VI Конгрес на микробиолозите на Македонија
со меѓународно учество
VI Congress of Macedonian Microbiologists
with international participation

FEMS-supported Symposium: "Emerging infections"

КНИГА НА АПСТРАКТИ

ABSTRACT BOOK

30.5 - 2.6.2018 год., Охрид, Р. Македонија
30.05 – 2.06.2018, Ohrid, R. Macedonia

VI КОНГРЕС НА МИКРОБИОЛОЗИТЕ НА МАКЕДОНИЈА СО МЕЃУНАРОДНО УЧЕСТВО

СИМПОЗИУМ ПОДДРЖАН ОД FEMS "EMERGING INFECTIONS"

VI CONGRESS OF MACEDONIAN MICROBIOLOGISTS WITH INTERNATIONAL PARTICIPATION

FEMS SUPPORTED SYMPOSIUM "EMERGING INFECTIONS"

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VI CONGRESS OF MACEDONIAN MICROBIOLOGISTS WITH INTERNATIONAL PARTICIPATION

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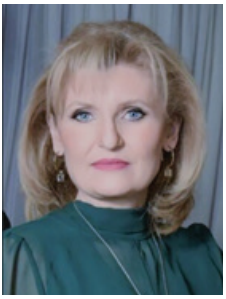

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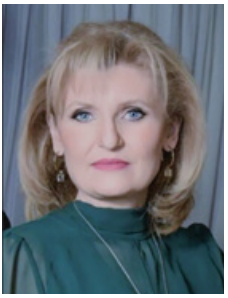

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ОБРАЌАЊЕ

Почитувани колешки и колеги,

Ни претставува особена чест и задоволство во име на Организациониот и Научниот одбор да Ви посакаме добредојде на **VI Конгрес на микробиолозите на Македонија со меѓународно учество** и **Симпозиум со наслов "Emerging infections", поддржан од FEMS (Federation of European Microbiological Societies)** кои ќе се одржат во периодот од 30 мај до 2 јуни 2018 година во хотелскиот комплекс Метропол- Белви во Охрид, Република Македонија, во организација на микробиолозите на Македонија, членови на МЛД.

Веруваме дека научната програма и можноста да се слушнат предавања од врвни експерти и научници од Балканот, Европа и пошироко, учеството во научни дебати и интерактивни дискусии, ќе бидат предизвик за вас и можност за размена на искуства, проширување на вашите знаења, вештини и техники од сите области на микробиологијата и современата дијагностика, превенција и терапија на инфективните заболувања, како и можност од воспоставување на нови професионални контакти.

Традиционално место на одржување е древниот град Охрид, колевка на македонската и словенската писменост.

Покрај научната, предвидена е и богата социјална програма и можност со вашите колеги-пријатели да го почувствувате гостопримството и богатото културно наследство на Охрид.

Се надеваме дека со вашето присуство и активно учество ќе придонесете за успешна реализација на предвидените активности.

Претседател на Организациониот одбор
Проф. д-р Ана Кафтанџиева

Претседател на Научниот одбор
Проф. д-р Никола Пановски

WELLCOME ADDRESS

Dear colleagues,

On behalf of the Organizing and Scientific Committees, it is our great honor and pleasure wellcome you to the **VI Congress of the Microbiologists of Macedonia with international participation,** and **FEMS supported Symposium entitled "Emerging infections"** which is held from May 30th to June 2nd, 2018 at the hotel complex Metropol-Bellevue in Ohrid, Republic of Macedonia, organized by the Microbiologists of Macedonia, members of MDS.

We believe that the scientific program and the opportunity to hear lectures of top experts and scientists from the Balkans, Europe and beyond, participation in scientific debates and interactive discussions will be a challenge for you and an opportunity to exchange experiences, expand your knowledge, skills and techniques from all areas of microbiology and contemporary diagnostics, prevention and therapy of infectious diseases, as well as the possibility of establishing new professional contacts.

Traditional event venue is the ancient city Ohrid, a cradle of Macedonian and Slavic culture and literacy.

In addition to the scientific program, we will provide a rich social program and opportunity for you and your fellows and friends, to feel the hospitality and rich cultural heritage of Ohrid.

We hope your presence and active participation will contribute to successful realization of the planned activities.

President of the Organizing Committee
Prof. Ana Kaftandzieva, MD, PhD

President of the Organizing Committee
Prof. Nikola Panovski, MD, PhD

FEMS-supported Symposium: "Emerging infections"

Prof. Bauke Oudega, FEMS President, Amsterdam, The Netherlands

Prof. dr. Bauke Oudega studied Chemistry at the University of Amsterdam, and he did his PhD at the Vrije Uniniversiteit of Amsterdam (VUA), The Netherlands (topic Bacteriocin mode of action). After a postdoc period in the USA, he worked as a Molecular Microbiologist at the VUA. His research focussed on Bacteriocins, Pili, Adhesion Factors and Fimbriae, and Protein Secretion Pathways in Gram-negative bacteria. He is author of around 150 publications, supervisor of over 25 PhD students and many undergraduate students. Later in his career, he became Dean of the Faculty of Earth and Life Sciences of the VU University and vice-rector of this University. At present, he is the President of the Federation of European Microbiological Societies.

FASCINATING PROTEINS AND THE FUTURE OF FEMS

B. Oudega

Authosummary of the speech of Bauke Oudega, FEMS President, Amsterdam, The Netherlands

My presentations deals with some structural aspacts of my early and later research, followed by early structural aspects of the Federation of European Microbiological Societies (FEMS), its present functioning and its future. I worked on several topics, all related to pathogenicity of bacteria and infections. First, the mode of action of bacteriocins was studied, than adhesion factors related to Enterotoxic E. Coli strains, and finally, protein secretion pathways of many pathogenic gram-negative bacteria. I will show and describe the structure of three proteins that I have wroked with. All amazing structures, like many bacterial proteins. Another amazing structure is FEMS. I will tell something about the start of this federation, its purpose and its development, and how important it is for many microbiologists in Europe. At the end of the presentation, I will discuss the present and future strategy of FEMS!

SESSION 1
CHALENGES OF ANTIMICROBIAL RESISTANCE

Prof.dr Eliora Z.Ron, Tel Aviv, Israel

Eliora Z. Ron got her PhD from Harvard University, USA, under the supervision of Professor Bernard D. Davis. Dr. Ron is author of more than 200 publications, including refereed research papers, review papers, books and chapters in books. Dr. Ron has several patents. The research activities of Dr. Ron focus on molecular genetics and genomics of bacteria with focus on antibiotic resistant septicemic E. coli. Dr. Ron is professor for Microbiology at Tel Aviv University and president of IUMS – International Union of Microbiological Societies. She was also president of FEMS – Federation of European Microbiological Societies (2004 - 2007). Among awards and memberships she has received during her carrier are: Honorary Doctorate from Ben Gurion University – 2010, 2007 EMET Prize (for excellence in arts, science and culture), Secretary General of the European Academy of Microbiology , fellow of the American Academy of Microbiology, fellow of the World Academy of Arts and Sciences, member of the Founding Committee of the European Microbiology Forum (EMF) and prize of the Council for Beautiful Israel by president Ezer Weizmann for the restoration of the north beach of Haifa after a severe oil pollution.

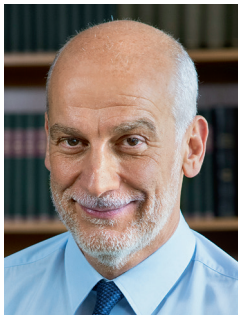
AMR1 MIPO TECHNIQUE IN THE TREATMENT OF HUMERAL SHAFT FRACTURES

E. Z. Ron

Traumatology Clinic UHC Sestre milosrdnice

Antibiotic resistant *Escherichia coli* are a major cause of mortality and morbidity and the main threat in hospital and community acquired infections. The emergence of septicemic, highly antibiotic resistant pandemic strains, such as E. coli sequence type 131 (ST131), which produces a CTX-M-type extended-spectrum-ß-lactamase (ESBL) constitutes a strong warning signal that warrants the search for new ways to fight such bacteria. Many of these bacteria survive in the serum and cause sepsis with a high rate of mortality. We used functional genomics (transcriptomics and proteomics), in order to understand the molecular mechanisms responsible for the ability to survive serum and will discuss potential avenues for combatting these bacteria.

Prof.dr Atanasios Tsakris, Athens, Greece



Athanasios Tsakris graduated as an MD from the Medical School of University of Athens, Greece and completed his training in Medical Microbiology at North Middlesex Hospital, London and the Central Public Health Laboratory, London. He received his PhD degree from the London School of Hygiene and Tropical Diseases, University of London. He is a Fellow in Medical Microbiology and Virology at the Royal College of Pathologists, London. He has spent sabbatical leaves and served as visiting Professor in the Division of Infectious Diseases, Department of Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA. Prof Tsakris is currently Professor and Head of the Department of Microbiology at the Medical School, University of Athens, Greece. He teaches various undergraduate and postgraduate academic courses at the University of Athens. He is member of the Scientific Advisory Board (SAB) of the Joint Programming Initiative on Antimicrobial Resistance (JPIAMR) in EU. His major interests include surveillance of multidrug-resistant Gram-negatives, development of phenotypic methods for the detection of carbapenemases, infection control activities and molecular mechanisms of antimicrobial resistance. He is an evaluator of grant applications for several organizations worldwide. Prof Tsakris is an author of more than 320 peer-reviewed research articles. He also serves as senior editor or editor of several major microbiology/infectious diseases journals.

AMR2 PHENOTYPIC METHODS AND INNOVATIVE TECHNOLOGIES TO DETECT MULTIDRUG-RESISTANT PATHOGENS

A. Tsakris

Department of Microbiology, Medical School, University of Athens, Athens, Greece

Antimicrobial resistance is a growing public health problem and in the next decades multidrug-resistant infections may become the leading cause of death worldwide. Consumption of antibiotics is considered the most important cause for the spread of antimicrobial resistance. Nevertheless, poor quality of health services, incorrect antimicrobial treatment practices and inadequate diagnostic testing for early detection of multidrug-resistant pathogens may contribute to its spread. Therefore, effective infection control and antimicrobial stewardship programs based on early and accurate pathogen detection may deliver a measurable impact in clinical practice. The harmonized introduction of innovative molecular technologies along with traditional and new phenotypic methods plays

a significant role in early pathogen diagnosis and accurate reporting of antimicrobial susceptibility testing. Phenotypic methods, such as combined-disk tests have been recently developed to meet current requirements in infection control activities. Several phenotypic assays were evaluated to detect ESBLs, AmpCs, various carbapenemase types and production of colistin-resistant mcr-1 gene. The accurate implementation of such assays can provide useful information for optimal antimicrobial use, active surveillance, infection control, patient cohorting and tracking of multidrug-resistant epidemics. The implementation of molecular antibiogram can also detect resistance genes and predict clinical resistance. In addition, syndromic diagnosis using multiplex molecular assays or pathogen class-specific multiplex molecular assays is a key element for appropriate use of antimicrobials. All these approaches may provide relevant information for personalized antimicrobial treatment, suitable introduction of new broad-spectrum antibiotics and infection control activities. Inspiration should be given to younger scientists to appreciate the significant role of clinical laboratory in infection control activities.

Prof.dr Tanil Kocagoz, Istanbul, Turkey

Tanil Kocagoz, M.D., Ph.D. studied in junior high school, Izmir Saint Joseph where he learned speaking French and in senior high school, Istanbul American Robert College where the curriculum was held in English. He received "Michael Hamilton Best Science Student Award" at graduation. Prof. Kocagöz graduated from Hacettepe University, School of Medicine, Ankara, in 1985. He worked as a director of a health center in Izmit for two and a half years as his conscription where he received a "Letter of Appreciation" from the Governer of Kocaeli for his important contributions to public health. Between 1988 and 1993 he completed Clinical Microbiology and Infectious Diseases residency program at Medical Microbiology Department of Hacettepe University Medical School, Ankara, Turkey, where he later became associate professor. Between 1992 and 1994 he worked as a researcher at University of California San Francisco, Microbial Pathogenesis Unit where he took part in cloning and sequencing of penicillin binding protein 2 (PBP2) of Staphylococcus aureus and identified mutations leading to fluoroquinolone resistance in Mycobacterium tuberculosis. After his return to Turkey he completed the Ph.D. program in biochemistry at the Medical Biochemistry Department of Hacettepe University. Development of new antimicrobials and rapid diagnostic tools for infectious diseases became his main areas of research. He moved to Istanbul in 2000 and dedicated himself to turn his inventions into products that will improve human health. For this purpose he took part in the foundation of R&D

companies and concentrated his efforts for building a bridge between universities and industry in biotechnology. In 2004, he received the degree of Professor at Yeditepe University where he worked as the head of the Department of Medical Microbiology for four years. Since 2008 he is working in Acıbadem Mehmet Ali Aydinlar University, Istanbul, Turkey. He is head of the departments of Medical Microbiology and Medical Biotechnology. He developed several original patented products in the area of diagnostic microbiology. "Bacit A" which lowers the time required for the diagnosis of Group A Beta Hemolytic Streptococci from two to one day, "Quicolor", a medium that enables antibiotic susceptibility testing in 4 to 6 hours instead of 24 hours, "Observable Real Time Electrophoresis –ORTE-", "Decomics", a decontamination and concentration method that eliminates the need for centrifugation and "TK Culture System", rapid culture system for the diagnosis of tuberculosis can be counted among these. In 2010, ORTE (Observable Real Time Electrophoresis) received the award of "Akin Cakmakci" presented by Turkish Technology Development Fund (TTGV). TK is a rapid culture system for the diagnosis of tuberculosis, which was supported by Foundation for Innovative New Diagnostics (FIND), received "Eurowards Turkey" entrepreneurship award. Prof. Kocagöz has more than sixty scientific papers published in international journals and several poster awards. In 2011, Istanbul Physicians Association, "Medical Service Award" for his important contributions to the diagnostic methods of tuberculosis that can be used in resource-limited settings.

AMR3 DEVELOPMENTS IN ANTIBIOTIC SUSCEPTIBILITY TESTS: PHENOTYPIC VERSUS GENOTYPIC

T. Kocagöz

Acibadem Mehmet Ali Aydinlar University, Istanbul, Turkey

Developments in molecular techniques enabled rapid identification of mutations leading to antimicrobial resistance. Thus it became possible to determine resistance to many antibiotics within hours. However, currently used rapid genetic methods cannot identify all mutations that create resistance to all antibiotics. Whole genome sequencing that can determine all mutations leading to antibiotic resistance is still to slow and too expensive to be used routinely in the laboratories. Phenotypic methods can determine resistance to all antibiotics independent of the mechanism of resistance. Standardized methods, like Kirby Bauer disk diffusion method, requires one day to give results. Matrix Assisted Laser Desorption Ionization – Time of Flight (MALDI-TOF) is a phenotypic method that enabled rapid identification of bacterial species so that the species of infecting bacteria can now be identified within the same day the microorganism is grown in culture. However, there is no currently available standardized method routinely used to determine antibiotic susceptibility to all antibiotics within

a few hours. There is a considerable effort to develop rapid phenotypic methods that can determine bacterial growth within hours, in the presence or absence of antibiotics. Determination of metabolic activity, optical evaluation of growth, flow cytometry, biosensor detection are among principles used for developing the new methods. Phenotypic and genotypic methods have different advantages over each other. Whichever method will win the race and become a routine method for same day antibiotic susceptibility determination is yet to be seen in near future.

Prof.dr Teres Alarcon, Madrid, Spain

Prof. Teresa Alarcón is Specialist Physician at Department of Microbiology in Hospital Universitario de la Princesa, Madrid, Spain. She is Associate Professor at the Department of Preventive Medicine, Public Health and Microbiology in the School of Medicine at the Autonomous University of Madrid. She obtained her degree in Biological Sciences at the Complutense University in 1986. She did her Thesis Degree and Doctoral Thesis about the diagnosis of the infection caused by Helicobacter pylori and the sensitivity of this bacteria to antibiotics under the direction of Dr. Lopez-Brea (who first started the cultivation of Helicobacter pylori in Spain in 1985). She obtained her Ph.D. in Biological Sciences in 1996 in the Complutense University. She did several training periods abroad, both pre-doctoral and post-doctoral. It is worth highlighting the collaborations with different international groups of investigation, such as Guillermo Perez-Perez of New York, Diane Taylor of Canada, Anthony Moran of Ireland and Sebastian Suerbaum of Germany. She is the leader of the group 52 of the Instituto de Investigación of the Hospital de La Princesa (IIP) and has written more than 115 papers both national and international. She is the author of 24 chapters in different books, more than 600 communications to national and international congresses. The group has also participated in different European multicenter studies for the investigation of the sensitivity of H. pylori to antibiotics. She has participated in numerous investigation projects highlighting FIS 05/2452 (Real time PCR for the rapid diagnosis of the infection by Helicobacter pylori) and FIS 08/1775 (Study of phages in Helicobacter pylori). She has been the director of 11 Doctoral Thesis, of 8 Research works for the abstention of the Diploma in Advanced Studies, 2 Master Thesis and 5 Degree Thesis.

AMR4 HELICOBACTER PYLORI AND ANTIMICROBIAL RESISTANCE: A GLOBAL PROBLEM

T. Alarcón

Department of Microbiology. Hospital Universitario de La Princesa, Madrid, Spain.

Helicobacter pylori is a Gram-negative and spiral rod that colonizes the human gastric mucosa in approximately half of the world's human population. The prevalence is lower in developed than in developing countries (from 25 to 80%). The bacteria are acquired early in life and colonization may persists for years and decades, although less than 10% of *H. pylori*-colonized individuals will develop a gastrointestinal disease such as chronic gastritis, peptic ulcer disease, gastric cancer or mucosa-associated-lymph-tissue (MALT) lymphoma. It is identified as a Group I carcinogen by the International Agency for Research on Cancer (from the World Health Organization) as there is sufficient evidence that the chronic infection with this bacterium causes non-cardia gastric carcinoma and low-grade B-cell MALT gastric lymphoma. *H. pylori* infection is also related to extradigestive diseases such as iron deficiency anaemia, idiopathic thrombocytopenic purpura, and vitamin B12 deficiency.

Numerous treatment regimens have been used to eradicate this microorganism using mainly six antibiotics: clarithromycin, amoxicillin, metronidazole, levofloxacin, tetracycline, and rifabutin. But antimicrobial-resistance is one of the main factors for treatment failure. The prevalence of resistance is rising and varies with the population. Clarithromycin-resistance is a great concern and seems to be higher in Western/Central and Southern Europe (higher than 20%) than in Northern European countries (lower than 10%) in a European multicentre study. The WHO published a list of bacteria for which new antibiotics are urgently needed, with 12 of them grouped according to the priority (critical, high, and medium), and clarithromycin-resistant *H. pylori* was included in the high priority group. Very high rates of metronidazole-resistance are also found in some countries. Other antibiotics such as tetracycline or amoxicillin have very low resistance rates and have remained stable for several years. Levofloxacin-resistance also varies according to country and population.

The infection with a resistant strain has clinical implications as patients may not receive the most appropriate treatment. Tailored culture-based therapy could be the best approach for all patients who undergo an endoscopy as guided therapy helps to reach higher eradication rates and avoids the misuse of antibiotics with the impact that this may have in gut microbiome and in the development of resistant bacteria. It is cost-effective in populations with more than 20% clarithromycin-resistance. In those cases in which tailored therapy is not possible, it is important to know local resistance data and consider some factors as sex and age of patient, previous history of macrolide consumption or patient disease in order to choose the most appropriate treatment.

Antimicrobial-resistance is mainly detected by phenotypic methods performed after culture such as E-test or agar dilution, although these methods are time-consuming and can take up to 2 weeks from the endoscopy. As resistance to clarithromycin in *H. pylori* seems to be mainly caused by specific mutations in a small region of the *23S rRNA* gene (A2142G, A2143G or A2142C), molecular methods have become an attractive alternative. Molecular techniques, also available for detection of resistance to levofloxacin, allows the selection of the most appropriate treatment for each patient when bacterial culture is not possible.

Detection of antimicrobial resistance, before starting the treatment, or at least after the first treatment failure, will lead to the most adequate antibiotic regimen for each patient so a more efficient treatment with fewer side effects will be achieved.

Prof.dr Ana Kaftandzieva, Skopje, R. Macedonia

Ana Kaftandzieva graduated at the Medical Faculty in Skopje with success in 1992. She was employed at the Institute of Microbiology and Parasitology, Medical Faculty in Skopje in 1995 and became the specialist of Medical Microbiology in 1999. In 2001 she was elected as an Assistant Professor of Microbiology. She became a Master of Science in 2004 and obtained her PhD in 2012. She was elected as a Professor in 2017. She participated in three scientific projects in the field of microbiology sponsored by the State Ministry of Science (1995-1998), two international project in the field of Dentistry (2009) and Pharmacy (2012), as well as in EuCAST-project (2013-2014) as a National expert. She continued her education at the Institute of Microbiology, Semmelweis University in Budapest, Hungary (FEMS Fellowship) and at the Institute fur Medizinische Microbiologie und Hygiene, at the University in Regensburg, Germany (2001-2002). She was in Clinical center Ljubljana in 2016, provided by Erasmus grant for exchange teaching staff. She was a member of the Organising Committee of all five Macedonian Congresses of Microbiology with international participation, FEMS symposium (2002) and Microbiologia Balkanica (2009). She is current President of the Macedonian Microbiological Society since 2016. She is a member of CAESAR (since 2013) and EUCAST teams (since 2015). Since 2001 she has been the Head of the laboratory of wound and soft tissue infections. Ana Kaftandzieva is currently Professor of Microbiology at the Medical Faculty in Skopje. Her interests include phenotypic and genotypic methods for detection of multidrug-resistant Gram negatives. She is an author of more than 120 articles.

AMR5 CARBAPENEM-RESISTANT *ENTEROBACTERIACEAE* - AN EMERGING THREAT

A. Kaftandzieva, E. Trajkovska-Dokic, Z. Cekovska, V. Kotevska, G. Jankoska, M. Petrovska, N. Panovski

Institute of Microbiology and Parasitology, Medical Faculty, UKIM Skopje

Carbapenem-resistant *Enterobacteriaceae* (CRE) occur due to the acquisition of carbapenemase enzymes or less commonly arise via other mechanisms. They include enzymes belonging to the Ambler classes A, B and D.

Aim: To determine the prevalence, susceptibility of CRE, the type of carbapenemases and to compare the findings with the previous study from 2014

Material and methods: All samples (urine, wound, blood, tubus/canila, sputum, tracheal aspirates) obtained from the hospitalized patients in the University Clinics of the „Mother Teresa" Campus, the City hospital „8th September" and the special hospital for surgical diseases „St. Naum Ohridski" in Skopje during one year period (January-December 2017) were processed at the Institute of Microbiology and Parasitology, Medical Faculty, Skopje. Standard microbiology techniques were used for isolation and identification of the strains. Determination of the susceptibility to 15 antimicrobial agents was done by disk diffusion method. Vitek 2 and E-tests were performed to determine MIC. CIM method for carbapenemase production and carbapenemase set were performed to all strains that showed smaller inhibition zones to carbapenems according EUCAST. PCR was performed for detection of four beta-lactamase genes (KPC, NDM, VIM and OXA-48).

Results: In that period, out of the total number of *Klebsiella pneumoniae*-Kp (215), carbapenem resistant were 45 (21%). 42 of them produced metallo-beta lactamases. The number of carbapenem resistant Kp from the total number of isolated Kp was as follows: 22/89 were from urine (24,7%), 16/89 were from wounds (18%), 2/7 were from blood cultures (28.5%), 3/15 were from tubus and 2/15 were from sputa (13%). Considering *Enterobacter cloacae*, 1/193 isolates (0,5%) from wounds and 2/13 isolated from blood culture (15,4%) were carbapenem resistant. All carbapenem resistant Kp were resistant to 9 tested antibiotics, 8, 2, 14 and 19 were intermediate susceptible to imipenem, meropenem, gentamicin, amikacin, respectively. 12, 3 and 4 isolates of Kp were susceptible to gentamicin, amikacin and ko-trimoxazole, respectively. 8/45 isolates of Kp (17,8%) showed higher MIC to colistin. Comparing these results to those from 2014, it can be noticed that in 8 out of 252 isolates of *Klebsiella pneumoniae* (3,17%), carbapenem resistance was detected. These 8 isolates were from six patients. The type of carbapenemases in all strains was KPC.

Conclusion: The percentage of carbapenem resistant isolates is increasing every year (particularly high percentage was observed in 2017), which is particularly worrying fact and it is the right time to pay attention to this matter more seriously and to perform all strategies to control the spread of CRE.

AMR6 PHENOTYPIC CHARACTERIZATION OF MULTIDRUG RESISTANT ACINETOBACTER BAUMANII

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INTRODUCTION: Multi and pandrug-resistant *Acinetobacter baumanii* (MDR*Ab* and PDR*Ab*) has emerged as a major nosocomial pathogen. One of the mechanisms of these types of resistance is carbapenemase producing capability. There are simple and inexpensive testing methods for screening of carbapenemase production and their use in laboratories is essential.

AIM: To screen and compare the results from different phenotypic methods for detection of carbanemase producing *Acinetobacter baumanii*.

MATERIAL AND METHODS: During the 6 months period (Sep 2017 – Feb 2018), 27 *Acinetobacter baumanii* (*Ab*) isolates from tracheal aspirates were collected. The specimens originate from the University Clinic of Pediatric diseases in Skopje, Clinical Campus ,,Mother Theresa" in Skopje. Identification and susceptibility tests were done with standard microbiological tests, also using automated VITEK 2 system. Isolates were subjected to screening for carbapenemase production by Modifed Hodge Test (MHT), Carbapenem Inactivation Method (CIM) using CLSI standards and disc diffusion method with combined discs (metallo-ß- lactamase (MßL) and AmpC ß-lactamase by penem with penem + MßL inhibitor discs and penem+AmpC inhibitor discs -Mast Group, United Kingdom).

RESULTS: Out of total of 27 *Ab* isolates , 22 were obtained from intensive care unit. MDR*Ab* were 25 species. Colistin appeared to be the most effective antibiotic with 100% in vitro susceptibility. MHT showed positivity at 11 species (41%) and only 2 of them were CIM positive (7%). With CIM, 8 isolates (30%) were intermediate according CLSI standards. MßL production was not detected with the presented methods, and only 2 (7%) of the isolates showed AmpC ß-lactamase production. Those isolates which were AmpC positive were not positive either with MHT, or CIM.

CONCLUSION: Most of the *Ab* isolates, 93% according CIM and 59% according MHT were not carbapenemase producers. For confirmation of intermediate CIM results, further investigations, including molecular tests, should be done.

Keywords: *Acinetobacter baumanii*, phenotypic methods, carbapenemase enzymes.

AMR7 COMPARATIVE IN VITRO ACTIVITIES OF FIRST AND SECOND GENERATION CATIONIC STEROID ANTIBIOTICS AND VARIOUS ANTIBIOTICS AGAINST CARBAPENEM-RESISTANT KLEBSIELLA PNEUMONIAE STRAINS

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Introduction: The ceragenins - cationic steroid antibiotic (CSA) designed to mimic the activities of antimicrobial peptides, are a new class of antimicrobial agent. These molecules have a number of advantages over antimicrobial peptides and most importantly they are resistant to proteases. *Klebsiella pneumoniae* can cause different types of healthcare associated infections, including pneumonia, bloodstream infections, wound or surgical site infections, and meningitis.

Objectives: This study was aimed to investigate the antimicrobial activities of first / second generations CSAs and various antibiotics against carbapenem-resistant *Klebsiella pneumoniae* strains

Materials/methods: The antimicrobial activities of first generation CSAs (CSA-13, CSA-44), second generation CSAs (CSA-131, CSA-138, CSA-142) and various antibiotics (ceftazidime, colistin, tobramycin, levofloxacin and meropenem) were studied against 30 strains of *K. pneumoniae* isolated in an intensive care unit. MICs were determined by microdilution method according to CLSI.

Results: According to our results, MIC_{50} and MIC_{90} values of CSAs differ from 8 to 32 µg/ml and 16 to 128 µg/ml. Overall, among CSAs, CSA-131 showed the lowest MIC_{50} and MIC_{90} values against all microorganisms. Furthermore, CSA-13 and CSA-44 as first generation CSAs; CSA-138 and CSA-142 as second generation CSAs have meaningful activities against tested microorganisms. The antimicrobial effects of studied CSAs were similar or better than tested antibiotics, except for colistin.

Conclusion: As a result, not only CSA-13 and CSA-44 as first generation but also CSA-131, CSA-138 and CSA-142 as second generation have significant antimicrobial effects on carbapenem-resistant *K. pneumoniae*. As far as we know, it is the first study to determine the first and second generation of CSAs on *K.pneumoniae*. Future studies should be performed to correlate pharmacodynamic and pharmacokinetic parameters of these molecules.

Table 1 : *In vitro* antimicrobial activities of first and second generation CSAs and various antibiotics.

Antibiotics	MIC_{50} (µg/ml)	MIC_{90} (µg/ml)
Ceftazidime	256	512
Colistin	0,25	64
Meropenem	32	128
Levofloxacin	32	64
Tobramycin	64	512
CSA-13	16	32
CSA-44	16	32
CSA-131	8	16
CSA-138	16	32
CSA-142	32	128

AMR8 EFFECTS OF VARIOUS ANTIBIOTICS ALONE OR IN COMBINATION WITH MEROPENEM AGAINST ACHROMOBACTER XYLOSOXIDANS STRAINS ISOLATED FROM CYSTIC FIBROSIS PATIENTS

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Introduction: *Achromobacter xylosoxidans* is an aerobic, non fermenting, Gram negative bacillus, innately *multidrug-resistant* microorganism and increasingly being recognized as an emerging pathogen in cystic fibrosis. *In our study, meropenem, colistin sulfate, tobramycin and levofloxacin were investigated for their in vitro activities* on 20 *A.xylosoxidans* clinical strains isolated from sputum samples of *cystic fibrosis patients. Furthermore,* colistin sulfate, tobramycin and levofloxacin alone and in combination with meropenem were investigated for their in vitro synergistic and postantibiotic effects (PAEs) on *A. xylosoxidans*.

Objectives: The aim of this study was to investigate the in vitro activity of various antibiotic combinations against *Achromobacter xylosoxidans* strains isolated from *cystic fibrosis patients.*

Materials/methods: The in vitro activities of meropenem, colistin sulfate, tobramycin and levofloxacin were determined by the broth microdilution method. The in vitro synergistic activities of tested antibiotics in combination with meropenem were determined using the

time kill curve technique. To determine the PAEs, *A. xylosoxidans* strains in the logarithmic phase of growth were exposed for 1 h to antibiotics, alone and in combination. Recovery periods of test cultures were evaluated using viable counting after centrifugation.

Results: By time–kill assays, all tested antibiotic combinations demonstrated additive or synergistic activity against at least tested strains at 1× or 4× MIC. Colistin sulfate produced a strong PAE ranging from 3.21 to 3.58 h in a concentration-dependent manner at 1xMIC or 4xMIC. At the same MIC concentrations, remarkable PAEs were induced by tobramycin (ranging from 2.34 to 3,47 h) , levofloxacin ranging from (1.54 to 2.27). Meropenem showed very weak PAE (ranging from 0,39 to 0,62) against all strains. In combination, meropenem scarcely changed the duration of PAE of each tested antibiotic alone.

Conclusion: The findings of this study may have important implications for the timing of doses during antimicrobial therapy with meropenem, colistin sulfate, tobramycin and levofloxacin.

AMR9 INVESTIGATION OF CEFTAZIDIME-AVIBACTAM SUSCEPTIBILITY IN ENTEROBACTERIACEAE AND PSEUDOMONAS AERUGINOSA ISOLATES BY DISK DIFFUSION AND GRADIENT METHODS

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Introduction
Ceftazidime-avibactam is a new cephalosporin and ß-lactamase inhibitor combination that has activity against multidrug-resistant (MDR) gram-negative bacteria including *Pseudomonas aeruginosa* and carbapenem-resistant *Enterobacteriaceae.*

Objective
The aim of this study was to investigate susceptibility of ceftazidime-avibactam in *Enterobacteriaceae* and *P. aeruginosa* isolates with a new (domestic) disk and gradient test, and compare the results.

Materials and Methods
65 *Enterobacteriaceae* and 11 *P. aeruginosa* isolates, isolated from different clinical specimens sent to our laboratory, were enrolled in the study.
Conventional methods and Phoenix (BD, USA) automated system were

used in identification of the isolates.
Zone diameter of ceftazidime-avibactam was detected by Kirby-Bauer disk diffusion ((Bioanalyse, Ankara, TR), MIC values, by gradient test (Liofilchem, Italy) methods.
Results were interpreted according to EUCAST 2017 criteria; (*Enterobacteriaceae* and
P.aeruginosa MIC≤8 (mg/L) sensitive, *Enterobacteriaceae* zone diameter ≥ 13 mm and
P. aeruginosa zone diameter ≥ 17 mm sensitive).

Results and conclusion
76 isolates, included in the study, were; 54 *Klebsiella pneumoniae*, 11 *Escherichia coli* and 11 *P. aeruginosa* Sensitivity and MIC ranges of ceftazidime-avibactam were as follows: *K. pneumoniae* 89% (0.032-256 mg/L); *E. coli* 78% (0.094-256 mg/L) and *P. aeruginosa* 55% (0.50-96 mg/L). Categorical interpretation of both zone diameters and MIC values were in 100% harmony. In our study, the susceptibility rates of ceftazidim-avibactam were detected as higher in *Enterobacteriaceae* isolates than *P. aeruginosa* isolates.

Although ceftazidime-avibactam combination is recommended as a potential alternative to carbapenems for the treatment of multidrug-resistant gram-negative bacteria, further large scale studies, both in-vitro and in-vivo, and comparison of the results with broth microdilution, are warranted to assess its susceptibility and clinical efficacy against carbapenemase-producing *Enterobacteriaceae* or multidrug-resistant *P. aeruginosa.*

Key words: *Enterobacteriaceae*, *P. aeruginosa*, ceftazidime-avibactam

AMR10 INVESTIGATION OF THE EFFICACY OF CEFTOLOZANE-TAZOBACTAM AND CEFTAZIDIM-AVIBACTAM IN CARBAPENEM RESISTANT ENTEROBACTERIACEAE ISOLATES

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Introduction
Increased number of isolates carrying carbapenemases constitutes

a great challenge to public health. There is an urgent need to develop new antibiotics to overcome these rapidly spreading isolates; however few have been developed in recent years. Ceftolozane-tazobactam and ceftazidime-avibactam are amongst these newly developed agents.

Objective

The aim of this study was to investigate the combination of new ß-lactam-ß-lactamase inhibitors such as ceftolozane-tazobactam and ceftazidime-avibactam; and evaluate their susceptibilities to carbapenem resistant *Enterobacteriaceae* isolates including carbapenemase genes.

Material and methods

71 carbapenem resistant *Enterobacteriaceae* species isolated from various samples sent to our laboratory were included in the study.
Isolates were identified using Phoenix (USA) automated system. Ceftazidim-avibactam and ceftolozane-tazobactam minimum inhibitor concentration (MIC) values were determined by gradient test method (Liofilchem, Italy) and results were interpreted according to EUCAST 2017 criteria.

Results and conclusion

Of the 71 isolates (62 *Klebsiella pneumoniae*, 9 *Escherichia coli*), 36 (51%) were isolated from; urine, 17 (24%) blood, 11 (15%) wound and 7 (10%) tracheal aspirate cultures. Ceftazidim-avibactam susceptibility rates were 76% (0.094-256 µg/ml) for *K. pneumoniae*, and 78% (0.094-16 µg/ml) for *E. coli*, and the susceptibility rates of ceftolozane-tazobactam were; 52% (1-256 µg/ml) for *K. pneumoniae*, and 44% (0.38-16 µg/ml) *for E. coli.*
Carbapenemase gene positivities are shown in Table 1.
The development of novel agents that are highly resistant to gram-negative pathogens is very important in terms of providing therapeutic options.
In our study, the sensitivity rate of ceftazidim-avibactam was higher than that of ceftolozane -tazobactam. It has been found that ceftazidime-avibactam exhibits better activity against other carbapenem resistant isolates, except those carrying NDM-1 enzyme.
As a result, further studies are needed to determine the susceptibility to these antimicrobials using more isolates, to investigate the existence of different resistance mechanisms and to demonstrate the clinical efficacy of these antimicrobials.

Key words: Ceftolozane-tazobactam, ceftazidim-avibactam, *Enterobacteriaceae*

Table 1. Number of carbapenemase gene positivities of isolates

	Unspecified gene	OXA-48	VIM	NDM	OXA-48+ VIM	OXA-48+ NDM
K. pneumoniae (n=62)	15	32	4	6	3	2
E. coli(n=9)	4	4	-	-	1	-
Total (n=71)	19	36	4	6	4	2

AMR11 ANTIMICROBIAL SUSCEPTIBILITY PATTERNS OF GRAM-NEGATIVE BACTERIA ISOLATED FROM PATIENTS ADMITTED TO TERTIARY CARE HOSPITALS IN SERBIA

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Introduction: The increasing prevalence of infections due to multidrug-resistant (MDR) Gram-negative bacteria constitutes a serious threat to global public health due to the limited treatment options available. MDR strains of Enterobacteriaceae (especially *Klebsiella pneumoniae, Escherichia coli, Enterobacter* spp.), *Pseudomonas aeruginosa*, and *Acinetobacter* spp. have emerged as a particular healthcare concern.

Objective: The aim of this study was to analyze antimicrobial resistance patterns of Gram-negative bacteria isolated from patients admitted to tertiary care hospitals throughout Serbia from 2016-2017.

Materials and Methods: A total of 120 Gram-negative pathogens, identified by mass spectrometry MALDI-TOF (Bruker TM) were included in the study. Antimicrobial susceptibilities were done by broth microdilution using MicroScan® panels (Siemens, USA). Minimum inhibitory concentrations (MICs) of all antimicrobials, with the exception of tigecycline, were interpreted according to the Clinical and laboratory standards institute breakpoints. Tigecycline MICs values for all tested isolates were evaluated using European committee on antimicrobial susceptibility testing approved breakpoints for Enterobacteriaceae (no tigecycline breakpoints available for *Acinetobacter*).

Results: *E. coli*, *K. pneumoniae* and *P. aeruginosa* were the most common causative agents of the urinary tract infections, sepsis and respiratory tract infections, respectively ($p<0.05$). Resistance rate of *K. pneumoniae* to meropenem was 64%. Additionally, only one isolate was resistant to tigecycline (4%). All *E. coli* were susceptible to amoxicilin+clavulanic acid, piperacillin+tazobactam, amikacin, minocycline, tigecycline and meropenem, while resistance rate to cephalosporins was ≥24%. Seven out of 25 isolates (28%) were multidrug-resistant. All tested isolates of *Acinetobacter* were resistant to piperacilin+tazobactam, ceftazidime, and meropenem. The resistance of *P. aeruginosa* to ceftazidime and meropenem was 75% and 45%, respectively. A total of 60% isolates were multiresistant, while panresistance was recorded in only one isolate identified as *K. pneumoniae* (0.8%).

Conclusion: Tigecycline, meropenem and amikacin retain their *in vitro*

activity against the majority of the tested organisms, including those with multidrug resistance.

Keywords: Gram-negative bacteria, multidrug resistance, MICs, broth microdilution

AMR12 TUBERCULOSIS AND DRUG RESISTANCE 2009-2016

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Introduction
Tuberculosis (TB) accounts for over 40% of all mortality cases from communicable diseases in Europe. 30 countries worldwide are classified as high multi drug resistant (MDR) TB burden countries, nine are in the European Region. The emergence of combined resistance to rifampicin and isoniazid (MDR-TB) is a matter of global concern.

Objectives
We are aiming to detect and follow the incidence of mono and multi resistant TB in Republic of Macedonia.

Material and methods
Over a period of 8 years we examined patient specimens for TB. Culture-positive samples were subjected to identification, and those cultures identified as M. tuberculosis (MTB) complex were subjected to drug susceptibility testing (DST).

DST for first-line drugs was performed using proportional method on Lowenstein Jensen, and in recent years, rapid molecular techniques recommended by WHO (GeneXpert). DST for second line drugs was assessed using Geno Type MTBDRsl.

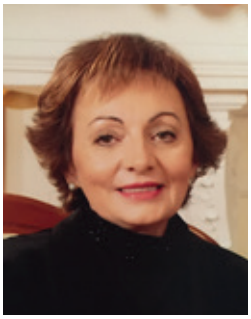
Results
In the period 2009-2016 we tested 1423 TB strains, 1333 (93,68%) were found to be sensitive and 90 (6,32%) resistant to first-line drugs. 69 strains (4,85%) were monoresistant, 15 (1,05%) of which were resistant only to Rifampicyn. Of the total 1423 strains, 21 (1,48%) were found to be MDR.

Conclusion
Currently a low 1,48% of our isolates are MDR. It is necessary to strengthen our TB laboratory capacity for early detection of drug-resistant TB. This is best done by using rapid molecular diagnosis as an initial method for all cases with clinical suspicion of TB.

