussion and Conclusions

Our results showed among the strains analysed a low biofilm producers suggesting the possibility to use the decontamination as the best strategies to prevent its colonization in water distribution systems.

On the bases of our results the use of MALDI-TOF-MS can offers a simple and rapid discrimination technique that could aid in the tracking of fast-spreading outbreaks of *L. pneumophila*.

P023 - Antimicrobial and antibiofilm activity of steroid derivatives against ESKAPE Pathogens

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Introduction

ESKAPE pathogens (*E. faecium, S. aureus, K. pneumoniae, A. baumannii, P. aeruginosa, E. cloacae*) are recognized to be responsible for the majority of difficult-to-treat community-acquired, healthcare-associated, and nosocomial infections. Bacteria ability to form biofilms on surfaces makes these pathogens a major cause of refractory biofilm-associated infections, since cells embedded in biofilm are often more tolerant to antimicrobials than planktonic ones. The emergence of antibiotic-resistant bacteria emphasizes the need for alternative therapeutic agents other than conventional antibiotics. Many natural steroids and their derivatives have been demostrated to exhibit broad biological functions. The aim of this study was to investigate the effects of novel steroid derivatives on on the growth of planktonic cells as well as on the formation and persistence of ESKAPE pathogens biofilm.

Materials and Methods

Six novel steroid derivatives (AG1113-1 to AG1113-6) and two known steroid drugs (prednisolone and deflazacort) were synthesized. The MIC values of these molecules against planktonic bacteria were measured by broth microdilution method, while the activity of steroid derivatives against bacterial biofilms at different development stages was tested by crystal violet, tetrazolium salt reduction assay and confocal laser scanning microscopy.

Results

All steroid derivatives exhibited moderate to significant antimicrobial and antibiofilm activity against all tested strains wherein compound AG1113-3 showed maximum activity. AG1113-3 was active against Gram-negative and Gram-positive bacteria, showing rapid time-dependent kinetics of bacterial killing. The biofilm inhibitory concentration for compound AG1113-3 was found to be 16-fold lesser than the MIC. AG1113-3 was able to induce a strong and dose dependent reduction in biofilm biomass, affect the biofilm thickness and disrupt pre-formed biofilms.

Discussion and Conclusions

These findings suggest that AG1113-3 acts as a potent antibacterial as well as antibiofilm agent and can be considered as a novel candidate molecule for the treatment of bacterial biofilm-related infections.

P024 - Detection and Characterization of Carbonic Anhydrases in the Outer Membrane Vesicles (OMVs) Released by Helicobacter pylori in the Planktonic and Biofilm Phenotypes

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Introduction: Biofilm represents a successful strategy for *Helicobacter pylori* survival. Current studies report that the EPS matrix of *H. pylori* biofilm is mainly constituted by proteomannans, LPS-related structures, extracellular DNA (eDNA), proteins and Outer Membrane Vesicles (OMVs). The OMVs are spherical bilayered structures (20-250 nm in diameter) which are released by the microorganisms during their growth and have a key role in *H. pylori* biofilm formation. A recent study analyzed the exoproteome of *H. pylori* at different growth phases in the planktonic phenotype. Among the 74 proteins selectively released by OMVs, the Carbonic Anhydrase (CA) was identified, however, no indication of the family was provided. Intriguing, the pathogen uses its CAs belonging to the alpha- and beta-families for the acid acclimation within the human stomach and thus, for the bacterial survival in the host. The aim of the present study was the detection and characterization of CA in the OMVs generated in the planktonic and biofilm phenotypes over time by *H. pylori*.

Materials and Methods: The strains used in the study were *H. pylori* NCTC11637 and 4 clinical strains, *H. pylori* 190, *H. pylori* F1 and *H. pylori* F4 characterized by a different antimicrobial susceptibility pattern. *H. pylori* biofilm formation was evaluated at 2, 6 and 10 days of incubation using Live/dead staining and Fluorescence microscopy. The OMVs from the planktonic (pMVs) and biofilm (bMVs) phenotypes were isolated by ultracentrifugation. The detection and quantification of CA associated with pOMVs and bOMVs were evaluated, at each time point, by protonography and mass spectrometry.
Results: *H. pylori* strains developed a mature biofilm already after 2 days of incubation. The presence of CA was detected in both pOMVs and bOMVs, however, the amount of CA in the pOMVs was greater than in the bOMVs except for the *H. pylori* 190. Furthermore, the content of CA increases over time in the pOMVs. The mass spectrometry analysis demonstrated that the CA delivered by OMVs in an isoform of the alpha-carbonic anhydrase family, which has a periplasmic localization.

Discussion and Conclusions: The detection and characterization of CA in pOMVs and bOMVs together with the novel finding of increased production of CA over time, shed new light on the role of this enzyme in the colonization, survival, persistence and pathogenesis of *H. pylori*.

P025 - The antimicrobial peptide lin-SB056-1 and its dendrimeric derivative inhibit biofilm formation by Pseudomonas aeruginosa in physiologically relevant models of chronic infections

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Introduction: Antimicrobial peptides (AMPs) have gained increasing attention as candidates for the development of novel antibiofilm drugs. Despite the large number of studies reporting the screening and optimization of AMPs, only a few of these evaluated the antibiofilm activity of AMPs in physiologically relevant model systems. Potent in vitro activity of AMPs often does not translate into in vivo effectiveness due to the interference of the host microenvironment with peptide stability and toxicity. Thus, mimicking the complex environment and in vivo host-pathogen interactions found in biofilm-associated infections is essential to predict the clinical potential of AMP-based antimicrobial agents. The present study examined the antibiofilm activity of the semi-synthetic peptide lin-SB056-
1 and its dendrimeric derivative (den-SB056-1) against Pseudomonas aeruginosa in relevant models of lung and wound biofilm-associated infections.

Methods: An in vivo-like three-dimensional (3-D) lung epithelial cell model was exploited to assess the ability of lin-SB056-1, alone and in combination with EDTA, and den-SB056-1 to prevent biofilm formation by P. aeruginosa. Association of P. aeruginosa with 3-D epithelial cells was determined by CFU counting and fluorescence microscopy. Lactate dehydrogenase assay was performed to analyze the effect of the tested compounds on 3-D epithelial cell viability. Biofilm-inhibitory activity of den-SB056-1 was also determined in an in vitro wound model (containing an artificial dermis and physiological concentrations of blood components).

Results: Although moderately active when tested alone, lin-SB06-1 was quite effective in reducing the formation of P. aeruginosa biofilms on 3-D epithelial cells in combination with host-compatible concentrations of EDTA. Den-SB056-1 demonstrated a stronger biofilm-inhibitory activity than its linear counterpart in the 3-D lung epithelial cell model, reducing the number of biofilm-associated bacteria up to 3-log units at non-toxic concentrations. Biofilm inhibition by den-SB056-1 was observed for P. aeruginosa PAO1 and cystic fibrosis isolates. A significant decrease in biofilm-like structures associated with 3-D cells was observed after peptide treatment. Efficacy of den-SB056-1 was also found in the wound model with a reduction of up to 1-log unit in biofilm formation by P. aeruginosa PAO1 and burn-wound isolates.

Conclusions: Screening of AMPs in conditions mimicking the host microenvironment represents an essential step to allow the advancement of antibiofilm peptides from the bench to the clinic. Overall, these results highlight the utility of clinically relevant biofilm model systems to compare and select potentially useful AMPs to be included in animal studies and clinical trials.

P026 - A new challenge in cystic fibrosis antimicrobial therapy: activity of electrochemically synthesized silver nanoparticles against planktonic and biofilm cells of bacterial pathogens

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Introduction: Staphylococcus aureus and Pseudomonas aeruginosa are the most prevalent respiratory pathogens in Cystic Fibrosis (CF) however other species, such as Burkholderia cepacia and Stenotrophomonas maltophilia, can cause a severe decline in lung function due to their Multidrug-Resistant (MDR) phenotypes. Recently, several studies have reported silver nanoparticles (AgNPs) as a promising alternative to antibiotics because of their relevant bactericidal effects against a wide range of microorganisms. In the present study we evaluated, for the first time, the activity of a new AgNPs formulation against MDR strains isolated from CF patients.

Materials and Methods: the AgNPs were synthesized by a new electronic device using an electrochemical method. The AgNPs were assayed, comparatively to tobramycin, against MDR strains of P. aeruginosa, S. maltophilia, B. cepacia, and S. aureus for: i) in vitro activity against planktonic cells (MIC and MBC, and time-killing assay by using micro- and macrodilution method, respectively); ii) in vitro activity against preformed biofilm when tested at 1x, 2x and 4xMIC (viable cell count); iii) in vivo cytotoxic potential (Galleria mellonella wax moth larva). Finally, morphological changes induced in P. aeruginosa biofilm were monitored by Transmission Electron Microscopy (TEM).

Results: The AgNPs were particularly active against P. aeruginosa and B. cepacia planktonic cells (median MIC: 1.06 and 2.12 mg/ml, respectively) by a rapid, bactericidal and concentration-dependent effect. The AgNPs resulted particularly effective against P. aeruginosa and S. aureus biofilm causing a viability reduction ranging from 50% (1xMIC) to >99.9% (4xMIC). TEM showed that the AgNPs deconstruct extracellular matrix of P. aeruginosa biofilm and accumulate at the cell surface causing cell death secondary to membrane damage. Compared to tobramycin, the AgNPs showed comparable, or even better, activity against planktonic and biofilm P. aeruginosa cells. The AgNPs at concentrations effective against B. cepacia and P. aeruginosa were not toxic to G. mellonella larvae.

Discussion and Conclusions: Our silver-based formulation may be an alternative therapeutic strategy for the treatment of chronic lung MDR infections in CF patients. Further studies are necessary to confirm this therapeutic potential by using an animal model, and to evaluate the efficacy of the AgNPs in combination with antibiotics commonly used in the treatment of CF infections, in order to reduce the AgNPs toxic potential, to avoid the possible development of resistance and, above all, to strongly enhance the microbicidal effect of antibiotics against both planktonic and biofilm cells.

P028 - Conjugation of LasR quorum-sensing inhibitors with ciprofloxacin increases antibiotic susceptibility of Pseudomonas aeruginosa clinical strains

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Introduction: There is a small set of drugs commonly used to treat *P. aeruginosa* infection, including ciprofloxacin, tobramycin, gentamicin, ceftazidime, and imipenem. While *P. aeruginosa* has developed various levels of resistance to each of these, its response to ciprofloxacin is of particular interest because the drug is initially very effective, but *P. aeruginosa* rapidly acquires high-level resistance, rendering the drug impotent. *P. aeruginosa* is able to form a well-organized bacterial structure during infection, called biofilm, using a mechanism of gene regulation called quorum sensing (QS).

Materials and Methods: We analyzed the ability of QS inhibitors (QSI) designed on the scaffold of the N-(3-oxododecanoyl) homoserine lactone (3O-C12- HSL) QS molecule to inhibit *P. aeruginosa* biofilm formation in clinical strains, wild type or mutant for LasR, the natural receptor of 3O-C12-HSL (range of concentrations: 1.0-10.0uM). Clinical strains were evaluated for: i) biofilm formation by microtitration and Syto9 assay; ii) bacterial cell growth by optical density measurement at 600nm; iii) susceptibility profile (aminoglycosides, penicillins, cephalosporins, carbapenems and fluoroquinolones) by BD Phoenix automated microbiology system. The bacterial response to ciprofloxacin was tested by minimum inhibitory concentration (MIC) by Etest (AB bioMerieux, AB Biodisk).

Results: Biofilm quantification analyses showed that 100% of the clinical strains were biofilm producers and they had the following categories of biofilm production: 25% non-adherent, 40% weakly adherent, 25% moderately adherent, 10% strongly adherent. The non-adherent and weakly adherent strains were MDS while the moderately adherent and strongly adherent strains were MDR. The MDR strains showed a median MIC of 0.5ug/ml, the MDS of 0.35ug/ml. Two out of 4 QSIs reduced biofilm formation of 60-70% in both wild type and LasR mutant clinical strains, already at the concentration of 5.0uM. One out of two QSI/ciprofloxacin conjugates strongly reduced biofilm formation and decreased of two/three folds the median MIC (0.2ug/ml) of ciprofloxacin in both MDS and MDR strains ($p<0.001$).

Conclusions: These results uncoveres the possible use of this QSI/ciprofloxacin conjugate to increase *P. aeruginosa* susceptibility to ciprofloxacin reducing also biofilm formation, in both wild type and mutant for LasR clinical strains.

P029 - Occurrence of blaOXA-232 and blaVIM-1 genes in a clinical Enterobacter cloacae from an Italian ICU

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Introduction. Bacterial colonization is often a first step in the pathogenesis of health-care infections, whose empirical antibiotic therapy partially depends on the microflora resistance pattern. The aim of the study was to characterize at molecular level two carbapenem-resistant clinical isolates of *Enterobacter cloacae* and *Klebsiella pneumoniae* collected by surveillance rectal swab from an inpatient at Treviso Hospital.

Materials and Methods. The *E. cloacae* and *K. pneumoniae* isolates were both obtained on December 29th 2017, from a 70-years-old inpatient at cardio-surgical ICU.
Identification and susceptibility profiles were determined by MALDI-TOF MS (bioMérieux) and MICRONAUT-AST (Merlin) systems, respectively. Fosfomycin MICs were performed using the reference EUCAST agar dilution method. Susceptibility tests results were interpreted according to EUCAST clinical breakpoint Table v 8.1. XpertCarba-R System (Cepheid) was used to screen for carbapenemase determinants. Antibiotic-resistance genes were identified by Check-Points MDRCT103XL microarray and/or PCR and sequencing. The plasmid characterization was assessed by conjugation and PCR-based replicon typing. Molecular typing of the *K. pneumoniae* strain was carried out by Multi Locus Sequence Typing (MLST).

Results. Both the *K. pneumoniae* and *E. cloacae* isolates resulted extensively drug-resistant, being only colistin and tygecicline susceptible (MIC_{COL}: ≤ 0.5mg/L; MIC_{TGC}: 0.5 mg/L). Molecular characterization confirmed the co-presence of bla_{SHV-5}, $bla_{OXA-232}$ and bla_{VIM-1} determinants in *E. cloacae* and bla_{SHV-5} and bla_{VIM-1} genes in *K. pneumoniae*, respectively. Aminoglycosides and fluoroquinolones resistance was due to the *aac(6')-Ib-cr* gene presence in both strains. The transferability of bla_{SHV-5} and bla_{VIM-1} determinants was verified by conjugation; an A/C replicon type was detected in both donor and transconjugant strains. The *K. pneumoniae* clinical strain studied was assigned to the new ST3351.

Discussion and Conclusions. Plasmid-mediated horizontal transfer of multidrug resistance traits plays a key role in the dissemination of antimicrobial resistance genes. The study highlights the occurrence of an *in vivo* inter-species exchange of a plasmid harboring bla_{VIM-1} and bla_{SHV-5} determinants. The rapid detection of resistance determinants in inpatients microbiota provide useful information both on therapy and on the opportunity to implement infection control measures avoiding dissemination. To our knowledge, this is the first report on the occurrence of *bla*OXA-232 and *bla*VIM-1 genes in a clinical *E. cloacae* from an Italian ICU.

P030 - PBP2a mutations causing low-level ceftaroline and ceftobiprole resistance in clinical methicillin-resistant Staphylococcus aureus (MRSA) isolated in Italy

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Introduction. This study was undertaken to characterize the mechanism of resistance to fifth-generation cephalosporins, ceftaroline (CPT) and ceftobiprole (BPR), in Italian methicillin-resistant *Staphylococcus aureus* (MRSA) strains isolated during a recent national survey. Mutations in PBPs were sought and the question of a possible bactericidal loss, alone and in combination with daptomycin, was also addressed.

Materials and Methods. 10 not-related Italian rifampicin- and methicillin-resistant *S. aureus* (RIF-R-MRSA) isolates, previously characterized by MLST/SCC*mec*/*spa*-typing, were analyzed for their *in vitro* antibacterial and bactericidal activity to CPT and BPR, alone and in combination with daptomycin. The nucleotide sequences of *mec*A, *pbp*1, *pbp*2, *pbp*3, *pbp*4, and *gdp*P genes were analyzed.

Results. The 10 MRSA isolates showed resistance to CPT (MICs 4-8 mg/L) and BPR (MIC 4 mg/L). Sequence analysis of PBP2a identified four different missense mutations (N146K; N204K; T235I; E239K) uniquely present in the non-penicillin-binding domain (nPBD). ST228-I-t001/t041 was the predominant clone in CPT/BPR-R population (N=9), associated with single substitutions in PBP2a (N146K; E239K); only one isolate with ST239-SCC*mec*III-t037 displayed double mutations (N204K; T235I), including the novel T235I. Time-kill curve analysis demonstrated the maintenance of a potent bactericidal activity of both drugs (3-4 log reduction) at all concentrations tested. Synergism with daptomycin was observed among the DAP-R/hVISA strains, while the same effect was masked by the high activity of cephalosporins alone, towards the DAP-S ones.

Discussion and Conclusions. Our results revealed that resistance to fifth-generation cephalosporins, strongly correlated with the major Italian epidemic clone ST228-SCC*mec*I-t001/t041, was due to an altered PBP2a sequence, with diverse missense mutations, all in the nPBD. In particular, we detected a novel mutation (T235I) in one isolate belonging to ST239-SCC*mec*III-t037. Despite PBP2a mutations, both drugs retained their bactericidal activity, and showed synergistic effect towards difficult to treat DAP-R/hVISA isolates.

P031 - Resistance to Ceftazidime-Avibactam in a ST512 KPC-producing Klebsiella pneumoniae due to a Tn4401 duplication

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Introduction: The worldwide spread of beta-lactamase-producing Gram-negative pathogens is a worrisome public health challenge, because of the reduced number of available treatment options. Ceftazidime-Avibactam (CAZ-AVI) is a combination of a third-generation cephalosporin and an inhibitor of Ambler class A (e.g. KPC), class C and some class D beta-lactamases, which represents a new option against many Carbapenemase-producing Enterobacteriaceae (CPE). However, CAZ-AVI resistance has started to be reported in CPE isolates due to mutations in the enzyme (e.g. KPC-2 and KPC-3) or in porins (e.g. OmpK36) or an increased gene dosage of blaKPC-3. This work reports on the characterization of a CAZ-AVI resistant KPC-producing K. pneumoniae clinical isolate (KP-8788).

Materials and Methods: Antimicrobial susceptibility testing was performed by reference broth microdilution and interpreted according to EUCAST criteria v 8.1. The isolate was processed with two Real-Time PCR for the detection of blaKPC, blaVIM, blaNDM, blaOXA-48 like, blaGES, blaIMP genes. Meropenem-hydrolyzing specific activity was determined for KP-8788 and comparators strains by spectrophotometric assay. Whole-Genome Sequencing (WGS) was performed on KP-8788 to verify the presence of KPC and porins mutations. Electrotransformation experiments were carried out using E. coli DH10B and a KPC-non-producing clinical isolate of K. pneumoniae (KP-09C086), presenting the same porins content and Sequence Type of the donor strain.

Results: KP-8788 showed a CAZ-AVI and meropenem (MEM) MIC of >8/4 mg/L and >16 mg/L, respectively. In PCR assays, the isolate tested negative for metallo-beta-lactamase and positive for blaKPC. KP-8788 had MEM-hydrolyzing specific activity (nmol/min/mg protein) of 88±2, compared to 7±2 of a KPC-producing K. pneumoniae susceptible to CAZ-AVI, used as control. WGS analysis revealed that KP8788 belonged to ST512 and that two copies of a wild type blaKPC-3 gene (located on a Tn4401 transposon) were present on an IncFIB plasmid. The plasmid was successfully transferred to E. coli DH10B and KP-09C086, which showed a concomitant increase of CAZ-AVI MICs (≤1/4 vs 4/4 mg/L and ≤1/4 vs >8/4 mg/L, respectively) and of MEM (≤0.125 vs 16 mg/L and 0.25 vs >16 mg/L, respectively).

Discussion and Conclusions: We present a novel mechanism of CAZ-AVI resistance in a ST512 KPC-producing K. pneumoniae due to a duplication of Tn4401 transposon occurring on the same plasmid. A similar mechanism was previously described, but consisted in increased Tn4401 copy number on two different plasmids. The presence of two copies of blaKPC-3 on the same plasmid could facilitate the horizontal diffusion of CAZ-AVI resistance, but further conjugation experiments should be necessary to clarify this aspect.

P032 - Spread of mcr-1 driven Colistin resistance on surfaces of Italian hospitals

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1. Introduction

Among those appeared in the recent years, the detection of a plasmid-mediated colistin resistance, driven by the *mcr-1* gene represents a serious clinical concern, as colistin was considered so far as a last-resort drug against multidrug resistant (MDR) Gram negative bacteria.
Since its original isolation, the *mcr-1* gene was detected almost globally in a 0.1-2% of isolates from livestocks and patients, in different Gram-negative bacteria. The emergence of *mcr-1* in clinical *Enterobacteriaceae* isolates appears particularly alarming, as it frequently occurs in MDR strains, further limiting current treatment options for lethal infections sustained by carbapenem-resistant *Enterobacteriaceae* (CRE). In Italy, *mcr-1* driven colistin resistance was firstly reported in a *E. coli* strain in 2016, although colistin resistance had already been reported previously in CRE from different Italian peripheral laboratories. However, so far *mcr-1* gene was essentially searched and detected in infected subjects, but its presence in the microbial population persistently contaminating hospital environment is not known. On the other hand, it is accepted that surface contamination can contribute to the onset of the so-called healthcare-associated infections (HAIs), which are often sustained by MDR or even panDR strains.
This study was therefore aimed to check the diffusion of the *mcr-1* driven colistin resistance in the hospital environment.

2. Materials and methods

The presence of *mcr-1* gene was searched in a library of 300 *Enterobacteriaceae* strains collected from the surfaces of eight Italian hospitals between 2016-2017.
Surface samples were collected from three points in hospital rooms (floor, bed footboard and sink), grown in Mac Conkey broth to amplify the *Enterobacteriaceae* population, then total DNA was extracted by the bacterial suspension and analyzed for *mcr-1* gene presence by nested PCR. Each DNA sample was also analyzed by a universal pan-bacterial PCR, as a control of DNA amplification. Plasmid pBAD24::mcr-1 was used as a positive PCR control. Each PCR-positive sample was further culturally isolated on Mac Conkey agar plates, confirmed for *mcr-1* gene presence by nested PCR, identified by Maldi-Tof typization, and tested for drug susceptibility by disc diffusion and broth microdilution.

3. Results

The results showed that 25/300 (8.3%) of the total *Enterobacteriaceae* isolated from hospital surfaces harboured *mcr-1* gene.
Bacterial identification results allowed evidencing that different species harbored *mcr-1* gene, including *Klebsiella pneumonia* and *oxytoca*, *E. coli*, *Acinetobacter*, *Enterobacter cloacae* and *agglomerans*, *Citrobacter freundii*, *Pseudomonas aeruginosa* and *putida* isolates, suggesting that this gene is silently spreading to many Gram-negative bacteria responsible for infections in the clinical settings. All *mcr-1* carrying isolates were colistin resistant (Col-R) by microdilution test, with a MIC varying from 4 mg/L to over 16 mg/L. In addition, as judged by the results obtained by the disc-diffusion method, all Col-R isolates were also resistant to two or more antibiotics among those effective against *Enterobacteriaceae*, exhibiting a MDR phenotype.

4. Discussion and Conclusions

Our data show for the first time that *mcr-1* carrying *Enterobacteriaceae* can be detected with relatively high frequency (compared to clinical isolates) on hospital surfaces, indicating that this plasmid has the ability to spread, not only *in vitro*, in key human pathogens. Persistent surface contamination in hospitals might favor colistin resistance spread among Gram-negative bacteria, perhaps helped by the selective pressure exerted by some disinfectants (i.e. chlorhexidine). This might represent a potential reservoir of threatening nosocomial pathogens and favor their diffusion in hospitalized patients.

Based on this, we suggest that surveillance for *mcr-1* driven colistin resistance might include not only clinical samples but also environmental analyses, and all clinically relevant Gram-negative species, to control and counteract the increase of untreatable infections.

P033 - Functional characterization of a divergent IND-type metallo-beta-lactamase from a multidrug-resistant Chryseobacterium indologenes isolate from Senegal

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Introduction: *Chryseobacterium indologenes* is an increasingly important opportunistic pathogen, commonly associated with nosocomial or device-related infections. Its clinical relevance is further accentuated by its natural resistance to several beta-lactam antibiotics (including carbapenems), due to the production of a resident metallo-beta-lactamase, belonging to the IND subgroup. Here we report the characterization of a new IND-type variant from a *C. indologenes* isolate from Senegal, which showed a multidrug resistance profile and whose complete genome sequence was determined.

Materials and Methods: The $bla_{\text{IND-4924}}$ ORF was amplified by PCR and cloned into vectors pLB-II and pET-9a, yielding plasmid pLBII-IND-4924 and pET-IND-4924, respectively. The latter was used to transform *E. coli* BL21(DE3) for high-level production and grown in ZYP-5052 auto-inducing medium (24 hours at 37 °C). The IND-4924 enzyme was purified (purity, > 98%) by means of two cation-exchange chromatographic steps. Steady-state kinetic parameters for the hydrolysis of beta-lactam antibiotics were determined by measuring spectrophotometrically the initial reaction rates. Low K_m values were measured as K_i using a competitive inhibition model.

Results: NGS analysis of *C. indologenes* 4924 revealed the presence of a gene encoding a new metallo-beta-lactamase variant, named IND-4924 and showing 73-87% sequence identity with other IND-type metallo-beta-lactamases. IND-4924 was purified with an overall yield of 3 mg per liter of culture. The purified enzyme showed a broad substrate profile, with overall higher turnover rates than those measured with IND-2 and IND-5. High catalytic efficiencies (k_{cat}/K_m, > 10^6 $M^{-1}s^{-1}$) were measured with most tested substrates, including with some oxyiminocephalosporins, imipenem and ertapenem. However, cefepime, a zwitterionic cephalosporin, was hydrolyzed at a significantly lower rate. In agreement with its functional properties, the production of IND-4924 in a *E. coli* host was able to confer resistance to penicillins, some cephalosporins and carbapenems, but not to ceftazidime and cefepime, which behaved as poorer substrates.

Discussion and conclusion: A detailed biochemical analysis carried on the purified IND-4924 beta-lactamase revealed that the enzyme exhibited a broad substrate profile, typical of IND-type enzymes, being one of the most active IND-type enzyme identified so far.

P034 - Epidemiology and antimicrobial resistance patterns of community acquired uropathogens in Turin

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Introduction: Urinary tract infections (UTI) are one of the most common infectious diseases and are usually treated empirically, at least at first. Unfortunately , the rise in antimicrobial resistance has become an alarming issue in many countries, posing a challenge to effective treatment. The aim of this study was to examine the local distribution and the changes in the susceptibility patterns and trends of community uropathogens towards commonly used antibiotics, in order to suggest a prudent and regionally specific approach to antimicrobial empirical treatment.

Materials and Methods: We conducted this retrospective study examining the results of routine microbiological tests carried out at our laboratory on midstream urine samples collected from community outpatients over a 2-year period (2016-2018). Identification and antimicrobial susceptibility testing were performed using an automated system (MicroscanWalkAway plus - Beckman Coulter). Pearson's Chi-squared test was used to assess trends in antibiotic susceptibility over time and results were considered statistically significant with $p<0.05$.

Results: A total of 2627 isolates were studied. *Escherichia coli* was the most frequently isolated pathogen (59,6%) followed by *Klebsiella pneumoniae* (10,6%) and *Enterococcus faecalis* (9,6%). Enterobacteriaceae, and *Escherichia coli* in particular, revealed the highest susceptibility towards nitrofurantoin, aminoglycosides, fosfomycin and cephalosporines (99,3%93,3%,89,7% and 86,4% respectively) while cotrimoxazole and fluoroquinolones, with a resistance rate of over 30%, did not appear as a good choice for empirical first line treatment. Against Gram-positive bacteria, glycopeptides and ampicillin showed the strongest activity. Analysis of the resistance trends during the considered period, indicated a significant increase in the resistance to carbapenems among Gram-negatives and in the prevalence of Extended Spectrum Beta-Lactamase (ESBL) producing strains. A moderate, though not statistically significant, rise in the resistance to cephalosporines and fluoroquinolones was also observed.

Discussion and conclusions: Knowledge of the local epidemiology of UTIs and of the antibiotic susceptibility patterns of uropathogens can be helpful in choosing the most appropriate empirical therapy. It can also be useful to monitor the ever increasing spread of multi-drug resistant strains in the community.

P035 - CRISPR-Cas system characterization and functionality to deeply know the Multi-Drug Resistant S. epidermidis strengths and weakness

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Introduction: Antibiotic resistance is worldwide an increasing problem for the public health. Scientists are working to better know the features of Multi-Drug Resistant (MDR) bacteria to develop new strategies to overcome the antibiotic resistance. The presence of CRISPR-Cas (Clustered Regularly Interspaced Short Palindromic Repeat and CRISPR-associated genes) systems in MDR pathogenic strains is an important trait to evaluate, because it can decrease the spread of plasmids carrying antibiotic resistance (AR) genes. At the same time, it appears to be related to the acquisition of some mobile elements carrying the AR genes such as SSC*mec*, and it can also gain resistance to phage infection (PI), a trouble in the phage therapy development.

Material and Methods: 10 Italian pathogenic MDR *Staphylococcus epidermidis*, epidemiologically not related, were characterized for their CRISPR-Cas system and susceptibility to PI. The entire CRISPR-Cas locus of selected strains was sequenced, and functionality tested by conjugation.

Results: All *S. epidermidis* strains harbored at least the *cas*6, 2 strains showed an isolated *cas* locus, whilst 3 strains carried the entire type III-A CRISPR-Cas with the identical genetic organization of the RP62A *S. epidermidis* CRISPR-Cas locus, but having more spacers. The anti-plasmid activity of the CRISPR-Cas system, tested in only one strain due to its antimicrobial resistance profile, was defective. Only three strains resulted sensitive to PI with three different phages of the Staphylococcal phage family.

Discussion: CRISPR-Cas system characterization highlighted a surprising prevalence of *cas*6, necessary for the CRISPR mediated immunity, showing a spread of CRISPR genes as a common trait in MDR pathogenic *S. epidermidis*. The occurrence of just *cas*6 or an isolated *cas* locus let us to speculate that antibiotic pressure, promoting the acquisition of foreign elements, decrease genome stability and increase plasticity, justifying the broken CRISPR-Cas system in pathogenic MDR strains. In addition, the acquisition of new spacers attested multiple adaptations useful to protect bacteria against phages. Notwithstanding the perfect match with the RP62A CRISPR-Cas, the CRISPR-Cas system of our tested strain seems to lack of anti-plasmid activity indicating a permissive plasmid acquisition.
Finally, the predominant resistance to PI represents an important challenge to consider in the design and the development of a phage therapy as new and innovative strategy to overcome antibiotic resistance.

P036 - Rapid detection of bacteria antimicrobial resistance by MALDI-TOF mass spectrometry and Accelerate phenoTM system

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Introduction. Rapid bacterial antibiotic susceptibility test (AST) and minimum inhibitory concentration (MIC) measurement are important to improve clinical care but also to help reduce both the widespread misuse of antibiotics and the growing drug-resistance problem.
In recent years, matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry (MS) has become established as a first-line diagnostic tool in the identification of microorganisms, including those producing human infections. Rapid detection of antimicrobial resistance is one of the future applications of this technique with the greatest likelihood of success.
In this study, different protocols of meropenem hydrolysis assay (MHA) by MALDI-TOF MS were applied in order to develop a valid tool for the phenotypic discrimination between carbapenemases-producing and -non-producing Gram-negative bacteria. Furthermore, we evaluate the performance of the innovative Accelerate Pheno™ System (APS, Accelerate Diagnostics) for ID and AST directly from positive blood cultures (BCs) in comparison to conventional methods (CM).

Materials and Methods. MHA was applied to 1185 *Enterobacteriaceae* strains for the phenotypic detection of carbapenemases. In addition, a preliminary study on 24 *Enterobacteriaceae* strains was performed to evaluate the possibility of detecting carbapenemase-producing strains by directly analysis of the MALDI-TOF spectra obtained for the identification of microorganisms, avoiding the meropenem hydrolysis assay. Finally, the performances of the APS were evaluated on 23 positive blood cultures and 10 challenge isolates.

Results. The MHA was successfully applied to detect carbapenemase activity in 981 well-characterized *Enterobacteriaceae* strains producing KPC or VIM carbapenemases, and in 146 carbapenem fully susceptible strains. This assay, applied also to NDM and OXA-48-producing strains and to CRE with resistance mechanisms other than carbapenemase production, has proved to be able to distinguish between carbapenemase-producing and -nonproducing *Enterobacteriaceae*.
APS performed on 10 challenge isolates showed an ID concordance of 100% as well as 9 AST, in one case AST wasn't provided due to very high clones count by APS. The evaluation on 23 positive blood cultures showed a ID sensitivity and specificity of 94.7% and 99,7%, respectively. The AST performance showed 88.3% of essential agreement, 85.6% of categorical agreement, 0% of very major error, 5.8% of major error, and 9.9% of minor error.

Discussion and Conclusions. In our experience, MHA demonstrated to be able to reveal the hydrolyzation of meropenem in all KPC or VIM carbapenemases, whereas no false positive results were obtained from fully susceptible *Enterobacteriaceae* strains.
In our hands APS showed noteworthy errors both for ID and AST, despite the high sensitivity and specificity observed for ID, and the AST performance is encouraging by the absence of very major error. However, the reduction in time-to-result represents one of the main advantage of this assay.

P037 - Long-term occurrence of antibiotic resistance genes and class 1 integrons in plankton-associated bacteria

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Introduction
Massive use and misuse of antibiotics in human care settings and animal farming led to the release of high amounts of antibiotics in aquatic environments, causing the selection and spread of antibiotic resistant bacteria (ARB). While the role of water and sediment in hosting and spreading ARB is well known, no information are available about the involvement of phyto- and zoo-plankton in this respect. This study aims to investigate if marine plankton community could be a reservoir of antibiotic resistance genes (ARGs) and class 1 integrons (considered proxies of both e anthropogenic pollution and ARB spread) analysing historical Continuous Plankton Recorder (CPR) samples by Droplet Digital PCR (ddPCR).

Materials and Methods
Twenty-nine formalin-fixed CPR samples, collected from different locations (Southern North Sea, Irish Sea, Newfoundland and North Atlantic) were used. CPR is a high-speed plankton sampler designed to be towed from ships over long distances: the CPR archive is one of the longest and geographically most extensive collections of marine samples in the world. Extracted DNA was analysed in duplicate by both qPCR and ddPCR for the abundance of *sul*2 (sulphonamide resistance gene) and class 1 integron integrase gene (*intI*1). Furthermore, the abundances of the two genes were correlated with the measures of biotic (*i.e.* copepods, phytoplankton, and *Vibrio* spp. abundance) and abiotic (*i.e.* sea surface temperature) factors. A specific spatiotemporal pattern for *sul*2 and *intI*1 genes was also analysed.

Results
The results from qPCR and ddPCR were discordant with the latter more sensitive than the former, *i.e.* 6 and 15 samples were positive but not quantifiable by qPCR *vs* 14 and 22 positive samples by ddPCR for *sul*2 and *intI*1, respectively. The presence of *sul*2 gene was not affected by the sampling site and/or date, while *intI*1 gene was significantly more abundant in the Southern North Sea than in the other sampling sites (p= between 0.007 and 0.011) and its abundance was higher in 1990-1994 than in 2000-2005 (p=0.011). The abundances of both *sul*2 and *intI*1 genes did not correlate with the measured biotic and abiotic factors.

Discussion and Conclusions
This study showed that ddPCR technique can be successfully applied to CPR DNA samples overcoming the problems of the low sensitivity and PCR inhibition recorded using traditional qPCR. Furthermore, ddPCR clearly revealed the long-term occurrence of ARGs and class 1 integrons in the plankton-associated microbial community. In addition, this work showed that class 1integrons are good markers of anthropogenic pollution being the Southern North Sea highly impacted by human activity if compared with the other sampling sites. This study was supported by the EU H2020 project “VIVALDI”.

P038 - Tn6349: a novel cfr- and poxtA-carrying transposon of enterococcal origin in a linezolid-resistant MRSA

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1. **Introduction**
   The spread of methicillin-resistant *S. aureus* (MRSA) is a worldwide public health concern, and percentages from invasive infections in our country have been constantly above 30% during the past decade. Linezolid is among the last resort agents for the treatment of MRSA infections, and resistance to this antibiotic, despite being uncommon, should be carefully monitored. Different resistance mechanisms to linezolid were described, including chromosomal mutations affecting the ribosomal target and acquired resistance mediated by genes such as *cfr*, which encodes a 23S rRNA methyltransferase, and *optrA* and *poxtA* which encode ABC-F ribosomal protection proteins. In this study we characterized the genetic context and stability of the first described *cfr*- and *poxtA*-carrying transposon in a linezolid-resistant MRSA strain of clinical origin.
2. **Materials and Methods**
   The genetic context of *cfr* and *poxtA* was investigated by bioinformatic analysis of whole-genome sequencing (WGS) data of *S. aureus* AOUC-0915, in conjunction with a PCR and Sanger sequencing approach to solve repeated regions. Gene transfer experiments were carried out using laboratory strains of *S. aureus* and *Enterococcus faecalis* as recipients. Stability of the genetic context of selected resistance genes was evaluated with inverse PCR experiments and curing experiments.
3. **Results**
   WGS analysis of MRSA strain AOUC-0915 revealed the presence of a 48,350 bp composite transposon, *named Tn6349*. This *transposon showed* 99% of identity over 84% of its length (ca. 40 kb) with pEF12-0805, a pRE25-like plasmid from *Enterococcus faecium* carrying the *cfr* and *optrA* resistance genes. Tn*6349* contains the *erm*(B), *cfr*, *poxtA* and *fexB* resistance genes and was inserted into a phiN315-like prophage element integrated in the bacterial chromosome. The *cfr* gene was bracketed by two identical IS*Enfa5* insertion sequences that were in the same orientation. The *poxtA* and *fexB* resistance genes were flanked by IS*1216* elements and were located in a region of enterococcal origin at the transposon 3' end. Tn*6349* and the genetic context of *cfr* gene proved capable of undergoing excision in circular form, despite not being transferable to *S. aureus* and/or *E. faecalis* hosts.
4. **Discussion and Conclusions**
   This study reports on the genetic structure of Tn*6349*, a novel chromosomal *cfr*- and *poxtA*-carrying composite transposon, and represents the first description of the genetic context of the recently described *poxtA* resistance gene. The high similarity of Tn*6349* with regions of plasmids from *Enterococcus* spp. suggests the likely acquisition of such structure through a recombination event involving an ancestral enterococcal plasmid and the *S. aureus* AOUC-0915 chromosome.

P039 - Search for and characterization of carbapenemase-producing Escherichia coli in Italy: evidence of a multiresistant non-ST131 isolate co-producing VIM-1 and KPC-2

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Introduction. Carbapenemase-producing *Enterobacteriaceae* (CPE) currently represents one of the major public health challenge worldwide. In Italy, as elsewhere in the world, a recent increase in the prevalence of CPE, primarily in the hospital setting, essentially results from the spread of high-risk pandemic clones like *E. coli* ST131: the major epidemic clone causing extraintestinal infections. This study aims to characterize all carbapenemase-producing *Escherichia coli* (CP-Ec) isolates recovered between August 2015 and October 2017 from patients admitted to a regional hospital in Italy.

Materials and Methods. Eight *E. coli* isolates (CP-Ec1 to CP-Ec8) resistant or with reduced susceptibility to carbapenems were collected. The isolates were extensively characterized for antibiotic susceptibilities, typing features, carbapenemase and ESBL genes, and their transferability by conjugation and transformation. Transferred plasmids were classified for their incompatibility (Inc) group and analyzed for carbapenemase genes by S1-PFGE and hybridization assays.

Results. All eight isolates exhibited an MDR phenotype and shared susceptibility to fosfomycin, tigecycline and colistin. Six isolates belonged to ST131 and were KPC-3 or VIM-1 producers. The remaining two (CP-Ec3 and CP-Ec6), both (and the sole) recovered from rectal swab, belonged to different STs (ST1266 and ST1011, respectively). CP-Ec3 exhibited the highest carbapenem MICs and co-expressed carbapenemases KPC-2 and VIM-1 (first report in Italy). CP-Ec6 produced KPC-3 and was the only isolate positive for $bla_{\text{CMY-2}}$. $bla_{\text{VIM-1}}$ or $bla_{\text{KPC-3}}$ proved to be transferable from all donors, except from the isolate (CP-Ec3) which harboured both determinants. Their transfer consistently occurred in association with an ESBL gene: (i) $bla_{\text{VIM-1}}$ and $bla_{\text{SHV-12}}$ were carried by a self-transferable plasmid belonging to the IncN2 group; (ii) $bla_{\text{KPC-3}}$ and $bla_{\text{CMY-2}}$ were co-transferred by a IncI1α plasmid.

Discussion and Conclusions. The isolation of different carbapenemase-producing *E. coli* STs in our region and the identification of several conjugative plasmids is of concern and underscores the need for an even more careful epidemiological surveillance, also aimed at monitoring the spread of plasmid-mediated carbapenem resistance in Enterobacteriaceae.

P040 - Multi-drug resistant clinical isolates: a retrospective study

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Introduction: Surveillance of antimicrobial drug resistance is fundamental to guide an empirical but reasoned treatment. We evaluated resistance patterns of ESKAPE isolates, which include the most common bacteria often associated with a profile of antibiotic resistance: *Enterococcus faecium/faecalis*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Escherichia coli* (as the main representative of the *Enterobacteriacae* family), during a seven years period at the University Hospital of Catanzaro (Southern Italy).

Materials and methods: In this retrospective study, we evaluated the frequency of ESKAPE isolates with different resistance patterns (group 1=low-resistant bacteria; group 2=multi-drug and extremely drug-resistant bacteria; group 3=pan-resistant bacteria), stratified by year (2011-2017), hospital units (intensive care units, medical and surgical units) and by sample type (urine, blood, wound swabs, respiratory samples, other samples). Pure bacteria cultures and antibiotic susceptibility test were obtained through automated VITEK system (bioMérieux). Susceptibility to antibiotics was evaluated through the breakpoints of European committee on antimicrobial susceptibility testing or EUCAST.

Results: Amongst 4213 isolates, *E. coli* (36%) was the most frequent, followed by *K. pneumoniae* (16%), *P. aeruginosa* (14%), *S. aureus* (13%), *E. faecalis* (10%), *A. baumannii* (8%) and *E. faecium* (3%). From 2011 to 2017 bacterial species that presented multi-resistance and pan-resistance are, respectively, *A. baumanni* and *K. pneumoniae ssp. pneumoniae*. Frequency of isolates in group 2 remained stable for *A. baumannii* (70%), while in group 3 there is an increasing trend for *K. pneumoniae ssp. pneumoniae* (dall'1% al 16%). Frequency of isolates in group 2 plus 3 was higher in intensive care units for *K. pneumoniae ssp. pneumoniae* and *A. baumannii*. It was also higher from blood samples than from different sources for most species.

Discussion and conclusions: Our results provided indications for an empirical and reasoned antimicrobial choice. Importantly, antibiotic resistance showed declined in recent years, probably due to optimized infection control and therapeutic algorithms, but still remain a significant problem. In particular, *A. baumannii* and *K. pneumoniae ssp. pneumoniae* were the most difficult to treat, but also the frequency of methicillin-resistant *S. aureus* appeared to be on the rise. In our hospital, surveillance and efforts to reduce multidrug resistant bacteria should be enforced, particularly focusing on these species, and in specific settings (i.e., intensive care units).

P041 - Klebsiella pneumoniae carbapenemase (KPC) producing: a year and a half of surveillance in Sicily

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Introduction
The worldwide dissemination of Carbapenemase-producing Entrobacteriaceae (CPE) has become a serious health problem in the community and in hospital, also in Italy these strains are increasing as has been documented. This is, in part, due to the propensity of microorganism to acquire genetic material through horizontal gene transfer and to the easy transmission of microorganism among people (in particular through contaminated hands). Guidelines from European Centre for Disease Prevention and Control, to prevent and control the spread of these strains, have been established. In Italy, there is an surveillance of system with the aim to report all cases of bloodstream infection due to Carbapenem-resistant *Klebsiella pneumoniae* (CRKPs). In this study we report the analysis of 380 CRKPs from bloodstream infection of hospitalized patients in different Sicilian hospitals and collected by the Regional Reference Laboratory for the Surveillance of infection by CPE, based at the Department of Science for Health Promotion and Mother-Child Care "G. D'Alessandro" in Palermo.

Materials and methods
From December 2016 until now, the Regional Reference Laboratory has collected clinical isolates of CRKP from five different hospitals of Palermo (Policlinico, Ismett, Buccheri La Ferla, Civico and Villa Sofia), one hospital of Caltanissetta, another one of Ragusa and the last one of Messina. One undred and one strains were isolated from Intesive Care Unit (ICU) while 279 strains were isolated from various hospital wards. The 380 CRKP non replicative isolates from blood and with antimicrobial MICs > 8 µg/ml for meropenem and/or imipenem-resistance according to EUCAST clinical breakpoints, are submitted to a rapid molecular typing. Detection of the epidemic ST-258/512 clone was performed by a multiplex PCR assay to detect three presumably-unique genes ST-258/512 clone that are *pilv-l*, *is-66* and *prp*.

Results
During the study 74/380 CRKP isolates belonged to the clonal complex 258 (CC-258) and in particular, among these, 25/101CRKP isolates from ICU belong to CC-258. While the others 306/380 CRKP isolates not belonged to CC-258. In Sicily, the prevalence of CC-258 is 20%.

Discussion and conclusion
The low prevalence of CC-258 suggests a change of the epidemiology of CRKP population which has moved from a substantially monoclonal circulation of ST-258 towards a more complex polyclonal spread, as demonstrated previously by data obtained only from Palermo hospitals. Our findings support the need to a continuous surveillance system and the application of an antibiotic stewardship programs.

P042 - Virulence of MRSA USA300 is enhanced by sub-inhibitory concentration of different classes of antibiotics

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1. Introduction

Community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) USA300 is responsible of many kinds of infections of skin and soft-tissue. Antibiotic resistance, biofilm formation and the ability to adhere and invade are virulence factors that contribute to MRSA pathogenesis.

In some cases, decreased bioavailability of antibiotics in systemic circulation could result; in these conditions sub-therapeutic levels of the antibiotics may be established, exposing bacteria to sub-inhibitory concentrations. In the present study, we investigated this process by measuring the effects of low doses of different classes of antibiotics on some virulence features of MRSA USA300 isolate, like the ability to adhere and invade eukaryotic cells.

2. Materials and Methods

Minimal Inhibition Concentrations (MIC) of ampicillin and amikacin were performed on *S. aureus* USA300, according to NCCLS, respectively.

HeLa cells were infected with *S. aureus* USA300, grown in the presence and in the absence of sub-MIC levels of each antibiotic, at a multiplicity of infection (MOI) of 10:1. After incubation, adhered and internalized bacteria were calculated by diluting cell lysates on TSA plates opportunely incubated overnight incubation at 37^{o}C.

3. Results

First we evaluated the in vitro bacteriostatic and bactericidal activities of ampicillin and amikacin, respectively on *S. aureus* USA300. Considering the high concentration necessary to inhibit bacterial growth we can establish that the tested strain is resistant to both tested antibiotics.

Starting from this value, the plasmatic concentration of each antibiotic was used (10 μg/ml ampicillin and 2 μg/ml amikacin, respectively), to challenge *S. aureus* invasive features.

In order to investigate the effect of sub-MIC concentration of ampicillin and amikacin on adhesion and invasion of USA300 on HeLa cells, we performed an antibiotic protection assay.

Our results show that the adhesion efficiency of USA300 is not impaired by the presence of antibiotics. Surprisingly the invasion ability of *S. aureus* is increased when bacteria grew in presence of each antibiotic even if the concentration adopted is much lower than MIC values.

