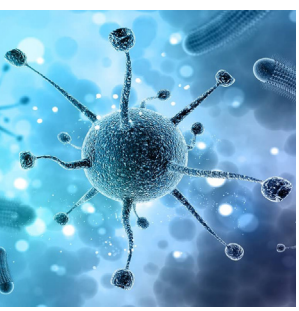
Poster Presentation

Medical Microbiology 2019





4th International Conference on

Medical Microbiology

May 20-21, 2019 | Vienna, Austria

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Konstantina Kontopoulou, Microbiol Curr Res, Volume 3
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Detection of colonization-infection by multi-drug resistant microorganisms in patients with previous hospitalization

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Objective: Detection of rectal and pharyngeal colonization by multi-drug resistant microorganisms (MDR) in patients with previous hospitalization in other hospitals.

Material-methods: 564 patients admitted to our hospital with prior hospitalization in other hospitals were screened for MDR microorganisms by rectal and pharyngeal swabs within 24h of their admission. The study did not include ICU patients. The patients were monitored for the development of any signs of possible infection. *Pseudomonas aeruginosa*, *Acinetobacter baumannii* complex and *Klebsiella pneumoniae* resistant to carbapenems, methicillin resistant *Staphylococcus aureus* (MRSA) and vancomycin resistant *Enterococcus spp*. (VRE) were concerned as MDR. The swabs were directly inoculated onto chromID CARBA prototype medium (bioMerieux, Marcyl'Etoile, France). Identification and susceptibility testing were performed by VITEK 2 automated system (bioMerieux, Marcy l'Etoile, France). The MICs of imipenem, meropenem, ertapenem, tigecycline, vancomycin and teicoplanin were determined using E-tests (bioMerieux, Marcy l'Etoile, France) following the Clinical and Laboratory Standards Institute (CLSI) guidelines and interpretative criteria. Detection of KPC and VIM resistance genes was done via combined-disk tests using meropenem with and without phenylboronic acid (PBA), EDTA or both, as recommended by EUCAST.

Results: 51 patients (9%) were colonized by one or two MDR microorganisms. Particularly, 20 (3.5%) were colonized with *K. pneumoniae*, 21 (3, 7%) with *A. baumannii* complex and 10 (1,8%) with *P. aeruginosa* resistant to carbapenems. All strains of *K. pneumoniae* were KPC. 4 (0,7%) patients were colonized with MRSA and 7 (1, 2%) were colonized with 2 MDR microorganisms. Cohorting was applied in all patients. 10 colonized patients developed an infection during their hospitalization with a microorganism with the same resistant phenotype as the colonization strain. Table 1 shows the rates of colonization and infection by the responsible microorganisms, while Table 2 indicates the type of infection.

Table1: Patients with colonization and corresponding infections

	A.baumanniicplx	*P.aeruginosa*	*K.pneumoniae*	MRSA	VRE
Colonization	21	10	20	4	-
Infection	3	2	5	-	-

Table 2: Type of infection

	Bloodstream infection	Urinary tract infection	Wound Infection
A. baumanniicplx	2	1	-
P. aeruginosa	1	-	1
K. pneumoniae	2	2	1

Conclusions: Screening of colonization by multi-drug resistant microorganisms in patients with previous hospitalization in another healthcare institution is considered necessary for the timely apply of patients cohorting and strongly implementation of contact precautions to prevent and limit the spread of multidrug-resistant microorganisms.

Speaker Biography

Konstantina Kontopoulou has done her master's in public health from University of Macedonia, Greece and doing her PhD at Aristotle University of Thessaloniki, Greece. She is specialized in medical biopathology and worked as a chief of microbiology department at Interbalkan Medical center, Thessaloniki and now she is working as a senior registrar of microbiology department at Gennimates general hospital, Thessaloniki. She has attended many conferences and has marked her imprint of research by winning awards under various categories. She also worked as a sub investigator for various clinical trial and research projects. She is currently an active member of various committees such as Medical Society of Thessaloniki, Greek society for Infection Control, Hellenic Microbiology Society.