Key words: tuberculosis, resistance, MDR

SESSION 2
BACTERIAL INFECTIONS AND ZOONOTIC DISEASES

Prof.dr Elena Trajkovska Dokic, Skopje, R.Macedonia



Dr Elena Trajkovska-Dokic has graduated at the Faculty of Medicine, University „St. Cyril and Methodius" in Skopje, R. Macedonia, with success (9.1) in 1986. Since 1988 she has been employed at the Institute of Microbiology and parasitology in Skopje, R. Macedonia. She has completed the specialization in medical microbiology and parasitology in 1994. She has received her PhD degree in medical microbiology in 2002. In 2012 she has been elected as a professor of microbiology and parasitology. She has participated at 4 projects in medical microbiology and at one project in pharmacy. Her field of interest include: Enterobacteriaceae, Helicobacter pylori, Molecular microbiology. She has participated at many scientific congresses, training courses and seminars in medical microbiology as part of her continuous medical education. She has published many papers in national and international journals. She has been an invited lecturer at the 7th and 8th Balkan Congress of microbiology, at the Symposiums for preventive medicine in Serbia, at the Congresses for laboratory diagnostic in Croatia etc. Dr Elena Trajkovska-Dokic has gained a lot of her medical knowledge during her stay at the Institut fur Klinische Mikrobiologie und Immunologie, Frei Universitat, Berlin, Germany, in the period 1989-1990. Six years later, she took advantage of the opportunity to gain knowledge and skills for several new techniques in microbiology during her stay at the Medical University Southampton in 1996-1997. Realizing of her objectives would not be able without the FEMS support which enabled her to stay the first three months and to work as a research fellow at the Medical University Southampton and General Hospital Southampton. Working at the institutions where microbiology was on a quite high level, she was inspired to continue her stay for the next 10 months and to learn as much as possible. After her coming back to her native country she has included a lot of her knowledge and skills at her everyday work. She is an active member of: Macedonian Medical Society, Doctor Chamber of R. Macedonia, Society for Macedonian microbiologists, Federation of European Microbiological Societies (FEMS) and Balkan Society for microbiology.

BI1 NEW CHALLENGES AND SOLUTIONS IN DIAGNOSTIC MICROBIOLOGY

E. Trajkovska-Dokic

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Until the end of the XX-th century, many human infections were undiagnosed due to the microbiologist's limitations in detecting of different microorganisms. Currently, we are facing the development of "Molecular Revolution", which offer many advantages, especially in diagnostic microbiology. There are several changes that have occurred in microbiology laboratories so far.

The syndrome-based and diseased-based sampling of patient's specimens, has optimized their type and number and also has simplified the laboratory prescription for the physician. Processing of clinical samples is the second very important change in microbiology laboratories. Traditionally, the gold standard for diagnosis has been culture, but in suffers from several limitations. Currently, molecular methods are emerging as the frontrunners in the detection of particular pathogens. The rapid molecular detection and characterization of the pathogen, offer an opportunity to clinician to tailor antimicrobial therapy, to identify an asymptomatic disease, and to facilitate the monitoring of treatment which may promote new drug design and development. Such improvements in patient's treatment will reduce the spread of antimicrobial-resistant organisms and the adverse effects of broad spectrum drugs.

The application of real-time PCR (RT-PCR) to the rapid screening and detection of common antibiotic resistance genes such as *mecA, vanA, shv, tem, ctx, ndm1* etc. has revolutionized the detection of antimicrobial resistance. Applications of genome sequencing as a strategy offer rapid detection, identification and genotyping, as well as designing the culture media for fastidious microorganisms, the assessment of antibiotic resistance and virulence properties. Conventional PCR using broad-range targets combined with sequencing remains an irreplaceable tool for the identification of new pathogens in clinical specimens. Diagnostic molecular biology is the fastest growing area in currant laboratory-based medicine, and has the potential to change the course of clinical medicine dramatically over the next decade.

Prof.dr Ian Clarke, Southempton, United Kingdom

Prof. Ian Clarke graduated in Microbiology from the University of Leeds,UK in 1979 and completed his PhD in Molecular Virology at the University of Warwick in 1982 studying genomic variation in rotaviruses. He worked as a research scientist in the food industry before appointment to a 'new blood lectureship' at the University of Southampton in 1984. Subsequently he has held various positions at Southampton including Director of Division. He was appointed to the Chair of Molecular Microbiology/Virology in 1998 at the Faculty of Medicine, University of Southampton, UK. Ian leads a research team that is interested in host-pathogen interactions with special emphasis on intracellular pathogens and diseases for which there are currently no effective vaccines. He has particular focus on Chlamydia and enteric viruses. The Chlamydia research group is specialized in chlamydial molecular biology, genomics and evolution. In Chlamydia research Ian collaborates with Prof. Bentley Fane (BIO5 Arizona, USA), Dr. Peter Marsh (Southampton), Prof. Kyle Ramsey (Midwestern, USA), Dr. Suneeta Soni (Brighton), and Prof. Nick Thomson (Sanger, Cambridge). Together with Prof. Thomson Ian has established the first global biobank of C. trachomatis isolates with matched genome sequences.Ian has also retained interests in human noroviruses with the aim of understanding their role in foodborne diseases and in developing a norovirus vaccine. The Southampton virus was the first norovirus to be characterised at the molecular level (published in Science). The group have subsequently developed the first reverse genetics system for noroviruses and the long term aim is to adapt human norovirus to growth in cell culture. Ian collaborates with Dr. Chris McCormick (Southampton) on DNA-based delivery of viral genomes, with Drs. Morgan Herod (Leeds) and Prof. Vernon Ward (Otago, NZ) on the properties of the non-structural proteins. Ian was chair of the ICTV Calicivirus study group (2009 – 2015). His research group is based within the Faculty of Medicine and comprises both clinical and non-clinical scientists with postgraduate students.

BI2 THE CHLAMYDIA PLASMID: ROLE IN TROPISM AND VIRULENCE

I. N. Clarke

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Chlamydia have two extrachromosomal elements - a small single-stranded DNA microvirus (chlamydiaphage) with a 3,200nt circular genome and plasmid of 7,500 bp which encodes eight genes. The recent development of a plasmid-based transformation protocol for Chlamydia species in our laboratory has led to renewed interest in the role and function of the plasmid and its individual genes and in the use of the chlamydiaphage as a vector. The study of natural variants, sequence comparisons and phylogenetic analyses has revealed insights into plasmid gene function. These observations coupled to detailed gene deletion studies have guided improvements in vector development. Despite these significant advances we still know very little about gene regulation in the plasmid. The plasmid in C. *trachomatis* can naturally transfer between strains although there appear to be significant barriers to plasmid transfer between chlamydial species. Plasmid recombination and evidence that the plasmid has a role in virulence will be discussed.

Prof. dr Franz Allerberger, Vienna, Austria

Franz Allerberger is Head of the Division of Public Health at the Austrian Agency for Health and Food Safety (AGES) in Vienna, Austria. After gaining a medical degree from the University of Innsbruck, Austria, he completed a residency at the Hospital of Tamsweg in Austria. Subsequently Professor Allerberger completed an international residency at the Mayo Clinic in Rochester, USA, followed by a sabbatical year in the Department of Immunology and Infectious Diseases at Johns Hopkins University, Baltimore, USA. He became qualified to teach hygiene, microbiology and preventive medicine in 1992, before becoming a professor in 2006. He is a registered specialist for clinical microbiology and for infectious diseases. Professor Allerberger was a staff member at the Institute for Hygiene and Social Medicine at the Medical University of Innsbruck, before he assumed his current position in 2003. Also in 2003, he became Associate Editor of Clinical Microbiology and Infection, the official publication of the European Society of Clinical Microbiology and Infectious Diseases (ESCMID). In 2005 he was

nominated Austrian Alternate-Member of the Scientific Advisory Board of the European Centre for Disease Prevention and Control (ECDC). Since 2015 he acts as Secretary of the Section Medical Microbiology of the Union Européenne des Médecins Spécialistes (UEMS-SMM).

BI3 TRENDS IN FOODBORNE ZOONOSES: AUSTRIA VERSUS EUROPE

F. Allerberger

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The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2016 of the European Food Safety Authority and the European Centre for Disease Prevention and Control presents results of the zoonoses monitoring activities carried out in 2016 in 37 European countries (28 Member States (MS) and nine non-MS, including the Former Yugoslav Republic of Macedonia). Campylobacteriosis was the most commonly reported zoonosis and the increasing European Union (EU) trend for confirmed human cases since 2008 stabilised during 2012–2016. The number of human listeriosis confirmed cases further increased in 2016, despite the fact that Listeria seldom exceeds the EU food safety limit in ready-to-eat foods. The decreasing EU trend for confirmed yersiniosis cases since 2008 stabilised during 2012–2016, and also the number of confirmed Shiga toxin-producing Escherichia coli (STEC) infections in humans was stable. In total, 4,786 food-borne outbreaks, including waterborne outbreaks, were reported. Salmonella was the most commonly detected causative agent – with one out of six outbreaks due to S. Enteritidis – followed by other bacteria, bacterial toxins and viruses. Salmonella in eggs continued to represent the highest risk agent/food combination. The report further summarises trends and sources for bovine tuberculosis, brucellosis, trichinellosis, echinococcosis, toxoplasmosis, rabies, Q fever, West Nile fever and tularaemia. Direct comparison among various countries can be severely hampered by profound differences in the respective national disease-specific surveillance systems. The incidences of travel-related illness (e.g. salmonellosis) have been shown to be a reliable proxy for the real prevalence of certain foodborne zoonoses in Europe, as has been the prevalence of salmonella in laying hen flocks in the case of human salmonellosis [3]. Assuming a constant environment, e.g. no change in policy and control of salmonellosis, campylobacteriosis and listeriosis, a Belgish study predicted the incidence of salmonellosis and listeriosis to remain stable, while the incidence of campylobacteriosis would almost double until 2020 [4]. In addition, the diagnostic environment presently is changing due to the crescent implementation of commercial multiplex PCR systems in routine stool diagnostics [5]. Classic culture methods

directed at the isolation of specific pathogens are increasingly becoming second-line tools, being deployed only – in some institutions being omitted at all - when rapid molecular tests give positive results. While this optimizes the yield from stool examinations and dramatically improves the timeliness of diagnosis, the availability of an isolate still poses – with only a few exceptions - a prerequisite for molecular typing of pathogens. Molecular typing complements traditional epidemiological surveillance by providing appropriate discriminatory analyses to foster rapid and early detection of dispersed clusters or outbreaks and to facilitate detection and investigation of transmission chains.

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Maja Rupnik is head of Department for microbiological research at National laboratory for health, environment and food (NLZOH) in Maribor, Slovenia. Since 2005 she has been a Professor for Microbiology at Faculty of Medicine, University of Maribor. She is a worldwide recognized expert on Clostridium difficile and one of the main organizers of traditional event International *Clostridium difficile* Symposium (www.icds.si). Her research interest is focused on molecular biology and epidemiology of Clostridium difficile, molecular typing techniques and on gut microbiota. The main interest of her group are the variant C. difficile strains. Her publications include more than 80 original papers, reviews and book chapters including high ranking

journals such as Nature, Nature Rev Micro, NEJM. She is associate editor for Anaerobe journal. The honors and awards she obtained for her work include Alexander von Huboldt grant , ESCMID/bioMerieux Award for Advances in Clinical Microbiology and national award Zois Certificate of Recognition for exceptional scientific achievements in microbiology.

BI4 HOW TO APPROACH CLOSTRIDIUM DIFFICILE AS AN EMERGING ZOONOSIS

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Clostridium difficile, recently renamed *Clostridioides difficile*, is an important human pathogen and causes intestinal infections ranging from mild diarrhoea to colitis and pseudomembranous colitis. The main group at risk are still elderly hospitalized patients, but the incidence in community is steadily increasing. Even for hospitalized cases it was shown that two thirds are not associated with another related hospital case. Therefore, community reservoirs for C. difficile infection, including animals, are starting to be increasingly recognized.

Animals are often colonized with *C. difficile*. Particularly well studied are pigs and cattle. The bacterium has been detected also in diverse range of foods, including meat, seafood, vegetables and fresh produce.

While postulated zoonotic and foodborne transmission has not yet been directly shown, there are several observations to support it. Animal and food isolates include PCR ribotypes (subtypes within *C. difficile* species) that are common among human isolates. Whole genome sequencing, a more discriminatory fingerprinting than PCR ribotyping, has detected pairs of identical *C. difficile* strains between human and pig isolates either on a single farm or over large geographical regions. Identical isolates were found also in households on dog paws, shoes and slippers. A large community outbreak in Australia was discussed to be potentially food related.

To better understand zoonotic potential of *C. difficile* is important to recognize and correctly diagnose community cases of *C. difficile* infection and if possible to collect strains from animals, environment and food. Good coverage of all diverse reservoirs will enable better comparison, understanding of transmissions and thereby zoonotic potential. This could have implications in controlling the *C. difficile* infection.

Prof.dr Vaso Taleski, Shtip, R.Macedonia

D-r Vaso Taleski is professor of microbiology at the Faculty of Medical Sciences, University „Goce Delchev" in Shtip, Republic of Macedonia. He is author of about 100 publications and papers. His research activities are related to microbiological diagnosis of human brucellosis. Among other activities D-r.Taleski was secretary of Macedonian Microbiological Society (ZMM) 1991-1993, president of ZMM 1993-1997, delegate of ZMM in FEMS 1997-2002, 2011-2015, member of FEMS Executive Committee (GRANTS Secretary) 2005-2012, member of the FEMS Grants Board 2000-2003, 2012-2017, member of organizing committees of FEMS European congresses of microbiologists (2003, 2006, 2009, 2011, 2013, 2015, 2017), president of congress grants committees of FEMS European microbiology congresses (2009, 2001, 2013, 2015, 2017) and is currently FEMS Director of events and internationalization.

BI5 NEW BRUCELLA STRAINS TOWARDS RE-EMERGING TRENDS OF BRUCELLOSIS

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Introduction

Brucellosis is a worldwide zoonotic disease that has a significant economic, social and public health impact. Significant changes of global ecological map identifying new species, hosts and reservoirs includes brucellosis permanently in-group of re-emerging diseases. Nevertheless, disease have been eradicated successfully in most of developed countries, still remains endemic in Mediterranean region, Middle East, Asia, and Central and South America.

Aim

To emphasize the importance of new, recently identified *Brucella* species, hosts and reservoirs that raise awareness of high potential of brucellosis as a re-emerging disease and further complication to control the spread of the disease in endemic areas and worldwide.

Material and Methods

Presentation of most recent reviews and published data of reported and confirmed potential new (amphibian) b*rucella* strains in cold-blooded hosts and reservoirs.

Discussion
Genus *Brucella* until recently, represented a genetically homogeneous and clonal group of bacteria. Numerous new members were reported in recent years. Species genetically highly related to each other (> 99 %) associated with infections of numerous warm-blooded animals and humans, are classified as: 1. Terresterial mammalian hosts (Classical strains: *B. melitensis*, *B. abortus*, *B. suis*, *B. canis*, *B. ovis*, *B. neotomae*), 2. Marine mammals (*B. ceti* and *B. pinnipedialis*), and 3. „Atypical", (*B. microti*, *B. inopinata*, *B. papionis* and *B. vulpis*).
In addition, recently isolated *brucellae* from cold-blooded, worldwide-distributed exotic frogs (amphibian *brucellae*) were reported. These new *brucellae* species are capable to cause localized manifestations to generalized infections of frogs. Genetically highly diverse, might represent several new *brucella* species or link between free living soil saprophytes and the pathogenic *brucella. Therefore, f*rogs represents new and ecologically significant natural host and reservoir.

Conclusions
Identification of new, amphibian, *brucella* species and new hosts and reservoirs (frogs), have significant contribution to new approach of understanding of evolution of the genus *Brucella* from a soil-associated motile bacterium to a host-adapted pathogens. Frog's isolates to date do not represent a zoonotic treat because, still, there is no evidence for that. Advices for precaution to avoid contacts with amphibians that maight be infected are very useful.
In addition, of existing, new amphibian-*brucella* species, new hosts and reservoirs (frogs) increase the concerns for successful control and keeps Brucellosis permanently on the list of re-emerging diseases.

Key words: brucellosis, new *brucella*, re-emerging, trends, reservoirs.

Ass.Prof.dr Liljana Labachevska-Gjatovska, Skopje, R.Macedonia

Liljana Labachevska-Gjatovska was born in 1981 in Skopje. She graduated at the Medical Faculty – University "Ss Cyril and Methodius" of Skopje, obtaining MD degree in 2006. In 2007 she passed the official exam and gained a license to practice medicine after which she enrolled the 4-year medical microbiology residency training gaining the specialist diploma at the Academic Specialist Studies of Microbiology and Parasitology in 2011. In November 2016 she successfully defended her PhD dissertation under the title: "Biofilms

as a factor for recurrent urinary infections" gaining the title doctor of medical sciences. Presently d-r Labachevska is responsible for the Laboratory for genito-urinary infections at the Public Health Institution-Institute for Microbiology with Parasitology. In 2014 she was on a three months medical research at the Medical school, University of Michigan, Ann Arbor (laboratory for clinical microbiology, laboratory for molecular diagnostics). In 2015 she gained a scholarship by the Washington State University for participation in a summer school for fundamentals of biofilm research, biofilm structure and image analysis organized by the research group of Associate Professor Haluk Beyenal at Gene and Linda Voiland School of Chemical Engineering and Bioengineering. Liljana Labachevska-Gjatovska is currently employed on a Full Time Basis as a MD, microbiologist and as ass.professor at the Institute of Microbiology and Parasitology, Medical Faculty, University of "Ss Cyril and Methodius", R.Macedonia.

BI6 BIOFILMS: A ROLE IN RECURRENT URINARY TRACT INFECTIONS

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Introduction: Biofilms are defined as functional consortiums of microorganisms that are attached to a surface enclosed in extensive hydrated polymeric matrix. Between the microorganisms in biofilm and free floating microorganisms of the same species there are essential differences regarding: specific gene expression, ability for intercellular communication via biochemical signaling molecules, antimicrobial resistance. These biofilm features play a key role in the development of chronic and recalcitrant to treatment infections. The ability of these bacteria to cause relapses of UTI directly correlates with their *genetic capability* for biofilm production on the urinary catheter surface or the uroepithelium.
Despite the advances in the biofilm research world wide, bacterial biofilm remains a common and inadequately treated condition in the Republic of Macedonia and the surrounding regions, with few strategies for prevention or cure.
In the *present study our objectives were to investigate* the diference between patients with recurrent UTI and those with a first time UTI in terms of their ability for biofilm production and the microorganisms resistance to antibiotics in relation to biofilm expression phenotype.

Materials and methods: The study included urine samples from outpatients, from both genders, over 18 years of age, with suspected UTI, referred for microbiological *examination* of *urine* at the Institute

of microbiology and parasitology, Medical Faculty, Skopje. A total number of 400 bacterial species, including 200 isolates from patients with first-time symptomatic UTI and 200 from patients with a history of recurrent UTI were examined.
For biofilm cultivation and biofilm biomass determination, adherence assay on 96-well microtitre plate and semi-quantitative spectrophotometric method were used, respectively.
For 25 selected strong biofilm producers, antibiotic susceptibility to ciprofloxacin and sulfametoxasole trimetoprime of planctonic cells was tested by microdilution assay and compared with the antimicrobial sensitivity of bacterial biofilms (performed with the Calgary Biofilm Device)

Results: Biofilms of strains isolated from patients with recurrent infections had significantly *greater biomass* (for $Z=2,79$ and $p<0,01$; $p=0,005$) compared to biofilms of isolates from first time UTI patients with *E. coli* being most prevalent uropathogen in both groups of patients followed by *Klebsiella spp*. There was statistically significant diference in the antibiotic susceptibility of planctonic cells vs their sessile counterparts.

Key words: biofilm, slime, recurrent urinary infections, antimicrobial resistance, *E. coli,* minimal biofilm eradication concentration.

Ass.Prof. Didem Kart, Ankara, Turkey

Didem Kart is Assistant Professor at the Hacettepe University Faculty of Pharmacy, Department of Pharmaceutical Microbiology, Turkey since 2016, where she also graduated in 2005. In 2014 she completed her PhD "Determination of the Microbial Interactions in Polymicrobial Biofilms Acting as a Role in Susceptibility Against to Disinfectants Frequently Used in Hospitals with Phenotypic and Genotypic Analysis" at the University of Gazi, Faculty of Medicine, Department of Microbiology, Turkey.
She is a member of European Society of Clinical Microbiology and Infectious Diseases, Turkish Microbiology Society, Society of Pharmaceutical Sciences of Ankara and Disinfection Antisepsis Sterilization (DAS) Association. She has several publications in international scientific journals and also participated in many international congresses in the last decade.

BI7 METABOLITE CHANGES ENTEROCOCCUS FAECALIS CO-CULTURING WIT CANDIDA ALBICANS AND PROTEUS MIRABILIS WITHIN MIXED-SPECIES BIOFILMS

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Introduction: *Enterococcus faecalis* is one of the major agents in a variety of hospital acquired infections, and their ability to form biofilms is known to be a virulence factor. There are different dynamic relations and interactions in mixed-species biofilms rather than that of single-species. *E.faecalis* is frequently isolated from the samples of patients having biofilm-related infections with *C.albicans* and *P.mirabilis*.

Objectives: Our goal was to establish an poly-species biofilm model in vitro and to find out the impact of presence of the *C.albicans* and *P.mirabilis* on metabolite profile of *E.faecalis*.

Method: Polymicrobial biofilms were reproducibly grown, consisting of *C.albicans*, *E.faecalis* and *P. mirabilis* in a 96-well microtiter plate. Comparative metabolomic analysis of mono and polymicrobial biofilm samples was carried out based on the GC-MS metabolomic profiling to scan wide range of metabolites. Distribution of metabolites was analysed by the Kyoto Encyclopedia of Genes and Genomes metabolic pathways database.

Results: The number of E.faecalis sessile cells was found significantly higher in dual and triple species biofilms than single-species. The multivariate metabolomic analysis shows clear separation between mono and polymicrobial biofilm groups of *E.faecalis* (Figure 1). After deconvolution and aligned of the chromatograms, 189 mass spectral features have been detected and 118 of them were annotated using retention index libraries. The pathway analysis showed that aminoacyl-tRNA synthesis, nitrogen and amino acid metabolism and tricarboxylic acid (TCA) cycle significantly changed in multi-species biofilms compared to single-species biofilm.

A) B) C)

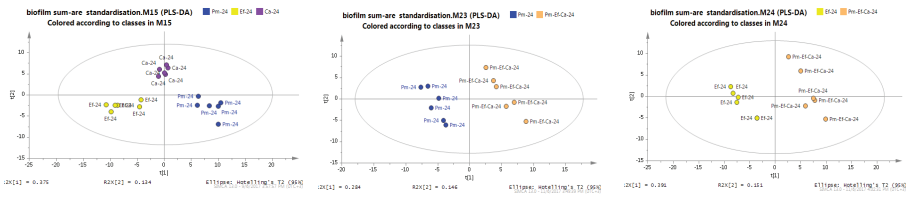

Figure 1:The metabolomic profile of *E.faecalis* in mono, dual and triple species biofilms.

Conclusions: The presence of P.mirabilis in triple biofilm resulted in increased pipecolic acid synthesis and decreased amino acid synthesis intermediates when compared to only E.faecalis biofilm, displaying a rerouting of metabolic pathways.

Keywords: *E.faecalis, P.mirabilis, C.albicans, mixbiofilm, metabolomic*

BI8 DETECTION OF BIOFILM PRODUCTION AND SUSCEPTIBILITY TO TIGECYCLINE AMONG CLINICAL ISOLATES OF ACINETOBACTER BAUMANNII

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INTRODUCTION: *Acinetobacter baumannii* is among the most clinically significant nosocomial pathogens due to its increasing presence in the hospital environment, which causes an extremely wide range of infections associated with high rates of mortality due to severe multiresistance.

OBJECTIVES: The aim of this study was to determine the ability of biofilm production and twitching and swarming type of motility through the substrate in the tested *A. baumannii* isolates, as well as the association of biofilm production with sample types and susceptibility to tigecycline.

MATERIAL AND METHODS: A total of 128 strains of *A. baumannii* isolated from blood (34), wound swabs (35), respiratory tract (39) and other clinical materials (20) were included in the study. Isolates were tested for the ability to produce biofilm in the microtiter plates at 26°C and 37°C for 24 hours incubation period. The presence of the pili was determined by the testing of the twitching and swarming motility in a semi-solid nutrient medium. Minimum inhibitory concentration (MICs) of tigecycline was determined by E-test and it was interpreted according to the recommended MIC values of tigecycline for Enterobacteriaceae - Susceptible: MIK≤1 μg/ml; Resistant: MIK>2 μg/ml.

RESULTS: The majority of isolates (over 90%) showed moderate or pronounced biofilm production capacity regardless of the experimental conditions. During the 24-hour incubation period, strains *producing strong biofilms* were recovered *more frequently* from wounds and respiratory tract than isolates from blood and other sites ($p<0.05$). No correlation between specific types

of motility and biofilm production was noticed. Tigecycline nonsusceptible isolates (90/128) were better biofilm producers than susceptible strains.

Conclusion: The biofilm production among *A. baumannii* isolates is a stable phenotypic trait, and along with resistance to antibiotics, is the crucial factors for survival *A. baumanii* in intrahospital conditions.

Keywords: *Acinetobacter baumannii*, biofilm, tigecycline , resistance, motility, pili

BI9 THE EVALUATION OF CIPROFLOXACIN AND VARIOUS AGENT COMBINATIONS ON DRIP FLOW REACTOR BASED P.AERUGINOSA BIOFILM MODELS

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Introduction: Poly-species biofilms are important problem in hospital acquired infections. *Pseudomonas aeruginosa*, *Candida albicans* and *Staphylococcus aureus* can coexist in mix-biofilms in a close relationship and display different antimicrobial susceptibility profile when grown in together. Quorum sensing is an alternative strategy in fighthing biofilm infections and some natural quorum sensing inhibitors (QSI) were found successful to interfere with the infections.

Objectives: Our aim is to set up dynamic mono and mix-biofilm models of *P.aeruginosa, S.aureus* and *C.albicans* in a repeatable style using Drip Flow Biofilm Reactor (DFR) and to evaluate the anti-biofilm effect of ciprofloxacine alone and in combinations with QSI and cyclic di-GMP inhibitors on *P.aeruginosa* cells when grown only and together with *S.aureus* and *C.albicans* in biofilms.

Material and Methods: Polymicrobial biofilms were reproducibly grown, consisting of *S. aureus, S.aureus, P. aeruginosa and C.albicans* in DFR. The antibiofilm effects of ciprofloksasin alone and combinations with some natural QSIs on sessile cells of *P.aeruginosa* in both mono and multispecies biofilm model in order to gain more insight into the role of biofilm composition on efficacy of the agents. Ciprofloksasin and QSIs combinations including cinnamaldehyde, resveratrol, L-canavanin, 4-nitropyridine N-oxide, p-benzoquinon, farnesol, epigallocatechin gallate, catechin hydrate, curcumin, baicain hydrate and esculin hydrate and combinations with cyclic di-GMP inhibitors such as sulfatiyazol and azathioprine were tested. Log reduction of

the antimicrobials were determined by plating method.

Results and Conclusion: After treatment of biofilm cells with ciprofloxasin, the number of *P.aeruginosa* cells in poly-species biofilms was found significantly higher than in poly-species biofilms. Ciprofloxasin and farnesol, sulfathiazole, resveratrol and Azathioprine combinations were found more effective to kill the *P.aeruginosa* cells in mono than poly-species biofilm. Except for resveratrol, sessile cells of *P. aeruginosa* in polymicrobial biofilm were found more susceptible to ciprofloxasin and QSIs combinations compared with the cells in only *P.aeruginosa* biofilm.

BI10 BACTERIAL PATHOGENS IN PATIENTS WITH CYSTIC FIBROSIS

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Introduction: Respiratory infections remain a major threat to cystic fibrosis (CF) patients. Detection and correct identification of bacteria implicated in these infections is critical for successful therapeutic management of CF patients.

The aim: was to retrospectively analyze the presence of bacteria in respiratory samples (tracheal aspirates, sputa) from CF patients treated at the University Clinic of Pediatric diseases in Skopje.

Material and methods: a total of 120 patients from the University Clinic of Pediatric diseases in Skopje were analyzed for the period of one year (January 2017 – December 2017). Specimens from respiratory tract from CF patients were investigated with conventional microbiological methods (culture on special media for bacteria and fungi).

Results: A total of 377 specimens were analyzed from 120 CF patients (average 3.1 per patients). Positive findings were registered in 79% of the analyzed specimens (297/377). From these, bacteria were detected in 82% (243/297), and in 18 % only fungi were identified (54/297). From the positive (243) bacterial findings, *P.aeruginosa* was detected in 32% (78), MSSA 32.5% (79), MRSA 17.3% (42), *P.aeruginosa* + MSSA 2.5% (6) and *P.aeruginosa* + MRSA 1.6% (4/243). Other Gram negative - 10.7% (26/243) and Gram positive bacteria - 3.3% (8/243) were also detected.

Conclusions: *P.aeruginosa* is still the major bacterial pathogen in CF

lung disease in parallel with methicillin sensitive *S.aureus*. We also detected a rising incidence of MRSA in our patients' CF samples, due to an increased awareness about this pathogen in the recent years, since it is associated with worse survival rates.

Key words: cystic fibrosis, infection, colonisation, *P.aeruginosa, S.aureus*

BI11 FOLLOW UP OF BLOOD CULTURES WITH QUALITY INDICATORS AFTER IN-SERVICE TRAINING

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Introduction
Because of its importance, blood culture requires close monitoring with indicators.

Objectives
The aims of this study are:
1) Monitoring 3 quality indicator parameters;
a) pre-analytical (appropriate volume)
b) analytical (match of Gram stain morphology with the final culture identification)
c) post-analytical (notification and documentation of critical value) aspects of blood cultures sent to our laboratory during a 6 months period after in-service training and various regulations
2) Raising the quality standards of the blood cultures.

Material and Methods
The study was carried out between January-June 2017, at blood culture bottles which were incubated in Bactec (BD, USA) and gave positive signal:
- Notification of Gram stain result and documentation as critical value
- Match of Gram stain results with the final culture identification
- Obtaining data from BD EpiCenter System and LIS
- Analysis of data; time of loading bottles, time/duration of positive results
- Examination critical value notification time
- Monitoring the differences between results within working hours and overtime

- Analysis of bacteria according to positive signaling times
- Indicators were compared after in-service training

Results and Conclusion

- 5200 blood culture samples were accepted in our laboratory during study period.
- Mean value of blood per bottle was 6 ml (between 3,2-8,4), previous 6.6 ml (4,5-8,8).
- 1228 (23,6%) blood cultures yielded positive signal
- The longest duration of positivity time was in diphteroid bacilli (25,71 hours) (previous 35,7 hours), the shortest duration in *Pseudomonas* spp. (19,27 hours) (previous *Enterobacter* spp. 12 hours)

Findings obtained before and after in service trainings an regulations are shown on Table 1.

After the regulations, decreased rate of mismatches in Gram stain, shortened time of notification of critical values, reporting all critical values were considered as positive improvements. Along with improvement in analytical and post-analytical periods, going on trainings also covering pre-analytical period are planned.

Key words: Blood culture, in-service training, quality indicator

BI12 INVESTIGATION OF ANAPLASMA / EHRLICHIA SPECIES WITH MICROSCOPIC AND MOLECULAR METHODS IN CATTLE IN KARAMAN PROVINCE*

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Introduction: *Anaplasma* and *Ehrlichia* species are microorganisms that cause subclinical and clinical infections in domesticated and wild animals and transmitted by ixodid ticks.

Objectives: This study was conducted to determine the A / E species causing infections in cattle in Karaman province by microscopy, Polymerase Chain Reaction (PCR) and Reverse Line Blotting (RLB) methods.

Material and Methods: Blood samples and thin blood smears were collected from 150 apparently healthy cattle from 21 locations of Karaman province in Turkey in 2016. After amplification the hypervariable V1 region of the 16S rRNA gene of A/E species a

reverse line blot (RLB) assay was performed using species-specific probes. Since some samples gave positive signals only to A/E catch-all probes samples analyzed in terms of major surface proteins (MSPs) of *Anaplasma marginale*.

Results: *Anaplasma*-like bodies were detected in four (2.66%) animals via microscopic examination. *Anaplasma centrale* was detected in eight (5.33%) animals via RLB. When we examined the samples in terms of *Anaplasma marginale* msp4 gene with nested PCR, a total of nine (6.00%) animals (six of them (%4.00) were positive for *A. centrale* with RLB) were found to be positive for *A. marginale*.

Conclusion: According to these results, it was determined that *A. marginale* and *A. centrale* were found in cattle in Karaman province. It is considered that it is necessary to carry out related sequence analysis and advanced proteomic studies for the identified species.

Key words: *Anaplasma*, *Ehrlichia*, cattle, Karaman, PCR, RLB.

* This study is supported by the Scientific Research Commision of Karamanoğlu Mehmetbey University (Project No: 41-M-16).

BI13 A RARE AGENT OF BRAIN ABSCESS: NOCARDIA OTITIDISCAVIARUM AND ANTIMICROBIAL SUSCEPTIBILITY TEST RESULTS

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Introduction: Nocardiosis is an infection caused by gram-positive, aerobic, filamentous bacilli. *Nocardia* spp. usually causes infection in immunosuppressive patients. It can affect all systems by spreading circulatory system, including central nervous system.

Objective: This report describes a brain abscess, developed following masteidectomy in a 50- year- old male with multipl myeloma, due to *Nocardia otitidiscaviarum.*

Material and Methods: After detection of brain abscess, drainage and partial lobectomy were performed, and imipenem, vancomycin,

trimethoprim sulfamethoxazole were initiated. Abscess materials sent to the laboratory were plated on agar media and inoculated into blood culture set. Conventional methods and MALDI-TOF (Bruker, Germany) were used in identification. Antimicrobial susceptibility was determined by gradient test (Liofilchem, Italy) (Table 1). Minimum inhibitory concentration (MIC) values were evaluated to lead the clinician in treatment.

Results and conclusion: In the examination of Gram stained smears; numerous PNLs and filamentous-branched gram-labile microorganisms, and modified acid-fast stained smears, AFB bacilli were detected.

On the 6th day of incubation, star-shaped, beta hemolytic white colonies were detected. The isolate was identified as *N. otitidiscivarum.* Intravenous linezolid, trimethoprim sulfamethoxazole were started. Same growth was detected at the 68th hour in the aerobic blood culture. Patient responded to antimicrobial chemotherapy and was transferred to another center for at least 6 weeks intravenous therapy, with recommendation of oral trimetoprim-sulfamethoxazole and amoxicillin-clavulanate treatment completion to 12 months.

Nocardia is an opportunistic pathogen that can cause serious infections, especially in immunocompromised patients.

Identification of *Nocardia spp*. by biochemical tests are not valid, the reference method is molecular method. This strain was also confirmed with 16S ribosomal RNA (rRNA)-based molecular identification.

Antimicrobial susceptibilities should be performed on all *Nocardia* isolates as a guide to therapy. Susceptibility testing is particularly important in patients infected with *Nocardia* spp. known to have high frequencies of antimicrobial resistance

Key words: *Nocardia otitidiscaviarum*, brain abscess, antimicrobial susceptibility

BI14 SEROPREVALENCE OF BORRELIA IGG AND IGM ANTIBODIES IN RELATION TO REPORTED TICK BITES AND SYMPTOMS IN SKOPJE REGION

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Aim: To determine the seroprevalence of *Borrelia* IgG and IgM antibodies in serum and to relate it to reported tick bites.

Method: Serum samples from 80 patients were collected in a one year

period and a Borrelia VirClia Monotest (Vircell, Spain), was performed for IgG and IgM antibody detection. Each monotest contains wells for automatic sample addition plus the reagents necessary to perform the assay. Assays are automatically processed on the instrument (Gold Standard Diagnostics,USA) with VirClia processing and interpretation software. Samples with indexes above 1.1 are considered as positive. The sera were taken at least 14 days after confirmed or suspected thick bite. All patients answered a questionnaire regarding history of a thick bite and previous treatment.

Results: Seropositive were 25/80 (31.5%) patients. Concomitant *Borrelia* IgG and IgM seropositivity was noted in 3 cases and only IgG or only IgM seropositive were 9 and 13 patients, respectively. Previous tick bite had been noted in 45/80 (56.3%), and in 12 of the seropositive patients. Erythema migrans as a symptom was noted in 5 patients, all of them reporting a thick bite and being IgG positive. Approximately 50% of the patients bitten by a thick received antibiotic therapy.

Conclusion: Having into consideration that *B. burgdorferi* in this area is tested for only few years, the results demonstrate IgG and IgM antibody seroprevalence of 31.5%, half of these cases are associated with a tick bite. Few of these seropositive patients reported previous symptoms. Further seroprevalence studies on human, as well as on animals are needed for better health protection of the population.

Key words: *Borrelia, tick bite, Lyme disease.*

SESSION 3
EMERGING TRENDS OF HOSPITAL INFECTION AND CONTROL

HI1 MICROBIAL COLONIZATION OF MEDICAL DEVICES IN INTENSIVE CARE UNITS

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Introduction: Microorganisms that colonize medical devices are main cause of Intensive care unit (ICU)-acquired infections. These microorganisms are part from the endogenous flora of the patients or exogenously acquired microorganisms from the hospital environment: microorganisms from inanimate hospital surfaces, contaminated medical equipment, hands of the hospital workers and the air in the ICUs.

Aim: Review of microbial colonization of the medical devices in ICUs.

Material and Methods: As material for this research were used 200 swabs taken from tubes, cannulas, catheters and surgical drains. The samples were sent from ICUs to the Institute for Public Health of the R. Macedonia - Skopje, for microbiological examination, for a period of 10 consecutive years (2008-2017). Standard culture method was used and the identification of microorganisms was made with standard microbiological methods and automated identification system - VITEK 2 (bioMerieux, France).

Results: Microbiological isolates were recorded in 53% from the samples. Positive findings were detected in 80,7% of the tubes and cannulas, 67,3% of the catheters and 37,5% of the surgical drains. The most common isolate from tubes and cannulas was *Acinetobacter spp* (37,3%). *Acinetobacter spp*. and *Escherichia coli* were represented with 33,3% (each) of all microbiological isolates from surgical drains. *Enterococcus spp*. was the most common isolated microorganism from catheters (18,2%).

Conclusions: The presence of *Acinetobacter spp*. in ICUs is alarming because of its tendency to become endemic in the hospital environment. *Escherichia coli* and *Enterococcus spp*. indicate devices fecal contamination. Health workers should be aware that colonization precedes cross-transmission and nosocomial infections. Continuous microbiological monitoring of the medical devices and appropriate infection control measures are essential.

Key words: *colonization*, *microorganism, medical device*, *ICU*

HI2 CLOSTRIDIUM DIFFICILE RIBOTYPES IN HOSPITALIZED PATIENTS

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Introduction

Clostridium difficile is the most important cause of healthcare-associated diarrhea, pseudomembranous colitis and toxic megacolon. As a nosocomial pathogen, this bacterium has a potential to cause outbreaks in the hospital environment. The standard antimicrobial therapy for *C. difficile* infection are metronidazole and vancomycin. In the last decade, an emergence of reduced susceptibility towards both of these drugs has been reported. **The aim** of the study was to type *C. difficile* isolates, obtained from hospitalized patients within the period of two years and to identify their susceptibility towards the clinically most important antimicrobial agents (matronidazole and vancomycin).

Material and methods

In the study were included a total of 21 isolates of *Clostridium difficile* collected during 2015 and 2016 from patients hospitalized within different wards at the Clinical Centre in Skopje. PCR ribotyping of the isolates was performed as described by Janezic and Rupnik. Antimicrobial susceptibility testing was performed in all *C. difficile* isolates by E-test. The MICs were interpreted according to the EUCAST recommendations for *C. difficile*,Version 8.0, 2018.

Results

Ten out of twenty one isolates of *C. difficile* (48%) belonged to ribotype 001/072. Two *C. difficile* isolates belonged to PCR ribotype 017 and the following ribotypes: SLO 160, SLO 187, SLO 120, 255/258, 014/020, 046, 002, 070 and 027 were presented with only one isolate each. All *C. difficile* isolates revealed good susceptibility to vancomycin and metronidazole.

Conclusions

PCR ribotype 001/072 was the most frequently detected *C. difficile* ribotype in hospitalized Macedonian patients within a period of two years. Unlike in many other European countries, the hypervirulent PCR ribotype 027 was very rare detected. Resistance of *C. difficile* towards the two clinically important antimicrobials has not been detected yet.

HI3 BACTERIAL ISOLATES IN WOUND INFECTIONS AT A TERTIARY CARE HOSPITAL IN KOSOVA

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Intoduction: Wound infection is an important cause of morbidity and mortality in surgical patients. Information on local pathogens and sensitivity to antimicrobial agents is crucial for successful treatment of wounds.

Aim: To determine antimicrobial susceptibility pattern of bacterial isolates from wound infection at University Clinical Center of Kosovo.

Methods: A cross sectional study was conducted among patients with wound infection visiting University Clinical Center of Kosovo, from January 2016 to December 2017. Wound swab was collected using sterile cotton swabs and processed for bacterial isolation and susceptibility testing to antimicrobial agents, following standard bacteriological techniques. Biochemical tests were done to identify the species of the organisms. Sensitivity testing was done using Kirby- Baur disk diffusion method.