4. Discussion and Conclusions

Our results strongly support the importance of the respect of a correct dosage in antibiotic therapy, in order to avoid the insurgence of more virulent phenotypes, due to the expression of virulence factors like the invasive capability.

To the best of our knowledge, this is the first report on a significant increase of invasion efficiency due to sub-MIC concentration of antibiotic in a hazardous virulent strain responsible for community-acquired infection.

P043 - Assessement of antimicrobial susceptibility through direct rapid E-test on positive blood culture

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1. Introduction

Sepsis is an extremely widespread and severe disease with a high rate of mortality. It represents the second cause of death among patients hospitalized in ICUs (Intensive Care Units). The aim of this study was to evaluate the possibility of using the E-test methodology to obtain rapid results in terms of antimicrobial susceptibility, starting directly from broth culture positive for Gram-negative monomicrobic flora.

2. Materials and Methods

100 blood culture samples positive for Gram-negative rods at the microscopic examination have been collected. Identification with MALDI-TOF from film of growth on blood agar after 4h incubation and direct E-test from broth culture after inoculum standardization were conducted on every sample: a rapid MIC interpretation was performed after 5-6h incubation. The antibiotics examined were: meropenem (MP), ceftazidime (CAZ), cefepime (PM), gentamicin (GM), levofloxacin (LV) and colistin (CL). The resulting MIC values were compared with the results obtained with traditional methods (E-test from subculture) and 3 different classes of error were observed: “VME” (Very Major Error), “ME” (Major Error) and “mE” (minor Error).

3. Results

The MALDI-TOF analysis after 4h incubation showed a 100% concordance with the results obtained from traditional Microscan identification. The comparison between direct E-test and E-test from subculture gave the following results for the enterobacteria category: concordance range going from 96,3% to 100%; -VME: MP 0/9, CAZ 0/34, PM 0/15, GM 0/21, LV 0/20, CL 2/16; -ME: MP 0/70, CAZ 0/43, PM 0/29, GM 0/59, LV 0/25, CL 0/65; -mE: MP 2/81, CAZ 0/81, PM 1/45, GM 3/81, LV 0/45, CL 0/65. Instead, the results for the not fermenting group were: concordance range from 88,2% to 100%; -VME: MP 0/3, CAZ 0/4, PM 0/3, GM 0/2, LV 0/3, CL 1/1; -ME: MP 0/13, CAZ 0/13, PM 0/8, GM 0/14, LV 0/9, CL 1/16; -mE: MP 2/17, CAZ 0/17, PM 1/12, GM 1/17, LV 0/9, CL 0/17.

4. Discussion and Conclusions

Although the results of the microscopic examination together with the identification of the bacterial species with a mass spectrometer are helpful for the very beginning of a therapeutic path, the rapid communication of a profile of antimicrobial susceptibilty/resistance represents an important step to operate a correction of the therapy. Therefore, a rapid system such as the direct E-test from broth blood-culture has been evaluated. The data collected showed that the direct antibiogram can provide in short times and with good accuracy the information about bacterial susceptibility to tested antibiotics, but it cannot give the definitive MIC values.

P044 - Chromosomal Integration of blakpc-2 in P. Mirabilis Clinical Isolates

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Introduction Over these years, KPC-type beta-lactamases have become one of the most prevalent acquired carbapenemases in *Enterobacteriaceae*, with the highest incidence in Mediterranean countries, especially Italy and Greece. Historically, bla_{KPC} has been largely reported in high-risk clones of *K. pneumoniae* (KPC-Kp), which have spread rapidly in several areas worldwide.
Here we describe the first Italian detection of a small set of *Proteus mirabilis* clinical isolates (AOUC-001 to -006) harbouring bla_{KPC-2} from a setting of high-level endemicity of KPC-Kp. Multidrug-resistance in *P. mirabilis* is commonly associated with the production of extended-spectrum beta-lactamases or the AmpC-type cephalosporinase, but acquisition of bla_{KPC} is still sporadically reported to date.

Materials and Methods Antimicrobial susceptibility was determined by standard broth microdilution. All KPC-positive *P. mirabilis* were fully sequenced using Illumina MiSeq and, for two isolates (AOUC-001 and -004), the PacBio technology. *In silico* identification of antimicrobial resistance genes was obtained by ResFinder. Complete characterization of bla_{KPC} genetic context was achieved by NGS data validated by PCR mapping and Sanger sequencing.

Results *P. mirabilis* AOUC-004 was virtually resistant to all tested antibiotics (i.e. PDR) while the others (AOUC-001 to -006) exhibited resistance to all except for carbapenems. Genome sequencing revealed that 5 out of 6 strains were strictly related each other, while AOUC-004 showed a higher genetic distance. In addition to bla_{KPC-2}, the 5 related strains also carried the *armA* and bla_{CMY-16} genes. Strikingly, such strains harbored two copies of bla_{KPC-2}, both embedded within two Tn*4401a* transposons. Conversely, AOUC-004 did not possess bla_{CMY-16} and had a double copy of bla_{KPC-2} aboard Tn*4401a* derivative elements. In all studied strains, acquisition of bla_{KPC} occurred through the chromosomal integration of a KPC-encoding plasmid strictly related to pKpQIL and pKPN101-IT, although targeting different *loci* within the genome of AOUC-004 and the remaining 5 related strains.

Discussion and Conclusions Here we characterized six clinical isolates of *P. mirabilis* encoding KPC-2 and explored the different genetic mechanisms at the basis of the chromosomal integration of bla_{KPC}. These results expand the current knowledge about the acquisition and integration of carbapenemase-encoding genes in *P. mirabilis* that, differently from what is generally observed among *Enterobacteriaceae*, is not an uncommon event. From a clinical perspective, acquisition of carbapenem and aminoglycosides resistance in *P. mirabilis* could represent a serious threat, considering its intrinsic resistance to several molecules such as tigecycline and colistin.

P045 - Putative enteroaggregative virulence and multidrug-resistance genes in Escherichia coli isolated from poultry in Algeria

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Introduction. Enteroaggregative *Escherichia coli* (EAEC) is an important agent of persistent diarrhoea in the developing world and of outbreaks of diarrhoea in the developed world. Several virulence factors such as *aggA*, *aggR* and *aap* are important in EAEC adherence to the intestinal mucosa and in stimulating biofilm formation. Several other genes, including *aatA*, *pet*, ShET1, *astA*, *irp2* and *shf* are also thought to be involved in EAEC pathogenesis. The aim of this study was to evaluate the enteroaggregative virulence genes in multi-drug resistant *E. coli* strains isolated from healthy broiler chicken and egg samples in Western Algeria.

Materials and Methods. A total of 47 *Escherichia coli* strains isolated from healthy broiler chickens and eggs, collected from Western Algeria, were analyzed regarding the presence of EAEC virulence genes (*aggR*, *aap*, *aatA*, *astA*, *pet*, *shf*, *irp2*, set1A) by polymerase chain reaction. Phylogenetic groups were assigned by a multiplex PCR detecting the genes *chuA*, a gene required for heme transport in enterohemorrhagic *E. coli* O157:H7, *yjaA* stress-induced protein and DNA fragment TspE4.C2, codifying a lipase.

Results. *E. coli* strains from chicken carried the EAEC probe sequence. Among these, two was *AggR*, *astA* and *irp2* probe positive strains, 9 was $astA^+$ and 10 was $irp2^+$. None of egg samples showed enteroaggregative virulence genes. The strains were identified mainly as phylogroup A, the most frequent commensal *E. coli* strains, 4 strains as B1 and one as E.

Discussion and Conclusions. Our data clearly indicates that the EAEC strains isolated from healthy broiler chicken samples present heterogenous combinations of putative enteroaggregative virulence genes. The *AggR* gene, important virulence factor in EAEC adherence to the intestinal mucosa, associated with *irp2* (yersiniabactin biosynthesis gene) and *astA* (gene encoding enteroaggregative heat-stable toxin 1, EAST-1) was harbored in two strains. The *irp2* and *astA* genes were both expressed in only one strain. Previously our study showed the resistance of the same strains to many of these drugs: nalidix acid, ciprofloxacin, amoxicillin, amoxicillin+clavulanic, cefotaxime, trimethoprim-sulfamethoxazole, tetracycline, chloramphenicol, neomycin, colistin and imipenem. Among these, six cefotaxime resistant strains expressed ESBL genes. Taken together all these results showed the coexistence of ESBL and virulence genes. Particularly, two strains (group B1 and E) harbored *irp2* and TEM, one (group B1) *irp2*, TEM and CTX-M-1, two (group A) *astA* and TEM and one (group F) was carrying simultaneously *astA*, *irp2* and *TEM*. In conclusion, this study revealed the presence of putative enteroaggregative virulence genes in multidrug resistant *E. coli* strains isolated from poultry.

P046 - Antimicrobial-resistant Enterobacteriaceae in colonized healthy dogs

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Background and aim

Antimicrobial resistant (AMR) mechanisms, are frequently reported in bacteria causing canine disease as well as in commensal bacteria, which could be a potential health risk for humans they come into contact with. Plasmid-mediated colistin-resistance mechanism gene (*mcr-1*) and ESBL-producing Enterobacteriaceae have emerged in both human and veterinary medicine. The aim of this study was to investigate the presence of *mcr-1* and ESBL-producers among *Enterobacteriaceae* isolated from dogs and to detected *mcr-1* and ESBL genes and analysis of *E.coli* phylogroups.

Materials and methods

Fecal samples from 92 healthy dogs belonging to different owners has been examined in 2018. The samples were collected by Veterinarian during routine parasitology screening, and they were also asked to complete a questionnaire related to medical history of the dog over the previous months including use of antimicrobials. Stools were collected in FecalSwab™ (Copan, Brescia It) analysed immediately or stored at -20°C within 24 hours. FecalSwab™ is a collection device that is able to preserve the viability of enteric pathogens up to 48h at room temperature. This device simplifies and standardizes stool sample collection, transport and processing by converting solid or semi-solid specimens into liquid phase, to facilitate automated fecal sample processing. To screening ESBL, 1 ml of each sample was suspended in 5 ml of BHI +1mg/l cefotaxime, and incubated overnight at 37°C, then 10 µl were streaked onto MacConckey agar+1mg/l cefotaxime. Plates were kept at 37°C for 24 h. Bacterial genomic DNA of each positive sample was extracted for identification of *mcr-1* and ESBL genes TEM, SHV, CTX-M, CMY, and analysis of *E. coli* phylogroups.

Results

29/92 (31,52%) samples were positive for ESBL screening (28 *E.coli*, 1 *E.fergusonii*) and 27/92 (29,34%) were confirmed by PCR. 2/92 (2,17%) samples were positive for *mcr-1*. The pattern of ESBLS genes were 10 CTXM1-TEM, 6 TEM, 6 CTXM1, 2 SHV-TEM-*mcr-1*, CTXM1-CMY, CTXM9-TEM. *E. coli* isolated strains were belonging to phylogroups: A (2/29), B1 (7/29), B2 (1/29), C (4/29), D (1/29), E (8/29) and F (3/29).

Conclusion

A high number of ESBLs genes were identified, including mobile-plasmid encoding resistance to colistin and *E.coli* phylogroups associated with extra-intestinal pathogenic *E. coli* (ExPEC). Considering the close contact that people have with dogs, the high levels of AMR *E. coli* in canine faeces may be a potential reservoir of AMR bacteria or resistance determinants.

P047 - MDR Gram negative and Carbapenem-resistant Enterobacteriaceae (CRE) at Neurological hospital

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Introduction

Inadequate working conditions and the exposure to risk factors such as high-risk medical interventions and invasive devices, failure of the hospital's efforts to prevent infections that require an individual investigation, are the most obvious problems concerning healthcare associated infections. HAIs, developed 48 hrs after hospital admission of the patient, are dependent on two key pathophysiological factors: decreased host defences and colonization by pathogenic organisms; and infected or colonized patients who frequently serve as reservoirs of bacterial infections for other patients. The implementation of preventive actions defined in CDC 2012 CRE TOOLKIT "Guidance for control of Carbapenem-resistant Enterobacteriaceae (CRE)" and generally for microorganisms transferred for contact were associated with a progressive reduction of incidence rate of alert microorganisms isolated in neurological and neurosurgical patients. In 2016 the total incidence of ICAs increased related to a more serious case mix.

Material and Methods

The study was performed at the Fondazione IRCCS Istituto Neurologico Carlo Besta, from 2015 to 2017 and was approved by the local ethics committee (protocol no. 25E/24/2015) The sample included patients of all ages affected by different neurological and neurosurgical pathologies, particularly the selection criteria of patients carrying multidrug resistance (MDR) or with specific resistance.
The outcome definitions are as follows: (1) microbiological surveillance after negativization of cultural examination and (2) evaluation of the assessment of actions taken to reduce risk factors and the fact that any antibiotic administration exerts selection pressure, modifying the bacterial sensitivity profile and promoting the acquisition of resistant and infectious organisms.

Results

From the 2016, concomitantly with an intensive preventive action to reduced SE, a strict selective antibiotic stewardship in ICU has allowed to an increase of antibiotic-free LOS days. Moreover, in concomitance there were a parallel reduction of ICAs and isolated alert microorganisms.
The trend of ICAs and alert microorganisms were the same for the three years (Fig.1).

Fig.1

	2015	2016	2017
Patients with infections	87	103	66
Number of infections	119	168	109
Pts - infections/1000 adms.	15,32	17,84	11,62
N° infections/10000 LOS	29,74	42,21	27,08
SE /1000 adms	12,25	14,2	9,15
SE/10000LOS	17,49	20,6	13,16
Legend:			
LOS= Length of Stay			
SE= Sentinel Event (microrganism)			

Discussion and Conclusions

The pool of preventive actions to reduce antibiotic- resistance in MDR and CRE microorganisms, associated at the control of antibiotic pressure and the careful stewardship, allowed to reduce the GRAM – alert but also to control ICAs both of alert/non alert microorganisms. At the Neurological hospital for the years considered we obtained an improved reduction of SE; this is very important because it allows also to restraint ICAs.

P048 - Role of male accessory gland infections in patients undergoing in vitro fertilization program

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Introduction: infections of the male genital tract, which represent 15% of infertility cases, including epididymitis, vesiculitis and prostatitis, are mainly due to germs sexually transmitted or to certain/occasional pathogens. The etiopathogenetic mechanisms are multiple but all result in a potential damage of sperm cells, due to pathogenic germs (Gram negative, *Chlamydia trachomatis*, *Ureaplasma urealyticum*, etc.), bacterial products (lipopolysaccharide, and others), toxic metabolites produced by microorganisms (H_2O_2, NH_3 produced by *U. urealyticum*), seminal leucocytes, or soluble factors, such as oxygen free radicals and cytokines. The Male Accessory Genital Infection (MAGI) is diagnosed when abnormal sperm parameters are found associated with, one or more of the following parameters: positive history for urinary infection, epididymitis and/or sexually transmitted disease, abnormal prostatic fluid and signs of ejaculate alteration. Recently, literature reports that MAGI increases the percentage of spermatozoa with fragmented DNA (%SDF) thus reducing the number of viable spermatozoa. The purpose of our study is well characterize MAGI in male infertility diagnosis, by 1) definition of a diagnostic workflow for the characterization of MAGI; 2) implementation of the classification of pathogens that cause MAGI; 3) evaluation of MAGI effects on sperm cells, including the DNA fragmentation.

Material and Methods: in this study, 30 samples from men (27-44 years) who had requested semen analysis (spermioculture, spermiogram and %SDF) were assessed. Before collecting the semen sample, the patients proceeded with urine collection in order to better differentiate the infection of the seminal tract from urinary tract infection. Once performed a dilution procedure, the seminal samples were spread on appropriate enriched, differential or selective culture media. Standard semen analysis was carried out according to WHO protocol after 2-4 days of sexual abstinence. An aliquot of semen sample was assessed using the Sperm Chromatin Dispersion Test (SCDt) for sperm DNA fragmentation analysis.

Results: The spermioculture allowed highlight the presence of pathogens in 36.6% of cases (13.6% not significant pathogens and 16.4% significant pathogens). The SDF% in semen samples, positive for pathogens, is between 50°-95° percentile.

Discussion and Conclusions: the data, although preliminary, as well as confirming the implication of MAGI in male infertility, provide opportunities to investigate the significance of other pathogens in addition to those currently recognized in MAGI. Furthermore, the correlation between %SDF and MAGI encourages in-depth studies on the mechanism of action of pathogens on spermatozoa.

P049 - Detection of Arcobacter spp. in food products and in clinical samples of humans by cultural and multiplex PCR based methods

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Introduction

During recent years, *Arcobacter* spp. has been identified as an emerging zoonotic pathogen with a wide geographical distribution (Ho et al, 2006), and associated with enteritis and abortion in animals and bacteraemia, gastroenteritis and diarrhea in humans (Collado and Figueras, 2011; Ramees et al, 2017). The genus *Arcobacter* was proposed in 1991 to group aerotolerant bacteria formerly classified in the genus *Campylobacter* (Vandamme *et al*, 1991). The presence of *Arcobacter* spp. is reported in different types of food such as meat, especially poultry, milk and dairy products, fish products such as bivalve molluscs, vegetables and contaminated water. A wide range of farmed animals, in which *Arcobacter* spp. has been frequently isolated in the intestinal tract and from faeces, are asymptomatic hosts and potential sources of contamination of water, food and environment (Collado et al., 2008; Giacometti et al., 2015). To date, 27 species of *Arcobacter* genus have been reported with a significant genetic diversity that have been isolated from different sources, including domestic and wild animals, birds, foods of animal origin (Ramees et al, 2017). The species *Arcobacter butzleri*, *A. cryaerophilus* and *A. skirrowii* have been more commonly associated with animal and human infections (Collado and Figueras 2011; Figueras et al, 2014). *A. butzleri* is the most important and prevalent species, classified as a serious hazard to human health by the International Commission on Microbiological Specifications for Foods (ICMSF, 2002); it is in fact a common isolation in the human cases reported in the bibliography (Arguello et al, 2015; Vandamme et al., 1992) and reported as an etiologic agent of traveller's diarrhea (Jiang et al, 2010). The main route of transmission to humans would be water and contaminated food products. Currently, the real presence of this pathogen in food is underestimated: most of the data refers to chicken meat, followed by pork, beef and raw milk. In the last years, DNA- based assays used for the identification of *Arcobacter* species have been developed, more rapid and with higher specificity than conventional identification methods, among which Multiplex PCR (Houf et al, 2000; Levican and Figueras, 2013). The present study was designed with an aim to know the occurrence of *Arcobacter* spp. in various food samples of animal origin and in clinical samples of humans collected in Sicilia region by utilizing both bacteriological and molecular tool of multiplex PCR (mPCR).

Materials and methods

A total of 262 samples were analyzed for the research of *Arcobacter* spp, of which n.124 of foods (meat products, raw milk, vegetables, fish products), taken from both production and marketing and n. 138 samples of human faeces of which n. 47 from hospitalized patients (adults and pediatric) and n. 91 from outpatients, between January 2017 and April 2018. Bacteriological and culture procedures for research and biomolecular methods for the identification of isolated *Arcobacter* strains have been developed and standardized, based on bibliographic data.

For isolation of *Arcobacter*, 25 g of samples (food products and humas samples) were aseptically inoculated in a 1:10 ratio in *Arcobacter* enrichment broth (Oxoid, UK) supplemented with Cefoperazone, Amphotericin B and Teicoplanin (CAT) selective supplement (SR0174, Oxoid, UK), homogenized with stomacher and incubated at 30 °C under microaerophilic condition for 48h. The skin of the neck, when it present, has been withdrawn by carcasses of poultry. Each raw milk sample was previously centrifuged at 3500 x g for 10 min at 22 °C, the upper phase removed and the sediment (20 ml) was added to 20 ml of enrichment broth and incubated as before.

Subsequently, 200 µl of the broth was filtered using 0.45 µm pore size nitrocellulose membrane filters (Sartorius), placed onto two selective agar plates: trypticase soy agar (TSA) supplemented with 5% defibrinated sheep blood and with CAT (Oxoid, UK) and modified Charcoal Cefoperazone Deoxycholate Agar (mCCDA) supplemented with CAT antibiotics. After a incubation for 60 min at 30 °C, the filters were removed and the plates were incubated under aerobic conditions at 30 °C for 48h. Subsequently, presumptive *Arcobacter* colonies (small colourless, translucent, convex with an entire edge) were picked, subcultured onto blood agar and incubated at 30 °C for 48h. Purified isolates were further confirmed morphologically by Gram staining and biochemical analysis (catalase, oxidase, urease tests and motility, indoxyl acetate hydrolysis, salt tolerance and growth on McConkey agar). The isolates referable at *Arcobacter* genus were stored in 20% (v/v) nutrient broth –glycerol at -80 °C, after molecular identification.

Total DNA from each characterized *Arcobacter* isolate was extracted according to the protocol developed by Houf et al (2000) and as also described in Ertas et al (2010). Five colonies of each strain grown on blood agar were suspended in 1 ml of sterile distilled water and centrifuged at 16,000 g for 10 min at 10 °C. Bacterial cell pellets were washed with 500 µl of sterile distilled water and then 100 µl of the suspension boiled in thermomixer for 10 min to lyse the cells. After another centrifugation (16,000 g for 10 min), 2 ml of each supernatant was used as the DNA template for the multiplex PCR assay. The primers and PCR assay conditions previously described by Houf et al (2000) were used for specific identification of *A. butzleri*, *A. cryaerophilus* and *A. skirrowii*. The primers amplify a 401-bp fragment from *A. butzleri*, 257-bp fragment from *A. cryaerophilus*, and a 641-bp fragment from *A. skirrowii* (Table 1). PCR reactions were performed in a 50 µL reaction mixture (2X PCR master mix Promega) contained: 2 µL template DNA, 5 µL of 10×PCR buffer, 1.25 mM MgCl2, 0.2 mM deoxynucleoside triphosphate mixture, 50 pmol of each of the primers, ARCO, BUTZ, CRY1, CRY2, 25 pmol of the primer SKIR and 1.5 U Taq DNA polymerase (Ertas *et al*, 2010). PCR amplification was performed in an automatic thermocycler (2720 Applied Biosystems) with an initial denaturation at 94 °C for 2 min, 32 cycles of denaturation (94 °C, 45 s), primer annealing (61 °C, 45 s) and final extension (72 °C, 30 s). The amplification products were then separated in 1.5% agarose gels with SYBR Safe DNA gel stain, at 100 V for 40 min and the bands were visualized with a UV transilluminator (GelDoc, Euroclone). DNA from reference strains *A. butzleri* (NCTC 12481), *A. cryaerophilus* (NCTC 11885) and *A. skirrowii* (NCTC 12713) were used as positive controls and sterile distilled water was used as negative control.

Results

The results obtained allowed to evaluate the diffusion of *Arcobacter* in the food products and in the clinical samples examined: *Arcobacter* spp was identified in 21/124 (17%) food samples of which 8.1% in poultry meat, 1,6% in meat samples, 7,2% in bivalve molluscs. As regards samples of human origin, *Arcobacter* spp. was isolated in a 1/91 human faeces sample (0.91%) from external patients. The identification of strain species referable to *Arcobacter* spp., performed by multiplex PCR, highlighted the prevalence of *A. butzleri* in food samples (81%), followed by *A. cryaerophilus* (9.5%) and *A.skirrowii* (9.5%). Molecular analysis performed on the only positive sample of human origin (1/138; 0,91%) revealed the characteristic amplicon of *A. butzleri*.

Discussion and Conclusions

Several reports showed that *A. butzleri* is the most common species of the genus *Arcobacter* and has been associated with human disease, such as enteritis, severe diarrhea, bacteremia and septicemia. *Arcobacter* spp. have been isolated from a variety of food products for human consumption (chicken, pork, beef meat, raw milk and dairy products, seafood, vegetables). Chicken meat particularly has been reported with highest prevalence for *Arcobacter* spp. followed by pork and beef. In general, the presence of these microrganisms in food processing environments, indicates possible persistence or cross contamination (Houf et al, 2002). Lehmann et al (2015) reported the prevalence of *Arcobacter* spp. as 27% from poultry meat and 2% from minced meat (beef and pork). Similarly, De Smet et al (2010) reported the presence of *Arcobacter* from pre- and post-chilled bovine carcasses indicating the need for hygienic practices to interrupt the transmission cycle. In all these works, *A. butzleri* was the species most frequently isolated. A study on chicken meat in Turkey report the prevalence of the species *A. butzleri*, followed by *A. cryaerophilus* and *A. skirrowii*: based on the type of samples, the carcasses resulted the most contaminated, followed by drumsticks (Molva et al, 2016). Several studies have shown the occurrence of *Arcobacte*r spp. in shellfish (Collado et al, 2009; Laishram et al, 2016; Mottola et al, 2016; Leoni et al, 2017). In Italy, Mottola et al (2016), report a prevalence of *Arcobacter* spp. in the 23,8% of mussel and in the 21,4% of clam samples, collected from local fish market in the Apulia region: the isolates were identified as *A. butzleri* (75%) and *A. cryaerophilus* (25%). A survey of the occurrence in mussels and in clams from the Central Adriatic Sea, has been detected *Arcobacter* spp. in 30% of samples (33% and 22% respectively) and *A. butzleri* shall be reported as the most common species (20%) followed by *A. cryaerophilus* (9%) and *A. skirrowii* (1%) (Leoni et al, 2017). Arcobacters have been also detected in fresh vegetables such as lettuces in Spain (Gonzales and Ferrus, 2011), in a spinach-processing plant (Hausdorf et al, 2013) and from pre-cut ready-to-eat vegetables (lettuces, spinach, rocket, valerian) (Mottola et al, 2016).

In our study, 21 samples of food (17%) were positive for *Arcobacter* spp. by culture method: the most contaminated samples were the chicken meat (8.1%) followed by shellfish (7.2%) and meat products (1.6%). As reported in the literature, the chicken meat, especially poultry carcasses, proved to be the most contaminated samples followed by pork and beef, especially minced meat. In this study, *Arcobacter* spp. were isolated from chicken carcasses and the samples of the most contaminated meat products were minced meat. Few samples were examined in this study, but the results obtained were similar to those reported by other authors.

Regarding bivalve molluscs, we instead found the clams more contaminated than mussels. *Arcobacter* spp. were not isolated from the samples of bovine raw milk and of fresh vegetables analyzed: probably a majority numbers of samples must be collected and among these latter, particularly the broad-leaved vegetables, should be more examined. Infact, according to some authors, the vegetables do not seem to be a reservoir for *Arcobacter* spp. but this type of food, broad-leaved vegetables particularly, can be contaminated through irrigation water as well as postharvest washing (Gonzales et al, 2017; Hausdorf et al, 2013).

However, the results of the work also show that the application of the implemented cultural method and the use of membrane filtration, as indicated by other authors, results to be more effective for the isolation of *Arcobacter* spp. The molecular analysis with multiplex PCR (m-PCR) allowed to confirm as *Arcobacter* spp. all the presumptive isolates.

In our study, the m-PCR method identified the species *A. butzleri*, *A. cryaerophilus* and *A. skirrowii* which were recovered from 81%, 9.5% and 9.5% of the samples, respectively. *A. butzleri* was the predominant species present in samples analyzed.

However, the m-PCR technique described by Houf et al (2000) is a sensitive assay targeting the 16S and 23S rRNA genes for the simultaneous identification of *A. butzleri*, *A. cryaerophilus* and *A.*

skirrowii. This PCR method has the advantage of 100% reliable identification of *A. butzleri*, but false positive reaction occurs for *A. cryaerophilus* and *A. skirrowii* (Levican and Figueras, 2013).

Therefore, other techniques for the correct identification of these species and of other potential pathogenic *Arcobacter* spp., should be used (Levican and Figueras, 2013; Ramees et al, 2017).

At the moment, preliminary data obtained show a lower prevalence in the food samples examined than bibliographic data and need further analysis.

It is important to highlight that the bacteriological method implemented during the present study, allowed to isolate of *Arcobacter* spp in 1/138 (0.91%) fecal samples collected: the strain was identified as *A. butzleri* with mPCR. At the moment, few studies on the presence and molecular characterization of *Arcobacter* spp in clinical samples of humans were performed in Italy. In summary, the preliminary results obtained in the present work, demonstrate that food products of animal origin can be vehicle of potential pathogenic *Arcobacter*. These results of the occurrence of *Arcobacter* spp. also can add new data available for this important zoonotic pathogen.

The present project allowed to collect a series of data and information of primary importance in the field of food safety. The epidemiological information obtained from the study led to a preliminary assessment of the risk related to the *Arcobacter* contamination along the food supply chain,

P050 - Rapid detection of Legionella pneumophila in environmental samples: validation of a novel LAMP-based kit

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Introduction. Legionella pneumophila is a waterborne pathogenic bacterium causing a severe pneumonia called the Legionnaires' disease. It is found ubiquitously in fresh water and survives within biofilms and free-living amoebae, even if it may be found at high levels also in man-made water systems such as hospitals, cooling towers and spas. The reference method used for its detection requires many days for isolation and identification, not detecting non-culturable bacteria and hence increasing the infection risk. Therefore, a new LAMP-based (Loop-mediated isothermal amplification) kit was developed, allowing a rapid, specific and sensitive detection of L. pneumophila.

Materials and Methods. Following filtration, rapid DNA extraction from membranes occurs through a specific ready-to-use extraction buffer. Afterwards, genetic amplification is carried out in the dedicated device ICGENE mini (Enbiotech Cat.N. EBT 801). The kit "Legionella pneumophila Glow" is under validation according to AFNOR NF148 (2015), hence different tests were performed, including sensitivity, inclusivity, exclusivity and various matrices contamination: in particular, hot water, mineral water and cooling tower water. Besides, LAMP performances were compared to cultural method by testing 45 real water samples.

Results. Regarding exclusivity all the other species tested resulted negative, while inclusivity results showed positive for all the L. pneumophila serogroups. As for sensitivity, the kit can detect up to 28 plasmid copies/µl. In addition, the 3 matrices used for the contamination experiments showed consistent results, as both contamination levels and all the samples typologies gave riproducible results. Real water samples showed a 100% agreement between the cultural and LAMP method, with 29 negative samples and 16 positive samples.

Discussion and Conclusions. The LAMP kit "Legionella pneumophila Glow" proved a useful option for a rapid, efficient and labour-saving screening of different typologies of water samples, offering significant advantages over the traditional method, as it is characterised by a high sensitivity, easiness of use for laboratory testing, and a large reduction in the analysis time, thus making a valuable asset to official controls.

P051 - A new laboratory workflow for the diagnosis of gastroenteritis in pediatric patients by using FilmArray® Gastrointestinal Panel

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Introduction. Pathogen-induced acute gastroenteritis is one of the leading cause of childhood morbidity and mortality worldwide. Conventional diagnostic methods for routine detection of enteric pathogens are time consuming, labor-intensive, often lack sensitivity and specificity, and leave undiagnosed cases. More recently, the introduction of the syndromic multiplex PCR systems has allowed to simultaneously detecting a wider range of enteric pathogens. This study represents a 2-year picture of the epidemiology of enteric pathogens in children suffering from gastroenteritis using the FilmArray® Gastrointestinal Panel (FA-GP), a multiplex molecular assay that allows to simultaneously detecting a large panel of pathogens independently of the etiological suspicion.

Materials and Methods. A total of 2128 stool samples, collected from children with clinical suspicion of bacterial and/or viral gastroenteritis attending the University Hospital of Parma, was submitted to the FA-GP and, when an adequate aliquot was available, to electron microscopy (n = 1494) for virus detection and to an enterovirus-targeting real-time PCR (n = 2110). Specimens with positive results for *Salmonella*, *Yersinia enterocolitica*, *Vibrio*, diarrheagenic *Escherichia coli/Shigella*, *Campylobacter*, *Plesiomonas shigelloides* by the FA-GP were also submitted to conventional diagnostic methods. When a parasite was detected by the FA-GP, the same sample and/or additional samples up to three per patient were submitted to conventional assays as confirmed.

Results. The FA-GP gave positive results in 1171 (55%) cases: 801 (68.4%) contained a single agent and 370 (31.6%) multiple agents, for a total of 1660 pathogens. Enteropathogenic *E. coli*, rotavirus, norovirus, toxigenic *Clostridioides difficile*, and sapovirus were the most commonly detected pathogens. A total of 970 additional agents (424 of which as single pathogen) was detected by the FA-GP and not included in the clinical suspicion. The overall recovery rate of the conventional methods from stools that resulted positive by the FA-GP was 39% for bacteria, 35.7% and 82.9% for *Giardia intestinalis* and *Cryptosporidium*, respectively, and ranged from 3.2% to 62.8% for viruses, if excluding all electron microscopy-negative astroviruses. Enterovirus, an agent not targeted by the FA-GP, was revealed in 9.2% (194/2110) of the examined samples, and in 73 cases it was the only agent detected.

Discussion and Conclusions. The results of this study allowed to extend the range of detectable pathogens independently of the clinical suspicion, to detect co-infections in almost one third of children positive for at least one agent and to show that conventional methods would have missed more than half of the enteric agents detected by the FA-GP.

P052 - Clostridium difficile infection in infant and children

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Introduction

Clostridium difficile (CD) is a spore-forming, obligate anaerobic, Gram-positive bacillus and is acquired from the environment or by the fecal-oral route. Toxins A and B are responsible for intestinal disease. CD is the most common cause of antimicrobial-associated diarrhea and is a common health care-associated pathogen. Clinical symptoms vary from a mild diarrhea to pseudomembranous colitis with bloody diarrhea, fever, and severe abdominal pain. Its significance in children is less well defined, but cases of *C. difficile* infection (CDI) appear to be increasingly prevalent in pediatric patients.

Materials and Method

105 samples stool collected from 77 pediatric patients of different age (0-10 years old) suspected of having C. difficile disease were tested by chemiluminescent immunoassay (CLIA) kit. *DiaSorin LIAISON® C.diff GDH Assay* is used as a screening assay to detect CD antigen, glutamate dehydrogenase. GDH positive samples were analyzed to identify toxigenic and non-toxigenic strains by *C.diff Toxins A&B Assay (DiaSorin LIAISON®).* Samples with discordant results between GDH and toxin A/B were tested by ***BD MAX™*** *C.diff assay* (Nucleic acid amplification tests).

Results

Presence of CD have been documented in 19 patients (19/77, 24.7 %). 5 out of 19 patients were preterm children (26.4 %); 6 were oncologic patients (31.5%), 8 were affected by other severe diseases (42.1%) for which were admitted to intensive care unit (n=5), surgical ward (n=1) and medical ward (n=2).

Discussion and Conclusions

During the first month of life CD is present in 50-70% of newborns. In spite of the presence of CD, newborns rarely develop CD-associated diarrhea. Gradually the bacteria is eliminated from feaces during the first year of life and only approximately 3% of children still harbouring the bacteria at 2 years of life. In the last decade there was an important increasing of incidence of CDI both in adult and pediatric patients. In the last year, we observed a very considerable incidence of CDI among hospitalized children. Testing in infants (younger than 12 month of age) is complicated by a high rate of asymptomatic colonization. We observed an important correlation between positive test and preterm and oncologic patients. Recognised risk factor in CDI are antimicrobial therapy, use of proton pump inhibitors, prolonged nasogastric tube insertion. Another important aspect to be considered is the gut microbiota. While in healthy newborns CD is antagonized by the presence of other bacterial species, in preterm and oncologic newborn CD became a real pathogen. Our purpose for the future could be describe the preterm's gut microbiota to understand if the lock or the low abundance of some species is the trigger for the switch from CD as colonizer to CD as pathogen.

P053 - Nanopore sequencing-based detection of Coxiella burnetii in acute Q fever endocarditis: a case report

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Introduction
Endocarditis is among the most common complications affecting patients after acute symptomatic Q fever or asymptomatic infection. Q fever is a zoonosis caused by *Coxiella burnetii,* an obligate gram-negative intracellular bacterium, causing severe and potentially fatal infective endocarditis (IE), if not diagnosed rapidly. Up to 31% of all cases of IE belong to the blood culture-negative infective endocarditis (BCNIE) category. In these cases, guidelines require time-consuming serological and/or molecular analyses from surgical materials. We report a clinical case involving a 54 years-old man with history of Bentall/Cabrol surgery, admitted for persistent fever. Cardiac imaging was suggestive of a peri-prosthesis abscess. Current methods led to *Coxiella burnetii* endocarditis diagnosis, and were compared with a new method for rapid detection.

Materials and Methods
Blood cultures were executed at admission and after 48 hours, but lack of indications led to serological analysis after collection of additional specimens. Samples were obtained from periaortic abscesses, periaortic fluid, and epicardial and pericardial tissue to perform molecular analyses. Bacterial DNA was isolated from 1.8 ml of blood cultures using a dedicated kit. Specific primers were used to amplify a 1500bp region of 16S by PCR, followed by nanopore-based sequencing. Taxonomic classification was assessed using a dedicated software.

Results
Blood cultures performed at admission and after 48 hours gave negative results. Similarly, molecular analyses conducted from bioptic tissues by PCR resulted in the same outcome. Following serological tests took six days after blood cultures, and showed positive results for *Coxiella burnetii*, with an IgG title > 1:4096. Treatment with specific drugs led to IE resolution. Parallel assessment of 16S species conducted by sequencing and starting from a pool of the same initial blood cultures, led to the detection of *Coxiella burnetii* in only three days. In particular, 58374 total reads were obtained after a 15 hours run, and taxonomic classification showed the presence of 132 *Coxiella burnetii* reads with 87.5% accuracy.

Discussion and Conclusions
Although serological diagnostic systems and/or gene amplification performed from bioptic tissue are the reference standards, the high probability of cross-reactivity with some similar intracellular pathogens and/or prolonged detection times may slow down the diagnosis. Conversely, nanopore-based sequencing using a minION seems to be promising for rapid diagnosis of *Coxiella burnetii,* leading to early endocarditis diagnosis. This method achieved the same results in less time, using a small amount of starting sample, without the need of additional collection and overcoming serological analysis limits.

P054 - Yersinia enterocolitica in Italy: a case of septicemia and abdominal aortic aneurysm infection

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Introduction *Yersinia enterocolitica* is a non-spore Gram-negative bacterium belonging to *Yersinia* genus and to the *Enterobacteriaceae* family. *Y. enterocolitica* is a heterogeneous group of bacterial strains, classified into 6 biogroups based on phenotypic characteristics, human/animal pathogenicity, and ecologic and geographic distribution. Serologically, *Y. enterocolitica* is further distinguished into more than 57 O serogroups, depending on their lipopolysaccharide-O-antigens. As a foodborne pathogen, *Y. enterocolitica* causes acute terminal ileitis and mesenteric lymphadenitis. Several septic complications have been described that can evolve, eventually, into endocarditis or infected aortic aneurysm, also known as mycotic aneurysm. Herein, we describe a case of *Y. enterocolitica* septicemia in a patient suffering from an abdominal aortic aneurysm.

Materials and methods We report a case of *Yersinia enterocolitica* septicemia in a 63-year-old patient admitted to the Vascular Surgery Department of Umberto I Hospital (Rome, Italy) for an abdominal aortic aneurysm. Three sets of peripheral blood and culture specimens collected from the aneurysmatic aortic wall were sent to the Microbiology laboratory. Two sets of blood cultures, after 24 hours of incubation in the automatic Virtuo BacT/ALERT, and the aneurysm specimens became positive. Microscopic examination revealed motile Gram-negative coccobacilli.

Results Biochemical tests of the colonies, using VITEK 2 System, identified *Yersinia enterocolitica/frederiksenii*. Accordingly, MALDI-TOF MS System confirmed the identification of *Y. enterocolitica*, with a score of 2.265. Finally, the 16S rDNA gene sequence analysis confirmed the *Y. enterocolitica* identification. Antibiotic susceptibility tests with VITEK 2 System and MICROSCAN WalkAway System 96 Plus revealed that the *Y. enterocolitica* isolate was susceptible to all antibiotics tested, except to amoxicillin/clavulanate. Serogrouping analysis using specific *Y. enterocolitica* antisera evidenced that the *Y. enterocolitica* isolate belonged to the O:9 serotype (biogroup 2). Finally, the presence of both *ail* and *yst* genes in the *Y. enterocolitica* isolate was detected using Real-time PCR.

Discussion and conclusions Our case contributes in enriching epidemiological data concerning *Y. enterocolitica* infections, which might represent severe complications in patients suffering from cardiovascular diseases. Moreover, this study, together with the others, should be regarded as valuable and useful tools for monitoring the rate of infections worldwide.

P055 - External Quality Assessment for Bacterial Identification: A 4-Year Multicentre Implementation Study

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Oneworld Accuracy Italia srl (1WA)

Aim of the Study
The accurate identification of bacterial species isolated from different biological sources (blood, urine, sputum, swab and wound) is of pivotal importance in the diagnosis and management of bacterial infections and, consequently, in preventing increased morbidity and healthcare costs. Regular participation to External Quality Assessment (EQA) schemes plays an essential role in ensuring accurate bacterial identification through monitoring aimed at improving labs' performance. The aim of this study was to analyze the results collected for Bacterial Identification EQA in a systematic manner and based on a yearly schedule (test events) and to identify potential benefits of participation to an EQA scheme as well as the common issues encountered by the participants.

Methods
The EQA scheme for Bacterial Identification was designed and implemented by Oneworld Accuracy, renowned EQA Provider, to test laboratories' proficiency. This study's focus was on 8 EQA test events that were conducted between 2014 – 2017 across Italian laboratories. In each EQA test event, five samples consisting of inoculated loops or KWIK-STIK™ Ampoule/Swab of various matrixes including blood, urine, sputum, swab, wound were provided to labs. Samples were challenged using both manual and automated methods from Becton Dickinson, Biomerieux, Liofilchem and Siemens. Data collected over the 4 years of EQA test events have been analyzed and segregated based on the evaluation's outcome (graded as Acceptable or Unacceptable). Samples have then been grouped according to source material and compared amongst each other. The established criterion was the identification of bacteria at the species' level. Labs that identified the bacterial species were thus employed to establish the overall performance.

Results
A total of 110 laboratories, with participation to all 8 EQA test events, were included in this study. Although participation rate varied based on sample matrixes, the average participation rate was 84% across the study arc. Overall, laboratories demonstrated good proficiency proving their ability to identify bacteria at the species' level in urine, sputum, swab and wound with high accuracy. Lower accuracy was shown when identifying bacteria from blood matrixes and in cases when anaerobic cultures were required.
Other issues encountered by laboratories, preventing them from meeting the study's criterion, included sample contamination, misidentification, wrong culture media employed, missed correlation between source material and clinical history.

Conclusions
Our data show that systematic participation to EQA schemes can be instrumental to improve labs' performance and detection of common issues so that corrective actions can be promptly identified and taken in a timely manner to restore high quality services.

P056 - Rapid identification of microorganisms in polymicrobial blood cultures by MALDI-TOF MS after short-term culture in selective liquid media

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Introduction: Rapid identification of the causative agent of bloodstream infection directly from positive blood culture (BC) has been made possible by rapid identification methods based on MALDI-TOF mass spectrometry (MS). These methods optimally apply to monomicrobial BC, whereas polymicrobial BCs require sub-culture on solid media before identification. The aim of the present study was to establish a rapid method for the identification of bacteria and yeast in polymicrobial BCs.

Materials and Methods: The proposed rapid method is based on the use of selective liquid media allowing growth of different categories of microorganisms, and rapid microbial identification by MALDI-TOF MS after short-term culture in the Alfred 60AST (Alifax) instrument. To this purpose, 56 polymicrobial BCs were analyzed with the rapid method, and the results were compared with those obtained by the routine method.

Results: The results obtained showed concordant identification for 43/50 (86%) microorganisms present in BCs containing two germs. In six out of six samples containing three or four different microorganisms, two of these were concordantly identified. Identification could be obtained after a mean incubation time of about 10.6 h for Gram-negative bacteria, 8.2 h for staphylococci, 3.7 h for streptococci/enterococci, and 13.6 h for yeast, compared to at least 24/48 h required by the routine method.

Discussion and Conclusions: To our knowledge, this is the first study specifically dedicated to rapid microbial identification in polymicrobial BCs. The results obtained indicate that this rapid method could be applied in the routine diagnostic practice, allowing a rapid identification of single germs and providing timely, useful information to streamline antimicrobial therapy.

P057 - Study of bacterial contamination of oxygen medical devises in chronic obstructive pulmonary disease patients

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Introduction

Chronic obstructive pulmonary disease (COPD) is a chronic inflammation of airways and lungs that leads to persistent bronchial obstruction and includes bronchitis, pulmonary emphysema and bronchial asthma. COPD is cause of morbidity and mortality worldwide and results in an economic and social burden. Patients with COPD are usually subjected to respiratory rehabilitation during which they perform oxygen therapy. The aim of the study was to evaluate the contamination and the consequent infectious risk (patient-oxygen supply, oxygen supply-patient) of the medical device in COPD patients under continuous oxygen therapy. Moreover, the antibiotic susceptibility profile was determined for all isolated strains. A screening for biofilm-forming ability was also performed.

Materials and methods

Patients affected by COPD admitted at Dept. of Respiratory Rehabilitation, San Raffaele Pisana, Roma, were enrolled from October 2016 to May 2018. All patients received continuous high-flow nasal oxygen therapy. Respiratory sputum specimens were analyzed at patient admission (T0) and at hospital discharge (T3), whereas devices and water samples at T0, after 7 and 14 days (T1, T2) and at T3. Microorganisms were identified by Vitek2 (Bio-Merieux) and mass spectrometry (MALDI-TOF Bruker). Antimicrobial susceptibility of bacterial isolates was determined by Vitek2 and disk diffusion test (Kirby-Bauer method). Biofilm forming ability was evaluated by the Congo Red agar plate assay.

Results

The analysis of the water within the bubblers revealed a high-load contamination of Gram-negative bacteria after two/three weeks of use (T1/T2/T3). In few cases, water samples were found contaminated at T0. Noteworthy, 50% of nasal cannulas were found contaminated at T1, mainly by β-lactam resistant Gram-positive bacteria. Remarkably, *Candida albicans* was the prevalent microorganism isolated from the sputum specimens (50%) both at T0 and T3. However, also a high percentage of Gram-positive and –negative bacteria were retrieved from sputum specimens. It is important to highlight that *Staphylococcus aureus* isolates presenting an identical antibiotype were found both in sputum specimens and nasal cannulas in 22% of patients.

Conclusions

Overall, this study indicates that there is a high risk of endogenous and exogenous contamination of medical devises after one week of oxygen therapy. Moreover, the high rates of isolation of *C. albicans* demonstrate that COPD patients are immunocompromised. Therefore, special care should be taken in handling oxygen medical devices in order to avoid bacterial contamination by nosocomial microorganisms. It would be high advisable the periodic replacement of the nasal cannulas, especially for immunocompromised patients.

P058 - Deadly puppy infection caused by an MDR ST58 E. coli O141 blaCTX-M-15, CMY-II, DHA-1 and aac(6′)-Ib-cr -positive in a breeding of Central Italy

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Introduction. Companion animals are considered as emerging reservoirs of antibiotic-resistant bacteria, but few epidemiological data on Beta-Lactamases (BLs)-producing *Escherichia coli* in pet dogs are available in Italy. The most prevalent acquired BLs in both companion and breeding animals are CTX-M-1 and CMY-II, with CTX-M-15 and DHA-1 being rarely identified in veterinary medicine in Europe. The aim of this study was to assess the presence of Extended-Spectrum BL, AmpC and fluoroquinolone/aminoglycoside resistance genes among *E. coli* from a Bulldog puppy in a dog breeding located in Pesaro area, Central Italy.

Materials and Methods. A total of five *E. coli* were collected in September 8th 2017, from biopsy specimens of the same two-weeks old dog. Species identification and serotyping were performed by API-20E system (bioMeriéux) and hot tube agglutination with specific sera. Antibiotic susceptibilities were obtained by AutoScan4 System (Beckman Coulter); the results were interpreted according to EUCAST 2017 guidelines: epidemiological cut-offs or clinical breakpoints for those drugs for which epidemiological cut-offs have not been made available. Check-MDR CT103XL (Checkpoint) microarray and/or PCR and sequencing were used for resistance genes investigation. Conjugation experiments were performed using *E. coli* J53 as a recipient. Molecular characterization of plasmids and clones was accomplished using PBRT Kit (Diatheva), Pulsed-Field Gel Electrophoresis (PFGE), Phylogroup identification and Sequence Typing (ST).