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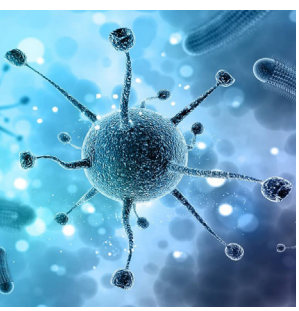

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Infection of mammalian liver by the malaria parasites relies of a network of parasite kinases

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There are 240 million cases of malaria leading to nearly 400,000 deaths each year. It is caused by five species of the protozoan parasite, *Plasmodium*, transmitted to humans by mosquitoes, in the form of 'sporozoites'. Sporozoites invade hepatocytes where they develop intracellularly into 'liver stages'. Liver stages exit the hepatocytes in membrane-bound vesicles, termed 'merosomes', that disintegrate in the blood-stream. There, liver stages infect erythrocytes and initiate the symptomatic step of malaria. Blocking *Plasmodium's* liver cycle could prevent disease and a better understanding of the key pathways at this step can identify drug targets for malaria chemoprophylaxis. We report that sporozoite entry into hepatocytes requires the parasite's cGMP-dependent protein kinase (PKG) and Calcium-Dependent Protein Kinases 1, 4 and 5 (CDPK1, CDPK4 and CDPK5). PKG and CDPK5 are also required for the parasite's egress from the hepatocyte. Chemical inhibition of *Plasmodium* PKG abolishes sporozoite motility by preventing secretion of proteins that enable adhesion of sporozoites to the extracellular matrix. Depletion of CDPK1, 4 and 5 also decreases sporozoite motility, but without significantly affecting their adhesion to the substrate. Since motility is required for sporozoites to a) disseminate from the site of deposition in the dermis, migrate through cell- and tissue-layers to enter the blood stream and c) enter a hepatocyte, its inhibition significantly decreases sporozoite infectivity. Chemical inhibition or knockdown of PKG and CDPK5 has a second effect – inhibiting either the formation or release of merosomes. Mice treated with a PKG inhibitor are significantly less susceptible to infection by sporozoites, providing preliminary evidence that chemical inhibition of parasite PKG can block infection in animals. By revealing the requirement for PKG, CDPK1, 4 and 5 in *Plasmodium* invasion of and egress from hepatocytes, our work provides biological and chemical validation for targeting these *Plasmodium* kinases for chemoprotection against malaria.

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The pathogenesis and characteristics of congenital affliction associated with zika virus - What to do

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Zika virus infections have exploded on the international scene with little warning due in part to the genetic changes in the virus over time in different geographic areas resulting in variable epidemiology, host-virus immune interactions, pathology and clinical manifestations. These will be reviewed with an emphasis on the genomic evolution and pathogenesis. Comparisons will be made to other pathogens that are associated with congenital affliction. Worldwide experience with attempts to care for these children will be discussed and the creation of national programs to respond to this public health threat including the development of antivirals and vaccines.

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Relationship between overcrowding, poverty and community acquired methicillin resistant *Staphylococcus aureus*

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Ongoing infections with CA-MRSA in deprived and overcrowded areas of London was described. There was continued arrival of this strains to the hospital setting. Disparities in CA-MRSA was not explained by racial causes but was associated with overcrowding. We diagnose sporadic cases of wound infection caused by CA-MRSA post cesarean interventions and frequent cases of SSTI (skin and soft tissue infections). We studied familial SSTI at community level and found association of it with overcrowding and location of the home at the poorer neighborhoods. We found 41.3% of the homes had history of SSTI. 22,3% of households had extreme overcrowding. In the poorer neighborhoods 66 of 129 households (51.2%) had a history of SSTI. In the richer neighborhoods the history of SSTI appeared in 37 of 119 (31.1%) (p=0.0019). Presence of CA-MRSA should always be suspected in infections associated with overcrowding and living in poor neighborhoods. A history of SSTI can be easily correlated with the presence of CA-MRSA. We suggest: SSTI should be treated with non beta-lactamic antibiotics, investigate and treat familial dissemination of the infection, explain measures of hygiene and control to block the reentry of the organism. Community sepsis should be treated with antibiotics that cover CA-MRSA, especially in front of personal or family history of SSTI or an overcrowded home or placed in disadvantaged socioeconomic zones. Restrict caesarean births. In the event of a family history of SSTI, extreme overcrowding or living in an area of disadvantaged socioeconomic zone, contemplate adding vancomycin to antibiotic prophylaxis. This recommendation should be evaluated in depth in each programmatic area.