Results: In this study 426 bacterial isolates were recovered from 670 specimens showing an isolation rate of 63.5%. The predominant bacteria isolated from the infected wounds were Escherichia coli in 157 cases (36.8%), followed by Enterobacter spp. 123 (28.8%), Klebsiella pneumoniae 91 (21.3%). All isolates showed high frequency of resistance to ampicillin, penicillin, cephalothin and tetracycline. The overall multiple drug resistance patterns were found to be 85%.

Conclusions: The infections were polymicrobic and multidrug resistant. Gentamicin, norfloxacin, ciprofloxacin, meropenem and amikacin were the most effective antibiotics.

HI4 AN OVERVIEW OF THE MOST COMMON ISOLATES FROM WOUND SPECIMENS

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Aim: To determine the most common microorganisms isolated from various wound specimens, as well as the percentage of resistant isolates.

Material and methods: In a period of one year (2017) a total of 3463 wound samples obtained from the hospitalized patients in the University Clinics of the „Mother Teresa" campus, and the City hospital „8th September" in

Skopje were processed at the Institute of Microbiology and Parasitology, Medical Faculty, Skopje. All specimens were inoculated onto standard agar media (Columbia agar with 5% sheep blood, Schaedler agar, glycose broth, and CALB agar for the isolation of yeasts). The aerobic plates were read within 24 hours and the anaerobic plates at 48 hours. Any growth was subsequently identified by standard microbiological methods. Automatized Vitek system was used for identification of all anaerobes and confirming the identification of the aerobes.

Results: Out of a total of 3463 samples, 2068 (59,7%) were positive by culture with 2971 isolated microorganisms. Of these, 2758 were aerobic bacteria: 1420 Gram-positive, 1338 Gram-negative, 100 were anaerobic bacteria, 108 were yeasts and only 5 were molds. The total number of specimens negative by culture was 1395 (40,3%). Out of wound swabs-2444, punctates-817, exudates-45, tissue-26, drain swabs-49, breast swabs-71, umbilical swabs-7, catheter swabs-4, positive were 1701 (59,60%), 282 (34.52%), 4 (8.89%), 8 (30.77%), 33 (67,35%), 33 (46,48%), 4 (57,14%), 3 (75%), respectively. The most frequently isolated Gram-positives were: *Staphylococcus aureus*-537 isolates, of which MRSA-were 130 (24,2%), *Enterococcus*-439, of which VRE-27 (6,15%), Staphylococcus coagulase negative-245, of which meticillin-resistant were 178 (72,6%). The most frequently isolated Gram-negatives were: *Pseudomonas aeruginosa*-325, *E.coli*-273, of which ESBL+ were 83 (30,4%), Acinetobacter spp-198, *Enterobacter cloacae*-193 of which ESBL+ were 25 (13%) and 1(0,5%) was carbapenem resistant. *Klebsiella pneumoniae*-89, of which ESBL+ were 20 (22,5%) and 16 (18%) were carbapenem resistant.

In conclusion, monitoring of the change in the microbial flora in the wounds, in particular the presence of resistant bacteria, could be important in guiding possible choice of antimicrobial chemotherapy or implementation of appropriate infection control measures.

Key words: wound specimens, resistant bacteria

HI5 VENTILATOR ASSOCIATED PNEUMONIA CASES CAUSED BY DELFTIA ACIDOVORANS

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Introduction: *Delftia acidovorans*, although believed to be non pathogenic usually, catheter-associated bacteriemia, peritonitis,

ocular infection, endocarditis cases have been reported.

Objective: In this study, two cases of *D. acidovorans* pneumonia and probable source of dissemination were investigated.

Material and Methods: Tracheal aspirate specimens, sent from two patients hospitalized at neurosurgical intensive care unit, were both Gram stained and cultured. Phoenix (BD, USA) and MALDI-TOF MS (Bruker, Germany) automated systems were used in identification of the isolates. Phoenix automated system and gradient test were used in antimicrobial susceptibility testing. Results were evaluated according to CLSI M100 Table 2B-5 for 'other non-*Enterobacteraceae* species'. Patients' clinical history and treatment were acquired. Due to probability of a nosocomial infection spread, environmental cultures of intensive care unit were collected.

Results and conclusion: Case one: 42-year-old female patient was hospitalized with cerebellar malignancy. Meropenem, linezolid and colistin were used in therapy.

Case two: 61-year-old immunocompetent male patient was operated due to acute subdural hematoma. Ampicillin sulbactam, ceftazidime, amikacin were used in therapy.

In the examination of Gram stained smears of tracheal aspirate specimens of patients abundant leucocytes and intracellular and extracellular gram negative bacilli were detected.

Isolates grown purely on plates were identified as *D. acidovorans* with Phoenix and MALDI-TOF automated systems. Antimicrobial susceptibility test results are reported in Table1. Any pathogen couldn't be isolated from environmental cultures. Respiratory infections have rarely been reported in the literature due to *D. acidovorans.* As the clinical, laboratory and radiological findings of our cases indicate infection, and the results of culture and Gram stain are compatible, agents were accepted as significant. *D. acidovorans* is resistant to commonly used antimicrobials such as aminoglycosides and polymyxins in the published articles, although, usually they are accepted as non pathogenic, they should be considered carefully when isolated from clinical specimens.

Key words: *Delftia acidovorans*, ventilator associated, pneumonia

Table1. Antimicrobial susceptibility test results of isolates

Antibimicrobial agent	Patient1	Patient2
Amikacin	Resistant	Resistant
Gentamicin	Resistant	Resistant
Ciprofloxacin	Resistant	Resistant
Levofloxacin	Resistant	Resistant
Colistin	Resistant	Resistant
Ceftriaxone	Sensitive	Sensitive
Ceftazidime	Sensitive	Sensitive
Piperacillin tazobactam	Sensitive	Sensitive
Trimethoprim-sulfamethoxazole	Sensitive	Sensitive

HI6 A KNOWLEDGE AND PERCEPTION ASSESSMENT OF NURSES TOWARDS HAND HYGIENE IN NORTHERN CYPRUS

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Introduction: Hand hygiene (HH) is generally accepted as the most important measure to avoid the transmission of harmful germs and prevent healthcare-associated infections (HAIs). To the best of our knowledge, there is no literature data regarding the epidemiology of HAIs for North Cyprus. Hence, exploring HH knowledge is a crucial concern in order to bridge the gap between theory and practice. On the other hand, World Health Organization (WHO) Multimodal Hand Hygiene Improvement Strategy is not implemented in N. Cyprus.

Objectives: Herein, we aim to describe the level of knowledge and perceptions of HH among nurses in N. Cyprus for the first time.

Material: A cross-sectional study was designed with a cross-cultural translation and adaptation of previously published questionnaires of WHO.

Methods: Data were collected through self-administered questionnaires with basic demographics, knowledge and perception sections about HH from nurses employed at two state hospitals. Questionnaires were delivered in April-May, 2017 period.

Results: We received response from 125 of 200 (62.5%) delivered questionnaires. Only 5.6% of the nurses had good HH knowledge, while the majority (80.8%) had moderate knowledge, and 13.6% had poor knowledge (Mean score out of 25=14.92 ± 2.26). Country of education was found to affect overall knowledge significantly (p=0.001). Likewise, "in-service education" was found to be strongly related with overall knowledge scores (p=0.037). The participants gave varied answers (Average Knowledge Score=58.8%) for questions regarding routes of cross-transmission, source and prevention of potentially harmful germs. Similarly, responses to questions regarding hand-rub/soap use, HH-methodologies, HH-cosmetics interactions were varied (Average Knowledge Score=60.21%). Overall, positive perception towards HH was demonstrated by 104 (83.2%) participants. Effectiveness of HH was perceived strongly but less important among other patient safety issues. Availability of alcohol-based hand-rub and promotion of HH by leaders/senior managers were perceived as most useful actions for improving adherence to HH (Scores ≥6 on a 7-point Likert-type scale: 95.2% [CI95%90.4, 98.4] and 94.4%, [CI95%90.4, 97.6] respectively).

Conclusion: Despite most of the nurses exhibited positive perceptions towards HH, participants had a moderate level of HH knowledge. Improvements in the existing training programs are needed as our study highlights the importance of HH education both in occupational and in-service education.

SESSION 4
VIRAL INFECTIONS

Prof.dr Jean-Claude Manuguerra, Paris, France

Jean-Claude Manuguerra (JCM) was originally qualified as a veterinarian and was trained in virology at Institut Pasteur in order to get his PhD. He spent two years as a post doctoral fellow at the National Institute for Medical Research (London, United Kingdom). He was then co-director of the National Influenza Centre for Northern France and of the WHO Collaborative Centre for Research and Reference on Influenza Viruses and Other Respiratory Viruses, one of the 13 laboratories included in the WHO Collaborative Multi-Centre Laboratory Network on SARS (1994-2003). He belongs to the French team sent to Hanoi for the control of the SARS outbreak at the Hanoi French Hospital in March 2003. Since then, he participated to a number of missions during outbreaks. Recently, in 2014 and 2015, he went to the Ebola diagnostic laboratory in Donka Hospital in Conakry and in the Ebola Treatment Center in Macenta in Guinea respectively. From 2000 to present, JCM has been a member of the steering committee of the Global Alert and Response Network coordinated by the WHO and chaired it from 2011 to 2013. JCM was Secretary General of the French Society for Microbiology from 1998 to 2001. Since December 2005, he has been corresponding member of the French Academy of Veterinary Medicine. Since 2014, JCM is the workpackage leader of the human virology network of the Medilabsecure EU funded project. JCM heads the Environment and Infectious Risks expertise and research Unit (ERI) which harbours the Laboratory for Emergency Response to Biological Threats, which was created in 2002.

VI1 MOLECULAR DETERMINANTS OF THE STABILITY OF INFLUENZA VIRUSES OUTSIDE THEIR HOSTS

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The transmission routes of influenza A viruses (IAVs) sbmit viral particles to a wide range of environmental conditions that affect their stability and ultimately their transmission. In water, temperature,

salinity, and pH are important factors that modulate virus persistence in a strain-dependent manner. Previously in our laboratory, we demonstrated that the loss of infectivity is not due to the degradation of the viral genome, and the viral factors responsible for the persistence phenotype of the IAVs remained to be described. To this end, we used an innovative method based on a real-time cellular analysis system to quantify viral loss in a controlled environmental model. We identified viral haemagglutinin (HA) and neuraminidase (NA) as the main proteins responsible for the persistence phenotype by comparing the inactivation slopes of several reassortant viruses. In order to identify the molecular determinants of environmental stability, we introduced synonymous and non-synonymous mutations in the HA or NA genes that modulated the persistence of IAVs. In total, our results demonstrate that the level of HA expression, the stability of the HA, as well as the stability and activity of the NA, are molecular determinants of viral persistence.

In addition, we observed that IAV particles retain their ability to attach to target cells but can not trigger membrane fusion after loss of infectivity in the environment, highlighting the importance of the HA and the NA. for virus survival outside the host.

Prof.dr Angel Galabov, Sofia, Bulgaria

Prof. Angel S. Galabov, MD, DSc, virologist, works in the Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences (BAS). He is Regular Member of BAS since 2008. His research focuses on antivirals, mainly on inhibitors of replication of enteroviruses, influenza viruses, adeno- and herpesviruses. Another fields of his work as researcher are biological response modifiers (interferon and its inducers, antioxidants) as antiviral agents, Balkan endemic nephropathy, virucidal microbicides, genetic characteristics of contemporary Bulgarians, of Protobulgarians and Thracians. He is author of 285 publications and 39 patents, supervisor of 20 PhD students. He was director of the Stephan Angeloff Institute during 17 years (1995 -2011) and associated this Institute in the International Network of Pasteur Institute. Prof. Galabov is the founder of the Balkan Society for Microbiology (BSM) in 1998, and actually is the President of BSM. He initiated the first in Europe symposium chain on antivirals (1974) and is active member of the International Society for Antiviral Research.

VI2 CYCLURIDINE: A NOVEL ANTIVIRAL EFFECTIVE AGAINST FLAVIVIRUSE

A. Galabov

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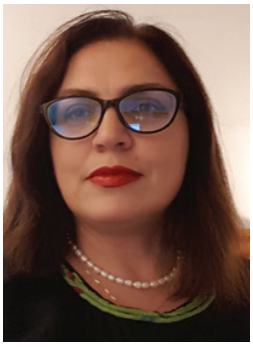
This report describes the contemporary state of research for antivirals effective against flaviviruses, especially focusing on inhibitors of the pestivirus causative agent of bovine viral diarrhoea virus (BVDV). We highlight cycluridine (CU), an originally synthesized Mannich's base [a tetrahydro-2(1H)-pyrimidinones derivative], as a highly effective antiviral possessing a strong inhibitory effect on BVDV replication. CU was active against replication of a wide variety of BVDV strains in cell cultures. The drug-sensitive period in the BVDV replication cycle included the latent period and the exponential phase; a 90-min delay in the peak of viral RNA synthesis was observed. CU administered orally manifested a pronounced protective effect in calves with natural mucosal disease/viral diarrhoea and calves experimentally infected with BVDV. Its magnitude of activity and selectivity places CU in the lead among all known substances with anti-BVDV activity. Additionally, CU applied subcutaneously showed anti-tick-born encephalitis virus (TBEV) activity, manifesting a marked protective effect in mice infected with TBEV. CU could be a prospective antiviral in veterinary and medical practice for the treatment of BVDV and other flavivirus infections.

Ass.Prof.dr Golubinka Boshevska, Skopje, R.Macedonia



Dr. Golubinka Boshevska, Assistant Professor, was born in 1971 in Kocani, Macedonia. She graduated at the Ss. Cyril and Methodius University, Medical Faculty in Skopje in 1995. Since 1999, she works at the Institute for Public Health, R. of Macedonia, where she has been appointed for head of the Laboratory for Virology and Molecular Diagnostics since 2009. From 2017 she is elected Assistant Professor at the Faculty of Medical Sciences, University "Goce Delchev" in Stip for the teaching-scientific field of Microbiology. Dr. Boshevska is national coordinator for antimicrobial resistance since 2008, alternative observer in the Forum of the National Coordinators for Microbiology at ECDC (2013), contact person for laboratory reporting of measles and rubella and Influenza in the WHO, national coordinator for laboratory containment of wild polio virus and laboratory biosafety (2005). Dr. G. Boshevska is a member of the Macedonian

Medical Association; The Doctors' Chamber of Macedonia; Macedonian Association of Microbiologists; Macedonian Association for Control of Intra-Hospital Infections; European Science Group on Influenza; EPISOUTH PLUS Project; European Network for the Diagnosis of "Imported" Viral Diseases (ENIVD); European Network of Laboratory Experts on the Detection and Monitoring of New Virus Disease Threats (EVD-LabNet); SECID network of Southeast European countries; member of CORDS- Connecting Organizations for Regional Diseases Surveillance, FEMS. She is a contact person for R. Macedonia MediLabSecure network of countries in the Mediterranean and the Black Sea region for monitoring viral causes in humans and animals. She has been coordinating projects, international and national, for influenza and HIV testing and surveillance. She has published 27 papers in PubMed, 11 in Macedonian journals, and has 65 presentations on international and national symposiums/congresses.

VI3 RE-EMERGENCE OF HANTAN VIRUS IN MACEDONIA

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Introduction: Hantan viral diseases are widely distributed in Europe, including our neighboring countries. In last 10 years only two cases were confirmed, one in 2009 and one in September 2016, out of 5-10 suspected cases per year. Hemorrhagic fever with renal syndrome (HFRS) is a notifiable disease since 2004. Responsible lab for detection of human cases is the laboratory for virology and molecular diagnosis, IPH.

Objectives: To present re-emergence Hantan virus cases in Macedonia, and their characteristics and control measures.

Material and methods. We analyzed epidemiological and lab reports for all reported cases, as well as containment measures.

Results: The first outbreak started in April 2017 with lab detection of Hantan IgM of the index case. In the period 03.04-15.05.2017 we detected 4 cases of Hantan out of 9 suspect cases. 6 of them, including the index, were shepherd from the same sheep farm in village Novak, near city of Debar, western part of Macedonia. All of them were male with age from 36-44 years (average 40,33 years). Hantan IgM and IgG positive were 3 cases (50%) (including index), one was only Hantan IgG positive without symptoms, and 2 cases were negative for Hantan IgM and IgG. 3 suspect cases in the same period were not connected

with the outbreak among shepherd, and were coming from different cities. All were male. Among them only one case of a hosier from Kochani city in Eastern part of Macedonia. was Hantan IgM and IgG positive. The second outbreak started in July among shepherd father and son, from the village Simnica, near Gostivar city, western part of Macedonia. Due to rapid development of the symptoms and fatal end of the two index cases clinician suspect Crimean-Congo haemorrhagic fever (CCHF). After negative result on the test for detection of CCHF nucleoprotein antibodies and CCHF IgM, 11 cases from the same sheep farm, were tested for Hantan IgM and IgG. Hantan IgM positive were 6 (54,54%), 1 was IgM equivocal and IgG positive; other 4 cases were negative for IgM and IgG. All were male. Immunoblot test on the two index cases was positive for Dobrava. A team of epidemiologist visited the sheep farm and the village. They made deratisation, education of the farm workers and village citizens, shared printed educational materials. Out of 11 additional single cases with HFRS, 6 (54,54%) were IgM and IgG positive. Sequence analysis was performed on 8 cases. 2 cases were positive in S and M segment and 1 positive only in M segment.The sequences cluster together with those detected in Albania and in Western Greece. In total, from April until November 2017 there were 31 suspect cases with 16 (51,6%) positive cases of Hantan virus. All cases had contacts with rodent excreta.

Conclusion: There is increasing number of Hantavirus detection in Macedonia. They start with the spring, affecting male shepherd. The sequences analysis confirm geographical distribution of Hantan viruses in Balkan countries.

Dr.Maja Bogdanic, Zagreb, Croatia

Dr. Maja Bogdanic graduated medicine at the Zagreb University, Croatia in 2010. After 3 years at the Department of Cytopathology, Clinical Hospital Merkur, (since 2015) she is resident in Clinical Microbiology at the Croatian Institute of Public Health. She is working on two scientific projects: "Efficient response to highly dangerous and emerging pathogens at EU level – EMERGE"(Head: Vladimir Draženović, MD) and "Prevalence and molecular epidemiology of emerging and re-emerging neuroinvasive arboviral infections in Croatia" (Head: Assist.Prof. Tatjana Vilibić Čavlek, MD, PhD). Dr. Maja Bogdanic is member of Croatian Society for Clinical Cytology.

VI4 PREVALENCE OF TICK-BORNE ENCEPHALITIS IN ENDEMIC REGIONS OF THE CROATIAN MAINLAND, 2017

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Arbovirus study group; [1]Collaborators on the project Croatian Science Foundation: IP-2016-06-7456: "Prevalence and molecular epidemiology of emerging and re-emerging neuroinvasive arboviral infections in Croatia" (CRONEUROARBO); [2]Collaborators of the Reference Center for Diagnosis and Surveillance of Viral Zoonoses Croatian Ministry of Health; Croatian Institute of Public Health, Zagreb, Croatia; [3]Institute of Microbiology and Immunology, Medical Faculty University of Ljubljana, Slovenia; [4]Veterinary Faculty University of Ljubljana, Slovenia

INTRODUCTION: Tick-borne encephalitis virus (TBEV) is the most important re-emerging arthropod-borne virus in Europe and Asia. In recent decades, the number of human cases in endemic regions of Europe has increased, endemic areas have spread to higher altitudes and new foci have emerged. The main vectors of TBEV in Europe are ticks *Ixodes ricinus*. Several studies showed that virus detection in ticks is not a sensitive indicator for risk assessment of human TBEV infection. Various animals have been used as sentinels in TBEV endemic areas, including horses in which asymptomatic TBEV is common with seroprevalence rates of up to 26%.

OBJECTIVES: To analyze the prevalence of TBEV in humans and horses in endemic regions of the Croatian mainland.

MATERIAL: From January to December 2017, a total of 90 patients with neuroinvasive disease (meningitis/encephalitis), 172 asymptomatic persons and 560 horses were tested for the presence of TBEV RNA and/or TBEV antibodies.

METHODS: TBEV IgM/IgG antibodies in human and horse sera were detected using a commercial ELISA. TBEV RNA was detected using a real-time and nested RT-PCR protocol.

RESULTS AND CONCLUSION: TBEV infection was confirmed in 11/90 (12.2%) patients with neuroinvasive disease (8 males and 3 females aged 21-68 years) by detection of TBEV IgM and low avidity IgG antibodies. TBEV RNA was detected in one patient. The majority of patients reported risk factors such as frequent visiting forest areas (7/63.6%), rural area of residence (7/63.6%) or frequent tick bites (6/54.5%). Cases showed seasonal distribution (April-November). Four (2.3%) asymptomatic persons were found to be IgG seropositive. In one participant, recent TBEV infection was documented by borderline

IgG avidity. TBEV IgG antibodies were detected in 80/560 (14.3%) sentinel horses. Our results highlight the need of multidisciplinary ("One health") surveillance of this re-emerging arboviral zoonosis.

VI5 MediLabSecure: A VIROLOGY AND ENTOMOLOGY LABORATORIES NETWORK FOR A ONE HEALTH APPROACH OF VECTOR-BORNE AND EMERGING VIRUSES IN THE MEDITERRANEAN AND BLACK SEA REGIONS

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INTRODUCTION: Countries of the Mediterranean and the Black Sea face common public health issues and among those, vector-borne infectious diseases represent a particular challenge to health authorities.

OBJECTIVES: Recent viral outbreaks highlight the necessity to strengthen laboratory capacities, increase inter-sectoral collaborations and enhance common preparedness and joint response to upcoming viral threats. As such, since 2014, MediLabSecure, a One-Health network of Public Health institutions, has been working to select, include and actively connect laboratories of animal virology, human virology and medical entomology with support from public health institutions for the European Union neighbouring countries.

METHODS: Today, the MediLabSecure network represents a matrix for awareness, risk assessment, monitoring and control of emerging viral diseases, mainly vector-borne, that are pathogens for humans and/or animals. It is an operational framework of 55 laboratories and 19 public health institutions across 19 non-EU countries of the Mediterranean and Black Sea regions. Among our actions, we organize inter-regional and inter-sectoral meetings, training sessions either specific to one scientific field or transversal and also general laboratory biosafety workshops. These training activities enabled the network to (1) implement harmonized and up-to-date diagnostic techniques for vector-borne viruses such as West Nile, Rift Valley fever, chikungunya and Zika viruses; (2) develop capacity building

in medical entomology and vector surveillance and (3) foster interdisciplinary collaboration for surveillance integration in the framework of One Health. Joint meetings are allowing the network partners and coordinating institutions to meet and exchange on the objectives and future steps of the project, their experiences, needs and expectations.

CONCLUSION: On the long run, MediLabSecure aims, through tailored training sessions, inter- sectoral meetings and a set of tools, at establishing a platform for an optimized implementation of standardized diagnosis, integrated risk assessment of diseases and harmonization of responses in case of epidemics in the Mediterranean and Black Sea regions.

VI6 PREVALENCE OF HIGH RISK HPV GENOTYPES AMONG WOMEN WITH CERVICAL CANCER IN MACEDONIA IN THE LIGHT OF NINE-VALENT HPV VACCINE

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Macedonia is in the second place for prevalence of cervical cancer in Europe. This is a disease that can be prevented by vaccination. So far, four-valent human papillomavirus (HPV) vaccines have been used, and a nine-valent vaccine should be soon introduced. The new nine-valent vaccine against HPV includes the four HPV genotypes (6, 11, 16, and 18) that are targeted by the quadrivalent HPV vaccine, plus five additional oncogenic types (31, 33, 45, 52, and 58).

Aim: In order to ensure a successful introduction of available nine-valent HPV vaccine, there is a need to identify pre-vaccination HPV genotype prevalence of unvaccinated subjects in Macedonia.

Material: This study, counter from March, 2017 to April , 2018, was composed of 253 women, who were positively screened for opportunistic cervical cancer by pap smears and attend family gynecologist for HPV testing.

Method: After extraction of DNA from the speciments by PureLink Genomic DNA Kit, Invitrogen, presence of HPV was detected by Real Time PCR Kit for detection and genotyping of HPV(16,18,31,33,35,3 9,45,51,52,56,58,59,66 and 68), Sacaceae, Biotechnologies, Italy on Real Time 7500 Applied Biosystems.

Results: Of the 253 cases, 50 (20%) were positive for HPV DNA. 33 of HPV positive women have an infection with single HPV genotype, and 17 have Multiple HPV infection. Prevalence of HPV 16 was 24.65% and for HPV 18 was 4.10%. Excluding types 16 and 18, the prevalence

of additional five high-risk genotypes was 39.75% (17.80% for HPV31, 6.84% for HPV 33 and HPV52, 5.47% HPV58 and 2.80% for HPV45).

Conclusion: The nine-valent HPV vaccine is likely to give the greater protection of women in Macedonia, because, in the absence of genotype 16 or 18 infection, these five genotypes on their own remained significantly associated with high-grade cervical lesions.

Key words: Real time PCR, HPV prevalence, vaccines

VI7 MDM2309 (rs2279744) POLYMORPHISMS AND RISK FOR HPV PERSISTENCE AND CERVICAL INTRAEPTHELIAL LESIONS AND CERVICAL CANCER DEVELOPMENT IN MACEDONIAN WOMEN

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Introduction: Persistent infection with high risk Human Papillomavirus (HPV) infection is the main factor in initiation of cervical intraepithelial lesions (CINs) and cervical cancers (CCa). Still only small percentage of HPV positive the lesions progresses to higher CINs or CCa. The host factors as single nucleotide polymorphisms (SNPs) in tumor suppressor genes have influence on cancer development. SNP rs2279744 within MDM2 promoter gene are plausible factors enabling HPV persistence and CIN or CCa development due to increased attenuation of p53 pathway.

Objective: We analyzed association of HPV positive infection in different CINs grade and CCa with presence of this polymorphism. The study group consisted of 131 selected HPV positive women with histological confirmed CIN or CCa and 110 controls (cytological negative women with no HPV infection).

Methods: The rs2279744 was genotyped using SNaPShot analysis and HPV detection and genotyping was performed using Seeplex HPV4A ACE (Seegene Inc., Seoul, Korea).

Results: The study showed no association between rs2279744

polymorphism and susceptibility to HPV infection in CIN and CCa, but we find that TT genotype and T allele of MDM2 309 has significantly lower frequency in HPV positive CIN2+ and CCa groups compared to HPV positive CIN1 [G vs T p=0.02, OR=0.52; GG vs TT; p=0.04, OR=0.29; TT vs TG+GG; p=0.007, OR=0.34] after stratification the cases in these two groups. So T allele and TT genotype of rs2279744 are associated with HPV persistence and progression of CIN1 to CIN2+ or CCa.

Conclusion: This SNP could be used as prediction markers in CCa management in Macedonian women, but the clinical relevant warrants further validation in large and well-designed studies.

VI8 RESPIRATORY SYNSITIAL VIRUS IN HOSPITALIZED PATIENTS WITH ACUTE RESPIRATORY TRACT INFECTION

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Introduction and objectives: Respiratory syncytial virus (RSV) is the most common cause of acute respiratory infections, especially acute bronchiolitis and viral pneumonia in all age groups, especially in infants <2 years. The aim of the study was to determine RSV frequency, demographic characteristics and risk factors in children and adult patients admitted to the hospital with acute respiratory tract infection.

Materials and Methods: From April 2013 to September 2017, demographic characteristics and risk factors of children and adult patients with acute respiratory infection due to RSV were evaluated. The presence of RSV in 1907 nasopharyngeal swabs were tested using multiplex real-time PCR. Patients data were evaluated retrospectively from hospital database.

Results: The frequency of RSV was found to be 11.2%. Of the patients, 59.3 % were male and 40.7 were female, while 1521 (79.8%) were children, and 386 (20.2%) were adults. The mean age of the patients were 14.14±22.79 (0 to 92) years. The rate of RSV positivity was significantly higher in 0-2 age group than others and female predominance was observed in the same group. Forty eight (22.4%) of the patients had at least one comorbidity and the coinfection rate was 32.2%. Of those, asthma (52%), chronic renal failure (8.3%) anemia (8.3%), *chronic obstructive pulmonary disease* (8.3%) and hematological malignancy (6.2%) were the most common

concomittant diseases. The rate of co-infection was 32.2% and it was found to be higher in children than in adults. The most common co-pathogens were rhinovirus, coronovirus, and parainfluenza virus.

Conclusion: In this study, RSV has been shown to be the most frequent viral agent in patients with respiratory tract infections, especially under 2 years of age. The detection rate was decrease with advancing age. It should be kept in mind that RSV may be associated with other respiratory viruses and concomittant diseases.

SESSION 5
PARASITIC INFECTIONS AND MYCOLOGY

Prof.dr Eduardo Pozio, Rome, Italy

Edoardo Pozio has more than 25 years of experience in basic and applied research in the field of parasitic zoonoses caused by Trichinella, Echinococcus, Cryptosporidium, Giardia and Leishmania. He has been in service at the Instituto Superiore di Sanità, Rome, Italy since 1981, and in 1986 he was appointed director of the Department of Helminthology, and since 2004 Director of the Department of Parasitic, Gastroenteral and Tissue Diseases. During his research activity, he has been responsible for numerous international and national research projects on parasitic infections caused by opportunistic pathogens (Cryptosporidium and Toxoplasma), Trichinella and Echinococcus. Since 1988 he has been conducting the International Trichinella Reference Center of the International Committee on Trichinellosis (ICT), which he chaired from 2000 to 2004. Since 1992 he has been responsible for the Trichinellosis Reference Laboratory of the World Organization for Animal Health (OIE), and in 2006 he was appointed Director of the Community Reference Laboratory for Parasites. He is an expert for trichinellosis for the European Commission and EFSA, vice-president of the Italian Society of Parasitology, and member of several scientific societies and of the editorial board of international and national scientific journals. He has published more than 230 articles in international journals and 14 books or book chapters.

PIM1 PARASITIC DISEASES IN A CHANGING WORLD: EPIDEMIOLOGY AND DIAGNOSTIC PROBLEMS

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At the beginning of the third millennium, parasitic diseases still affect millions of people, primarily in the South of the world. On the contrary, people living in the northern hemisphere experienced a strong reduction of parasitic diseases. This scenario, considered consolidated at the end of the 20th century, is changing due to three important factors: the massive migration of people from the South to the North of the world, the globalization of humans, animals and goods, and climate change. In

industrialized countries, many diseases, not just of parasitic origin, are now considered emerging or re-emerging, and their diagnosis is challenging. In addition to this epidemiological picture, there is the increasing importance of medical entomology, since tropical and subtropical arthropods are colonizing new world regions moving to the North and acting as vector of serious viral diseases such as dengue, chikungunya, and yellow fever. In Europe among vector borne parasitic diseases, leishmaniasis, traditionally considered a disease limited to the Mediterranean basin, is increasing its distribution area to Central Europe, and new foci have been described in Switzerland and Germany in the last years. Although the prevalence of malaria is decreasing in endemic regions, global warming can favor the introduction of new and more efficient mosquito vector species in temperate regions, where infected immigrants and travelers from endemic regions can trigger local transmission, as frequently documented. Schistosomiasis, always considered a tropical disease, has appeared in the European Union with an important focus in the Corsica island, due to the concomitance of three factors, human migration from endemic countries, climate change and presence of a susceptible snail vector. The uncontrolled increase of the wild boar population in Europe, caused by the reduction of the human population devoted to agriculture and the illegal introduction by hunters of more prolific breeds, resulted in an increase of trichinellosis due to the consumption of game meat. Changes in eating habits, a sign of globalization, are favoring the consumption of raw fish, which in turn resulted in outbreaks of opisthorchiasis in Italy and an increase of anisakidiosis cases all over the world. Since the transmission routes and epidemiology of parasites are extremely different, globalization and climate change may impact differently on them. Illegal importation of meat and freshwater fish products can be the source of human outbreaks when introduced by personal baggage. The climate change may have a direct influence on parasite cycles increasing/decreasing their survival in the environment. For example, increased humidity favors the survival of parasite eggs and cysts, whereas increased temperature can reduce their survival in the environment. Rainfall intensity increases the spread of eggs, oocysts and cysts by water. Increased drought periods reduce parasites' survival. Investigations at international European airports have shown that tons of meat, fish and other food are illegally imported daily in personal baggage from extra-European countries into the European Union without any veterinary

control. The strong reduction in the prevalence of parasitic diseases in the 20th century resulted in the loss of diagnostic expertise among physicians, microbiologists, biologists and entomologists. At the same time, little investments were made to develop new drugs and diagnostic tools for parasitic diseases. A plethora of commercial kits for the diagnosis of parasitic infections is on the market, however, no one of these kits have been validated by an independent body, resulting in very poor diagnostic power. This scenario represents important gaps to address today's problems related to migration, climate change and globalization.

Prof.dr Olgica Djurkovic-Djakovic, Belgrade, Serbia

Olgica Djurkovic received her medical degree in 1980, her MS degree in neonatology and her PhD degree in parasitology in 1990, all from the University of Belgrade, then Yugoslavia. Following a 2-year post-graduate fellowship at the Dartmouth Medical School in Hanover, NH, USA, she became Head of the Department of Parasitology at the Institute for Medical Research, University of Belgrade, where she has been a Research Professor since 1998. Under her leadership, the Department became a national Centre of Excellence in 2011, today for Food- and Vector-borne Zoonoses. She is also Head of the National Reference Laboratory for Toxoplasmosis. Olgica has been the President of the Serbian Society for Parasitology since its revival in 2012. She is also the General Secretary of the European Federation of Parasitologists (EFP), and will organize the next EFP congress, EMOP 13, in Belgrade in 2020. Her research focuses on Toxoplasma gondii infection and other food-borne parasites. Olgica has published more than 100 peer-reviewed papers and edited two monographs/books, and has a citation record of around 1.000 (source Scopus).

PIM2 IS TOXOPLASMOSIS AN EMERGING DISEASE? NEW INSIGHTS

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Known for over a century, *Toxoplasma gondii* is a globally distributed parasite that infects one third of the world population. The clinical significance of *T. gondii* infection has long been defined by the disease it may cause in the developing fetus in case of maternal infection in pregnancy, congenital toxoplasmosis (CT), and as an opportunistic infection in immunosuppressed individuals. Thus, prevention strategies involve prevention of acute infection in pregnant women (thereby preventing CT), and of infection (mainly reactivation) in the immunosuppressed population. However, information that has emerged in the past decades suggests it may be time to re-define some existing concepts. Much of this comes from the insight into the *T. gondii* population structure, characterized by clonal lineages (designated as types I, II and III) predominating in Europe and North America (and a fourth one in the latter), and by a higher frequency of non-clonal, atypical strains in South America and Africa, which has had important implications for the understanding of *T. gondii*-induced clinical entities. Indeed, atypical strains have been associated

with severe ocular toxoplasmosis, atypical presentations, and even life-threatening disease in both immuno-competent and immunosuppressed individuals. Reinfection with atypical strains has explained (the few) cases of CT in babies born to immunized mothers. In addition, the recent emergence of information on the association of *T. gondii* infection with neurological and psychiatric diseases, of which an association with schizofrenia, through the influence on dopamine metabolism, seems established beyond doubt, alters the view of latent infection as completely latent and innocuous. On the other hand, the WHO and FAO have recently established toxoplasmosis as a foodborne infection of global concern, with a disease burden similar to that of classical foodborne illnesses such as salmonellosis and campylobacteriosis. A similar exercise in Europe has also placed toxoplasmosis at the top of the list of foodborne parasites at the European level, second only to *Echinococcus multilocularis,* and even heading the list in Western Europe. This has increased attention to toxoplasmosis and to attempts at redefining strategies for the control of *T. gondii* within the food chain, all the more at this time of global climate change and intense human movement (migrations and tourism), which both favour risk of infection. The widely varying geographically-dependent prevalence of infection and the geographic differences in the *T. gondii* population structure, determine toxoplasmosis as a travel risk. Moreover, globalization of food including importation of meats from areas of a highly diverse *T. gondii* population may also present a risk factor for severe infections. Therefore, all these issues emerging from the changing understanding of *T. gondii* infection in a changing world call for re-thinking the strategic approaches in both management and prevention.

Dr. Maria Angeles Gomes-Morales, senior researcher, PhD, Rome, Italy

Maria Angeles Gomes- Morales graduated pharmacy in 1980 at the University of Granada,Spain, where she received her PhD and specialization in microbiology and parasitology. From 2006 she is senior researcher in charge of the immunology section of the European Union Reference Laboratory for Parasites, Department of Infectious Diseases, Instituto Superiore di Sanita, Rome, Italy. Dr. Gómez-Morales has more than 30 years experience in basic and applied research in the field of human and animal parasitology. Her main activities regard the characterization of the immune response to parasites and the study of the humoral and cell-mediated immunity to parasitic infections.

PIM3 ARE FISH-BORN PARASITIC DISEASES EMERGING IN EUROPE?

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Among the infectious diseases, fish-borne parasitic diseases (FBPD) have generally been limited to populations living in low and middle-income countries. However, the geographical limits and populations at risk are expanding because of growing international markets, improved transportation systems, globalization of the food supply and demographic changes, and the increasing popularity of raw seafood products. In Europe, FBPD primarily include those caused by nematodes belonging to the family Anisakidae, trematodes (*Opisthorchis felineus*, and *Methorchis bilis*) and cestodes (*Diphyllobotrium latum*, *D. dendritricum*). Other FBPD can occur and they can be caused by the ingestion of parasitized fish that has been imported. In Europe (EU), anisakid nematodes are the most relevant group of parasites in terms of consumer health risk and product quality, with *Anisakis* and *Pseudoterranova* as the genera of greatest concern because several species are considered a human health hazard. Although zoonotic flatworms represent an important source of infections due to freshwater fish consumption in many parts of the world, in the EU this consumption is relatively low and geographically localized. After the world war second, the trematode *Opisthorchis felineus* and the cestode *Diphyllobotrium latum* have been the causative agents of FBPD. More than 200 *O. felineus* infections due to consumption of marinated tench fillets, were documented in Italy, since 2003. *D. latum* is the etiological agent of about 80-90 infections per year in the EU due to the consumption of raw or undercooked fillets of perch and salmon in Estonia, Finland, France, Italy, Lithuania, Poland, and Romania. Anisakid nematodes are either causing infection following ingestion of viable parasites, or allergic (hypersensitivity) reactions against parasite antigens. For EFSA, the only parasite in fishery products that is implicated in allergic reaction is the nematode *Anisakis simplex* and the primary initiator of the different forms of allergy is via infection by live larvae. Once sensitization has occurred, response to nematode allergens can be highly aggressive and generate severe allergic disease. Some authors have shown that an infection can provoke a concurrent allergic episodes in a sensitized individual and they claim this is the principal mechanism for disease. However, others consider that allergic episodes can not only be elicited by infection, but also by exposure to allergen remaining in food with no viable larvae. Since there are inadequate systems for routine diagnosis and monitoring or reporting for many of the FBPD, the incidence of human disease and parasite occurrence in food is underestimated. Consequently, the process by which priorities in national public health systems are developed, is generally handicapped by the lack of good data on health and economic impacts.

Jelena Srbljanovic, Belgrade, Serbia

Jelena Srbljanovic graduated at the School of Pharmacy, University of Belgrade, Serbia in 2011. Since 2011 she is on doctoral studies, (module pharmaceutical microbiology) at the same university. Jelena is employed at the University of Belgrade, Institute for Medical Research, Centre of Excellence for Food- and Vector-Borne Zoonoses, National Reference Laboratory for Toxoplasmosis. She is taking part in numerous scientific projects. She is member of the Serbian Society of Parasitologists. In 2016 she had received the European Federation of Parasitologists Young Scientist Award.

PIM4 NOVEL ANTIMALARIALS: AMINOQUINOLINES AFFORDING MURINE SURVIVAL WITH HUGE LEVELS OF PARASITEMIA

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Introduction. Our previous work focused on the antimalarial potential of adamantane aminoquinolines (AQ), while here we explore the activity of novel benzothiophene, thiophene and benzene AQ.

Objectives. To examine the effect of new compounds in both *in vitro* and *in vivo* conditions.

Material and Methods. The series of 11 AQ, with modifications at the linker and at the quinoline nucleus were synthesized at the Faculty of Chemistry, University of Belgrade. Lactate dehydrogenase assay, used for *in vitro* testing, was performed in a chloroquine (CQ) sensitive (3D7) and a CQ-resistant (Dd2) strain of *P. falciparum* with CQ as a control. *In vivo* activity was assayed in modified Thompson test, using C57BL/6 mice infected with *P. berghei* ANKA strain. Cure was defined as survival beyond day 31, with parasite clearance. The presence of residual parasitemia was examined by quantitative PCR (qPCR) of survivors' blood and liver tissues.

Results. Of the series of 11 compounds, even 10 (90.9%) had IC_{50} lower than CQ against Dd2 strain *in vitro* and thus qualified for further *in vivo* evaluation. Six compounds were available, and since none showed toxicity in preliminary experiments, subsequent

examination for efficacy showed that virtually all prolonged survival of treated vs. untreated mice (P<0.05). Most importantly, ClAQ8 (160 mg/kg/day) afforded cure of 100% mice, with qPCR confirmation of parasite clearance, while ClAQ9 and ClAQ13 both allowed survival of treated animals (1/4 and 1/5, respectively) beyond day 31, but with parasitemia. Interestingly, thiophene ClAQ11 and benzothiophenes (ClAQ7, ClAQ9) allowed mice to survive parasite burdens of up to 86%.