Results. All the n=2 (from liver) and n=3 (from intestine) dog specimens resulted *E. coli* O141 positive. The isolates revealed resistance to ampicillin, piperacillin, third generation cephalosporins, aztreonam, ciprofloxacin, gentamycin and moxifloxacin. The bla_{DHA}, $bla_{\text{CMY-II}}$ and $bla_{\text{CTX-M-15}}$ genes were detected by microarray, while *aac(6')-Ib-cr* were always identified by PCR. Sequencing of $bla_{\text{DHA-type}}$ genes showed the presence of $bla_{\text{DHA-1}}$ variant. Conjugation experiments confirmed the transferability of $bla_{\text{CTX-M-15}}$, bla_{DHA}, and *aac(6')-Ib-cr* genes; plasmid characterization detected IncHI2, IncX3, IncX1, IncFII. The strains showed an identical PFGE profile, of phylogenetic group B1 and ST58.

Discussion and Conclusions. This is the first Italian report on a two-weeks old dog invasive infection caused by a MDR ST58 *E. coli* O141 $bla_{\text{CTX-M-15}}$, $bla_{\text{CMY-II}}$, $bla_{\text{DHA-1}}$ and *aac(6')-Ib-cr* positive. MDR *E. coli* clone here characterized was probably the cause of death of all the eight dogs belonging to the same litter. The ST58 reservoir could be the water tank-stored used to prepare the milk-based litter meal. As *One health* approach, the dissemination of MDR bacteria in companion animals' environment should be regularly monitored to avoid cross-contamination.

P059 - Bacteriuria in a hunting dog caused by Salmonella enterica subspecies enterica serovar Thompson, first report

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Background

Salmonella enterica subspecies *enterica* serovar Thompson (*S.* Thompson) is one of the less frequently serovars isolated from animals and humans. This serovar is isolated from animals, mostly poultry, food of animal origin and environment, like surface water.

Case Presentation

A 2 years old male intact "Segugio Italiano" breed dog that lives in the countryside, regularly vaccinated and fed with commercial food and occasionally raw meat, was presented with a history of fever and severe bronchitis not responsive to empirical therapy with enrofloxacin and FANS. Abdominal ultrasound revealed an irregularity of the urinary bladder wall and cystitis was suspected. Urine collection was performed by cystocentesis and culture revealed presence of Gram-negative rods. Bacterial strain were identified as *Salmonella* spp by MALDI-TOF and further identified by serotyping according to the ISI 6579:2014 Part 3 as *S.* Thompson (antigenic formula 6,7, 14:k: 1,5) a serovar of the O:7 (C_1) serogroup.

Results

Antimicrobial susceptibility testing was conducted by *in vitro* broth microdilution method, to determine the MIC using commercial plate for companion animals and readed by automatic scanner. *S.* Thompson was intermediate to fluoroquinolones, susceptible to beta-lacatams and trimethoprim/sulfamethoxazole. Antibiotic therapy with trimethoprim/sulfamethoxazole was started for 4 weeks with rapid improvement of the general condition of the patient, and control urinalysis after treatment were negative.

Discussion

Infections of the urinary tract due to *Salmonella* species are uncommon, and in human beings they are mainly associated with immunosuppressive therapy or urological abnormalities.
Salmonella Thompson is one of the less frequently serovars isolated from humans and animals and there are only few reports about isolation of this serovar in a dogs without any information about the infection site given. To the authors' knowledge, *S.* Thompson has never been isolated from the urine of another animal species and this report represents the first case of bacteriuria in a dog caused by *S.* Thompson. Generally *Salmonella* species can infect the urinary tract due to haematogenous spread or by ascending urethra. Furthermore, contamination of the urine through faecal shedding might appear and to prevent any occurrence of bacterial contamination during the medical procedure, urine collection was performed by ultrasound-guided cystocentesis; therefore, faecal contamination of the sample can be excluded in this case.

P060 - Staphylococcal infection in companion animals: prevalence of methicillin-resistant Staphylococci and usefulness of eMRSA broth in the presumptive screening of Staphylococcus aureus

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Background:
MDR staphylococci is a problem of global proportions that presents serious challenges to the successful treatment of staphylococcal infections of companion animals. Staphylococci may have genes (mecA-mecC and mup) that give them the ability to resist to many drugs. Staphylococcal infection in the dog and cat is mainly caused by Staphylococcus Intermedius Group (SIG) and rarely S.aureus, but companion animals that are in close contact with their owners are at the greatest risk of increased colonization with S. aureus (SA). Pets with access to or sleeping in the bedroom or animals of health-care workers were had increased carriage levels. Aim of the study was to evaluate the prevalence of Methicillin- and Mupirocin-Resistant Staphylococcus, and performance of eMRSA™ broth in the presumptive screening of S. aureus, in a pet population.

Materials/methods:
A total of 216 flocked swabs (FLOQSwab™ COPAN Italia) from 174 dogs, 40 cats and 2 rabbits with staphylococcal infection were taken from different anatomic sites (skin and soft tissue) and seeded in blood agar and Mannitol Salt Agar, incubated overnight and the colonies identified by MALDI-TOF. Samples were analyzed by two commercial (Xpert®MRSA NxG, Xpert®SA Cepheid) and one "home brew" Real Time PCR assays. Samples were also inoculated in eMRSA™ broth and subsequently incubated at 35°C for 24 h. eMRSA™ is an enrichment broth containing a specific dye which changes color from yellow to green when SA is present.

Results:

174/216 samples were identified as SIG, 18/216 *S. felis*, 14/216 SA, *4/216, S. Schleiferi, 2/216 S. capitis, 2/216 S. simulans , 1/216 S. lentus, 1/216 S. epidermidis*, 1/216 *S. sciuri*. Real Time PCR, test Xpert® MRSA NxG and Xpert®SA nasal complete identified 65/216 samples positive for gene *mecA* (51 SIG, 10 SA, 2 *S. felis, 1 S. epidermidis, 1 S. lentus),* no samples were positive for gene *mecC* and *mup.* SA were correctly identified by eMRSA™ broth with clear change to blue-green-color.

Conclusions:

SIG is most frequently isolated in infections followed by *S. felis* and *S. aureus*, we also observed that the 30% of Staphylococcal strain are either MRSP/MRSA. The p*revale*nce of MRSA in dog were 2,87% and 10% in house hold cats, this results show that the pet cats is more likely to be a reservoir of *S. aureus* with genetic determinants of antibiotic resistance.This prevalence of MDR staphylococci causing infection in pets creates important therapeutic limitations in veterinary medicine. eMRSA™ broth allowed to complete the screening of veterinary clinical samples identifying a suspicious infection caused by *S. aureus*. This method, in association with molecular technique able to identify MecA-MecC gene, allow the identification of MRSA/MSSA within 24 h from sampling.

P062 - Evaluation of growth dynamics of Listeria monocytogenes in the RTE fruits

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Introduction: *Listeria monocytogenes (L.m.)* is a ubiquitous Gram-positive foodborne pathogen. *L.m.* causes many diseases, ranging from mild gastroenteritis to severe blood and central nervous system infections. Commonly, processed ready-to-eat (RTE), cold-stored meat and dairy farm are considered high-risk foods for *L.m.* infections. A food product can become contaminated with *L.m.* anywhere along the pathway of food production (harvesting, packing, distribution, serving). Because of the high case fatality rate associated with *L.m.* infections, FDA has established a zero-tolerance for *L.m.* in ready-to-eat foods, including processed fresh-cut fruits and vegetables. The aim of this work was to study the growth and survival dynamics of *L.m.*, artificially introduced in minimally processed, fresh cut fruit and stored at 4°C and in conditions of thermal abuse (8°C). Moreover, we evaluated its growth potential during commercial shelf-life (3-6 days) and up to 25% over.

Materials and Methods: To study the behavior of *L.m.* in RTE, challenge tests were performed, at different times, on 4 types samples (melon, coconut, fruit salad and pineapple) following the procedure reported by AFSSA (Agence Française de Sécurité Sanitaire des Aliments) - EU Community Reference laboratory for *L.m.* Furthermore were assessed the total mesophilic count, the presence of Enterobacteriaceae, Salmonella and E. coli, in order to obtain more knowledge on the hygienic-sanitary situation of the considered products. The pH and aW were also recorded.

Results: Preliminary results showed the growth of *L.m.* at both temperatures 4 and 8°C, but particularly at 8°C. This behavior was highlighted in melon, where extreme growth occurred early. In coconut and pineapple samples, *L.m.* survived during all the tested period at 4°C but did not show substantially increase in growth. A weak replication is recorded only after 6-8 days post inoculum (p.i.). In fruit salad *L.m.* grew slowly at 4°C but showed a steady increase particularly after 4-6 days p.i.. The growth rate of *L.m.* resulted pH-dependent while aW values seems all favorable for its survival.

Discussion and Conclusions: RTE exposed tissue may be colonized more easily by pathogenic bacteria than intact produce. This is due to the higher availability of nutrients on cut surfaces and the greater potential for contamination because of the increased amount of handling.

We found that the inoculated *L.m.* survived and increased only slightly on fresh-cut coconut stored at 4°C but increased early significantly on fresh-cut melon stored at 4 and 8°C. The present study provides useful data for understanding the behavior of *L.m.* and an effective tool for predicting the pathogen trend during the manufacture, distribution, and storage of RTE fruits.

P063 - Environmental and epidemiological investigations of Legionella in prisons and schools in wester Sicily

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Introduction
Legionella is a Gram-negative annoying bacillus found in freshwater, but under the right conditions, the organism is able to proliferate in water systems created by man. From 2015 to 2017 the Regional Reference Laboratory of Clinical and Environmental Surveillance of Legionellosis of Palermo monitored Legionella in prisons and schools of western Sicily. This was a project proposed and funded by the Regional Health Department. In particular, this study evaluates Legionella contamination in water distribution systems of nine prisons located, four in Palermo, three in Trapani, two in Agrigento, and five schools of Palermo. Furthermore, here we present the first Italian and European study regarding the monitoring and molecular typing of *Legionella* spp in water systems of prisons and schools of western Sicily.

Materials and Methods
Our investigation focused on water systems (eg storage tanks, showers, washbasin) of prisons and schools of western Sicily. We collected 94 water samples in prisons and 40 in the schools. This samples were processed according to the procedures described in the Italian Guidelines of 2015. The Legionella strains were serologically identified by latex agglutination tests and the *Legionella pneumophila* serogroup 1 and serogroup 6 strains were molecularly analyzed through SBT *(*Sequence Based Typing), using the standard protocol of the EWGLI (European Working Group for Legionella Infections).

Results
Legionella pneumophila serogroup 6 was the most prevalent serogroup in prisons, and 12 samples were positive for *Legionella pneumophila* serogroup 1. But most of all, in four penitentiary institution we found an high bacterial load (10^4 UFC/L). While in the schools, on five structures investigated only one was positive for *Legionella pneumophila* serogroup 6 and the bacterial load was 10^3 UFC/L. About the molecular investigation were found, among serogroups 1, ST (Sequence Type) 1 and a new ST, that was submitted to the EWGLI SBT-database and has been assigned the ST 2451, while, among serogroups 6 the following ST: ST 93, ST 292, ST 461, ST 728, ST 1317, and ST1362.

Discussion and Conclusions
In this study, we described our results obtained from environmental and epidemiological investigations of *Legionella pneumo*phila isolated from prisons and schools of western Sicily.
The incidence of Legionnaire's disease is rising and the mortality rate remains high, particularly for immunocompromised patients. We recommend a complete and constant monitoring of water systems also in prisons because penitentiary populations contain vulnerable people.

P064 - Performance of three different coagglutination commercial kit used to serogrouping Legionella pneumophila

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Introduction

Legionella is a microorganism responsible of Legionnaires' disease characterised as an acute febrile respiratory illness that often can evolve in fatal pneumonia. This pathological finding is mainly caused by *Legionella pneumophila* serogroup 1. It spreads through water, air-conditioning units and several water systems. The Legionnaires' disease occurs in both epidemic and endemic form and in mild illness or fatal pneumonia. For all these reasons, it's important to diagnose the infection and identify the microorganism so fast. One of the method for the rapid identification and serogrouping of *L. pneumophila* is the coagglutination test. This type of test allows to identify Legionella without any crossmatch with other microorganisms. In this study we report the performance of three different kits, used to identify and serogrouping *L. pneumophila*. In our laboratory experience using of kit showed a cross reactivity with the *Stenotrophomonas maltophilia*.

Materials and methods

One hundred strains of *S. maltophilia* were tested with three commercial coagglutination kit. All the strains were isolated from different samples: urine, blood, sputum and central venous catheter. All colonies were tested by three kit.

After that all the *S.maltophilia* suspensions were heated at 100 °C for 15 minutes to prevent cross coagglutination reactions and they were tested again.

Results

68/100 strains of *S.maltophilia* agglutinated with all reagents of *L. pneumophila* serogroups 1, *L. pneumophila* serogroups 2-14 and *Legionella species.*

After that the heated suspensions were tested again with all antibodies of the different serogroups of Legionella spp and they did not show any agglutination. Probably because the heat prevented coagglutination reactions with *S.maltophilia*, while confirmed Legionella spp agglutination.

Conclusions

The coagglutination method is characterized by its rapidity, simplicity and feasibility. It is a useful and convenient means for the rapid detection and serogrouping of *L. pneumophila*. But it's recommended to use a preliminary heating of all the colonies of doubtful morphology to avoid cross coagglutination reaction.

P065 - Monitoring of opportunistic bacteria in the Animal Assisted Therapy circuit

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Introduction. The beneficial effect of dogs on the human psychological and physical rehabilitation is well known. The Animal Assisted Therapy (AAT), greatly alleviated symptoms in different human psychological disorders. Literature data reported that AAT has a significant benefits for the treatment of elderly affected by Alzheimer disease (AD) and other types of dementias. The frequent co-occurrence of cognitive and mood disorders, make elderly persons especially suitable for treatments based on affective emotional motivation and psychological stimulation. Even if, the criteria for the inclusion or exclusion of dogs in the AAT are clearly defined, as well as the appropriate measures to be taken in order to prevent zoonoses, there are few scientific data focused on zoonotic transmission of different bacteria species that can occur during the AAT. The aim of our study was to evaluate the presence of opportunistic bacteria in the areas used for AAT. In particular, we analyzed the presence of *Staphylococcus spp.*, *Acinetobacter baumannii*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, that could represent opportunistic pathogens in emerging zoonoses.

Materials and Methods. In order to monitor the presence of bacteria in the AAT circuit, several environmental samplings using a sterile swab were carried out in the spaces used for this therapy. To identify the bacterial species of interest, each swab was spread on appropriate enriched, differential or selective culture media. Methicillin Resistant (MR) *Staphylococci* were confirmed by susceptibility test with oxacillin disc. For gram negative isolates, the carbapenemase resistance mechanisms have been also identified. The identification of all bacterial isolates was confirmed by mass spectrometry using the Matrix Assisted Laser Desorption/Ionization (MALDI) mass spectrometer.

Results. We isolated about 334 bacterial species. *Staphylococci* represent about 50% of the total bacteria, of which 18% are MR while 32% are Methicillin Sensitive (MS) strains. Otherwise, the gram negative isolates represent about 9% of all isolated germs.

Discussion and Conclusions: AD is one of the most common neurodegenerative disease and even if its aetiology is unknown, the irreversible neurodegeneration leads to the loss of cholinergic neurons. Literature data reported that anticholinesterase drug therapy has limited efficacy and it is associated to an increased risk of adverse events. To overcome the collateral effects of drug therapy, a non-pharmacological treatment like AAT involving therapy dogs is nowadays commonly used with reported significant benefits. Our study can be a starting point for AAT innovation in order to ensure that participating animals are at low risk of exposing AD patients to zoonotic pathogens.

P066 - Bioinformatic analysis of pre- and post-processing RNA-seq data on COL-R/COL-S Acinetobacter baumannii transcriptomes

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Introduction: Bioinformatics represents a crucial point for the researcher biologist's work. The computational approach can enrich biological knowledge about bacteria including the antibiotic resistance. The Next Generation DNA Sequencing (NGS) has in deep impacted molecular biology research and among NGS strategies, RNA-sequencing (RNA-seq) is becoming an instrument to perform high-throughput transcriptome analysis.

Materials and Methods: The transcriptomes of two isogenic strain pairs of Italian clinical colistin susceptible/resistant (COL-S/R) *Acinetobacter baumannii* (*Ab*), epidemiologically not related, were sequenced by RNA-seq, using Illumina Mi-Seq system, and bioinformatically analyzed. Two replicates were performed using two different library: a Short-Insert library (SI) -an A Single end strand library with reads of 50 bp- and a Tru-Seq Library (TS) -an A Paired end library with reads of 150 bp and average insert size of 350/400 bp-. The raw data were qualitatively control checked by FastQC and processed with Trimmomatic v.0.33.2 having different options to remove the adapters, to trim sequences and to set the trimming parameters. Rockhopper tool was used to annotate the reads on two reference genomes, *Ab* ATCC 17978 and *Ab* ACICU. It quantified the transcripts, normalized read counts for each sample using the upper quartile gene expression level reported as RPKM value (Reads Per Kilobase per Million mapped reads), and calculated differentially expressed genes by computing *q*-values ≤ 0.01 with a false discovery rate of $<1\%$. Finally, computational filtering analysis were performed to select only the RNAs present in both COL-R strains with the same expression trend (over/under-expression) to determine their signatures.

Results: During the trimming we set the command lines. The number of surviving reads was different related to the set of specific parameters. Looking at the coding RNAs, our data showed 5 over-expressed mRNAs in COL-R *vs* COL-S annotated on *Ab* ATCC 17978, 3 derived from the SI library (A1S_0938, A1S_2651, A1S_2752) and 2 from the TS (A1S_2027, A1S_2230), and 4 over-expressed mRNAs mapped on *Ab* ACICU, 2 derived from the SI library (ACICU_02907, ACICU_03004) and 2 from the TS (ACICU_01518, ACICU_02436). Comparing the obtained results, only 2 homologous pairs of mRNAs were detected in the both annotations (A1S_2752/ACICU_03004 and A1S_2230/ACICU_02436).

Discussion: Our analysis evidenced that several methodological factors, e.g. number of replicates, different type of NGS libraries, the setup of trimming and the choice of the reference genome led to different results. Hence, a consistent biological and bioinformatic background is required to elaborate and rightly interpret the huge amount of big data resulting from RNA-seq.

P067 - Introduction of NGS in environmental surveillance for healthcare-associated infections control

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Introduction: Healthcare-associated infections (HAIs) and antibiotic resistance are the most frequent and serious cause of complications related to health care. The monitoring of hospital environmental surfaces takes place with cultural isolation, with some performance limitations. Hence the urgency of implementing environmental surveillance systems using new generation methods. In this pilot study, next generation sequencing (NGS) technologies were evaluated for hospital environmental surfaces microbiome characterization, compared with traditional and molecular PCR methods.

Materials and Methods: Three randomized rooms were selected for two consecutive environmental sampling including floor, footboard and sink for a total of 36 critical points. NGS and comparison culture isolation and a custom molecular qPCR panel kit were performed. Resistome, using a panel of 84 resistance genes, was additionally evaluated.

Results: *Pseudomonas*, *Acinetobacter*, *Streptococcus*, *Staphylococcus*, *Corynebacterium*, and *Propionibacterium* represented the most frequently bacterial area profile. Of them only *Acinetobacter*, *Staphylococcus*, and *Pseudomonas* were isolated with cultural methods, *Acinetobacter:* 33/36 NGS *vs* 4/36; *Staphylococcus:*36/36 NGS *vs* 36/36, *Pseudomonas*: 34/36 NGS *vs* 1/36. Comparison with custom PCR analysis showed a rate of identification of *Acinetobacter:*10/36, *Staphylococcus:*36/36, and *Pseudomonas:* 29/36. Other microorganisms were detected from 1 to 29% for a total of 8 phyla and 58 different genera, showing the sink as the most representative site. To note, *Streptococcus*, detected by NGS in many samples, was not sought with traditional methods. Basically resistome identified mainly genes conferring resistance to macrolides, aminoglycosides, fluoroquinolones and beta-lactams.

Discussion and Conclusions: Data from this study proved as NGS can be an effective method in monitoring hospital indoor microbial communities. NGS data together with resistome provide a deeper understanding of the development of HAIs leading to create new sanitation protocols.

P068 - Complete genome sequence of Streptoccocus mitis integrating nanopore and Illumina data

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Introduction:
De-novo assembly is the computational process in which sequence reads are joined to reconstruct the genome sequence. Complete genome of two strains of *Streptoccus mitis*, S022-V3-A4 and S022-V7-A3, was sequenced integrating Illumina and nanopore technologies. Illumina generates short reads with an high quality score, while nanopore generates long but less accurate reads.

Materials and Methods:
High Molecular Weight DNA was obtained using a chemical lysis DNA extraction protocol. DNA was ligated to barcodes and sequencing adapters and subjected to nanopore sequencing. DNA was also sequenced with Illumina Hiseq generating 100 bp x 2 reads. Albacore basecaller was used to filter low quality nanopore reads and to produce fastq files. Illumina and nanopore fastq reads were assembled using Unicycler (v. 0.4.0), which integrates different bioinformatic tools to build a scaffold with nanopore long reads and then corrects errors with Illumina reads. Assembled genomes were automatically annotated with Prokka (v. 1.13). The capsular locus was manually annotated. Comparative genome analysis was performed using bedtools (v. 2.27).

Results:
For strain S022-V3-A4, nanopore sequencing generated 38.428 reads with a mean length of 3.713 bp, for a total of 142.789.394 bp, while 126.060 reads with a total length of 263.360.813 bp (mean 1.639) were generated for S022-V7-A3. Illumina sequencing produced about 10 million reads for each strain. Illumina and nanopore reads were assembled with Unicycler. The chromosomes were 2.087.048 bp in length with 39.75 % GC content and 2.033.398 bp with 40.24% GC content for strains S022-V3-A4 and S022-V7-A3, respectively. Comparative analysis showed that 1.515.275 bp (about 75 % of the genome) were highly homologous between the two strains. 25 % of the genome was unique and possibly belongs to the mobilome. Analysis of the capsule locus (*cps*), located between the conserved *dexB* and *aliA* genes, showed that it was 22.935 bp in S022-V3-A4 and 20.715 bp in S022-V7-A3. The 14 predicted *cps* gene products of the two strains are >97% identical except for the Wzx flippase (78% identical) and Wzy polymerase (31% identical). S022-V3-A4 presents an additional gene, *aliC*, at the 5' end of the *cps* locus. These *cps* loci are not present in sequence databases. However 12 out of 14 gene products are homologous (25-87%) to predicted proteins of the type IV *cps* locus of *S. mitis* strain SK637.

Discussion and Conclusions:
Despite an high coverage Illumina sequencing could not assemble a closed genome. Nanopore sequencing generated a large amount of sequence data in 3 hours of sequencing. The combination of Illumina and nanopore sequencing allows the rapid and accurate assembly of complete bacterial genomes.

P070 - Fighting antibiotic resistance, it's in your hands: Mobile phones a fertile ground for microorganism's growth

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Introduction
Nowadays approximately 66.5% of the world population uses mobile phones. In Italy this rate is higher than 67.9%, and the clinical environment it's not an exception. Mobile phones have been identified as a fertile ground for bacterial growth and even though smartphones have outnumbered keypad phones, decreasing the chance for bacterial survival, they still are a major source of contamination. Being in close contact with the body repeatedly throughout the day, smartphones act as reservoirs of infection and vehicles for spreading contaminating bacteria across many different clinical and non-clinical environments. Nosocomial infections are an important problem in all modern hospitals, indeed some epidemiological studies regarding environmental surfaces and the transmission of bacteria demonstrated that pathogenic microorganisms could be transferred to the patients by the contaminated hands of healthcare workers (HCW). Smartphones are used routinely all day long but not cleaned properly, as HCW may do not wash their hands as often as they should. For this reason, this study was performed during a campaign for hands cleaning awareness, and is aimed at evaluating the contamination of smartphones for the estimation of potential risks of diffusion of antibiotics resistant pathogens between doctors, patients and healthcare professionals.

Materials and method
During the campaign day for hands cleaning awareness, 107 swabs were collected from the surface of smartphones belonging to volunteer HCW of Integrated University Hospital of Verona. Most of the participants were clinicians: Orthopaedic, Infectious diseases, Hygiene specialist, Microbiologist, Surgeons. All the samples were cultured and colonies identified using mass spectrometry technology (MALDI-TOF MS, Biomerieux®). After sampling, each smartphone was decontaminated with alcohol-based disinfectant wipes (Neo Sterixidina, Golmar, Italy).

Results
All 107 smartphones tested (100%) harboured culturable bacteria in the range of 1-20 CFU/cm^2, with a mean bacterial count of 4.5 CFU/ cm^2 (median 1 CFU/ $cm^{2)}$ 36 smartphones had only one genera, 68 had between two to four genera and only in 3 mobile phones grew more than four bacterial species. Twenty-two different bacterial species were isolated. Coagulase negative Staphylococci and Bacilli were the prevalent bacteria founded (77 isolates, 34%), other Gram Positive bacteria where 36 (16%), Gram Negative Pathogenic Bacteria 15 (7%), *Staphylococcus aureus* 13 (7%) and other Gram Negative Bacteria 4 (2%). The most clinical relevant species were: 1 *Klebsiella pneumoniae* (3%), 2 *Escherichia coli* (6%), 4 *Pseudomonas* (11%), 5 *Enterococcus* (14%), 8 *Acinetobacter* (23%) and 13 *Staphylococcus aureus* all of them susceptible to methicillin (MSSA) (43%)

Discussion and Conclusions
The use of Mobile phones in hospital settings it's a source of transmission of a variety of bacterial agents including multidrug-resistant pathogens as *S. aureus* resistant to methicillin (MRSA). The surface spread method is an easy and useful tool for detection and estimation of bacterial contamination of mobile phones. Previous studies demonstrated that mobile phones in the clinical environment provide a reservoir for bacteria known to cause nosocomial infections. We carried out

this study to evaluate the level of contamination on touch screen mobile phones within our hospital, to emphasize that hospital-acquired infections can be associated with lacking hands hygiene of medical practitioners. With a contamination rate of 100% identified on the screen of mobile phones sampled, we confirmed that mobile devices are a potential reservoir for bacteria. However, no drug resistant bacterial strains were isolated, even though the percentage of pathogenic bacteria was high (16%). Bacteria harbored on mobile phones, as showed in our study, are the same of hand commensal bacteria. Our study strengthens the need of a rigorous adherence to hand hygiene guide lines as the most effective mean of reducing potential spread of pathogenic bacteria in hospital setting and highlight the need for a more comprehensive approach to reduce nosocomial infections, which in addition to promoting hand hygiene also focus on cleanliness of mobile phones. In our knowledge no available napkins are produced specifically to clean mobile phones, and only a few clinicians clean daily their mobile phones. Further research is needed in order to provide evidence of the mobile phone contamination during the ward clinical practices and multidrug resistant bacteria prevalence.

P071 - Risk assessment of water used in healthcare facilities

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Introduction

The incidence of hospital infections by opportunistic microorganisms has increased considerably in recent decades. Among the various sources of pathogens, water is probably the most undervalued. It may contain low concentrations of microorganisms that can cause infections to more vulnerable individuals through different routes of exposure in healthcare facilities.

Materials and Methods

Monthly sampling (January-June 2018) consisted in the collection of 1 liter of cold water and 1 liter of hot water. Water samples were collected at pre-selected hotspots in three hospitals (i.e. 6-9 water samples per site). In addition to the determination of microbiological water quality parameters, the samples were analyzed for OPPPs (Opportunistic Premise Plumbing Pathogens).

Results

The environmental survey showed that the water present in these healthcare facilities complied with the quality requirements set by the current legislation. However, the presence of some bacteria, which exposure could lead to infections in particularly vulnerable individuals, was found. The identified species were: *Pseudomonas aeruginosa*, *Burkholderia cepacia*, *Acinetobacter junii*, *Stenotrophomonas maltophilia*, filamentous mycetes such as *Aspergillus brasiliensis*, *Aspergillus flavus*, *Fusarium oxysporum*, *Fusarium solani*, and lievitiformes mycetes such as *Rhodotorula mucillaginosa* and *Cryptococcus albidus*. The presence of *Legionella* serogroup 2-14, at levels > 1000 CFU, was found only in one hospital.

Discussion and Conclusions

The problem of waterborne nosocomial infections is still underestimated. This preliminary study demonstrated the presence in water of pathogens that are not targeted by current regulations. However, due to the small number of samples and the lack of epidemiology studies of infection cases of water origin, at this point it was not possible to establish a relationship between the presence of OPPPs and infection cases. This objective will be pursued in a further prospective study. Our work aims to put the spotlight on the importance of extending environmental research to opportunistic pathogens for a better assessment of the microbiological quality of water. Additionally, these data should be combined with the intensive epidemiological surveillance of infection cases caused by waterborne pathogens. This will enable to establish effective corrective measures to limit the spread of pathogens and minimize the risk of water-related infections in healthcare facilities.

POSTER VIROLOGIA

P072 - An in vitro model to study the sensitivity of JC Polyomavirus to type I IFN in kidney COS-7 cell line

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Introduction. John Cunningham virus (JCV), the etiological agent of the Progressive Multifocal Leukoencephalopathy, establishes a persistent infection in the kidneys but the mechanism by which the immune system is involved in JCV persistence is not well understood. Since interferons (IFNs) are the primary antiviral cytokines that limit the spread of infection and modulate the innate immune response, the aim of our study was to investigate the sensitivity of JCV to IFNs considering possible variations in viral replication. The data were compared with the trend of JCV replication in COS-7 cells not treated with type I IFN and with the trend of encephalomyocarditis (EMC) virus replication in the same cell line.

Materials and methods. COS-7 cells not treated with type I IFN, were directly infected with JCV virions corresponding to 1.5×10^5 genome equivalents per milliliter (gEq/ml). In parallel, other COS-7 cells were first stimulated with 100-1000-10000 U/ml of natural IFN α (10^5 U/mL) and Avonex IFN β (10^5 U/mL) and, after 24 hours, infected with the same supernatant. Extracted DNA and relative supernatants, harvested after 3, 7, 14 and 21 days post-infection (d.p.i.), were analyzed by Q-PCR. To validate the sensitivity of the same preparations of IFNs on EMC replication, experiments were performed on the same cells and evaluated by electron microscopy.

Results. COS-7 cells infected by JCV and not treated with type I IFN revealed a progressive increase of viral replication. At 3 d.p.i. a JCV viral load average of $5.83x10^4$ gEq/cell DNA content was detected, reaching the maximum value of $7.81x10^6$ gEq/cell DNA content at 21 d.p.i. In parallel, JCV replication, evaluated in the supernatants, showed the same increased trend observed in COS-7 cells. On the other hand, at 3 d.p.i type I IFN induced a low reduction of the JCV viral load in COS-7 cells stimulated with 100 U/mL of IFN α (1.05×10^4 gEq/cell DNA content) and IFN β (7.00×10^3 gEq/cell DNA content) with 1000 U/mL of IFN α (8.2×10^3 gEq/cell DNA content) and IFN β (3.01×10^3 gEq/cell DNA content) and with 10000 U/mL of IFN α (6.74×10^3 gEq/cell DNA content) and IFN β (2.62×10^3 gEq/cell DNA content), followed by a progressive increase of JCV viral load until 21 d.p.i. As expected, we didn't observe any cytopathic effect on COS-7 cells stimulated with the same concentration of type I IFN and infected with EMC virus.

Discussion and conclusions. Our study could synthesize a methodological approach useful tool to investigate the role of innate immune system on JCV replication. A low response of JCV to type I IFN, observed in an *in vitro* model, could contribute to the establishment of persistent infection in COS-7 cells that representing a human common site of viral persistence.

P074 - Potent and selective inhibition of Thymelaea Hirsute extracts against human immunodeficiency virus (HIV)-1

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Introduction: Historically, natural products have been the most successful source of inspiration for development of new drugs. Members of the Thymelaeaceae family have been of interest owing to their excellent medicinal value. Different classes of natural products have been isolated from these species, including flavonoids, coumarins, and diterpenoids. Given the successful history of natural product-based drug discovery, extracts from the aerial parts of Thymelaea Hirsuta, were evaluated for their anti-HIV activity.

Materials and Methods: Ethyl acetate extracts from leaves (71B) and branches (72B) of Thymelaea Hirsuta were tested in cell-based assays for their cytotoxicity and anti-HIV-1 wt, resistant strains and HIV-1 BaL properties. The mode of action of 72B extract, chosen as lead, was characterized evaluating potential virucidal activity, its ability to inhibit HIV-1 reverse transcriptase (RT), protease activity and syncytia formation.

Furthermore, safety profile of the extract was determined on viability of Lactobacillus sp. and transepithelial resistance (TER).

Results: Both leaves and branches extracts showed potent and selective activity against HIV-1 wt (EC_{50} = 0.8 microgram/ml) at not cytotoxic concentrations (CC_{50} = >100 microgram/ml). They proved to be active against HIV-1 variants carrying clinically relevant NNRTI and NRTI mutations at low concentration (0.3-4 microgram/ml range) and against the M-tropic strain HIV-1 BaL. 72B extract prevented syncytia formation at low concentration (0.4 microgram/ml). 72B did not affect the Caco-2 epithelial cells monolayer integrity. The extract tested up to 100 microgram/ml did not reduce the viability of Lactobacilli.

Discussion and Conclusions: In our studies, ethyl acetate extracts from Thymelaea Hirsute turned out interestingly active against HIV-1 with either one of the two main T- and M-tropic viruses that use as co-receptor for cell entry CXCR4 or CCR5, respectively. Extract 72B was not virucidal, since exposure of HIV to high concentration did not affect virus infectivity. Furthermore, extract turned to be potently active against resistant strains and it was not able to inhibit the RT and Protease enzymatic functions. The potent inhibitory effect on the syncytia formation in co-cultures showed that 72B inhibits an early event in the replication cycle of HIV. Pre-clinical safety profile of this extract showed no adverse effect on the growth of Lactobacilli, and non-toxic concentration of the extract did not influence the TER. These observations are encouraging and further safety and efficacy *in vitro* and *in vivo* studies will be performed in order to identify the chemical constituents of these extracts and to better define the mechanism of action.

P075 - Temporin L derived peptides: new potential anti-HIV agents

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Introduction. Temporins are small (8-17 amino acid residues), C-terminally alpha-amidated peptides with a weak cationic charge that are widely distributed in frogs belonging to the Ranidae family. They are among the largest families of antimicrobial peptides (AMPs) with more than 100 different isoforms. Most of these peptides adopt an amphipathic helical-like conformation in a hydrophobic environment. The first isolated and well characterized are the isoforms A (TA), B (TB) and L (TL). They are principally active against Gram-positive bacteria, and only weakly active against Gram-negative bacterial strains, with the exception of temporin L. TL is the strongest antimicrobial peptide, but it is toxic on human erythrocytes and this fact makes the design of synthetic analogues with a higher therapeutic index vital. Several interesting TL derived peptides have been identified, called TL34, TL48 and TL49, which preserved yet the antimicrobial activity albeit with reduced cytolytic effects *in vitro*. Therefore, we evaluated their potential as anti-Human Immunodeficiency Virus type 1 (HIV-1).

Materials and Methods. We determined the cytotoxicity of TL34, TL48 and TL49 on TZM-bl and peripheral blood mononuclear cells (PBMCs), cell lines suitable for HIV-1 infection, through 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) assay. Then, we evaluated TL derived peptides' activity to inhibit HIV-1 infection in pre-treatment and post-treatment assays on the same cellular models. Finally, to determine the infectivity, the enzyme-linked immunosorbent assay (ELISA) HIV $p24^{gag}$ was used.

Results. In order to determine a not cytotoxic concentration useful to the antiviral tests, a MTT assay was performed. TZM-bl cells resulted more sensitive than PBMCs to the treatment with the three peptides after 48 hours. The peptides showed a relevant antiviral effect against HIV-1. In detail, TL49 was able to reduce more than half of the viral infectivity at 5 micromolar in post-tratment assay on PBMCs. On the other hand, TL48 could inhibit HIV entry at 10 micromolar in pre-treatment assay on TZM-bl.

Discussion and Conclusions. Recently, we found that some TL analogous peptides had a high anti-herpetic activity, both against HSV-1 and HSV-2. It is well known that HSV-2 is associated with incident HIV infection facilitating its transmission. Therefore we analysed the anti-HIV potential of TL34, TL48 and TL49. The results seem to be an attractive topic for the future research with the aim of discovering a drug with a dual antiviral potential.

P077 - The amphibian antimicrobial peptide temporin L inhibits in vitro herpes simplex virus type 1 infection, a continuous story

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Introduction: Antimicrobial drugs is lacking of innovative therapies leading the possibility to develop resistance mechanism in the major part of microorganism. In this scenario the World Health Organization (WHO) defines as priority the discovery of new drugs able to fight the superbugs that are emerging. For instance the Herpes Simplex Virus type 1 (HSV-1) strain resistant to acyclovir and its modifications are nowadays a reality. In this scenario the characterization and the develop of innovative antimicrobial therapies represent a mission for the researcher. Among them an important class of source as new antimicrobial agent is represented by the peptides (AMP). These peptides share the amphipathic nature, the positive charges and the presence of hydrophobic amino acid residues. Concerning the fact that several peptides among this class were full characterized for their antibacterial activities, we investigate the potential of a peculiar AMP of Temporin group: Temporin L (TL). These peptides are an huge class of AMPs that count around 100 members with a defined range of size.

Materials and Methods: TL34 has been modified at glycine residue in position 10. Analogs are synthesized using solid-phase Fmoc chemistry method, followed by purification by reversed phase HPLC. The cytotoxic activity was determined via MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. The antiviral activity was evaluated against HSV-1 through co-treatment, cell pre-treatment, virus pre-treatment and post-treatment assays in a range of concentrations between 100 and 1 microM.

Results: Temporin L is, until today, the only temporin that showed antibacterial activity against Gram negative and has, unfortunately, also a high hemolytic property. For this reason several modification in the primary structure of TL were achieved in order to increase the antibacterial activity with the counterpart in toxicity reduction. Here we reported the Temporin L derivate ([Pro3, DLeu9] TL1) and its modified (indicated as 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12) evaluation as inhibitor of HSV-1 infection in in-vitro model. Then we evaluated the membrane fusion properties defining the phase of infection interferences of these peptides and prelude to the mechanism of action.

Discussion and Conclusion: Innovative treatment for HSV infection is nowadays mandatory. This emerging problem is due to the develop of HSV-1 acyclovir resistant strains. In this scenario the possibility to treat the HSV-1 infection via peptide therapy is a new opportunity that we have to pursue with tenacity in order to translate this study in a product.

P078 - Multivalent-Zanamivir analogs as potent Influenza Virus Inhibitors

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Introduction: Worldwide, influenza causes 3–5 million cases of severe illness, and 250–500 thousand deaths annually. Vaccination is the primary strategy for influenza prevention, however, there are a number of situations for which vaccination is not adequate and effective antiviral therapeutics would be of utmost importance. Vaccine production by current methods cannot be carried out with the speed required to stop the progress of a newly emerging strain of influenza virus. Consequently, development of new antiviral agents constitutes an important part of the approach and solution to epidemic and pandemic influenza. While several classes of anti-influenza compounds exist, the neuraminidase inhibitor class of compounds is currently the only option in most clinical settings, because of the high level of resistance to the adamantine class of antivirals. Emergence of resistance to currently approved antivirals is of great concern, leaving no antiviral drugs to be used in humans. A promising strategy to address this lack of new antiviral agents involves conjugating numerous copies of an inhibitor, such as Zanamivir (ZA), to a sequence-defined oligomeric chain. Recent structural studies of the influenza virus have shown that about 50–100 copies of tetrameric Neuraminidase (NA) are located on the surface of the virus particle and additional studies have shown that polyvalent ZAs can be active against influenza. In this work, we have designed multivalent inhibitors such as di-, tri-, tetra-, and poly-valent ZA analogs to interact with more than one of the NA's subunits in order to improve the antiviral activity.

Methods: Here we report the comparative study of multivalent ZA inhibitors on the infectivity of influenza virus A/Wuhan/359/95 (H3N2), examined using the plaque reduction assay.

Results: A multivalent ZA drug exhibited up to 27 fold improvement in anti-influenza potency compared with the commercial drug Zanamivir against human viral strain and in particular Tetravalent-ZA resulted able to reduce viral titer respect to monovalent ZA more than 120 times.

Discussion and Conclusion: The increased activity could be attributed to their ability to induce virus aggregation on the host cell surface rather than the cause of an increase in the intrinsic binding affinity. All these findings provide a basis for exploring new multivalent inhibitors as more efficacious therapeutics.

P079 - Antiviral thiazolides inhibit paramyxovirus replication by targeting the Fusion protein folding: role of glycoprotein-specific disulfide isomerase ERp57

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Introduction: *Paramyxoviridae*, a large family of enveloped viruses including important human pathogens as measles, mumps, respiratory syncytial virus (RSV), parainfluenza viruses, metapneumoviruses and henipaviruses, evolved a sophisticated membrane-fusion machine consisting of a receptor-binding protein (HN/H/G) and the F-protein, a member of class-I viral fusion glycoproteins playing a critical role in virus infectivity. There is no effective antiviral chemotherapy for most paramyxoviruses. The thiazolide nitazoxanide (NTZ) has been shown to possess antiviral activity against several RNA viruses. Herein we investigated the effect of NTZ and second-generation thiazolides (SGT) on paramyxovirus replication and explored the mechanism of the antiviral action.

Materials and methods: The prototype Sendai virus (SeV) was used as model. Monkey kidney AGMK cells and human A549 alveolar type II-like epithelial cells were infected with SeV under single-step and multi-step growth conditions. NTZ, tizoxanide and different SGT were dissolved in DMSO and diluted in culture medium before treatment. Virus yield was determined by hemagglutinin titration and infectivity assay, and cell viability was determined by MTT assay. Viral proteins were characterized by SDS/PAGE-autoradiography after [^{35}S]methionine/cysteine-labeling, Endo H-digestion, Western-blot, immunofluorescence analysis and Proximity Ligation assay. ERp57 disulfide reductase activity was determined by insulin reduction turbidometric assay and by dieosin glutathione disulfide reductase assay. For RSV-F transfection experiments, A549 cells were transiently transfected with the RSV-F/ORF/C-Flag construct or pcDNA3 empty vector as control. All transfections and siRNA interference experiments were performed using jetPRIME Transfection Reagent.

Results: Nitazoxanide and its circulating-metabolite tizoxanide act at post-entry level by provoking SeV and RSV F-protein aggregate formation, halting F-trafficking to the host plasma membrane. F-protein folding depends on the glycoprotein-specific thiol-oxidoreductase ERp57 for correct disulfide-bond architecture. We found that thiazolides behave as ERp57 non-competitive inhibitors (K_I = 1.5 µg/ml for tizoxanide). ERp57-silencing mimicked thiazolide-induced F-protein alterations.

Discussion and conclusions: Nitazoxanide is used in the clinic as a safe and effective antiprotozoal/antimicrobial drug; its antiviral activity was shown in patients infected with hepatitis-C virus, rotavirus and influenza viruses. The results suggest that nitazoxanide may be effective also against paramyxovirus infection and reveal a host-targeting antiviral strategy against paramyxoviruses by drug-directed misfolding of the fusion protein via ERp57 inhibition.

P080 - An innovative application of MALDI-TOF MS in clinical virology

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Introduction. Virus detection and/or identification is traditionally performed using cell culture, electron microscopy and antigen or nucleic acid detection. In this study, Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS), commonly used in clinical microbiology, was developed and tested as an innovative tool to be applied to virus identification by using two different approaches.

Materials and Methods. In the first approach, human polioviruses were selected as a model to evaluate the ability of MALDI-TOF MS to identify specific viral protein to be used as biomarkers of purified virus particles, followed by the serotypes identification. To this aim the Sabin reference strains (I, II, III) were firstly analysed and, subsequently, the results were then confirmed by a blind application of the assay to clinically isolated strains.
In the second approach, a protein profiles library was newly create to discriminate between uninfected and respiratory virus infected cell cultures after a viral proteins enrichment method. The library was built using different reference strains after an extensive modification of the MALDI-TOF MS pre-processing, MSP creation, subtyping MSP creation and identification default parameters setting.

Results. The very efficient technique adopted to obtain highly purified poliovirus allowed us to discriminate viral protein peaks from uninfected cells peaks and to detect specific poliovirus protein biomarkers. Moreover, MALDI-TOF MS analysis applied to the three Sabin poliovirus serotypes revealed characteristic peak profiles for each of them showing three independent clusters for the three serotypes. After a proper statistical investigation, the VP4 was used as a potential biomarker to identify poliovirus strains at the serotype level. On the bases of VP4 all clinical isolates were identified at the serotype level.
In the second approach, the spectra generated from virus infected cell cultures revealed the presence of some different peaks not overlap- ping those of uninfected cell cultures for all the reference virus infected cell cultures. The parameters for the creation of the Main Spectrum Profile (MSP) for each of the reference virus infected cell cultures were set on the basis of these peaks. The obtained MSP spectra were used to create a new respiratory viruses library in our Bruker Daltonics database in order to blind identify viruses isolated from biological samples after a cell culture step. The spectra obtained by 58 additional cultured strains correctly match with the new database demonstrating its reliability.

Discussion and Conclusions. In conclusion, this study could be considered a starting point for further evolutions of the developed system, since the differences observed comparing the spectra obtained from virus infected cell culture suggest the possibility to apply these approaches to the identification of other viruses including other picornavirus such as enterovirus and coxsackievirus and viruses responsible for respiratory infections, as well as to viral agents causing infections of other body sites.

P081 - Overproduction of IL-6 and Type I IFN in a Lethal Case of Chikungunya Virus Infection in an Elderly Man during the 2017 Italian Outbreak

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Introduction: Chikungunya fever is a mosquito-borne viral disease caused by Chikungunya virus (CHIKV) and is generally considered self-limiting non-fatal disease. However, severe clinical presentations with high mortality rate (48%) are reported associated with the presence of several underlying medical conditions. Recently, a CHIKV epidemic occurred in Italy, involving 270 confirmed cases in Lazio and Calabria regions. Here, we report the virus characterization and an abnormal pattern of circulating cytokines in a lethal case of CHIKV during the 2017 Lazio region outbreak.

Materials and Methods: In September 2017, a 77-year-old male with underlying cardiac diseases was admitted for acute neurological syndrome to the Santa Maria Goretti Hospital in Latina, Rome, and he died after 9 hour admission in sub-intensive unit care for acute cardiac arrest. Due to the ongoing CHIKV outbreak, RT-PCR and indirect immunofluorescence assays were performed to assess a possible infection. The virus was isolated on BHK-21 cells, and the near-complete genome sequencing was performed. Circulating IFNs and inflammatory cytokines were evaluated and quantified using ELISA, and the levels compared to those detected during the very early stages of the infection in four non-fatal cases belonging to the same outbreak.

Results: Laboratory tests showed CHIKV infection, based on positive RT-PCR test (Ct: 12), in the absence of CHIKV-specific antibodies. The sequence of the isolate (CHIKV/ITA/Lazio-INMI2-2017) showed only one nucleotide difference (synonymous substitution) as compared to the outbreak prototype strain (CHIKV/ITA/Lazio-INMI1-2017), and clustered with recent isolates of the Indian Ocean sublineage of ECSA in the maximum-likelihood phylogenetic tree. The evaluation of the inflammatory response showed that IFN-alfa, IFN-beta and IL-6 levels were extremely high as compared to non-fatal cases, while IFN-gamma and TNF-alfa levels were in the same range. The elevated levels of IFN-alfa, IFN-beta and IL-6 seemed not directly correlated to elevated levels of CHIKV-RNA.