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Socio-environmental factors and diarrheal diseases in under five-year old children in the state of Tocantins, Brazil

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Diarrhoea is a waterborne disease that affects children, especially those under 5 years of age. The objective of this study was to identify the spatial patterns of distribution of diarrheal disease in under 5-year-old children in the State of Tocantins, Brazil, from 2008 to 2013. Geoprocessing tools were used to carry out an epidemiological study, to prepare thematic maps in the TerraView 4.2.2 software based on secondary data. General indicators of the disease, presence of spatial dependence through the Global Moran's Index (I) and the Spatial Association Index (LISA) were described. There were 3,015 cases of under 5-year-old children hospitalized for diarrhoea, with an average annual rate (AAR) of 4.10/1,000 inhabitants (inhab.). Among the main characteristics were: increasing rates in under 1-year-old children (6.16 to 9.66/1,000 inhabitants); children aged 1 to 4 full years (63%); males (55%); 8deaths of under one-year-old children (75%); county of Araguaína (67%); incidence in the county of Nazaré (63.97/1,000 inhab.); prevalence and incidence in the Araguaína microregion (45%, AAR 9.38/1,000 inhab.). The presence of a cluster with spatial autocorrelation was found in the Araguaína microregion, which was statistically significant (I = 0.11, p-value < 0.03), with priority of intervention (Moran Map). There was an increase in the number of hospitalizations for diarrhoea in under 5–year-old children in the state of Tocantins. The spatial analysis identified clusters of priority areas for measures of maintenance and control of diarrheal diseases.

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Predictive value of microRNAs for decreasing CD4 T cell count among HIV-1-infected patients who spontaneously control viral replication (HIV controllers)

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Background: A small group of HIV-1-infected individuals (5-15%) control disease progression for several years in the absence of any antiretroviral therapy. Among this group, elite controllers spontaneously control HIV-1 replication (below 50 HIV-1 RNA copies/ml); nevertheless, they are still susceptible to have several aspects of the immune response deregulated, especially elevated immune activation and inflammation. Homeostatic factors contribute to maintain a stable pool of T cells in this situation where T cell apoptosis is enhanced. This situation promotes the release of micro vesicles, such as exosomes that are released by the cells and are present in blood, urine and saliva. This content includes miRNAs, small non-coding RNA capable of recognizing specific mRNA and inhibiting its translation into proteins. These molecules may thus promote hematopoietic stem cells and regulate the immune system and inflammatory processes that could influence the homeostasis cell equilibrium. HIV could interfere with the exosomal pathway. The direct influence of exosomal miRNAs on the cells of the immune system during HIV infection is a topic that is still poorly understood. Since exosomes can modulate immune responses and may affect HIV pathogenesis, we conducted this cross-sectional study of quantification of selected miRNAs in HIV elite controllers. We also investigated the association of plasma-derived exosome miRNA levels with both soluble cytokine levels and cellular immune activation.

Methods: Two groups of elite controllers were analysed, i.e., those that during the follow up had stable or increasing CD4 T cell count (SEC, N=21), and those who had significant decline of CD4 T cell count (DEC, N=11). Plasma-derived exosomes were used to determine the expression of miRs and determine their association to soluble cytokine markers and cellular immune activation.

Results: Plasma exosome-derived miR-16 and miR-21 are downregulated in DEC group, while miR-221 was upregulated compared to SEC group. Only miR-21 was independently associated with CD4 T cell decline in elite controllers (p=0.049; odds ratio 0.369, IC95 [0.137-0.994]). On the other hand, negative correlation between plasma exosome-derived miR-21 and MCP-1 was found (p=0.020). No correlation between expression of miRs and cellular immune activation markers was found.

Conclusion: Exosome-derived miR-21 might be used as a valuable prognosis soluble biomarker to define HIV-1 elite controllers who will have significant decay in their CD4 T cell counts.

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A novel immobilized method for isolation of phage display library-derived scFv antibody specific to *Listeria monocytogenes*

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We developed a novel simple and cost-effective immobilization method for bio panning of a phage-display library. We used Light Expanded Clay Aggregate (LECA) as biomass support matrix to isolate phage display library-derived scFv antibody specific to *Listeria monocytogenes*, a bacterium causing serious illness in human and animal. Four rounds of positive selection against LECA-immobilized *L. monocytogenes* and an additional subtractive panning against *L. innocua* were performed. This panning scheme in combination with our novel immobilization method allowed us to isolate the phage clones bind to *L. monocytogenes* without cross-reactivity toward ten other non-*L. monocytogenes* bacteria. One of the selected phage clones was able to specifically recognize three major pathogenic serotypes (1/2a, 1/2b and 4b) of *L. monocytogenes* and 11 tested *L. monocytogenes* strains isolated from foods. This scFv antibody has potential use in development of immunoassay-based methods for rapid detection of *L. monocytogenes*. In addition, the LECA-immobilization method described here offers an efficient, simple and cost-effective bio panning strategy to isolate specific monoclonal antibodies against any given species of pathogenic bacteria from phage-display libraries.