Conclusion. Better *in vitro* activity against a CQ-resistant strain, and the ability of some of the examined AQ to afford survival of infected mice, particularly of benzothiophene ClAQ8 which afforded cure, show significant antimalarial potential of this AQ series. In addition, mouse survival in the presence of huge levels of parasitemia, which may indicate compound impact on parasite pathogenicity/virulence, merits further investigation.

Prof.dr Mine Doluca Dereli, Izmir, Turkey

Mine Doluca Dereli, M.D., Ph.D. studied in Izmir "American Collegiate Institute" high school, where the curriculum was held in English. Prof. Doluca Dereli graduated from Ege University, Faculty of Medicine in 1987. She completed her Ph.D. education in Microbiology at Medical Microbiology Department of Dokuz Eylul University Health Sciences Institute in 1993. She worked as an associate professor in Dokuz Eylul University Faculty of Medicine, Department of Medical Microbiology between 1996 and 2002. She worked as a researcher on anaerobe bacteria and *Clostridium difficile* pathogenesis in Edinburgh University Medical Microbiology Anaerobe Reference Laboratory both in 1995 and 2000. She became a Microbiology Professor in Dokuz Eylul University Faculty of Medicine, Department of Medical Microbiology in 2002 and since then she has been working in the same position. She did research on molecular genetical analysis of antifungal resistance in pathogenic yeasts in Drexel University Faculty of Medicine Department of Microbiology and Immunology. She also worked as a researcher in Lousanne University Microbiology Institute on azole antifungal resistance in *Candida albicans* isolates in 2008. Her main research areas are virulence factors of *Candida* species, antifungal susceptibility testing and antifungal resistance mechanisms in *Candida* species. She is a member of both national and international Microbiology Societies. She has published nearly 135 articles and chapters both in national and international peer-reviewed journals and books.

PIM5 EMERGING FUNGAL INFECTIONS

M. Doluca Dereli

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Incidence of invasive fungal infections has been increased during the last decade due to the increased survival rate and time of critically ill patients or patients with impaired immune status. As a result of the widespread and increased use of antifungals, resistance especially "multidrug resistance" (MDR) in fungal pathogens has been reported and many novel resistance patterns have been observed in recent years.

Antifungal resistance originating from environmental sources in *Aspergillus* and *Candida* species and emergence of simulatenous resistance to many antifungal classes in *Candida* species has been alarmingly reported recently. Many reports suggested that intrinsic resistance might also be emerging as shown in *C. parapsilosis* and *C. tropicalis* isolates for fluconazole. Fluconazole and echinocandin resistance were documented to be increased from 9 to 14% and 4.9 to 12.3%, respectively in *C. glabrata* in some countries. Many reports of MDR in *C. auris* cases has been published. MDR had also been documented in *C. albicans, Candida lusitaniae* and *Yarrowia (Candida) lypolitica*. *C. haemulonii* complex isolates usually showed high MIC values for amphericin B and some strains showing echinocandin resistance have been reported. *C. guilliermondii* complex showed decreased susceptibility to azoles and echinocandins intrinsically which might cause problems in the treatment. *Pichia kudriavzevii* (*C. krusei*) and *Kluyveromyces marxianus* (*C. keyfr*) have been reported to develop MDR after exposure to antifungals. Low susceptibility to polyenes and azoles has been a concern for *C. rugosa*.

Azole resistance in *A. fumigatus* has also been reported to be reaching 6-27% depending on the geographical area probably due to the increasing prevalence of azole-resistant isolates in the environment. Acquired azole resistance in intrinsically amphotericin B resistant *A. terreus* and *A. flavus* species has been observed. Fluconazole resistance in *Cryptococcus neoformans* strains especially isolated from AIDS patients has been documented to be another important issue.

At the same time with antifungal resistance, many changes and shifts had been observed in the etiology and epidemiology of fungal infections. Additionally new pathogens are being isolated from the clinical specimens of the patients.

Candida auris has been reported to be an emerging MDR fungal pathogen causing nosocomial and invasive infections with high mortality rates. It was first isolated in 2009 from an external ear discharge of a patient in Japan. After this, three cases of nosocomial fungemia was reported which was followed by infections across five continents. Due to the emergence of *C. auris* worldwide and its resistance pattern, this fungi

became a great concern to public health agencies. Almost all *C. auris* isolates were resistant to fluconazole, up to 1/3 to amphotericin B, 7% to echinocandins. Other azoles showed variable activity against this microroganism. Forty one percent of the isolates were resistant to two antifugal classes while 4% to three classes.

Candida infanticola and *Candida spencermartinsiae* were reported to be possible emerging species in cancer patients. They were suceptible to new azoles however fluconazole and caspofungin had high MIC values.

Previously most aspergillosis cases were caused by *A. fumigatus* however the etiology of invasive mould infections have shifted towards non-*A. fumigatus Aspergillus* spp and other moulds especially Mucorales species, *Fusarium* spp., *Scedosporium* spp. were reported to become progressively more important. All Mucorales species are not affected by voriconazole and most are moderately resistant to echinocandins. *S. aurantiacum* was reported to be resistant to many azoles, amphotericin B and echinocandins and *S. prolificans* was a MDR microorganism and all antifungals exhibited high MIC values.

It can be concluded that new emerging fungi has been identified and antifungal resistance as well as MDR has been reported during the last decade which can have important influences on the patient therapy.

Ass.Prof.dr Gordana Mirchevska, Skopje, R.Macedonia

Dr Gordana Mirchevska has graduatedat the Faculty of Medicine, University "Sts. Cyril and Methodius", Skopje, Macedonia, in 1997, obtaining her medical degree with success (9.13). In September 1999, she has been employed at the Institute of Microbiology and parasitology in Skopje, Macedonia. She has been approved a specialization in medical microbiology and parasitology in 2001, that has been completed in 2004. She has received Master and PhD degree in medical mycology in 2011 and 2017, respectively. She has been elected as an Assistant in microbiology and parasitology in 2002, and re-elected in 2010. In December 2017, she has been elected as an ass.professor of microbiology and parasitology. She has participated in 3 projects in medical microbiology. She has also published numerous papers in national and international journals. She has participated at many scientific congresses, training courses and medical seminars in medical mycology. She has been awarded several awards and grants for attendance at scientific events. She has pursued her 3-month FEMS Fellowship in medical mycology at the Department of medical mycology, Institute of Medical microbiology, University Zurich, Switzerland, in 2006. In 2011, she has received her grant from the American Society of Microbiology to participate at the ASMCUE Conference for

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PIM6 EVALUATION OF METHODS FOR DIAGNOSIS OF INVASIVE CANDIDIASIS

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Introduction: *Candida* species are an increasing cause of invasive bloodstream infections, associated with high morbidity and mortality, in both neutropenic and non-neutropenic critically ill patients. The rapid detection of *Candida* in primarily sterile specimens is essential for prompt antifungal treatment and favorable clinical outcome.

Aim: To prospectively evaluate the diagnostic performance of serological (mannan and anti-mannan) methods in comparison with conventional methods (blood culture), for diagnosis of invasive infections caused by *Candida* species.

Material and methods: Blood and sera from 60 patients divided in 2 groups (immunocompromised and critically ill patients) were examined for diagnosis of invasive *Candida* infections. Blood was analyzed with conventional methods (blood culture with automated BactAlert system), and mannan antigen and anti-mannan antibodies were detected with ELISA test (Platelia *Candida* Ag-plus and Platelia *Candida* Ab-Plus (BioRad, France)).

Results: Positive blood culture was registered in 23.33% and 43.33% in the first and second group, respectively. A positive ELISA test for mannan antigen was detected in 60% and 30%, and for anti-mannan antibodies was positive in 31.82% and 35.71% in both groups, respectively.

Conclusion: Conventional and serological methods should be used in combination, for easier detection of invasive fungal disease caused by *Candida* species, especially in patients with primary immune deficiencies.

Key words: *Candida*, candidemia, blood culture, mannan, anti-mannan

PIM7 CLINICAL AND HOSPITALIZATION FEATURES OF CYSTIC ECHINOCOCCOSIS CASES IN KARAMAN, TURKEY

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Introduction: Cystic echinococcosis, an important parasitic disease with high treatment cost, caused by the larval form of *Echinococcus granulosus*, characterized by cysts in internal organs of intermediate hosts.

Objectives: It was aimed with this study to investigate the clinical and hospitalization features of cystic echinococcosis (CE) cases in Karaman province from 2010 to 2017 using data from the provincial state hospital.

Material and Methods: Hospitalization status of patients by gender, age group, year and residence and correlation between duration of hospitalization and age of hospitalized patients were investigated. Number (n), percent (%), mean ($\overline{X}$), standard deviation (SS), median (M) and interquartile ranges (IQR) were used for the analyzing of data.

Results: Over eight years, 60 patients with CE were hospitalized. Hospitalization rates were 16.3% in males and 10.4% in females. There was no statistically significant difference between the hospitalization times and gender, age and place of residence ($p > 0.05$). The mean and median hospitalization times of hospitalized patients were 7.3±3.99 and 7±4 days respectively. Highest hospitalization rate was found in the 0-20 age group and the lowest in 41-50. There was a statistically significant positive correlation between age of hospitalized patients and duration of hospitalization ($p < 0.05$). Also, hospitalization times of patients reside provincial center is significant lower than those of patients reside district centers ($p<0.05$). Hospitalization times in 2014 and 2015 were significantly lower than in 2012 and 2013 ($p < 0.05$). Of the patients 73.2% were admitted to general surgery, followed by internal diseases (8.7%), gastroenterology (7.5%), thoracic surgery (3.5%) and other clinics.

Conclusion: Cystic echinococcosis is thought to cause a significant burden on the economy and labor force as a result of hospitalization.

Key words: Cystic echinococcosis, hospitalization, Turkey.

PIM8 STATUS OF ANTIPARASITIC DRUG APPLICATION OF ANIMAL BREEDERS

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Introduction: Conscious antiparasitic medication in animals is important not only for the protection of animal health but also for the prevention of zoonotic diseases transmitted to humans from animals.

Objectives: In this study, it was aimed to evaluate the antiparasitic drug application status of the animal breeders in Dinar, Afyonkarahisar according to their education levels and professions.

Material and Methods: Data were collected by face-to-face interview method from 95 animal breeders in 2015-2016. Individuals were classified as 'primary school', 'secondary school' and 'high school and above' according to their education levels and 'those engaged only in animal breeding', 'those engaged in farming in addition to livestock' and 'those engaged in other professions in addition to livestock' according to their professions.

Results: The rate of those who did not apply antiparasitic drugs to dogs was 77.5%. While there was no significant difference in the application of antiparasitic drugs to dogs according to education level and professions ($p > 0.05$) a statistically significant difference was found between the expected and observed frequencies in terms of drug administration of all individuals ($p < 0.05$). The rate of those who applied antiparasitic drugs to cattle, sheep and goats was 61.1% and no significant difference was found according to education level and professions ($p > 0.05$) and between the expected and observed frequencies of all individuals ($p < 0.05$). Twenty-five percent of animal breeders reported that they used antiparasitic medication every 3 months, 37.5% every 6 months and 37.5% annually to their dogs and no statistically significant difference was found between expected and observed frequencies according to periodic frequencies ($p > 0.05$).

Conclusion: It has been determined that animal breeders are insufficient regarding to administering antiparasitic drugs to dogs which may lead to an increase in some zoonotic diseases in the region.

Key words: Antiparasitic drug, Animal breeder, Questionnaire.

PIM9 CLINICAL SIGNIFICANCE OF MOLECULAR DIAGNOSIS OF TOXOPLASMOSIS IN HSCT RECIPIENTS

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Introduction. Although generally mild and self-limiting in immunocompetent individuals, *T. gondii* infection may cause life-threatening disease in patients treated with haematopoietic stem cell transplantation (HSCT). Hence, prompt and precise laboratory diagnosis of toxoplasmosis is a prerequisite for timely introduction of specific treatment in these high-risk patients.

Objectives. To evaluate the clinical significance of molecular, qPCR-based methods in the diagnostic algorithm of reactivated toxoplasmosis (RT) following HSCT.

Material and Methods. During the 2012-2014 period, we have performed the pre-HSCT serological screening of 23 patients which allowed us to identify those seropositive (n=17) and to conduct further monitoring of RT by serology and qPCR. The qPCR protocol was based on detection of the *AF147529* sequence within the *T. gondii* 529bp-gene in peripheral blood (PB) samples, optimized in our laboratory to a detection limit of 1 parasite/ml of PB.

Results. RT was detected in three (17.6%) seropositive recipients, all of them receiving allogeneic stem cells from seronegative donors. Antiparasitic treatment was given to all three patients, of which two recovered, with clearance of parasitemia only a few days upon initiation of specific treatment. One patient was diagnosed late, at the time of associated bacterial infection, and despite energetic antimicrobial treatment, succumbed to multiorgan failure at day 39 post-HSCT. While the dynamics of specific IgM and IgG did not contribute to the diagnosis of RT (though specific IgG doubled within days after the first positive qPCR in one patient), molecular methods were able to show even miniscule parasitemia (6 parasites/ml in one patient) and were beneficial for the monitoring of treatment effect.

Conclusion. These first results of post-HSCT monitoring of RT which show that regular qPCR follow-up allows for precise and timely diagnosis of reactivation, providing clinicians with more options and time for therapeutic intervention, resulted in the introduction of weekly qPCR monitoring of RT into the routine management of HSCT patients ever since.

PIM10 PATHOGENIC YEASTS DETECTED OF FRUITS AND VEGETABLES ON SURFACE COLLECTED FROM ISTANBUL, PENDIK, TURKEY

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INTRODUCTION: Fruits and vegetables contain important vitamins and minerals, but surface on them may have pesticide residue. Fungicide is a specific type of pesticide that controls fungal disease. Fungicides are used in agriculture for inhibiting fungus growth on plants.The incorrect use of fungicides is dangerous for human health.

OBJECTIVES: Yeast is a fungus and needs a supply of energy for its living and growth. Sugar supplies this energy. Many yeasts are obtained from environmental samples containing high sugar. Example: apple, grape, etc. The purpose of this study is to determine the yeasts on collected from different supermarkets in Istanbul.

MATERIAL AND METHODS: This study was conducted in March 2018. From 50 samples including apples(29), pears(5), tomato(1), potato(2), soybean sprouts(2), lettuce(7), cucumber(3), and grape(1), only nine yeast strains could be isolated on Sabouraud Dextrose Agar (SDA). The strains were identified both by MALDI-TOF –MS (bioMérieux, France) and by API ID32C (bioMérieux, France).

RESULTS: A total of 50 samples taken from 5 different vegetable and 3 different fruit types. In 9 samples we isolated yeasts colonies, in 41 samples there was no growth. Among yeasts isolated there were *Cryptococcus albidus, Cryptocoocus laurentii, Candida membranifaciens, Rhodotorula mucilaginosa, Candida norvegica, Candida guilliermondii* and *Kleockera apiculata* species that known to cause disease in humans. (Table.1.)

VI Конгрес на микробиолозите на Македонија
со меѓународно учество

VI Congress of Macedonian Microbiologists
with international participation

СЕСИЈА 1/SESSION 1
БОЛНИЧКИ ИНФЕКЦИИ И НИВНА КОНТРОЛА/ HOSPITAL INFECTIONS AND CONTOL

HAI1 HEALTH CARE-ASSOCIATED INFECTIONS IN KOSOVA: CHALLENGES AND SOLUTIONS

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Healthcare-associated infections (HAIs) constitute an important public health problem in both developing and transitional countries and pose a major threat to patient safety. Despite considerable progress in the development of infection control programmes in some countries with limited resources, programmes in most developing world settings are either non-existent, poorly adapted or insufficiently funded by governments.

Lack of financial funds, inadequate infrastructure and management, improper use of antimicrobials, poorly functioning laboratory services and shortage of trained staff are key constraints for effective infection control in the hospitals of developing countries. As a consequence, these countries are facing the challenges of higher rates of healthcare-associated infections, frequent outbreaks, unsafe care and spread of infections in the community.

Kosova, as low-middle income country, is not an exception to this rule. Many challenges in the field of infection prevention and control lie ahead of Kosova. Kosova has only 80 € of Governmental expenditures per capita in health care and so far is without health insurance system.

A multicenter study of the prevalence rate was performed in all hospitals in Kosovo during 2016. The overall countrywide prevalence rate of HCAIs was 4.9%. The highest rate was noticed at the tertiary care level in the University Clinical Centre of Kosovo (UCCK) (7.2%). These rates are below the average infection rate in the EU, even though this is likely to be an underestimate. A possible explanation was the case mix and complexity of inpatients and the lack of diagnostic capabilities in small hospitals of the country. On the other hand, antimicrobial prescribing is very high in Kosovo hospitals, especially cephalosporins.

The best solutions for an effective infection control program entail introduction of prevention bundles, greater governmental commitment,

improvement of compliance with hand hygiene, surveillance, prudent use of antimicrobials, translation of research results into practice and upgrading the capabilities of microbiology laboratory. Infection control guidelines from developed countries are often perceived as a standard for the developing world, but have to be modified to take into account differences in local needs.

Focusing on infection control, countries with limited resources can improve the quality of healthcare in the future.

Key words: developing countries, infection control, Kosova

HAI2 DETECTION OF ENVIRONMENTAL SOURCES OF CLOSTRIDIUM DIFICILE IN ICU ON CLINIC OF NEUROSURGERY

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Introduction*: C. difficile* is very sensitive to oxygen even to low level in the environment, but it can persist in the environmeth as metabolically inactive spore. Laboratory manipulation of *C. difficile* requires a no oxygen contamination e.g.controlled anaerobic conditions and enriched nutrient media with reductive agents (Thioglycolate and L-cistine) and antibiotic mixture (cycloserine as inhibitor of Gram positive bacteria and cefoxitin to inhibit growth gram negative and Gram positive bacteria).

Aim of study: Recovery of *C.difficile* from hospital environment in from ICU on Clinic of neurosurgery unit since there have been detected patients with CDI or CD colonization confirmed previously.

Material and methods: Samples were colected with dried ryon swabs in ICU where CDAD were detected. 60 dried ryon swabs from patinet's beds, and 24 wet ryon swabs were used for sampling from patinet's hands. Swabs were inoculated by Edwards (1) in pre-reduced anaerobic selective media Thioglycolate broth (TB) (stored in anaerobic atmosphere for 24h before sampling), taped around the tube's lids and imidiatly put into previously prepared anaerobic jars. Incubation period was for 48h/ 37°C. Replacing one loop over selective CD selective media. Wet ryon swabbing was performed also on CD selective media priviosly prepared in anerobic conditions for two hours, taped around the lids and incubated in priviosly prepared anaerobic jars.

Resultes and discussion: 24,5% (N=50) samples colected with ryon swabs, from patient beds were positive on facultative anaerobic microorganisms: Klebsiella aerogenes, Enterococcus spp , MRSA and Candida albicans. Only 6,6 samples in TSB have presented germinative shapes of CD spores. None of patients han samples was positive for opportunistic microorganisms but normal skin flora.

Conclusion: Pre-reduced anaerobic selective media in combination with strictly controlled anaerobic conditions during sampling can be effective in detecting some spores of C. difficile in environmenthal samples as possibility to replace of anaerobic chamber if need to.

Key words: *Clostridium dificile*, environment, anaerobic cultivation

HAI3 NOSOCOMIAL INFECTIONS IN INTENSIVE CARE UNIT

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Nosocomial or healthcare acquired infection is a localised or systemic condition resulting from the presence of an infectious agent(s) or toxin(s). In majority of the cases there is no evidence that the infection was present or incubating at the time of admission in Intensive Care Unit - ICU. Infection is common in critically ill patients and often results due to the severity of the patient's illness. Nosocomial infections appear between 9.1% and 48.7% in critically ill patients. Central venous catheterisation, urinary catheterization, mechanical ventilation, stress ulcer prophylaxis and increasing length of ICU stay are significant risk factors for nosocomial infections in critically ill patients hospitalized in ICU. The most commonly observed nosocomial infections are respiratory in origin, following by urinary tract infections and dual infections. Ventilator-associated pneumonia (VAP) presents an indicator of quality of care and it is a frequent infection in the ICU, with a mean incidence exceeding 30%. Since ICU patients are frequently exposed to broad spectrum of antimicrobials, they are susceptible to infections by multidrug-resistant microorganisms like Pseudomonas, Acinetobacter and MRSA. Despite adequate antimicrobial treatment, nosocomial ICU infections can significantly affect ICU stay and cause an increase in the patient mortality. The aim of this study was to determine the prevalence of nosocomial infections in our ICU. The study also analysed common microorganisms associated with these infections, their antibiotic

sensitivity profile and the impact of these infections on ICU stay, patient mortality and the prevention of infection.

Keywords: nosocomial infection, critically ill patient, ventilator associated pneumonia

НОЗОКОМИЈАЛНИ ИНФЕКЦИИ ВО ЕДИНИЦИТЕ ЗА ИНТЕНЗИВНА НЕГА

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Нозокомијалните инфекции се локализирана или системска состојба која произлегува од присуството на инфективен агенс (и) или токсин (и). Во најголем број од случаите нема докази за присуство на инфекција или период на инкубација при приемот во Единиците за интензивна нега. Инфекциите се чести кај критично болните пациенти и се во корелација со тежината на болеста на пациентите. Нозокомијалните инфекции се јавуваат помеѓу 9,1% и 48,7% кај критично болните пациенти. Централна венска катетеризација, уринарна катетеризација, механичка вентилација, профилакса на стрес улкус и зголемување на должината на лекување во Единиците за интензивна нега се значајни фактори на ризик за развој на нозокомијални инфекции кај критично болни пациент

и хоспитализирани во Единиците за интензивна нега. Најчесто забележани нозокомијални инфекции се со потекло од респираторниот тракт, следни се инфекциите на уринарниот тракт како и комбинираните инфекции. Вентилатор-асоцирана пневмонија (VAP) претставува показател за квалитетот на лекување и е честа инфекција во Единици за интензивна нега, со просечна инциденца над 30%. Поради промените што се случуваат согласно критичната болест, како и изложеноста на различни антимикробни лекови од широк спектар пациентите во Единиците за интензивна нега често се подложни на инфекции од страна на микроорганизми кои се резистентни на повеќе лекови, како што се Pseudomonas, Acinetobacter и MRSA. И покрај адекватниот антимикробен третман, нозокомијалните инфекции во Единиците за интензивна нега можат значително да влијаат врз должината на болничкото лекување, како и врз морталитетот на пациентите. Целта на оваа студија е да се утврди преваленцата на нозокомијални инфекции во нашата Единица за интензивна нега. Во студијата, исто така, се

следат микробиолошките причинители поврзани со овие инфекции, нивниот антибиотски профил на сензитивност, влијанието на овие инфекции врз должината на болничкото лекување и морталитетот како и стратегијата за спречување на инфекциите.

Клучни зборови: нозокомијални инфекции, критично болни пациенти, вентилатор асоцирана пневмонија

HAI4 ЦЕНТРАЛНИ ВЕНСКИ КАТЕТЕРИ ЗА ХЕМОДИЈАЛИЗА И РИЗИКОТ ОД КАТЕТЕР ПОВРЗАНИ ИНФЕКЦИИ ПРЕДИЗВИКАНИ ОД ГРАМ ПОЗИТИВНИ КОКИ

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ВОВЕД

Централните венски катетери (ЦВК) често се користат како привремен или траен васкуларен пристап (ВП) кај пациентите на хемодијализа. Катетер-поврзаните инфекции (КПИ) се многу честа компликација која влијае на преживувањето, морбидитетот и морталитетот, како и честите хоспитализации кај пациентите на хемодијализа. Најчест предизвикувач на КПИ се Грам позитивните коки, од кои *Coagulasa-negative staphylococci* (CoNS) и *Staphylococcus aureus* се почести, а присутен е и *Enterococcus* но значително помалку. Пациентите на хемодијализа се особено изложени на колонизација но и инфекција со Methicillin-resistant *Staphylococcus aureus* (MRSA) и воопшто се изложени на зголемен ризик од бактериемија предизвикана од Грам-позитивни патогени. Антибиотскиот третман на Грам-позитивната бактериемија е голем предизвик, бидејќи зголемениот број на видови стануваат отпорни на антибиотици.

МАТЕРИЈАЛ И МЕТОДИ

На Клиниката за нефрологија во Скопје, беше направена ретроспективна анализа на 650 пациенти на ХД, кои имале вкупно 821 привремен феморален катетер (ФК) во период од 3 години. Феморалните катетери беа следени од поставувањето, до нивното вадење, а анализирани беа оние кои имаа КПИ предизвикани од Грам позитивни коки. Кај овие пациенти се анализираа и основната ренална болест(посебно со ДМ), акутна/хронична бубрежна болест, времетраењето на катетерите и антибиотската терапија, како и клиничките (покачена температура над 38Ц, треска..) и лабораториските знаци за инфекција (покачени Ле, ЦРП). При сомнение за КПИ (посебно во тек на ХД) беа земани хемокултури од ФК и периферна вена. КПИ според одредени критериуми беше

поделена на „дефинитивна„, „веројатна„ и „ можна„ инфекција. Антибиотската терапија беше дадена парентерално, емпириски при секое сомнение за постоење на КПИ, а по добивањето на антибиограмот беше адаптирана според него. Податоците беа обработени со дескриптивни статистички методи, АНОВА и др.

РЕЗУЛТАТИ И ДИСКУСИЈА

Од сите анализирани ФК, 136 имаа позитивен микробиолошки наод за Грам позитивни коки. ФК беа поделени во 3 групи според причината за започнување со ХД .Гр.1 – започнување со ХХП, Гр.2 – акутно бубрежно оштетување лекувано со ХД и Гр.3- пациенти на ХХП со проблеми со ВП. Вкупното времетраење на ФК беше 5997дена (просечно 44 дена). *Staphylococcus aureus* беше изолиран кај 63 ФК (29 од нив MRSA), CoNS кај 48 (12 од нив метицилин резистентни), *Enterococcus* кај 25 ФК. Само 58 пациенти имаа клинички знаци за инфекција (треска, покачена температура..). Најчесто користени антибиотици беа: Цефтриаксон, Ванкомицин и Ципрофлокацин. Сигнификантна статистичка разлика беше најдена меѓу групите за „дефинитивна„ КПИ инфекција. Независна варијабла за КПИ беа клиничките знаци за инфекција, високата леукоцитоза и ЦРП. Сигнификантна статистичка разлика беше најдена меѓу групите (АНОВА p=0,046) за времетраењето на ФК. Бројот на отстранетите ФК под сомнение за КПИ беше сличен со потврдените КПИ.

ЗАКЛУЧОК

Справувањето со КПИ предизвикани од Грам позитивни коки е од исклучително значење заради можноста за појавата на соеви на бактерии со помала осетливост кон антибиотиците (MRSA, *Coagulasa-negative staphylococci*- Methicillin-resistant, *Vancomycin-resistant enterococci -VRE*) особено ако се знае дека дел од нив нормално живеат на кожата. Сепак, во последните 30 години, тие се признати како еден од најчестите патогени одговорни за нозокомијална инфекција, со висока стапка на морталитет. Превенцијата на КПИ преку преземање на мерки за спречување на инфекциите е од посебно значење заради последиците кои би произлегле од честото давање на антибиотска терапија и појавата на ризик од антимикробна резистенција.

HAI5 MULTIRESISTANT STRAINS OF BACTERIAL ISOLATES IN STEM CELLS TRANSPLANT RECIPIENTS

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Background: Multidrug resistant (MDR) bacteria are increasingly frequent in HSCT recipients, but significant differences in etiology of bacterial infections and prevalence of resistant strains exist between different transplant centers Extended-spectrum beta-lactamase producing *Escherichia coli, Vancomicyn resistant enterococci, Penicillin-resistant Pneumococci Methicillin-resistant Staphylococcus aureus and* are major problem to manage.

Aim: to evaluate the frequency of multidrug resistant bacteria in our center. Our clinic is a part of CAESAR network for multidrug resistant bacterial isolates in Europa.

Material and method: during a 17 years period we have transplanted 440 patients with different hematological malignant and nonmalignant diseases. All patients were treated in sterile room conditioned with HEPA filters and low bacterial diet. Antibacterial prophylaxis consisted Ciprofloxacine 1,0gr/day. As empirical antibiotics regimen we administered combination of third-generation cephalosporin + amynoglicoside, while as second line therapy Imipenem/ Cilastatin+Vancomycin.

Results: in every patient 3 times a week we monitor: blood culture, central venous catheter culture, sputum, urine culture. From all 288 bacterial isolates the distribution of MDR bacteria were: ESBL positive Escherichia coli 3 (0,10%) VR Enterococcus 2(0,06%) MDR Pseudomonas aer. 1 (0,03%) Klebsiella aerogenes 2 (0,06%) MRSA 17(0,06%) Acinetobacter baumanii 2(0,06%) Stenotrophomonas Maltophilia 2(0,06%). There were fatal outcome due to sepsis in 3 patients (VRE 1, MDR Pseudomonas 1, Stenotrophomonas maltophilia 1).

Conclusion: the monitoring of local microflora is crucial for every transplant center. Empirical first-line therapy must be individualized and according to ECIL 4 escalation and de-escalation strategy is necessery for better outcome in this group of immunocompromised patients.

МУЛТИРЕЗИСТЕНТИ СОЕВИ НА БАКТЕРИИ КАЈ РЕЦИПИЕНТИ НА МАТИЧНИ ХЕМАТОПОЕТСКИ КЛЕТКИ

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Вовед- зголемена е изолацијата на мултирезистентните бактериски соеви кај реципиентите на матични хематопоетски клетки, иако значителни разлики постојат во видот и тежината на бактериските инфекции во различни трансплантациски центри. Извонредно тешко е да се третираат инфекциите предизвикани со соеви на Ешерихија коли која произведува проширен спектар на бета лактамази, Ванкомицин резистентни Ентерококи, Пеницилин резистентни Пнеумококи и Метицилин резистентен Стафилококус ауреус.

Цел на трудот- да се евалуира фреквенцијата на мултирезистентни бактерии во нашиот транспламтациски центар.

Материјал и методи-во периодот 2000-2017 година трансплантирани се вкупно 440 пациенти со различни малигни и немалигни хематолошки заболувања. Сите пациенти се кондиционирани во стерилни единици опремени со ХЕПА филтрација на воздухот и низко-бактериска диета. Антибактериската профилакса е спроведена су Ципрофлоксацин 1,0гр./дневно. Емпириската антибиотска терапија се состои од Трета генерација Цефалоспорин во комбинација со Аминогликозид. Второлинискатa терапија е Карбапенем со Ванкомицин. Кај секој пациент три пати неделно е мониторирана локалната микрофлора со хемокултури, култура од централен венски катетер, уринокултура и спутум. Од сите 288 бактериски изолати- дистрибуцијата на мултирезистентни бактерии е следна- ESBL positive Escherichia coli 3 (0,10%) VR Enterococcus 2(0,06%) MDR Pseudomonas aer. 1 (0,03%) Klebsiella aerogenes 2 (0,06%) MRSA 17(0,06%) Acinetobacter baumanii 2(0,06%) Stenotrophomonas Maltophilia 2(0,06%). Фатален исход поради бактериска сепса е присутен кај 3 пациенти (VRE 1, MDR Pseudomonas 1, Stenotrophomonas maltophilia 1).

Заклучок- мониторирањето на локалната микрофлора е мандаторна за секој трансплантациски центар. Терапијата треба да биде индивидуализирана во согласност со препораките на ЕЦИЛ 4.

HAI6 MICROBIOLOGY OF CARDIAC IMPLANTABLE ELECTRONIC DEVICE INFECTIONS

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Introduction: Cardiac implantable electronic device (CIED) infection is rare, but potentially dangerous complication. Literature data shows mortality of 30-35% in CIED infections and 20-25% in valve endocarditis.

Objectives: The aim of the study was to describe a tertiary care center experience with the microbiology of CIED infections. We described clinical characteristics, type of microorganism, complications and outcome of CIED infection in ten patients.

Material and Methods: In our hospital between January 2006 and November 2017 CIED infection was identified in ten patients, 6 male and 4 female, age 24-80 years, mean age 62.1 years. Criteria for CIED infections are related to clinical findings, positive blood cultures with causative microorganism or echocardiographic evidence of vegetation associated with transvenous leads or right-sided heart valves.

Results: Clinical features of patients are fever in 10, heart failure in 2, pulmonary embolism in 1, and chills in 3 patients. Echocardiography demonstrated lead vegetations in 8 patients, 2 patients had concomitant tricuspid valve vegetations. All patients presented with recurrent fever and were treated as outclinic patients before addmittance to our hospital. Mean interval between device implantation and first findings of infection was 3.3 years. Blood culture was positive in 9 patients (90%). Isolated causative organisms in blood cultures and extracted leads are: staphylococci in 4 (40%), of which 2 were MRSA, streptococci in 2 (20%), *Pseudomonas aeruginosa* 1 (10%), *Corinebacterium* spec. 1 (10%), and *Enterococcus* in 1 patient (10%). Treatment with a combination of antibiotics and surgical lead extraction was performed in 7 pateints while treatment with placement of new epicardial leads and LVAD explantation was performed in 2 patients. Concomitant valve surgery was performed in two patients.

Conclusion: *Staphyloccocus aureus* and *S. epidermidis* were the most common causative organisms in CIED infections. Twenty percent of CIED infections involved methicillin-resistant staphylococci which are more likely to be acquired in health care environments.

HAI7 OCCURRENCE OF CATHETER-ASSOCIATED URINARY TRACT INFECTION OF TRAUMA PATIENTS IN ICU

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Introduction:
Urinary Tract Infection is the most common hospital- acquired infection. About 80% of nosocomial UTIs are related to urethral catheterization and are acquired after 48-72 hours of hospital admission.

Objectives:
To identify microbial pathogens associated with urinary tract infection (UTI) in catheterized patients from Intensive Care Units (ICU) and to determine the susceptibility pattern of these isolates to antimicrobial agents.

Materials and Methods:
Consecutive urine samples of 234 catheterized trauma patients admitted in ICUs were included in the study. They were processed by standard microbiological procedures.,in blood agar and McConkey, then incubated for 24 hours and proceeded with biochemical tes.t Isolated organisms were speciated, and antibiotic susceptibility performed as per standard guidelines.

Results:
From the 234 urine samples of catheterized patients, 173 showed no growth and were found to be sterile. Bacterial growth was seen in 38 patients and fungal growth in 23 patients. Among the bacterial isolates, Enterobacter spp. were isolated in 13,11% of cases,Pseudomonas aeruginosa was isolated in 18,03% , Acinetobacter spp. were isolated in 8,19% , Klebsiella pneumonia was isolated in 4,91%, Staphylococcus aureus in 3,28%, Enterococcus spp.in 13,11%, E*scherichia coli* were isolated in1,64%, of cases. *Candida albicans* was the most common isolate,with 37,7% of cases. Resistance rates were relatively high: ampicillin 73,9%; gentamicin 70,83%; co-trimoxazole 70%;nitrofurantoin 75,8%; imipenem 57,89%; ceftazidime 81,25%; ciprofloxacine 67,64%; norfloxacine 86,2%; chloramphenicol 67,64%.

Conclusion:
Development of CAUTI is frequent in trauma patients Most episodes of short-term catheter-associated bacteriuria are asymptomatic and are caused by single organisms, while long-term catheterisation promotes multibacterial infections and colonization. Chronic indwelling catheters are an important reservoir of different multiresistant gram-negative organisms, therefore they are frequently isolated from CAUTIs. Emphasis should be placed on good catheter management and reducing the duration of catheterization

rather than prophylaxis in order to reduce the incidence of catheter-related UTI. Culture and susceptibility testing play a vital role in the management if UTI occurs.

Keywords: multiresistant gram-negative organisms, catheter, Intensive Care Unit, urinary tract infection,antibiotic resistance.

HAI8 INFECTION AFTER ANTERIOR CRUCIATE LIGAMENT RECONSTRUCTION

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BACKGROUND: Infection is a rare but potentially devastating complication after anterior cruciate ligament (ACL) reconstruction.

PURPOSE: To report the incidence of infections after ACL reconstruction and to describe the treatment of this complication.

METHODS: All primary ACL reconstructions performed in our institution between January 2010 and January 2018 were included in this study. We made retrospective analysis including time from initial surgery, clinical symptoms and culture results of all infected patients. Local debridement, screw removal, curettage and irrigation of the infected part was performed immediately after a diagnosis of infection was made. Postoperative intravenous antibiotics were used for at least 21 days (range, 21-31 days). Patients were evaluated with Lysholm and Tegner activity knee score and measurements with Rolimeter and Gonimeter two months later.

RESULTS: Infection occurred in 3 of 480 patients (0.62%). In all patients the graft harvesting site and tibia tunnel were affected. In two cases infection occurred 4 weeks after the initial surgery (range, 3-5 weeks) and in one case it occurred after 7 days from the initial surgery. Local debridement, screw removal, curettage and irrigation of the infected part was performed only in the first two patients. At the final follow-up all patients had full range of motion, side to side difference of anterior tibia displacement less than 3 mm, the mean Lysholm score was 92 and the mean Tegner score was 6.

CONCLUSION: Infection after ACL reconstruction mostly occurs on the graft harvesting site and in the tibia tunnel. Fast and adequate treatment can provide good and excellent results in this situation.

Keywords: ACL reconstruction, Postoperative infection, Treatment, Clinical results.

ИНФЕКЦИИ ПО РЕКОНСТРУКЦИЈА НА ПРЕДЕН ВКРСТЕН ЛИГАМЕНТ НА КОЛЕНО

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ВОВЕД: Инфекцијата претставува ретка но катастрофална компликација по реконструкција на преден крстен лигамент (ПВЛ) на колено.

ЦЕЛ: Целта на студијата беше да се презентира инциденцата на инфекции по реконструкција на ПВЛ на колено и да се опише третманот на оваа постоперативна комликација.

МЕТОДИ: Во студијата беа вклучени сите пациенти со реконструкција на ПВЛ на колено оперирани во периодот од јануари 2010 до јануари 2018 година. Кај сите нив беше направена ретроспективна анализа која го вклучуваше периодот од операција до појава на инфекција, клиничките резултати како и микробиолошкиот наод на земениот матерјал.Локален дебридман, отстранување на шрафот, киретажа и испирање на инфицираниот дел беше спроведено веднаш по дијагностицирањето на инфекција. Кај сите пациенти беше ординиран интравенски антибиотски третман во траење од најмалку 21 ден (21-31 ден). Кај сите пациенти по 2 месеци од лекувањето беше спроведна клиничка евалуација на функционалноста на зафатеното колено со користење на прашалниците по Lysholm и Tegner и мерење со ролиметер и гониметер.

РЕЗУЛТАТИ: Инфекција по реконструкција на ПВЛ се појави кај 3 од 480 пациенти (0.62%). Кај сите пациенти инфекцијата беше локализирана во тибијалниот коскен тунел и на местото од каде се вадеа тетивите за графтот. Кај 2 пациенти инфекцијата се појави по 4 недели од оперативната интервенција (3-5 недели) , а кај еден пациент инфекцијата се појави по 7 дена од оперативната интервенција. Локален дебридман, отстранување на шрафот, киретажа и испирање на инфицираниот дел беше спроведено само кај првите два пациенти. На крајната контола сите три пациенти имаа полн опсег на движења во коленото, разлика во предната тибијална транслација меѓу двете колена помала од 3мм, 92 поени од Lysholm прашалникот и 6 поени од Tegner прашалникот.

ЗАКЛУЧОК: Инфекцијата по реконструкција на ПВЛ најчесто е локализирана во тибијалниот коскен тунел и на местото од каде се вадат тетивите за графтот. Брзиот и адекватен третман обезбедува добри резултати по појава на оваа постоперативна комликација.

КЛУЧНИ ЗБОРОВИ: Реконструкција на ПВЛ на колено, Постоперативна инфекција, Третман, Клинички резултати.

HAI9 УРИНАРНИ ИНФЕКЦИИ ПРЕДИЗВИКАНИ ОД ИНТРАХОСПИТАЛНИ СОЕВИ НА БАКТЕРИИ НА КЛИНИКАТА ЗА НЕФРОЛОГИЈА ВО СКОПЈЕ

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ВОВЕД

Уринарните бактериски инфекции (УИ) се меѓу најчестите инфекции предизвикани од интрахоспиталните соеви на бактериите. Многу студии покажуваат дека дека на нив отпаѓа 23-49% од интрахоспиталните инфекции. Најчести предизвикувачи се Грам негативните бактерии и тие значително придонесуваат за зголемување на морбидитетот, морталитетот и трошоците на лекувањето. Како фактори на ризик за настанување на УИ се вбројува женскиот пол, уролошки интервенции, други присутни инфекции, дијабетес мелитус, Хронична бубрежна болест/ хемодијализа, антимикробна терапија и др.

МАТЕРИЈАЛ И МЕТОДИ

На Клиниката се нефрологија во Скопје се направи ретроспективна студија каде се анализираа позитивни уринокултури за интрахоспитални соеви на бактерии кај пациенти кои беа хоспитализирани на клиниката од различни причини, во период од 2 години. Во студијата беа анализирани вкупно 84 пац (45 жени и 39 мажи, просечна возраст 55г). Анализирани податоци: основна болест (посебно ДМ и карциноми) и коморбидитети, пол, возраст, клинички и лабораториски знаци за инфекција, уринокултури, уролошки интервенции, дадените антибиотици и антимикробната резистенција кај дел од нив.