Discussion and Conclusions: The analysis of inflammatory cytokines revealed a remarkable and strong increase of circulating type I IFN, as well as of the IL-6 pro-inflammatory cytokine, suggesting a possible role of type I IFN in cytokine storm, which may be correlated with unfavourable prognosis of CHIKV infection. Further studies are necessary to investigate the relationship between clinical severity and host factors like elderly, comorbidities, inflammatory and immune responses in CHIKV infection.

P083 - Diagnostic usefulness of the bone marrow cytomegalovirus viral load in management of graft failure in allogeneic stem cell transplant

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Introduction. Regarding the viral reactivation cytomegalovirus (CMV) infection is still a significant issue in haematopoietic stem cell transplant (HSCT) patients. In fact, myeloablative conditioning regimen preceding HCST opens a "window of opportunity" for latent CMV. Depending on the precise conditions of HCST, CMV infectes bone marrow (BM) stroma, reduces homing of transplanted hematopoietic stem and progenitor cells, impaires cell lineage differentiation that can lead to "graft failure" (GF). In consequence, uncontrolled virus spread causes morbidity and mortality. We reported rare clinical cases of two HSCT-patients who showed GF secondary to very early reactivation BM-CMV. To our knowledge, we are the first to show an association between early BM-CMV reactivation and GF in allogenic-HSCT-patients.

Materials and Methods. Patients (1 female and 1 male) suffered from myeloblatic leukemia received HLA sex-matched related donor peripheral stem cells but ABO mismatched group. Donors and recipients were CMV seropositive (D+/R+). CMV viral load was detected and quantified by qPCR (ELItechGroup. Kit) in serial BM and peripheral blood (PB).

Results. At 15 days post-transplant, diagnosis of GF was made when the recipients had an absolute neutrophil count $<0.5\times10^9$/L and 0% of donor haematological chimerism. Patients with GF showed uncommon early CMV reactivation only in BM samples and not in PB ones. Viral load detected in BM was > 23.000 copies/ml in female patient and > 11.000 copies/ml in male patient. He showed PB-CMV viral load positivity >10.000 copies/ml one week after BM-CMV reactivation. Both patients with GF had poor prognosis with high risk of mortality. Immediately after CMV infection diagnosis, therapy with ganciclovir (5 mg/kg, intravenous, twice daily) was started together with granulocyte-colony growth factor administration. The clearance of DNA-CMV viral load coincided with the engraftment of whole trileneage blood components of donor origin. Both patients improved clinical status showing increase of donor chimerism and AB0 group blood switch. Actually, the patients are surviving.

Discussion and Conclusion. The aim of these case reports is to discuss diagnostic workup of CMV reactivation in critically ill immunodeficient patients. We highlight the importance of best follow-up for high risk patients such as HSCT. In our opinion, BM is the better informative sample than PB . Workup should include viral BM detection at +15 days post-HSCT at the end to prevent transplant failure or delayed engrafment.

P084 - Commercial anti-HCV assays comparison and GBV-C cross-reactivity in HCV low positive samples

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Introduction:
Hepatitis C virus (HCV) serological screening represents the main tool to prevent viral transmission. Several full automated test are available , with high specificity and sensitivity, however a significant number of results give borderline signal–to-cut-off (s/co) ratio, with a uncertain interpretation. The aim of this work was to compare the performance in three different assays in HCV samples resulted "low positive" with HCV test screening Architect (Abbott), furthermore we assessed the possible GBV-C cross-reactivity.

Material and Methods:
Fifty-nine consecutive samples showing a low positivity (1≤S/CO≤4) with currently used screening test Architect® (HCV-Ab_{low})(Abbott) were analyzed. They were tested with standard confirmatory immunoblot assay (Deciscan®HCVPlus,BioRAD) and with i) LIAISON® XL Murex HCV-Ab (Diasorin) and ii) VIDAS® HCV-Ab (Biomerieux). The prevalence of GBV-C RNA was analysed by home-made RT-PCR in the sera from HCV-Ab_{low}, in 55 HCV-$_{neg}$ and 66 HCV-$_{pos}$ subjects.

Results
A significant correlation was observed between Architect® and LIAISON® XL (r^2=0.36, p<0.0001) and Architect® vs. VIDAS® (r^2=0.16, p=0.0004). Notably, the median of S/CO values obtained by LIAISON®XL and by VIDAS® was higher in samples with a positive Deciscan® than in those with a negative Deciscan® test (p<0.05). Among HCV-Ab_{low}, negative samples frequency observed with LIAISON® XL (44.1%), VIDAS® (42.4%) was similar to that reported using Deciscan® (42.4%). The frequency of samples showing positive results with LIAISON® XL (55.9%) and VIDAS®(57.6%) was similar to that showing positive/indeterminate result with Deciscan® (57.6%). Whether we consider Deciscan® as the gold standard, the specificity of LIAISON®XL and VIDAS® were 56% and 64%, the sensitivity were 80% and 100% respectively. GBV-RNA positivity observed in HCVAb_{low} was 8.4%, 7.2% in HCVab_{neg} and 13.6% among HCV Ab_{pos} samples. Since no significant differences were observed in GBV-C RNA positivity between HCV-Ab_{neg} and HCV-Ab_{low} population, we could exclude that GBV coinfection could be responsible of HCV Ab_{low} positive results.
Interestingly, all GBV-RNA positive samples HCV-Ab_{low} resulted Ab negative in Liason and Vidas assay.
Phylogenetic analysis data revealed that fifteen GBV-positive patients were infected with genotype 2, one patient showed 93.29% similarity with genotype 1 from Uganda, and one patient, anti-HCV negative and GBV-RNA positive, had 93.30% identity with a genotype 3 strain from China.

Discussion and Conclusion
A comparable diagnostic performance of LIAISON® XL, VIDAS® and Deciscan® in identifying non-confirmed HCV-Ab_{low}. The frequency of GBV-C RNA was similar in HCV Ab_{low} patients and anti-HCV negative subjects suggesting no cross-reactivity between these viruses in antibody anti-HCV test.

P085 - Antibodies Avidity Index calculation allows for the detection of recent HIV infections in Palermo

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Introduction: Detecting recent HIV infections allows to individualize a rapid initiation of treatment, but also to evaluate HIV incidence and changes in factors that influence the acquisition of the infection. Among the laboratory methods proposed to discriminate recent HIV infections, the avidity index (AI) of anti-HIV antibodies has emerged as one of the most accurate, being able to reliably discriminate HIV infections established within 6 months. Purpose of the study was to evaluate the usefulness of an AI test based on third-generation EIA to identify recent HIV infections, applying a restriction algorithm recently developed in order to identify and exclude from the test long-standing/established infection.

Materials and methods: We conducted a longitudinal retrospective study on 82 serum samples with HIV-positive or -undetermined results at the WB test, collected from April 2015 through February 2017 at the "P. Giaccone" University Hospital in Palermo, Italy. In the samples surviving a restriction algorithm, developed modifying previous "Recent Infection Testing Algorithm" flow chart (RITA), the anti-HIV AI was measured with a third-generation immunoassay (Vitros Anti-HIV 1+2, Ortho Clinical Diagnostics, Raritan, NJ), which detects anti-HIV1/2 antibodies. Each serum sample was divided into two aliquots and diluted 1:10 with PBS (untreated sample) or guanidine hydrochloride (treated sample), a caotropic agent that interferes with antigen-antibody binding. The AI for each specimen was calculated as: (OD of treated sample/OD of untreated sample) x100.

Results: When the restriction algorithm was applied, only 19 of the 82 samples tested survived the exclusion parameters. For the 19 samples eligible for recent infection the AI was calculated and, in all samples, (19/19, 100%) the resulting OD ratio was indicative of low avidity (AI ≤75%). According to the results of the AI test, the frequency of recent infections (≤6 months) was 23.2% (19/82) in our 23-months WB-reactive samples collection. Avidity test results were combined with the Fiebig staging of the sera analyzed. Low avidity (≤75% AI) result correctly classified as recent infections 100% (7/7) of the p31-negative sera (Fiebig stages <VI).

Discussion and Conclusions: The anti-HIV AI test can be a useful tool to estimate the incidence of HIV infection, when combined with the available clinical and laboratory data. The AI test, as we have applied, allows to determine reliably recent HIV infections, permitting the public health authorities to correctly perform epidemiological evaluations and helping the infectious diseases specialists to select the contacts requiring partner notification and contact training.

P086 - Performance evaluation of a newly developed molecular assay for the accurate diagnosis of Norovirus infection

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Introduction: Gastroenteritis remains an important cause of morbidity and mortality and accounts for significant economic and societal losses. Rapid and affordable laboratory tests for Norovirus (NoV) infection are essential for the correct management of patients and to control NoV outbreaks. In this study, the performance of a newly proposed fully automated cassette based sample-to-results solution for the detection of NoV, InGenius Norovirus ELITe MGB® test, was evaluated for the first time.

Material and Methods: An archival collection of 120 stool samples from children hospitalized for acute gastroenteritis was screened by InGenius NoV ELITe MGB® assay and the results compared to a reference NoV real-time reverse transcription RT-PCR method. All stored fecal specimens were previously tested also for group A Rotavirus (RVA), Astrovirus (HAstV) and Adenovirus (AdV). The fecal sample collection included 50 NoV-positive samples of different genotypes and variants and 70 NoV-negative, including two subsets: 50 samples tested-negative also for RVA, HAstV and AdV, and 20 stools tested -positive for at least one of the latter viruses.

Results: InGenius NoV ELITe MGB® assay had a substantial agreement with the real-time RT-PCR reference method, assessed through the Cohen's kappa (k=0.95), combining an optimal diagnostic accuracy with an excellent sensitivity (100%) and specificity (95.7%). Overall, the Ct values obtained by InGenius were significantly lower than those produced by the reference method ($p<0.05$). Three additional NoV-positive samples were found by InGenius among the NoV-negative samples (late-positives; 30.95 to 37.65 Ct). The InGenius NoV assay was able to correctly detect the entire wide panel of epidemiologically relevant NoV genotypes used in this study.

Discussion and conclusions: The results of this study suggest an higher analytical sensitivity for the detection of NoV genome of the InGenius commercial test evaluated when compared to the reference method. An affordable correlation of NoV viral loads in stools to severity of disease remains to be established. Overall, the InGenius NoV assay can be recommended as a valuable detection method for an accurate diagnosis of NoV infection for both sporadic enteritis and outbreaks investigation.

P087 - Torquetenovirus (TTV) load is associated with mortality in Italian elderly subjects

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Introduction. An age-related dysregulation of immune response, known as immunosenescence, contributes to increased susceptibility to infections, frailty and high risk of mortality in the elderly. Torquetenovirus (TTV), a circular, single-stranded DNA virus, is highly prevalent in the general population and it may persist in the organism, also in association with other viruses such as Cytomegalovirus (CMV), causing chronic viremia. The relationship that TTV establishes with the immune system of infected hosts is not clear. It is known that TTV encodes microRNAs (miRNAs) that might contribute to immune evasion and that the highest viral loads are found in peripheral blood cells. Moreover, it's suspected that TTV infection lead to increased production of inflammatory mediators, thus playing a role in immunosenescence.

Materials and Methods. We investigated the association of TTV load and miRNAs expression with inflammatory and immune markers and the influence of TTV load on mortality within a cohort of 379 elderly subjects (age range 60–105 years) who were followed up for 3 years. Presence and load of TTV DNA were determined in polymorphonuclear leukocytes (PMNLs) by a single step TaqMan PCR assay designed on a highly conserved segment of the untranslated region of the viral genome. miRNA expression was analysed and quantified with a commercial quantitative RT-PCR miRNA assay using primers targeting the miRNA mature region of TTV genome.

Results. TTV DNA load in PMNLs was slightly positively correlated with age and negatively associated with serum albumin levels and NK cell activity. A mild positive correlation between TTV DNA load, monocytes and IL-8 plasma levels was found in females and males respectively. TTV DNA copies $\geq$ 4.0 log represented a strong predictor of mortality (Hazard ratio = 4.78, 95% CI: 1.70-13.44, after adjusting for age, sex and the main predictors of mortality rate) and this association remained significant even after the CMV IgG antibody titer was included in the model (HR = 9.83; 95% CI: 2.48-38.97; N=343 subjects). Moreover, multiple linear regression model showed that TTV miRNA-t3b of group 3 was inversely associated with triglycerides, monocytes and C-reactive protein, and directly associated with IL6.

Discussion and Conclusions. Overall these findings suggest a role of TTV in immunesenescence and in the prediction of all-cause mortality risk in Italian elderly subjects. Further studies are needed to fully understand the pathogenic mechanisms of TTV infection during aging.

P089 - Hepatitis A outbreak in Sicily during Spring/Summer 2017: circulation of viral strains spreading in MSM community across Europe

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Introduction: Acute hepatitis A (AHA) is transmitted mainly via the faecal-oral route and/or contaminated aliment. Furthermore, several outbreaks in the men who have sex with men (MSM) population classified hepatitis A as a sexually transmitted disease.
From January to December 2017, 24 EU/EEA countries reported 15.040 of AHA cases with an overall M/F gender ratio of 3.6. Outbreak-confirmed cases involved 3.781 residents, M/F ratio of 6.8 and > 70% of males were MSM. All of them resulted infected by hepatitis A virus subgenotype IA (HAV- IA) with ≥99.3% homology to VRD_521_2016, RIVM-HAV16-090 or V16-25801 strains (eCDC data).

Materials and methods: Data about symptoms onset, gender, age, HIV status of acute hepatitis A cases occurred in Sicily in 2017 were collected by UOC Epidemiology and Prevention of Catania and "P. Giaccone" General Hospital or "G. Di Cristina" Paediatric Hospital of Palermo. HAV-RNA was isolated from available serum or faecal samples and a PCR fragment of about 460 bps, encompassing VP1/2A region, was amplified and sequenced by an *"in-house"* protocol. Sequences analysis was conducted using MEGA 6 software and reference sequences were retrieved from GenBank.

Results: A total of 130 cases of acute hepatitis A (M/F: 6; median age: 28 years) were recorded in Sicily during 2017. Of them 10 subjects were children (aged 3-14 years old), 102 (78%) males (aged 18-56 y) and 18 females (aged 20-44 y). The epidemic studied involved mainly Sicilians young men (< 45 years old) of which 22% were also HIV positive. The most of cases (97/130, 74%) occurred between May to September 2017 spreading to children and women too. Nucleotide sequences of HAV VP1/2A region were obtained from samples of 24 patients admitted to Hospitals of Palermo. Sequence analysis revealed the presence of HAV- IA in 21/24 samples and 19 sequences showed 100% of homology to strains (VRD-521-16 or RIVM_HAV16-090) reported in ongoing more than 3000 cases in 22 European countries. Molecular investigations demonstrated the transmission of a VRD-521-16 strain to two children aged 8 and 10 years by intra-familiar contact with their uncle, a young MSM who had become infected during a summer trip to Malta. Four patients had eaten seafood 2-4 weeks before symptoms onset, for the others sexual activity remains the most probable risk factor.

Discussions and Conclusions: This study showed that the increase of AHA cases occurred in Sicily in 2017 is closely related to a large continental outbreak which is spreading in MSM community, leading to infection of children and women. To prevent HAV circulation there is a need to promote anti-HAV vaccination starting from MSM up to the general population who is at a high risk of infection during epidemics through the mainly faecal-oral route.

P090 - Environmental surveillance of Hepatitis A Virus: evaluation of urban sewages and shellfish farms contamination during the ongoing outbreak in Sicily

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Introduction: Hepatitis A virus (HAV) is an enteric picornavirus, typically transmitted by the fecal-oral route through exposure to contaminated food (shellfish, strawberries, etc) or water, that causes acute hepatitis in humans. HAV is classified in 6 genotypes (I to VI), divided into subtypes A and B. Recently, several outbreaks due to HAV-IA have been reported in men who have sex with men (MSM), demonstrating new transmission routes in industrialized countries. From 2017, Italy is involved in a large HAV outbreak (2017 incidence: 5,8 x 100.000 inhabitants). Also in Sicily the number of cases has considerably increased in 2017 (126 cases notified) and apparently the infection is spreading from MSMs to the general population, also involving women and children.

Materials and Methods: From May 2017, HAV genome has been searched on urban sewages, collected by the Acute Flaccid Paralysis Sicilian Regional Reference Centre from wastewater treatment plants of the main Sicilian towns. Sewage samples have been concentrated following the guidelines for environmental surveillance of polioviruses (WHO 2003) and HAV VP1/2A region has been amplified by nested-PCR using pan-genotypic primers. At the same time, shellfish samples were collected from harvesting areas, dispatch centres, restaurants, fish markets and supermarkets, during official monitoring programs, and tested according to ISO/TS 15216-2:2013 at the Experimental Zooprophylactic Institute of Sicily "A. Mirri".

Results: HAV-RNA was detected in 10/16 (62%) urban sewages collected from May to September 2017 in Trapani (1/1), Messina (3/3), Catania (2/5) and Syracuse (4/7). Sequence analyses demonstrated the co-circulation of two HAV-IA strains, named VRD-521-16 and RIVM-HAV16-090, currently involved in a large European outbreak in MSM population (>3000 confirmed cases in 22 EU/EEA countries). During Fall/Winter only sewages collected from Trapani and Catania were still contaminated. Starting from March 2018, HAV genome reappeared in wastewater from Syracuse, but nucleotide sequences were related to older HAV-IA clones or to the HAV-IA V16-25801 variant, recently spreading in Germany, UK and Spain among MSMs. Surveillance on shellfish showed the presence of HAV genome in only 1/23 samples analyzed. The HAV-positive

shellfish sample was collected in Syracuse on May 2017 and the strain belonged to HAV-IB subtype.

Discussion and conclusions: Environmental surveillance demonstrated that the widespread circulation of HAV-IA strains in Sicily, is probably not linked to marine and shellfish contamination but to person-to-person transmission, including MSMs sexual contact, as recently reported in other European countries.

P091 - Differential in vitro characteristics of enteric (F species) vs respiratory adenoviruses

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Introduction: Adenovirus (AdV) is widely considered to be among the most important viral agents causing gastroenteritis in young children, mainly due to the F species 40 and 41 serotypes.

Traditional methods for the laboratory diagnosis of adenovirus infection are based on electron microscopy (ME) and conventional cell culture (CCC), even though the latter is not considered the best for detecting enteric AdV. Indeed, they are notoriously difficult to cultivate in vitro, in contrast with respiratory strains which are very easy to grow; this is of great significance considering the high prevalence and severity of infections caused by these viruses.

The principal aim of the present study was to evaluate the actual circulation of AdV 40 and 41 in a representative cohort of children with acute gastroenteritis by molecular methods. Furthermore, DNA sequencing of genetic loci possibly involved in the differential behaviour observed in their infectious cycle has been done.

Materials and Methods: Among the faecal specimens (n=4,996) collected from paediatric patients admitted with acute gastroenteritis to the University-Hospital of Parma during the years 2010-2015, analyzed by conventional methods (ME and CCC) at the Virology Unit (and then stored at -80C°), 205 had been found positive for AdV. The latter were subjected to PCR amplification and restriction endonuclease analysis (RFLP) to identify the F species (40 and 41 serotypes). Their cultivability in a human intestinal cell line (Int 407) was compared to that of AdV5 reference respiratory strain. DNA sequencing and analysis of the early genetic locus E1A was performed using an automated sequencer.

Results: Among the AdV strains identified from the considered samples, 118 belonged to F species, 91,5% of which were AdV41; surprisingly, about half of them were cultivable strains, even though with a peculiar behaviour, showing a different cytopathic effect and a lower number of infected cells, when compared to the respiratory strain. The DNA analysis of the E1A coding sequence revealed significant differences between AdV41 and AdV5.

Discussion and Conclusions: Among the F species, the 41 serotype largely prevailed in the considered paediatric population in accordance with data from other Countries. Moreover, the presence of AdV41 cultivable in human intestinal cells, even though with very different characteristics compared to the respiratory AdV5 strain, could reflect an increased ability of this viral serotype to infect enteric cells in vivo and deserves further investigation to shed light on molecular mechanisms accounting for the observed viral fitness.

P093 - Human cytomegalovirus infection in an unexplored "at risk" category of subjects

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Introduction: Human cytomegalovirus (HCMV) is a highly prevalent opportunistic agent in the world population, which persists as a latent virus after primary infection and can cause severe diseases in immunocompromised individuals upon the symptomatic reactivation of the virus belonging to specific genotypes. In particular, HCMV envelope glycoproteins (g) N and O possess hypervariable regions, resulting in a number of genetic variants in circulating clinical strains that could have a key role as virulence factors, most likely in combined patterns. Literature data is also accumulating about HCMV reactivations in non-immunocompromised adults with critical illness that may impair clinical outcomes. Indeed, the interplay between HCMV and immune surveillance is supposed to become unbalanced in advanced age with expanded anti-HCMV immune responses subsequent to subclinical reactivations. Accordingly, more in-depth studies concerning HCMV reactivation in non-immunocompromised adults are needed, including unexplored "at risk" categories of subjects such as elderly patients facing an acute life-threatening disease, where it is reasonable to expect cases of HCMV reactivation: elderly subjects with ischemic stroke represent one of the possible unexplored candidates for this kind of study.

Materials and Methods: An observational prospective study was performed in a cohort of 105 elderly (≥65 years) patients admitted to the Stroke Care Units of Parma University-Hospital for major acute ischemic stroke. Plasma samples from patients tested positive for anti-HCMV IgG, collected at 10±2 days from hospital admission, were analyzed for the presence of viral DNA by Real-Time PCR; gN and gO genotyping was performed by RFLP in case of positive DNAemia.

Results: HCMV DNA was detected (few hundred copies/mL) in 11.7% of the analyzed samples. The gN and gO genotypes were characterized in 77.7% of the positive samples for HCMV-DNAemia. In particular, the gN3 genotype was found to predominate (57.1%); with regard to gO, the recurrent genotypes were gO1 (57.1%) and gO2 (42.9%). Furthermore, these data suggest that combined gN3-gO1 and gN3-gO2 genotypes could be the recurring virulence factor patterns associated with viral DNAemia.

Discussion and Conclusions: This study focused on the role of HCMV infection in acute ischemic stroke. Overall, the results suggest that HCMV tends to escape immune surveillance in some elderly patients in this clinical setting. The quite low viral genome copies could be associated with localized, rather systemic, viral reactivations and involved specific HCMV envelope genotype combinations. The impact of these data will be further evaluated with respect to clinical outcomes and HCMV-specific T cell responses of the studied population.

P094 - EBV seroprevalence and primary infection at the University Hospital Luigi Vanvitelli of Naples from 2007 to 2017

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Introduction: The Epstein-Barr virus (EBV) is a common herpesvirus that may cause asymptomatic infection or various diseases such as mononucleosis, lymphoprolipherative diseases and multiple cancers. EBV has specific human tropism in adults infected predominantly during the first decade of life but also cause acute asymptomatic primary infection in young children and severe infection in adolescence.According to previous studies the seroprevalence increases with age and varies across population, geographic region, ethnicity, and socioeconomic status. We analysed the data obtained from the serological analysis of the patients hospitalized in the University Hospital of Naples Luigi Vanvitelli from January 1, 2007 to January 1, 2017. The aim of this study wasto evaluate the baseline information about the EBV infection prevalence in our country compare to the other regions.

Materials and methods: Our database wascomposed of 7881 patients with details on: picking data, sex, date of birth, anti-VCA IgM, anti-VCA IgG, anti-EBNA IgG and EBV agglutination test. Samples were analysed with BiomérieuxVidas for IgM anti-VCA IgG anti-VCA and IgG anti-EBNA. Instead, for agglutination test we used a fast indirect emo-agglutination on a slide for the search for heterophilic antibodies M.N.I. TEST by Techno Genetics a specific and sensitive assay.

Results: The EBV seroprevalence, in our country, increased gradually with age. Female (50,6%) had a slightly higher seroprevalence compared to male (49,3%), indicating a non-correlation with sex. The seropositivy for primary infection was higher in patients of about 5 years old while seropositivity for past infection was predominant in patients of about 35 years old.

Conclusions: Our analysis, on the patients hospitalized in the University Hospital of Naples Luigi Vanvitelli, have shown the correlation between the EBV prevalence and the age that represent the higher risk factor.For these reason the vaccine development should be an important strategy to reduce the impact of EBV.

P095 - EBV-DNA as a diagnostic and follow-up parameter for nasopharyngeal tumors in the western Sicilian population

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Introduction: Nasopharyngeal carcinoma (NPC) is not common; globally representing only 0.7% of all tumors. More than two thirds of patients are diagnosed only late, during advanced disease, because primary submucosal lesions often escape endoscopic examination. In recent years, several factors have been identified that play a role in the determinism of NPC: environmental risk factors, genetic susceptibility and the Epstein-Barr Virus (EBV). Therefore, we sought to further explore the etiologic link between NPC and EBV in a low-risk population, such as in western Sicily, by researching and quantifying EBV-DNA in biopsy and serum samples.

Material and methods: The study was divided into two parts: in the first 40 paraffin samples of histological preparations were analyzed retrospectively for the determination of the viral load of EBV; in the second a prospective study was conducted on a 30 patients that underwent nasopharyngeal biopsy for suspected NPC, aimed at evaluating EBV-DNA concentrations in biopsy and serum samples. In this last group the detection of anti-EBV antibodies (IgG-VCA, IgG-EA, IgG-EBNA, IgA-EA-D) was performed in the serum.

Results: In the first group 30 were tumors and 10 controls. EBV-DNA was detected in 25/40 samples analyzed. The EBV-DNA was found in 18/19 cases of undifferentiated non-keratinizing squamous cell carcinoma, in all 3 cases of lymphoepithelioma, and in 4/6 cases of lymph node metastasis of undifferentiated non-keratinizing squamous cell carcinoma. We found a high number of genomic copies ($5{,}7x10^5$-$5{,}9x10^7$) in 16/19 (84.2%) cases of undifferentiated carcinoma and in 3/4 (75%) cases of lymph node metastasis and in 3/3 of lymphoepithelioma. In the control case, the number of genomic copies was very low. In the second group of patients 9 were tumors, 1 severe dysplasia and 20 controls. The presence of EBV-DNA at lesion level was found in the 5 patients with NPC with a constantly high viral load; in 4 of the 5 a high serum load was found too. In all cases of NPC, an antibody reactivation pattern was highlighted. The case of severe dysplasia and the case of simple lymphoid hyperplasia, both with a high EBV-DNA load either at tissutal and serum level, were histologically positive for NPC at 6-month biopsy follow-up.

Discussion and Conclusions: The results indicate that the number of genomic copies of EBV can be a useful tool for assessing the onset of the disease in high-risk individuals defined serologically. It was possible to highlight the almost complete correlation between the high EBV-DNA loads and the histological diagnosis. A closer follow-up, associated with the assessment of viral load, should be implemented in order to identify early NPC onset.

P096 - Temporal dynamics of hepatitis C virus (HCV) genotypes over a three-year hospital-based survey in Northern Italy

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Introduction: HCV infection is one of the major public-health burden. Persistent HCV infection may cause liver cirrhosis, hepatocellular carcinoma, and death. HCV prevalence changes between geographic areas, age groups, transmission routes, and risk factors. Italy has the highest HCV infection prevalence in Europe, with 1.4 million infected-people. HCV genotypes 1-3 show a worldwide distribution, while genotypes 4-7 are limited to specific areas. This study aims to investigate the pattern of HCV genotypes and its association with viral load over three years (2013-2015) in the area of Parma (Northern Italy).

Materials and Methods: serum/plasma from 825 individuals attending the University Hospital of Parma were subjected to HCV RNA quantification by COBAS AmpliPrep/COBAS TaqMan HCV v2.0 (Roche) and genotyped by VERSANT HCV Genotype 2.0 LiPA (Siemens) assays. Of these, 454 were males (55%; median age: 51 years), 371 females (45%; median age: 65 years), 738 (89.5%) Italians, and 87 (10.5%) foreigners mostly coming from Eastern Europe (59.8%) and Africa (19.5%).

Results: HCV genotype (G) 1 (51.4%) prevailed, followed by G2, G3, G4, and G6 (22.9%, 17.4%, 8.2%, and 0.1%, respectively); subtypes 1b, 3a, and 1a (34.7%, 16.7%, and 16.5%, respectively) predominated. Among foreigners, subtypes 1b, 3a, 1a, 4a/4c/4d, 2a/2c, 4 not-subtypable (n-s), 2 n-s, 2b, and unusual subtypes 4e and 6 n-s (49.4%, 21.8%, 8.1%, 8.1%, 3.5%, 3.5%, 2.3%, 1.1%, 1.1%, and 1.1%, respectively) were found. Subtypes 1a and 3a predominated in males ($P<0.0001$). Subtype 1a prevailed in subjects aged less than 61 years ($P<0.0001$), while 3a in subjects aged less than 31 years ($P<0.05$). Subtypes 1b, 2a/2c, and 2 n-s prevailed in females ($P<0.0001$, $P<0.05$, and $P<0.005$, respectively), and in subjects older than 60 years ($P<0.0001$). The prevalence of subtype 1b increased during the study period, contrarily to that of subtypes 1a and 3a ($P<0.05$). Higher viremic HCV RNA titres were found in males infected with subtypes 2a/2c, 4a/4c/4d, and 4 n-s.

Discussion and Conclusions: different HCV genotypes were observed in the area of Parma and subtypes 1b, 3a, and 1a prevailed. The high prevalence of subtype 3a found in young people suggests its epidemiological relevance in the future. The less-represented G4 and G6, mostly found in foreign residents, may become more frequent due to increasing migratory flows from endemic areas. Higher HCV viral loads assessed in males infected with subtypes 2a/2c, 4 n-s, and 4a/4c/4d evidence a greater viral replicative efficiency. The obtained results reinforce the need for structured epidemiological studies in order to better understand the dynamics of HCV circulation and improve preventive strategies.

P097 - Epidemiology of human immunodeficiency virus (HIV) infection in a tertiary care hospital in Northern Italy

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Introduction. Virus detection and/or identification is traditionally performed using cell culture, electron microscopy and antigen or nucleic acid detection. In this study, Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS), commonly used in clinical microbiology, was developed and tested as an innovative tool to be applied to virus identification by using two different approaches.

Materials and Methods. In the first approach, human polioviruses were selected as a model to evaluate the ability of MALDI-TOF MS to identify specific viral protein to be used as biomarkers of purified virus particles, followed by the serotypes identification. To this aim the Sabin reference strains (I, II, III) were firstly analysed and, subsequently, the results were then confirmed by a blind application of the assay to clinically isolated strains.
In the second approach, a protein profiles library was newly create to discriminate between uninfected and respiratory virus infected cell cultures after a viral proteins enrichment method. The library was built using different reference strains after an extensive modification of the MALDI-TOF MS pre-processing, MSP creation, subtyping MSP creation and identification default parameters setting.

Results. The very efficient technique adopted to obtain highly purified poliovirus allowed us to discriminate viral protein peaks from uninfected cells peaks and to detect specific poliovirus protein biomarkers. Moreover, MALDI-TOF MS analysis applied to the three Sabin poliovirus serotypes revealed characteristic peak profiles for each of them showing three independent clusters for the three serotypes. After a proper statistical investigation, the VP4 was used as a potential biomarker to identify poliovirus strains at the serotype level. On the bases of VP4 all clinical isolates were identified at the serotype level.
In the second approach, the spectra generated from virus infected cell cultures revealed the presence of some different peaks not overlap- ping those of uninfected cell cultures for all the reference virus infected cell cultures. The parameters for the creation of the Main Spectrum Profile (MSP) for each of the reference virus infected cell cultures were set on the basis of these peaks. The obtained MSP spectra were used to create a new respiratory viruses library in our Bruker Daltonics database in order to blind identify viruses isolated from biological samples after a cell culture step. The spectra obtained by 58 additional cultured strains correctly match with the new database demonstrating its reliability.

Discussion and Conclusions. In conclusion, this study could be considered a starting point for further evolutions of the developed system, since the differences observed comparing the spectra obtained from virus infected cell culture suggest the possibility to apply these approaches to the identification of other viruses including other picornavirus such as enterovirus and coxsackievirus and viruses responsible for respiratory infections, as well as to viral agents causing infections of other body sites.

P099 - LTR-5' genetic variability correlates with HIV-DNA burden during cART

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Introduction: Previous in vitro studies show that an increased genetic variability in HIV-1 LTR-5' U3 (containing critical transcription factor binding sites) promotes HIV-1 transcriptional activity. Here, we investigate the correlation between U3 heterogeneity and viro-immunological parameters in HIV-1 infected patients (pts) receiving antiretroviral therapy (cART).

Materials and methods: This longitudinal prospective study includes 32 newly diagnosed HIV-1 infected pts (diagnosis: 2015-2017), receiving a first line modern cART (NRTI+INI, N=21; NRTI+NNRTI, N=4; NRTI+PI, N=2; OTHER, N=5). For all pts, viremia, total HIV-DNA, $CD4^+$T and $CD8^+$T cells are evaluated at baseline (BL), at virological success (VS, viremia<50 cps/ml), and at 6 months after virological success (6mVS). Total HIV-DNA (log cps/10^6CD4^+) and residual viremia (defined as viremia at VS and at 6mVS, cps/ml) are measured by ddPCR. BL LTR5' U3 is analyzed by NGS (Illumina). Shannon Entropy weighted for the intra-patient prevalence of viral species (Sn, range: 0 [single haplotype] to 1 [presence of >1 haplotype with the same intra-patient prevalence]) defines U3 heterogeneity. Pearson correlation and Mann-Whitney test are used to correlate U3 heterogeneity with HIV-DNA, CD4/CD8 ratio, and viremia at BL, VS, and 6mVS.

Results: At BL, median(IQR) viremia, CD4/CD8 ratio and total HIV-DNA are 4.8(4.4-5.3) log cps/ml, 0.40(0.20-0.62), and 4.1(3.4-4.7) log cps/10^6CD4^+, respectively. Median U3 entropy value (Sn) is 0.82(IQR, 0.75-0.93). VS is achieved after a median time of 7.9(4.7-19.3) weeks. Median(IQR) total HIV-DNA reduction is 0.60(0.83-0.33) log cps/10^6CD4^+ from BL to VS, and 0.35(0.74-0.11) log cps/10^6CD4^+ from VS to 6mVS, while residual viremia is 6.5(1.6-26) cps/ml at VS and 1.5(0-24) cps/ml at 6mVS. Correlating U3 Sn and viro-immunological parameters at BL, we find that higher U3 entropy values correlate with higher HIV-DNA burden (rho=+0.41, P=0.01), higher viremia (rho=+0.35, P=0.04) and lower CD4/CD8 ratio (rho=-0.37, P=0.04). Moreover, while at VS total HIV-DNA does not differ between pts characterized by a U3 Sn>0.8 and pts characterized by a U3 Sn<0.8 (median[IQR] value: 3.21[3.01-3.70] and 3.44[3.28-3.72] log cps/10^6CD4^+, respectively; p=0.26), from VS to 6mV higher U3 entropy values significantly correlates with slighter HIV-DNA reductions (median[IQR] delta HIV-DNA: 0.15[0.11-0.30] for Sn>0.8 vs. 0.24[0.10-0.69] for Sn<0.8, P<0.01, rho=-0.44, P=0.02). No significant correlations are found for CD4/CD8 ratio and residual viremia at both VS and 6mVs.

Discussion and Conclusions: This proof-of-concept study shows that LTR-5' U3 heterogeneity correlates with HIV-DNA burden, viremia and CD4/CD8 ratio at cART initiation, and may be a factor influencing total HIV-DNA decay during cART.

P100 - Potential impact of the nonavalent HPV vaccine in male population living in Sicily

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Introduction: Human papillomavirus (HPV) is the most common sexually transmitted disease. In contrast to the wealth of information available on the rate of and risk factors for acquisition of HPV infection in women, much less is known about such factors in men. More recently, vaccination in boys and young men has been recommended as well and gender-neutral immunization programs have been proposed. The aim of this study was to assess HPV genotype distribution among men with and without clinical manifestations and to evaluate the potential impact of a candidate nonavalent HPV vaccine on HPV infection compared with the presently utilized quadrivalent HPV vaccine, in a male population living in Sicily.

Material and methods: The analysis involved 901 men who had come to the Virology laboratory at the of the Polyclinic, Palermo University, between January 2009 and December 2016, with a request for HPV testing. The common reasons for requesting an HPV test included men having an HPV positive partner; men having a risky sexual contact in the prior 2 months; men with clinical lesions. Mean age was 36.7 (range 19-77 years). Samples were collected from penis and urethra. After HPV testing and evaluation of clinical symptoms three series of genital samples were selected and examined as follows: 177 genital samples of men with presence of clinical symptoms; 247 genital samples of men with a risky sexual contact (STD) and 422 genital samples of men with HPV positive partner.

Results: Of 846 genital samples β-globin positive 479/846 (56.6%) samples tested were HPV positive. Oncogenic types were found in 376/479 (78.5%) positive samples. Multiple HPV type infections were shown in 235/479 (49%) samples of whom 111 (23.1%) had two genotypes, 63 (13.1%) three genotypes, 38 (7.9%) four genotypes, 18 (3.7%) five genotypes, 3 (0.6%) six genotypes and then only 2 (0.4%) eight genotypes. 95.7% (225/235) of multiple infections contained at least one high-risk genotype; 62.7% (153/244) of single infections contained HR. Altogether 245/479 (51,1%) samples harboured at least one of the four HPV types covered by the current quadrivalent vaccine (HPV 6, 11, 16 and 18), while 399 (83,3%) samples harboured at least one of the nine genotypes included in nonavalent vaccine, implying a significantly higher estimated coverage of HPV infection from the nonavalent vaccine than the current quadrivalent vaccine (83,3% *vs* 51.15%; $p<0.001$).

Discussion and conclusions: The prevalence of HPV infection in the population object of the study was relatively high (56.6%), in line with other published reports. Implementation of nonavalent vaccination programs could become thus a cost effective public health prevention approach, basing on its potential to produce substantial incremental benefits.

P101 - Epidemiological study of cervical HPV infection in three women age groups in Southern Italy

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Introduction: Human papillomavirus (HPV) is a small non-enveloped, double-stranded DNA virus that infect mucosal and cutaneous epithelia in humans. In order to discriminate HPV genotypes involved in the development of cervical cancer, HPV have been classified into Low-Risk (LR) (6, 11, 26, 40, 42, 43, 44, 53, 54, 61, 62, 64, 67, 69, 70, 71, 72, 73, 81, 82, 83, 84, IS39 and CP6108) and 14 High-Risk (HR) (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68). Several studies assessed that the prevalence of HPV infection varies across populations and according to a woman's age. In Italy, HPV vaccination, started since 2008, involved 12-year-old girls. In this study we evaluated the distribution and the prevalence of different HPV genotypes in our territory from 2011 to 2017, focusing on the distribution of age. We aim to assess HR-HPV prevalence and the possible variations in the age-distribution of HPV prevalence for a better understanding of the impact of HPV program of vaccination on the local female population.

Materials and Methods: A total of 1265 patients were recruited from January 2011 to December 2017; the samples collected in the ThinPrep Pap Test/PreservCyt®Solution, were performed in the Laboratory of Virology and Microbiology-Section of Molecular Microbiology. DNA extraction, amplification and hybridization were performed according to the manufacturer's instructions (Roche Molecular Diagnostics, Milan, Italy) to evaluate the presence of one or more HPV genotypes. The test's sensitivity and specificity are 96% and 99% respectively.

Results: Out of a total of 1265 specimens we identified 563 positive and 702 negative samples. On 563 patients we found 579 HR-HPV and 519 LR-HPV. The prevalent HR-HPV genotypes were the following: 16 (23.76%); 31 (12.06%); 18 (6.91%); 51 (7.45%); 58 (7.45%); 59 (7.62%); 66 (7.98%). In the context of LR-HPV, HPV 53 was the most frequently found (12.41%). In the years 2011-2017 no substantial differences were found in the incidence of HPV genotypes in relation to three age groups (<23; 23-29; >30), especially for HPV 16 and18 identified as a vaccine target.

Discussion and Conclusions: Genotyping all 38 genotypes of the ano-cervical area, we provided a complete epidemiological data not underestimating the incidence of LR-HPV genotypes such as HPV 53 which is already classified as a potential high risk. Unfortunately our data show a poor adherence to the vaccination strategies on our territory especially in the age group < 23.

P102 - Seroprevalence of Herpes viruses in a retrospective study in Southern Italy

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Introduction: Human Herpes viruses (HHVs) are pathogens affecting the worldwide population with an ubiquitous distribution. There are two types of HSV, HSV1 and HSV2 transmitted by direct contact with infected secretions and sexually respectively. As a regards primary varicella infection in late life, could reactive and cause herpes zoster (VZV) while human cytomagalovirus (CMV) is a highly prevalent herpes virus that infect a large part of population worldwide. Monitoring the epidemiology of this viruses help to the prevention of neonatal herpes and the other associated diseases. The present study assess the spread of HSV1-2, VZV and CMV seroprevalence in a random population.

Materials and Methods: A seroprevalence retrospective study from a general population was carried out using sera collected from patients of all ages (1-70 years) hospitalized at the University Hospital Vanvitelli in Naples Italy for different causes. During a period from 1/01/2016 to 30/06/2017, 1854 patients's sera were analysed for detection of type-specific. HSV1-2 antibodies using Elisa (Kit Instituit Virion/ Serion GmbH), CMV antibodies using enzyme immunoassay Biomerieux Vidas, VZV antibodies using chemiluminescence immunoassay (Gemini Combo Alifax) following manifacturer's instructions.

Results: The seroprevalence of IgG antibodies towards three of common Human Herpes viruses were respectively investigated. Within total group of 1854 patients, 1149 were analized for HSV1-2, 895 for VZV and 1529 for CMV. In our population seroprevalence for HSV1-2 increase from childhood to adolescence and decrease in adults. Furthermore, the multiple logistic regression demonstrated that, for HSV1-2, gender was statistically significant while lower significance was associated with age. The results for VZV and CMVs antibodies detection show that seropositivity is higher in women than men but not significant while in CMV the gender was significant for acquiring infection. A final analysis about the seropositivity's patient (HSV1-2, VZV, CMV), by the gender and the age, show that gender is now the more significant risk factor.

Conclusions: This study is the first to report the comparative seroepidemiology among HHV in Southern Italy. Agreeing with previous studies in other country we found that for HSV1-2 females had a higher risk of infection compared to males. VZV seroprevalence shows rapid increase during the first decade of life, in fact affect mainly adolescents and adults. On the other hand CMV increases gradually at old age.The high proportion of different ages groups detected in our study, underlines vaccine recommendations and screening not only for women before pregnancy but also for all the susceptible individuals.

P103 - Designing of pan-genotyping primers able to identify the novel GII.P16 pandemic recombinant Norovirus

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Introduction: Noroviruses (NoVs) are leading cause of acute gastroenteritis across all age, causing 685 million cases/year. They are classified in 7 distinct genogroups (GI-GVII) and NoVs GI, II and IV, able to infect humans, have been divided in more than 30 genotypes based on polymerase (ORF1) and capsid protein (ORF2) sequences. GII.4 NoVs caused the majority of outbreaks and sporadic cases worldwide and usually new variants emerge every 2 to 3 years, resulting in epidemics and sometimes global pandemics. Norovirus evolution is driven by antigenic drift and intra/inter genotype recombination. From 2016, new recombinant NoV variants are spreading worldwide and an update of PCR protocols was necessary to amplify ORF1 diagnostic fragments of GII.P16 genotype.

Materials and methods: From January to December 2016, 98/464 (21%) stool samples collected from children hospitalized with acute gastroenteritis at the ''G. Di Cristina'' Children's Hospital in Palermo were detected NoV-positive by Real-time PCR, targeting ORF1/ORF2 junction. NoV dual-typing was performed by sequencing two genomic portions, encompassing region A-B of ORF1 (pol) and regions C-D of ORF2 (cap), using JV12A/JV13B and COG2F/G2SKR primers, respectively. Concomitantly, ORF1 was amplified with new degenerate primers JV12Y/JV13RN, designed using a dataset of ORF1 sequences belonging to all NoV genotypes.

Results: With standard protocols complete genotyping (pol + cap) was obtained for 52/98 (53%) NoV-positive samples. Twenty-one samples were typed as GII.P4_GII.4 Sydney 2012 variant, 12 GII.Pe_GII.4 Sydney 2012, 7 GII.P17_GII.17, 10 GII.P2_GII.2, 1 GII.P21_GII.3 and 1 GII.P21_GII.4 Sydney 2012. Lacking ORF1 fragment amplification, partial ORF2 genotyping was obtained for 14 GII.2 and 1 GII.4 strains. ORF2 phylogenetic analysis showed that these strains were strongly correlated to the new recombinant GII.P16_GII.2 and GII.P16_GII.4, spreading worldwide since 2016. When a wide panel of complete NoV ORF1 sequences was retrieved from GenBank and aligned with JV12A/JV13B primers sequences, GII.P16 sequences showed multiple nucleotide mismatches with the PCR primers used. Therefore, degenerate primers JV12Y and JV13RN were created. The use of JV12Y/JV13RN degenerate primers allowed to obtain the 15 missing ORF1 sequences and to confirm the circulation of GII.P16_GII.2 and GII.P16_GII.4 recombinant strain in Palermo since September and December 2016, respectively.

Discussion and conclusions: NoVs rapid evolution is related to their ability to cause large outbreak and re-infection in both adults and children. Up to date methods are essential for molecular surveillance of NoVs, for monitoring emerging strains and to study the impact of NoV disease on public health.

P104 - Nationwide hospital-based surveillance reveals increasing non-GII.4 Norovirus prevalence in Italy

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Introduction: Noroviruses (NoVs) are one of the leading causes of gastro-enteric diseases worldwide in all age groups. Novel epidemic NoVs with GII.P16 polymerase and either GII.2 or GII.4 capsid type have emerged worldwide in the late 2015 and in 2016. We performed a molecular epidemiological study of the NoVs circulating in Italy to investigate the emergence of new NoV variants.

Material and methods: During two consecutive years 2015-2016, the Italian Study Group for Enteric Viruses (ISGEV; https://isgev.net/) surveillance for NoV analyzed by real-time reverse transcription (RT)-PCR assay a total of 2184 and 2179 stool samples of diarrheic children, respectively. All NoV-positive specimens were genotyped by sequence analyses in the diagnostic region A (included in ORF1) and C (ORF 1-ORF2 junction) and genotypes were determined using the Norovirus Typing Tool database (http://www.rivm.nl/mpf/norovirus/typingtool).

Results: Hospital-based surveillance in Italy revealed increased prevalence of NoV infection in children in 2016 (14.4% versus 9.8% in 2015) and the emergence of GII.P16 strains in the late 2016, with an overall yearly prevalence of 23.0%. The majority of the strains with a GII.P16 ORF1 showed a GII.2 capsid genotype (79.5%). The mean and median age of GII.2 patients was significantly ($P<0.005$) higher (5.7 vs 3 and 4.6 vs 2 years, respectively) than that of GII.4 cases (Mann-Whitney U Test, two-tailed).

Discussion and conclusion: Such a consistent circulation of GII.2 viruses had never been signaled before by NoV surveillance in Italy and was mainly sustained by the novel recombinant GII.P16_GII.2 strain. GII.17 NoVs remained epidemiologically relevant. A tendency to infect older patients has been observed for non-GII.4 NoVs when being newly introduced. These observations seem to be consistent with a lack of herd immunity in the population, allowing the novel viruses to infect older patients more easily than GII.4 strains. The emergence and global spread of non-GII.4 noroviruses pose challenges for the development of vaccine strategies.

P105 - Role of Sicily in the Global Polio Eradication Plan: Relevance of Syndromic and environmental surveillance network

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Introduction

Since 1988 WHO is strongly committed in Polio Eradication to be achieved through immunization activities, syndromic and environmental surveillance. Polio is a highly infectious disease caused by wild poliovirus (WPV), transmitted mainly through the fecal-oral route, causing irreversible paralysis and death. At present WPV is still endemic in 3 countries (Afghanistan, Pakistan, Nigeria) but Italy can be considered at risk of importing WPV or circulating vaccine derived poliovirus (cVDPV). Indeed Sicily, due to its geographical position, is the closest landing destination for immigrants from endemic areas. The Sicilian Regional Reference Center (SRRC) monitors Acute Flaccid Paralysis (AFP) cases in 0-14 years old children, investigates the presence of WPV and cVDPV in sewage (environmental surveillance) and carries on seroprevalence surveys to investigate the immunity levels of the local population.