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Influenza vaccination of patients with autoimmune rheumatic disease

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Objectives: Compared to the healthy population, patients suffering from autoimmune rheumatic diseases have a significantly increased risk of various infections.

Methods: Our study includes three groups of patients (99 in total) with stable diseases status, suffering from: 30 patients with systemic lupus erythematosus (SLE), 37 with rheumatoid arthritis (RA) and 32 with Sjögren's Syndrome (SjS). 46 patients were immunized with an inactivated trivalent split vaccine (15 µg HA A/California/7/2009 (H1N1), 15 µg HA A/Pert/16/2009 (H3N2) and 15 µg / HA B Brisbane / 60/2008) whereas 52 patients did not accept the proposed vaccination. These three groups of patients were divided into two subgroups depending on vaccination: Vaccinated - SLE1 (19), RA1 (15) and SjS1 (14), and unvaccinated - SLE2 (11), RA2 (22), SjS2 (18). In the following six months parameters of disease activity and the titer of antibodies against influenza A H1N1 were monitored. We used hemagglutination inhibition test (according to the method of the Center for Disease Control and Prevention (CDC) with antigen A/California/7/2009 influenza virus (H1N1), and turkey erythrocytes for the detection of antibodies against the A H1N1.

Results: The incidence of viral and bacterial infections among vaccinated patients was significantly lower, compared to the non-vaccinated group. Influenza occurrence was significantly associated with previous respiratory infections (p=0.001). ST levels for all vaccinated patients (84.17) were significantly higher than in non-vaccinated patients (8.80) (p=0.008) and were associated with last vaccination in all patients and in SLE group (p=0.012, p=0.039 respectively). Seroprotective rate for all vaccinated patients was 48% compared to 15% in unvaccinated (p=0.014) and it was highest among SLE patients (53%) (p= 0.049).

Conclusions: Based on several years of monitoring respiratory infections in our patients, it is clearly visible that a high risk for exacerbation of the underlying disease was linked to viral or bacterial infection and practically never to the vaccination itself.

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Hand hygiene assessment of staff working in food processing units by detecting germs of the genus *Staphylococcus*

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Staphylococcus aureus is commonly worn by seemingly healthy people (usually on the nasal mucosa and on the skin). Frequency of wearers is higher among people who present or have experienced boils, panartists, or various wounds in the skin. The main danger of food contamination with bacteria of the genus *Staphylococcus*, consists in the development by some strains (coagulase - positive) of an enterotoxin capable of causing acute gastroenteritis in humans. *Staphylococcal enterotoxin* is thermostable, which is why it is difficult or even impossible to inactivate it after its development. Produces intoxications with a short incubation period even after 30 minutes after ingestion, generally after 3-6 hours. The risk of intoxication increases because the growth of these bacteria does not necessarily cause changes in the taste and smell of the food even when the number is high. The collection of sanitation samples was made from several food processing units from hard-to-reach points (30%) and from the surfaces with which the food came into direct contact (work tables, tops, walls, etc.) (70 %). Depending on the surface, the number of samples taken was 7, for areas less than 1000 m^2 and 12 for areas larger than 1000 m^2. Randomized samples were also taken from the hands of the workers involved in the different processing steps, accounting for 5% of the total number of employees. On Baird - Parker, typical colonies of *Staphylococcus aureus* presented a blackish - gray, bright, convex color, approximately 1.5-2 mm in diameter after 48 hours of incubation, surrounded by a clear or sometimes opalescent area. Atypical colonies also considered "background flora", have the same characteristics but without the presence of clear or opalescent areas. For confirmation, gram stained smears were performed and examined by microscope with a 100x immersion objective. The number of positive samples was 3.7%, of which 0.6% coagulase positive *Staphylococcus* were isolated. Of the total positive samples, 87% were taken from the hands of the staff, 6% from the worktops, 3% from the floors and 4% from the other surfaces. Staff working in the food industry is the main potential source of microbiological contamination with bacteria of the genus *Staphylococcus*, which is why the proportion of sanitation samples taken from food processing units must be the hands of the staff.