РЕЗУЛТАТИ И ДИСКУСИЈА

Кај најголем број од пациентите имаше само еден причинител на инфекцијата (90,7%), додека кај 9,3% од болните имаше два причинителa на УИ. Покачени вредности за Ле и ЦРП, како и фебрилност беше регистирано кај поголемиот дел од пациентите (80%). Уросепса беше регистрирана кај 7 пациенти. Дијабетес мелитус имаа 16 пациенти (19%); пациенти со трансплантиран бубрег (15 пац -17,8%) кај кои и кога немаше позитивна уринокултура се даваа антибиотици заради имунолошката супримираност; опструктивна нефропатија и суспектен/потврден малигни процес (дел од пациентите беа со JJ сонди) имаа 12 пац (16,6%). Најчести предизвикувачи: *Escherichia coli* (39,1%,најголемиот дел ЕСБЛ+), *Klebsiella spp* (18,7%, дел од нив ЕСБЛ+), *Pseudomonas aeruginosa* (12,5%),а многу помалку *Proteus mirabilis, Enterococcus faecalis* и др. Најголемиот дел од инфекциите беа лекувани со Имипенем/ Меропенем, Тазобактам или Колистин (според антибиограм).

Добиени беа неколку резултати каде постоеше осетливост само на еден антибиотик. Ванкомицин Резистентен Ентерокок беше регистриран во 2 случаи и беше лекуван со Линезолид.

ЗАКЛУЧОК

Испитуваните пациенти во голем дел имаа ризик фактори на кои не можеше да се влијае (основна болест, коморбидитети, возраст..) но постојат и фактори на кои може да се влијае. Пред се тоа е спроведување на сите мерки за превенција од интрахоспитални инфекции – рационално поставување на уринарен катетер, уролошки интервенции и рационално давање на антибиотска терапија.

HAI10 EPIDEMIOLOGICAL SURVEILLANCE OF HOSPITAL HYGIENE AT THE CLINICAL HOSPITAL BITOLA IN 2017

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INTRODUCTION

Hospital acquired infections are considered as major causes of mortality, emotional stress and enhanced morbidity in hospitalized patients.These also account for significant economic loss and additional burden on health care institutions.The environment in the hospital plays an important role in the occurrence of hospital acquired infection.

OBJECTIVES

The objective of this study was to present the results of the epidemiological surveillance on the hospital hygiene in the Clinical Hospital Bitola in 2017.

MATERIALS AND METHODS

Descriptive analysis of the results of swabs and air samples, taken for the period from January 1, 2017 to December 31, 2017 in the Clinic hospital in Bitola by the Epidemiological department of Centar for public health Bitola. Swabs were taken in various wards and intensive care units (High risk areas). Air samples were taken from the operating rooms with a sedimentation method or a volumetric air sampler.

RESULTS

Out of 853 swabs taken from the objects for general use, 19 (2,23%) were positive (department for orthopedics, department for pediatrics, department for eye diseases, department for surgery, neonatology, department for anesthesiology and reanimation with intensive treatment, the department for urology, internal diseases and the department for gynecology with obstetrics). Out of 162 swabs

taken from sterile material, 1 (0.62%) was positive (department for orthopedics). Out of 241 air samples taken, 2 (0.83%) were positive (from the department for orthopedics and from the department for anesthesiology and reanimation with intensive treatment). The most common microbiological isolates were molds, Micrococcus luteus, Acinetobacter baumannii and coagulase-negative staphylococci.

CONCLUSION

Epidemiological surveillance has a esencial importance for effective infection control programs in health care organizations.

ЕПИДЕМИОЛОШКИ НАДЗОР НА ХИГИЕНА ВО БОЛНИЧКА СРЕДИНА ВО КЛИНИЧКА БОЛНИЦА БИТОЛА ВО 2017

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ВОВЕД

Болнички стекнатите инфекции се сметаат како една од водечките причини за морталитет и морбидитет кај хоспитализираните пациенти. Тие исто така претставуваат причина за големи економски загуби на здравствените институции пред се поради продолжување на времетраењето на хоспитализацијата и трошоците за лекување. Хигиената во болничката средина има важна улога за преносот и одржување на микроорганизмите кои се причинители на болнички стекнатите инфекции.

ЦЕЛ

Целта на овој труд беше да се прикажат резултатите од спроведените епидемиолошки увиди за чистота на болничка средина во Клиничка болница Битола во 2017 година.

МАТЕРИЈАЛ И МЕТОДИ

Дескриптивна студија на микробиолошките наоди на брисеви од предмети за општа употреба, стерилен материјал и примероци на воздух во Клиничка болница Битола во периодот од 01.01.2017-31.12.2017 земени од страна на епидемиолошката служба од Центар за јавно здравје Битола. Брисевите беа земени од различни оддели, а примероците на воздух беа земени од операциони сали со седиментациона метода или волуметарски семплер.

РЕЗУЛТАТИ

Од вкупно земените 853 брисеви од предмети за општа употреба, 19 (2,23%) беа со позитивен микробиолошки наод (од одделението за ортопедија, за детски заболувања, за очни заболувања, за хируршки заболувања, за неонатологија, службата за анестезиологија и реанимација со интензивно лекување, одделението за уролошки

заболувања, за интерни заболувања и одделението за гинекологија со акушерство). Од вкупно земените 162 брисевите од стерилен материјал, 1 (0,62%) беше со позитивен микробиолошки наод (од одделението за ортопедија). Од вкупно земените 241 примерок на воздух, 2 (0,83%) беа со позитивен микробиолошки наод (од одделението за ортопедија и од службата за анестезиологија и реанимација со интензивно лекување). Најчесто се изолирани мувли, Micrococcus luteus, Acinetobacter baumannii, Staphylococcus koagulaza negativen.

ЗАКЛУЧОК

Континуираните епидемиолошки увиди кои се спроведуваат во здравствените установи се од големо значење. Преку нив се дава и стручно – методолошка помош, која има есенцијално значење за контрола на чистота на болничка средина, а со тоа и спречување на болнички стекнатите инфекции.

HAI11 THE BRISTOL STOOL SCALE AND ITS RELATIONSHIP TO *CLOSTRIDIUM DIFFICILE* TESTING RESULTS IN MEDICAL AND SURGICAL DEPARTMENTS

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Introduction. We conducted a prospective study of all stool specimens collected for *C. difficile* testing from adult inpatients at the Clinical Center of Serbia, a tertiary-care academic hospital, from April 2013 through December 2013.

Objectives. When *C. difficile* infection is suspected, unformed stool specimens should be tested on presence of the microorganisms and its toxins. We aimed to estimate how stringently surgical and medical departments comply with this rule and to examine the ratio of stools of inadequate consistency that have been sent for testing.

Materials and Methods. We accepted all stools for testing although the recommendation is to test unformed stools only. To evaluate the consistency of stools, we used Bristol stool scale, which is graded visual scale of stool density. Semiformed stools are defined as those with Bristol scores 5 or 6; liquid stools are defined as those with a Bristol score of 7. Our *C. difficile* testing algorithm includes culture on

selective CLO agar plates and rapid tests for toxins' detection.

Results. During the study 3698 specimens were tested for *C. difficile*. Of 750 stools that tested positive on this microorganism 29.87% were scored by less than 5 on Bristol stool scale, 36% were evaluated 5 and 6 and 34.13% were scored 7. Generally, unformed (semiformed and liquid) stools prevailed (70.13%), and they came from surgical departments more often, but there was no statistical difference (p=0.43) between medical and surgical departments in terms of consistency of stools sent for testing that tested positive.

Conclusions. Considering the fact that unformed stools are the right specimens for testing on *C. difficile* infection, surgical departments followed that rule more stringently than medical ones, but the difference between them was not statistically significant.

HAI12 PREVALENCE OF *STAPHYLOCOCCUS AUREUS* IN HOSPITALIZED PATIENTS IN TIRANA

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Introduction: *Staphylococcus aureus* is a significant pathogen in human medicine. Common pathogen Methicillin-resistant *S. aureus* (MRSA) in a different group of patients; causes different clinical syndromes and different antimicrobial susceptibility patterns. The study aim is to evaluate the prevalence of *S. aureus* and MRSA in Clinical specimens hospitalized to "Mother Theresa" Hospital Center.

Method: About 356 clinical specimens were collected by patients to diagnose the infection in wound, pus/exudates, blood, urine, sputum and indwelling medical devices during 2 years. We isolated and identified *S. aureus* by using standard tests, Also for further accurate microbial identification we have use the VITEK® 2 system. The samples were tested to detect the presence of MRSA by a slide latex agglutination kit for the rapid detection of PBP2.

Results: The overall prevalence of *S. aureus* in patients was 34% (121/356). Out of all 93 cases isolated with *S. aureus*, 29 (31.2%) from urine infections; 26 (27.9%) from skin and soft-tissue infections cases; 15 (16.2%) from vaginal and urethral swab; 13 (14%) from nasal and ear swab cases and 10 (10.75%) from blood stream and catheter-associated infections. The prevalence of methicillin-resistant S. aureus (MRSA) was 20.6% (25/121) cases. Of the MRSA isolates

identified in this study 6 (24%) were susceptible to antibiotics 10 (40%) demonstrated intermediate resistance and 9 (36%) were multi-drug resistant with resistance to six classes of antibiotics

Conclusions: The rate of *S. aureus* in hospitalized patients on this study was 34% and the MRSA 20.6%. These results indicated that this type of infection is a significant concern for health services and patients included. The highest percentage of *S. aureus* found in surgical and non surgical wound suggest that further investigation should be implemented. A screening of all hospitalized cases can lead to reduce the incidence of this infection in the hospital environment and also to control the risk factors.

Keyword: *S. aureus*; urine infections, skin and soft-tissue infections, nasal and ear swab nasal infections, Hospitalized patients

HAI13 COMPLIANCE OF HEALTHCARE WORKERS TO HAND HYGIENE PRACTICES IN NEONATAL INTENSIVE CARE UNITS: OVERT OBSERVATION

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BACKGROUNDS
Hand hygiene (HH) has been identified as the single most important factor in minimising hospital acquired infections. However, compliance to handwashing guidelines has remained low.

The objective of this study was to assess the compliance of hand hygiene of healthcare workers (HCWs) in the neonatal intensive care unit in a tertiary university hospital in Istanbul.

METHODS.
Observational HH data were collected during a month, day and night 1-hour observations period in Istanbul, Marmara University, Pendik Training and Research Hospital, Neonatal Intensive Care Unit, from 5 March to 30 March 2018. HH compliance of HCWs for "My Five Moments for Hand Hygiene" (MMH) of the World Health Organization (WHO) was recorded.

The total bed capacity was 15, the number of sinks was 4, the number of alcohol-based disinfectants was 15, and the number of active health workers was 36.

Among these HCWs 20 nurses (56%), 8 doctors (22%) and 8 health

personels (22%) and 34 (94%) were females(94%), while 2(6%) were males. All nurses and health personels were female.

RESULTS

A total of 739 HH opportunities were collected during the study period. Overall HH compliance was 51,2 % (378/739) . Compliance differed by role was as follows: nurses 52 %, health personnel 50% and doctors 42 %. The compliance and the technique of hand hygiene are shown in the table.1

28% of the male and 8% of the female doctors washed their hands with soap and water and 14% of the male and 32% of the female doctors used alcohol-based disinfectant .

21% of the nurses washed their hands and 31% used alcohol-based disinfectant . Health personnel preferred only hand washing for disinfection.

CONCLUSION

The hand hygiene compliance was found to be low. Effective training programs should be applied to improve hand hygiene compliance.

Table.1. The compliance and the technique of hand hygiene

	NURSES		DOCTOR		HEALTH PERS.		*TOTAL*	
	Hand washing with soap and water	Hand hygiene with disinfectant	Hand washing with soap and water	Hand hygiene with disinfectant	Hand washing with soap and water	Hand hygiene with disinfectant	Hand washing with soap and water	Hand hygiene with disinfectant
Prior to patient contact	11,70%	42,30%	9,10%	45,50%	10%	50%	11%	42%
Prior to a clean or aseptic procedure	26,40%	26,40%	50%	0	0	0	27,70%	25%
After contact with body fluid	21,40%	42,80%	0%	0	0	0	20%	40%
After patient contact	37,80%	32,90%	25%	37,50%	16,60%	33,30%	37%	27%
After contact with the patient environment	15,80%	26,70%	4,80%	23,80%	5%	25%	14,50%	27%
TOTAL	%20.5	32,90%	11,60%	30,30%	8,20%	32,40%	19,60%	31,50%

HAI14 CANDIDA COLONIZATION IN PATIENTS WITH RISK FACTOR FOR INVASIVE FUNGAL INFECTIONS IN THE ICU AND ITS CONNECTION TO CANDIDEMIA

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The aim of the study was to determine colonization by Candida in patients at risk for developing invasive fungal infections and its connection with Candidemia.

Material and methods: In the study we selected 264 patients with risk factor for invasive fungal infections. All patients had used antibiotics 264 (100). 203 (76.9%) patients were found with central venous catheter, 42 (15.9%) patients were with diabetes, 40 (15.2%) patients were with cancer, 33 (12.5%) patients had surgical interventions and 92 (34.8%) patients were with parenteral nutrition.

Results: It is noted that colonization was identified in 92 (45.3%) of 203 samples taken from the central venous catheter; to 156 (59.1%) of 264 patients who used antibiotics; to 34 (81%) out of 42 patients with diabetes; to 17 (42.5%) out of 40 patients with cancer; to 6 (18.2%) out of 33 patients with surgical interventions; to 73 (79.3%) out of 92 patients with parenteral nutrition. Prevalence of colonization on samples taken by diabetic patients was statistically significant with other types of samples ($x2 = 63.5$, $p < 0.01$). Candidemia was identified in 39 (14.8%) patients (95% CI 10.7 - 19.7). We observed a significant relationship of Colonization with Candidemia in the patients that used central venous catheter ($x2 = 63.5$, $p < 0.01$), diabetes ($x2 = 4.1$, $p = 0.04$) and parenteral nutrition ($x2 = 4.4$, $p = 0.03$).

Conclusion: Candida colonization is associated with risk factors for invasive fungal infections and Candidemia. Thus, this means that candida colonization may be used as a helpful parameter to prevent invasive Candida infections.

Keywords: Colonization, Candidemia, parenteral nutrition.

HAI15 ANTIMICROBIAL RESISTANCE PATTERN OF PSEUDOMONAS AERUGINOSA IN A TERTIARY CARE HOSPITAL IN KOSOVO

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Background
Pseudomonas aeruginosa is one of the most relevant pathogens causing nosocomial infections and standard antibiotic regimes are increasingly becoming ineffective due to the rise in drug resistance.

Aim
The aim of this study was to evaluate the antimicrobial resistance pattern of *Pseudomonas aeruginosa* isolated in a tertiary care hospital in Kosovo.

Materials and Methods
The study was conducted at Department of Microbiology within the National Institute of Public Health of Kosovo from January 2015 to December 2017. The clinical samples were processed by standard procedures. Antimicrobial susceptibility was performed by the disk – diffusion method according to EUCAST. Colistin E-test was performed only for multidrug resistance isolates.

Results
There were a total of 5216 samples presenting significant growth, of which 3706 (71%) were Gram negative bacilli. *P.aeruginosa* was the second most frequently isolated pathogen in 607 samples (16.3%). Majority of the samples were tracheostomy swabs 219 (36%), followed by wounds 135 (22.2%) and endotracheal tubes 105 (17.2%). *P. aeruginosa* was predominant pathogen from ICU with 302 samples (49.7%). In tracheostomy tubes resistance rates to gentamicin, ciprofloxacin and piperacillin-tazobactam were 75.7%,64.8% and 26.9% respectively. In endotracheal tubes resistance rates for gentamicin and piperacillin-tazobactam were 54.2% and 27.6%. For blood culture highest resistance was for meropenem 25 (58.1%), gentamicin 25 (58.1%) and imipenem 24 (55.8%). Neonatology unit and ICU displayed highest resistance rate towards carbapenems 55%. Isolates tested with colistin E-test displayed resistance as follows: blood culture 3 patients (6.9%), tracheostomy tubes 2 (0.91%) and endotracheal tubes 1 (0.95%).

Conclusion
The results showed high resistance rates towards carbapenems in both Neonatal and adult ICU. Differences in resistance rate

corresponded to prescription practices and antibiotic use in different units. Inappropiate use of antibiotics is responsible for emergence and spread of resistance among *P. aeruginosa* strains.

HAI16 NEONATAL SEPSIS - OUR EXPERIENCE IN NEONATAL INTENSIVE CARE UNIT

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Background:
Neonatal sepsis remains a serious complication, especially among preterm infants. Neonatal sepsis is divided into early- and late-onset sepsis, based on timing of infection and presumed mode of transmission. Early - onset sepsis is defined by onset in the first week of life, to infections occuring in the first 72 hours due to maternal intrapartum transmission of invasive organisms. Late - onset sepsis is defined as infection occurring after one week and is attributed to pathogens acquired postnatally.

Materials and Methods:
We have investigated neonatal sepsis in our ICU from 1 January till 31 December 2017 a one-year period, to determine mortality associated with sepsis and identify the dependent predictors for morbidity and mortality. A total 216 infants were admitted in the ICU. Data base were collected regarding, primary reason for ICU admission and infection, infecting agent, and length of ICU stay.

Results: In 31 infants (14,3 %) was detected early - onset sepsis. In 2 infants (0,9%) was detected late - onset sepsis after one week. Premature infants 19 (61 % ; range 27- 37 gestational age) were more exposed to sepsis than term infants 12 (39 % ; range 38-40 gestational age) . The most frequent isolates were Staphylococcus coagulase negative 16 (52 %), followed by Acinetobacter 6 (19 %), Seracia 6 (19 %) and Pseudomonas aureginosa 3 (10 %). Late - onset sepsis was significantly more common in premature infants.We confirmed that late - onset sepsis resulted with increase in duration of ICU stay and duration of antibiotic treatment. Early diagnosis, followed by appropriate antibiotic treatment, short hospital stay and restricted use of invasive devices should be the aims to reduce the risk of late - onset sepsis during the stay in the ICU.
Conclusion: Neonatal sepsis is a major cause of death in infants despite sophisticated neonatal intensive care. Early and adequate antibiotic therapy decrease the risk of morbidity of hospitalized patients.

Keywords: sepsis, ICU, morbidity, antibiotic treatment, risk factor

СЕСИЈА 2/SESSION 2 МЕДИЦИНСКА БАКТЕРИОЛОГИЈА/ MEDICAL BACTERIOLOGY

MB1 THE INFLUENCE OF SURFACTANTS ON THE BIOFILM PRODUCTION OF ACINETOBACTER BAUMANII CLINICAL ISOLATES

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Acinetobacter baumannii is one of the most notorious bacterial pathogen in hospital environment. Its clinical significance, especially over the last 15 years, has been propelled by its antibiotic resistance, literally to all known antibiotics, making this bacterium a superbug. Possible explanation for intrahospital infection is that an epidemic strain is most commonly introduced by a colonized patient. Once on a ward, the strain can then spread to other patients and their environment. *A. baumannii* can survive in dry conditions and during outbreaks has been recovered from various sites in the patients' environment, including bed curtains, furniture and hospital equipment.

There are three major factors possibly contributing to the *A. baumannii* persistence in the hospital environment, i.e., resistance to major antimicrobial drugs, resistance to desiccation, and resistance to disinfectants.

Besides bacterial intrinsically resistance to a number of commonly used antibiotics (including aminopenicillins, first- and second generation cephalosporins and chloramphenicol), acquired resistance mechanism to all known antibiotics are abundant and diverse (enzymatic cleavage, multidrug efflux pump, modification of target binding site or reduced drug influx).

Resistance to desiccation and disinfectants is probably consequence of biofilm production on live and artificial substrates, in nature, humans (in tissue or in medical devices) or nonliving hospital equipment. A structured consortium attached on a living or inert surface formed by microbial cells sticked to each other and surrounded by the self-produced extracellular polymeric matrix is known as biofilm. The formation of biofilm is considered an adaptation of microbes to hostile environments. Aggressive and intensive antibiotic treatment is usually helpful to control the exacerbations

of chronic biofilm infections induced by dispersed bacteria and reduce the biofilms, but can not eradicate the biofilm infections, because the minimal concentration of antibiotic for eradication of mature biofilm is difficult to reach in vivo. Therefore, once a bacterial biofilm infection established, it becomes difficult to eradicate.

Several authors concluded that resistance to currently used disinfectants is probably not a major factor favoring the epidemic spread of *A. baumannii*, since all disinfectants inhibited growth of all *A. baumannii* isolates when concentrations and contact times recommended by the respective manufacturer were used. Our experiments also suggest these findings, with emphasis on antibiofilm effect. We performed experiments with 70% ethanol and hypochlorite solution on biofilm formed in microtiter plates and on urinary catheters. In our experiments, both substances decreased biofilm production after 5 and 10 minutes of action, but hypochlorite solution achieved better antibiofilm activity. However, in routine clinical practice, a substantial number of viable bacteria remained if contact times were less than 30 s or if diluted agents were used. This could be explanation for *A. baumannii* persistence in hospital environment.

Since we are entering in postantibiotic era, new antimicrobials and pharmaceutical substances are extensively developed to take place as a substitute or addition to usual antibacterials.

Microemulsions and nanoemulsions are isotropic, thermodynamically stable and transparent (or translucent) systems of oil, water and surfactants with antimicrobial activity. Their long -term stability, ease of preparation (spontaneous emulsification) and high solubilisation of drug molecules make microemulsions potential antibacterial agents as well as drug delivery tools. Microemulsions cause damage to the bacterial cell wall or cell membrane resulting in complete cell disorganization and disruption, presence of peripheral cytoplasmic condensations, irreversible loss of viability, increase in cell membrane permeability and partial solubilization of the cell membrane by fatty acids, leading to release of membrane proteins. Experiment with nanoemulsion of cetylpyridinium chloride, a quaternary ammonium salt, shows the disruption of *A. baumannii* biofilm, as consequence of the destabilizing and damage of the cell membrane. Our experiment with emanon and polisorbate microemulsion with different water content shows that both microemulsions have better antimicrobial effect on planktonic and sessile isolates, if microemulsions have higher percentage of water (60%).

Another surface bioactive substances are polymeric nanoparticles, that can be engineered for applications in medicine and pharmaceutical industry as drug carriers or new generation of antimicrobial drugs. Nanoparticles are smart molecules and exhibit unique physical, chemical and biological properties due to small size and possess high surface area-to-volume ratio. These characteristics render them highly effective in biological applications and make them potential candidates for development of novel nano-antibiotics. Several different nanoparticles (silver, gold, selenium,

NO) alone or in combination with antibiotic show excellent effect on *A. baumannii* biofilm, but mechanism of action is still unknown. In near future our goal is to performed experiment with titanium dioxide nanoparticles on planktonic and sessile isolates of *A. baumannii*.

In conclusion, it is obviously to expect that *A. baumannii* is going to become "the superbug" in the near future, and we have to try to develop alternative substances to eradicate bacteria from hospital environment, as the most useful preventive measure.

MB2 EFFECT OF LACTOBACILLUS ON BIOFILM PRODUCTION BY *GARDNERELLA VAGINALIS*

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Introduction

Bacterial vaginosis (BV) is a highly prevalent vaginal dysbiosis that has been linked to adverse pregnancy outcomes and enhanced transmission of sexually transmitted infections (STIs). The precise pathogenesis of BV remains unclear. However, depletion of vaginal *Lactobacillus* and overgrowth of anaerobes (often dominated by *G.vaginalis*) and a pH > 4.5 are thought to be key characteristics of the disease process. Currently, is consensual that BV also involves the presence of a dense, structured and polymicrobial biofilm, primarily constituted by *G.vaginalis* clusters, strongly adhered to the vaginal mucosal surface.

Biofilms are communities of microorganisms attached to a surface and encased in a polymeric matrix of polysaccharides, proteins and nucleic acids. Due to the fact that bacteria within biofilms are not effectively eliminated by the immune system or fully destroyed by antibiotics, biofilms appear to contribute to persistence and a high rate of relapse and recurrence of BV.

On the other hand, coaggregation of probiotic microorganisms to pathogens generates a hostile environment for the pathogens implying the reduction of their growth and re-establishment of indigenous microbiota. Reduction of the adhesive and biofilm forming capacity activity of *G. vaginalis* bacteria by *Lactobacillus* strains is a well-known and desired effect of strains for potential vaginal probiotic application.

The objectives of the present study were to evaluate in vitro the effect of *Lactobacillus* on biofilm production by different species of *G.vaginalis* isolated from women with bacterial vaginosis (BV).

Material and methods

A total of 36 isolates from women with BV identified as *G.vaginalis*

were tested for their biofilm-forming capacity as monocultures and in bacterial coculture with confirmed non-biofilm producing strain of Lactobacillus, in a ratio of 1:!. The ability to form biofilm was investigated using the microtiter plate assay, a standard test for detection of biofilm production. As part of this assay biofilm biomass quantification was done with crystal violet assay and the isolates were classified as: non-biofilm producers, weak-, moderate- and strong-biofilm producers.

Results and conclusion

Lactobacillus strain in our study was capable of interfering with the growth of *G. vaginalis* biofilms to different degrees.

According to the criteria for biofilm-forming ability, after 24-h incubation of the monocultures 25%, 27%, 22% and 25% of *Gardnerella* strains were strong, intermediate, and negative biofilm producers, while in cocultures of *Gardnerella* and *Lactobacillus* the percentages were 5.5%, 13.8 %, 19% and 47%, respectively.

Our results indicate the potential of lactobacilli as probiotics, since they effectively reduced the adheration and biofilm formation of the tested *Gardnerella* species which is a well-known and desired effect of strains for potential vaginal probiotic application. The elucidation of the antagonistic mechanisms as well as their effect on human cells may be useful in providing insight into the clinical situation in which probiotic and indigenous vaginal lactobacilli interfere with *Gardnerella*'s presence and reduce the risk of bacterial vaginosis and enlighten the importance of development of new products containing such microorganisms or products secreted by them.

MB3 EFFECTS OF *PSEUDOMONAS AERUGINOSA* ON GROWTH AND BIOFILM PRODUCTION OF *STAPHYLOCOCCUS AUREUS* CO-ISOLATED FROM CYSTIC FIBROSIS PATIENTS

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INTRODUCTION

Staphylococcus aureus is an important pathogen which colonizes and infects respiratory tract of cystic fibrosis (CF) patients. This bacterium can be also co-existed with *Pseudomonas aeruginosa* in later periods of the disease. Both pathogens produce various virulence factors including exotoxins, enzymes and cell wall proteins.

OBJECTIVES

We aimed to characterize dual-species interaction by exploring whether *P. aeruginosa* influences the growth and biofilm production of co-isolated methicillin resistant *S. aureus* (MRSA) from sputum

samples of CF patients.

MATERIAL, METHODS

Experiments were performed with 11 couples of MRSA and *P. aeruginosa* isolates from sputa of CF patients. Each MRSA isolate was grown in monoculture or in culture supplemented (1:1 volume) with 8h and 24h culture supernatants, heat-killed whole cells, and cell lysates of co-isolated *P. aeruginosa* in Luria-Bertani medium in microplates. Bacterial growth was determined by optical density measurements (OD600) at selected time points (4h, 8h, 12h, and 24 h). Biofilm production was measured using the crystal violet assay after 48h incubation at 37°C.

RESULTS AND CONCLUSION

Growth of MRSA isolates was increased by 8h culture supernatants of *P.aeruginosa* (mean growth was increased 15.4% ±8.5SD) and inhibited by heat-killed whole cells of *P.aeruginosa* (mean growth reduction was -30.3% ±31.4SD). Biofilm formation by MRSA strains at 48h was increased with the presence of 8h and 24h culture supernatants, cell lysates and heat killed cells of co-isolated *P.aeruginosa*; the corresponding biofilm mass increment rates in comparison to monoculture were 293%, 240%, 203% and 152%.

Our results highlighted that 8h culture supernatants of *P.aeruginosa* strains had an inducible effect on the growth of MRSA. All culture supplements prepared from *P.aeruginosa* were exhibited a positive effect on the biofilm production of MRSA. We suggest that our findings may facilitate the understanding of inter- and intraspecies bacterial interactions in CF.

MB4 СЕКСУАЛНО ПРЕНОСЛИВИ ИНФЕКЦИИ КАКО ГОЛЕМ ГЛОБАЛЕН ЗДРАВСТВЕН ПРОБЛЕМ

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Повеќе од 30 различни бактерии, вируси и паразити предизвикуваат сексуално преносливи инфекции, се пренесуваат преку сексуален контакт, вклучувајќи вагинален, анален и орален секс. Осум од триесет познати патогени сè со најголема инциденца. Од овие осум инфекции, успешно можат да се лекуваат четири: сифилис, гонореја, хламидија и трихомонијаза. Другите четири: хепатитис Б, херпес, ХИВ и ХПВ се вирусни инфекции и како такви сè уште се неизлечиви, но може да се ублажат или да се модулира нивниот тек.

СПИ сеуште се игнорирани иако се еден од најголемите проблеми на

јавното здравство, пратени со голем број на смртни случаеви и огромни економски чинења. Според СЗО во последниве години само од четирите излечиви болести во возрасната група која е сексуално најактивна (15-49 години) годишно има околу 400 милиони ново инфицирани: *Chlamydia trachomatis* 132 милиони*, Neisseria gonorrhoeae* 85 милиони, syphilis 6 милиони, и *Trichomoniasis vaginalis* 141 милиони. Пандемијата со ХИВ се стабилизира на околу 2 милиони новоинфицирани годишно, и исто толку умрени пред воведувањето на антиретровирусната терапија. Во 2015 година бројот на умрените е околу еден милион. Над 350 милиони во светот живеат со хронична хепатитис Б инфекција, и 1,5 до 2 милиони умрени секоја година. Повеќе од 290 милиони жени имаат ХПВ инфекција, од нив 530 000 добиваат рак на грлото на матката, а 275 000 од рак на грлото на матката умират секоја година. Од 400-500 милиони во светот живеат со ХСВ2 инфекција. Голем проблем предствуваат резистенцијата на *Neisseria gonorrhoeae,* големиот број на деца родени со конгенитален сифилис (300.000 умрени со конгенитален сифилис и уште околу 215.000 со ризик за смрт поради предвремено породување) и навременото дијагностицирање на хламидија инфекциите. Во Република Македонија се најдобра евиденција има за ХИВ инфицирните. Останати заболувања се чини се следат со недоволно внимание. Од почетокот на пандемијата во 1987 до 2016 година вкупно се регистрирани 311 лица со ХИВ/СИДА (179 лица болни од СИДА и 131 ХИВ позитивни). Дистрибуцијата по пол покажува дека 258 лица се од машки пол, а 53 лица се од женски пол. Во овој период умрени се 83 лица од СИДА, а во 2016 година во РМ 227 живеат со ХИВ/СИДА. Во 2017 година има 39 новооткриени случаи на ХИВ/СИДА. Во анализираната 2016 година во Републик Македонија има регистрирано 133 инфицирани со хлмидија, 112 со хепатитис тип Б, 4 случаи со гонореа и 4 случаи со сифилис. Најголем е бројот на пријавени со шуга, 217 случаи. Во 2017 година има пријавено 4 случаи со гонореа 4 случаи со сифилис и 137 случаи со хлмидија (99% кај жени).

СЗО на својата сесија од 05.10.2015 ја донесе глобалната стратегија за ХИВ, вирусен хепатитис и сексуално преносливи инфекции за периодот од 2016-2021 (како континуитет на стратегијата за контрола и намалување на СПИ 2009-2016 година). Како таргети за ХИВ инфекцијата во овој 5 годишен период се поставени: намалување на новоинифицирните на помалку од 500.000 годишно; помалку од 500.000 умрени годишно; нула новоинфицирани доенчиња; 90% тестирани; 90% од позитивните да бидат третирани; кај 90% од третираните да има успех (вирусно потиснување). За ова се предвидуваат 20 милјарди американски долари во 2016 година а за 2020 се предвидуваат 30 милјарди долари. Стратегијата за останатите СПИ ќе оди во насока на намалување на ранливоста, ризикот и пренесување на СПИ; навремено дијагностицирање и лекување, следење н арезистенција на лекови. кко посебни таргети до 2030 година се: 90% намалување на инциденцата со *T. pallidum* во споредба со 2018 година; 90% намалување на

инциденца со гонореа; помалку од 50 случаи со вроден сифилис на 100.000 живородени деца во 80% од земјите; 80% покриеност со вакцина против ХПВ кај адолесценти 9-13 години во 80% од државите. Заова во првите 5 години се предвидуваат 18 милијарди американски долари.

MB5 *UREAPLASMA UREALYTICUM* IN SPERMOCULTURE - DETECTION AND IMPORTANCE

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Introduction: *Ureaplasma urealyticum* and *Mycoplasma hominis* are the most often isolated genital mycoplasmas in humans. Mycoplasmas show an affinity for mammalian cell membranes. The damage to host cells is due to several mechanisms: the release of H_2O_2, superoxide radicals, and the activation of macrophages and the release of cytokines. The biochemical activity of *Ureaplasma urealyticum* leads to the release of ammonium ions and cytopathological changes. The adherence of the spermatozoids decreases their mobility and leads to morphological changes.

The **objective** of our study was to detect the presence of *Ureaplasma urealyticum* and other bacteria in spermocultures.

Material: Material for our analysis was semen samples, which were received for a routine diagnosis at the Institute of Microbiology and Parasitology, Medical Faculty, Skopje, during one year period (2016). The most common diagnosis in our patients was sterility.

Methods: The samples were inoculated on Columbia blood agar for isolation of aerobic bacteria and Schaedler agar for anaerobic bacteria. Identification of isolates was done by classical and automated biochemical tests. Direct microscopic smear for presence of leukocytes and bacteria was also done from each sample. For isolation, identification, enumeration and susceptibility of ureaplasmas was used Mycoplasma IES - Autobio.

Results: A total of 1380 spermocultures, during one year, were examined. The presence of bacteria was detected in 386 samples (28%). *Ureaplasma urealyticum* was the most often found bacteria, in 117 sperms (30.3% of positive spermocultures). *Mycoplasma hominis* was isolated in only one patient. Other common isolated bacteria were: *Escherichia coli*, *Enterococcus* and *Streptococcus agalactiae.* Most often ureaplasma was present as the only isolate, in 105 cases (27.2%). In 12 patients, beside *Ureaplasma urealyticum*, there were isolated other bacteria: *Streptococcus agalactiae* 4, *Escherichia coli* 3, *Enterococcus* 2, anaerobes 2 and *Klebsiella pneumoniae* 1 isolate.

Conclusion: Patients usually come for microbiological examination of sperm during sterility or infection. According to the literature data and our previous findings the successful treatment of ureaplasma was followed by significant improvement in the sperm motility. Detection of *Ureaplasma urealyticum* beside other bacteria in sperm is of particular importance.

MB6 CULTURE AND MOLECULAR METHODS FOR DETECTION OF *MYCOBACTERIUM TUBERCULOSIS COMPLEX*

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Introduction
Tuberculosis(TB) is a disease with worldwide significance. Effective treatment of tuberculosis requires rapid detection and identification of *Mycobacterium tuberculosis complex*(MTC). The gold standard for TB diagnosis is through the culture but it is time-consuming and can take 4 to 8 weeks . On the other side, nucleic acid amplification techniques due to its rapidity and sensitivity may aid early diagnosis.

Methods
In this study, we retrospectively analysed the data of our mycobacteriology laboratory. BACTEC MGIT 960 (Becton Dickinson, USA) system was used for isolation and Ziehl Neelsen and Auramine-Rhodamine staining were used for microscopic evaluation.
Molecular diagnosis was performed with GeneXpert MTB/RIF (Cepheid,USA) in respiratory tract specimens and FluoroTypeMTB (Hain Diagnostics, Germany) in non-respiratory specimens.

Results
We retrospectively reviewed 15,172 samples that accepted to our laboratory for culture from January 2011 to September 2017. Samples from respiratory tract consisted 54,5% and non-respiratory specimens 45,5% of total samples with 3.4% and 2.2% positivity rates respectively (Table). Direct examination was positive in 147 of the 426 isolates in which mycobacteria were detected. 355 (2.3%) of the isolates were detected as MTC, and 71 (0.46%) as non-tuberculous mycobacteria.
We retrospectively reviewed 7087 samples accepted to laboratory for molecular methods from January 2015 and December 2017. Our hospital serves as a reference center for molecular methods and samples from the chest diseases hospital were also included in analyses. (Table). Molecular detection rate for MTC is very similar with the culture results in our hospital. More striking finding was 10 times higher positivity rate

for chest diseases hospital compared to our hospital.

Conclusions
Although rapid identification of tuberculosis is important, molecular methods are quite expensive systems. This study has shown that in cases where clinical suspicion is strong the likelihood for positivity of molecular methods is higher. When clinical suspicion is weak and no radiological evidence, the positivity and cost effectiveness of the molecular test is reduced.

Table :Culture and molecular detection rates of *Mycobacterium Tuberculosis Complex*

Year	**Location**	**CULTURE RESULTS**	
2011-2017		**Pulmonary** Total/positive(%)	**Extrapulmonary** Total/positive(%)
	Marmara University Hospital	8282/277(3.4)	6890/150(2.2)
		MOLECULAR RESULTS	
		Pulmonary Total/positive(%)	**Extrapulmonary** Total/positive(%)
2015-2017	Marmara University Hospital	3116/100(3.2)	1128/75(6.6)
	Chest Diseases Hospital	2725/861(31.5)	118/13(11)

MB7 FROM COUGH TO TUBERCULOSIS - A SPUTUM'S WAY THROUGH OUR LABORATORY

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Introduction
Too many people have undetected TB for too long; late detection of TB increases their risk of transmitting the disease to others, having poor health outcomes, or that they and their family will suffer distress and economic hardship. A high-quality laboratory system that uses modern diagnostics is a prerequisite for the early, rapid and accurate detection of TB and drug resistance.

Objectives
This presentation aims to demonstrate the capacity and protocol of our National Reference Laboratory for Tuberculosis. We believe this presentation will clarify the methods we use for TB detection. It will also fill any gaps in the communication between our personnel and clinical doctors, also we will repeat our protocol for the colleagues working in other fields in microbiology.

Summary
Uptake of TB diagnostic technologies requires appropriate laboratory infrastructure, sufficient human resources and adequate policy reform at country level to enable their effective use in TB screening and diagnostic algorithms.

The WHO periodically issues an algorithm for diagnosis and follow up of patients with TB and MDR-TB. Currently this algorithm includes rapid molecular tests (such as GeneXpert MTB/RIF), microscopy, culture, first and second line - line probe assays (FL-LPA, SL-LPA) when necessary, and solid or liquid phenotypic drug susceptibility testing. We have modified this algorithm to fit our needs and capability considering laboratory and clinical personnel, financial issues and equipment availability.

Conclusion
We need to implement continuous efforts in order to follow WHOs diagnostic protocols for TB. More focus should be placed towards educating clinical doctors and especially family doctors to suspect TB and recommend appropriate additional testing. Fortifying our laboratory with personnel and sufficient funds and equipment should be considered among countries top priorities.

MB8 IDENTIFICATION OF MYCOBACTERIA WITH BIOCHEMICAL METHODS IN THE PAST

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Introduction: Nontuberculous mycobacteria (NTM) are environmental opportunistic pathogens causing mycobacteriosis among immunocompromised and immunocompetent patients. Pulmonary mycobacteriosis accounts for abouth 90% out of all infections with NTM. Due to their environmental persistence, not all clinical clinical isolates of NTM are clinically relevant and only 20-60% of all isolates of NTM species that are known to cause lung infection meet criteria for NTM pulmonary disease. Followed by this it is very important to correctly identify mycobacterial species in patient's sample for achieve

high success rate of treatment.

Aim of our study was to compare biochemical methods with molecular methods (GenoType Mycobacterium CM, AS, NTM DR (Hain Lifesciences, Nehren, Germany)).

Materials and methods: For this study, 25 different Mycobacterium kansasii (MK) isolates were used growing on solid media Loewenstein-Jensen (LJ). Isolates were tested using four different tests (nitrate reduction test, growth on solid Middlebrook 7H10, iron uptake, and pigment production). Reduction of nitrates is demonstrated colorimetrically by the addition of two reagents, as some mycobacteria are capable of reducing nitrates to nitrites. The morphology of mycobacteria was observed with microscope on the medium 7H10. Some mycobacteria are able to reduce iron, creating brownish substances. By using the pigment test, we distinguish non-chromogenic, chromogenic, scotochromogenic and photochromogenic mycobacteria from other mycobacteria by placing one pair of media in the dark and one pair on the light.

Results: MK is one of the mycobacteria representing 7.4% out of 4575 isolates NTM in Slovenia, in the period 2000 - 2016. From 25 different MK isolates tested reduction of nitrates, 80% were positive and 20% contaminated. On the 7H10 solid media, the colonies were rough, yellow photochromogenic in 96% and 1 (4%) isolate was non-photochromogenic (white colonies). The same results were obtained at iron uptake.

Conclusion: We have found that biochemical tests are much more time consuming, results often quite subjective, less accurate and reliable, otherwise cheaper than molecular tests. However, these tests are still used to describe new species, and some of them are additional test to today's molecular tests. Especially colony morphology on the Middlebrook 7H10 medium is useful and frequently used in many laboratories. With the decline in TB in Slovenia, the number of clinically significant mycobacteriosis is slowly increasing. Therefore, accurate identification with molecular biological methods and some biochemical tests is very important for daily routine work.