Materials and methods

From 1991, following the National Institute of Health (ISS) and the Italian Ministry of Health guidelines, active AFP surveillance is performed in Sicily by the SRRC, in collaboration with 67 hospitals included in the Sicilian AFP surveillance Network. Viral isolation from fecal samples and determination of antibody titers was performed on every AFP case signaled. From 2013, environmental surveillance was carried on in 7 Sicilian immigrants detention centers (IDCs), but following changes in immigrants management political directions, from 2017 environmental surveillance is performed on wastewaters collected at treatment plants in the main Sicilian cities: Catania, Messina, Siracusa, Trapani. All samples collected were analyzed according to WHO/V&B/03.03 protocol.

Results

According to the results of cell-culture viral isolation tests on fecal samples, Poliovirus etiology was excluded for all the 113 AFP cases reported from 2002 to 2017, confirming Guillain-Barré syndrome as the main cause of AFP in Sicily. All patients showed a protective immunity as a result of vaccination. The analysis of the 64 sewage samples collected in IDCs revealed no WPV contamination, although in four sewage samples, collected in December 2013, the Sabin-like PV2 was isolated. A total of 92 sewage samples was collected from municipal wastewater treatment plants in 2017 and 2018 and no WPV was isolated in the samples analyzed.

Conclusions

Sicily is considered an important gateway to Europe for immigrant or refugees coming from Africa and Middle East and traveling the "Central Mediterranean route". The risk of polio neurovirulent strains reintroduction is real since most of refugees come from countries that are still endemic or where polio has recently circulated. Therefore additional efforts for surveillance activities are necessary until the achievement of the global polio eradication goal.

P106 - Molecular epidemiology of Rotavirus in Pavia, Northern Italy, 2015-2018

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Introduction: Rotaviruses belonging to genogroups A, B, C and H are associated with human infections and the viruses belonging to genogroup A are the most frequently implicated in human gastrointestinal infections. Rotaviruses A are further classified into different genotypes, based on genetic differences in RNA segments 7 and 4, encoding respectively for VP7 and VP4 proteins, which form the basis of the dual nomenclature system used for species A Rotavirus strains, whereby glycoprotein (G or VP7) and protease-cleaved protein (P or VP4) subtype are differentiated. In the present study we report the molecular characterization of Rotavirus in hospitalized patients in the period 2015-2018 in Pavia, Northern Italy.

Materials and methods: A retrospective study was conducted on 122 stool samples stored at the Fondazione IRCCS Policlinico San Matteo of Pavia, Italy. The samples were collected in the period September 2015-April 2018 from 122 patients hospitalized (115 pediatrics and 7 adults), with Rotavirus infection. The samples tested positive for the presence of Rotavirus with the routine immunochromatographic assay (RIDA®QUICK Rotavirus, R-Biopharm AG, Darmstadt, Germany) were stored at - 80°C and retrospectively analyzed with Reverse Trascriptase Polymerse Chain Reaction and sequencing analysis for strain characterization.

Results: Rotavirus RNA was detected in 111 samples out of 122 (90%) and genotyping was obtained in 108 of the 111 (97.3%) positive samples, 98.1% (106/108) were collected from pediatric patients, whereas 1.9% (2/108) from adults. About VP7 genotyping detection, the most frequent G-type was G3 (30.6%, 33/108), followed by G1 (27.8%, 30/108), G9 (19.4%, 21/108), G12 (13.9%, 15/108) and G2 (7.4%, 8/108), while G6 (0.9%, 1/108) was identified at lower rate. Concerning VP4 genotyping, the main P-type was P8 (87.9%, 95/108), whereas P4 (11.1%, 12/108) and P9 (0.9%, 1/108) were observed in a minor number of cases. Eight G and P type associations were identified. G3P[8] and G1P[8] were the most detected strains with a rate of 30,6% (33/108) and 27,8% (30/108) respectively, followed by common Rotavirus genotypes G9P[8], G12P[8] and G2P[4] that circulate, whereas uncommon Rotavirus genotypes as G9P[4], G6P[9] and G12P[4] were sporadically observed.

Discussion and conclusions: Differences in the prevalence of Rotavirus genotypes circulation were observed comparing our results with data from other Italian and European groups. In particularly, the genotype mostly detected in our study was G3P[8], whereas it was identified less frequently in other studies conducted in Italy in the same period.

P107 - Emerging equine-like G3P[8] rotavirus in children hospitalized with acute gastroenteritis in Palermo

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Introduction: Group A rotaviruses (RVAs) are the most common cause of acute gastroenteritis (AGE) among children less than five years of age. RVAs are classified into G and P genotypes based on the sequence diversities of the two external proteins: VP7 and VP4. From 2013, a novel equine-like G3P[8] RVA human/animal reassortant has emerged as the predominant strain in different geographical areas. In Palermo, laboratory surveillance of gastroenteritis and molecular characterization of RVA isolates was conducted to monitor their genetic variability and the circulation of novel strains in the human population.

Materials and methods: Stool samples, collected from children hospitalized for AGE at the "G. Di Cristina" Children Hospital of Palermo, were screened for RVA by RT-RealTime-PCR. To determine the G and P genotypes, positive specimens were analyzed by multiplex hemi-nested RT–PCR using type-specific primers for VP7 and VP4, respectively. RVA-positive samples were genotyped and nucleotide sequences were compared with sequences available in GenBank.

Results: During 2017, 85 of 669 stool samples (12.7%) tested by RT-RealTime-PCR were positive for RVA. Hemi-nested RT-PCR was able to G/P genotype 80% (68/85) of the RVA-positive samples, indicating G9P[8] as the prevalent genotype (50.6%), followed by G2P[4] (27.1%), G1P[8] and G3P[8] (1.2%). Unexpectedly, 11.8% (10/85) of stool samples had a P[8] VP4 type but were G-non-typable. Sequence analysis of first step PCR VP7 gene amplicons showed that 6/10 (60%) of the P[8]/G-non-typable samples belonged to an atypical equine-like cluster of G3 RVAs, including reassortant strains circulating in different regions of the world.

Discussion and conclusions: Based on VP7 sequence analysis, six G3 strains isolated in Palermo in 2017 showed a reassortant Human/Equine nature. G3 RVA strains have a broad host range, including humans, cats, cows, dogs, horses, pigs, rabbits, sheep, monkeys and bats. The segmented RVA genome facilitates both intra and inter genogroup reassortment events. Reassortment between animal and human strains is a powerful mechanism driving the evolution of RVAs. The identification of equine-like RVA genes in the human population highlights the potential for the emergence of novel rotavirus strains. Uninterrupted RVA surveillance is important to understand the diversity of the strains circulating within the human population over the time and to ensure the effectiveness of vaccine programs.

P108 - Impact of the introduction of anti-rotavirus vaccination on for acute gastroenteritis at the children's hospital "G. Di Cristina"of Palermo

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Introduction: Rotavirus (RV) is the most common cause of severe acute gastroenteritis (AG) in childhood with over 400,000 deaths/year, concentrated in developing countries. In Italy, RV is the first cause of diarrhea hospitalization within 5 years of life. In Sicily, free vaccination against RV for all infants was introduced in 2013.

Materials and methods: A survey was performed on children aged 1 month to 14 years (average 42.09 months, median 31.91 months) hospitalized for AG at the G. Di Cristina Children's Hospital Palermo, from March 2017 through February 2018. For each case a stool sample was collected for the detection of antigen and/or RV genome and clinical and epidemiological data were acquired. The prevalence of RV infection was compared with the pre-vaccination period (2011-2012).

Results: Of the 328 samples collected, 67 were positive for RV (prevalence: 20.4%). Only 16 of the RVAG cases had a possible source of infection in family members. In the week before the presentation of RVAG only 6% (4/67) of the children reported a previous admission to another ward of the same hospital. Among clinical symptoms, mild-severe dehydration (77.6%) and vomiting (86.6%) predominated, often lasting more than three days (16.4%). The majority (46.2%) of children hospitalized for AG had received a complete anti-RV vaccination cycle, 18.6% had a single dose, 49.6% were not vaccinated and in 4.2% vaccination status was undetermined. However, only in 2 (3.2%) of 63 vaccinated children AG was caused by RV. On the contrary, among the unvaccinated children the prevalence of the RVAG was 24.5%, producing a significant difference between two cohorts (p= 0.001).

Discussion and conclusions: Surveillance activities are strategically important for monitoring the protective effects of vaccination. Our study showed that vaccination reduced admissions for RVAG at the Children's Hospital of Palermo compared to the pre-vaccination period (20.4% Mar-Feb 2017-2018 vs 26% Mar-Feb 2011 -2012). Nosocomial infections appear to be very limited. The severity of symptoms was higher in the RVAG patients than in AGs with other causes (Median Vesikari score: 7 vs 5). The relative risk of developing RVAG was 18 times higher (RR= 18.7) in the cohort of unvaccinated children admitted with AG compared to the vaccinated cohort (p = 0.0023). Our preliminary results encourage to the widespread use of vaccination.

P109 - Norovirus detection performance of bionexia® noro/rota-adeno immunochromatographic (ict) test compared to realtime rt-pcr

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Introduction: Noroviruses (NoVs) are the main agents of sporadic cases and outbreaks of viral gastroenteritis (GE), affecting both children and adults. NoVs are non-enveloped RNA viruses whose genome includes 3 Open Reading Frames (ORF1-3), encoding for RNA polymerase, the major viral capsid protein VP1 and minor structural protein VP2, respectively. NoVs can be classified into 7 genogroups (GI-GVII) on the basis of VP1 sequence. Genogroups GI-II and IV occur in human infections and can be divided into more than 30 genotypes based on the sequences of polymerase and capsid proteins. GII.4 genotype is associated with the majority of NoV related GE cases worldwide, but novel variant and recombinant strains are continuously produced. Molecular techniques represent the gold standard for NoV detection in stool samples but Immuno-cromatographic Tests (ICTs), revealing viral antigens, are attractive due to simplicity, low cost and short turnaround time. In this study the performance of an ICT test for NoV detection was evaluated using Real-time PCR as reference.

Materials and Methods: A total of 100 fecal samples collected from February to March 2017 at the ARNAS Civic Hospital from children hospitalized with acute GE were tested for NoV detection with the BIONEXIA® Noro/Rota-Adeno ICT test (bioMérieux) and results were compared to a RealTime reverse transcription (RT)-PCR assay able to differentiate between GI and GII NoVs. NoV-positive specimens were genotyped by sequence analyses in region A (ORF1, Pol) and C (ORF1-2 junction, Cap).

Results: The ICT test identified only 2 NoV-positive samples while Real-time PCR allowed to increase the number of NoV-positive samples to 34. Among the NoV-positive samples, 29 had low Cycle Threshold values, indicating high viral loads. Therefore, comparing the performance of the two tests, ICT showed only 5.9% sensitivity. Molecular characterization of NoV isolates allowed to identify some of the genotypes most recently introduced in our country, including GII.P16/GII.4 Sydney 2012 (10 samples), GII.P16/GII.2 (9), and GII.P16/GII.4 (2), while 3 isolates were only partially genotyped (2 GII.P16 and 1 GII.2). For 11 NoV-positive samples no genotype could be assigned.

Discussion and conclusions: ICTs are generally preferred in diagnostic laboratories not equipped for molecular investigations for rapid diagnosis of NoV infection. However, in the present study BIONEXIA ICT assay showed unsatisfactory sensitivity for NoV detection, possibly due to the novelty of the viral types circulating in the latest period, reflecting the high genetic/antigenic variability of NoVs. The continuous updating of diagnostic test, taking into account the newly circulating strains, is essential for correct assessment of the etiology of GE.

P110 - Could Parvovirus B19 infection modulate intracellular redox state?

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Introduction: Several studies have demonstrated that an alteration of the intracellular redox balance, which occurs during different viral infections, is associated with the progression of diseases. The redox imbalance is mainly due to an overproduction of reactive oxygen species (ROS) and/or decrease of reduced glutathione (GSH), the most plentiful intracellular antioxidant. The pro-oxidant conditions are used by viruses for their own replication. Parvovirus B19 (B19V) is a human pathogenic virus of the *Parvoviridae* family, responsible for a wide range of clinical manifestations. B19V has a strictly tropism for eryhtroid progenitors cells (hEPCs) of bone marrow, but it was found to persist in several tissue types. Nowadays the correlation between B19V infection and the changes in intracellular redox state is unknown.

The aim of our study was to set up an *in vitro* model of infection with B19V aimed at evaluating whether the infection was able to induce alteration of intracellular redox state. In detail we wondered to characterize redox imbalance by analysing GSH levels and redox sensitive cell pathways activation.

Materials and Methods: In this work, we tuned an infection model for B19V using human leukemia erythropoietin-dependent cells (UT7/EpoS1). Cells were infected with a B19V viremic serum in order to obtain a multiplicity of infection of 10^4 genomes/cells. The amount of viral DNA present within infected cells at different time from infection was evaluated by qPCR assay. In parallel, we performed a colorimetric Glutathione assay to measure GSH levels in infected cells compared to uninfected (Ctr) ones.

Results: Our data showed that B19V DNA accumulated within infected UT7/EpoS1 cells in a time-dependent manner during a 48h-course of infection. The virus led to a reduction of GSH levels respect to Ctr cells.

Discussion and Conclusions: Our results indicate that B19V induces redox changes in infected cells suggesting a possible role of pro-oxidant state in activating specific redox-regulated signalling useful for viral replication. Further studies are in progress to clarify the mechanisms by which redox state alteration could influence B19V replication.

P111 - Antiviral activity assessment of a new class of polyoxometalates with a focus on zika virus

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Introduction

Polyoxometalates (POMs) are discrete polynuclear metal oxides with a large structural and compositional variety, and a multitude of properties resulting in applications in fundamental and applied science. In the search for new antiviral compounds, a minilibrary of three recently synthesized POMs was screened in vitro against a panel of viruses, chosen as representative of different virus characteristics, such as the presence or absence of lipid envelope, a DNA or RNA genome. Subsequently, in order to perform a preliminary study of the mechanism of action, we focused on zika virus (ZIKV), a virus still lacking of specific antivirals and vaccines and representing an emerging issue to the global health system.

Materials and Methods

The antiviral activity of three POMs, one containing tellurium and two containing titanium, was tested against eleven different viruses by in vitro antiviral assays and virus inhibition assays. The POM with the greater selectivity index (SI) was selected and the step of ZIKV replicative cycle putatively inhibited was investigated by specific antiviral assays, as pre-treatment, binding, entry and post-entry assays.

Results

The titanium-containing POMs were active against human rhinovirus (HRhV), respiratory syncytial virus (RSV), vesicular stomatitis virus (VSV), ZIKV, adenovirus (ADV), human papillomavirus 16 (HPV), herpes simplex virus 1 and 2 (HSV1, HSV2), human cytomegalovirus (CMV) and vaccinia virus (VACV), but not against human rotavirus (HRV). By contrast, the tellurium-containing POM showed antiviral activity only against ZIKV and herpes simplex viruses. No one displayed virucidal activity. The titanium-containing POM having the most favorable SI targets the entry step of ZIKV replicative cycle altering virus penetration into the host cell.

Discussion and Conclusions

In this study we identified a new class of POMs with a broad spectrum antiviral activity and for the first time a POM endowed with a strong antiviral activity against the human ZIKV. This titanium-containing POM is able to inhibit the entry process of ZIKV infection and could be the basis to develop an efficient ZIKV antiviral drug.

P112 - COS-7-based model: a reliable system able to support a productive John Cunningham virus infection

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Introduction John Cunningham virus (JCV) is a human neurotropic Polyomavirus whose replication in the Central Nervous System (SNC) induces the fatal demyelinating disease, Progressive Multifocal Leukoencephalopathy (PML). JCV propagation and PML investigation have been severely hampered by the lack of an animal model and cell culture systems to propagate JCV have been very limited in their availability and robustness. We previously confirmed that archetype JCV efficiently replicated in COS-7 cells as demonstrated by the progressive increase of viral load by quantitative PCR (Q-PCR) during the time of transfection and that archetypal regulatory structure was maintained, although two characteristic point mutations were detected during the viral cycle. Hereby we reported an important extension of our previous efforts in defining our reliable model culture system able to support a productive JCV infection.

Materials and methods Supernatants collected from previous COS-7 transfected cells have been used to infect freshly seeded COS-7 cell line. Cells and supernatants were collected once a week until 35 days post-infection (d.p.i.). Viral DNA was extracted from 1×10^6 COS-7 cells. DNA extracted and relative supernatants were quantified using Q-PCR for TAg and used for Western blot analysis. Immunofluorescence (IF) was used to detect VP1 intracellular localization. The NCCR region from cells and supernatants, was amplified and sequenced.

Results An infectious viral progeny was obtained as confirmed by Western blot and by IF. Within COS-7 cells Western blot showed that VP1 protein as early as expressed 7 d.p.i. and its level increased during the infection experiments, reaching the maximum expression at 21 d.p.i. In parallel, Western blot analysis in the supernatants detected a VP1 expression at 14 d.p.i. as consequence of the release of final viral products via host cell lysis. Moreover, VP1 protein was detectable in the cells and in the supernatants up to 35 d.p.i. IF experiments revealed JCV VP1 both in cell cytoplasm and in the nucleus in all time course infection experiment. Finally, during infection, the archetype regulatory region was conserved. The 37 T to G nucleotide transversion within the binding site for the cellular transcription factor Spi-B in box B and the 217 G to A nucleotide transition in box F at the level of the binding site for the cellular transcription factor NF-1 already observed during transfection were maintained.

Discussion and conclusions Importantly, in this study we developed an improved culture system to obtain a large scale production of JC virus in order to study the genetic features, the biology and the pathogenic mechanisms of JCV that induce PML.

P113 - New developments in the prognosis of congenital cytomegalovirus infection

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Introduction: Congenital cytomegalovirus (CMV) infection is the most prevalent infection-related cause of congenital neurological disability and sensorineural hearing loss. Literature data report that CMV-specific cell-mediated immunity (CMI) plays a crucial role in the control of CMV infection. Several authors have suggested that the QuantiFERON®-CMV (QFN®-CMV) assay may be a useful tool in the clinical management of CMV infection in the post-transplant setting. The aim of this study was to evaluate the QFN®-CMV assay in congenitally infected newborns in order to identify a reliable prognostic immunological CMV-specific marker in compliance with severe neonatal CMV disease.

Materials and methods: Twenty neonates with congenital CMV(cCMV) infection were enrolled in the study. Thirteen out of 20 (65%) neonates were asymptomatic and 7/20 (35%) neonates were symptomatic at birth. QFN®-CMV (Qiagen-USA) assay was performed on blood samples collected within the end of the second week and second month of life. At these two specific time-points, quantification of CMV-DNA by real-time PCR assay (ELITechGroup-Italy) was also performed. The results of the immunological CMV-specific assay were correlated with whole blood CMV-DNAemia as well as with neonatal and long-term outcome. The QFN®-CMV assay measures CMV-specific CMI evaluating IFN-gamma secretion by CMV-specific CD8 positive T-cells. The assay and the interpretation of IFN-gamma response was performed as per manufacturer's instructions.

Results: Among the 13 asymptomatic infants, the 92.3% (12/13 infants) had positive QFN®-CMV results at both time-points or at the second time-point. All the symptomatic infants (100%) at birth (neuroimaging abnormalities, hearing loss and disseminated disease) had negative/indeterminate QFN®-CMV results at both time-points. At the second time-point, the infants with negative/indeterminate QFN®-CMV results showed significantly higher median level of CMV-DNAemia compared to infants with positive QFN®-CMV results (P=0.04).

Discussion and Conclusions: QFN®-CMV assay seems to be reliable in evaluating CMV-specific CMI in cCMV infection. The presence of CMV-specific CMI appears to correlate with asymptomatic infection at birth and during follow-up (study in progress).

P114 - Citrullination during Human Cytomegalovirus infection: implications for autoimmune diseases

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Introduction: 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. Autoimmune diseases (AD) are characterized by chronic inflammation due to an abnormal immune response against the body's own tissue. In genetically predisposed patients, HCMV has been shown to be relevant in the pathogenesis of AD but whether it initiates or supports the development of AD is still not known. Citrullination is a post-translational modification (PTM) catalyzed by peptidyl arginine deiminases (PAD) that convert peptidylarginine into peptidylcitrulline, whose dysregulation has been associated with a spectrum of ADs, cancer, and neurodegenerative disorders. Against this background, the goal of this project is to characterize PTMs such as citrullination during infection with Herpesviruses.

Materials and methods: We investigated the PADs expression in Human Foreskin Fibroblasts (HFF) during HCMV infection using both Real Time quantitative PCR and Western Blot analysis. We also used an in vitro antibody-based assay in order to measured the PAD activity. Furthermore we determined the pattern of citrullination during infection using a citrulline-specific rhodamine phenylglyoxal (RhPG)-based probe.

Results: We demonstrate that HCMV triggers PAD2 expression in HFF both at mRNA and protein levels. Viral replication rate of the HMCV is strongly impaired in the presence of Cl-amidine, a specific pan-PAD inhibitor, indicating that citrullination is required for HCMV replication. Consistently, the depletion of PAD2 by siRNA technology during infection showed decreased infectious yields compared to the control. We then measured the PAD activity in infected HFFs, a striking increase of PAD2 activity was detected at 48 and 72 hpi that was inhibited by Cl-amidine. Interestingly, using the citrulline-specific probe, we determined the overall pattern of citrullination during infection that changes consistently at different time points during infection.
Discussion and conclusion: These findings may shed light on the role of HCMV in the pathogenesis of ADs and provide possible medical interventions for their treatment.

P115 - High cytomegalovirus replication in the foetal hippocampus

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Introduction. Cytomegalovirus (CMV) causes congenital abnormalities in the central nervous system that are irreversible and persist for life. To date, the neuropathogenesis of CMV-induced neural injuries has yet to be fully understood. This study aimed to analyse brain tissues from CMV-infected foetuses to identify the distribution of the CMV-infected cells and tissue viral load in 9 different brain areas.

Materials and Methods. Eight foetuses at 21 weeks of gestation, with documented CMV infection (CMV-DNA on amniotic fluid) were studied. Specifically, tissues from brain cortical areas and underlying white matter, thalamus, hypothalamus, hippocampus, basal ganglia and cerebellum, were analysed by immunohistochemical staining for the CMV early antigen (ppUL44) expression. The histological damage and the tissue viral load were evaluated by haematoxylin-eosin and real-time PCR (ELITechGroup, Italy), respectively.

Results. CMV-positive cells and histological damage were observed in the brain of 6/8 (75%) foetuses. Considering the extension and the severity of pathological findings, in these cases, different degrees of overall brain damage were found: severe (n=1), moderate (n=3) and mild (n=2). For each foetus, the pathological alterations were not localised in a specific brain area but uniformly distributed. Conversely, a higher viral load in 4 brain areas was detected in correlation with severe/moderate brain damage. In particular, the highest median viral load was identified in the hippocampus (197 copies/5 ng DNA; range:10-910 copies/5 ng DNA). In the same area, the highest number of CMV-positive cells was also found.

Discussion and conclusions. It is known that during foetal life, the hippocampus is the brain region with the highest proportion of neural stem cells. Our results suggest that CMV has a preferential tropism for immature and proliferating neural cells.

P116 - The role of HSV1 infection in brain epigenetic aging

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Introduction. Aging is naturally characterized by time-dependent deterioration of multiple biological and cellular functions, and complex molecular changes, including epigenetic post translational modifications (PTMs), are hallmarks of aging. Deregulation in the acetylation levels may be involved in various pathologies, including cancer, viral infections and neurodegenerative diseases.
Herpes Simplex Virus type-1 (HSV-1), a neurotropic virus capable to establish a latent infection in the host trigeminal ganglia and able to reach the brain following reactivations, is known to interact with host epigenetic complexes, but several aspects have yet to be clarified. This study was aimed to investigate the influence of HSV-1 on the host epigenetic aging in the brain, by evaluating specific PTM levels in *in vitro* and *in vivo* experimental models of acute and recurrent HSV-1 infection.

Materials and Methods. Primary cultures of neuronal cells were obtained by E17 rat embryo brains and, after 7 days *in vitro*, infected with HSV-1(0.1 multiplicity of infection) or MOCK solution, then analysed by Western Blot (WB) at 24h (acute infection) or 8 days post-infection (p.i.) to evaluate H3K56 and H4K16 acetylation levels. Long-term infection was obtained treating neurons with Acyclovir (before, during and after infection) to induce virus latency.
Entorhinal cortex homogenates from BALB/c mice were analysed in WB for H3K56 and H4K16 acetylation levels. These mice were HSV1 or MOCK inoculated at 2 months of age, and then subjected to several hyperthermia cycles to induce virus reactivations over their life and sacrificed at 13 months of age. The virus presence in the brain was tested by PCR and RT-PCR analysis of viral TK and ICP4 genes, and IF analysis of gB protein expression.

Results. We found that HSV1 modulates the levels of H3K56 and H4K16 acetylation during acute infection in neuronal cell cultures. Furthermore, a decrease in acetylation levels of H3K56 was found 8 days p.i. and similar effects were found following recurrent infections in mice. On the contrary, H4K16 acetylation was found only slightly altered in both models.

Discussion and Conclusions. These results indicate that HSV-1 infection induce a modulation in H3K56 acetylation during long-term infections, and suggest that the virus may promote epigenetic aging. Further studies will be focused on characterizing how the virus could act on age-related epigenetic hallmarks.

P117 - Role of exosomes in HSV-1-driven neuronal damage

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Introduction: Several evidence, including those from our group, support the role of Herpes Simplex Virus-1 (HSV-1) infection in Alzheimer disease (AD) pathogenesis, a neurodegenerative disorder characterized by the accumulation of misfolded proteins in the brain such as beta-amyloid peptides (Abeta) and neurofibrillary tangles. These are mainly composed by hyperphosphorylated forms of tau (ptau), an intracellular protein able to bind and stabilize microtubules only when in unphosphorylated-native form. Recent studies have demonstrated transfer of Abeta and spread of tau/ptau between neurons in the brain of transgenic mouse models of AD, indicating that these pathological proteins can be propagated in the brain. Specifically, these proteins have been found in neuron-derived exosomes, small extracellular vesicles that recently emerged as key players in cellular communication in both health and diseases, including viral infections. Herein, we investigated whether HSV-1 infection in the brain could promote tau spreading among neurons via exosomes.

Methods: Brain slices from HSV-1- and Mock-infected BALB/c mice undergone several cycles of hyperthermia to induce virus reactivation over their life were analysed in confocal immunofluorescence for viral glycoprotein B (gB) expression and ptau levels, as well as for NeuN expression and DAPI to detect neuronal and cell nuclei, respectively. Human neuroblastoma (SH-SY5Y), glioblastoma (A172) cells and primary cultures of rat neurons were HSV-1- and Mock-infected for 24-48h, then exosomes were isolated from cell supernatants, analysed in western blotting (wb) or incubated on uninfected cells (neurons/glia) for 24h following UV-treatment. Cell lysates were then analysed in wb for ptau content and compared to untreated cells.

Results Exosomes derived from HSV-1-infected cells contained: a) viral gB protein, suggesting that the virus exploits these vesicles for its spreading among cells; b) increased levels of ptau with respect to those isolated from Mock-infected cells, indicating that the virus can promote ptau propagation among neurons via exosomes. Accordingly, cells incubated with exosomes isolated from HSV-1 infected cells showed: a) the occurrence of HSV-1 productive infection, as demonstrated by virus titration in their supernatant; b) higher levels of ptau with respect to those detected in untreated cells, indicating that exosomes have released their cargo into the cells.

Conclusion

Overall, these data indicate that the virus can promote ptau propagation between neurons via exosomes, as well as its own spreading, and support the hypothesis that repeated HSV-1 reactivations into the brain may concur to neurodegeneration.

P118 - Helix aspersa muller mucus (Helixcomplex ®) interferes with Herpes simplex 1 virus infection

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Introduction: Snail mucus is a mixture of active substances commonly thought to have healthy properties for the treatment of skin disorders. Although snail mucus is an ingredient of various cosmetic and para-pharmaceutic products, the biological effects are still unknown.

Materials and Methods: Crude extract from *Helix aspersa muller* mucus (HelixComplex®) was obtained (Patent N: 102017000117547) and tested on *in vitro* experimental models of Herpes simplex 1 virus (HSV-1) infections (0.01 PFU/cell) on Vero cell line (ATCC-CCL81).

Results: In size separation experiments, we observed a peculiar protein band at 50-60 kDa. Protein band was extracted and analyzed by ESI-Q-TOF-HPLC-MS instrument: the protein band in the range of 50-60kDa was composed by a protein complex. After 2 hours from HSV-1 infection of Vero cells, the treatment with 3mg/ml of the protein complex was able to reduce 3 log the HSV-1 titre, similar to the effect obtained with acyclovir 10^-5 M. Interestingly, the anti-viral effect is dose-dependent and it is evident already 24 hours after HSV-1 infection. To be sure that the anti-viral effect was dependent on protein activity, we pre-incubated the protein complex at 60°C for 1 hours. This treatment abolished the anti-viral activity of the *H. aspersa* protein complex and corresponded to a denaturated protein pattern at SDS-PAGE analysis.

Conclusions: These results identify the *H. aspersa* snail mucus protein complex at 50-60 kDa as a suitable anti-viral compound for the treatment of HSV-1 infections, where we need to block HSV-1 spreading. Interestingly, we obtained efficacy results with a natural product that is comparable with pharmaceutical treatments as acyclovir.

P119 - Epigenetic impact in HSV-1 infection via small molecules regulation

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Introduction: Until few years ago we were used to define the epigenetic role in the life as: "The term 'epigenetic' fuses old and new concepts that refer to the modulation of gene expression in cellular heritability, fate, development and programming-reprogramming other than the DNA sequence itself. Epigenetic control of transcription is regulated by enzymes that mediate covalent modifications at gene-regulatory regions and histone proteins around which chromosomal DNA is wound"[1]; actually we have to implement this sentence with the role of epigenetic mechanism in virus infection control, progression and fate. In this scenario, the small molecules that are well known and characterized to modulate the epigenetic mechanism could represent an innovative therapeutic and study approach in virus treatment and dissemination. We investigated the effect of several epigenetic modulators in the HSV infection. Based on their mechanism of action we highlighted the possibility to modulate in positive and negative manner the HSV infection.

Materials and methods: The antiviral activity was evaluated onHSV-1 through cell pre-treatment assay. Cell pre-treatment provides on early incubation of Vero cells with inhibitors MC2884, GSK126 and other. The inhibitors were dissolved in dimethyl sulfoxide (DMSO) to a stock solution of 10 mM and 5 mM, respectively, and were diluted to working concentrations with Eagle's Minimum Essential Medium (EMEM).Vero cell monolayers were treated in a range of concentrations between 30 and 3 µM for 5h at 37°C before being infected with HSV-1 for 1h for all the compounds tested.

Results: Principal target of the epigenetic modulator used are the Histone Acetylation Transferase and the histone methyltransferase EZH2 that are involved in the regulation of the lytic-latency cycle of HSV-1 For this purpose, we have tested inhibitors such as: MC2884, GSK126 and other. Our results underlined that the epigenetic regulation is direct involved in virus replication/infectivity and it is virus MOI (Multiplicity Of Infection) dependent.

Conclusions: In our knowledge this is the first study that showed the HAT/EZH2 inhibitor MC2884 uponHSV-1 infection. The ability to modulate in positive manner the HSV-1 infection should be the base for further studies that should be able to decrypt the mechanism of action of this biological evidence.

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P120 - Human Herpesvirus 6A and 6B inhibit in vitro angiogenesis by induction of Human Leukocyte Antigen G

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1. Introduction

We have previously reported that human herpesvirus 6 (HHV-6) infection of endothelial cells (ECs) induces the loss of angiogenic properties, through the expression of HHV-6 U94, likely associated to the release of a soluble mediator.
On the other hand, the soluble isoform of HLA-G is known to exhibit an anti-angiogenic function, important in implantation, neoplastic and transplantation settings.
This study was therefore aimed to analyze the expression of HLA-G in HHV-6 infected ECs, to evidence any potential role in virus-induced anti-angiogenic activity.

2. Materials and methods

To evaluate HLA-G induction, human umbilical vein endothelial cells (HUVECs) were infected with HHV-6A or 6B, or nucleofected with plasmids expressing virus U94 gene, or treated with recombinant U94 protein. HLA-G induction was analyzed, by RT-PCR, flow cytometry and ELISA. Ability of virus genes to directly induce HLA-G expression was investigated by luciferase reporter assay. Induction of cell transcription factors was analyzed by RT-PCR microarray analysis. Angiogenesis ability was analyzed by capillary-like formation on basal membrane extract.

3. Results

Results showed that both HHV-6A and HHV-6B infection induce a potent up-modulation of HLA-G expression in infected ECs, including both membrane and soluble isoforms. Interestingly, HHV-6A and HHV-6B induced preferentially different isoforms of HLA-G. The virus-induced increase of HLA-G is likely due to the expression of the U94 virus gene, which reproduced the effect of whole virus. U94 effect is mediated by the induction of the human transcription factor ATF3, which recognizes a consensus sequence on the HLA-G promoter, inducing its activation.
Notably, the virus-induced inhibition of ECs angiogenic ability was directly correlated to HLA-G expression and release, since the addition of an anti-HLA-G antibody restored the angiogenic properties of HHV6-infected ECs.

4. Discussion and Conclusions

Our data show for the first time that HHV-6A and 6B infections induce up-modulation and release of HLA-G in human endothelial cells, and that this remodulation, and in particular the release of the soluble HLA-G isoform from infected cells, is directly related to the inhibition of angiogenetic properties observed in ECs upon HHV-6 infection. Furthermore, the results indicates for the first time that virus infection induces ATF3, which is able to interact directly with the HLA-G promoter, finally inducing the HLA-G production associated to virus infection.
It will be interesting to analyze the virus effects also in different cell types, since these might be important in diverse clinical conditions involving not only the regulation of angiogenesis but also the development of immune response and inflammation.

P121 - From bench to business. MicroNature srl an innovative startup of Unicampania

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Introduction: In the last years there was a growing interest toward the exploitation of agro-industrial wastes as natural sources for the production of high added-value compounds. Usually, these residues are burned or used for composting, although they still contain valuable bioactive molecules that can be used for several purposes in many sectors. Even though the early research on active molecules was mainly directed towards pure compounds, an alternative trend focuses on mixtures of natural molecules; indeed, it has been established that natural compounds' blends are more active than the isolated molecules thanks to their synergic activity. Moreover, the increasing demand for biologically active molecules has encouraged the research in new sources of natural compounds. In this perspective, the large amount of agro-industrial residues produced every year represents an exploitable and valuable resource for the production of bioactive molecules. MicroNature srl an innovative Spin-Off of University of Study of Campania Luigi Vanvitelli, has developed a new treatment against Herpes simplex virus type 1 and 2 (HSV-1 and 2) infection through the exploitation of waste in the agro-industrial chain.

Material and Methods: The antiviral activity was evaluated against HSV-1 and HSV-2 through co-treatment, cell pre-treatment, virus pre-treatment and post-treatment assays in a range of concentrations between 0,01microg/ml of solution and 100 microg/ml. The toxicity of natural extract was evaluated via MTT assay at 24h and 48h post stimulation. The Chemical composition was evaluated via filtration, HPLC and Mass Spectrometry.

Results: The natural extract was not toxic on the cellular system used even at maximum concentration of 1 millig/ml. The antiviral activity achieved was in the range of 0,2-2 microg/ml in the virus pre-treatment assay for both HSV-1 and HSV-2. This widespread therapeutic window leads the possibility, *bonafide,* to translate the extract in a commercial product.

Conclusion and Discussion: MicroNature srl is in patent pending for the extraction mixtures from agro-industrial waste with high added value in the field of microbiology. HerpeStopNow is under human phase test. The results achieved lead the possibility to conquer a part of anti-herpetic treatment market.

P122 - A preliminary study on reactive oxygen metabolites in alphaherpesvirus-seropositive Mediterranean buffaloes (Bubalus bubalis)

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Introduction

In recent years, oxidative stress has been postulated to be an important factor in pathogenesis and development of diseases. Reactive Oxygen Species (ROS) are well known for being both beneficial and deleterious and have long been known to be a component of the killing response of immune cells to microbial invasion. In nature, alpha herpesviruses are mostly associated with a single host species. Italian Mediterranean buffalo (*Bubalus bubalis*) is susceptible to bubaline herpesvirus 1 (BuHV-1) as well as to bovine herpesvirus type 1 (BoHV-1). The aim of this study was to determine the association between these two alpha herpesvirus infections and serum reactive oxygen metabolites (ROM) in *Bubalus bubalis*.

Materials and Methods

Five years old buffalo calves were divided in three groups by ELISA results: (1) - buffalo calves seropositive to BuHV-1, (2) - buffalo calves seropositive to BoHV-1 and (3) - seronegative animals. The presence of antibodies against BuHV-1 or BoHV-1 was investigated in blood samples by enzyme-linked immunosorbent assay (anti-gB/gE blocking ELISA). Through the combined use of gB-gE ELISA tests, we assigned a specific infection status, for the BuHV-1 infection status (gB-pos/gE-neg), and for the BoHV-1 status (gB-pos/gE-pos). To assess serum total oxidant and antioxidant levels, d-ROMs and anti-ROM tests were utilized. As indicator of the degree of oxidative stress, the oxidative stress index (OSI) was calculated using the following formula: OSI = d-ROM/anti-ROM x 100.

Results

The results indicated a significant increase of d-ROMs in seropositive BuHV-1 animals compared with seropositive BoHV-1 ($p<0.05$) and seronegative animals ($p<0.01$). An increase, but not significant, was highlighted in seropositive BoHV-1 animals compared with seronegative animals. No significant difference was detected in comparing the anti-ROM values of the experimental groups. OSI was significantly higher ($p<0.05$) in group 1 than in control group 3, whereas no significant difference was detected between the groups 2 and 3.

Discussion and Conclusion

The interplay between oxidative stress and herpesviruses infection has been extensively studied neither in human nor in veterinary medicine. In particular, very few information exist about pathological aspects of biological relationship in *Bubalus bubalis* infected by alpha herpesviruses. In the present study our results suggest that in Bubalus bubalis the BuHV-1 seems to induce a worsening balance in ROMs levels with respect to BoHV-1. Taken together, our results provide for the first time insight into the ROS modulation induced by herpesvirus in buffalo.

P123 - Measles virus and CNS adaptation

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1. Introduction. Measles disease is generally self-limited however can lead to life-threatening complications related to the transient immune suppression and to central nervous system (CNS) invasion, which may lead to severe neurological sequela such as measles inclusion body encephalitis (MIBE) and subacute sclerosing panencephalitis (SSPE). A recent report has shown that SSPE caused by measles virus (MV) mutants that persist in the brain despite high levels of neutralizing antibodies, is more common than previously thought. Paramyxoviruses, including the human pathogen MV, enter host cells by fusing their viral envelope with the target cell membrane. This fusion process is driven by the concerted action of the two viral envelope glycoproteins: the receptor binding protein (H) that recognizes specific proteinaceous receptors on host cells, and the fusion protein (F). However, mechanisms by which MV enters the CNS and triggers the disease remain unclear.

2. Materials and Methods. We have applied fundamental research tools to investigate features responsible for neurotropism in these early brain lesions.

3. Results. We found that CNS-adapted viruses had amino-acid substitutions in the ectodomain of the F protein and functional analyses of the fusion machinery indicate that promote fusion with less dependence on interaction with known MV receptors. In addition, recombinant viruses expressing mutated fusion machinery spread in the absence of known MV receptors.

4. Discussion and Conclusions. Our findings indicate that alterations to the fusion machinery that promote fusion and enable cell-to-cell dissemination without the need to engage regular MV receptors, which are not present on neurons, may be a key change that is responsible for neuroinvasion. However, the existence of an alternative MV receptor present notably on neurons is highly suspected. In conclusion, we hypothesize that in the absence of effective cellular immunity a MV variant bearing fusion machinery that enabled efficient spread in the CNS underwent a intra host evolution of the fusion machinery that leads to neuropathogenicity.

P127 - Molecular analyses on HPV infections in semen

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Introduction: Human Papillomaviruses (HPVs) are non-enveloped double stranded DNA viruses classified in different genera and several different genotypes. Depending on the genotypes, HPV infections can be asymptomatic or can cause from warts to malignant tumours. Some genotypes, such as HPV18 and HPV16, are considered high risk (HR) HPVs and they are the major cause of cervical cancer. HPV is highly tissue-tropic and infects epithelial cells, but it can also binds other cell types. It is known that HPV can be found in semen but its effects on spermatozoa and male reproductive system are not completely clarified.

Materials and Methods: In order to study the HPV infection in semen, we developed a new molecular approach, based on a differential cell lysis step and DNA extraction, which allows to evaluate virus localization in the different semen components. Samples are genotyped by reverse hybridization assay and DNA presence and quantity in sperms, somatic cells and semen plasma are evaluated by nested PCR and type-specific real time PCR. Moreover, RNA is also extracted from the separated semen fractions and the expression of selected genes is assessed by RT-qPCR. Seminal parameters are also carefully analysed.

Results: Results show that HPV can be identified in every fraction of semen. Different samples can contain the virus in different fractions and more than one HPV genotype can be found in the same fraction. Additionally, our data suggest that only when HPV DNA is detected in spermatozoa can cause a reduction of sperm cell motility that resulted proportional to the viral load. Moreover, preliminary results suggest that samples infected by HR-HPVs show a low viral load. Interestingly, the viral load of the low risk (LR) mucosal HPVs is often much higher. RT-qPCR results suggest that HPV infection could modify sperm maturation pathway causing the impairment of motility, altering the expression of some maturation genes. Indeed, in LR-HPV infected samples the

Discussion and Conclusions: Our results highlight the heterogeneity of semen infections: all semen components can contain viral DNA and viral load can be very different for HR- and LR-HPVs; some samples may carry HPV either in cells or in sperms and other samples in both. Only when viral DNA is localized in the sperm fraction, sperm motility is impaired, otherwise, the infection of ductal cells exfoliating in the semen could influence the expression of genes important for sperm maturation. Therefore, these data confirm that HPV infection in semen, even if asymptomatic in the man, can decrease male fertility and prompt new possible consequences of the viral infection in semen.

P128 - G6PD deficiency and the redox imbalance: new insights into the susceptibility and the immune response to influenza A virus

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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. It has been reported that G6PD-deficiency could increase the susceptibility to viral infections even if the mechanisms are not well elucidated. Our preliminary data showed that in permissive cells influenza virus caused a reduction of G6PD expression and activity, suggesting a mechanism to promote viral replication, while little is known on its role in immune infected cells.

The aim of this study was to deep inside the mechanisms throw which the virus determines the decreased expression of enzyme and its potential correlation with G6PD-related intracellular redox state changes and the evaluation of the role of G6PD on influenza virus infection in innate immune cells.

Materials and Methods: Human epithelial lung cells (A549) and murine macrophage cells (RAW) were infected with influenza A PR8/H1N1 virus. At different time of infections, the expressions of G6PD and mRNA level were evaluated by western blot and real-time PCR. The enzymatic activity of G6PD was evaluated through a colorimetric assay kit. G6PD-silenced A549 cells and normal cells were subjected to influenza virus infection and the expression of G6PD protein by western blot analysis and viral titer on infected cells by TCID50 assay were evaluated. GSH level was evaluated by using a colorimetric assay kit.

Results: We found that the expression level of G6PD and activity decreased in infected cells compared to uninfected ones in both cell lines. Interestingly macrophage cell line showed less activity of enzyme after the infection compared to epithelial cell line. Furthermore A549 cells silenced for G6PD and infected with influenza virus showed an increased expression of influenza viral proteins relative to control-infected cells. TCID50 assay showed a higher viral titer in the supernatants of silenced and infected cells relative to normal infected cells. As expected, virus induced a drop in GSH level, but it decreased even more in silenced and infected cells.

Discussion and Conclusions: G6PD may contribute to virus-induced redox imbalance and viral replication of influenza virus in epithelial cell line and innate immune cells. Further studies are in progress to clarify the mechanisms by which G6PD deficiency could influence the spread of viral infections and the host response against virus.

P130 - Colostrum from mothers of preterm infants contains exosomes endowed with antiviral activity

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Introduction

Human colostrum, also known as first milk, is the secretion that all mammals provide for their newborns during the initial days post-parturition, and represents a unique source of highly concentrated nutritional components and growth factors for the gastrointestinal development. Most importantly, it also plays a peculiar role in newborn protection against allergic, chronic, and infectious diseases. The aim of this study is to test the antiviral activity of human colostrum from mothers of preterm newborns against two viral pathogens clinically relevant for infants, namely respiratory syncytial virus (RSV) and human rotavirus (HRV). In search of non immunological components of human colostrum, we explored the antiviral potential of subcellular vescicles – also known as exosomes - secreted in human colostrum.

Materials and methods

Samples of human colostrum were collected from mothers of preterm newborns. Exosomes were purified and characterized by western blot and electron microscopy. The antiviral activity of exosomes against RSV and HRV was assessed in vitro by standard plaque assays or focus reduction assays, along with the one of colostrum. The step of viral replicative cycle targeted by colostrum-derived exosomes was investigated with specific assays.

Results

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

Discussion and Conclusions

Human colostrum contains several protective factors against infectious diseases. Immune and non-immune components, including maternal antibodies, lactoferrin, lysozyme, cytokines, and lipid compounds have been reported to protect newborns from viral infections. The results of this study confirm the wide spectrum antiviral activity of human colostrum, and they disclose the antiviral activity of colostrum-derived exosomes against RSV and HRV, highlighting their role and contribution in mediating the protective effect of colostrum against viral infections.

P131 - Inhibition of human DDX3 helicase reduces replication of Coxsackie B viruses

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Introduction: Coxsackie B (COXB) viruses are small, naked icosahedral viruses with a positive single-stranded RNA genome and belong to *Picornaviridae*, enterovirus genus. They comprise B1 to B6 serotypes and usually establish subclinical infections in humans that, in neonates and children, may yield a variety of syndromes such as myocarditis, pericarditis, aseptic meningitis, encephalitis, respiratory distress. Recently, inhibitors of human DEAD-box protein 3 (DDX3) helicase exhibited some antiviral activity against several DNA and RNA viruses including HIV, HCV. DDX3 RNA helicase plays a key role in several cellular processes including gene expression, mRNA export and translation, and cell cycle regulation. Since neither vaccines nor specific antivirals are available for COXB, we wanted to assess whether DDX3 inhibitors also hamper replication of COXB5.

Materials and Methods: As part of UNAVIR, a collaborative project funded by Tuscany region, the DDX3 inhibitors UVR01, 02, 03, 05, 08, 10, 11, and 12 were synthesized and provided by Prof. Botta, University of Siena. COXB5 strain, routinely used for serum neutralization assay, was expanded *in vitro* on Kb cells and titrated by limited dilution and plaque assay. Cytotoxicity was determined with standard methods and antiviral activity by plaque assay using 10 $TCDI_{50}$.

Results: Up to 50 microM UVR compounds were well tolerated although UVR03 and 12 reduced cell proliferation. Except UVR02, which showed no activity, all compounds reduced COX B5 replication with IC_{50} from 0.35 to 12.50 microM, at levels similar to Ribavirin, the reference drug for enteroviruses.

Conclusion: DDX3 inhibitors showed remarkable antiviral activities against COXB5 virus and low cytotoxicity and are therefore promising candidates for the development of antiviral drugs against these viruses.

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P132 - Characterization of pre-treatment risk factors associated with failure in HCV-infected patients naive to direct acting antivirals: particular focus on natural HCV resistance

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Introduction: This study aimed to evaluate the presence of natural resistance-associated-substitutions (RASs) and other pre-treatment risk-factors for failure in a National cohort group of

HCV-infected patients naive to direct-acting-antivirals (DAA) starting a first-line treatment with a NS5A inhibitor-containing regimen.