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Molecular characterization of antibiotic resistant *Escherichia coli* isolates recovered from food samples and outpatient clinics

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Multidrug-resistant *Escherichia coli* is one of the most important public health concerns worldwide that can be transferred through the food of animal origin to human being causing serious infection. The genetic responsibility of such resistant genes (Plasmids, integrons and transposons) can be easily transmitted from the resistant strain to another. Therefore, the main objectives of the study is the molecular characterization of the resistant *Escherichia coli* isolates recovered from food samples and human isolates collected from outpatient clinics, KSA especially the resistance strains against aminoglycoside resistance genes which are responsible for the resistance against gentamicin and the resistance caused b-lactamases genes. Examination of food samples revealed 120 *Escherichia coli* isolates (22.22%) (30 strains O26: K60,28 strains O128: K67, 20 strains O111: K58, 18 strains O126: K58, 10 strains O55: K59, 9 strains O86: K61 and 5 strains O157: H7). All the strains were highly resistance to penicillin, amoxicillin-clavulanic and erythromycin with a percentage of 100%, while the resistance to gentamicin, ampicillin, oxytetracycline, chloramphenicol, norfloxacin, trimethoprim, and nalidixic acid were 83%, 75%, 65.3%, 55.8%, 36.5%, 30.7% and 26.9% respectively. On the other hand, 59.6% of tested strains were sensitive to ciprofloxacin. Positive amplification of 896 bp fragments specific for aacC2 genes were observed by PCR designated for the detection of the aminoglycoside resistance genes. Meanwhile, multiplex PCR designed to detect the ampicillin and amoxicillin-clavulanic acid resistant *E.coli* isolates revealed positive amplification of 516 bp fragments specific for BlaTEM gene with all the resistant strains to ampicillin and amoxicillin clavulanic acid. Moreover, positive amplification of 392 bp fragments specific for BlaSHV resistant gene were observed with (60.52%) of *E.coli* isolate. While all the tested strains were negative for amplification of BlaOXA_1.

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Blastocystis sp. - a harmless commensal to a harmful pathogen

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The talk will highlight a long quest to search for answers surrounding one of the most enigmatic organisms called *Blastocystis* sp. Many aspects of the organism were constantly enshrouded with questions. With every new finding there arose conflicting reports, confusing data and more importantly discrepancies especially on the parasite's role in causing disease. Hence *Blasocystis* sp. was dismissed and considered only to be another commensal along with million other organisms in the human gut. Paradoxically, it is one of the most commonly found organism in any stool survey. The talk details key highlights since 1990 on our contributions made to unravel the biology, life cycle, prevalence, genotypic expressions, phenotypic characteristics, pathogenicity, drug treatment, apoptosis and association to irritable bowel syndrome and colorectal cancer. In the early 90's, there were scanty and sporadic number of reference papers, lack of expertise to provide guidance and most of all the lack of encouragement to proceed this path from sceptics who were sure that this *Blastocystis* sp. quest would be unprofitable. The presentation will trace the historical development of research on *Blastocystis* sp. and at the same time highlights the thought processes and paradigm shifts which contributed to the newer insights on this organism. The talk will highlight how this once considered harmless commensal has been associated with colorectal cancer, stress and irritable bowel syndrome.

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Knowledge and attitudes about antibiotic use and resistance: A cross-sectional study among primary healthcare center attendees in an urban area, Alexandria, Egypt