Key words: Mycobacterium kansasii, nontuberculous mycobacteria, solid medium LJ, solid medium 7H10

MB9 TUBERCULOSIS IN CHILDREN-9 YEARS EXPERIENCE IN IDENTIFICATION OF MYCOBACTERIAL SPECIES

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Tuberculosis in children is often missed or overlooked due to nonspecific symptoms and difficulties in diagnosis.

The aim of study was to follow up the percentage of children suffering from tuberculosis with bacteriological confirmed diagnosis.

This study was based on the analysis of the 847 case histories from tuberculosis patients treated in our Institute in period of 9 years(2009-2017).

Acidoalchocolo resistant bacilli were detected in 15 cases(1,8%),Lowenstein Jensen cultures were positive in 27(3,18%).

In the investigation period culture and slide positive cases were founded as follows:

year	No. of patients	No. of positive cultures	%	No. of positive slides	%
2009	89	4	4,4	3	3,3
2010	97	5	5,1	2	2,06
2011	89	3	3,3	2	2,06
2012	74	1	1,3	/	0
2013	92	1	1,08	/	0
2014	97	4	4,1	2	2,06
2015	95	2	2,08	2	2,0
2016	111	4	3,6	3	2,7
2017	103	3	2,19	1	0,09

Culture of MBT is the gold standard for diagnosis of TB, that is a much more sensitive test than smear examination.

Having in mind that TB is particularly difficult to diagnose in children,the XpertMTB/RIF assay is the best way for diagnosis of pediatric TB.

MB10 TUBERCULOUS PLEURAL EFFUSION–CASE REPORT

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Introduction: Parapneumonic effusion (PE) are seen in up to 57% of patients with pneumonia.The majority of these effusions are noninfected and resolve with standard antibiotic treatment for the associated pneumonia. PE are seenduring the acute bacterial pneumonia and

rarely in assotiation with viral pneumonia and tuberculouspleuritis. Tuberculosis (TB) is an infectious disease that usually affects the lungs.Extrapulmonary TB affecting mainly the lymph nodes and pleura. Abdominal tuberculosis is diagnostic challenge because of the nonspecific features of the disease.

Case report : A 22 year old man presented to our institution with complaints of cought, haigh grade fevers, right side pleuritic chest pain, abdominal pain, nausea/vomiting, malaise and 2-week history of weight loss. Physical examination was unremarkable.

Laboratory tests showed a mild leucocytosis of 14,5x 10 3cells/ mL(normal 3,8- 10,6), ESR was elevated at 110 mm/hr (normal, 0-20), CRP was 86,6 mg/dL (normal 0- 5).

A chest x-ray showed a right pleural effusion and parenhchymal disease in the right lower lobe.

A5 day course of ceftriaxon and moxifloxacin resulted only a mild symptom relief.During a laparoscopic biopsy done six days after hospitalization because of strong abdominal pain, were noted extensive infiltration of the peritoneum between the omentum, bowel, and abdominal well. Histological demonstration of biopsy showed granulomatous inflammation consistent with tuberculosis.Pleural fluid analysis revealed a lymphocytic predominant exudates.

Conclusion: It is important to consider the possibility of tuberculouspleuritis in patients with parapneumonic effusion. Clinicians must keep a high degree of suspicion of tuberculosis peritonitis in patients with insidious abdominal signs.In our case we reviewed our experience with pulmonary TB, and concomitant peritoneal tuberculosis.

ТУБЕРКУЛОЗЕН ПЛЕВРАЛЕН ИЗЛИВ- ПРИКАЗ НА СЛУЧАЈ

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Вовед: Парапнеумоничните изливи (ПЕ) се срекаваат кај повеке од 57 % од пациентите со пневмонија. Поголемиот дел од овие изливи се повлекуваат со стандарден антибиотски третман на асоцираната пневмонија. ПЕ се присутни најчесто кај акутните бактериски пневмонии а ретко се срекаваат и кај туберкулоза и вирусните пневмонии. Туберкулозата (ТБ) е инфективна болест која најчесто ги зафака белите дробови. Екстрапулмоналната ТБ ги зафака главно лимфните чворови и плеурата. Абдоминалната туберкулоза е дијагностички предизвик заради неспецифичните знаци на болеста.

Приказ на случај: 22 годишен маж беше хоспитализиран на нашето одделение со симптоми на кашлица, висока температура,

десностранa болка во граден кош, абдоминална болка, гадење/повраќање, малаксаност и податок за губиток на телесна тежина во последните 2 недели.Физикалниот наод при преглед беше некарактеристичен.
Лабораториските анализи покажаа леукоцитоза 14,5x 10 3/mL(референтни вредности 3,8- 10,6), покачени вредности на СЕ 110 mm/hr (0-20) и ЦРП 86,6 mg/dL (0- 5).
Рентгенграфскиот наод покажа плеурален излив лево и бронхопнеумоничен процес во базални партии на лево белодробие. Терапијата во тек на 5 дена беше со ceftriaxoni moxifloxacin, но без евидентно подобрување. Поради интензивирање на абдоминалната болка шестиот ден по приемот направена е лапароскопска биопсиа при што е видено инфилтрација на висцералниот и париеталниот перитонеум. Хистолошкиот наод од биопсијата беше туберкулозен грануломатозен перитонитис. Анализата на плевралниот излив покажа ексудат со предоминација на лимфоцити.

Заклучок: Важно е да се мисли на можноста на туберкулозен плеуритис кај пациентите со парапнеумонични изливи. Клиничарите треба да се сомневаат на туберкулозен перитонитис кај пациенти со подмукла абдоминална болка. Во нашиот случај укажуваме на пулмонална туберкулоза со плеврален излив и перитонеална форма на абдоминална туберкулоза.

MB11 ISOLATES OF *MYCOBACTERIUM TUBERCULOSIS* FROM SPUTUM IN MICROBIOLOGICAL LABORATORY – CLINICAL HOSPITAL BITOLA IN THE PERIOD 2013 – 2017

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Introduction: According to the National Tuberculosis Control Program in Macedonia, the diagnosis of tuberculosis requires investigation of sputum of acidealcoholic resistant bacilli (ARB) in a direct preparation as well as the proving of *Mycobacterium tuberculosis* in culture of the Levenstein – Jensen substrate.

Aim: To see the number of isolates of *Mycobacterium tuberculosis* in sputum of patients with pulmonary tuberculosis in the Microbiological Laboratory of the Clinical Hospital–Bitola in the period 2013–2017.

Materials and Methods: Standard procedures for proving ARB (acidoalcoholic resistant bacilli) have been used in a direct microscopic colored Cil – Nilsen preparation and a culture of Levenstein – Jensen pads made of sputum.

Results: In the year 2013, in the Microbiological Laboratory, 428 analyzes were performed for 206 patients suspected of pulmonary tuberculosis, with 14 (6.79%) positive isolates diagnosed, in 2014, 356 analyzes of 184 patients were performed and 10 were diagnosed (5.43%), in 2015, 256 analyzes were performed out of 144 patients, 6 isolates (4.16%) were diagnosed, in 2016, 248 analyzes are performed of 139 patients and 4 (2.87%) isolates were diagnosed, and in 2017, 198 analyzes were performed for 120 patients and 5 (2.52%) isolates of *Mycobacterium tuberculosis* were isolated. They have been confirmed in the Reference Microbiological Laboratory for Tuberculosis at the Institute for Pulmonary Diseases and Tuberculosis in Skopje with developed resistance tests.

Conclusion: The number of isolates of *Mycobacterium tuberculosis* in 2013 compared to 2017 has been reduced from 14 to 5 isolates The improved standard and living conditions are possible factors for reducing the number of patients with pulmonary tuberculosis.

ИЗОЛАТИ НА *MYCOBACTERIUM TUBERCULOSIS* ОД СПУТУМ ВО МИКРОБИОЛОШКАТА ЛАБОРАТОРИЈА- КЛИНИЧКА БОЛНИЦА БИТОЛА ВО ПЕРИОДОТ 2013-2017 ГОДИНА

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Вовед: Согласно Националната програма за контрола на туберкулоза во Македонија, во дијагноза на туберкулоза се бара испитување на спутум на ацидоалхохолно резистентни бактерии (АРБ) во директен препарат како и докажувањето на *Mycobacterium tuberculosis* во култура на Левенштајн-Јенсен подлога.

Цел: Да се согледа бројот на изолати на *Mycobacterium tuberculosis* во спутум на пациенти со белодробна туберкулоза во Микробиолошката лабораторија при Клиничка болница-Битола во периодот на 2013-2017 година.

Материјал и методи: Користени се стандардни постапки за докажување на АРБ (ацидоалкохолнорезистентни бацили) во директен микроскопски препарат обоен по Цил- Нилзен и култура на Левенштајн- Јенсен подлога изработени од спутум.

Резултати: Во периодот на 2013 година во Микробиолошката лабораторија извршени се 428 анализи за 206 пациенти суспектни за белодробна туберкулоза при што се дијагностиковани 14 (6.79 %) позитивни изолати, во 2014 извршени се 356 анализи за 184 пациенти и дијагностиковани се 10 (5,43 %), во 2015 извршени се 256 анализи за 144 пациенти дијагностиковани се 6 изолати(4,16%), во 2016 извршени се 248 анализи за 139 пациенти

при што се дијагностиковани 4 (2,87 %) изолати, и во 2017 година извршени се 198 анализи за 120 пациенти и изолирани се 5 (2,52%) изолати на *Mycobacterium tuberculosis*. Истите беа потврдени и во Референтната Микробилошка лабораторија за туберкулоза при Институтот за Белодробни заболувања и туберкулоза во Скопје со изработени тестови на резистенција.

Заклучок: Бројот на изолати на *Mycobacterium tuberculosis* во 2013 година во однос на 2017 година е намален од 14 на 5 изолати. Подобрениот стандард и условите за живеење се можните фактори за смалување на бројот на пациенти со белодробна туберкулоза.

MB12 BIOSAFETY EQUIPMENT AND LABORATORY ACQUIRED INFECTIONS IN THE MACEDONIAN PUBLIC HEALTH MICROBIOLOGICAL LABORATORIES

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Introduction: Microbiological laboratories are special, often unique working environments with clearly recognizable risk of infectious diseases for the employees. World Health Organization recommends all medical and diagnostic laboratories to be designed for minimum 2 or higher level of biological safety.

Aim: To determine the condition of public health microbiological laboratories in R. of Macedonia, in terms of implementation of measures to protect against biological risks and to summarize data of laboratory acquired infections (LAI) and possible reasons that lead to LAI.

Material and methods: A cross-sectional study was conducted in a period of two months (March-April 2014) by distributing a specially designed questionnaire with total of 71 questions to 187 employees in these laboratories. Data processing was done using the SPSS for Windows 13,0. Statistical significance of differences was tested by Pearson Chi-Square test and Student-s t test.

Results: According the answers of the respondents, 37% believe they work in the laboratories having first level of biosafety and 30% that have a second level. There are no positive answers for existing third or fourth level of equipment in any of the microbiological laboratory in R. of Macedonia. Biological safety cabinets (BSC), have 6 institutions

out of 19 (30%). Other laboratories in the country have no BSC. Of the total number of examined workers, 19% gave information about a history of occupational disease associated with their workplace. The most frequent reports of LAI are from laboratory technicians and employees with longer working experience.

Conclusion: Macedonian microbiological laboratories are applying a range of measures against biological harmful agents, but not all, not everywhere and not completely. There is a clear need for continuous education of the personnel, raising levels of biosafety and biosecurity, as well as implementation of mandatory preventive care.

Keywords: microbiological laboratories, biosafety, laboratory acquired infections.

MB13 ГЕНИТАЛНИ ИНФЕКЦИИ СО STREPTOCOCCUS AGALACTIAE ВО РЕПРОДУКТИВНИОТ ПЕРИОД НА ЖЕНИТЕ

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ЗУ ЦЈЗ Прилеп

Вовед: Во репродуктивниот период на жените Streptococcus agalactiae може да се најде како колонизатор или предизвикувач на инфекции опасни за животот на новороденчето.

Цел: Изолација на Streptococcus agalactiae од гениталните патишта на жените на возраст од 18 до 45 години во период од 01.01.2016 до 31. 12.2017 година.

Материјал и методи: Бактериолошкаобработка на брисеви од vagina на следниве подлоги:COS agar, Chromid Streoto B agar i Strepto plus B kit.

Резултати: Вкупно испитани вагинални брисеви во наведениот период се 7009. Од нив во, во 339 (5,5%) е изолирана Streptococcus agalactiae како предизвикувач на инфекции. Осетливоста кон антибиотици е одредена со диск-дифузиона метода и е следната: најголема осетливост бактеријата има кон: Vancomycin89%, Nitrofurantoin 83% Penicillin (Ampicillin, Amoxiklav) 79%, Levofloxacin77%, Ciprofloxacin 77%; најмала кон Clindamycin56%, Eritromycin53% i Trimetoprim –sulfametoxazol 15%.

Заклучок: Многу значајна е навремена детекција и лечење на инфекциите кај жените во репродуктивниот период предизвикани со Streptococcus agalactiae. Со тоа се намалуваат инфекциите и смртноста кај новородените при пороѓај.

MB14 *SALMONELLA* DETECTION IN OUT-CLINIC PATIENTS IN SKOPJE AND ITS SURROUNDING

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Introduction

Every year *Salmonella* causes an estimated 94 million illnesses and 155 000 deaths worldwide. Thousands of cases are never diagnosed because they are passed off as stomach flu or something much less severe. *Salmonella enterica* subspecies *enteritidis* is the most common isolated serotype in Europe and our country.

Objectives

Our aim was to detect and follow the incidence of human *Salmonella* isolates in our patients in a period of 3 years

Materials and methods

We analysed faecal specimens from out-patients with gastrointestinal symptoms, mostly from Skopje, Tetovo and Kumanovo for period of 3 years (2015-2018). Standard procedures and selective culture media as SS/XLD agar and Hectoen agar (Oxoid UK) were used in order to isolate *Salmonella*. The serologic identification of O and H antigens was performed with agglutination anti-serums (Bio-Rad, Germany).

Results

In the period of three years, we tested 1578 specimens. A total of 183 specimens out of 143 patients were found positive with *Salmonella* spp. We found that 92% out of all positive patients were *Salmonella enterica* subspecies *enteritidis*. 8% were other *Salmonella*: 11 and 1 isolates of *S typhy-murium* and *S paratyphy,* respectively. Furthermore, 82% of *Salmonella* cases were isolated from patients younger than 7 years.

Conclusion

As a major pathogen of food-borne disease, especially among young children and out-clinic patients, there is need for precise identification of *Salmonella* as a key for solving and prevention of outbreaks. In order for more successful diagnosis of *Salmonella* infections we encourage the doctors to have in mind the possibility of *Salmonella* infection even in less severe cases of gastro intestinal symptoms.

MB15 THE MOST COMMON AGENTS OF URINARY INFECTIONS IN PATIENTS TREATED IN PHI RE-MEDICA IN 2018

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Introduction: Urinary infections are second in the frequency of all infections in the human population and have a high clinical significance.
Aim: To show the presence of isolated bacterial agents of urinary infection in patients treated in PHI Re-Medica in 2018.

Material and methods: 1503 patients were examined in the microbiological laboratory of the PHI Re-Medica - Skopje, within 2 hours after taking the analysis. Urinals were planted using the standard quantitative microbial method of the Columbia agar and the chromogenic selective substrate chromID CPS, incubating at 37 °C for 24 hours. The identification of bacteria was carried out with standard microbiological methods and with the help of automated System VITEK 2 Compact 15.

Results: Of the total of 1503 examined, 88.4% were from women and 11.6% from men. In 47.2% of patients urine was positive, and in men at 87 (49.7%). Of the positive urine cultures, most women were isolated in *Escherichia coli* with 44.2%, *Enterococcus species* 22.8%, *Streptococcus agalactiae* 17.3%, *Klebsiella pneumoniae spp pneumonia* 4%, *Proteus mirabilis* 3.6% and *Staphylococcus aureus* 2.1%, *Candida species* 1.8%, while *Morganella morganii*, *Pseudomonas aeruginosa*, *Enterobacter cloacae complex, Enterobacter aerogenes, Citrobacter koseri, Citrobacter freundii* and *Proteus vulgaris* were represented with less than 1%. Most of the men isolates were *Escherichia coli* with 31.7%, *Enterococcus species* 30.8%, *Streptococcus agalactiae* 16.3%, *Proteus mirabilis* 9.6%, *Morganella morganii* 4.8%, *Klebsiella pneumoniae spp pneumoniae* and *Citrobacter koseri* 2.9% and *Pseudomonas aeruginosa* less than 2%. In the examined patients the most common were infections with one isolate, while in 22.5% of the women two isolates were detected, and in men 13.8%. In women, *Escherichia coli* was most commonly combined with *Enterococcus species* and *Escherichia coli* with *Streptococcus agalactiae*, while in men *Escherichia coli* with *Morganella morganii*.

Conclusion: Gram-negative bacilli dominate from isolated organisms, of which *Escherichia coli* is the most commonly isolated bacterial agent of urinary infections. If there is a mixed infection in women it is a combination of Gram-positive cocci with Gram-negative bacilli and in men a combination of two Gram-negative bacilli.

Keywords: urinoculture, bacterial infection, *Escherichia coli*

НАЈЧЕСТИ ПРИЧИНИТЕЛИ НА УРИНАРНИ ИНФЕКЦИИ КАЈ ПАЦИЕНТИ ЛЕКУВАНИ ВО ПЗУ РЕ-МЕДИКА ВО 2018 ГОДИНА

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ПЗУ Ре-Медика, Скопје

Вовед: Уринарните инфекции се на второ место по зачестеност на сите инфекции во хуманата популација и имаат големо клиничко значење.

Цел: Да се прикаже застапеноста на изолираните бактериски причинители на уринарна инфекција кај пациенти лекувани во ПЗУ Ре-Медика во 2018 год.

Материјал и методи: Беше испитувана урина од 1503 пациенти во микробиолошката лабораторија на ПЗУ Ре-Медика – Скопје, во период од 2 часа по земање за анализа. Урините беа засадувани со стандарден квантитативен микробиолошки метод на крвна подлога - Columbia agar и хромогена селективна подлога - *chromID CPS, со инкубација на 37°C 24 часа*. Идентификацијата на бактериите се изведуваше со стандардни микробиолошки методи и со помош на автоматизиран System VITEK 2 Compact 15.

Резултати: Од вкупно испитани 1503 примероци, 88.4% беа од жени, а 11.6% од мажи. Кај 47.2% пациентки уринатa беше позитивна, а кај мажи кај 87 (49.7%). Од позитивните уринокултури, кај жени во најголем процент беа изолирани: *Escherichia coli* со 44.2%, *Enterococcus* species 22.8%, *Streptococcus agalctiae* 17.3%, *Klebsiella pneumoniae ssp pneumonia* 4%, *Proteus mirabilis* 3.6% и *Staphylococcus aureus* 2.1%, *Candida* species 1.8%, додека *Morganella morganii*, *Pseudomonas aeruginosa, Enterobacter cloacae complex, Enterobacter aerogenes*, *Citrobacter koseri*, *Citrobacter freundii* и *Proteus vulgaris* беа застапени со помалку од 1%.

Кај мажите најзастапени беа: *Escherichia coli* со 31.7%, *Enterococcus* species 30.8%, *Streptococcus agalactiae* 16.3%, *Proteus mirabilis* 9.6%, Morganella morganii 4.8%, *Klebsiella pneumoniae spp pneumoniae* и *Citrobacter koseri* 2.9% и *Pseudomonas aeruginosa* помалку од 2%.

Кај испитаните пациенти најзастапени беа инфекциите со еден изолат, додека кај 22.5% жени беа детектирани по два изолата, а кај мажи 13.8%. Кај жените најчесто беа комбинирани *Escherichia coli* со *Enterococcus* species и *Escherichia coli* со *Streptococcus agalactiae*, додека кај мажите *Escherichia coli* со *Morganella morganii*.

Заклучок: Од изолираните микроорганизми доминираат Грам-негативните бацили, од кои *Escherichia coli* е најчест изолиран бактериски причинител на уринарни инфекции. Доколку постои мешана инфекција кај жени тоа е комбинација на Грам-позитивни коки со Грам-негативни бацили, а кај мажи комбинација од два Грам-негативни бацили.

Клучни зборови: уринокултура, бактериска инфекција, *Escherichia coli*

MB16 ENTEROCOCCUS FAECALIS КАКО ПРИЧИНИТЕЛ НА УРИНАРНИ ИНФЕКЦИИ И НЕГОВАТА СЕНЗИТИВНОСТ ПРЕМА АНТИБИОТИЦИ

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Одделение по микробиологија при Центар за Јавно Здравје Прилеп Македонија

Цел на ова труд е да се прикаже нашето искуство со изолатите од уринарниот тракт (UTI) во овој случај Enterococcus faecalis (E faecalis) како често изолирана бактерија кај уринарните инфекции и нејзината осетливост према одредени антибиотици.

Материјал и методи: Во овој труд ние ги прикажуваме уринокултурите испитани во нашата лабораторија во последните 2 години (2016-2017) следејќи ги препораките од EUCAST. Примероците на урина беа засадувани на KP плочи (Oxoid/ BioMerieux) и CPS (UTI)(BioMerieux/ Oxoid) плочи. Колониите беа идентификувани со аглутинација со Latex Anti-Streptococcus group D аглутинацискитест и Esculin тест, додека антибиограмот беше работен на крвен Mueller Hinton blood agar со диск дифузиона метода.

Резултати: Од вкупно 12793 уринокултури кои беа испитани во нашата лабораторија 2002 беа позитивни (15,4%). Од вкупниот број на позитивни уринокултури, 229 (14.9%) беа идентификувани како E. faecalis ,73% од вкупниот број изолирани E. faecalis беа од примероци на урина од женска популација, 84% од сите се од група над 18 години и 74.5% со дијагноза . Cystitis и инфекции на уринарен тракт.

Осетливоста на E. faecalis позитивните изолати беа испитувани на следните антибиотици: Ampicillin, Ciprofloxacin, Levofloxacin и Nitrofurantoin. Најмногу осетлив од испитуваните антибиотици е Ampicillin со 90.0% потоа Nitrofurantoin со 68.9%, Levofloxacin со 61.9% и најмалку осетлив е Ciprofloxacin со 55.2%.

Заклучок: Осетливоста на испитуваните примероци на E. faecalis изолирани од уринокултури следејќи го EUCAT е прифатлив. Антибиограмот сам ни покажува кој антибиотик треба да го одберат докторите.

MB17 *ENTEROCOCCUS FAECALIS* AND *ENTEROCOCCUS FAECIUM* IN URINE SAMPLES AND ANTIMICROBIAL SUSCEPTIBILITY

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Introduction
Urinary tract infections are considered to be the most common bacterial infections. Genus *Enterococci* are part of the normal intestinal flora of humans and animals. They have been long recognized as important human pathogens and are becoming increasingly so. The genus *Enterococcus* includes more than 17 species, although only a few cause clinical infections in humans. More important are two species *Enterococcus faecalis* and *Enterococcus faecium.*

Aim
The aim of this study is to evaluate the presence of *Enterococcus faecalis* and *Enterococcus faecium* and antimicrobial susceptibility in urine samples which are send from Primary Health Care and University clinical center of Kosovo (Tertiary Health Care).

Material and methods
For isolating these microorganisms the cultivation is done in blood agar and MacConkey agar. Identification has done by biochemical methods and identification cards GP automatic system Vitek 2 Compact ,while the antimicrobial susceptibility is done using the disk diffusion method and susceptibility cards AST GP67 of Vitek 2 Compact (bio Merieux France).

Results
Microbiological characteristics analysis of all urine cultures received by the microbiologic laboratory during 2017 are conducted in the National Institute of Public Health in Pristina. During 2017, 34192 urine samples are received and analyzed, of which 26174 or 76.5% are negative and 8018 or 23.5% are positive. From all positive samples, 218 or 2.71% are *Enterococcus faecalis* and 45 or 0.56% are *Enterococcus faecium*. Although related to antimicrobial susceptibility, 2 or 0.91% *Enterococcus faecalis* are Vancomycin Resistant Enterococci, 5 or 2% are Ampicillin resistant and 24 or 11% are High Level Gentamicin resistance (HLGR), 19 or 42% *Enterococcus faecium* are Vancomycin Resistant Enterococci (VRE), 28 or 62% are Ampicillin resistant and 34 or 75% are HLGR.

Conclusion
From these results we conclude that species *Enterococcus faecalis* is the most common cause of urinary tract infections, although *Enterococcus faecium* are more resistant to antibiotics Vancomycin, Ampicillin and Gentamicin.

Key words: urine culture, *Enterococcus faecalis, Enterococcus faecium.*

MB18 EXCELLENT EXPERIENCE IN TREATMENT OF BACTERIAL URINARY INFECTIONS WITH COMBINATION OF ANTIBIOTIC AND URO-ANTISEPTIC

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INTRODUCTION: The bacterial urinary infections are common pathology among the population in Berovo and the surrounding. The most common bacterium, which causes those infections, is Escherichia Coli. The bacterium is very resistant to a large number of antibiotics and uro-antiseptics (urinary antiseptics) which is why the treatment lasts for a long time and it is according to an anti-bio gram made by a doctor-microbiologist.

PURPOSE: It is to show my excellent results in the treatment of urinary infections caused by the bacterium Escherichia Coli with the combination of medicines: antibiotic pills Amoxicillin + Clavulanic Acid 1, 0-gr and uro-antiseptic (urinary antiseptic) pills Pipemiolic Acid 400 mg.

MATERIALS AND METHODS: It is used health cards of 19 patients with a diagnosis - urinary infections caused by Escherichia Coli - (subjective problems: frequent urination, pain and laboratory). Blood and urine and urine cultures of the same number of bacteria 150.000 in ml, 250.000, 350.000 and 500.000 in ml) are anti -bio grams for administering therapy, made at microbiology at the Health Home – Berovo.

RESULTS: The treatment starts with an anti- bio gram in all 19 patients: one pill Amoxicillin + Clavulanic Acid 1, 0 gr every 12 hours. Six (31, 5%) patients take this antibiotic for one week and then one more week they drink urine antiseptic 1 pill Pipemiolic Acid 400 mg. After two- week therapy, at those 6 patients at 24 hours the control urine cultures are sterile (it has been reached the cause eradication). For 10 days, 7 (36, 8 %) patients take 1 pill Amoxicillin + Clavulanic Acid 1, 0 gr every 12 hours and 10 more days 1 pill Pipemiolic Acid 400 mg. daily the control urine cultures at all 7 patients are sterile (negative); 4 (21%) patients take the same antibiotic for 14 days and 14 more days the same uro-antiseptic. At all patients, the control urine cultures are negative. At only two (10, 7%) patients after 28 days the same therapy in the control urine cultures, the cause of infections persisted.

CONCLUSION: From the received results, at 19 patients with bacterial urinary infections caused by Escherichia Coli, at 17 (89, 3%) patients after administering the pill Amoxicillin + Clavulanic Acid 1, 0 gr and the pill Pipemiolic Acid 400 mg, the treatment has been reached (the length of therapy at patients depends on the number of bacteria - Escherichia Coli in ml). The combination of medicines: the antibiotic - 1 pill Amoxicillin + Clavulanic Acid 1, 0 gr and uro - antiseptic (urinary antiseptic) 1 pill Pipemiolic Acid 400 mg, showed to be a cure of choice in the treatment

of urinary infections caused by Escherichia Coli. Hence, there is need of cooperation among the doctors of Primary Health and the doctors – microbiologists for total patient curing.

ОДЛИЧНО ИСКУСТВО ВО ЛЕКУВАЊЕТО НА БАКТЕРИСКИТЕ УРИНАРНИ ИНФЕКЦИИ СО КОМБИНАЦИЈА НА АНТИБИОТИК И УРОАНТИСЕПТИК

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ВОВЕД: Бактериските уринарни инфекции се честа патологија меѓу населението во Берово и општината. Најчеста бактерија која е предизвикувач на тие инфекции е Escherichia Coli. Таа бактерија е многу резистентна на голем број антибиотици и уроантисептици и затоа лекувањето трае подолго време и е според антибиограм направен од доктор – микробиолог.

ЦЕЛ: Да ги прикажам моите одлични резултати во лекувањето на уринарните инфекции предизвикани од бактеријата Escherichia Coli со комбинацијата на лековите: антибиотикот табл. Amoxicillin + Clavulanic Acid 1, 0 gr и уроантисептикот табл. Pipemiolic Acid 400 mg.

МАТЕРИЈАЛ И МЕТОДИ: Користени се здраствените картони на 19 пациенти со дијагноза: уринарни инфекции предизвикани од Escherichia Coli (субјективни тегоби: често мокрење, печење и болка и лабораторија: крв и урина и уринокултурите на истите (број на бактерии150,000 во ml, 250,000, 350,000 и 500,000 во ml) со антибиограми за ординирање на терапија, направени на микробиологија во Здравствениот дом – Берово.

РЕЗУЛТАТИ: Лекувањето го започнуваме според антибиограм кај сите 19 пациенти: 1 табл. Amoxicillin + Clavulanic Acid 1, 0 gr на 12 часа. 6 (31, 5 %) пациенти го земаат овој антибиотик 1 недела, а потоа уште една недела пијат уроантисептик 1 табл. Pipemiolic Acid 400 mg. По две недели терапија кај тие пациенти на 24 часа контролните уринокултури се стерилни (постигната е ерадикација на предизвикувачот); 7 (360 8%) пациенти земаат 10 дена табл. Amoxicillin + Clavulanic Acid 1, 0 gr на 12 часа и уште 10 дена 1 табл. Pipemiolic Acid 400 mg. дневно и контролните уринокултури кај сите 7 пациенти се стерилни (негативни); 4 (21%) пациенти го земаат истиот антибиотик 14 дена и уште 14 дена истиот уроантисептик и кај сите контролните уринокултури се негативни. Само кај 2 (10, 7%) пациенти по 28 дена иста терапија во контролните уринокултури перзистирал причинителот на инфекциите.

ЗАКЛУЧОК: Од добиените резултати кај 19 пациенти со бактериски

уринарни инфекции предизвикани од бактеријата Escherichia Coli, кај 17 (89, 3 %) пациенти по ординирањето на табл. Amoxicillin + Clavulanic Acid 1, 0 gr и табл. Pipemiolic Acid 400 mg, е постигнато излекување (должината на земање терапија кај пациентите зависи од бројот на бактериите - Escherichia Coli во ml). Комбинацијата на лековите: антибиотикот табл. Amoxicillin + Clavulanic Acid 1, 0 gr и уроантисептикот табл. Pipemiolic Acid 400 mg, се покажа како лек на избор во лекувањето на уринарните инфекции предизвикани од Escherichia Coli. Значи, потребна е заедничка соработка меѓу докторите од примарно здраство и докторите – микробиолози за целосно излекување на тие пациенти.

MB19 BACTERIAL ISOLATES IN AMBULANTORY PATIENTS WITH URINARY INFECTIONS IN MICROBIOLOGICAL LABORATORY AT CLINICAL HOSPITAL – BITOLA IN THE PERIOD OF 2017

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Clinical Hospital - Biola

Introduction: Proving of bacterial isolate causing urinary infections is important for determining the duration and length of treatment.

Aim: To show the presence of bacterial isolates in ambulant patients with urinary infections from the Clinical Hospital – Bitola in the period of 2017.

Materials and Methods: Urinocultures were standardly processed on a blood agar and a chromogenic Uri-select substrate by making an antibiogram for the positive isolates and the total number of live bacteria in 1ml of urine.

Results: The total number of isolates was 157 (38.38%) of 409 examined samples. The most common isolates were *Escherichia coli* – 81 (48.50%), *Enterococcus species* – 53 (31.73%), *Klebsiella species* – 19 (11.37), *Proteus mirabilis* – 12 (7.18%), *Acinetobacter species* – 2 (1.19%) isolates.

Conclusion: The presence of aetiological isolates in urinary infections is different and indicates the predominance of *Escherichia coli*. Studies of isolates with an antibiogram prepared for them and the total number of live bacteria in 1ml urine is important for the proper treatment of these infections and the length of therapy.

БАКТЕРИСКИ ИЗОЛАТИ КАЈ АМБУЛАНТСКИ ПАЦИЕНТИ СО УРИНАРНИ ИНФЕКЦИИ ВО МИКРОБИОЛОШКАТА ЛАБОРАТОРИЈА ПРИ КЛИНИЧКА БОЛНИЦА БИТОЛА ВО ПЕРИОДОТ ЗА 2017 ГОДИНА

В. Радевски, Б. Илковска, С. Штаковска, Р. Трајковска, М.Паспалова, А. Насева, Т. Арифи

Клиничка болница- Битола

Вовед: Докажувањето на бактериските изолати предизвикувачи науринарни инфекци се значајни за одредување на терпијата и должината на лекувањето на истите.

Цел: Да се прикаже застапеноста на бактериските изолати кај амбулантски пациенти со уринарни инфекции од Клиничката болница Битола во периодот на 2017 година.

Материјал и методи: Уринокултурите беа стандардно обработени на крвен агар и хромогена подлога Ури-селект со изработка на антибиограм за позитивните изолатииивкупен број на живи бактерии во 1 ml урина.

Резултати: Вкупниот број на изолати беше 157(38,38%) од 409 испитани примероци. Како најчести изолати беа *Escherichia coli* -81(48,50%), *Enterococcus* species-53 (31,73%), *Klebsiella species*19 (11,37%), *Proteus mirabilis*-12(7,18%) *Acinetobacter species*-2 (1,19%) изолати.

Заклучок: Застапеноста на етиолошките изолати кај уринарните инфекции е различена и укажува предоминација на *Escherichia coli*. Испитувањата на видовите изолати со изработен антибиограм за нив како и вкупниот број на живи бактерии во 1 ml урина е важна за правилното лекување на овие инфекции и времетраење на терапија.

СЕСИЈА 3/SESSION 3
ИНФЕКЦИЈА И ИМУНИТЕТ/ INFECTION AND IMMUNITY

IN1 INFECTIONS IN TRAUMA SURGERY – ARE WE GETTING ANY BETTER?

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Introduction
Surgical site infections represent serious complication following surgery that may alter the clinical and radiological result and call into question the liability of the operator. Traditionally, risk-factors have been recognized as patient-related, surgical related and operative room-related. The aim of the present study was to verify the influence of surgical-related and operative room-related risk-factors on incidence and etiology on surgical site infections following surgery for skeletal trauma.

Materials and methods
Two-phase prospective study was conducted at the University clinic of traumatology and Institute of microbiology and parasitology – Medical faculty of Skopje. The study consisted of identifying incidence and etiology, as well as antimicrobial susceptibility of surgical site infections. In between, the program of prevention strategy regarding surgical-related and operative room related risk-factors focused on surgery residents, nurses, operative room personnel and patients was conducted. All the participants were followed for at least 3 months and the influence of the program of prevention strategy was evaluated by comparing the results of two separate phases.

Results
The results of our study showed that the incidence of surgical site infections remains close in two separate phases. In the first phase, there was predominance of Gram+ bacteria – 54% (*Staphylococcus aureus*, *Enterococcus*, *MRSA*). The microbiology results of the second phase showed that the predominance of Gram+ bacteria was much pronounced and represented 69%, while the findings of Gram – bacteria were significantly lower.

Conclusion
While the overall incidence of surgical site infections remained the same in two separate phases, the only difference that can be noticed is a change in bacterial flora (increase of Gram positive bacteria and decrease of Gram negative). The implemented educational program did not affect over the results on short term. The process decreasing the rate and severity of surgical site infections has many facets that should be followed diligently.

IN2 COMPARATIVE ANALYSIS OF CLINICAL AND MICROBIOLOGICAL FINDING IN PATIENTS WITH FASCIITIS NECROTICANS

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Fasciitis necroticans is a rapidly progressive inflammatory infection of the fascia, with secondary necrosis of the subcutaneous tissues.

Fac. necroticanshas also been referred to as hemolytic streptococcal gangrena, acute dermal gangrena, hospital gangrena and synergistic necroticans cellulitis.

Risc factors include poor immune function such as from diabetes or cancer, obesity, alcoholism, intravenous drug use, and periferal vascular disease.

At the Clinic for Plastic and reconstructive surgery, Skopje, in the period from 2013 to 2018, 6 patients with nec. fasciitis were treated. 2 of them was due to intravenous drug injection, 3 due to diabetes and one due to poor immune function.

Immediately we start with high doses of antibiotics, Lendacin and Klindamicin. We take culture immediately from the wound and second one when surgical debridement was taken. Culture results was Staphylococcus aureus, Streptococcus pyogenes and Enterococci. In one of the patient, Clostridium perfringens was involved. Because of MRSA in two of the patient, we changed with Vancomycin. HBO as adjuvant therapy was also involved.

Surgical debridement was therapy of choice, but always with antibiotics support.

IN3 THE DIFFRENCE BETWEEN FREQUENCY, CLINICAL MANIFESTATIONS AND THERAPY OF *CAMPYLOBACTER* AND *SALMONELLA* IN REPUBLIC OF MACEDONIA

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Introduction
The world wide incidence of the diarrhoeal infectious disease in 2015 is around 2.4 biliion with 1.3 million death cases. Diarrhoeal diseases account for 1 in 9 child deaths worldwide, making diarrhoea the second leading cause of death among children under the age of 5. In the report of EFSA and the European Centre for Disease Prevention and Control are presents the results of the zoonosis monitoring activities and food born outbreaks carried out in 2015 in 32 European countries. Campylobacteriosis was the most commonly reported and the increasing European Union trend for confirmed human cases since 2008 continued.
If this is the case, we got interested why the situation in our country is significantly different. Why despite the relatively constant number of S*almonella* present among the isolated bacteria, there is an extremely lower number of confirmed infections of *Campylobacter*. Namely, if the isolated bacteria in patients treated at the Clinic for Infective Diseases and Febrile Illnesses in Skopje reflect the situation in the whole country as it is the case with the other pathogens.

Methods and Materials
Comparisons between the two groups and parameters at admission and exit were performed with Student t-test, chi-square test and Mann-Whitney U test. Multivariate linear regression analysis was performed for determination of independent predictors of duration of hospitalization. The seasonal distribution of the two group was presented using histogram. SPSS statistical software (version 22.0 SPSS, Inc., North Castle, NY) was used for the analysis; two-tailed $P < 0.05$ was considered significant. Data are shown as mean ± standard deviation if not otherwise stated.

Results
The 57 patients with gastrointestinal infection were divided into two groups according to the cause of the infection diagnosed using stool culture: *Campylobacter* group of 26 patients and *Salmonella* group of 29 patients. The age of all studied patients ranged from 0.5 to 65 years, with mean of 13.5 years and a median of 6 years. There

was no significant difference between the age of *Salmonella* and *Campylobacter* infected patients. Most patients were from urban areas 83%. We have divided the examined parameters of the two groups into 3 sections: demographic and clinical, biochemical and antibiotics usage. In only 3 out of 35 parameters the differences between the two groups was borderline significant: leukocyte count at admission, blood urea and sodium. Leukocyte count at admission and sodium was higher in *Campylobacter* patients and blood urea in *Salmonella* patients. We investigated the progression of the infection disease through the change of 6 hematological parameters. The parameters were compared at admission in the ward and on discharge. Reduction rate was calculated as a division of the admission value with the exit value of the given parameter. The reduction rates showed significant decrease of leukocyte count (p=0.02) and neutrophils (p=0.01) and increase of lymphocytes (p=0.01) in *Campylobacter* during the hospital stay. Whereas in *Salmonella* patients only neutrophils showed significant decrease (p=0.01) and lymphocytes (p=0.01) showed significant increase. Furthermore we compared the rate of change (reduction rate) between the two groups of patients and did not find any significant difference .

In the linear regression analysis, we found that univariate positive correlation exists between duration of hospitalization and number of comorbidities and negative between duration of hospitalization and urban area, neutrophils at exit, erythromycin therapy, hemoglobin reduction rate and erythrocytes count reduction rate. In the multivariate linear model, as independent predictors of duration of hospitalization were found living in urban area and erythromycin therapy .

Discussion and Conclusion

In the Republic of Macedonia the number of the human salmonella infections varied from 184 cases in 2010 to 254 cases in 2016, or 348 in 2015 which indicates an incidence proportion of 17.4 cases per 100 000 people. This number is relatively constant in the last 15 years with an exception of occasional outbreaks of epidemic manifestations in certain towns. According to the statistics by the Public Health Institute, the number of the humane *Campylobacter* infections in the last 15 years has not surmounted 8 cases per year with an incidence proportion not higher than 0.1 to 0.4 cases per 100 000 people. After all we only can say that the number of Campylobacteriosis in our country is not well established.

IN4 NON-CYSTIC FIBROSIS BRONCHIECTASIS (NCFB) – HETEROGENEITY, MICROBIOLOGICAL FINDINGS AND THERAPEUTIC APPROACH

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The heterogeneity in the approach to NCFB is reflected in the various radiological and clinical assessments, the existence of different scores for the assessment of the severity of the disease, different etiological associations, diversity in the microbiological findings, difference in lung function, the existence of comorbidities... Hence a multidisciplinary approach for the patients with NCFB is needed. The heterogeneity of the patients with NCFB opens the question of phenotypization of the patients and the need for individual treatment approach.