Materials and methods: RASs in NS3/NS5A/NS5B (N=1588/1267/1031) were analysed by Sanger-sequencing in 1686 DAA-naïve patients. 476 had an outcome after first-line of a recommended NS5A inhibitor-containing regimen and a baseline NS5A-test. Differences between the sustained-virological-response (SVR) and virological-failure (VF) group by Fisher's exact test and multivariable logistic-regression analysis, to define risk-factors associated to treatment-response were assessed.

Results: Overall, 519/1686 (30.8%) patients showed at least 1 natural RASs, with NS5A-RAS prevalence of 18.5%. 476 patients (GT1a[166]-GT1b[147]-GT2c[19]-3a[117]-4a/d[27]) had an outcome (432 SVR and 44 VF) after NS5A-regimens: daclatasvir(DCV)/ledipasvir(LDV)/ velpatasvir(VEL)+sofosbuvir(SOF)+/-ribavirin(RBV) N=125/130/45, 3D/2D (paritaprevir/ritonavir +ombitasvir±dasabuvir)+/-RBV N=124/12, grazoprevir(GZR)+elbasvir(EBR)+/-RBV N=39. A different distribution of baseline risk-factors for failure was observed among SVR-patients vs VF-patients, with ≥2 risk-factors more frequently observed at baseline among VF (63.6%) compared to SVR (42.6%, P<0.001). Particularly, natural NS5A-RASs were observed before treatment more in VF-patients 34.1% vs SVR-patients 7.4%; (P<0.001). Furthermore, by multivariable logistic-regression, baseline HCV-RNA>800.000IU/ml (BL hVL), cirrhosis and presence of at least 1 natural RAS regimen-related were all negatively associated to SVR (adjusted odd ratios [95%C.I.]: 0.38 [0.16-0.88], P=0.024; 0.49 [0.25-0.97], P=0.041, 0.80 [0.68-0.94], P=0.008; respectively). No other risk-factors were associated to SVR. Particularly, BL hVL and natural RAS regimen-related were frequently observed before treatment in GT1 3D+/-RBV-failures (71.4%, 71.4%) and in GT3 DCV+SOF+/-RBV-failures (80.0%, 30.0%) vs SVR-patients (GT1-3D: 47.7%, 32.1%; GT3-DCV+SOF: 42.2%, 4.4%, respectively, P<0.05). No specific associations were found in GT1/GT4 LDV+SOF+/-RBV treated patients (113 SVR, 17 VF). All treated patients GT2 (12 DCV+SOF, 6 VEL+SOF+/-RBV), 38 GT1 (GZR+EBR+/-RBV), 39 GT1-3 (SOF+VEL+/-RBV) achieved SVR regardless of baseline risk-factors and length duration. The majority of these patients were without or only 1 risk-factor.

Discussion and Conclusions: The presence of specific pre-treatment risk factors, such as RAS regimen-related (mostly NS5A-RAS), BL hVL and/or cirrhosis, was associated with virological failure for some specific regimens in GT1 or GT3.

P133 - Selection of Resistance Associated Variants in patients experienced Direct Acting Antiviral therapy failure

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Introduction: New therapies based on Direct Acting Antivirals (DAAs) represents a milestone for HCV eradication and many inhibitors against NS3/4A protease, NS5A RNA-binding phosphoprotein and NS5B RNA-dependent RNA polymerase (RdRp) were included in interferon-free combination therapies. DAAs regimes achieved HCV eradication in >90% of cases. The choice of treatment strategy depends on HCV genotype/subtype and on the presence of resistance-associated variants (RAVs) in DAAs-experienced patients.

Materials and Methods: HCV NS3, NS5A and NS5B sequences were obtained from serum sample of 12 patients HCV/1b or HCV/4 (1/12) infected at start of DAAs therapy (To) and at virological rebound (Tr). Patients had been treated with Ledipasvir plus Sofosbuvir (5/12), Paritaprevir, Ombitasvir plus Dasabuvir (4/12), or with the new regimes Velpatasvir plus Sofosbuvir (2/12) and Grazoprevir plus Elbasvir (1/2). No patient studied has a history of failure at previous therapy with DAA. HCV sequences were analyzed by visual inspection and by geno2pheno tools (https://hcv.geno2pheno.org). HCV HLA-I restricted epitopes were obtained from Immune Epitope Database and Analysis Resource (IEDB, www. iedb.org) and binding score variations were evaluated through IC50 Artificial Neural Network (IC50 ANN) score.

Results: Sequence analysis confirmed the belonging to genotype 1b or 4d of HCV isolated as previously determined. NS3 sequences from patients treated with Paritaprevir showed at T0 the RAV S122T in only 1/4 isolates while at Tr, the RAVs Y56H plus D168A/V were selected in all four cases. Instead, NS3 sequences from the patient who had failed Grazoprevir therapy showed the selection of none of mutations described, except the polymorphism A150V.
Pattern of multiple RAVs, including mutations at 93th residue of NS5A proteins (Y93C/E/H/Q) were detected in all isolates obtained at Tr. Prior therapy 4/12 isolates showed Y93H often with other RAVs as Q30R, L31M, Q54H, Q62E/K. In NS5B gene, an isolate (1/7) showed the RAV S282T after Sofosbuvir therapy while M204V was selected in 2/4 isolates under Dasabuvir pressure. HCV epitopes map revealed the overlap to RAVs positions in NS3 and NS5A proteins and mutations as Y56H, D168A/V in NS3 and Y93C/E/H in NS5A may affect HLA-binding leading to immune- escape.

Discussions and Conclusions: DAAs therapy failure is rare, often due to genotype misclassification and to selection of RAVs within HCV quasispecies. HCV rapid adaptation may lead to the emergence of variants that can simultaneously escape to host immune response and to therapy reducing the probability of recovery.

P134 - Influence of the HIV-1 integrase genetic background on the resistance pathways observed in vivo in patients treated with integrase inhibitors

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Introduction

The introduction of integrase inhibitors has represented an important step for antiretroviral therapy. Raltegravir was the first representative of a class of integrase strand transfer inhibitors (INSTIs), followed by elvitegravir and more recently dolutegravir and bictegravir. Resistance against these compounds is generally associated with 3 main mutation pathways: Y143C/R, Q148H/R/K orN155H. Of these, the former affects only raltegravir. The molecular mechanisms that drive the selection of a specific pathway arestill poorly understood. The aim of this study was to investigate the influence of the viral genetic background in the integrase gene on the selection of a specific resistance pathway.

Materials and Methods

Four patients treated with INSTIs whose viral isolates naturally developed drug resistance mutations (either N155H or Q148H/G140S) were enrolled in this study. Integrase genes were amplified from plasma samples and cloned into an HIV molecular clone (*pNLmodΔintGFP*) suitable for the phenotypic study of integrase sequences. Site directed mutagenesis was applied to these clones to reproduce the wildtype genotype and both the natural resistance pathway (observed *in vivo*) and the alternative pathway for each patient. After transfection of all clones in packaging cells, the supernatants were used to infect a permissive cell line. Fluorescent emission of the reporter GFP was measured during *in vitro* replication of recombinant viruses to calculate the replicative capacity (RC) and the 50% inhibitory concentration (IC50) of raltegravir and dolutegravir.

Results

The combination G140S/Q148H displayed in all clones a higher IC50 compared to N155H, for both raltegravir and dolutegravir. The RC of widtype clones was variable from patient to patient, and he introduction of resistance mutations greatly reducedit, albeit again with a high degree of variability, depending on the viral genetic context. The mutation Q148H determined the maximum loss of RC. However, in clones from patients where it was naturally selected by treatment, its association with the G140S secondary mutation allowed a higher recovery of RC compared to those from patients where the N155H pathway was selected. The mutation N155H had generally less impact on RC, even less so in clones from patients where the N155H was naturally selected.

Discussion and Conclusions

The genetic background of HIV integrase gene at baseline is likely to have influenced the resistance mutational pathway naturally observed in these patients. In particular, despite the lower resistance conferred by N155H, this mutational pathway may be selected in some patients with a peculiar viral genetic background, as conferring a better overall replicative capacity at the drug concentrations obtained *in vivo*.

P135 - Clinical severity in bronchiolitis is influenced by the different RSV-A strains and by the patient Interferon response

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Introduction: Respiratory syncytial (RSV) subtype-A is thought to have a more severe clinical course than –B in bronchiolitis but to what extent the clinical severity depends on the RSV-A infecting genotype remains controversial. Moreover, while patient innate immune system has a key role to clear RSV infection, it participates in disease pathogenesis as well. The RSV-A genotype distribution in Italy has changed over the past 12 epidemic seasons; genotype NA1 predominated up to 2011-2012, replaced in 2012 by the novel genotype ON1. We sought to test the hypothesis that genotype-specific viral loads and clinical severity were related to the Interferon (IFN) response elicited by the specific RSV strain NA1 or ON1.

Material and Methods: Previously healthy infants hospitalized for bronchiolitis in the Paediatric Emergency Department, "Sapienza" University of Rome, from 2005-2006 through 2016-2017 were prospectively tested for 14 respiratory viruses using molecular techniques. Samples positive to RSV only were sequenced. Quantitative expression of RSV RNA (viral load) and of two IFN-stimulated genes, MXA and ISG56 (markers of type I and III IFN activation) were measured in nasopharyngeal washings from 96 full-term infants infected by RSV-A NA1 (N=50) or ON1(N=46). Clinical and patient data obtained with a structured questionnaire were analyzed in correlation with viral loads and gene expression values using SPSS.

Results: ON1 patients had higher viral loads, despite presenting a milder clinical course with respect to NA1. In neither groups, the amount of RSV load correlated with clinical severity. Significantly higher levels of both MXA and ISG56 genes were found in NA1 than in ON1 patients.

Discussion and Conclusions: Infants with the ON1 genotype had a milder clinical course than those with NA1, despite having higher viral loads. On the contrary, a more elevated level of MXA and ISG56 was observed in infants infected with NA1. Similarly to other viral infections, IFN activation may be effective in controlling the amount of RSV replication but may cause detrimental effects on the host.

P136 - Real-word efficacy of HCV subtype 4d treatments: clinical cases

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Introduction: Despite direct-acting antivirals (DAAs) success, treatment failure is related to safety/effectiveness and resistance-associated substitutions (RASs). Genotype 4 (HCV4) has long been considered one of the more difficult genotypes to be treated. Additionally, clinical trials and real-life data are limited about it. We reported two clinical cases of HCV4 positive patients, which failed first line DAA contained regimens.

Materials and Methods: Serum samples of patients were collected at baseline and during follow-up. NS3, NS5A and NS5B genes were sequenced by Sanger method (cut-off 15%). HCV genotype/subtype were confirmed using subtyping tools and phylogenetic analysis. Newly generated sequences at several steps point of therapy and references sequences were aligned and manually edited. Maximum-likelihood was inferred by PhyMLv3.0. Genetic variability analysis to detect RAS, resistance-associated polymorphism (RAP), and polymorphisms was performed by Geno2pheno tool.

Results: The two patients were experienced to peg-interferon (PEG-IFN)/ribavirin (RBV) and infected by HCV subtype 4d. Phylogenetic analysis identified the reappearance of viral strain at week 48 and at week 96 after failure. Patient 1 (liver stiffness 6.7Kpa), receiving 12-week treatment with triple therapy PEG-IFN/RBV plus simeprevir (SIM), had virological breakthrough. In a second line regimen patient 1 received SOF + velpatasvir (VEL), achieving sustained virologic response (SVR). At baseline and at breakthrough samples, we found just NS5A M31L RAP in dominant viral population, which reduces susceptibility to ombitasvir (OMV). Patient 2 had a history of hepatocellular carcinoma (HCC) and failed SMV + sofosbuvir (SOF) therapy. After liver transplantation, in a third line regimen, patient 2 was treated with SOF + ledipasvir (LDV) without success. At baseline of DAA therapies, using population sequencing, S282A RAP related to polymerase inhibitor resistance was detected. In all sequences of patients NS3 116V, NS5A T58P, D105N, D126E and NS5B T130N polymorphisms, not yet drug resistance related, were detected.

Discussion and Conclusions: Viral sequencing of genomic target regions play a role to select the most appropriate regimen for retreatment. Therefore, no RASs were detected at baseline of new therapies. Additionally, phylogenetic analysis excluded a re-infection event. In conclusion patient 1 failed SMV triple therapy, which demonstrated SVR rate of 86% in prior relapsers. Patient 2 failed SOF+SIM (SVR rate of 92%) and SOF+LDV (SVR rate of 95%) therapy before and after liver transplant, respectively. Genotype 4 could have some virological characteristics related to failure, such as lowest inter-genotype diversity (29.03%) than others genotypes.

POSTER RAPPORTI MICRORGANISMO-OSPITE

P139 - Combined anti-mycobacterial and anti-inflammatory response following in vitro stimulation with Hydroalcoholic extract from Origanum vulgare

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Introduction. Plants may provide molecules with possible therapeutic value against infectious as well as non-infectious diseases and cancer and still today represent the main health care source for most of human population. In this study, we analysed the immunomodulatory effects of hydroalcoholic extract gained from *Origanum vulgare*, a plant traditionally known for its anti-oxidative properties, on innate immune cells and evaluated them in terms of activation of anti-microbial and anti-inflammatory response.

Materials and Methods. Hydroalcoholic extract of *Origanum vulgare* (HyE-Ov) was tested on human monocyte derived dendritic cells (DC), type-1 macrophages (M1) and type-2 macrophages (M2) infected or not with *M. bovis* Bacille Calmette-Guerin (BCG) and its immunomodulatory effects were evaluated in terms of phagosome acidification, ROS generation, intracellular mycobacterial viability and TNF-alpha, IL-12, and TGF-beta production. Finally, in order to identify the presence of specific metabolite/s responsible for the observed biological effects of oregano sample, total extract was separated into eight fractions by High Performance Liquid Chromatography with Diode-Array Detection, HPLC-DAD.

Results. The stimulation of BCG-infected DC, M1 and M2 with HyE-Ov significantly reduced intracellular mycobacterial viability, which was associated with ROS generation in all cell types and enhanced phagosome acidification in M1 and M2, only. Moreover, the observed HyE-Ov induced reduction of intracellular mycobacterial viability was dependent by phagosome acidification and by ROS generation, but not by mitochondrial ROS, in all cell types. The stimulation with HyE-Ov of BCG-infected DC significantly reduced TNF-α and IL-12 production and increased TGF-β levels. Finally, the separation of HyE-Ov in eight different fractions by HPLC and their analysis in terms of antimicrobial response on the three cell types and of cytokine production on DC shows that the activation of anti-microbial and anti-inflammatory response is induced by different fractions, suggesting the presence of more than one bioactive molecule within the hydroalcoholic extract.

Discussion and Conclusions. Altogether, these results show that HyE-Ov promotes anti-mycobacterial innate immunity by limiting inflammatory response *in vitro* and suggest that this plant extract may be exploitable as phytocomplex for novel anti-inflammatory and anti-infective host-directed therapeutic approaches.

P140 - Blood transcriptomic analysis of the H56-CAF01 Mycobacterium tuberculosis vaccine shows how recall innate responses are modulated by adjuvant at priming

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Introduction. Transcriptomic profiling of the immune response induced by vaccine adjuvants is of critical importance for the rational design of vaccination strategies. In the present study, we investigated how the vaccine adjuvant used for priming modifies the way the immune system responds to the re-exposure to the H56 *Mycobacterium tuberculosis* vaccine antigen alone.

Materials and Methods. mRNA sequencing was performed on blood samples collected (1, 2 and 7 days after priming and after boosting) from mice primed with the *Mycobacterium tuberculosis* chimeric vaccine antigen H56 administered alone or with the liposomal adjuvant system CAF01 and boosted with the antigen alone. A very low antigen dose was used for the boost in order to select antigen-specific clones of T and B cells and to mimic the challenge with the pathogen. Transcriptomic data were analysed together with immunological data characterizing by both cellular (antigen-specific CD4+ T cells and germinal center B cells in draining lymph nodes) and humoral responses (quantification of H56-specific IgG up to 7 weeks after boost).

Results. Gene expression analysis 2 days after priming showed that the CAF01 adjuvanted vaccine induced a stronger upregulation of the innate immunity modules compared to the unadjuvanted formulation. The immunostimulant effect of the CAF01 adjuvant, used for priming, was clearly seen also one day after boosting, with activation of blood transcription modules related to innate immune response, such as monocyte and neutrophil recruitment, activation of antigen presenting cells and interferon response. Seven days after boost, differential expression of innate response genes faded while a moderate differential expression of T cell activation modules was appreciable. The analysis of the immune response showed a higher frequency of H56-specific CD4+ T cells and germinal center B cells in draining lymph nodes and a strong H56-specific humoral response of mice primed with H56 + CAF01. Transcriptomic analysis of H56-specific $CD4^+$ T cells isolated from lymphoid organs was also conducted to profile gene expression in the mature antigen-specific helper T cell population upon vaccination.

Discussion and Conclusions. Taken together, these data indicate that the adjuvant used for priming strongly re-programs the immune response that, upon boosting, results in a stronger recall of the innate response essential for shaping the downstream adaptive response.

P142 - Microbiome metabolites in biological samples may impact the female and male reproductive health

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Introduction: The genital tract microbiome plays an important role in determining many facets of reproductive health. Nevertheless, its implication in the outcome of assisted reproductive technology (ART) procedures is not widely explored.

Materials and Methods: Using the Ion Torrent PGM, we sequenced 188 samples including the cervical-vaginal lavage, the follicular fluid, the seminal fluid and the embryo culture medium from 47 infertile couples attending the ART. The QIIME 1.9.1 and Galaxy platforms were used for data analysis.

Results: According to LEfSe, *Lactobacillus crispatus* is associated with a positive outcome (LDA score 4.5), *Streptococcus* with the absence of embryo transfer (LDA score 3.1) and *Staphylococcus* with the ICSI procedure (LDA score 3.4). These results are complemented by the prediction of the functional features of the community composition. According to PICRUSt, *Lactobacillus* genus contributes to the methane metabolism which is increased in the positive outcome (LDA score 2.9) while *Streptococcus* genus to the pyruvate metabolism which is increased in the absence of embryo transfer (LDA score 2.6), and *Staphylococcus* to pyrimidine metabolism which is increased in the ICSI procedure (LDA score 2.2). To note, some follicular fluids and embryo cultures show the presence of bacteria such as *Prevotella*, *Ureaplasma*, and *Chlamydia* not identified at the time of sampling in the seminal fluid and vaginal lavage of the matched infertile couple.

Discussion and Conclusions: The study provides insights into the nature of microbiome in ART outcome and suggests the ability of microbial community to drive the local metabolism. *L. crispatus* utilizes methane to produce lactate which maintains the physiological and protective acidic vaginal pH, favouring the positive outcome. Conversely, a pathogen such as *Streptococcus*, associated with the failure of embryo transfer, is able to massively metabolize pyruvate which is essential for the embryo implant. Similarly, *Staphylococcus*, associated with the ICSI procedure, can exploit and redirect cellular nutrients and metabolic pathways towards the bacterial nucleotide biosynthesis, causing the shutdown of the host cellular metabolism and the decrease of pregnancy rate. Furthermore, the microbial colonization of embryo culture medium and follicular fluids strongly confirms the retrograde ascent to the upper genital tract and persistence of previous bacterial infections. Our data highlight that bacterial metabolism is central to the human reproduction and suggest that its modulation could hugely improve the quality of clinical infertility management.

P143 - Gut microbiota dysbiosis and analysis of fecal SCFAs levels in a cohort of toddlers/preschoolers at early diagnosis of autism

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Introduction. Autism spectrum disorder (ASD) is a serious neurodevelopmental condition and studies evidenced a possible role of gut microbiota (GM) in ASD pathophysiology. Up to date assigning a specific microbial signature to ASD is quite difficult also because of the lack of age-restricted cohort studies. We analyzed GM composition and fecal short chain fatty acids (SCFAs) levels in a cohort of young children (2-4 years of age) with ASD at first diagnosis.

Materiel&Methods. Study subjects:11 children with ASD and 14 healthy controls were enrolled for the study. Fecal microbiota analysis: V3-V4 16S rRNA gene sequencing by IlluminaMiSeq platform and data analysis. Bacterial species absolute quantification: droplet digital PCR on the Bio-Rad QX200 platform. Fecal butyrate and propionate quantification*:* gas chromatography. Analysis and prediction of GM functions: PICRUSt analysis.

Results. Among all data collected, we observed a striking decrease of OTUs assigned to *Bifidobacterium longum,* one of the dominant bacteria in infant GM and, conversely, an increase of *Faecalibacterium prausnitzii,* a late colonizer of healthy human gut and a major butyrate producer. Metagenomic data were validated by absolute quantification using ddPCR. PICRUSt analysis showed increased metabolic pathways associated with SCFAs biosynthesis and utilization in accordance to detected increased levels of fecal butyrate in ASD patients.

Conclusion. Our findings indicate unbalance of GM composition and shift in colonization of gut beneficial bacterial species in ASD patients as off early childhood.

P144 - The role of microbioma in endometriosis: a Review

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Introduction: In the third International Consensus Workshop on Research Priorities in Endometriosis in São Paulo, 2014, following the 12th World Congress on Endometriosis at the number 37 of fisiopathology's study reccomandation we find "Role of the microbiome in endometriosis;Metagenomic studies should be undertaken of the microbiome of the reproductive tract and /or the gut in women with or without endometriosis.

Materials and methods: A web search of the literature was conducted on Pub Med,Plos,Hindawi,Google scholar search term as "microbiota'' " estrobolone" "endometriosis" "microbioma endometriosis" ,according PRISMA statement we selected 16 publications .

Results: A first study in 2002 showed rhesus monkeys with endometriosis have higher negative bacteria concentration ,lower lactobacilli concentration and intestinal dysbiosis.In a recent study, the Firmicutes/Bacteroidetes ratio was elevated in mice with endometriosis.The dysbiosis disrupts homeostasis through a reduction of bacterial diversity .Alpha diversity analyzed complexity and species diversity on six indices,species complexity was studied with Beta diversity analysis increased.The estrobolone dysfunction causes a dirsuption of the estrogen metabolism with a elevated beta-glucuronidase producing bacteria .Estrogens are responsible of a Fox3p -regolatory T cells (treg) alteration and only in secretory phase, as mechanism that mediate a tolerance versus ectopic endometrium .The strong correlation with dysbiosis was confirmed by a Danish cohort study on 37661 women with endometriosis had an increased risk of intestinal bowel disease (IBD)overall standardised incidence ratio (SIR) =1.5; 95% confidence intervall (CI)1.4 to 1.7 and of ulcerative cholitis and Crohn's disease. The 16s rRNA genomic sequencing in endometrial samples of women with endometriosis identified in a study ;Streptococcaceae ,an increase in Moraxellaceae and a decrease in lactobacillus spp.- acid lactic producer responsive of high Ph and an increase of E.coli.Also bacterial vaginosis have a decrease in Lactobacilli spp. and an increase in anaerobic bacteria, including Gardnerella vaginalis and Atopobium vaginae.

Discussion and Conclusions: The microbioma of the entire reproductive tract was rarely undertaken in the woman with endometriosis. The host-microbioma immunologic interaction is a target of the complex fisiopathology of the endometriosis.Ulterior studies in estrobolone dysfuntion could confirm the dysbiosis of gut and reproductive tract and a reduced host immunological capability.Estrogens regulate homeostasis of various tissues in a balanced bacterial population.

P145 - Effects of Fecal Microbial Transplantation on gut microbiota, metabolomics, microbial translocation and T-lymphocyte immune activation, in recurrent Clostridium difficile infected patients

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Introduction: Fecal microbiota transplantation (FMT) has been described to restore resistance to recurrent *Clostridium difficile* infection (R-CDI). The desired effect of FMT should be the reconstitution of a healthy intestinal microbial ecosystem. Less known are the complex mechanisms leading to FMT-CDI clinical remission of the extra-intestinal inflammatory reactions induced by microbial translocation (MT) and their metabolites. The study aimed to assess the effect of FMT in R-CDI patients on gut microbiota composition, MT, immune activation of T lymphocytes and metabolomics profiles.
Materials and Methods: *Donors and fecal samples preparation:* At the Policlinico Umberto I Hospital in Rome, healthy volunteers donors (<30 years of age) were subjected to microbiological and parasitological screening to evaluate their suitability as donors. R-CDI patients no responsive to antibiotics treatment, were selected too. *Microbiota characterization:* fecal microbiota composition was evaluated before and after the FMT procedure in all patients and in healthy donor. To this purpose fecal microbiota was characterized through 16S rDNA V3-V4 targeted sequencing. *SCFAs/polyamine profiles*: Short Chain Fatty Acids (SCFAs) and polyamine were analyzed by Gas chromatography–mass spectrometry (GC-MS). *T Cell phenotype:* lymphocyte surface phenotypes were evaluated before (T0) and after (T1) by flow cytometry using fresh peripheral blood. For the activation analysis of T cells CD4+ and CD8+, the following fluorochrome-labeled antibodies were used: Pacific Blue-CD3 (BioLegend, 500 uL), PerCp/Cy5.5-HLA DR (BioLegend, 500 uL), Pe/Cy7-CD8 (BioLegend, 2 mL), CD38-APC (BioLegend, 2 mL), APC/Cy7-CD4 (BioLegend, 2 mL).
Results: FMT was performed successfully in four R-CDI patients. Proteobacteria relative abundance, were significantly decreased and beneficial phylum was increased after FMT. In these preliminary observations, the reduction of LPS binding protein (LBP) appears to be a marker of MT in subjects receiving FMT for R-CDI. After FMT, fecal metabolomics profiles reveal a strong increase of acetyl-putrescine and spermidine, and of short chain fatty acids, acetate and butyrate. The levels of immune activation of T cells $CD4^+$ and $CD8^+$and LBP were decreased and showed a trend for an inverse correlation with $CD8^+$ HLA-DR^+ $CD38^+$.
Discussion and Conclusion: After FMT in R-CDI patients there is the reconstitution of gut microbiota, the improvement of short chain fatty acids profiles, a change of polyamines profiles, and a reduction of LBP, as an expression of MT. FMT reconstitution of *eubiosis* is not only helpful as a radical cure of CDI, but also as an immune-modulatory mediator able to positively affect the degree of MT and T-cell immune-activation.

P146 - Gut Microbiota Analysys in Hav infected patients with or without HIV coinfection

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Introduction

Increasing evidence have been accumulated about the importance of gut microbiota in basic metabolic processes, as well as in the pathogenesis of several viral infection. A number of studies have been performed on the influence of gut microbiota on chronic hepatitis diseases progression, while many reports highlighted that in HIV infection gut microbiota dysbiosis is correlated to a persistent systemic immune activation. Aim of the present work is to describe the gut microbiota composition in patients infected with an acute hepatitis virus (HAV) in subjects with or without HIV co-infection.

Materials and Methods

Fecal samples were collected from seven HAV mono-infected patients and from 2 HAV/HIV co-infected subjects under successful cART therapy (median CD4 667 IQR 427-907 cells/µl). They were all Italian males with a median age 33 (IQR 29.5-38.5) All but one HAV/HIV co-infected patient, displaying only antibody positivity to HBV core antigen, were HBV and HCV negative. Fecal samples underwent nucleic acid extraction with an automated platform (QiaSymphony, Qiagen), then on extracted DNA V3 and V4 regions of the 16S rRNA gene were amplified using Illumina protocol to prepare 16S Ribosomal RNA library to be sequenced on the Illumina MiSeq platform. A specific tool for Illumina 16S data (IMNGS) was used to assess taxonomy for each correct read. Alpha diversity was evaluated calculating the Shannon index for each genus and was further stratified by phyla. Comparison among groups was performed using Mann-Whitney test.

Results

A median of 155,700 (IQR 124,703-204,431) reads were obtained among samples. Of these, the taxonomy tool identified a median of 38,669 (IQR 28,477-49,531) reads for sample.

In all but one patient, Bacteroidetes phylum was the most represented, being Prevotella genus the most abundant in 5 out of 7 gut microbiota in HAV mono-infected and in all HAV/HIV co-infected patients.

Median alpha diversity was 1.4 (IQR 1.0-1.8) in HAV mono-infected and 2.3 (IQR 2.2-2.4) in HAV/HIV co-infected patient ($p=0.11$); when the Shannon index was stratified by phyla, Bacteroidetes alpha diversity was 0.4 (0.2-0.8) in HAV mono-infected and 0.5 (0.1-0.8) in HAV/HIV co-infected patient ($p>0.99$); Firmicutes alpha diversity was 1.8 (1.7-1.9) in HAV mono-infected and 2.1 (1.9-2.2) in HAV/HIV co-infected patient ($p=0.22$)

Discussion and Conclusions

The present data, obtained on a small group of subjects, may suggest that in patients infected with an acute hepatitis virus (HAV) gut microbiota remain quite heterogeneous, with Prevotella as the most represented genus. In addiction, HAV infection seemed not to alter gut microbiota of successful treated HIV co-infected patients. Additional experiments are needed to further address these points.

P147 - Distinct signatures of the gut microbiome in an italian cohort of patients with parkinson's disease

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Introduction: Parkinson's disease (PD) is a neurodegenerative disorder characterized by the accumulation of mutated α-synuclein abnormally aggregated (Lewy bodies) at various levels of the cerebral axis. Interactions between the intestine and the brain are significantly modulated by the gut microbiota through immunological, neuroendocrine and neurological mechanisms: several studies show the close association between the alterations of the intestinal microbiota and PD, which may also precede or occur during the course of the disease.

Materials and Methods: The composition of the microbiota in fecal extracts of 63 PD patients and 58 controls was determined. Degenerate primers targeting the V3 and V4 hypervariable region of the bacterial 16S rRNA gene were utilized for PCR amplification and libraries construction. Analysis of the data generated on the Miseq System was carried out using the BaseSpace 16S Metagenomics App (Illumina), whereas operational taxonomic unit (OTU) mapping to the Greengenes database (V.13.8) was performed using the Quantitative Insights Into Microbial Ecology (QIIME) platform (V.1.8.0).

The PICRUSt and KEGG software packages were used to predict metagenome functional content from 16S rRNA genes. GraphPad Prism (version 7.01) and SPSS softwares were utilized to perform univariate and multivariate statistical data analysis.

Results: The relative abundances of the *Bacteroidetes*, *Actinobacteria*, *Proteobacteria*, and *Verrucomicrobia* phyla showed significant differences between patients and healthy controls using univariate analysis. Differences were also detected in the *Bacteroidaceae*, *Lachnospiraceae*, *Lactobacillaceae*, *Prevotellaceae*, and *Verrucomicrobiaceae* families, such as in the *Akkermansia*, *Bacteroides*, *Bifidobacterium*, *Blautia*, *Clostridium*, *Escherichia*, and *Lactobacillus* genera. Finally, abundance of several species (among these *Akkermansia muciniphila*, *Bacteroides rodentium*, *Bacteroides uniformis*, *Bacteroides vulgatus*, *Escherichia albertii*, and *Prevotella copri*) was significantly different in patients with PD and in controls. The multivariate analysis showed further distinctive differences when various covariates were considered (e.g. age, BMI, coffee, smoking, constipation, disease phenotype, treatment). Functional predictions revealed distinctive pictures in numerous metabolic pathways, according to the disease phenotype and the type of pharmacological treatment.

Discussion and Conclusions: In agreement with previous results, the study highlights altered abundance of several taxa and distinct functional pathways and, in particular, shows independent effects of PD medications on the microbiome. The findings provide interesting hypotheses on the pathophysiology and treatment of PD.

P148 - Mucosa-associated microbiota impact on epigenetics factors influencing genes expression in Crohn's disease patients in remission status

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Introduction. Crohn's Disease (CD) is a chronic inflammatory disorder of the gastrointestinal tract. The impact of epigenetic modifications to disease pathogenesis has recently been considered. Recent studies have shown that the interaction between host genome and environment play an important role in the phenotypic expression of diseases. Since the importance of this interaction in the onset of Inflammatory Bowel Disease (IBD), it has been hypothesized that environmental factors, such as gut microbiota and its metabolites, could act as epigenetic modulators influencing host genes expression, associated with disease development. One of the main problems in CD is the maintenance of the remission status, once has been reached. The aim of the present study was to evaluate the impact of the mucosae associated microbiota (MAM) on methylation level of gene promoters related to inflammation and immunity.

Materials and Methods. Ileal biopsies were collected from 21 CD patients in remission (CD) and from 27 healthy control (HC) matched for sex and age. Total DNA extracted was used to: 1) characterize the MAM by Next Generation Sequencing (NGS); 2) evaluate the methylation level of gene promoters related to inflammation and immunity in 12 CD patients and 12 HC. Furthermore we evaluated the expression of genes related to methylation process of the inflammation pathways, by RNA extraction from a second bioptics samples. All data collected were analysed by means of cross-correlations and networks.

Results. Patients with CD in remission showed: i) a still persistent *dysbiosis status*; ii) a significant decrease in methylation level of IL13Ralfa1 gene promoter; iii) an increased expression of IL13Ralfa1 gene (corroborated by the lower level of promoter methylation), JAK1 and DNMT3B, and a significant decrease in the expression of the CECAM6 gene.

Discussion and Conclusions. The analysis of data collected indicates that: the methylation level of the IL13Ralfa1 gene promoter was positively related to a specific bacterial consortium in CD patients. The existence of bacterial consortia impacting on the activation/or suppression of inflammation genes expression, could open the way for new therapies. The modulation of the intestinal microbiota could influence the inflammation status preserving, once it has been reached, a CD remission status.

P149 - Mapping and comparing bacterial microbiota in the anterior nares of allergic subjects

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Introduction. Anterior nares are an important niche for bacterial colonization by both commensals and opportunistic pathogens. Although abnormalities in the nasal human microbiota have been associated with the etiology of allergic disease, to date, no studies have comprehensively compared bacterial communities over time. This study investigated the temporal dynamics and variations of the global nasal bacterial community across 94 subjects (age range 3-14 years) with allergic rhinitis (AR), adenoid hypertrophy (AH), and healthy controls.

Materials and Methods. Bacterial microbiota was characterized by sequencing the V3-V4 region of the 16S ribosomal RNA (rRNA) gene. Nasal swabs were sampled during different seasons and the relationship of mucosa-associated microbiota with disease occurrence, different clinical variables such as inflammation markers and rhinomanometry measures were analyzed. After filtering raw data, a bioinformatic pipeline was carried out to analyze biodiversity, microbial composition, and correlations with collected clinical variables.

Results. Results showed no differences in alpha and beta-diversity among groups and for each group over time. Significant differences in biodiversity respect to vaccine administration and exposure to smoke as well as significant correlations between biodiversity and spirometric values have been observed. For what concerns microbial composition, a high interindividual variation was observed in the relative abundance of taxa in contrast with high individual composition stability over time. *Proteobacteria*, *Actinobacteria*, and *Firmicutes* represented the major phyla within nasal microbiota while *Pseudomonas*, *Acinetobacter*, *Moraxella*, and *Corynebacterium* were the most abundant genera constituting a stable core microbiota. Although representing low relative abundance, the genus *Haemophilus* was significantly more present in healthy subjects respect to AR suggesting a possible association to the healthy status. Network analysis evidenced significant correlations between taxa among the whole studied population as well as within groups, suggesting microbial interdependencies in nasal microbiota.

Discussion and Conclusion. Recent studies evidenced a significant association between microbial composition and the onset and progression of allergic or nonallergic inflammation. In this study, we found that although individuals have their own unique bacterial fingerprint, it is possible to delineate a stable nasal core bacterial community. Moreover, the results suggest that vaccination and exposure to smoke rather than allergic disease occurrence affect the composition of mucosa-associated microbiota. Thus, the role of bacteria in the pathogenesis of allergic diseases remains uncertain.

P150 - Epinecrotic and thanatomicrobiome in human postmortem interval

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Introduction: Forensic microbiologists are developing new application to investigate the dynamic and coordinated changes in microbial activity that occur when a human host dies. The components of the human postmortem microbiome include the thanatomicrobiome (the microbiome of internal organs of cadavers) and epinecrotic microbial communities (the microbiome on surfaces of decaying remains). Our study provides a critical examination of the structure and function of these post-mortem microbiomes in order to clarify how time elapsing postmortem affects the bacteria communities and to presumptive estimate of postmortem interval (PMI) in low temperature conservation.

Materials and Methods: To characterize the culturable aerobic and anaerobic bacterial communities during the decomposition, we swabbed with sterile cotton swabs the eye, ear, nose, buccal cavity and rectum (epinecrotic distrects) and brain, spleen, liver, heart (thanatomicrobiome) of 20 human cadavers. Moreover on the basis of the time elapsed postmortem, the samples were divided into 3 groups: 1-3 days; 4-7 days and > 7days. Bacterial swabs were streaked on different culture media plates. The isolated colonies were identified using matrix- assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF)

Results: The bacteria cultured from the epinecrotic and thanatomicrobiome districts were subdivided in 3 groups, consisting of Phyla Proteobacteria, Firmicutes and Actinobacteria. In each anatomic area, distinct compositional changes were observed at the phyletic level, according to variation of the PMI.

Discussion and conclusions: Our data show that the culturable aerobic and anaerobic bacteria communities change over time. In particular we hypothesized the initial postmortem microbiome would be a reflection of the host microbiome preceding death in a manner that correlates with health status, which could be valuable for comprehensive human microbiome surveillance.

P151 - Quality of dietary carbohydrates affects gut microbial community of phenylketonuric subjects

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Introduction - Low-phenylalanine diet, the cornerstone treatment for phenylketonuria (PKU), has been shown to increase glycemic index (GI) and glycemic load (GL), affecting the availability of substrates for microbial fermentation. Indeed, changes in the PKU gut microbiota and in microbial metabolites have been previously reported. Here we compared gut microbial communities of children with PKU and with mild hyperphenylalaninemia (MHP, unrestricted diet).

Materials and Methods - Forty-two children (21 males/21 females, 9–13 years old) were enrolled in the study. We assessed dietary intake and performed gut microbiota analysis by next-generation sequencing using V3–V4 hypervariable 16S rRNA genomic region.

Results - While alpha-diversity analysis revealed no significant differences between PKU and MHP groups, phylogenetic analysis highlighted a significant separation of gut microbiota according to both unweighted (p=0.008) and weighted Unifrac distances (p=0.03). Major differences were seen within the *Firmicutes* phylum. Indeed, PKU children were depleted in *Faecalibacterium* spp. and enriched in *Blautia* spp. and *Clostridium* spp. We found a divergent response of members of the *Firmicutes* phylum with respect of daily glycemic index, higher in PKU children. *F. prausnitzii,* unclassified *Ruminococcaceae* and, to a lesser extent *Roseburia* spp. negatively correlated with GI, whereas other *Lachnospiraceae* (unclassified) were positively associated.

Indicator species analysis suggested *Faecalibacterium prausnitzii* to be related to MHP status, whereas *Ruminococcus bromii* to be associated to PKU.

Discussion and Conclusions - Despite PKU children have a higher vegetables and fiber intakes, considered good substrates for beneficial microbes, the quality of carbohydrates ingested seems to particularly affect *F. prausnitzii* abundance, considered a biomarker for a healthy status. It still remains to evaluate whether an improvement of current free-amino acid formulas could rebalance the microbial community.

P152 - Cultivating gut microbiota: a 3D in vitro model

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Introduction: *In vitro* modeling of the gut microbiota is of upmost importance for studying the interaction between microorganisms and the host in health and disease. In this study, a 3D *in vitro* model of the gut microbiota was reproduced on electrospun gelatin structures**.**

Materials and Methods: The 3D model of gut microbiota was developed *in vitro* by growing microorganisms on gelatin structures. These structures were obtained by electrospinning a solution of gelatin, acetic acid and (3-Glycidoxypropyl)trimethoxysilane as crosslinking agent with a Linari apparatus. Structures were dried to ensure solvents evaporation and then sterilized with 70% ethanol and UV light exposure. The gut microbiota was recovered from a fecal sample collected from an healthy donor and treated following the recent European Guidelines for fecal microbiota transplantation. Gelatin structures were laid on round cover glasses (∅ 30 mm) into 6-wells plates, covered with broth medium and inoculated with an aliquot of filtered fecal suspension. Plates were incubated at 37°C in anaerobic atmosphere. At different time intervals, samples were analyzed by confocal laser scanning microscopy (CLSM) of DAPI-stained preparations and by the crystal violet 0.1% adhesion assay. Diversity of the microbial populations grew on the gelatin structures was evaluated by amplicon sequencing of the16S rDNA.

Results: Fecal microorganisms were able to grow, adhere to the gelatin structures, and form biofilm consortia. 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 within the structures. Results of the microbial diversity analysis will be shown.

Discussion and Conclusions: Stable 3D microbial consortia potentially useful as a model for the gut microbiota were obtained, indicating that the electrospun gelatin structures can constitute an appropriate substrate to reproduce the 3D gut environment. This model could be used for studying microbial interactions in the gut environment, production of metabolites, and activity of drugs and probiotics, as well as in more complex biological platforms for investigating the physiological and pathological interactions between gut microbiota and the host.

P153 - Gastrointestinal microbial population and impact of antibiotic treatment in early infants.

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Introduction. The gastrointestinal microbial community is dynamic during the first years of life, before stabilizing to an adult-like state. However, little is known about the impact of antibiotic treatment on the developing human gut microbiome. Here we report a microbiological study on the gut composition of infants, approximately half of whom received multiple courses of antibiotics during the six months of life.

Material and Methods. Fecal samples were collected from 56 infants about six months age, hospitalized at the Regina Margherita Children Hospital and affected by various diseases (bronchiolitis,urinary tract infections,pneumonia,etc).Specimens were processed at Public Health and Pediatrics Dept., Microbiology Section.Two groups were selected according to the antibiotic therapy (A=32) or not (B=24). Stool samples were collected from diaper and cultured on selective media to detect lactobacilli, bifidobacteria, enterococci, *Enterobacteriaceae* and yeasts. The qualitative analysis was performed using Gram staining and by biochemical methods (API System). Moreover, we considered the number of different taxa (species or strains) identified after antibiotic treatment (ceftriaxone, amoxicillin+clavulanate acid, clarithromyicin).

Results. On average,the infants not treated with antibiotics had a richer microbial community compared to the infants who received antibiotics: *Enterobacteriaceae* (B=$3.6x10^8$ *vs* A=$1.2x10^6$ CFU/gr), aerobial total count (B=$4.7x10^{10}$ *vs* A=$2.5x10^8$ CFU/gr), bifidobacteria (B=$3.6x10^5$ *vs* A=$1.4x10^3$CFU/gr).We observed that lactobacilli were detectable less frequently in the guts of infants treated with antibiotics than in the control group (B).
The data obtained show that in A, breast or artificial feeding contribute to re-establish an intestinal bacterial balance similar to B group. Moreover, the investigation revealed that the gut microbiota of the clarithromyicin and ceftriaxone-treated infants had significantly higher proportions of enterococci than the controls ($3x10^9$, $1.2x10^9$ *vs* 9.4 $x10^8$, respectively),while the *Bifidobacterium* genus was predominant in ceftriaxone and amoxicillin+clavulanate acid groups compared to untreated group ($7.8x10^9$,$1.6x10^9$*vs* $8.4x10^7$). A larger percentage of subjects receiving antibiotics (12%) was colonized by *Candida spp.* than the controls (8%).

Discussion and Conclusions. Our findings showed that antibiotics treatment in early infants affects all aspects of gastrointestinal microbial population, including species richness, diversity, community structure and the abundance of colonizing bacteria genera. However, the subsequent lactation with breast milk or artificial milk restore the altered population of lactobacilli and bifidobacteria to values comparable to those of children not treated with antibiotics

P154 - The oropharyngeal microbiome diversity in healthy individuals and in celiac disease patients

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Introduction: Celiac disease (CD) is a chronic immune-mediated enteropathy of the small intestine triggered by gluten in genetically predisposed individuals expressing the HLADQ2/DQ8 molecules. Literature data reported that gut microbial alteration has been hypothesized to contribute to the CD pathogenesis. The gut microbiota has a crucial for the well-being of the human host, and its dysbiosis has been suggested to play a pathogenic role in several diseases, including autoimmune and gastrointestinal disorders. In this context, we recently profiled duodenal microbiome in active (a-), gluten-free diet (GFD), celiac disease (CD) adult patients and controls finding higher levels of the Proteobacterium *Neisseria flavescens* in a-CD patients than in the other two groups. *N. flavescens* was able to induce inflammation in both murine and human dendritic cells, and it was observed in a-CD microbiome with respect to those of the other two groups. Here, as gastrointestinal tract could be considered a single ecosystem extending from the oral cavity to the rectum, we investigate the oropharyngeal microbiome in CD patients and controls to evaluate whether this niche share microbial composition with the duodenum.

Material and Methods: We characterized the oropharyngeal microbiome in 56 subjects by 16S rRNA gene sequencing: 14 a-CD, 22 GFD patients and 20 controls. Sequences were analyzed using QIIME v. 1.9.1. bioinformatics tool and the microbial functions prediction by PICRUSt. Aerobic/facultative anaerobic microbiota were also cultured, isolated and identified by mass spectrometry. **Results:** Our results highlight interesting similarities between the duodenal and oropharyngeal microbial alterations in a-CD patients. Proteobacteria abounded in a-CD and *Neisseria* species mostly accounted for this abundance, whereas Bacteroidetes were more present in control and GFD microbiomes. Culture-based oropharyngeal microbiota analysis confirmed the greater abundance of species belonging to the *Neisseria* genus in a-CD than in either GFD and controls. The prediction of the microbial functions indicated a greater metabolic potential for degradation of aminoacids, lipids and ketone bodies in a-CD microbiome than in control and GFD microbiomes, in which polysaccharide metabolism predominated.

Discussion and Conclusions: Our results confirm the increased presence of *Neisseria* strains in a-CD oropharyngeal microbiome suggest a continuum of a-CD microbial composition from mouth to duodenum. We may speculate that microbiome characterization in the oropharynx, which is a less invasive sampling than the duodenum, could contribute to investigate the role of dysbiosis in CD pathogenesis.

P155 - Evaluation of bacterial flora of cranial vagina in fertile and infertile bitches

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Background and Aim

In the literature few data are present about the effect of vaginal bacteria overgrow on pregnancy rate. *Mycoplasma* of the reproductive tract are currently considered to be opportunistic, but their possible pathogenic role can be hypothesized. In this cross-sectional study we evaluated the bacterial overgrowth in the cranial vagina in bitches with a history of pregnancy failure (IG-infertile group) or not (FG-fertile group), identifying the most common species of *Mycoplasma* and *Ureaplasma* and their antimicrobial susceptibility.

Material and Methods

Ninety-five bitches have been evaluated for ovulation timing before mating/insemination with fresh semen. During the gynaecological examination microbiological samples were collected from the cranial vagina using a sterile uterine culture swab, which was then added in solid amies medium and stored at 4°C for 24-36h. The samples were cultured for *Enterobacteriaceae*, *Pseudomonadaceae*, *Streptococcus* spp, *Staphylococcus* spp, *Enterococcus* spp, *Ureaplasma* spp, *Mycoplasma* spp and considered positive when Colony-Forming Unit/sample (or Colour-Changing Units for Mollicutes) $>10^2$. Bacterial strains were tested for antimicrobial susceptibility and, if positive, bitches were treated with specific antibiotics before or just after mating/insemination. Following pregnancy diagnosis and evaluation of fetal viability, the number of pups delivered for each bitch was registered.

Results

Twenty-four out of 35 and 39/60 bitches were positive for culture in FG and IG, respectively. The logistic regression model evidenced that, the Odds Ratio of the pregnancy failure, regardless of culture positivity, in IG was 3.1 (95% C.I. 1.05 – 9.20; $p<0.05$). DNA sequencing identified *Mycoplasma spumans* and *Ureaplamsa diversum* as the main isolated *Mollicutes* in cranial vagina. Antimicrobial susceptibility test carried on *Mollicutes* revealed that the highest sensitivity was to doxycycline and a high numbers of bacterial strains were resistant to macrolides.

Conclusions

No significant differences between groups in regard with bacterial overgrowth or any specific positivity for bacteria and eventual treatment were found. A moderate tendency that IG being positive for *Ureaplasma* spp (Odds Ratio 4.0; p=0,10) was observed. *Streptococcus* spp and *Ureaplasma* spp are significant risk factor for pregnancy failure in both groups despite treatment. Further evaluations are ongoing to expand the caseload.