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Antibiotic misuse and bacterial resistance are a significant public health concern worldwide. Egypt lacks policies and regulations concerning medication prescriptions. The study explores the knowledge and attitudes regarding antibiotic use and resistance among adults attending a primary healthcare center in an urban area of Alexandria. The researcher conducted a single point, descriptive cross-sectional study on 87 adults aged ≥18 years attending a primary healthcare center in an urban area of Alexandria in January 2019 using a semi-structured questionnaire to gather data about the knowledge and attitudes of using antibiotics and resistance through face-to-face interviewing method. The data were analyzed by descriptive statistics to explore the respondents' level of knowledge with respect to the use of antibiotics and resistance. High level of knowledge was assigned as > 66.7% of the total score. About 52.8 % of the respondents (63.2% of them were females) lack adequate knowledge about the use and resistance of antibiotics. Almost 65.6 % of males had less restrictive knowledge about the use of antibiotics and resistance than 45.5 % of the females. Simultaneously, 47.1 % of the respondents erroneously believed that antibiotics work on both bacterial and viral infections and 14.9 % thought it just fights viruses. Approximately, 66.7 % of them were unaware of the meaning of antibiotic resistance. Moreover, 33.3 % stated they have no role to play against bacterial resistance. 83.9 % of respondents knew that vaccination could prevent bacterial resistance. The findings display poor knowledge and attitudes of proper antibiotic use and resistance among respondents. Healthcare providers should utilize these findings to educate the public on how to rationally use antibiotics and the health hazards of bacterial resistance.

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The potenial effect of probiotic bacteria against resistant-carpabenem *Acinetobacter baumennii*

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Infections caused by carbapenem resistance *Acinetobacter baumannii* (crab) are continually a focus of significant attention since the limitation of therapeutic options. Probiotic bacteria always have an essential role in dairy products and fermented food and promoted the positive health image as the treatment of microbial infections. Here, we evaluated the potential of antimicrobial agent produced by probiotics for the protection against crab infection. Tracheal aspirate specimen from 150 patients at Egyptian hospitals were recognized as Ab by PCR detection of blaOXA-51. Antimicrobial susceptibility was studied. Positive crab isolates with blaOXA-24 and blaOXA-58 incidence were undergoing for screening using probiotics that have been isolated from dairy products & food supplement. Probiotic have highest antagonistic activity was identified and its bioactive compounds were purified & characterized by studying physiochemical characters. Nearly ninety-six of the cases were crab & 37.5% of cases harboring blaOXA-24 & only one case has blaOXA-58. *In vitro*, significantly 80% (P<0.05) of crab remarkably inhibited by four probiotics. *Bifidobacterium bifidum* strain that showed the highest activity against crab has been identified, with significant inhibition levels reaching 83.33% in the case of the supernatant and even 97% inhibition of supernatant purified by column chromatography. Purified BbV1 was heat stable with amino acid content as identified by LC-MS/MS and belonged to bacteriocins-like compounds. Our finding demonstrated that natural BbV1 provides a protection against *Acinetobacter* infection *in vitro*. *In vivo*, further studies were applied using immunological and histological studies for application as nutritional and pharmaceutical use.

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Susceptibility to Hepatitis B infection, Hepatitis B/HIV co-infections and Hepatitis B immunity in HIV positive patients starting HAART in Durban, South Africa

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Background: HIV/HBV co-infection remains a global threat to HIV management despite the available effective Hepatitis B vaccine and Hepatitis B covering Antiretroviral therapy. Many studies done in South Africa and internationally showed high prevalence of HIV/ Hepatitis B co-infection which mandated routine screening for both infections before initiating HAART. Fewer studies highlighted the prevalence of Hepatitis B susceptibility in the general population starting HAART and most of them were limited to children and high-risk groups. The aim of this study was to demonstrate the extent of Hepatitis B susceptibility, Hepatitis B/HIV co-infections and Hepatitis B immunity in the general HIV infected patients.

Method: This was a retrospective review of randomly sampled 1066 files of patients initiated on HAART between January 2012 and December 2014 at two Durban hospitals. Data collection included demographic characteristic, CD4 counts and Hepatitis B serology. Data was analysed for the prevalence of Hepatitis B susceptibility, HIV/HBV co-infection and Hepatitis B immunity, while correlations between age, CD4 count and these three groups were demonstrated. Statistical analysis was performed using SAS version 9.3

Results: Total prevalence of HBV susceptibility was 69.7%, HBV immunity was 26.9% and true chronic HIV/HBV co-infection was 3.4%, while HBVsAg positivity accounted for 8.4% of the participants. Adults were more susceptible to HBV than children, with median age of 36 years. Stratified for age, children were more immune (90%) to HBV than adults.

Conclusion: This study demonstrated a significantly high number of HIV infected persons who were susceptible to Hepatitis B infection in Durban, South Africa, where both HIV and HBV are endemic, co-infection is high and safe and effective HBV vaccine is available. We recommend Hepatitis B vaccination of the Hepatitis B susceptible patients initiating HAART in South Africa to prevent further HIV/HBV co-infection.

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