The microbiological findings in patients with NCFB differentiate in whether they were analyzed in the exacerbation phase or in the stable phase and there were also differences in the studies which were associated with the geographical position of the researched pool. A special interest exists lately for the presence of nontuberculous mycobacteria and anaerobic bacteria as well as fungal colonization. Colonization, especially with Pseudomonas aeruginosa, has a great impact in the prognosis of the disease in patients with NCFB.

At the Clinic of Pulmonology and Allergology we analyzed retrospectively 614 hospitalizations of 366 patients with NCFB, in a time period of 78 months. The statistical analysis was done with the computer program SPSS Statistics 20. 53.6% men and 46.4% women were analyzed, with an average age of 61.3 years. The most common comorbidity associated with NCFB was chronic obstructive pulmonary disease (61.2%). 63,2% of the patients didn't have their sputum taken for analysis. 55,31% of the patients that did have their sputum taken for analysis displayed a positive sputum culture. In these samples, separately or combined with other microorganisms, Candida albicans was the most commonly isolated microorganism (49,6%) followed by Pseudomonas aeruginosa (34,4%). Next, ordered according to frequency of incidence were Acinetobacter species, Streptococcus pyogenes Gr. A, Streptococcus pneumoniae. Rarely isolated were: Aspergillus niger, Aspergillus fumigates, Staphylococcus aureus, Klebsiella pneumoniae, Enterobacter aerogenes, Escherichia coli, Stenotrophomonas maltophilia. On average, the patients with NCFB were hospitalized for 10.3 days.

The therapeutic approach in the patients with NCFB is primarily aimed at treating the etiologically associated condition and an attempt of

eradication of the microbiologically detected agent. Chronic antibiotic and/or immunomodulatory treatment should be considered. Special attention in the therapy of patients with NCFB is brought to the inhaled antibiotic treatment when Pseudomonas aeruginosa is detected. Supportive measurements are a necessary part of the therapeutic approach.

It could be concluded that there are a few potential conditions in patients with NCFB which could be treated and in that way the prognosis of the disease could be influenced. The factors that affect mortality are different from the ones that affect the quality of life and the exacerbations in patients with NCFB. The management of patients with NCFB in the future will be directed at developing tests for early etiologically-associated diagnosis, development of new biomarkers for the presence of neutrophilic inflammation, early specific etiologically-aimed treatment, usage of new inhaled antibiotic medicines, new medicines for inflammation control, measurements for hygiene and infection prevention, immunization and vaccines, and prevention and dealing with exacerbations and complications.

БРОНХИЕКТАЗИИ НЕАСОЦИРАНИ СО ЦИСТИЧНА ФИБРОЗА (БНЦФ) - ХЕТЕРОГЕНОСТ, МИКРОБИОЛОШКИ НАОД И ТЕРАПИСКИ ПРИСТАП

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Хетерогеноста во пристапот кон БНЦФ се огледа во различната радиолошка и клиничка проценка, постоење на различни скорови за проценка на тежината на болеста, различна етиолошка асоцираност, диверзитет во микробиолошкиот наод, разлика во белодробната функција, присуството на коморбидитети ...). Поради тоа е неопходен мултидисциплинарен пристап кон болните со БНЦФ. Хетерогеноста на болните со БНЦФ го отвара прашањето за фенотипизација на болните и потребата за индивидуален пристап во третманот.

Микробиолошкиот наод присутен кај пациентите со БНЦФ се разликува според тоа дали е анализиран во фаза на егзацербација или во стабилна фаза а постојат и разлики во студиите кои се често асоцирани и со географската позиција на истражувачкиот пул. Особен интерес во последно време постои за присуството на нетуберкулозни микобактерии (NTM)и анаероби како и за можната фунгална колонизација. Колонизацијата, особено со Pseudomonas

aeruginosa, има огромен импакт во прогнозата на болеста кај болните со БНЦФ.

На Клиниката за пулмологија и алергологија ретроспективно анализиравме 614 хоспитализации на 366 пациенти со БНЦФ, во временски период од 78 месеци. Статистичката анализа е направена со компјутерскиот програм SPSS Statistics 20. Беа анализирани 53.6% мажи и 46.4% жени, со просечна возраст од 61.3 година. Најчест коморбидитет асоциран со БНЦФ беше хроничната опструктивна белодробна болест (61,2%). Кај 63,2% не бил земен спутум за анализа. Кај лицата кај кои е земен спутум за анализа позитивна култура на спутум беше најдена кај 55,31%. Во овие примероци самостојно или во комбинација со други микроорганизми Candida albicans беше најчесто изолиран микроорганизам (49,6%) и Psaudomonas aeruginosa (34,4%), По честота на излолација следи Acinetobacter species, Streptococcus pyogenes Gr. A, Streptococcus pneumoniae. Ретко се изолирани: Aspergillus niger, Aspergillus fumigates, Staphylococcus aureus, Klebsiella pneumoniae, Enterobacter aerogenes, Escherichia coli, Stenotrophomonas maltophilia. Просечно, пациентите со БНЦФ беа хоспитализирани 10.3 дена.

Терапискиот пристап на болните со БНЦФ пред се е насочен кон третман на евентуалната етиолошки асоцирана состојба и обид за ерадикација на микробиолошкот детектиран агенс. Следи примена на хроничен антибиотски и/или имуномодулаторен третман. Особено акцент во терапијата на болните со БНЦФ се посветува на инхалаторната антибиотска терапија при детектиран Pseudomonas aeruginosa. Супортивните мерки се неоподен дел од терапискиот пристап.

Може да се заклучи дека постојат повеќе потенцијални состојби кај болните со БНЦФ кои може да се третираат и на тој начин да се влијае на прогнозата на болеста. Причините кои влијаат на морталитетот се различни од оние кои влијаат на квалитетот на животот и егзацербациите кај болните со БНЦФ. Менаџмент на пациентите со БНЦФ во иднина ќе се усмери кон развивање на тестови за рана етиолошк- асоцирана дијагноза, развој на нови биомаркери за присуство на неутрофилна инфламација, ран специфичен етиолошко-усмерен третман, употреба на нови инхалациони антибиотски препарати, нови лекови за контрола на инфламацијата, мерки за хигиена и превенција на инфекцијата, имунизација и вакцинации, и превенција и справување со егзацербациите и компликациите.

IN5 CHANGES IN CYSTIC FIBROSIS AIRWAY MICROBIAL INFECTIONS: AN UPDATE

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Cystic fibrosis (CF) is the most common potentially fatal genetic disorder in Caucasians, with autosomal recessive heredity, affecting around 1 in 2.500 of live births. CF is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. This results in dysfunction of the apical membrane CFTR protein, which is a chloride ion channel involved in maintaining the water and ion homeostasis on epithelial cell surfaces in the ductus of the affected organs. The clinical consequences include multi-system disease characterized by progressive pulmonary damage leading to respiratory failure, pancreatic dysfunction, liver disease that may progress to cirrhosis, gut motility problems, and elevated sweat electrolytes. Virtually all men with CF are infertile due to atresia or complete absence of the vas deferens.

Despite the various complications linked to the disease, the main cause of morbidity and mortality in CF is lung disease. In CF lungs hydration of the airway surface liquids (ASL) is diminished, resulting in thick and sticky mucus, which provides the perfect environment for bacteria to infect and propagate. In the less hydrated periciliary layer, the cilia are flattened and the ability to clear bacterial infection reduced. This impairment of the noninflammatory defence mechanism of the respiratory tract leads to early recruitment of the inflammatory defence mechanisms, dominantly with polymorphonuclear leukocytes (PMN). Therefore, from early childhood, CF patients have recurrent and chronic respiratory tract infections, resulting in an exaggerated pro-inflammatory response. Despite the overwhelming nature of this inflammatory response, it remains insufficient to eradicate infection, resulting in a vicious cycle of infection, inflammation, and mucus hypersecretion/dehydration that causes blockage of airways, progressive remodeling and destruction of the airways.

The use of medications to slow the progression of lung disease and organized management of patients in specialized CF centers has led to significant improvement in survival during the last 3decades. The mean life expectancy for patients now approaches 40 years. In parallel to the changing epidemiology of patients, recent reports have highlighted the changes that are occurring within the spectrum of organisms causing infection in CF. A relatively limited number of bacteria have been involved in lung infections and the prevalence of different pathogens varies according to the patient's age; first being usually represented by *Staphylococcus aureus* and *Hemophilus influenzae* in young children and by *Pseudomonas aeruginosa* thereafter. Several emerging pathogens have been described as responsible for severe lung infections, including *Burkholderia cepacia*

complex, *Achromobacter xylosoxidans*, *Stenotrophomonas maltophilia*, *Ralstonia* and *Pandoraea* species, methicillin resistant *S. aureus* (MRSA) and nontuberculous mycobacteria (NTM). Aspergillus fumigatus, also contribute to morbidity and mortality in CF patients. With aging, recurrent episodes of pulmonary exacerbation cause lung function decline. Infections caused by *Pseudomonas aeruginosa*, the *Burkholderia cepacia* complex (mostly *B. multivorans* and *B. cenocepacia*) and *Achromobacter xylosoxidans* persist as chronic infections and lead to respiratory failure in the terminal stage of the disease. Because of intensive antibiotic pressure, increasing rates of multidrug-resistant (MDR) bacteria are isolated over time and now represent a major concern. In the absence of adequate isolation measures, bacterial outbreaks have been reported. *Burkholderia cepacia* complex outbreaks are associated with poor outcomes and sustained mortality rates.

The driver of these changes in CF airway microbial infections is unknown, mechanisms postulated include: improved cultivation and identification, the selective pressure of antimicrobials, infection transmission and infection control practices, increasing prevalence of individuals with milder disease, and the improved survival. As lung disease and respiratory infections continues to be the hallmark feature of CF and is primarily responsible for the attributable morbidity and mortality, understanding the spectrum and role of organisms involved in CF airways disease is of paramount importance.

ПРОМЕНИ ВО МИКРОБНИТЕ ИНФЕКЦИИ НА ДИШНИТЕ ПАТИШТА КАЈ ЦИСТИЧНАТА ФИБРОЗА: СОВРЕМЕНИ СТАВОВИ

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Цистичната фиброза (ЦФ) е најчеста потенцијално фатална генетска болест во белата раса, со автосомно рецесивен начин на наследување, засегајќи 1 на 2.500 живородени. ЦФ е поредизвикана од мутации во цистичнофиброзниот трансмембранозен спроводнорегулаторен (ЦФТР) ген. Тоа резултира со дисфункција на ЦФТР протеинот во апикалната мембрана, кој претставува хлорен канал значаен за одржување на хомеостазата на вода и јоните на површината на епителните клетките во дуктусите на зафатените органи. Клинички последици се мулти-системска болест, која се карактеризира со прогресивно белодробно оштетување кое води до респираторна инсуфициенција, панкреасна дисфункција, хепатална болест која може да прогадира до цироза, проблеми со цревниот моталитет и покачени електролити во потта. Практично сите мажи со ЦФ се инфертилни поради атрезија или комплетно отсуство на vas deferens.

И покрај различните компликации поврзани со болеста, белодробната болест е главна причина за морбидитет и морталитет кај ЦФ. Во ЦФ белодробието намалена е хидрацијата на течноста која ги обложува дишните патишта, резултирајќи со густ и леплив мукус, кој претставува одлична средина за инфекција и прогресија на бактериите. Во помалку хидрираниот перицилијарен слој, цилиите се зарамнети и редуцирана е нивната способност за чистење на бактериските инфекции. Ова нарушување на неифламаторниот одбрамбен механизам на респираторниот тракт, води до рано регрутирање на ифламаторните одбрамбени механизами доминантно од полиморфо-нуклеарни леукоцити. Затоа, од раното детство, пациентите со ЦФ имаат повторувачки и хронични респираторни инфекции, што резултира со претеран проинфламаторен одговор. И покрај преобемната природа на овој инфламаторен одговор, тој останува инсуфициентен во ерадикација на инфекциите, резултирајќи со затворен круг инфекција, инфламација, хиперсекреција/дехидрација на мукус кој ги блокира дишните патишта, прогресивно ремоделирање и деструкција на дишните патишта.

Користењето на лекови кои ја успоруваат прогресијата на белодробната болест и организираното водење на пациентите во специјализирани ЦФ центри, доведе до значајно подобрување на преживувањето во последните 3 децении. Очекуваното средно преживување сега се приближува до 40 години. Паралелно со изменетата епидемиологија на пациентите, новите соопшенија ја истакнуваат промената во спектрумот на организми кои предизвикуваат инфекции кај ЦФ. Релативно ограничен број на бактериски видови беше вклучен во белодробните инфекции и нивната преваленција вариираше во зависност од возраста: прво главно претставени со Staphylococcus aureus и Hemophilus influenzae кај малите деца, а потоа со Pseudomonas aeruginosa. Се опишува и појавува на неколку патогени одговорни за тешки белодробни инфекции, вклучувајќи ги Burkholderia cepacia complex, Achromobacter xylosoxidans, Stenotrophomonas maltophilia, Ralstonia и Pandoraea соеви, метицилин резистентен S. aureus (MRSA) и не-туберкулозен микобактериум (NTM). Aspergillus fumigates исто така придонесува за морбидитет и морталитет кај пациентите со ЦФ. Со возраста, повторувачките епизоди на пулмонални егзацербации предизвикуваат опаѓање на белодробната функција. Инфекциите причинети од Pseudomonas aeruginosa, Burkholderia cepacia complex (најчесто B. multivorans и B. cenocepacia) и Achromobacter xylosoxidans перзистираат како хронични инфекции, водејќи до респираторна инсуфициенција во терминалниот стадиум на болеста. Поради интензивен антибиотски притисок, со текот на времето се зголемува стапката на изолирање на мултирезистентни бактерии, што сега претставуваат голема загриженост. Во отсуство на соодветни мерки за изолација, се соопштува појава на епидемии, како на пр. со Burkholderia cepacia complex, асоцирани со лош исход и одржлив морталитет.

Факторот кој ги предизвикува овие промени во микробните инфекции на дишните патишта е непознат, но претпоставени механизми се: подобреното култивирање и идентификација, селективен притисок на антимикробните средства, трансмисија на инфекциите и практиките за контрола на инфекциите, зголемената преваленција на лица со поумерена болест и подобрување на преживувањето. Бидејќи белодробната болест и респираторните инфекции продолжуваат да бидат главна карактеристика на ЦФ и се првенствено одговорни за припишаниот морбидитет и морталитет, разбирањето на спектарот и улогата на организмите вклучени во ЦФ белодробната болест е од огромно значење.

IN6 ANTIMICROBIAL RESISTANCE IN NON-INVASIVE *STREPTOCOCCUS PNEUMONIAE* STRAINS

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The antimicrobial resistance of *Streptococcus pneumoniae*, or pneumococcus, is a global problem. In our study, we examined antibiotic susceptibility to beta-lactams, macrolides and fluoroquinolones, of 15653 non-invasive pneumococcal isolates, recovered from respiratory samples (nasal swabs, sputa, tracheal aspirates, bal) from patients (hospital/outpatients) treated at the University Clinics of the Mother Theresa Clinical Campus Skopje, during 20-years period (1997-2016). Susceptibility to antibiotics was determined by disc diffusion method on Mueller-Hinton agar. Penicillin resistant pneumococci (PRP) were screened with oxacillin (1μg/ml). The minimal inhibitory concentration (MIC) of 145 isolates of *Streptococcus pneumoniae* (2006-2008) was examined with Vitek-2 System and for 34 isolates we performed comparative evaluation between Vitek-2 System and E test Vitek-2 System and E test (*bioMerieux,* France) (2017-2018). Interpretation was according to Clinical and Laboratory Standards Institute (CLSI) and European Committee on Antimicrobial Susceptibility testing (EUCAST) recommendations. Resistance of pneumococcal isolates to penicillin (disc diffusion method) in the period 1996-1998 was 13.5%, 1999-2000 15.1%, 2001-2005 26.2%, in 2006-2008 30.8%, in 2012 40.3%, in 2013, 40.5%, in 2014, 29.7%, in 2015 26% and in 2016 21%. Erithromycin resistance increased from 6.1% in 1996-98, 7.7% in 1990-2000, 15.1% in 2001-05, 28.9% in 2006-08, in 2012 30.8%, in 2013, 27.1%, in 2014, 30.5%, in 2015 30.8% and in 2016 37.7%. Pneumococci have not demonstrated statistically significant resistance to ceftriaxone (1.5% in 1996-98, 2.8% in 1999-2000, 0.9% in 2001-05, 1.1% in 2006-08, 0.7% in 2012, 0.1% in 2013, 0.6% in 2014, 0.1% in 2015, 001% in 2016).

Resistance to ciprofloxacin was 2.7% in 1996-1998, 1.5% in 1999-2000, 1.6% in 2001-2005, 3.1% in 2006-2008 and resistance to moxifloxacin was 0.4% in 2012, 0.1% in 2013, 0.8 in 2014, 0.3 in 2015 and 0.7 in 2016. Determination of MIC (CLSI) to penicillin showed that, during the period 2006-2008, out of 145 pneumococcal isolates 20.6% were resistant, 43.4% intermediate susceptible and 35.8% were susceptible. The overuse and misuse of antibiotics for the treatment of respiratory tract infections has been considered one of the major reasons for the emergence of resistance in bacteria against antibiotics. Surveillance of antibiotic resistance in *Streptococcus pneumoniae* is important for the appropriate choice of empirical therapy, to detect new resistance developments in a timely manner, as to monitor the effect of campaigns on resistance rates and prevention with vaccines.

Key words: *Streptococcus pneumoniae*, antibiotics, antimicrobial resistance

АНТИМИКРОБНА РЕЗИСТЕНЦИЈА НА НЕ-ИНВАЗИВНИ ИЗОЛАТИ НА *STREPTOCOCCUS PNEUMONIAE*

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Антимикробната резистенција на *Streptococcus pneumoniae*, или пневмококот, е глобален проблем. Во нашата студија, испитувавме осетливост кон бета-лактами, макролиди и флуорокинолони, на 15653 не-инвазивни пневмококни изолати добиени од респираторни примероци (назални брисеви, спутуми, трахеални аспирати, бал) од пациенти (болнички/амбулантски) лекувани на Универзитетските клиники на Клиничкиот кампус "Мајка Тереза" Скопје, во период од 20 години (1997-2016). Осетливоста кон антибиотиците беше испитувана со диск дифузиона метода на Милер-Хинтон агар. За скрининг на резистенцијата на пневмококите кон пеницилин (ПРП) користевме оксацилин (1μg/ml). Минималната инхибиторна концентрација (МИК) на 145 изолати на изолати на *Streptococcus pneumoniae* (2006-2008) година беше испитувана со Vitek-2 систем, а кај 34 се спроведе компаративно испитување со Vitek-2 системот и Е тест (bioMerieux, Франција) (2017-2018). Толкувањето на МИК беше во согласност со препораките на Институтот за клинички и лабораториски стандарди (CLSI) и Европската комисија за тестирање на антимикробна осетливост (EUCAST). Резистенцијата на пневмококите на пеницилин (дифузиона метода) во периодот 1996-1998 година изнесува 13,5%, 1999-2000 15,1%, 2001-2005 26,2%, во 2006-2008 30,8%, во 2012 година 40,3%, во 2013 година 40,5% , во 2014 година, 29,7%, во 2015 година 26%, а во 2016 година 21%. Резистенцијата на еритромицин

се зголемила од 6,1% во 1996-1998, 7,7% во 1990-2000, 15,1% во 2001-05, 28,9% во 2006-08, во 2012 година 30,8%, во 2013 година 27,1%, во 2014 година 30,5% 2015 30,8%, а во 2016 година 37,7%. Пневмококите не покажаа статистички значајна резистенција кон цефтриаксон (1.5% во 1996-98, 2.8% во 1999-2000, 0.9% во 2001-05, 1.1% во 2006-08, 0.7% во 2012, 0.1% во 2013, 0.6% во 2014 година, 0,1% во 2015 година, 001% во 2016 година). Резистенцијата кон ципрофлоксацин изнесуваше 2,7% во 1996-1998, 1,5% во 1999-2000, 1,6% во 2001-2005, 3,1% во 2006-2008 и кон моксифлоксацин 0,4% во 2012 година, 0,1% во 2013 година, 0,8 во 2014 година, 0,3 во 2015 и 0,7 во 2016 година. Според CLSI, резистентни на пеницилин беа 20,6%, умерено осетливи 43,4% а осетливи беа 35,8%. Прекумерната употреба и злоупотребата на антибиотици за третманот на инфекции на респираторниот систем се смета за една од главните причини за појава на резистенција на бактериите кон антибиотици. Следењето на резистенцијата на антибиотици кај *Streptococcus pneumoniae* е важно за соодветен избор на емпириска терапија, за навремено откривање на новите случувања во однос на појавата на резистенција, како и за следење на ефектот од кампањите за антибиотска контрола или превенција со вакцини.

Клучни зборови: *Streptococcus pneumoniae*, антибиотици, антибиотска резистенција

IN7 EVALUATION OF THE SPUTUM QUALITY ASSESSMENT AND ITS CORRELATION WITH THE ISOLATED RESPIRATORY PATHOGENS

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Introduction The results of bacteriological examination of expectorated sputum specimens are difficult to interpret, especially when young children are inquest.

Aims To analyze the correlation between the sputum quality assessment and isolation of the respiratory pathogens

Methods In a period from 01.07.2017 to 30.12.2017, a total of 1485 sputa were quality assessed after slides of the specimens had been gram- stained and microscopically examined at 100x magnification. The sputum quality was judged by the relative number of squamous

epithelial cells (SEC) which suggests oropharyngeal contamination, and inflammatory cells, which suggests material derived from the site of an active infection. "+" Q-score indicated predominant inflammatory cells, whereas"–/0" Q -score- predominant SEC or equivocal presentation of the both cell types. The cultures of all sputum specimens were compared with the assessment of their quality.

Results There was a statistically significant connection between the positive cultures for respiratory pathogens and the + Q-score quality of the sputa, and the most isolated bacteria were *S. pneumoniae*, *M. catarrhalis*, *H. influenzae* with 36.1%, 16.5% and 15.8%, respectively. Of those with -/ 0 Q-score, the most often isolated were *E. coli*, *S. aureus*, *P. aeruginosa*. Of all isolated *E.coli*, ESBL-producers were 86%, and of all isolated *S.aureus*, MRSA were 37%.

Conclusion Sputum quality assessment is a useful tool for recognizing possible colonizing resistant bacteria, while the actual antibiotic treatment should not be highly considered.

ЕВАЛУАЦИЈА НА КВАЛИТЕТОТ НА ПРИМЕРОЦИТЕ ОД ДОЛНИТЕ ДИШНИ ПАТИШТА И НЕГОВА КОРЕЛАЦИЈА СО ИЗОЛИРАНИТЕ РЕСПИРАТОРНИ ПАТОГЕНИ

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Вовед Експекторираните примероци се контаминирани од орофарингеалната флора во која може да се присутни и потенцијални патогени, правејќи ја микробиолошката интерпретација доста комплексна. Добивањето на квалитетен искашлок е особено тешко кај детската популација.

Цел Анализа на корелацијата помеѓу проценката на квалитетот на спутумите и изолацијата на респираторни патогени.

Материјал и методи Во периодот од 01.07.2017 до 30.12.2017, од вкупно 1485 примероци од долните дишни патишта беа направени размаски обоени по Грам и микроскопирани на мало зголемување (100x). Проценка на квалитетот на примероците беше направена преку анализа на целуларните компоненти, при што површните епителни клетки (SEC) сугерираа на орофарингеална контаминација, додека инфламаторните клетки сугерираа на материјал добиен од место на активна инфекција. "+" Q-score значи предоминација на инфламаторни клетки, додека"-/0"Q-score предоминација на SEC или подеднакво присусво на двата целуларни типа. Пораснатите култури беа споредувани со микроскопската проценка на квалитетот на спутумите.

Резултати Постоеше статистичка сигнификантност помеѓу примероците со "+" Q-score и изолацијата на респираторни патогени, при што најчесто изолирани беа *S.pneumonie* со 36,1%, *M.catarrhalis* со 16,5% и *H.influenzae* со 15,8%. Од оние со "-/0"Q-score, најчесто беа изолирани *E.coli, S.aureus* и *P.aeruginosa,* при што од сите изолирани *E.coli,* ESBL-продуцирачки беа 86%, додека од изолатите на *S.aureus,* MRSA беа 37%.

Заклучок Микроскопската проценка на квалитетот на спутумите помага во дефинирањето на веројатна колонизаторска флора, која во голем процент е мултирезистентна, додека од друга страна, потребата од антибиотик е крајно дискутабилна.

IN8 SEROLOGIC IMMUNE RESPONSE TO RESPIRATORY PATHOGENS, DIFFERENCES IN CHILDREN AND ADULTS

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Clinical bacteriological techniques are insufficient in determination of the entire palette of respiratory pathogens. Attention has been pointed on serological diagnosis, especially indirect immunofluorescence

Aim: The aim of this study was to analyze the results obtained examining the presence of most often detected specific IgM and IgG antibodies towards 9 causes of atypical pneumonia.Two groups were examined , children and adults

Material: The study comprised of sera collected during 2015- 2017 (3years), a total of 11445 sera. The number of sera that were analysed from children was 2841 (24,9%), and from adults 8604 (75,1%).. All sera were diluted adequately for detection of IgM and IgG classes of antibodies.

Method: Indirect Immunofluorescence (IIF) was performed for detection of specific abs to 4 bacterial (*Legionella pneumophila, Mycoplasma pneumoniae, Coxiella burnetii* and *Chlamydia pneumoniae*) and 5 viral ags (Adenovirus, Respiratory syncytial virus - RSV, Influenza A v., Influenza B v., Parainfluenza v. 1, 2, 3) ags, placed on a slip (Pneumoslide Vircell)

Results: From the total number of 11445 sera examined for the 3 years period,

5298 (46,2%) were positive to at least one class of antibodies, IgM or IgG and 6147 (53,8%) negative for specific IgM or IgG antibodies to the mentioned bacterial and viral antigens. The analysis of IgM antibodies

in children pointed *Mycoplasma pneumonie* abs as the most frequently detected antibacterial abs- 17,3%, and Infl.B virus abs 2,5% as for antiviral abs. In adults for bacterial ags most often detected were abs to ***Mycoplasma pn-*** 9,1%, and *Legionella pn*. 4,9%, and for viral ags to **Infl.B** virus- 1,06%. The incidence of the detected IgG abs in children showed predomination of ab to Adenovirus (2,1%). The analyses in adults have shown that **IgG** abs to RSV (20,1%) and Adenovirus (8,5%) predominated throughout examined period.

Conclusion: The immune response to respiratory infections in children and adults pointed following conclusions:

- Detection of IgM antibodies has indicated *predominance of antibacterial abs* in both examined groups, but from different origin*Mycoplasma pneumoniae* for children and *Mycoplasma pneumoniae* and *Legionella pneumophila* for adults.
- Detection of IgG antibodies has indicated *predominance of antiviral abs* in both groups throughout the examined 3 years.

Key words: respiratory diseases, atypical pneumonia, bacterial and viral causes of respiratory infections

IN9 ROC ANALYSIS OF SERUM IGA AND IGG ANTIBODY LEVELS ON THE CHLAMYDIAL MOMP ANTIGEN

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INTRODUCTION: The real challenge for diagnosing acute chlamydial infection is the asymptomatic nature of the infection, as well as the specific developmental cycle of *Chlamydia trachomatis*. Although PCR is the only recommended method for the diagnosis of acute chlamydial infection, it seems that for most laboratories in low-income countries this standard is still unavailable due to high costs with technical complexity in relation to space, equipment and human resources.

OBJECTIVE: Improving the diagnostic efficacy of the immunoassay test (ELISA) by ROC analysis of the serum level of IgA and IgG antibodies to the chlamydial MOMP antigen.

MATERIAL: The study included 225 sexually active respondents of both sexes who tested for chlamydial infection in Institute of Public Health Kragujevac.

METHODS: For the detection of an acute chlamydial infection among other tests, an immunoenzyme test (ELISA) was used to detect the serum IgA and IgG antibody levels on the chlamydial MOMP antigen (Euroimun, Lubeck, Germany). Diagnostic efficiency of the test was determined in relation to the results obtained by the gold standard RT-PCR method (Sacace Biotechnologies, Como, Italy).

RESULTS AND CONCLUSION: Based on the *cut-off* values recommended by the manufacturers, IgA (sen: 44,4%; spec: 94,2%; PPV: 26,7%; NPV: 97,3%) and IgG (sen: 66,6%; spec: 85,4%; PPV: 18,1% NPV: 98,2%) show low sensitivity and positive predictive value with satisfactory specificity and negative predictive value. The values of the Youden's index are low in both cases, but IgG (52.6%) has slightly higher values than IgA (38.7%). *Cut-off* values (IgA: S / Co ≥ 0.87; IgG: Ru / ml ≥ 17.57) are defined based on the characteristics of the ROC curve (IgA: AUC = 0.952; IgG: AUC = 0.930). Using new *cut-off* values, we have shown that with superior sensitivity (100%) and satisfactory specificity (84%) it is possible to correct the diagnostic efficiency of the IgG test. Also, with a sensitivity of 77.8% and a specificity of 90.2% of IgA, there is a good balance of sensitivity and specificity, which is also confirmed by the Youden's index, which is 84%. ROC analysis of the serum level of IgA and IgG antibodies to the chlamydial MOMP antigen and the definition of new *cut-off* values has significantly improved the diagnostic efficacy of these tests.

IN10 INVESTIGATION OF ETIOLOGY BY MULTIPLEX PCR METHOD IN AUTOPSY CASES CONSIDERED FOR LOWER RESPIRATORY TRACT INFECTION: A 5-YEAR STUDY

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INTRODUCTION: Lower respiratory tract infections (LRTI) caused by viral agents; is one of the leading causes of morbidity and mortality, especially in children, elderly and immunocompromised patients. Postmortem examination of LRTI cases can increase the overall

proportion of cases with a definitive diagnosis, and importantly, provide information that increases our understanding of the causes of LRTI. In our study, we aimed to search etiology by Multiplex PCR method in autopsy cases considered for LRTI, with histopathological findings to make sure that the identified viruses were the actual infectious agents or not and to evaluate any consideration in death causes.

MATERIAL-METHOD: In this study, we included a total of 836(367 female, 469 male) cases consisting of sudden death cases from infantile-pediatric age group and autopsy cases considered for lower respiratory tract infection in our laboratory between January-2013 and May-2017. Distribution of cases according to age and gender were shown in Table1.Tracheal swab samples in 731 (87.4%) cases taken from autopsy and paraffin embedded lung tissue samples in 105 (12.6%) cases considered for lower respiratory tract infection by histopathological examination were studied by usage of FTD Respiratory 21(Fast-track Diagnostics Luxemburg)kit, in multiplex PCR method. Paraffin embedded tissue samples were adjusted to deparaffinization before analysis.They were evaluated for Influenza A/B, Influenza A(H1N1)swl, RSVA/B, adenovirus, hMPV A and B, coronaviruses 229E/NL63/OC43/HKU1, parainfluenza viruses 1-2-3-4, rhinovirus, enterovirus, parechovirus, humanbocavirus and *Mycoplasma pneumoniae*.

RESULTS: Whereas at least one virus was detected by PCR in 380 (45.5%) of total 836 cases, any viral agent wasn't identified in 456 (54.5%) of cases (Table 2). Only one viral agent was detected in 259 (30.95%), two viral agents were in 104 (12.5%) and three viral agents were in 17 (2.05%) cases. Rhinovirus [hRV; 161 (42.3%)] and Adenoviruses [AdV; 73 (19.2%)] were most prevalent.Furthermore, postmortem histopathological examination of the lung tissues are shown in Table 3.

CONCLUSION: The systematic implementation of a postmortem respiratory viral diagnosis combined with autopsy and histological examinations thus seem evident. The multidisciplinary studies where microbiologists, pathologists and forensic medicine experts take part would both increase the success of postmortem microbiology and contribute to preventing epidemics, thereby creating a healthy population.

Table 1: The distribution of cases according to gender and age group

	Gender					
	Female		Male		Total	
Age group	n	%	n	%	n	%
0-1 mounth	89	10.6	106	12.7	195	23.3
1 mounth-18 age	262	31.3	329	39.4	591	70.7
>18 age	16	1.9	34	4.1	50	6
Total	367	43.9	469	56.1	836	100

Table 2: The distribution of cases in which at least one viral agent is detected		
	Virus	**Number of cases (%)**
At least one agent	Rhinovirus	157 (18.8)
	Adenovirus	39 (4.7)
	RSV A/B	37 (4.4)
	Influenza A	27 (3.2)
	Humanbocavirus	20 (2.4)
	Coronavirus 229	17 (2.0)
	Parainfluenza 3	14 (1.7)
	Coronavirus 63	11 (1.3)
	hMPV	11 (1.3)
	Enterovirus	11 (1.3)
	Coronavirus 43	10 (1.2)
	Parainfluenza 4	8 (1.0)
	Mycoplasma pneumoniae	8 (1.0)
	Coronavirus HKU	3 (0.4)
	Influenza B	3 (0.4)
	Parainfluenza 2	2 (0.2)
	Parainfluenza 1	1 (0.1)
	H1N1	1 (0.1)
Negative		456 (54.5)
Total		836 (100)

Table 3: Postmortem histopathological examination of the lung tissues:		
	n	**(%)**
No infection found	318	38
Interstitial pneumonia	168	20.1
Lobular pneumonia, purulent bronchitis	187	22.4
Neonatal pneumonia	27	3.2
Diffuse alveolar damage, early stage lobular pneumonia	46	5.5
Allergic bronchitis, bronchiolitis	3	0.4
Necrotizing granulomatous inflammation	2	0.2
Neonatal hyaline membrane disease	8	1
Superimposed bacterial infection	38	4.5
Autolysis	8	1
Could not be reached	31	3.7
Total	836	100

IN11 BRUCELLOSIS A NEGLECTED DISEASE, STILL A SERIOUS CONCERN FOR PUBLIC HEALTH, A REVIEW AT THE UNIVERSITY HOSPITAL CENTER "MOTHER TERESA" IN TIRANA, ALBANIA

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Introduction: Brucellosis is an important re-emerging zoonosis with a worldwide distribution. Brucellosis is also called the disease of the Mediterranean, and Albania is the country with the highest number of affected (in the Mediterranean).

Aim: To take a look at the prevalence of the disease and link the clinic to the lab.

Methods: In this study retrospectively analyzed the data available at the Microbiological Laboratory of QSUT for the period 2012-2017. During this period were analyzed 360 samples of patients who had clinical indications for brucellosis. These samples were subjected to Wright test.

Resultats: A total of 360 samples were enrolled, 135 (37.5%) women and 225 (62.5%) men, 86 (23%) had a positive Wright test. Among the samples 20% had a titer 1:1280, 3% higher than 1:1280, 19% 1:40, 17% 1:80, 15% 1:40, 10% 1:320, 9% 1:640. The units that have sent the largest number of samples are infectious (194 samples, with 64 positive), neurological (46, with 8 positive) and pediatric (49, with 3 positive cases) units. It is also noted that the number of cases that this test has needed has, increased from 2012 to July 2017.

Conclusions: 23% of the samples were positive for brucellosis. Previals titre 1: 1280 in 19% of cases. Wright test's clinical evidence of positivity is in 82% of cases. It is important to draw attention to the fact that brucellosis is very active, as well as in the important liaison of the clinic with the laboratory in order to take the necessary measures in the immediate and proper intervention of the disease.

Keywords: brucellosis, Wright test, microbiology.

IN12 CLINICAL ASPECTS OF THE EMERGENCE AND TREATMENT OF OPHTHALMIC INFECTIONS

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Introduction: Chronic ophthalmic diseases require the use of multicausal therapy, which in most diseases is associated with the use of medications in the form of eye drops, creams and gels that are ordinated subconjunctivaly or intraocularly. The long-term use of these drugs, due to non-compliance with the instructions of the used medicines and the incorrect application of these drugs is inevitably associated with the appearance of secondary superinfection of the eyes.

Aim of the study: The main purpose in the creation of the study was to see the frequency of primary and recurrent infections in people with low vision and blind people, which have been conservatively treated with local ophthalmic preparations such as eye drops, creams and gels for a long time.

Material and methods: The research represents an analytical cross-sectional study conducted on 311 blind and persons with severe visual impairment who used local ophthalmic drugs for a period of 5-15 years due to primary ophthalmic disease. The examinees suffered from various ophthalmic diseases and the research is part of an epidemiological study of blindness conducted in the Skopje region.

Results: From a total of 311 participants in the research, in 182 (58.5%) are registered primary or recurrent ophthalmic infections. Of those, from 63 people (34.6%) conjunctival swab is taken, in 45 (71.4%) are isolated microbial agents, of which the most common agens were Staphylococcus aureus, epidermidis and saprophyticus and Streptococcus. In the remaining 129 (41.5%) examined persons, according to the anamnestic data and the available documentation, ophthalmic infections are not registered.

Conclusion: The local application of ophthalmic medications in the form of eye drops, creams and gels requires strict adherence to the accompanying instructions, which explicitly require their application within a period of 21-30 days of initial use, with strict respect to the principles of antisepsis in relation to hand hygiene and the local drug application.The results of the study indicate that during any ophthalmic infection prior to the administration of the local medicines, a previous microbiological investigation with swabs with an antibiogram is necessary and correction of the ordinated antibiotic is required depending on the results of the antibiogram.

Key words: ophthalmic infections, people with low vision, blind people

КЛИНИЧКИ АСПЕКТИ ОД ПОЈАВАТА И ТРЕТМАНОТ НА ОФТАЛМОЛОШКИ ИНФЕКЦИИ

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Вовед: Хроничните офталмолошки заболувања налагаат примена на мултикаузална терапија, која во најголем број од заболувањата е поврзана и со примена на медикаменти во вид на капки, масти и желеа кои се ординираат субкоњуктивално или интраокуларно. Долготрајната примена на овие лекарства, заради непридржување кон упатствата на применетите лекови и неправилната нивна апликација, неминовно е поврзана со појава на секундарни суперинфекции на очите.

Цел на трудот: Основна цел при изработката на трудот ни беше да ја согледаме зачестеноста на примарни и рецидивирачки инфекции кај слабовидни и слепи лица, кои долготрајно конзервативно се лекувани со локални офталмолошки препарати во вид на капки, масти и желеа.

Материјал и методи: Трудот представува аналитичка студија на пресек (cross-sectional study), спроведена врз 311 слепи и лица со тешко оштетеување на видот, кои во временски период од 5-15 год., користеле локални офталмолошки препарати заради основното офталмолошко заболување. Испитуваните лица боледувале од различни офталмолошки заболувања, а истражувањето представува дел од епидемиолошка студија за слепилото спроведена во Скопскиот регион.

Резултати: Од 311 учесници во истражувањето, кај 182 лица (58,5%) се регистрирани примарни или рецидивирачки офталмолошки инфекции. Од нив, кај 63 лица (34,6%) земен е коњуктивален брис, при што, кај 45 лица (71,4%) се изолирани микробиолошки агенси, од кои најчести причинители се Staphylococcus aureus, epidermidis и saprophyticus и Streptococcus. Кај останатите 129 (41,5%) испитувани лица, спрема анамнестичките податоци и достапната документација, офталмолошки инфекции не се регистрирани.

Заклучок: Локалната примена на офталмолошки медикаменти во вид на капки, масти и желеа налага стриктно придржување кон приложените упатства, кои изричито налагаат нивна примена во временски период од 21-30 дена од првичната употреба, со стриктно почитување на принципите на антисепса во однос на хигиена на рацете и локалната апликација на лекот. Резултатите укажуваат дека при секоја офталмолошка инфекција пред апликацијата на локални медикаменти потребно е предходно микробиолошко иследување со брис со антибиограм и корекција на ордираниот антибиотик во зависност од резултатите од антибиограмот.

Клучни зборови: офталмолошки инфекции, слабовидни лица, слепи лица

IN13 ATYPICAL BACTERIAL AND VIRAL PROFILE IN RESPIRATORY TRACT INFECTIONS

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Introduction: Atypical bacterial and viral pathogens play an important role in respiratory tract infections. Since the conventional methods can identify only 30-50% of these infections, the serological tests are used commonly for laboratory diagnosis of these agents.

Objective: The aim of study was to assess the identification of atypical bacterial and viral profile of respiratory tract infections.

Material and methods: The survey was conducted during 2016 and 2017 at the private microbiology laboratory "Mikrobiologjia" in Prishtina, Kosovo. Study included blood samples from 139 patients referred from both community and hospital care level. Samples were tested with commercial indirect immunofluorescent assay (IFA, Pneumo-slide, Vircell SL, Spain) in which nine different antigens were fixed onto a slide.

Results: A total of 139 cases were processed with Pneumoslide during two year of survey period. Of total number of patients, 72 (51.8%) of them were female. The predominant age group affected with atypical agents was between 20-64 years (71.2%) and > 65 years in 17.3% of cases. Coinfection was noticed in 16 cases (11.5%).

IgG and IgM positivity rates for the agents were as follows, respectively; 0 and 6.5% for *Legionella pneumophila* and *Coxiella burnetii*, 34.5% and 24.5% for *Mycoplasma pneumonia* and 16.5% and 18.7% for *Chlamydia pneumonia*. IgG and IgM positivity rates among viral pathogens were as follows, **23.7**% and 0 for adenovirus, 51.8% and 4% for Respiratory syncytial virus, 23.2% and 0 for Influenza A virus, 28.1% and 4.9% for influenza B virus; 10 cases were positive for IgM for parainfluenza viruses type 1-3.