P156 - Quality of probiotic formulations containing Bacillus spores

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Introduction. Spores of several *Bacillus* species have long history of consumption and safe use as probiotics, defined as live microorganisms that, when administered in adequate amounts, confer a health benefit on the host. Considering the importance of quality control for probiotic products for human consumption and the difficulties in the identification of *Bacillus* species, we analyzed the content of ten probiotic formulations marketed in Italy and claiming in the label to contain *Bacillus* strains, by bacterial enumeration and identification with different methods.

Material and Methods. Microbial enumeration was performed by the plate count method. For species identification, we compared the performance of biochemical tests based on the BCL2 Vitek card and MALDI-TOF mass spectrometry, using 16S rDNA sequencing as the reference technique. Molecular typing was performed by RAPD-PCR using eight different primers. The virulence potential of *Bacillus cereus* isolates was evaluated by PCR for virulence-encoding genes and by *in vitro* assays for phospholipases.

Results. Microbial quantification showed that the number of viable cells contained in many of the analyzed products differed from the labeled content. In some formulations, the recovered species were different from those declared. In addition, contaminant bacteria were sometimes detected (*Lysinibacillus fusiformis*, *Bacillus cereus*, *Acinetobacter baumannii*, *Bacillus licheniformis*, and *Bacillus badius*). Characterization of the *B. cereus* isolate showed the presence of many virulence determinants this species possesses. Overall, our data show that the content of only two of the ten analyzed formulations is quantitatively and qualitatively concordant with the label.

Discussion and Conclusions. Quality control of probiotics is the focus of numerous organizations worldwide, with the ESPGHAN recently highlighting the importance of a more stringent control of commercial probiotic products for human use. The finding that most of the analyzed formulations do not fulfill the labeled content suggests that appropriate identification and typing techniques should be applied to the *Bacillus* genus and that quality controls for this class of products should be revised. In fact, in the clinical context, the administration of probiotic products that do not comply quality requirements most likely leads to reduced/absent efficacy of the preparation and represents a potential infective risk for patients, if pathogens or opportunistic pathogens are present.

P157 - Functional analysis of FlhF, a key protein for Bacillus cereus virulence

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1. Introduction. The multidomain (B-NG) protein FlhF is the third paralog of the signal recognition particle (SRP) GTPases Ffh and FtsY. In *Bacillus cereus*, FlhF is involved in controlling motility and is required for microbial pathogenicity in an *in vivo* model. FlhF depletion alters the secretion of several virulence proteins, also causing a significant reduction in the amount of the L_2 component of hemolysin BL (HBL), one of the most potent toxins *B. cereus* produces. Herein we describe the functional characterization of *B. cereus* FlhF as SRP-like protein, its homodimerization, physical interaction with L_2, and protein domains involved in protein/protein interactions.

2. Materials and Methods. qRT-PCR was used to analyze the transcription level of the L_2-encoding gene (*hblC*) in *B. cereus* (WT) and in its *deltaflhF* mutant. *In vivo* interactions between FlhF/FlhF, FlhF/L_2, and between the B and NG domains of FlhF and L_2 were analyzed using the *in vivo* bacterial adenylate cyclase two-hybrid (BACTH) system. Protein/protein interactions were evaluated on solid media and by the quantitative beta-galactosidase assay. Poly-histidine pull-down was used as independent *in vitro* method to confirm FlhF/L_2 interaction. Site-directed mutagenesis was used to produce amino acid substitutions in FlhF.

3. Results. The transcription level of *hblC* was not altered in the *deltaflhF* mutant, indicating that the reduced secretion of L_2 by the strain was not the result of an expression defect. In BACTH screens, *B. cereus* FlhF directly interacted with itself and with L_2, and both FlhF domains were required for FlhF/L_2 interaction. FlhF and L_2 were also found to interact *in vitro*. The results of FlhF point mutations on protein function will be shown.

4. Discussion and Conclusions. As previously shown for *B. subtilis*, *B. cereus* FlhF is able to dimerize *in vivo*. In addition, FlhF interacts with the L_2 component of HBL *in vivo* and *in vitro*, and both the B and NG domains of FlhF are able to contact L_2. Further experiments are still ongoing to understand if FlhF dimerization is dependent on the GTP-load and if the formation of stable homodimers is crucial for FlhF/L_2 interaction. In conclusion, our data indicate that FlhF acts as SRP-like protein involved in the targeting/recruitment of L_2 to the plasma membrane and underline the key role of this protein in modulating *B. cereus* virulence potential.

P158 - Ability of rifampicin- methicillin-resistant Staphylococcus aureus isolates belonging to different genetic backgrounds to invade human osteoblast

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Introduction: Methicillin-Resistant *Staphylococcus aureus* (MRSA) has been reported as one of the main human pathogens. It is able to infect every organ, causing severe infections as recurrent osteomyelitis (OM) and prosthetic joints infections (PJIs), that require prolonged antimicrobial treatment, having a dramatic impact on the patient quality of life and surgical interventions. Rifampicin is frequently used in the therapy of these infections, and rifampicin-resistant MRSA (RIF-R MRSA) strains have been frequently associated to OM and PJIs. We would like to access the nature of altered host-pathogen interactions correlated to rifampicin resistance, due to mutations in the "rifampicin resistance-determining region" (RRDR) and genetic backgrounds.

Materials/methods: The internalization frequency was evaluated in *ex-vivo* model of infection within human MG-63 osteoblasts cells at a multiplicity of infection (MOI) of 100:1, for 10 molecularly characterized RIF-R MRSA isolates, 8 RIF-susceptible (RIF-S) strains belonging to identical genetic backgrounds included as comparison, 2 isogenic strains in which RIF-R was acquired during therapy, and ATCC12598 invasive isolate as reference strains. Quantification of intracellular bacteria was determined by: extracellular bacterial lysis with lysostaphin, lysing host cells, plating the lysate on agar plates and counting the colonies. Statistical analysis was carried out using GrapPad Software.

Results: The ability and the predisposition to internalize are correlated to the genetic backgrounds of the strains and we observed different invasion rates: ST8-SCC*mec*IV strains, showed the best ability to internalize (rate of internalizations of 0.45-0.55 CFU/cell, P value 0.014) and RIF-R isolates were also associated with new mutations in RRDR. ST228-SCC*mec*I - the major epidemic HA-MRSA clone, involved in severe infection in Italy - is not able to internalize (rate of internalization of 0.025-0.057 CFU/cells, P value 0.0051). ATCC12598 internalization rates were 0.28 CFU/cell in accordance with bibliographic data. The isogenic couple in which rifampicin resistance was acquired under therapy, showed a statistical significant difference for the RIF-S (P value 0.0039).

Conclusions: We demonstrated that the ability to invade osteoblasts is correlated to genetic background: ST8, ST239/241, ST5 and ST22 showed higher internalization efficiency. Among the same genetic background, RIF-R isolates show higher internalization rates, with respect to the corresponding RIF-S clones. These novel findings could provide an insight into host-pathogen successful interaction of specific RIF-R-MRSA clones and contribute to the development of a new knowledge-based-approach for treatment of chronic infections.

P159 - Type I IFN is involved in Streptococcus pneumoniae infections

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Introduction. Type I IFN is known to interfere with viral replication. However, type I IFN may affect certain bacterial species. Lymphocytes are rendered more susceptible to listeriolysin O, for example, causing increased apoptosis during *Listeria monocytogenes* infection. In contrast, IFN-α inhibits the intracellular replication cycle of *Chlamydia trachomatis* and *Legionella pneumophila*. Similarly, group B Streptococcus induces type I IFN via TLR7, resulting in resistance to infection and group A Streptococcus induces type I IFN in macrophages. Pneumococcal pneumonia is a leading cause of mortality worldwide. The inflammatory response to bacteria is necessary to control infection, but it may also contribute to tissue damage. The discovery of innate immunity receptors involved in the recognition of this microorganism and the role played by some important mediators such as type I IFNs may be important to designate alternative therapies.

Materials and Methods. In order to establish the role played by type I IFNs during pneumococcal pneumonia, we used an experimental mouse model of lung infection. In this study, we used genetically defective mice for 3D, lacking a protein "chaperone" called UNC93B1 that regulate the "trafficking" of endosomal TLRs from endoplasmic reticulum to endosome, IFN-beta and C57BL/6 wild-type (WT) as controls. The mice were intranasally infected with a sublethal dose of *S. pneumoniae* D39 strain and mortality was observed. Furthermore, at different time intervals, lungs and brain were taken and tested for CFU and IFN-beta levels. As for *in vitro* experiments, bone marrow cells from WT and 3D mice were differentiated in vitro in macrophage cells. These cells were injeced with different infection multiplicities of D39 and IFN-beta levels were measured.

Results. 3D mice were more susceptible to *S. pneumoniae* infection than WT and had higher bacterial counts in the brain and lungs. Furthermore, these mice showed decreased levels of IFN-beta in the same organs. In vitro differentiated macrophages cells produced also significantly smaller amounts of IFN-beta in 3D than the C57BL/6J.

Discussion and Conclusion. Our results showed that mice lacking of UNC93B1 were more susceptible to pneumococcal infection and this susceptibility correlated with a decreased production of IFN-beta. In vitro experiments demonstrated that 3D macrophages cells infected with *S. pneumoniae* infection also produced less IFN-beta.

P160 - Role of endosomal Toll-like (TLR) receptors in the etiopathogenesis of pulmonary infections caused by Streptococcus pneumoniae

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Introduction.Microrganism recognition by the host immune system occurs through a limited number of receptors present on the cellular surface. Toll-like receptors (TLR) family is the most studied receptor category. Endosomial TLRs recognize both PAMP expressed on intracellular microrganisms and nucleic acids released after pathogen degradation and it has been highlighted that the "trafficking" of endosomal TLRs from endoplasmic reticulum to endosome is regulated by a protein "chaperone" called UNC93B1. *S. pneumoniae* is the most virulent bacteria causing pneumonia with high mortality in children and elderly. The studies carried out to date on the involvement of the innate immune system in pneumococcal infections showed the possible role of two membrane TLRs, such as TLR2 and TLR4. Little is known about the role played by endosomal TLRs in the field of these infections. It is therefore important to investigate TLRs involvement in the pathogenetic mechanisms of this infection.

Materials and Methods. To investigate the role played by TLRs during pneumococcal pneumonia, we set up a lung infection experimental murine model. To this end, we used genetically defective mice for the different TLR receptors and C57BL/6 wild-type (WT) as controls. The mice were intranasally infected with a sublethal dose of *S. pneumoniae* D39 strain and mortality was observed. Furthermore, at different time intervals, lungs and brain were taken and tested for CFU and cytokine levels. As for *in vitro* experiments, we isolated 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. 3D mice showed a most severe phenotype accompanied from CFU elevated number in the lungs and brain at 24 and 48 h following *S. pneumoniae* infection. A 100% mortality rate was observed in 3D mice. In contrast, 30% lethality occurred in $TLR7^{-/-}$, $TLR9^{-/-}$ and $TLR13^{-/-}$ mice. The absence of endosomal TLR seems to cause both the lungs and the brain to produce less production of MIP-2 and KC. Differentiated macrophages and dendritic cells produced significantly smaller amounts of MIP-2 and KC in 3D than the C57BL/6J.

Discussion and Conclusion. Our data showed that 3D mice were particularly susceptible to pneumococcal infection. The absence of endosomial TLRs alone could not reproduce 3D phenotype. Furthermore, endosomal TLR absence caused less 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. Finally, our data demonstrated that macrophages and dendritic cells are involved in *S. pneumoniae* infection.

P161 - Formyl peptide receptors are required for high-level chemokine responses in neutrophils stimulated with Streptococcus agalactiae

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Introduction: Neutrophil recruitment to sites of infection is a critical first step in the innate immune response to *Streptococcus agalactiae* (Group B streptococcus, GBS), a major causative agent of perinatal systemic infections. Previous studies have indicated that neutrophils can amplify their own recruitment to sites of bacterial infections by producing keratinocyte chemoattractant (KC or chemokine (C-X-C motif) ligand 1, CXCL1) and macrophage inflammatory protein 2 (MIP-2 or chemokine (C-X-C motif) ligand 2, CXCL2. A better understanding of the mechanisms underlying this process is crucial to designing improved therapies against GBS.

Materials and Methods: Murine bone-marrow-derived neutrophil preparations were exposed *in vitro* to live and heat killed GBS (HK-GBS) and supernatants were collected for cytokine measurements by ELISA at 24 hours after stimulation.

Results: Neutrophils produced ten to twelve-fold-higher levels of CXCL1/2 after recognition of live, as compared with heat-killed, bacteria, while producing only slightly higher amounts of TNF-alpha. The formyl peptide receptor 1 (Fpr1) and 2 (Fpr2) antagonist t-Boc-FLFLF (also termed Boc-2) and the Fpr2-selective peptide inhibitor Trp-Arg-Trp-Trp-Trp-Trp-$CONH_2$ (WRWWWW, WRW4) prevented increased CXCL1/2 production in response to live bacteria. Fpr agonists, however, were unable to induce significant chemokine responses when used alone.

Discussion and Conclusions: Our results suggest that neutrophils are capable of discriminating between live and dead bacteria by sensing formylated peptides or other Fpr2 ligands, which strongly synergize with endosomal TLRs for high-level production of CXCL1/2, thereby *amplifying neutrophil recruitment* to bacterial infection sites.

P162 - Update concerning the possibility to infer the group of pathogens in blood cultures, based on the different levels of serum PCT

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1. Introduction

In a recent study we showed that procalcitonin levels were significantly different based on some blood culture isolates found in the same samples. To further address such interesting issue, we decided to update our previous data with the latest 12month values.

2. Material and Methods

This retrospective observational clinical study was conducted using clinical and routine laboratory data collected from June 2016 to June 2018. Blood cultures (BC) and serum samples were serially collected from each patient with suspected sepsis, defined according to the most recent guidelines (Sepsis-3). Only the first sample, obtained during the first septic episode, was evaluated for each patient. Samples for blood cultures were obtained under aseptic conditions in all patients, incubated and monitored for seven days by BacT/Alert 3D system or BacT/Alert Virtuo (bioMérieux, France). The identification of organisms and drug resistance phenotype characterization were assessed by conventional microbiology methods or automated systems. Serum PCT levels were measured by ELFA technique (VIDAS B.R.A.H.M.S. PCT, bioMérieux, France), in the samples of enrolled patients, as well as in samples of a group of non-bacteremic controls. Statistics were obtained using ANOVA, as well as PLSD post hoc test. A $p < 0.05$ was considered statistically significant.

3. Results

Our findings suggest that different levels of PCT were found in different patients when stratified by the microorganism isolated in their blood cultures. More in details, PCT levels in patients infected by an Enterobacteria isolate was very significantly ($p < 0.0001$) different from patients associated with Gram-positive sepsis. Also, we found that concentrations of PCT in non-fermentative Gram-negatives were significantly lower ($p = 0.0033$) versus Enterobacteria-caused sepsis. Finally, PCT exhibited a significantly lower ($p = 0.0034$) levels in sepsis caused by yeast in comparison to sepsis caused by Enterobacterial strains.

4. Discussion and Conclusions

During our previous investigations, we found that another sepsis biomarker presepsin, is differentially affected by different type of blood culture isolates, whose Enterobacteria were associated with very high presepsin levels. Similarly, in this study, appeared significantly higher PCT concentrations in blood cultures positive for Enterobacteria strains. In conclusion, we wish to put forward the use of PCT to estimate the group of bacteria responsible for sepsis episodes.

P163 - In vitro interaction of Pseudomonas aeruginosa biofilms with human peripheral blood mononuclear cells

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Introduction: Human host immune cell response to intact bacterial biofilms is a poorly investigated but an important research topic. Several reports in the literature investigate neutrophil or monocyte response to biofilms while only few studies focus on human peripheral blood mononuclear cells (PBMC)-biofilm interactions. The low viability of human cells upon incubation with intact pre-formed biofilms is one of the hurdles that makes it difficult to study host immune cells-intact biofilm interactions. Thus, in the present study we aimed to establish an *in vitro* host cell-biofilm interaction model that could sustain the viability of host cells for at least 24h, and to investigate PBMC response to *Pseudomonas aeruginosa* (PA) biofilms.

Materials and Methods: PA biofilms were obtained in 96 well plates by 24h incubation of bacteria in complete RPMI medium with 10% human plasma. PBMC obtained from healthy donors were added to pre-formed PA biofilms. Following 24h incubation: i) the supernatants were taken from the wells, sterile filtered and kept frozen for further use, ii) the PBMC were harvested to assess PBMC viability and activation, and iii) biofilm forming bacteria were collected after washes and CFU counts performed. In further experiments, the supernatants obtained in (i) were added to the pre-formed PA biofilms for 24h, and the biofilm derived bacteria counts were assessed.

Results: The trypan blue exclusion assays, performed at 2h, 6h, 10h, and 24h upon incubation of PBMC with PA biofilms showed that cell-death increased with incubation time, but was <15% even at 24h. PBMC incubated for 24h with pre-formed PA biofilms were significantly more activated compared to PBMC incubated in the absence of biofilm. Interestingly, the CFU number of PA was increased in biofilms incubated with PBMC as compared to biofilms incubated without cells. To test the hypothesis that PBMC products could stimulate the growth of PA biofilms, we performed experiments where pre-formed PA biofilms were incubated in the presence or absence of supernatants obtained from the PBMC+biofilm wells (see (i) above). The CFU count of biofilm derived PA, incubated in the presence of the supernatants, resulted 2-10 times higher than those of biofilms incubated alone ($P<0.05$).

Discussion and Conclusions: These results demonstrate that i) it is possible to establish PA biofilms *in vitro*, in conditions optimal to human immune cells. The developed human cell-biofilm co-incubation model is suitable to study human immune response to biofilms; ii) in the presence of PBMC and/or PBMC components PA biofilm formation is enhanced. This may indicate a successful bacterial defensive/persistence strategy against immune response.

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P164 - Apyrase, the Shigella flexneri virulence factor downregulates caspases activity through the degradation of intracellular ATP

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Introduction

Shigella flexneri is a facultative intracellular pathogen that causes the bacillary dysentery in humans. Its pathogenicity relies on the expression of the type III secretion system that injects effector proteins inside host cell. These effectors induce the bacterial invasion of host cell and are the main players of the innate immune response modulation, observed during *Shigella* infection. Apyrase is a periplasmic ATP-diphosphohydrolase required for *Shigella* actin-based motility, however the role of its catalytic activity has not been defined. Apoptosis is a genetically programmed cell death process, which is triggered by external and internal signals. This form of cell death plays an essential role in tissue development, homeostasis, and also in the elimination of pathogen infected cells. It requires energy and is regulated by the intracellular levels of ATP. Here we show that in infected epithelial cells intracellular ATP levels and caspase activity are controlled by *Shigella* apyrase.

Materials and Methods

Shigella flexneri serotype 5a strain M90T, the *phoN2* mutant strain HND115, and the complemented strain HND115 pHND10 were plated on Trypticase soy broth agar (TSA) plates (BBL Microbiology Systems) containing 0.01%Congo Red (CR) and grown on Luria-Bertani (LB) broth. The human cell line Caco-2 was cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and grown in the presence of 5% CO_2 at 37◦C. *S. flexneri* strains were used to infect semi-confluent monolayers at a multiplicity of infection (MOI) of 100, using the gentamicin protection assay. At selected time points, cells were treated with trypsin, collected and assayed for caspase activity using the Vybrant FAM poly Caspase Assay Kit, and for intracellular ATP using the ATP determination kit (Molecular Probes).

Results

We quantified the intracellular ATP levels in Caco-2 cells infected with M90T, HND115 and HND115 pHND10 strains. We observed an increase of intracellular ATP levels in cells infected with HND115 compared to cells infected with M90T and the complemented strains at 2 hours post infection. Interestingly, cells infected with M90T and the complemented HND115 pHND10 strains displayed ATP levels similar to the non-infected control cells. This result indicates that PhoN2 is necessary to maintain the physiological levels of intracellular ATP during *S. flexneri* infection. The analysis of caspases activity, in a time course infection experiment, showed a prominent increase in caspases activation in cells infected with HND115 strain compared to the cells infected with M90T and the complemented strains, and the non-infected cells.

Conclusion

Our data indicate that the *S. flexneri* virulence factor PhoN2 is important for conditioning cell sensitivity to cell death at the early stage of infection.

P165 - Immunogenicity of a vaccine against Salmonella enterica serovar Typhimurium based on Generalized Modules for Membrane Antigens (GMMA)

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Introduction

Salmonella enterica serovar Typhimurium (STm) is one of the predominant causes of invasive nontyphoidal *Salmonella* (iNTS) disease. Currently, there are no licensed vaccines against iNTS. The O-antigen portion of STm lipopolysaccharide (O:4,5) has been recognized as an important target for vaccination. In this work, we characterized the murine immune response to a vaccine against STm based on the GMMA (Generalized Modules for Membrane Antigens) technology as a delivery system for O:4,5.

Materials and methods

C57BL/6 mice were immunized at weeks 0 and 10 with Alhydrogel formulated STmGMMA by subcutaneous (SC) or intranasal (IN) route, using 0,63 µg or 10 µg quantified based on O-antigen of vaccine. Systemic and local O:4,5-specific antibody responses induced by STmGMMA vaccine in serum, fecal and intestinal washes were evaluated by ELISA, and bactericidal activity in serum was assessed by L-SBA assay. The cellular immune response was estimated by multiparametric flow cytometric assay of splenocytes.

Results

After primary immunization, higher levels of O:4,5-specific serum IgG antibody were observed following immunization with STmGMMA administered by SC route (both doses) and with the higher dose by IN route. Boosting at 10 weeks induced, in all groups, an increase of O:4,5-specific IgG with higher levels when administered SC compared with IN administration, and serum bactericidal activity against the homologous strain. Analysis of IgG subclasses showed a balanced Th1/Th2 response in mice immunized by the SC route, while the IN route elicited the production of higher IgG2b (Th1-related). The analysis of intestinal antibodies showed that only the higher dose of GMMA vaccine delivered SC elicited fecal IgG. Similar levels of antigen-specific IgG were observed in feces and intestinal washes after boosting. In contrast, intestinal IgA were induced especially by the IN route with higher levels observed in fecal than in intestinal wash samples. A significant production of IL-2, IFN-gamma, and IL-17A by $CD4^+$ T cells was observed in splenocytes of mice immunized with STmGMMA using both doses and administration routes, and *in vitro* restimulated with STmGMMA.

Discussion and conclusions

These data demonstrate the ability of the STmGMMA vaccine to induce local and systemic, humoral and cellular, immune responses. The study also highlights the modulation of the immune response driven by different routes of immunization.

P166 - Adhesion of KPC-producing Klebsiella pneumoniae strains to HT-29 colon adenocarcinoma cells

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Introduction

KPC-producing *Klebsiella pneumoniae* (KPC-KP) high-risk clones pose distinct clinical challenges due to their ability to acquire multiclass antibiotic resistance and disseminate in the hospital settings, producing invasive infections with high mortality.
Epidemiological studies have revealed that the initial step of KPC-KP infection is colonization of the gastrointestinal tract. Furthermore, the gut of colonized patients is the principal reservoir of KPC-KP in the hospital setting.
K. pneumoniae adhesion to biotic surfaces is mediated by two types of adhesins: Type I mannose-sensitive and Type III mannose-resistant fimbriae, encoded by *fim* and *mrk* genes clusters, respectively. In this work we studied the *mrk*-mediated adhesion to HT-29 colon adenocarcinoma cells of KPC-KP clinical strains.

Materials and Methods

Five KPC-KP were used: KKBO-1, KK207-1, KPC157, DG5544 and DG5546, belonging to ST258 clade II, ST258 clade I, ST512 (CG258), ST101 and ST2502 (CG101), respectively. Confluent monolayers of HT-29 cells were maintained in RMPI medium in presence or absence of antibiotics (streptomycin and penicillin), obtaining cells with two different morphologies: differentiated (polarized) and undifferentiated (apolarized). Morphology of HT-29 cells was verified by transmission electron microscopy (TEM) observation. Bacterial inocula were prepared in LB medium and $1\pm0.2x10^8$ CFU of bacterial strains were added to each culture plate well, in presence of 2% D-mannose (inactivation of *fim* fimbriae). Each strain was tested with polarized and apolarized cells, respectively, in quadruplicate. Bacterial cells were allowed to adhere at 37°C in a CO_2 atmosphere for 1 hour. After incubation, each well was rinsed three times with PBS and adherent bacteria were released by addition of Triton X-100. Adherent bacteria were quantified by plating appropriate dilutions on LB agar medium.

Results

All the analyzed strains adhered to differentiated HT-29 cells ($1x10^5$ to $1x10^7$ recovered bacterial cells). For KKBO-1, KK207-1 and KPC-157 a detectable level of adhesion was documented with undifferentiated cells also, although significantly lower. By contrast, no adhesion was observed with the two CG101 strains and undifferentiated cells. Analyzing the *mrk* gene cluster of studied strains we observed that the two CG101 strains had a common mrkD variant (the adhesin) different from those found in the other strains.

Discussion and Conclusions

We demonstrated that KPC-KP strains, belonging to CG258 and CG101 high-risk clones, adhere significantly to differentiated colon adenocarcinoma HT-29 cells. However, the CG101 strains only were unable to adhere to undifferentiated cells. This behavior could be attributed to the diversity existing in the *mrk*-genes cluster of KPC-KP.

P167 - Isolation, characterization and analysis of pro-inflammatory potential of Klebsiella pneumoniae outer membrane vesicles

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Introduction: *Klebsiella pneumoniae* has emerged as an important opportunistic pathogen mostly causing nosocomial infections. *Klebsiella pneumoniae* produces outer membrane vesicles (OMVs) like other pathogenic Gram-negative bacteria. These vesicles contain lipopolysaccharide (LPS), outer membrane, periplasmic and cytoplasmatic proteins, lipids, DNA and RNA. In this study, we examined the production and the characterization of OMVs from *Klebsiella pneumoniae* and determined the induction of the innate immune response against *Klebsiella pneumoniae* OMVs.

Material and Methods: OMVs were purified, by ultracentrifugation at 150000g for 90' at 4°C, from *Klebsiella pneumoniae* ATCC 10031 culture supernatants**.** To characterize the OMVs we performed Trasmission Elettron Microscopy (TEM), Dynamic Light Scattering (DLS) and 10% sodium dodecyl sulphate poly-acrylamide gel electrophoresis (SDS-PAGE). The protein bands were removed from the gel, digested with trypsin and the derived peptide mixtures analyzed using LC-MS/MS techniques. Protein identification occurred through the MASCOT database. To define whether *Klebsiella pneumoniae* OMVs induce a proinflammatory response *in vitro*, BEAS-2B cells were treated with different amount of OMVs, 0.5-1-5 μg/ml, for 24h and 0.1 μg/ml LPS was used as positive control. The expression of proinflammatory cytokine genes was analyzed by Reverse Transcriptase PCR (RT-PCR).

Results: TEM has identified spherical vesicles of a narrow range of size, without any contamination. DLS measured the particle size of 79.53 nm in diameter and identified a homogeneous size distribution proving by the polydispersion index of 0.211. SDS-PAGE/digestion in situ (on gel)/LC-MS/MS showed the presence of outer membrane porin protein C and A. *Klebsiella pneumoniae* OMVs induced proinflammatory cytokine gene expression in a dose-dependent manner in BEAS-2B cells.

Conclusions: Our study suggests that the OMVs of *Klebsiella pneumoniae* act as strong immune-modulators to induce a pro-inflammatory response, thus contributing to its pathogenesis. Despite rapidly increasing clinical importance of *Klebsiella pneumoniae* infections, it is crucial to further investigate the virulence potential of *Klebsiella pneumoniae* OMVs.

P168 - In patients with Helicobacter pylori and Epstein-Barr virus coinfection: there is a correlation with IL-10 and IL1RN polymorphism?

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Introduction
Recently *Helicobacter pylori* and Epstein-Barr virus (EBV) co-infection has been associated with gastric diseases. However polymorphisms in genes encoding cytokines, such as interleukin 10 (IL-10) and interleukin 1 Receptor (IL-1RN), have been correlated with a different level of cytokines; appearing to contribute to the risk of developing gastroduodenal diseases.
To our knowledge, this is the first preliminary study speak to the link of co-infection with *H. pylori* and EBV and their correlation with genetic predisposition in development of gastric diseases.

Methods
In this study 96 patients with different gastric diseases were enrolled. From each patients two gastric biopsies were taken. *H. pylori* and EBV infection was diagnosed by the detection of the *ureaseA* and BAMHI-W respectively. The virulence factors, resistance to clarithromycin of *H. pylori*, IL-1RN penta-allelic variable number repeats and IL-10 (at position 1082) polymorphism were detected by polymerase chain reaction.

Results
In our results, within in the patients with gastric cancer the rate of co-infection was higher than that within in patients with normal gastric mucosa, active chronics gastritis and Malt lymphoma. About the characterization of *H. pilory* strains, the polymorphism *s1m1i1* of *vacA* gene, was most frequent in patients with Malt lymphoma in comparison to others, while the polymorphism *s2m2i2* was most frequent in patient with normal gastric mucosa. Furthermore, the patients positive to *cagA* gene and *oipA* gene with status ON were patients with gastric cancer. Among the different polymorphism, there was only a significant association between polymorphism of IL-1RN and gastric disease, particularly within patients with Polymorphism IL-1RN: 1/2 was significantly less frequent in patients with Malt lymphoma .

Conclusion
According to our analysis none correlation was found between co-infection and polymorphisms in genes encoding IL-10 and IL-1RN. We conclude that the various factors can be enrolled in development of gastric diseases.

P170 - Replication ability of influenza A viruses in macrophages: possible correlation with intracellular redox state

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Introduction

During influenza virus infection the first line of defence is represented by airway macrophages, whose response is essential in determining the outcome of infection. There are disaccording data about the ability of this virus to infect and replicate into macrophages; different cellular blocks against viral life-cycle have been proposed. It is well known that mild changes in the intracellular redox state towards pro-oxidant conditions favor influenza virus replication. Glutathionylation, i.e. formation of mixed disulfides between protein cysteines and glutathione (GSH) cysteine, is a mechanism by which the redox state of the cell can modulate the function of several targets, including proteins involved in viral infection and host response. So far, there are no evidences on the possibility for influenza virus proteins to undergo glutathionylation and the potential relationship of the process with viral replication and inflammatory response. The aim of this study was to evaluate the replication ability of different influenza A strains in macrophages and investigate whether it was correlated to changes in the redox state of the cells, particularly to the formation of glutathionylated proteins.

Materials and Methods

Mouse macrophage cell line RAW264 was infected with the following influenza A virus strains: human A/Puerto Rico/8/34 H1N1 (PR8), A/NWS/33 H1N1 (NWS) and pandemic A/California/04/09 H1N1 (pH1N1) strains or avian Parrot/Ulster/73 H7N1 (ULSTER) strain. Virus production was determined in the supernatants of infected cells 24 and 48 hrs p.i., by measuring the hemagglutinating units (HAU). Viral mRNA and proteins expression were analysed by RT-PCR and Western Blotting (WB), respectively. GSH levels were measured by HPLC. To evaluate glutathionylation of viral proteins, they were immunoprecipitated from biotinylated glutathione (BioGEE)-preloaded and infected cells with an antiinfluenza antibody and were analysed on a non-reducing SDS-PAGE followed by WB with streptavidin peroxidase.

Results

We found that all the analysed strains were able to infect and to replicate in macrophages, as we were able to detect non-structural protein NS1, consistent with the *novo* protein synthesis and virions in the supernatants. The strains pH1N1 and NWS showed the higher efficiency in replication. All the strains induced a GSH depletion. WB with streptavidin peroxidase revealed that viral nucleoprotein and matrix protein1 were glutathionylated.

Discussion and Conclusions

These results indicate that the analysed strains are able to replicate productively in macrophages. Moreover, they induce a GSH depletion that could be due, at least in part, to the formation of mixed disulfides with viral proteins, an event that could be important for viral replication.

P171 - Trichodysplasia Spinulosa in a kidney transplant recipient with concurrent JC, BK, and TSPyV viruria and decoy or degenerated cells

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Introduction: Trichodysplasia spinulosa (TS) is a rare, disfiguring skin disease of severely immunocompromized patients characterized by papules and spines on the face, alopecia of eyebrows and lashes, and thickening of the affected skin layers. Here, we present the first reported Italian case of TS in a 8 year-old kidney transplant recipient who has been already transplanted twice as he suffers from a congenital nephrotic syndrome caused by mutations in the *NPHS1* gene that encodes nephrin. He developed the typical TS clinical picture in September 2017. The lesions that had first been noticed on his face gradually extended to the neck, back, and extremities.

Materials, Methods and Results: Histopathological examination showed keratotic masses expanding from the hair follicles, with widened infundibuli and inner follicular layers containing increased cellularity of nucleated eosinophilic inner root sheath cells with trichohyalin granules. PCR analysis of the DNA extracted from keratotic spines revealed high viral load values for TSPyV ($6x10^6$ copies per cell) while only 2 copies per cells were detected for Merkel cell polyomavirus (MCPyV) and none for HPyV6 and 7. HPV21 and 92 genotypes from the beta genus were also found in the DNA extracted from the skin swabs. Analysis of serum plasma samples collected about the time of TS diagnosis revealed the presence of JC DNA (approximately10^3 copies/mL). PCR analysis for TSPyV was not performed at that time. Blood samples collected in March 2018 confirmed the positivity for JC DNA (500 copies/mL) and resulted also positive for BK DNA (600 copies/mL) while TSPyV was always undetectable. Urine samples harvested in February 2018 displayed high viral loads for TSPyV ($4x10^5$ copies/ml), BK ($2x10^8$ copies/ml) and JC ($1x10^9$ copies/mL). A huge number of decoy cells with enlarged ground-glass nuclei were observed alongside many other cells with degenerative changes in the cytoplasm and large hyaline granules very much resembling those observed in TS skin biopsy. All these cells were stained with antibodies against SV40 large T antigen. Electron microscopy is underway.

Discussion and conclusion: Because of the JC viremia and a transient increase in serum creatinine levels, the immunosuppressive therapy was reduced; JC viremia progressively reduced, the renal function was good, while the skin disorder worsened.

P172 - Clade B and C HIV Tat proteins display different immunomodulatory actions

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Introduction: the clade C HIV is responsible for about half of all HIV infections, followed by subtypes A and B. HIV-1 subtypes are characterized by differences in progression rate and virulence. However, mechanisms explaining the different progression rates between distinct viral clades remain unknown. As the Tat protein of HIV contributes to immune activation (a major T lymphocyte impairment experienced by HIV-infected individuals), we compared immunomodulatory properties between clade B and C Tat proteins.

Materials and Methods: CD4+ and CD8+ T cells were cultured in resting and activated conditions in the absence or presence of Tat (clade B or C). The production of pro-inflammatory cytokines and of master regulator of transcription were measured by gene expression analysis and flow cytometry.

Results: both clade B and clade C Tat boost the activation of anti-CD3/CD28 stimulated CD4+ and CD8+ T cells, favoring the release of pro-inflammatory cytokines and the induction of Tbet and Eomes. However, effects performed by clade C Tat were observed at earlier time points than those mediated by clade B Tat, suggesting a faster way of action. In addition, while clade B Tat does not activate resting T cells, clade C Tat induces the expression of pro-inflammatory molecules from CD4+ and CD8+ T cells in the absence of simultaneous TCR-induced stimulation.

Discussion and Conclusions: clade C Tat displays pro-inflammatory effects on CD4+ and CD8+ T cells not observed in clade B Tat, which instead activates lymphocytes only if a simultaneous TCR-mediated stimulation occurs. This suggests that, while both Tat variants could contribute to immune activation during the acute phase, the presence of Tat C may interfere with the latent phase, promoting CD4+ T cell activation. This may partially explain differences observed in both acute and latent phases between clade B and C HIV infections.

P173 - Response to antiretroviral treatment of tumor cells expressing human endogenous retroviruses

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Introduction: In our genomes there are thousands of copies of human endogenous retroviruses (HERVs), retro transposable elements originated from integration of ancestral retroviruses that infected human germ line cells million years ago. HERVs expression is mainly regulated by epigenetic mechanisms and is subjected to temporal and spatial regulation under different microenvironmental conditions. The altered expression of these elements contributes to the onset, progression and acquisition of aggressiveness features in many kinds of cancers. It's well known that cellular plasticity and stemness are fundamentals requirement for tumor progression and metastasis: from therapy resistance via self-renewal, proliferation, differentiation and immune evasion abilities of cancer cells. We already demonstrated that, under pressure of microenvironmental changes, HERV-K reactivation in human melanoma cells is strictly required to maintain cell plasticity and to sustain the expansion of a cancer cell subset with stemness features. Moreover, we had already established that treatment with antiretroviral drugs could inhibit HERV enzymes and affect cancer cells. In this context, to widen knowledge and therapy opportunity, we used antiretroviral drugs to assess the effect on different cancer cell lines under microenvironmental stresses.

Materials and Methods: TVM-A12, Hep-G2, Caco-2, A549, MCF7, MCF7-DX cell lines were cultured in standard or stem cell media. For treatment were used antiretroviral drugs zidovudine and efavirenz. Apoptosis analysis were assessed by flow cytometry and the expression of HERV-K and HERV-H were evaluated by RT-Real time PCR.

Results: When cultured in stem cell medium, depending on changes of culture conditions, all the cell lines showed modifications in cellular morphology, with generation of non-adherent cell aggregates like spheres. Microenvironment modifications induced an increase of HERV-K and HERV-H expression. Interestingly, flow cytometry analysis showed the increase of apoptosis in antiretroviral-treated cells cultured in stem cell medium.

Discussion and Conclusions: The responsiveness of HERVs to external stimuli attributes to these genetic elements a high relevance in the crosstalk between tumor and microenvironment. These new findings support our previous work on melanoma cell line; we founded that also in other cancer cells HERV-K is involved in the acquisition of plasticity and aggressiveness under the pressure of the microenvironment. Moreover, the responsiveness to antiretroviral treatment of cancer cells expressing HERVs suggests that the use of these drugs alone or in combination with chemotherapeutic regimens could be an alternative approach to treat aggressive tumors.

P174 - Role of post-translational modifications of cytoskeletal intermediate filaments on influenza A/NWS/33 virus (H1N1) infection in mammalian cells: focus on cytokeratin 8 phosphorylation

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Introduction: influenza virus causes epidemics and pandemics, with significant impact on public health. The cytoskeleton is hijacked by influenza virus to reach the replication site. Previous data assessed the role of actin- and tubulin-stabilization as restriction factors of human influenza A/NWS/33 virus (IAV) infection in rhesus monkey-kidney (LLC-MK2) cells (semi-permissive model). This study aims to investigate the involvement of cytokeratin 8 (K8) phosphorylation, a post-translational modification of intermediate filaments (IF), on IAV infection in LLC-MK2 and human type II alveolar epithelial (A549; permissive model) cells.

Materials and Methods: LLC-MK2 and A549 cells were infected for 24 h with IAV at a multiplicity of infection (MOI) of 0.1 or 2 plaque-forming units (PFU)/cell. IF-depolymerization was induced with acrylamide (5 mM), while IF-phosphorylation with okadaic acid (OA, phosphatase inhibitor; 0.05 microg/ml) and 12-O-tetradecanoylphorbol-13-acetate (TPA, protein-kinase activator; 25-50 nM), applied for different periods. Immunofluorescence and Western blotting assays were carried out using anti-viral nucleoprotein (NP) (Argene/BioMérieux), anti-K8 (Thermo Fischer Scientific), and anti-phospho-K8 (Thermo Fischer Scientific) antibodies. Secondary antibodies conjugated with isothiocyanate of rodamine/fluorescein or alcaline phosphatase were used. Fifty percent tissue culture infectious dose ($TCID_{50}$) assays were performed to evaluate viral yields.

Results: K8 showed a higher expression in A549 *vs* LLC-MK2 cells, while K8 phosphorylation was low in both models. IF-depolymerization with acrylamide raised NP expression in both cells, almost unchanging the viral yields. IF-phosphorylation with OA increased NP expression in A549 but not in LLC-MK2 cells, modifying the K8 pattern mostly in A549 cells. IF-phosphorylation with TPA decreased NP expression in LLC-MK2, while increased that of A549 cells, raising K8 phosphorylation mostly in A549 cells. Viral yields were consistent with the obtained results. IAV infection strongly modulated K8 organization and increased K8 phosphorylation in A549 cells, leaving almost unmodified LLC-MK2.

Discussion and Conclusions: the depolymerization of IF favours IAV infection in the examined models. The drug-mediated IF-phosphorylation promotes IAV infection in A549 cells, showing no significant effects in LLC-MK2. Accordingly, viral infection prevalently modulates K8 organization and phosphorylation in A549 cells. These results evidence the presence of different regulation mechanisms acting on IF post-translational modifications in the studied models. Understanding how specific cell functions/components regulate influenza virus replication may provide new starting points for innovative anti-viral researches.

P175 - Myriocin ameliorates defective killing of pathogens in cystic fibrosis patients

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Introduction- Chronic inflammatory diseases affecting airway mucosa, such as Cystic Fibrosis

(CF), are characterized by defective immunity and recurrent infections of pathogens organized in drug resistant microbial communities, named biofilms. *Aspergillus fumigatus* is the most prevalent filamentous fungus in the respiratory tract of CF patients, contributing to lung deterioration.

Myriocin, an orphan drug with pleiotropic action, reduces pro-inflammatory lipids accumulation, promoting anti-oxidant response to stress. This compound, administered *in vivo* by nanocarriers, has been demonstrated to reduce inflammation and to ameliorate, in a mice model of CF, host response to pathogens. Moreover, myriocin exerts a direct fungistatic activity against in vitro preformed biofilms of *A. fumigatus*.

Materials and Methods – The effect of myriocin on *A. fumigatus* phagocytosis and killing has been investigated in: i) IB3-1 cells, an adeno-associated virus-transformed human bronchial epithelial cell line derived from a CF patient (ΔF508/W1282X) and its isogenic C38 cells corrected by insertion of CFTR; ii) CF patient monocytes, isolated from peripheral blood.

We infected cells, either pre-treated or not with myriocin (10 μM, 2 h), with *A. fumigatus* (Af293, MOI 1:1). Non-internalized conidia were removed after 1 hour and cells allowed killing the internalized fungus for 4 hours. Still alive conidia were counted after osmotic cell lysis by colony forming unit (CFU count). For IB3-1 and C38 cells, the expression of pathogens receptors TLR2 and NOD2 were investigated by real-time PCR.

Results –CF epithelial cells displayed a markedly reduced ability to kill internalized fungi compared with controls. Myriocin corrected the defective IB3-1 killing ability, decreasing more than two folds the number of CFU/cell. Preliminary results on CF monocytes seem to confirm this data.

A basal significantly reduced expression of NOD2 and TLR2 in IB3-1 versus C38 was observed, suggesting that these proteins may account for IB3-1 defective fungal killing. Myriocin treatment significantly restored the TLR2 and NOD2 expression.

Discussion and Conclusions - Internalized *A. fumigatus* conidia remain alive in CF epithelial cells whereas are promptly killed by control cells. We demonstrated that myriocin corrects CF defective killing of internalized fungal conidia and restores the expression of pathogens receptors TLR2 and NOD2, which are downregulated in CF and correlate with infection susceptibility and enhancement of microbe-targeted autophagy.

P176 - Intrinsic immune response and phagocytosis in microglial cells infected with Chlamydia Pneumoniae

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Introduction: Chlamydia pneumoniae (Cp), obligate intracellular bacterium, is a common respiratory pathogen with a tendency to chronicize. The central nervous system (CNS) may be vulnerable to infectious agents during aging. Microglia are the resident phagocytic cells of the CNS and when stimulated react and produce inflammatory mediators able to mediate multiple aspects of neuroinflammation, including recognition of pathogens, phagocytosis, cytotoxicity through production of cytokines, and secretion of reactive oxygen, and nitrogen species. Recent data have suggested that Cp has been associated with several disorders of CNS. The current study was undertaken to delineate the inflammatory response of glial cells upon Cp infection by measuring the vital cellular parameters as the induction of cell proliferation, apoptosis, and NO modulation.

Materials and Methods: Mixed glial (MG) cells were prepared from the brains of CD-1 mice (P3-5) and U-87 MG cells, a human glioblastoma astrocytoma brain cell line were infected with Cp at a MOI of 4 inclusion-forming units/cell. For Cp adhesion, MG and U-87 MG cells were incubated for 1,2 and 3h, while for infectivity the cells were incubated for 24,48 and 72h. The human laryngeal carcinoma cell line HEp-2 cells were used as the positive control for each experiment. Cell viability was evaluated by MTT. The production of NO was measured by a Griess reaction. The determination of apoptosis was detected by TUNEL assay kit. To evaluate the gene expression of IL-6, and IL-8 was analyzed using both Real time PCR and ELISA.

Results: MG and U-87 MG cells infected with Cp showed many intracellular typical inclusion bodies. Cp adhered and infected MG and U-87 MG cells. Our data showed that MG and U-87 MG cells infected with Cp release NO compared with control cells. The TUNEL assay showed statistically differences in the percentages of apoptosis in infected cells compared to the controls. By Real time PCR we have analyzed the gene expression of IL6, and IL8. The data obtained demonstrate that infection with Cp is associated with increased levels of these proinflammatory cytokines, in particular of IL8, indicating that active role of Cp in the nervous system is due to the excessive activation of the microglia. These data were confirmed by ELISA assay.

Conclusion: Our data showed that infection with Cp of MG and U87-MG cells determined a significant increase in innate immune response. The infection in the brain of a patient affected by neurodegenerative disease could trigger the inflammatory cascade that results in damage to the central nervous system. Further studies are necessary to understand the mechanism of action, to evaluate, in the early stages of diagnosis of the disease, the presence of Cp in patients and to intervene with appropriate therapy.

P177 - Role of bovine lactoferrin-induced macrophagic phenotype in wound-healing

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Introduction: Macrophages are an essential component of innate immunity and have a broad role in the maintenance of tissue homeostasis. Two major polarization states have been described for macrophages, the pro-inflammatory type 1 (M1) and the anti-inflammatory type 2 (M2). The ratio between M1/M2 phenotype determines the progression and/or resolution of chronic diseases, including wounds healing. During the early stages of inflammation, around 85% of macrophages have an M1 phenotype, while 5-7 days post injury is primarily populated by M2 phenotype. In this respect, a better understanding of the macrophagic role in wounds-healing is still required in order to design more efficient therapeutic strategies.

Materials and methods: THP-1 cells, a myelomonocytic cell line, were differentiated in macrophages and treated with 10 pg/ml LPS from *E. coli* and 20 ng/ml IFN-γ. 100 µg/ml bovine lactoferrin (bLf) were added to the culture medium after 3 and 24 h from the stimulation. After 48 h of incubation, the supernatants were used for cytokines quantitation. The adherent cells were scraped used for western blot analysis. For the open label study, 26 patients suffering from bisphosphonate-related osteonecrosis of the jaws (BRONJ), after surgical removal of the necrotic bone, were divided in 2 arms and post-surgically treated with the classical or bLf treatment.

Results: Here, we demonstrate that bLf is able to modulate human macrophagic phenotype by regulating pro- and anti-inflammatory cytokines synthesis as well as the iron homeostasis system in THP-1 cells. In particular, it is known that, depending on M1 or M2 polarization, macrophages express at different extent a set of genes related to iron homeostasis, leading to an "iron-retention" or an "iron-release" phenotype, characteristic of M1 and M2 phenotypes, respectively. We prove that bLf treatment on M1 macrophages: i) induces a decrease of pro-inflammatory cytokines (IL-6 and IL-1β), and an increase of the anti-inflammatory IL-10, a M2 hallmark; ii) rebalances the iron homeostasis machinery switching macrophages from the "iron-retention" to the "iron-release" phenotype. This intriguing ability can be postulated to be one of the major mechanisms exerted by bLf in facilitate wound repair. In this respect, we demonstrate that bLf is able to accelerate the postsurgical wound-healing (1-2 weeks) in subjects suffering from BRONJ compared to the classical treatment (2-3 months).

Discussion: On the basis of our data, we can postulate that bLf, by regulating human macrophagic phenotype, can be considered a novel therapeutic approach able to accelerate wound-healing process as well as to avoid the wounds chronicization that could lead to higher susceptibility to bacterial infections and tissue damage.

P178 - Vitronectin/Integrin interactions are required for gbs adhesion and invasion of epithelial cells

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Introduction: *Streptococcus agalactiae* or group B streptococcus (GBS) is a frequent cause of sepsis and meningitis in neonates. A crucial feature in the pathogenesis of these infections is bacterial adherence to and invasion of epithelial cells. We describe here a novel mechanism whereby GBS accomplish these tasks by exploiting the vitronectin-integrin axis.

Materials and Methods: We examined various GBS strains for their ability to bind to vitronectin (Vtn) immobilized on inert surfaces or on the surface of epithelial cells. We also investigated the role of the cell wall protein PbsP in these interactions by using GBS *pbsP* deletion mutants and anti-PbsP antibodies.

Results: Deletion of *pbsP* in the BM110 strain significantly decreased the capacity of GBS to adhere to immortalized epithelial cells, including A549 (lung) and Caco-2 (colon), and this effect was reversed by genetic complementation. Moreover, the binding of several wild type strain to Vnt was significantly reduced by pretreatment with anti-PbsP antibodies. Pretreatment of epithelial cells with Vtn or anti-Vtn antibodies significantly increased and decreased, respectively, bacterial adhesion and invasion. Moreover, recombinant PbsP interacted with Vnt adsorbed on the surface of epithelial cells or on inert surfaces. Antibodies directed against the alpha(v) integrin subunit significantly reduced Vtn-mediated bacterial internalization.

Discussion and Conclusions: To mediate adherence to host cells, GBS expresses various surface-associated proteins, including PbsP. Our data suggest that PbsP is required for bacterial adherence, by virtue of its ability to interact with Vnt. This matricellular protein, in turn, is linked with alpha(v) integrins on the epithelial cell surface, which trigger an internalization response in epithelial cells. Our data may be use to develop novel means to control GBS infections by interfering with colonization and invasion of mucosal surfaces.

POSTER MICOLOGIA, PARASSITOLOGIA

P179 - Performance of Candida albicans germ tube antibodies (CAGTA) alone and in association with (1→3)-β-D-glucan (BDG) in the diagnosis of invasive candidiasis

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Introduction: invasive candidiasis (IC) plays an important role as severe infection, especially in elder population, immunocompromised individuals and intensive care unit patients. IC diagnosis and treatment are difficult because of the absence of pathognomonic signs and symptoms. In addition, culture-based examination (gold standard) is known to have low sensitivity and long time to report. All this often leads to unnecessary and costly empirical antifungal therapies, burdened also by the onset of drug-resistance and serious side effects for the patient. To partially overcome these problems, in recent years, novel non cultural markers are being investigated.

Materials and Methods: we retrospectively analyzed serum samples, previously assessed for BDG levels: 29 samples from proven IC episodes *(C. albicans* = 16; *C. glabrata* = 8; *C. parapsilosis* = 1; *C. pelliculosa* = 1; *C. tropicalis* = 1; 2 intra-surgery biopsies positive for *Candida* spp) and 28 from non-IC cases (non-IC), including 9 sera with positive blood cultures (*E. faecium* = 5; *S. pneumoniae* = 2; *P. aeruginosa* + *A. baumannii* = 2) and 19 negative blood cultures. The CAGTA immunofluorescence (IF) assay was performed using serial serum dilutions. According to the protocol, the samples were evaluated by an operator-dependent optical reading (IF positive/negative samples). In parallel, the IF images were captured and the data were expressed as arbitrary fluorescence units (AFU); then, the results were interpreted as positive or negative, by a cut-off (ROC curve, Youden index). The BDG values derived for a previous study.

Results: the operator-dependent reading and the AFU measuring protocol provided comparable data (IC and non-IC sera were correctly identified in most of the cases). Interestingly, the AFU reading enabled a semi-quantitative evaluation of the samples and an objective interpretation of the results, with sensitivity of 52% and specificity of 89%, while BDG had shown sensitivity and specificity of 90% and 75%; the overall accuracy was 70% for CAGTA and 83% for BDG. The association of the two markers, CAGTA and BDG, greatly increased sensitivity to 97% and accuracy to 84%. When excluding non-*C. albicans* episodes, the sensitivity of CAGTA increased from 52 % to 86 %, while it reached 100% when considering only *C. albicans* deep-seated cases. Finally, when considering colonization, BDG demonstrated the most relevant decrease in specificity (from 88% in non-colonized to 58% in colonized patients).

Discussion and Conclusions: with the exception of non-*C. albicans* episodes, CAGTA is a good marker of IC, particularly in presence of deep-seated candidiasis. The combination of BDG and CAGTA, greatly increases their performance.

P180 - Metabolic profiling of Candida albicans

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Introduction

In recent years, metabolomics has become an attractive strategy for the analysis of complex mixtures of small molecular weight compounds present in living systems. Microbial metabolomic analysis focused on extracellular and intracellular metabolites can be used for several purposes, including species identification. Currently, Yeast Metabolome Database, derived from *Saccharomyces cerevisiae*, is the only available database of fungal metabolites. The aim of this study was to outline the metabolic profile of *Candida albicans* SC5314 in different growth conditions. In particular, the metabolites of budding and germinating yeast cells were analysed by proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy.

Materials and Methods

Yeast cells from semi-synchronized cultures were suspended in fresh YPD and 199 medium and incubated at 30 and 37°C, respectively, for 1 and 6 h. Extracellular metabolites, separated by centrifugation followed by ultrafiltration, and intracellular metabolites, obtained by mechanical cells disruption, were profiled by ^{1}H-NMR spectroscopy. Spectra of the culture supernatants and lysates were acquired using a JEOL 600 MHz ECZ600R spectrometer. The metabolites were identified and quantified using Chenomx NMR suite 7.6.

Results

Different metabolic profiles were observed for *C. albicans* cells grown in the selected conditions. An increase in extracellular released metabolites has been observed over time (from 1 to 6 h). Around 50 intracellular metabolites were annotated. Among the major intracellular metabolites, ethanol, acetate, alanine, betaine, and pyruvate were present in different concentrations in budding and germinating yeast cells. These metabolites are involved in the energy production pathway. The presence of the genes coding for the enzymes involved in the metabolic pathways of all the identified metabolites has been confirmed through *Candida* Genome Database (http://www.candidagenome.org).

Discussion and Conclusions

The results obtained in this preliminary study contribute to increase the knowledge of *C. albicans* metabolic profile for the creation of its metabolites database. In addition, this strategy allows to unravel the pathways involved in the transition from budding to germinating phenotype. Furthermore, the characterization of *C. albicans* main metabolic pathways might favour the identification of hot spots possibly targeted by new antifungal agents.

P181 - Increasing antifungal resistance among Candida parapsilosis isolates causing candidemia at a large Italian teaching hospital

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Introduction: *Candida parapsilosis* was the second cause of candidemia in our hospital between 2005 and 2013, accounting for the 20.9% of *Candida* bloodstream infections (BSIs) . With regard to its antifungal resistance profile, isolates showed a low rate of resistance to fluconazole (2.3%) and full susceptibility to voriconazole, itraconazole, and posaconazole. The objective of the present study was to describe the trend of *C. parapsilosis* profiles of antifungal resistance in a time period subsequent to that of our previous study.

Materials and Methods: All the first episodes of BSIs due to *C. parapsilosis* identified between 2012 and 2017 were included in the study. Yeast organisms were isolated, after growth on Difco *Candida* bromcresol green agar plates, from cultures of patients' blood, which was collected as part of standard clinical care. Isolates were identified to the species level by MALDI-TOF mass spectrometry. Antifungal susceptibility testing was performed using the SYO panel. The CLSI resistance breakpoint for fluconazole was defined as an MIC of ≥8 μg/ml; the CLSI resistance breakpoint for voriconazole was defined as an MIC of ≥1 μg/ml. The CLSI resistance breakpoint for anidulafungin, caspofungin, and micafungin was defined as an MIC of ≥8 μg/ml. A linear regression analysis was used to test for the linear increase of *C. parapsilosis* BSI by year. We used a p-value below 0.05 for statistical significance. Biofilm formation was measured using the crystal violet biomass assay.

Results: Overall, 757 BSI episodes due to *Candida* species were diagnosed between 2012 and 2017; 199 (26.5%) were due to *C. parapsilosis*, ranging from 20.6% in 2012 to 29.7% in 2017 (p for trend not significant, NS). A significant increase of resistance to fluconazole was found over the study period, which spanned from 0 cases in 2012 up to 15 (34.1%) in 2017 (p <0.001). Of the 37 cases of fluconazole-resistant *C. parapsilosis*, 25 (67.5%) were diagnosed in patients admitted to medical wards, 6 to surgical wards (16.2%), 4 to the intensive care unit (10.8%), and 2 to oncology or hematology ward (5.5%) at the time of blood sample collection. Three cases of fluconazole-resistant *C. parapsilosis* were isolated in 2017 from the blood of two adults and one child, admitted to medical wards and ICU, respectively, that were cross-resistant to voriconazole. No other significant change in susceptibility were observed against echinocandins, itraconazole, posaconazole, amphotericin B, and flucytosine. Two high level fluconazole-resistant strains were biofilm producers.

Discussion and Conclusions: Epidemiology of candidemia is changing rapidly. Since 2014, isolation of fluconazole-resistant *C. parapsilosis* strains has become a growing threat in our hospital.

P183 - Anti-biofilm Activity of N-terminus of human lactoferrin against different Candida species in Lumen Catheters

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Introduction. *Candida species* are human opportunistic fungal pathogens responsible for superficial as well as systemic infections, mainly in immunocompromised individuals. The ability to grow as a biofilm community resistant to most antifungals is a common trait shared by several clinically relevant species. Among these, *C. albicans, C. parapsilosis, C. tropicalis, C. glabrata, represent* common causative agents of nosocomial infections associated with medical prosthetic devices. The high mortality rate associated to catheter related infections points to the pressing need for new antifungal drugs, which are able to eradicate biofilm-associated mycoses. This study aimed at analyzing the inhibitory activity of the synthetic N-terminal lactoferrin-derived peptide (hLF 1-11) against biofilms produced by clinical isolates of different *Candida* species in an *in vitro* model of catheter-associated biofilm production.

Materials and Methods. Catheter-associated *Candida* biofilm production was used to assess the hLF 1-11 ability to reduce biofilm formation in a catheter lumen by evaluating CFU/mL reduction and biofilm architecture changes using confocal laser scanning microscopy (CLSM). Peripheral venous catheters (PVC) of 1 mm diameter and 32 mm length were used for biofilm formation assays. Following a 24 h incubation at 37°C, control and treated catheters were washed in PBS and then sonicated; yeast suspensions were plated on SD agar for colony-forming unit (CFU) counting. Two different experimental conditions were used to assess the peptide anti-biofilm activity: 4-fold diluted RPMI in sodium phosphate buffer or 10% glucose solution, with hLF 1-11 concentrations ranging from 22 mg/L to 88 mg/L. R

Results. The results obtained showed that hLF 1-11 was able to induce a reduction of sessile cell viability starting from a peptide concentration of 44 mg/L in both experimental conditions. A more pronounced anti-biofilm effect was observed when a 10% glucose solution was used as experimental condition, mimicking the environment associated to parenteral nutrition, on both early and preformed *C. parapsilosis* and *C. albicans* biofilms. A less pronounced inhibitory activity was exerted by hLF 1-11 on *C. tropicalis* and *C. glabrata* biofilms, at the highest concentration only, while no activity could be detected on mature biofilm formed by these two species. C*LSM imaging confirmed the peptide activity on biofilm formation, with low number of viable fungal cells visible on the peptide-treated catheter lumen compared to untreated catheters.*

Discussions and Conclusions. The overall findings candidate hLF 1-11 as a promising agent to prevent biofilm formation by all the *Candida* species tested and to treat *Candida albicans* and *Candida parapsilosis* mature biofilms grown on PVC catheters.

P184 - Antifungal properties of newly designed carbonic anhydrases inhibitors selective for microbial enzymes

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Introduction
Resistance of relevant pathogenic microorganisms to available antimicrobials is one of the most serious current threats for global health and novel compounds able to escape existing mechanisms of resistance are greatly needed. Microbial enzymes belonging to the carbonic anhydrases (CAs) family (beta- and eta-CAs) have recently been explored as potential drug targets, since they are essential for the growth of many pathogens and their inhibition leads to growth impairment. However, the use of currently available CA inhibitors (CAIs) as antimicrobials is associated to a number of side effects due to their interactions with human alfa-CA isoforms. In this study, newly designed synthetic CAIs were assayed against a panel of enzymes to identify a sub-set of compounds highly selective for microbial CAs. Selected CAIs were then investigated against the important fungal pathogen *Cryptococcus neoformans*.

Materials and Methods
The kinetics of the CO_2 hydration reaction catalyzed by recombinant CA isozymes in absence and presence of each CAI (10 mM to 1 nM) was determined by stopped-flow spectroscopy (10 s, 25°C). Inhibition constants were obtained using the Cheng–Prusoff equation. The growth of *C. neoformans* ATCC 6895 in liquid medium added with 3 mM CAIs was evaluated spectrophotometrically after 72 h of incubation at 30°C. The number of fungal colonies grown in solid medium supplemented with 3 mM CAIs was counted after120 h of incubation at 37°C in air or 5.5% CO_2 atmosphere. The average colony diameter was also determined by an imaging software. Percent growth inhibitions were calculated in comparison with controls in the absence of CAIs.

Results
All the investigated CAIs were inactive towards the tested human CA isoforms, while displaying a good inhibitory activity on microbial beta- and eta-CAs. Two CAIs with high selectivity and affinity for the cryptococcal beta-CA confirmed to be active also on whole fungal cells, significantly inhibiting the growth of *C. neoformans* in liquid medium. One of these CAIs proved also to inhibit the fungal growth on solid medium when the plates where incubated in ambient air. The inhibition was not observed in 5.5% CO_2, when CA activity is not essential, thus confirming the enzymatic target of the investigated compound.

Discussion and Conclusions
This study reports on new synthetic compounds which preferentially interact with microbial CAs and inhibit the enzyme also in microbial cells growing *in vitro*, thus providing the proof of concept that CAs are targets that may be specifically engaged by molecules lacking host toxicity. The described compounds may thus represent the prototype of selective CAIs which may be used for the expansion of the current arsenal of antiinfective drugs.

P185 - First insights of fluconazole resistance in Candida metapsilosis

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Introduction
The growing incidence of infections due to the species belonging to the *Candida parapsilosis species complex* stresses the need for a better understanding of genetic and molecular determinants of virulence and antifungal resistance of these species. *Candida parapsilosis sensu latu* spp. differ in their prevalence and virulence, with *Candida metapsilosis* being associated to the lowest clinical prevalence (1.1 to 8.4% of the infections caused by the complex). In recent years, *C. parapsilosis* and *C. orthopsilosis* have been associated with azole resistance and the molecular mechanisms underlying drug resistant phenotypes are staring to be unraveled. Conversely, to date no studies are available on fluconazole resistance in *C. metapsilosis*. This study reports the molecular characterization of azole resistant clinical isolates of *C. metapsilosis*.

Materials and Methods
A panel of 7 *C. metapsilosis* clinical isolates with different azole susceptibility was isolated at the Microbiology Unit of Pisa University Hospital (Pisa, Italy) and Auckland City Hospital, New Zealand. 2 *C. metapsilosis* clinical isolates were characterized by a fluconazole resistant phenotype (MIC=8 mg/L), two isolates were susceptible to fluconazole (MIC=2 mg/L), and three had a fluconazole MIC of 4 mg/L. We evaluated the involvement of efflux multidrug transporters in the development of fluconazole resistance by gene expression analysis as well as the presence of amino acid substitutions in the orthologue gene of *C. albicans ERG11* gene (cytochrome P-450 lanosterol 14alpha-demethylase *ERG3* gene (Delta 5,6 sterol desaturase) and *RTA3* gene (7-transmembrane receptor protein).

Results
Since *C. metapsilosis* genome has been sequenced but not yet annotated, in silico tests allowed us to identify the presence of orthologous genes of *C. albicans CDR1*, *SNQ2*, *MDR1* and *RTA3* encoding for efflux pumps or *ERG11* and *ERG3* genes encoding for ergosterol targets. Expression analysis did not indicate a significant increased expression of efflux pump genes under basal growing condition. Sequencing analysis did not detect any polymorphisms in *ERG* and *RTA3* genes. N° polymorphisms were identified in *ERG11* gene, although they were in heterozygous state and detected both in fluconazole resistant and susceptible *C. metapsilosis* strains.

Discussion and conclusions
This work represents the first attempt to characterize fluconazole resistant strain in *C. metapsilosis* clinical isolates. Interestingly, none of the mechanisms known to be involved in azole resistance in closely related species such as *C. parapsilosis* and *C. orthopsilosis* have been found in *C. metapsilosis*, suggesting that alterative molecular mechanism may be related to azole resistance in this species.

P186 - Evaluation of Micronaut-AM yeast susceptibility test panel, first EUCAST commercial antifungal susceptibility panel: preliminary data on reproducibility performed on ATCC C.parapsilosis and C.krusei

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Introduction: Invasive Candidiasis (IC) is the most common Invasive Fungal Infection (IFI) among non-neutropenic patients. Antifungal agents for candidemia treatment are few molecule (azoles, echinocandins, flucytosine, and polyenes), and treatment failures have been reported. Antifungal susceptibility tests will be performed, particularly when this failure occurs. Two standardized reference methods are available, CLSI and EUCAST, but the preparation of the plate is cumbersome and time consuming. The Micronaut-AM (MAM) colorimetric yeast susceptibility test is the first commercial broth microdilution method that produces MIC data for *Candida* spp. which use EUCAST interpretive criteria for interpretation of MIC. We evaluate the reproducibility of the MIC results using ATCC QC *C.parapsilosis* 22019 and *C. krusei* 6258, using two different inoculum preparation modality.

Materials and Methods: Fresh quality control strains of ATCC 22019 and 6258 where cultivated onto Sabouraud-glucose 2%-agar for 24h and inoculum was prepared in NaCl 0.9% until the turbidity matches a McFarland of 0.5. Each strain was then prepared with two different dilution methods: A- two steps dilution: first 1:20 dilution pipetting 200 µl of the yeast suspension into 4 ml NaCl 0.9% , and later a 1:50 dilution pipetting 200 µl of the 1:20 dilution into 11.5 ml MICRONAUT-RPMI-1640 Medium. B- one step dilution: pipetting 10 µl of the yeast suspension into 11.5 ml MICRONAUT-RPMI-1640 Medium. Methylene blue solution was added to *C.krusei* to facilitate reading of MIC to reduce trailing effects. MAM plates were incubated at 37°C for *C.krusei* and at 30°C for *C.parapsilosis* for 24h. Growth of yeasts was indicated by a colour change from blue to pink mediated by the AST indicator, and MIC was established with visual reading. The test was repeat six different days and the results recorded by a second operator.

Results: *C.parapsilosis* 22019 and *C.*krusei 6258 showed 100% agreement with the recommended MIC/antifungal ranges for all the antifungals tested.
The comparation of dilution method A and dilution method B showed overall for *C.parapsilosis* and for *C.krusei* a MIC agreement of 92 and 91% (range 77-100%) respectively for all the antimicotics.

Discussion and conclusion: Candida susceptibility testing is recommended to support therapeutic options in clinical setting. Standardized reference test are very complex for clinical microbiology laboratories. The Sensititre Yeast One is a commercial microdilution method widely used in clinical laboratories but it need to be interpreted with CLSI criteria. MICRONAUT- AM is the first antifungal susceptibility test that uses EUCAST interpretative criteria, and no data are available on the clinical performance. Our data with ATCC strains showed an excellent performance on reproducibility and respect of the recommended MIC range. The manufacturer recommended a complex procedure to prepare the yeast inoculum, with two step dilution before inoculating the plate. In our study we compare it to a simplest “one step dilution” inoculum preparation to validate the results. The two inoculum procedures showed an excellent agreement, giving the opportunity to more easy approach to susceptibility test for yeast. Further study are necessary to validate the MICRONAUT-AM panel on clinical yeast strains.

P187 - CRISPR-Cas9 system as an efficient tool for the simultaneous editing of an entire gene family in Candida orthopsilosis

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Introduction
The agglutinin-like sequence (*ALS*) gene family of the fungal pathogen *Candida orthopsilosis* encodes for three cell-wall glycoproteins (*CORT0B00800*, *CORT0C04210* and *CORT0C04220*) involved in adherence to host surfaces. The role of each *ALS* gene has been extensively studied in *Candida albicans* through the creation of single knock-out strains. However, this approach can be time-consuming, especially when dealing with multi-gene families. Here we describe a plasmid-based CRISPR method for an efficient gene-targeting technology aimed at the simultaneous mutagenesis of all *C. orthopsilosis ALS* genes, enabling the creation of triple mutant strains in which the entire *ALS* gene family was inactivated.

Material and Methods
The sequenced strain of *C. orthopsilosis*, 90-125, together with a clinical isolate, Co124, previously shown to be highly adherent to human buccal epithelial cells (HBECs), were selected as parental strains. Plasmid pRibo-CoALS harbouring the *CAS9* gene and a gRNA able to simultaneously target 20 nucleotides shared among each member of the *ALS* gene family, was constructed and transformed in *C. orthopsilosis*. A repair template, containing two stop codons as well as a unique restriction site, was included in the transformation, enabling us to disrupt the open reading frame of each *ALS* gene and quickly genotype the transformants. The resulting CRISPR-edited clones, 90-125-3ed and Co124-3ed, were characterized for their ability to adhere to biotic surfaces (HBECs).

Results
CRISPR-edited clones lacking functional copies of the entire *CoALS* gene family were successfully obtained in two different *C. orthopsilosis* genetic backgrounds. The genotype of the resulting CRISPR-edited strains was confirmed by PCR, digestion and sequencing. Wild type and triple-mutant strains did not show any growing defect on liquid and solid media. As expected, our findings revealed that the deletion of the entire *ALS* gene family had a striking effect on the adhesion ability of *C. orthopsilosis* to HEBCs. In fact, when compared with their respective parental strain, both 90-125-3ed and Co124-3ed, showed a significant reduction in the adhesion ability, with more than 80% and 90% reduction in the adhesion index, respectively.

Discussion and conclusions
Our study demonstrates for the first time that the CRISPR-Cas9 system can be used for the efficient inactivation of an entire gene family with a one-step process in *C. orthopsilosis*, significantly speeding up the creation of multiple edited strains. Moreover, adhesion experiment indicated that the *ALS* gene family plays a pivotal role in the adhesion process of *C. orthopsilosis* to HBECs.

P188 - Novel 3-(4-benzylpiperidin-1-yl)-1-(1H-indol-3-yl)propan-1-one derivatives were effective in vitro against clinical isolates of Candida spp. and Aspergillus niger

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1. Introduction

A series of 3-(4-benzylpiperidin-1-yl)-1-(1H-indol-3-yl)propan-1-one derivatives were synthesized and previously evaluated as tyrosinase inhibitors [1]. Over the last few years, incidence of *Candida sp*. infections have significantly increased, with higher mortality rates and hospital acquired infections. The aim of the present study was to investigate the effectiveness of some of our indole derivatives against clinical isolates of *Candida spp* and *Aspergillus niger*.

2. Materials and Methods

The synthesis of the selected 3-(4-benzylpiperidin-1-yl)-1-(1H-indol-3-yl)propan-1-ones was obtained using the appropriate 3-acetyl derivative and the corresponding secondary amine (4-benzylpiperidine or 4-(4-fluorobenzyl)piperidine) hydrochloride.
All the obtained compounds were tested against 3 clinical isolates of *Candida glabrata*, 4 clinical isolates of *Candida parapsilopsis*, 3 clinical isolates of *Candida albicans* and *Aspergillus niger* ATCC 16404. Strains were isolated at the IRCCS (Centro Neurolesi "Bonino Puleio"), Messina. The MIC and MBC determinations were performed according to the EUCAST guidelines.

3. Results

Derivatives were active against *Candida* sp. and *A. niger* at concentrations ranging between 125 and 1000 μg/ml. The most active compounds contained fluoride (F) and methoxy groups (MeO).

4. Conclusions

Indole derivatives were effective against clinical isolates of *Candida* spp. and *Aspergillus niger*. Their use could potentially be explored *in vivo* as topical agent. Their effectiveness in combination with existing drugs could also be further explored.

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P189 - Is the increase of the sCD74 and MIF in sera of Echinococcus–infected patients associated with helminth–mediated immunodepression?

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1. Introduction. Cystic echinococcosis (CE) is a zoonotic disease caused by the larval stage of the species complex *Echinococcus granulosus sensu lato*. Diagnosis of such severe disease is very often an accidental feature during radiological investigations for other reasons. Therefore the role of laboratory in such diagnosis must be improved. The aim of this investigation was the evaluation of some pathogenetic mediators of the human echinococcosis: MIF (Macrophage Migration Inhibitory Factor) and the soluble form of its receptor CD74 (sCD74) and the assessment of their possible role in patients bearing a hydatid cyst. Were also valuated serum IgG1, IgG4 and IgE at different times (both pre- and post-therapy) and the C-reactive Protein (CRP).

2. Materials and methods In this study we enrolled forty patients, 18 echinococcus-positive patients, 11 control patients with non-parasitic cysts and 11 healthy control. Echinococcus IgG, MIF and sCD74 were evaluated by ELISA test. Chemiluminescent assay was used to assess total IgE. Nefelometry was used to measure IgG1, IgG4 and CRP. Difference between groups were estimated with ANOVA plus Fisher's LSD test.

3. Results MIF and sCD74 levels of Echinococcus-positive patients were significantly ($p < 0.05$) lower than the same parameters found in subjects with no parasitic cysts and healthy controls. IgG4 concentrations were similarly increased ($p < 0.05$) in Echinococcus patients vs control groups, while the same value significantly dropped after surgical or medical therapy. Very similar to the above parameters was the trend of IgE, which was significantly increased in echinococcus patients, but decreased after therapy in this group of subjects.

4. Discussion and conclusions In conclusion, the novel findings of this study was the increase of MIF and sCd74 in Echinococcus-infected patients vs healthy controls and subjects with non-parasitic cyst. Further assessment of larger group of patients, as well as multivariate analysis might support the use of such parameters as biomarkers in echinococcus disease.

P190 - MALDI-TOF mass spectrometry as innovative tool applied to parasites identification

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Introduction
Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) is increasingly utilized as a rapid technique to identify microorganisms by their molecular fingerprint and/or by biomarker detection and it represents a first-line method for the accurate routinely identification of bacteria and fungi; its application in parasitology is on the contrary very limited. In this study MALDI-TOF MS was used to identify *Trichomonas vaginalis*, *Dientamoeba fragilis* and to differentiate *Entamoeba histolytica* and *E. dispar*.

Materials and Methods
In this study, an aliquot of one cultured reference strain for each parasite were submitted to formic acid/acetonitril protein extraction and to MALDI-TOF MS analysis. The spectrum obtained for *T. vaginalis* was suplemented in the Bruker Daltonics database (Bruker Daltonics, Germany) and a new identification method was created by modifying the range setting for the MALDI-TOF MS analysis in order to exclude the overlapping of peaks derived from the culture media used in this study. The spectra obtained for *E. histolytica*, *E. dispar* and *D. fragilis* were analysed and subsequently imported into the ClinProTools software version 2.2. (Bruker Daltonics) to perform a statistical analysis in order to check the presence of specific peaks for each parasite. To verify the reliability of the system 21 *T. vaginalis*, 6 *E. histolytica*, 8 *E. dispar* and 13 *D. fragilis* clinical isolates, respectively, were used.

Results
After implementation and modification of the paramenters' setting, the protein spectra of *T. vaginalis* clinical isolates were correctly identified. Five discriminating peaks between *E. histolytica* (2 peaks) and *E. dispar* (3 peaks) and 6 discriminating peaks for *D. fragilis* were found, respectively. When the spectra belonging to the clinical isolates were analysed, all the identifications matched those obtained by a specific Real-time PCR, except for one *E. histolytica* strain.

Dicussion and Conclusions
Although the massive number of entries in the available commercial database, the absence of reference spectra of parasites does not allow their identification. Our study demonstrated that MALDI-TOF MS can be applied to the identification of parasites by using two different approaches: i) the comparison of the obtained spectra with a database that can be suitably implemented also modifying the parameters setting, ii) the detection of specific protein biomarkers.
For the unique discordant result regarding a *E. histolytica* strain isolated from a patient with dysentery also positive for *E. histolytica* antibodies, the presence of amino acid/posttranslational differences as compared to the reference strain could be hypothesized.

P191 - new tools in the diagnosis of cutaneous leishmaniasis, a multidisciplinary approach

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Introduction: Leishmaniasis is a vector-borne protozoan infection whose clinical spectrum ranges from asymptomatic infection to fatal visceral leishmaniasis. In Europe, there are risks of emergence or re-emergence of leishmaniasis which include the spread of visceral and cutaneous leishmaniasis caused by *Leishmania infantum (L. infantum)* from the Mediterranean area of Europe, increased number of immunosuppressed people, and high prevalence of asymptomatic human carriers of *L. infantum.*. Sicily is one of the major islands of the Mediterranean Basin and is considered to be a sub-endemic area for cutaneous leishmaniasis. *L. infantum* is the most common species on the island. Clinical examination of suspected cases, parasitological diagnosis by skin scraping test and immunodiagnosis are the routine methods available for the diagnosis of leishmaniasis. Monoclonal antibodies have long been available for the identification of *Leishmania* species but they are not frequently used.

Materials and Methods:. Between April 2015 and April 2018 total of 80 patients with suspect of cutaneous leishmaniasis were enrolled.

Results: Sixty-one patients were positive; by RT-PCR method revealed the presence of single Leishmania to 7,6*10^6 parasites in the biopsies from patients; while dermoscopic evaluation revealed an erythematous macula, central ulceration that showed the characteristic called "white starburst-like pattern", "yellow tears" and vascular structures (i.e. dotted vessels, polymorphous ⁄ atypical vessels).

Discussion and Conclusions: Molecular techniques in diagnosis of leishmaniasis has become increasingly relevant due to their remarkable sensitivity, specificity and possible application to a variety of clinical samples. Amongst these, real-time PCR has become increasingly popular in the last years, not only for detection and quantification of Leishmania species. However, despite RT-PCR methods which were proven to be very effective in the diagnosis of leishmaniasis, a standardized method does not yet exist. Dermoscopy, is a non-invasive evaluation to aid in the visualization of the epidermis and dermis. Furtheremore, there has been increasing evidence that dermoscopy can also be useful in the diagnosis of some skin infections. Dermoscopy may be a promising non-invasive tool to predict the clinical course in cases of cutaneous leishmaniasis.

P192 - Comparison of different serological kits for thedetection of antibodies against Leishmania sp

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Introduction: in Mediterranean basin, there are fourspecies of Leishmania: the zoonotic *L. major* and the anthroponotic *L. tropica*, both causing exclusively cutaneous Leishmaniasis (CL), *L. infantum*, which usually cause zoonotic CL but can occasionally be responsiblealso for visceral leishmaniasis (VL) and *L. donovani*, which causes VL. Leishmaniais transmitted by the sand-fly bite and, after a variable incubation period (from 2 months to some years), the parasite can migrateto visceralorgans or disseminate in the skin. It has been demonstrated that, if untreated, any CL can lead to visceral manifestation in the long term.Moreover, the lack of prompt diagnosisand treatment of visceral leishmaniasis leadsto irreversible damagesor even to patientdeath.Leishmaniasis diagnosis is currently doneusing different laboratory tests,such as serology, microscopy, culture and PCR.The microscopy is considered the gold standard but not always is possible to carry it on.If the diagnosis is done by a serology, the positivity has to be confirmed at least by two different tests that use two different antigens. Nowadays, there are many serological kits, based on different *L. infantum* or *L. donovani*antigens, specific for the detection of IgG, IgM or both.WHO recommend to use the rK39 antigen but, based on literature, the sensitivity and specificitycan vary. Meanwhile in not-endemic countries it showed very low sensitivity, in endemic countries it gave false positives due to cross-reactivity with other protozoans. The aim of the present study was to compare different serological kit, in order to substitute the IFI routinely used in our lab and no more produced.

Material and Methods: in this study, we perform the assay using 5different kitsand compared with BiomerieuxIFI kit: 2 ELISA (Bordier and Vircell) 2 IFI (Vircell and Euroimmune) and 1 WB (LD-BIO). We processed 10 serum specimenscollected from patients attended to Center for Tropical Disease or sent to our laboratory from other hospitals. The assayswere performed following the manufacturer's instructions.

Results: among 10 sera, only 2 were positive according to our routine-adopted test and resulted to be positive also in all the tested kits. Out of the other 8 samples, only 2 were confirmed as negative by all the tests. The remaining 6 samplesgave positive results to at least one test. The Cohen's k, calculated comparing each of the 5 tested kits*versus* the IFI Biomerieux, showed a variability ranging from 0,1 to 1.

Discussion and Conclusions: The results showed a high variability among the tested kits, endorsing the necessity of a second confirmatory test and an accurate clinical analysis. Further efforts should be applied to develop more sensitive and specific serological tests.

P193 - Levels of total IgE and total IgA in Ascaris patients exhibit an intriguing divergence between stool positive and stool negative subjects

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1. Introduction

Intestinal helminth infections, including ascaridiasis, are associated with activation of T helper 2 lymphocytes (Th2 cells). However the regulatory T cells (Treg cells) have been reported to inhibit a protective response/eradication, therefore leading to chronic infection. Our aim was to establish the role of humoral immunity, evaluated as IgA and IgE levels, and of cellular immune response estimated by lymphocyte-released cytokines in Ascaris patients, with previous or present infection by such nematode.

2. Materials and Methods

Several Th1, Th2 and Treg cytokines were evaluated by "Evidence Investigator" semi-automatic instrumentation by the panel "high sensibility cytokines kit"(Randox Laboratories Ltd., Crumlin, UK). Chemiluminescent assay was used to assess total IgE. Nefelometry was used to measure IgA. Stool samples were collected for parasite search and microscopic evaluation was carried out by direct methods (saline and iodine wet mounts) and by Ritchie's concentration technique with formalin-ether. Difference between groups were estimated with ANOVA plus Fisher's LSD test.

3. Results

We observed a significant increase ($p < 0.05$) of IL-4 (Th2) and IL-10 (Treg) serum levels in ova-free patients with increased level of IgG anti-Ascaris versus patients with ova-positive stool samples. Similarly, IgA and IgE levels were significant higher ($p < 0.05$) in ova-free serum positive patients in comparison with patients exhibiting ova of Ascaris in fecal specimens.

4. Discussion and conclusion

Presence of Ascaris, demonstrated by the ova in fecal samples, appeared to significantly reduce humoral immune responses (measured as IgA and IgE levels) and cellular immune cascade (evaluated as lymphocyte-released cytokines) studied during chronic infection with such nematode.

P194 - "Micro-Young-Group: Advancement in Parasitology": a parasitology course for young microbiologists from basis to practice

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Introduction: In the current era of globalization, the massive movement of populations causes the spread of parasitic infections to several developed countries, and therefore, an increased attention to neglected tropical diseases (NTDs) has recently been encountered in places where such diseases are generally considered unusual. This brought to light the difficulties in diagnosis, control and treatment of NTDs, especially regarding the issues of an adequate formation of the healthcare personnel. From this perspective, the SIM and SOIPA scientific societies united their efforts, backed by the 'giovani SIM' association, to establish an 'ad hoc' course especially designed for young microbiologists named 'Parasitology from basis to practice'. The course aims in building up a solid core in basic parasitology for the diagnosis of NTDs along with the aim to create a network of italian specialists named "Micro-Young-Group: Advancement in Parasitology "(MicroYG). This network of young specialists, guided by their senior referents, will be monitoring the prevalence and diffusion in Italy of human intestinal parasitic diseases, both autochthonous and immigration-based.

Materials and methods: The course, free for 20 parasitologists, was held at the CREMOPAR centre, Eboli (SA) in 5 sessions starting from the 23rd October 2018; the course was articulated both with lecture seminars and laboratory activities. Nationally-renown experts gave lectures on pathogenesis, epidemiology and diagnosis of the most important human intestinal parasitosis.

Results: Practical activities included correct preparation and assessment of the specimens: i) Malaria haemoscopy; ii) diagnosis of intestinal parasitosis with the innovative FLOTAC technique; III) *Acanthamoeba* cultural and microscopic diagnosis.

Discussion and Conclusions: This was the first course promoted by the "Giovani SIM" association under the auspices of SIM and SOIPA societies. The course was a significant moment of aggregation and professional enrichment for the next generation of microbiologists. The basis for a correct approach to human intestinal parasitosis represent the basis to create a national network on a national scale and represents only the first of several initiatives specifically addressed to young microbiologists promoted by the two scientific societies.

P196 - Evaluation of the chemosensitivity of asexual and sexual stages of P. falciparum field isolates by pLDH assay

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Introduction: The number of malaria cases reported in Italy is low (500-600 per year). The L.Sacco Hospital is the reference infectious disease hospital of the metropolitan area of Milano. Cultivation and *in vitro* adaptation of fresh clinical isolates of *Plasmodium falciparum* is challenging and even more demanding if the growth of both sexual and asexual stages is attempted. This is crucial for *in vitro* screening of novel antimalarials and transmission blocking agents, as requested by the ongoing global elimination/eradication efforts. The aim of the present work was to attempt the long term cultivation and gametocytes differentiation of the *P. falciparum* isolated from patients in the L. Sacco Hospital independently from their geographic origin.

Materials and methods: During the study period, 20 blood samples from malaria patients were received. The parasites were immediately put in culture using RPMI 1640 medium, sodium bicarbonate, hypoxanthine, HEPES and glutamine, in the presence of 1% AlbuMAX II (lipid-rich bovine serum albumin). Growing and stabilized cultures were then cultivated in the presence of 10% naturally clotted heat-inactivated human serum to allow gametocyte production. Gametocytogenesis was triggered by diluting the cultures to 0.5% parasitemia, and changing medium daily without the addition of erythrocytes. When a parasitemia of 5% was obtained and the parasites were stressed by nutrient deprivation, the cultures were treated with N-acetylglucosamine to clear residual asexual parasites and gametocyte differentiation was followed for 9-10 days. Chemosensitivity tests were done on both asexual and sexual stages of parasites using a panel of known antimalarial drugs in a modified version of the pLDH method.

Results: The results indicated that 65% of fresh isoltates of *P.falciparum* adapted successfully to asexual culture. Moreover, 69% of the adapted isolates were able to produce mature gametocytes. Both asexual parasites and gametocytes were obtained in almost all cases in adequate quantity to perform the chemosensitivity assays. Within the field isolates, in the asexual stage, the distribution of results indicates a 100% susceptibility to DHA, atovaquone and mefloquine, whereas the response to CQ and quinine was differently distributed among the samples. The response of gametocytes was within the expected range seen using laboratory strains.

Discussion and conclusions: We demonstrated that the pLDH method can be easily adapted to evaluate the chemosensitivity of field isolates to known or novel antimalarials, including transmission-blocking compounds. This confirms that the pLDH method has several advantages over other methods, it is reproducible, low cost, useful for both sexual and asexual stages and for field isolates.

P197 - Diagnosis of perinatal malaria in the globalization era

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Introduction

Perinatal malaria can represent an important issue for screening and diagnosis of malaria, especially for detecting and monitoring *Plasmodium* infection in early stages.

Materials and methods

A 2-month-old male newborn was admitted to the Academic Department of Pediatrics of the Bambino Gesù Children's Hospital due to anemia and exposure to HIV. He was born prematurely in Italy by cesarean section at 34 weeks' gestation after a bicorial, biamniotic pregnancy by migrant woman from Nigeria (30-years-old). He was the first of non-identical twins. Combined to anemia, a spleen enlargement was revealed, therefore malaria was hypothesized. Malaria laboratory routine panel was performed on the newborn, but also on mother and other twin blood samples, as follows: *i*) RDT; *ii*) microscopy of Giemsa stained thick and thin blood smears for *Plasmodium* spp. identification and parasitemia titer; *iii*) molecular screening and typing of *Plasmodium* spp. by multiplex qualitative PCR assay based on 18S rRNA gene. Genotyping of *P. falciparum* isolates responsible for infections of the mother and newborn was performed by amplification of a neutral microsatellite loci marker and five highly polymorphic markers.

Results, Discussion and conclusions

The RDT mother's sample was negative, while infant's sample resulted positive; both microscopy of blood smears and PCR confirmed infection with *P. falciparum.* Four genotypic molecular markers were amplified from mother and newborn DNA samples and one among these markers showed different allelic variants between the two samples. This difference could be explained plausibly by the multiplicity of infection of the mother during the pregnancy. Probably the mother harbored more than one isolates during the pregnancy, only one of them was transmitted to the newborn and one different isolate persisted in the mother's blood after delivery.

Because of the increasing number of pregnant women coming from endemic areas for malaria to non-endemic countries, an accurate anamnesis of infant's mother and the inclusion of *Plasmodium* spp research into the TORCH screening for mother and infant at birth should be performed, avoiding delay in diagnosis and reducing morbidity and mortality associated to the disease.

P198 - Metronidazole resistance in Trichomonas vaginalis: the role of the symbiont M.hominis

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Introduction: *Trichomonas vaginalis* is responsible for trichomoniasis, the most common non viral sexually transmitted disease. Up to 85-90% of *T.vaginalis* isolated in Europe are stably infected by the bacterium *Mycoplasma hominis.* Metronidazole (MNZ) is the drug of choice in the therapy of trichomoniasis, but resistances are constantly increasing. Several effects of the symbiosis between *T.vaginalis* and *M.hominis* on the pathobiology of the protozoon have been studied, but the role of the bacterium on the MNZ resistance of *T.vaginalis* is not clear, yet. In this study we describe the effect of the presence of *M.hominis* on the MNZ resistance of *T.vaginalis* isolates.

Materials and Methods: We tested 48 strains isolated in Italy and Mozambique from symptomatic women for the presence of the symbiont *M.hominis* by specific PCR, and we assessed their sensitivity to MNZ. We then created sets of isogenic *T.vaginalis* strains, with and without *M.hominis*, to exclude any possible strain to strain variability in metronidazole resistance. We cultivated protozoan cells in presence of serial dilutions of MTZ (range 200 µg/ml to 0.19 µg/ml) and we quantified their viability at 1, 24, and 48 hours.

Results: Among the 48 trichomonad isolates comprised in the study, we selected 8 strains that were naturally infected by *M.hominis*, and 8 mycoplasma-free. All of them resulted to be sensible to MNZ, even with different MIC, and no significative differences among the two groups of isolates was demonstrated. To highlight the possible role of *M.hominis* in the sensitivity to MNZ of protozoa independently from any strain-to-strain variability, we repeated the experiments on the isogenic sets of protozoa. The presence of *M.hominis* induce an increase of the minimal lethal concentration of about two times.

Discussion and conclusions: The presence of the symbiont *M.hominis* induce a slight but steady increase in metronidazole resistance in *T.vaginalis*. This finding has interesting implications on the trichomonad therapy, expecially in refractory infections. Further studies are needed to shed light on the mechanisms by which *M.hominis* induces an enhancement of MNZ tolerance in the protozoon.

P199 - Candidatus Mycoplasma girerdii: a new friend for Trichomonas vaginalis into vaginal microbiome communities

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Introduction: Bacterial vaginosis (BV) is associated with an increased risk of sexually transmitted infections, including trichomoniasis. Several studies have shown the association of *Trichomonas vaginalis* with *Mycoplasma hominis*, a bacterium involved in BV, with consequent modulation of host interactions and contribution to associated pathology. Through metagenomic analyses, a novel *Mycoplasma* species, *Candidatus Mycoplasma girerdii* has been identified in BV, only in association with *T.vaginalis* infection, suggesting a strict dependency on the protozoon.

In this study we attempted to shed light on some aspect of the relationship between *Ca.M.girerdii* with *T.vaginalis*, in the presence and in the absence of the symbiont *M.hominis.*

Materials and Methods: Quantitative PCR for *Ca.M.girerdii* was set up using the full-length of 16S rRNA gene as a control, to test the multiplicity of infection (MOI) in naturally infected *T.vaginalis* strains. Moreover, we created isogenic *T.vaginalis* strains with and without one or both Mycoplasma species, to assess the capability of *Ca.M.girerdii* to infect protozoan cells also in presence of *M.hominis*, and to evaluate the impact of infection on kinetics of growth of the protozoon. Finally, bacteria location was assessed by gentamicin protection assay and immunofluorescence.

Results: We demonstrated a stable infection of *T.vaginalis* by *Ca.M.girerdii*, even in the presence of *M.hominis*, and we obtained evidence that *Ca.M.girerdii* is located intarcellularly. Moreover, the number of *Ca.M.girerdii* in different *T.vaginalis* strains varied greatly, suggesting a specific MOI for each protozoan strain. Starvation assay and freezing stress in experimental and natural infected strains show a decrease of MOI, suggesting a high sensibility to environmental conditions by *Ca.M.girerdii*.

Ca.M.girerdii is not able to infect *T.vaginalis* when *M.hominis* is present; replication rate in isogenic *T.vaginalis* strains has been compared, showing a faster growth rate and higher cell densities in presence of both bacteria.

Discussion and conclusions: These findings confirm the association of *Ca.M.girerdii* with *T.vaginalis*, suggesting a stable intracellular relationship. Moreover, the presence of *M.hominis* in protozoa may inhibit the infection of *Ca.M.girerdii*. Further studies will better characterize the role of *Ca.M.girerdii* in the outcome of trichomoniasis.

P200 - A surveillance network of Cystic Echinococcosis in farm animals and in humans at risk of infection in the Campania region

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Introduction: Cystic Echinococcosis (CE), also known as hydatid disease, is caused by the larval stage of the tiny tapeworms *Echinococcus granulosus* (EG) *sensu lato.* The parasite is transmitted to dog (definitive host) when it ingests the organs of other animals that contain hydatid cysts, resulting actively transmitted in all pastoral regions where sheep, cattle, buffaloes, pigs predominate, including Italy. Infection in humans occurs accidentally through the oral ingestion of eggs, excreted by canides, and may cause the development of cysts in many organs, mainly liver and lung. The initial phase of the human infection has an asymptomatic disease course, with well-encapsulated, slowly enlarging cysts that often grow unnoticed and neglected for years. The real prevalence and diffusion of CE are difficult to estimate due to the high proportion of asymptomatic infected individuals. The Veterinary Sector of Campania Region, together with the 'Research Unit for the Monitoring of Intestinal parasitosis of Migrants of the Mediterranean area' (URPIM) of the University of Campania "Luigi Vanvitelli", the Regional Center for Monitoring Parasitic infection (CREMOPAR) and the Experimental Zoo-prophylactic Institute of Southern Italy, has developed a wide project that focused on the building of a surveillance network of CE in farm animals and in humans at risk of infection in the Campania region.

Materials and methods: A monitoring programmes in farm animals and diagnosis of human CE in farm workers and their relatives have been performed from 2015 up to now using serological assays (ELISA and IHA) and subsequently confirmed by molecular tests (Immunoblotting). Since the human diagnosis of CE relies mainly on findings by hepatic ultrasonography or other imaging techniques, from 2018 the hepatic ultrasonograpghy on the field has been introduced. Moreover all the data are evaluated using Geographical Information System (GIS).

Results: We have analyzed 1245 human serum samples: 14 samples are resulted positive at ELISA test, while only 3 samples at IHA. The positive samples at IHA test have been confirmed through immunoblotting.

Conclusions: CE is a preventable disease; periodic deworming of dogs, improved hygiene in the slaughtering of livestock (including the proper destruction of infected offal), public education campaigns and massive monitoring programs have been found to lower and prevent transmission and alleviate the burden of human disease. Our preliminary data on the prevalence of CE in farm animals confirm the importance of the surveillance upon free ranging dogs and those human subjects at risk of infection. Moreover, the introduction of liver ultrasonography should bring an improvement to our program of screening.

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