Conclusions: Atypical bacteria and viruses play an important role as etiological agents

Pneumoslide IgM is useful diagnostic rapid test. The most predominant agent was *Mycoplasma pneumonia*.

Key words: Atypical bacteria, viruses, pneumoslide

IN14 LEGIONELLA PLEUROPNEUMONIA IN CHILDHOOD - A CASE REPORT

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Introduction: Genus "Legionella" includes over 40 species with more than 60 serological types .The most important and pathogenic for humans is Legionella pneumophila (serotype 1). It is an intracellular bacteria resistant to beta-lactam antibiotics . It can be found especially on wet and warm surfaces. Clinically manifests itself from a milder form to severe pneumonia . Symptoms-fever, chills, sweating , headache, fatigue, muscle pain , cough , chest pain, dyspnea. Today the simplest and most useful method for diagnosing is Pneumoslide . The choice of therapy are macrolides.

Case report: Female 7 year old child with cough, decreased appetite, difficulty breathing. Status- afebrile, pale, with dyspnea, cough. On auscultation vesicular breathing, weakened left,with a pneumonic finding. No BCG scar. From the investigations: Blood count with leucocytosis, elevated sedimentation, CRP, sputum bacteriologically negative.Pneumoslide IF IgM : positive for Legionella pneumophila ser. 1, Influenza A and Influenza B. Immunochromatographic test for the qualitative detection of Legionella pneumophila antigen ser. 1 in urine: positive . Echo abdomen and thorax: Left supradiaphragmal inhomogeneous zone, expanded pleural space. The left diaphragmatic dome and pleura thickened, hyperechogenic, small anechogenous zone-effusion. Chest X-ray - pneumopneumonia leftsided. Therapy and decursus: antibiotic therapy with Azithromycin for 10 days , corticosteroid, oxygen support of 2 l / min, respiratory exercises. We followed improvement of general condition pulmonary findings and normalization of inflammatory markers, as well as control chest X-ray.

Conclusion: Legionella pneumonia is present in childhood. Thanks to rapid and accurate diagnostics with pneumoslide and qualitative detection of antigen in the urine, as well as timely effective macrolide therapy , the outcome of the disease is positive.

ЛЕГИОНЕЛА ПЛЕУРОПНЕВМОНИЈА ВО ДЕТСКА ВОЗРАСТ- ПРИКАЗ НА СЛУЧАЈ

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Вовед: Во родот "легионела" влегуваат над 40 вида со над 60 серолошки типа.Најважна и најпатогена за човекот е Legionella

pneumophila (серотип 1). Претставува интрацелуларна бактерија отпорна на бета-лактамски антибиотици. Ја има насекаде околу нас, а посебно на влажни и топли површини. Клинички се манифестира од поблаг облик па се до тешки пневмонии. Симптоми -покачена температура , треска ,потење ,главоболка, замор, болка во мускули, кашлица, болка во градите, диспнеа. Денес наједноставен и најкорисен метод за дијагностика е Pneumoslide. Прв лек на избор во терапијата се макролидите.

Приказ на случај: Женско 7 годишно дете со кашлица, намален апетит, отежнато дишење. Статус- афебрило, бледо ,диспноично со надразителна кашлица. Аукултаторно везикуларно дишење, ослабено лево, со пневмоничен наод .Белег од БеСеЖе нема. Од иследувањата: Крвна слика со леукоцитоза, забрзана седиментација, покачено ЦРП, спутум бактериолошки негативен. Pneumoslide IF IgM : позитивен за Легионела пнеумофила сер. 1, Инфлуенца А и Инфлуенца Б. Имунохроматографски тест за квалитативна детекција на антиген на Легионела пнеумофила сер. 1во урина : позитивен .Ехо абдомен и торакс : хепар, лиен, панкреас и бубрези уредни. Лево супрадијафрагмално инхомогена зона, проширен плеврален простор. Левата дијафрагмална купола и плевра задебелени, хиперехогени, мала анехогена зона-излив. Ртг пулмо - Наод во прилог на пнеуропневмонија лево. Терапија и декурзус: антибиотска терапија со Азитромицин 10 дена, кортикостероид, кислородна подршка од 2л/мин, респираторни вежби. Бележевме постепено подобрување на општа состојба, регресија на белодробниот наод и нормализирање на маркерите за инфламација,како и контролниот Ртг наод.

Заклучок: легионела пневмонијата е присутна во детска возраст. Благодарение на брзата и точна дијагностика со пневмослајд и квалитативна детекција на антиген во урина, како и навремената ефикасна терапија со макролиди, исходот од заболувањето е позитивен.

IN15 PROCALCITONIN GUIDED ANTIBIOTIC THERAPY IN LOW RESPIRATORY INFECTIONS- META ANALYSIS

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Infections in respiratory tract are the most important reason for antibiotic treatment and thereby contribute to the increasing rate of antibiotic multi-resistance. In 30-50% use of antibiotics are shown to be unnecessary. Multiple randomized controlled trials have demonstrated that procalcitonin (PCT) is a good biomarker for

bacterial systemic infections and for initiation and discontinuation for antibiotic treatment in low respiratory infections (LRI). Use of PCT is recently approved by FDA . In stabile patients with LRI, when the level of PCT is < 0,25µg/l can guide the decision to withhold antibiotics and recheck PCT after 6-24 hours. When PCT is > 0,25µg/l , start with antibiotics, repeat PCT after 3 -7 days, when PCT < 0,25µg/l in correlation with clinical improvement or decrease 80% of initial PCT level then stop therapy early. A 2012 Cochrane meta- analysis based on data from 14 randomized controlled trials found reduction in initial use of antibiotics, without an increase in mortality rate or failure treatment. In 2017 in Cohrane data base analysed 18 new randomized controlled trials to acsses safety and efficacy of using PCT for starting or stopping antibiotics. The mortality and treatment failure as primary endpoints and duration of antibiotic treatment for secondary endpoints. The results in primary endpoints shown decrease in mortality from 8,6 to 10,0% in control group . In secondary endpoints : 2,4 day reduction in antibiotic exposure and lower risk of antibiotic related side effects.

Authors conclusions : Update meta analysis of individual participant 6708 data from 12 countries shows that the use of PCT to guide initiation and duration of antibitic treatment results in lower risk of mortality, lower antibiotic consumptation and lower risk for antibiotic related side effects. Future research is needed to confirm the results in immunosuppressed patients and patients with non- respiratory infections.

ПРОКАЛЦИТОНИН АНТИБИОТСКИ ВОДИЧ КАЈ ДОЛНО РЕСПИРАТОРНИ ИНФЕКЦИИ - МЕТА АНАЛИЗА

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Инфекциите на респираторниот тракт се најчеста причина за употреба на антибиотска терапија. Во 30-50% употребата на антибиотици се покажува како непотребна. Продолжена антибиотска терапија води до колонизација со резистентни соеви на микроорганизми , зголемен ризик од несакани ефекти како и Clostridium dificile колитис. Повеќе рандомизирани студии покажаа дека прокалцитонин (ПЦТ) е добар биомаркер за системска бактериска етиологија, како и улогата на ПЦТ во отпочнување и прекин на антибиотска терапија кај долно респираторните инфекции. Неговата употреба е неодамна одобрена и од агенцијата за лекови во америка (FDA).

Кај стабилни пациенти со долно-респираторни инфекции, кога вредноста на ПЦТ <0,25 µg/l, може да се одложи употреба на

антибиотици во зависност од клиничката слика и контрола по 6 –24 часа. Кога ПЦТ >0,25μg/l се препорачува отпочнување со антибиотик и се повторува по 3 и 7 дена, се до пад на ПЦТ < 0,25 или 80% од почетната вредност на ПЦТ, кога препорака е да се прекине антибиотската терапија. Ова е најчестиот ПЦТ водич користен во студиите. Во 2012 Cochran-ova мета- анализа на 14 рандомизирани студии на 4221 пациенти кои го користеле ПЦТ како водич за антибиотска терапија покажано е значајно намалување на употреба на антибиотици како и времетраење на антибиотици, без зголемување на стапката на смртност или неуспешен третман. Во 2017та во Cohran-ова дата база презентирана е мета- анализа на нови 18 рандомизирани студиии, при што се анализирани морталитетот и неуспешен третман како примарна цел. Секундарна цел била времетраење на антибиотска терапија. Резултатот бил намалување на смртноста 8,6 % во однос на 10,0% во контролната група во примарната цел, а во секундарната цел намалување на времетраењето на антибиотски третман за 2,4 дена во однос на контролната група.

Авторите заклучиле во мета анализата на 6708 пациенти од 12 земји дека употребата на прокалцитонин антибиотскиот водич резултира со намалена стапка на смртност, намалена употреба на антибиотици и намален ризик од несакани ефекти. Натамошни дополнителни студии се потребни кај имунодефицитни пациенти и пациенти со останати инфекции.

IN16 EVALUATION OF ETIOLOGICAL AGENTS OF GASTROINTESTINAL INFECTIONS IN HOSPITALIZED PATIENTS AT THE UNIVERSITY CLINIC FOR INFECTIOUS DISEASES AND FEBRILE CONDITIONS IN SKOPJE

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Introduction: Bacteria are frequent causes of acute gastrointestinal infections worldwide. Acute diarrhea is defined as a sudden sudden passage of loose stools (more than three times per day) which may be associated with nausea, vomiting, fever, or abdominal pain and a duration of fewer than 2 weeks.

Aim: the aim of this study is to evaluate the most frequent bacterial causes for microbiological diagnosis of gastrointestinal bacterial infections and proof of bacterial carriage.

Material and methods: during a 2-year period (01.01.2016-

31.12.2017), a total of 2562 specimens from patients with clinical presentation of acute diarrhea, hospitalized at the University Clinic for infectious diseases and febrile conditions in Skopje, were analyzed at the laboratory for microbiology. Standard microbiological methods for isolation and identification of enteropathogenic bacteria, were used. Microbiological diagnosis of acute gastrointestinal infections was performed with analysis of feces and rectal swabs with coproculture. Selective and differential media were used (Columbia blood agar, McConkey selective agar, SS agar, *Campylobacter* selective agar and *Yersinia* selective agar, and Selenit F broth, Biomerieux, France). Definite identification of enteropathogenic bacteria was performed with serological method–slide agglutination with somatic O and flagellar H antigens.

Results: In our laboratory, during a 2-year period, a total of 2562 specimens from patients with clinical presentation of acute diarrhea, were analyzed. Positive findings of bacteria in coproculture were identified in 6.6% (170/2562). A dominant etiological agent was *Salmonella enteritidis* group D-77.6 % (132/170), *Salmonella* species – 2.4% (4/170) and *Salmonella paratyphi* group C-0.6% (1/170). *Shigella flexneri* group B 2.9% (5/170). *Campylobacter jejuni* 16.5% (28/170) was most frequently present, following the isolates of *Salmonella*. The highest percentage of proven bacterial infections was registered in male population-67% (114), and female patients were registered in 33% (56). Analysis of age showed domination of children from 1 to 10 years – 54.2% (92/170), while the others were older than 10 years – 45.8% (78/170). The highest percentage of proven *Salmonella* infections were registered during summer time.

Conclusions: Acute diarrhea in patients with acute gastrointestinal infections hospitalized at the University Clinic for infectious diseases and febrile conditions in Skopje, most often in summer. The most frequent causes of acute diarrhea in our patients are still *Salmonella* species.

Kew words: diarrhea, gastrointestinal infections, bacteria, *Salmonella*

ЕВАЛУАЦИЈА НА ПРИЧИНИТЕЛИ НА АКУТНИ ГАСТРОИНТЕСТИНАЛНИ ИНФЕКЦИИ КАЈ ХОСПИТАЛИЗИРАНИ ЛИЦА НА УНИВЕРЗИТЕТСКАТА КЛИНИКА ЗА ИНФЕКТИВНИ БОЛЕСТИ И ФЕБРИЛНИ СОСТОЈБИ ВО СКОПЈЕ

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Вовед: Бактериите се честа причина за акутни гастроинтестинални инфекции во целиот свет. Акутната дијареја се дефинира како ненадеен почеток на ретки столици (повеќе од 3 пати на ден), кое може да биде асоцирано со гадење, повраќање, треска, или абдоминална болка, а трае помалку од две недели.

Цел: Цел на овој труд е да се евалуираат најчестите бактериски причинители за поставување на микробиолошка дијагноза на гастроинтестинални бактериски инфекции и докажување на бацилоносителство.

Материјал и методи: Во период од две години (01.01.2016-31.12.2017), вкупно 2562 примероци од болни со клиничка слика на акутна дијареја, хоспитализирани при Клниката за инфективни болести и фебрилни состојби во Скопје, беа обработени во микробиолошката лабораторија. Се користеа стандардни микробиолошки лабораториски методи за изолација и идентификација на ентеропатогените причинители. Микробиолошката дијагноза на акутните гастроинтестинални инфекции се изведуваше со анализа на копрокултура од фецес и ректален брис. Се користеа селективни и диференцијални хранителни подлоги (Колумбија крвен агар, McConkey селективен агар, SS агар, *Campylobacter* селективен агар и *Yersinia* селективен агар, како и Селенит Ф бујон, Biomerieux, Франција). Дефинитивната идентификација на ентеропатогените бактерии беше изведувана со примена на серолошки метод–аглутинација на плочка со соматски О и флагеларен Н антиген.

Резултати: Во нашата лабораторија во период од две години вкупно беа обработени вкупно 2562 примероци. Позитивни наоди на бактерии во копрокултура, беа регистрирани кај 6,6% (170/2562). Од добиените резултати од копрокултура доминира *Salmonella enteritidis* група D-77,6 % (132/170), *Salmonella* species – 2,4% (4/170) и *Salmonella paratyphi* група C-0,6% (1/170). *Shigella flexneri* група B 2,9% (5/170). *Campylobacter jejuni* 16,5 % (28/170)

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беше најчесто застапена по салмонелите кои се предизвикувачи на цревни инфекции. Највисок процент на докажани бактериски инфекции беше регистрирана кај машката популација-67% (114/170), а женскиот пол беше застапен со 33% (56/170). Анализата на возраста покажа доминација на деца од 1 година до 10 години - 54,2% (92/170), а останати пациенти беа на возраст над 10 год 45,8% (78/170). Највисок процент на докажани салмонелози се регистрираше во летниот период.

Заклучок: Акутната дијареа кај пациентите со гастроинтестинални инфекции хоспитализирани на Клниката за инфективни болести и фебрилни состојби во Скопје е најчеста во летниот период. Најчестите бактериски причинители на акутна дијареја кај нашите пациенти сеуште припаѓаат на родот *Salmonella*.

Клучни зборови: дијареја, гастроинтестинални инфекции, бактерии, *Salmonella*

IN17 EPIDEMIOLOGICAL AND CLINICAL CHARACTERISTICS OF PATIENTS WITH A HIGH ASO TITER

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Introduction: Group A Streptococci are bacteria that produce streptolysin O. After infection the body produces antibodies against streptolysin O, whose levels rise during a period of 1 to 3 weeks, peak at 3 to 5 weeks and then normalize after a few months. This test is used as a parameter to follow infections with Streptococci.

Aim: To present the epidemiological and clinical characteristics of patients with a high ASO titer.

Materials and methods: A total of 270 patients were the subject of observation, during a period of 5 years. They were treated in the inpatient ward, as well as in the day hospital. A retrospective analysis was performed, utilizing data from the treatment logs.

Results: From a total of 270 patients, 109 patients are male (40.4%) and 161 are female (59.6%). From an urban environment are 203 (75.2%) and 67 patients (24.8%) are from a rural environment. According to age, division is as follows: 34 (12.6%) are preschool aged children, 53 (19.6%) were students, 100 (37%) are employed, 62 (23%) are unemployed and 21 (7.8%) are pensioners. According to year of infection, 63 (23.3%) patients were registered in 2013, 41 (15.2%) in 2014, 37 (13.7%) in 2015, 52 (19.3%) in 2016 and 77 (28.5%) in 2017. In regard to seasonal character, no significance was noted. According to

clinical presentation, 171 (63.3%) patients were asymptomatic and 99 (36.7%) presented with joint problems.

Conclusion: Continuous monitoring of patients with a high ASO titer, as well as timely treatment with beta-lactam antibiotics with sufficient duration, enables us to reduce subjective discomfort, as well as to prevent more severe complications, such as rheumatic fever and glomerulonephritis.

ЕПИДЕМИОЛОШКИ И КЛИНИЧКИ КАРАКТЕРИСТИКИ КАЈ ПАЦИЕНТИ СО ВИСОК ТИТАР НА АСО

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Вовед: Стрептококите од група А се бактерии кои продуцираат стрептолизин О. После инфекцијата телото продуцира антитела против стрептолизин О чие што ниво расте во тек на 1-3 недели, максимумот го достигнува 3-5 недели а се нормализира после неколку месеци.Овој тест се користи како параметар за следење на стрептококните инфекции.

Цел: Да се прикажат епидемиолошките и клиничките карактеристики кај пациенти со висок титар на АСО

Материјал и методи: Обработени се вкупно 270 пциенти, во период од 5 години, лекувани амбулантски на Инфективно одделение во Велес. Користени се податоци од амбулантски дневник и изработена е ретроспективна анализа.

Резултати: Од вкупно 270 пациенти,според полот, машки се 109 (40,4%) а женски се 161(59,6%).Според место на живеење, од град се 203(75,2%) а од рурално потекло се 67(24,8%). Од вкупниот број на лекувани, 34(12,6%) се деца од предшколска возраст,53(19,6%) се ученици, 100(37%)се вработени,62(23%) се невработени и 21(7,8%) се пензионери.Според година на заболување, во 2103год. се регистрирани 63(23,3%), во 2014 год. - 41(15,2%), во 2015 год.- 37(13,7%), во 2016 год - 52(19,3%) а во 2017 год 77(28,5%).Според сезонскиот карактер, нема сигнификантна разлика на појавувње. Според клиничките карактеристики , кај 171(63,3%)нема клинички симптоми а кај 99(36,7%) се регистрираат зглобни потешкотии.

Заклучок: Континуираното следење на пациенти со покачен титар на АСО, како и навремено започнување со антибиотски третман со бета лактамски препарати во доволно долг период, ни овозможува редуцирање на субјективните тегоби и превенирање на компликаци од потежок степен како реуматска треска и гломерулонефрит.

IN18 HAEMATOLOGICAL LEUKOCYTIC CHANGES IN OPHTHALMIC INFECTIONS

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Introduction: In parallel with clinical and microbiological investigations, the definitive diagnosis of infections caused by various microbial causes is confirmed by laboratory, haematological follow-up investigated parameters.

Aim of the study: Since ophthalmic infections in most cases represent local primary or recurrent diseases of the eye, the main goal in the creation of the study was to observe the haematological laboratory accompanying parameters that refer to the qualitative and quantitative changes of the hematopoietic cells of the leukocyte lineage in people with these infections.

Material and methods: The study analyzes haematological qualitative and quantitative changes in leukocyte lineage in individuals with ophthalmic infections of the anterior eye segment, in which bacterial microbial agents are diagnosed as causative agents of the infections.

The study involved 63 blind and persons with severe visual impairment, in whom microbiologically verified ophthalmic infections were diagnosed with a conjunctival swab with an antibiogram. The examinees suffered from various chronic ophthalmic diseases, and haematological analyzes were performed in parallel with microbiological investigations.

Results: From a total of 63 people with ophthalmic infections in the anterior segment of the eye, in 45 (71.4%) were isolated microbial agents from the group of staphilococcus aureus, epidermidis and saprophyticus and streptococcus.

Haematological investigations in these 45 individuals indicated that in 17 people (37.7%) in the blood picture concomitant leukocytosis appeared, and granulocytes dominate in the leukocyte lineage. In the remaining 28 people (62.3%), verified bacterial infections were not accompanied by haematological changes in the leukocyte lineage.

Out of 17 targeted individuals, 10 (22.2%) had anamnestic data for other parallel primary disease in addition to ophthalmic infection.

Conclusion: Bacterial ophthalmic infections of the anterior eye segment in the majority of cases represent local infections that do not cause generalized sensitization of the hematologic leukocyte lineage, due to which no quantitative and qualitative changes in the number of leukocytes appear in the blood picture, that are inherent in the generalized bacterial infections in the body.

Key words: ophthalmic infections, haematological changes, leukocytosis

ХЕМАТОЛОШКИ ЛЕУКОЦИТНИ ПРОМЕНИ ПРИ ОФТАЛМОЛОШКИ ИНФЕКЦИИ

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Вовед: Паралелно со клиничките и микробиолошките иследувања, дефинитвната дијагноза на инфекциите предизвикани од различни микробиолошки причинители се потврдува и со лабораториските, хематолошки пропратни иследувани параметри.

Цел на трудот: Со оглед на тоа дека офталмолошките инфекции во најголембројслучаипредставуваатлокалнипримарниилирецидиварачки заболувања на очниот апарат, основна цел при изработката на трудот ни беше да ги согледаме хематолошките лабораториски пропратни параметри кои се однесуваат на квалитативните и квантитавни промени на хематопоезните клетки од леукоцитната лоза кај лица заболени од овие инфекции.

Материјал и методи: Во трудот се анализирани хематолошките квалитативни и квантитавни промени во леукоцитната лоза кај лица со офталмолошки инфекции на предниот очен сегмент, кај кои што се дијагностицирани бактериски микробиолошки агенси како причинители на инфекциите. Во истражувањето беа вклучени 63 слепи и лица со тешко оштетен вид, кај кои се микробиолошки верифицирани офталмолошки инфекции дијагностицирани со конјуктивален брис со антибиограм. Испитуваните лица боледувале од различни хронични офталмолошки заболувања, а хематолошките анализи се вршени паралелно со микробиолошките иследувања.

Резултати: Од 63 лица со офталмолошки инфекции на предниот сегмент на очите, кај 45 (71,4%) се изолирани микробиолошки причинители од групата на Staphylococcus aureus, epidermidis и saprophyticus и streptococcus. Хематолошките иследувања кај овие 45 лица укажаа дека кај 17 лица (37,7%) во крвната слика се појавува пропратна леукоцитоза, а во леукоцитната лоза доминираат гранулоцити. Кај останатите 28 лица (62,3%) верифицираните бактериски инфекции не беа пропратени со хематолошки промени во леукоцитната лоза. Од 17 таргетирани лица, кај 10 (22,2%) постоеја анамнестички податоци за паралелно друго основно заболување покрај офталмолошката инфекција.

Заклучок: Бактериските офталмолошки инфекции на предниот очен сегмент во најголем број на случаи представуваат локални инфекции, кои не предизвикуваат генерализирана сензибилизација на хематолошката леукоцитна лоза, заради што во крвната слика не се појавуваат пропратни квантитативни и квалитативни промени во бројот на леукоцитите, кои се својствени за генерализираните бактериски инфекции во организмот.

Клучни зборови: офталмолошки инфекции, хематолошки промени, леукоцитоза

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СЕСИЈА 4/SESSION 4 АНТИМИКРОБНА РЕЗИСТЕНЦИЈА/ ANTIMICROBIAL RESISTANCE

AR1 ANTIMICROBIAL RESISTANCE IN REPUBLIC OF MACEDONIA – RESULTS FROM CAESAR NETWORK

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Introduction: CAESAR network (Central Asian and eastern European Surveillance of Antimicrobial Resistance) is a comprehensive system for monitoring the prevalence and trends of antimicrobial resistance. It includes countries that are not members of the European Union (EU), including the Republic of Macedonia. It was established by the World Health Organization (WHO), the National Institute of Public Health of the Netherlands (RIVM) and the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) in close collaboration with the European Center for Disease Control (ECDC). The CAESAR network targets 8 species of invasive bacteria (blood and cerebro-spinal fluid isolates).

Aim: To make an analysis of the activity of our country in CAESAR network (2013-2017) and to present the situation of antimicrobial resistance in that period.

Material and methods: CAESAR methodology includes: data collection from all participants in one unique system, their processing and full analysis (the network is fully compatible with EARS-Net - the results of the resistance of the isolates of the EU Member States). The official annual reports (2013 - 2017: web site www.euro.who.int/) were used. The following bacteria are under surveillance: *Staphylococcus aureus* (MRSA), *Streptococcus pneumoniae* (Pen R - PRP), *Escherichia coli* (ESBL +, carbapenemase +), *Klebsiella pneumoniae* (ESBL +, carbapenemaza +), *Enterococcus faecium and faecalis* (VRE), *Pseudomonas aeruginosa* (multiresistant) and *Acinetobater spp*. (multiresistant).

Results and discussion: R. Macedonia has been active since the beginning (01.01.2013) of this project included in the whole process of regular data collection, basic analysis, entry data into the electronic system at the national level (data base) and sent (again electronically) to RIVM (International Center) for their further processing and comprehensive

analysis. The data are finally evaluated and confirmed, if there is some unusual resistance is reported and back to the individual laboratories. During the period 2013-2017, many activities were organized: March 2013, the first meeting with the CAESAR network (representatives from RIVM, WHO), November 2014, Workshop organized by the COMBACTE team with international participation, November 2015 , the first national CAESAR meeting, June 2016, Workshop on EUCAST methodology, June 2017, Workshop for prevention and promotion of AMR (hand hygiene in the hospitals), etc. The total number of registered strains for this five-year period is 1148. The percentage of resistant strains is calculated from the total number of isolates. Accordingly, the percentage of resistance, summarized for all five years, is: 45.4% MRSA of the total number of *S. aureus* (277), 35.2% Pen R - PRP of 34 strains of *Streptococcus pneumoniae*, 1.7% VRE (from 108 strains of *Enterococcus faecalis*), 59% VRE (from 84 *Enteroccus faecium*), 67.8% ESBL positive of 302 *E. coli* strains, 87.6% ESBL positive from 145 strains of *Klebsiella pneumoniae*, and 76.9 % of 139 on *Acinetobacter spp*. and 34% of 59 strains of *Pseudomonas aeruginosa*, resistant to carbapenems. According to the official annual reports, our results are in category B - the data are not representative of the population concerned (a small number of samples are sent), but they are completely authentic. With EQA UK - NEQAS (United Kingdom Quality Assessment Service), the external control is performed once a year; all CAESAR laboratories are included - a confirmation that the laboratories have the capabilities to respond on the set tasks.

Conclusion: Macedonia is an active and successful participant in the CAESAR network from the very first beginning. The percentage of isolated and applied resistant strains is high but probably unrealistic due to the small number of samples submitted for analysis. In the future, it has to be worked on this plan, in order to achieve A level of confidence, but also for a comparative national base for the antimicrobial resistance of invasive isolates.

АНТИМИКРОБНА РЕЗИСТЕНЦИЈА ВО Р. МАКЕДОНИЈА – ИСКУСТВА И РЕЗУЛТАТИ ОД CAESAR МРЕЖАТА

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Вовед CAESAR мрежата (Central Asian and eastern European Surveillance of Antimicrobial Resistance) претставува сеопфатен систем за следење на преваленцата и трендовите на антимикробна резистенција. Ги опфаќа земјите кои не се членки на Европската Унија (ЕУ), вклучувајќи ја и Р. Македонија. Основана

е под иницијатива на Светската здравствена организација (WHO), Националниот Институт за јавно здравје на Холандија (RIVM) и European Society of Clinical Microbiology and Infectious Diseases (ESCMID) во тесна соработка со Европскиот центар за контрола на болестите (ECDC). CAESAR мрежата опфаќа следење на целна група инванзивни бактерии (изолати од крв и ликвор).

Цел на трудот е да се направи анализа на активноста на Р. Македонија во CAESAR мрежата (2013-2017) и да се прикаже состојбата на антимикробната резистенција во тој период.

Материјал и методи. CAESAR методологијата вклучува: колекција на податоците од сите учесници во еден единствен систем, нивно процесирање и целосна анализа (мрежата е целосно компатибилна со EARS-Net - резултати од резистенција на изолатите од земјите членки на ЕУ). Користени се официјалните годишни извештаи (2013 - 2017: веб страницата_www.euro.who.int/). Се следат изолатите: *Staphylococcus aureus* (MRSA), *Streptococcus pneumoniae* (Pen R - PRP), *Esherichia coli* (ESBL+, карбапенемаза +), *Klebsiella pneumoniae* (ESBL +, карбапенемаза +), *E. faecium* и *faecalis* (VRE), *Pseudomonas aeruginosa* (мултирезистентен) и *Acinetobater spp.* (мултирезистентен).

Резултати и дускусија. Р. Македонија од самиот почеток (1.01. 2013) е активна во целиот процес на редовна колекција на податоците, темелна анализа, внесување во електронскиот систем на национално ниво (база на податоци) по што ги испраќа (повторно електронски) до RIVM (Интернационален центар) за нивно натамошно процесирање и сеопфатна анализа. Податоците финално се евалуираат и конфирмираат и се издвојуваат невообичаените фенотипови на резистенција назад до поединечните лаборатории. Во периодот (2013-2017) организирани се многу активности: март 2013, прв состанок со основачите на CAESAR мрежата (претставници од RIVM, WHO), ноември 2014, Workshop организиран од COMBACTE тимот со интернационално учество, ноември 2015, прв национален CAESAR состанок, јуни 2016, Workshop за EUCAST методологија, јуни 2017, Workshop за превенција и промоција на AMP(хигиена на раце во болниците), итн. Вкупниот број на пријавени соеви за овој петгодишен период е 1148. Процентот на резистентни соеви е пресметан од вкупниот број на изолати. Според тоа, процентот на резистенција, сумирано за сите пет години изнесува: 45,4% MPCA од вкупниот број на *S. aureus* (277), 35,2% Pen R - PRP од 34 соја на *Streptococcus pneumoniae*, 1,7% VRE (од 108 соеви на *Enterococcus faecalis*), 59% VRE (од 84 *Enteroccus faecium*), 67,8% ESBL позитивни од 302 соја *E. coli*, 87,6% ESBL позитивни од 145 соеви на *Klebsiella pneumoniae*, како и 76,9% од 139 на *Acinetobacter spp.* и 34% од 59 соја на *Pseudomonas aeruginosa*, резистентни на карбапенеми. Според официјалните годишни извештаи, нашите резултати се во категоријата B - податоците не се репрезентативни за популацијата

на која се однесуваат (се испраќа мал број на примероци), но сепак се наполно веродостојни. Со EQA UK - NEQAS (United Kingdom Quality Assessment Service) надворешната контрола која се врши еднаш годишно досега сите тестови се поминати позитивно - потврда дека лабораториите ги имаат способностите (технички и стручно) да одговорат на поставените задачи.

Заклучок. Македонија е активен и успешен учесник во CAESAR мрежата од самиот почеток. Процентот на изолирани и пријавени резистентни соеви е висок, но веројатно нереален поради малиот број примероци испратени за анализа. Во иднина, мора да се работи на тој план, за да го достигнеме новото А, но и за пореална национална база за антимикробната резистенција на инванзивните изолати.

AR2 TRENDS IN PERCENTAGES OF ANTIMICROBIAL RESISTANCE OF INVASIVE ISOLATES IN EUROPE AND R.MACEDONIA IN THE PERIOD 2012-2107

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Introduction

Antimicrobial resistance (AMR) represents a global problem, particularly concerning in the countries with lack of adequate strong systems for its surveillance and control.

Aims of the study is to overview the percentages and trends of antimicrobial resistance (AMR) in R.Macedonia in the period 2013-2017, and to compare with that ones in EU-EEA countries.

Methods-source of data: The continuous surveillance of bacterial resistance is necessary condition for antimicrobial resistance control. EARS-Net (European Antimicrobial Resistance Surveillance Network), coordinated and funded by ECDC (European Center for Diseases Control), has produced annual reports on AMR in the EU countries, Iceland and Norway since 2002 for invasive bacterial strains. Such reports are also produced by the WHO and ever since 2013 functions the CAESAR (Central Asian and Eastern European Surveillance of Antimicrobial Resistance) system for surveillance of antimicrobial resistance in non-EU European and Central Asian countries. The reports (EARS-Net and CAESAR) from 2013, 2014, 2015, and 2016 were used for comparison of levels and trends of AMR. **:** EARS-Net performs surveillance of AMR in eight bacterial pathogens of

public health importance: *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Acinetobacter* species, *Streptococcus pneumoniae, Staphylococcus aureus, Enterococcus faecalis*

and *Enterococcus faecium.* The methodology of collecting, checking and processing of data in CAESAR system are in accordance with the EARS-Net. . Only data from invasive (blood and cerebrospinal fluid) isolates are included.

Results: 1148 invasive strains were reported from R.Macedonia during the five years period with the increasing trend of reported isolates (from 189 in 2013 to 269 in 2016). About 825.000 invasive strains were reported from all 28 EU Member states and two EEA countries (Norway and Iceland) in the same period. The number of participating laboratories and unique isolates has increased, indicating improved population coverage of the network. The number of isolated strains in R.Macedonia is about four times less then the EU-EARS average ones, according to the number of citizens. EARS-Net Report, indicates a stagnant and even reduced resistance of the Gram-positive bacteria, significantly decreased trends were detected in the percent of MRSA (from 18,1% in 2013 to 13,7% in 2016). In the same period the percentage of MRSA in R.Macedonia increased from 41,2% to 48%, and to 57% in 2017 (CAESAR network). For the first time there were a stagnation and even reduced resistance of the Gram-negative bacteria in EU-EEA countries, especially *K.pneumoniae* (fluoroquinolones, aminoglicosides, 3rd gen. cephalosporines) and *P.aeruginosa* (fluoroquinolones, aminoglicosides and carbapenems). Increasing trends were detected in *E.coli* (aminoglicosides and 3rd gen. cephalosporines) and in *P.aeruginosa* (ceftazidime). The percentages of resistance of invasive strains in R.Macedonia are two to four times higher then the average ones in EU-EEA countries with the increasing trends in this period for almost all eight bacterial species. The antimicrobial resistance situation in Europe displays large variations depending on bacteria, antimicrobial group and geographical region. For several antimicrobial group and bacterium combinations, a north-to-south and west-to-east gradient is evident in Europe. In general, lower resistance percentages are reported by countries in the north and higher percentages reported by countries in the south and east of Europe

Conclusion: Percentages of resistance of invasive strains isolated in R.Macedonia are significantly higher than the average ones in the EU and similar to those in South Europe and the Balkan region countries. There is stagnation or decreasing trends of the average percentages of resistance in EU-EEA countries and increasing trends of resistance in R.Macedonia. This is due to the long-lasting irrational antibiotic use.

Key words: antimicrobial agents, resistance, invasive strains

ТРЕНДОВИ ВО ПРОЦЕНТИТЕ НА АНТИМИКРОБНА РЕЗИСТЕНЦИЈА НА ИНВАЗИВНИТЕ ИЗОЛАТИ ВО ЕВРОПА И РЕПУБЛИКА МАКЕДОНИЈА ВО ПЕРИОДОТ 2013-2017 ГОДИНА

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Вовед

Антимикробната резистенција (АМР) претставуваеден глобален проблем, особено во земјите во кои недостасува целосно следење и контрола на истата.

Цел на трудот е да направи преглед на трендовите на процентите на резистенција на инвазивните изолати во Р.Македонија во периодот 2013-2017 и да ги спореди со истите во ЕУ-ЕЕА земјите.

Метод-извори на податоци – Годишните извештаи на EARS-Net (European Antimicrobial Resistance Surveillance Network), координирана и основана од ECDC (European Center for Diseases Control), објавува податоци за АМР во земјите на ЕУ, Исланд и Норвешка секоја година, почнувајќи од 2002 година за резистенцијата на инвазивните соеви. Такви извештаи објавува и СЗО почнувајќи од 2013 година за европските земји кои не се членки на ЕУ, како и за земјите од Централна Азија - CAESAR (Central Asian and Eastern European Surveillance of Antimicrobial Resistance). Двете мрежи користат иста методологија и се компатибилни, а ја следат АМР на 8 патогени бактерии кои имаат значење за јавното здравје: *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Acinetobacter* species, *Streptococcus pneumoniae, Staphylococcus aureus, Enterococcus faecalis* и *Enterococcus faecium*, исклучиво за соеви изолирани од крв или цереброспинален ликвор (инвазивни изолати). Извештаите од двете мрежи за 2013, 2014, 2015 и 2016 година се искористени за споредба на АМР во ЕУ со онаа во Македонија. За трендовите на процентите на резистенција за Македонија е користена и 2017 година, а за ЕУ извештајот за 2017 година се очекува во ноември 2018 година.

Резултати: 1148 инвазивни соеви се изолирани во Македонија за пет годишниот период, со тренд на зголемување на бројот на изолатите (189 во 2013 до 269 во 2016 година). Во истиот период во земјите на ЕУ-ЕЕА се пријавени околу 850.000 инвазивни изолати, со тренд на пораст на изолирани соеви од година во година. Бројот на изолати во Р.Македонија во просек е за околу четири пати помал

SESION 4

од оној во ЕУ-ЕЕА, споредено со борјот на жители. Европските извештаи индицираат стагнација, па дури и тренд на намалување на резистенцијата на Грам позитивните бактерии, особено на MPCA во однос на вкупниот број изолирани соеви на *Staphylococcus aureus.* Во истиот период процнтот на MPCA во Р.Македонија расте од 41,2% во 2013 до 48% во 2016 година, па дури до 57% во 2017 година. За прв пат е забележена стагнација, па дури и тренд на намалување на процентите на резистенција на Грам негативните бактерии во земјите на ЕУ-ЕЕА., особено на клебсиела кон флуорокинолони, аминогликозиди и цефалоспорини од трета генерација, како и на псевдомонас кон флуорокинолони, аминогликозиди и карбапенеми. Процентите на резистенција во Р.Македонија во истиот период се два до четири пати повисоки со растечки трендови кај скоро сите осум видови на бактерии.

Заклучок: Процентите на резистенција на инвазивните соеви изолирани во Македонија се сигнификантно повисоки од просечните во ЕУ и слични на оние во Јужна Европа и Балканот. Постои стагнација или намалување на резистенцијата во ЕУ, а зголемување во Р.Македонија. Сето ова е поврзано со долгогодишната нерационална употреба на антибиотици во Македонија.

Клучни зборови: антимикробни средства, резистенција, инвазивни соеви

AR3 ANTIMICROBIAL SUSCEPTIBILITY OF *CAMPYLOBACTER* ISOLATES

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Introduction

Campylobacteriosis are usually self-limited, but antimicrobial treatment is required for severe enterocolitis, enterocolitis in immunocompromised patients, and bacteriemia caused by *Campylobacter*. Very frequently acute enterocolitis due to *Campylobacter* is treated empirically with fluoroquinolones and macrolides. But, the rate of *Campylobacter* resistance to these drugs is increasing everywhere in the last decade.

The objective of this study was to determine the isolation rate of *Campylobacter* among hospitalized and outpatients and its

antimicrobial susceptibility patterns.

Material and methods

A total of 3820 stool samples obtained from patients hospitalized at the Pediatric Clinic, Clinic of Infectious Diseases and Febrile Conditions as well as from outpatients were included in this study. They were processed by standard microbiological methods for isolation and identification of pathogenic enterobacteria, including *Campylobacter*. Antimicrobial susceptibility of *Campylobacter* isolates to ceftriaxone, amoxicillin-clavulanic acid, erythromycin, ciprofloxacin, tetracycline and gentamicin was determined by disc-diffusion technique. Determination of minimal inhibitory concentration (MIC) was performed in all *Campylobacter* isolates for erythromycin and ciprofloxacin. Results were interpreted on the basis of EUCAST criteria. *Campylobacter jejuni* (ATCC 700819) and *E. coli* (ATCC 25922) were used as control strains. Chi-square and Fisher's exact tests were used for testing the differences between proportions, and p value less than 0.05 was considered statistically significant.

Results

The isolation rate of *Campylobacter* in 2016 was 2.20 and it increased to 2.90 in 2017. Eighty-five of 97 (87.7%) isolates were *C. jejuni* and 12/97 (12.3%) were *C. coli*. 52.57% of *Campylobacter* isolates were resistant to ceftriaxone, 35.06% to ciprofloxacin and 32.99% were resistant to tetracycline. Resistance to amoxicillin-clavulanic acid and gentamicin was detected in 18.55% and 8.24% of *Campylobacter* isolates, respectively. None of the isolates were resistant to erythromycin. Sixty-three (64.95%) of 97 isolates were susceptible to ciprofloxacin with MIC between 0.064 and 0.125 μg/ml, but 34 (35.05%) of 97 isolates were resistant to ciprofloxacin with MIC between 0.5 and > 32 μg/ml. Fifteen of 34 (44.11%), 6/34 (17.64%) and 13/34 (38.23%) were with MIC > 32 μg/ml, 3 μg/ml and 1 μg/ml, respectively. From the total of 85 *C. jejuni* and 12 *C. coli* isolates, 26 of 85 (30.58%) *C. jejuni* isolates and 6 of 12 (50.0%) *C. coli* isolates were producers of Extended spectrum β-lactamases.

Conclusion

The permanent increase of the isolation rate of *Campylobacter* gastroenteritis indicates the need for improving its laboratory confirmation. Erythromycin remains the most effective antibiotic agent for treatment of *Campylobacter* enterocolitis in our patients. Since high percentage of *Campylobacter* isolates revealed resistance to ciprofloxacin and ceftriaxone, it is very important to convey patients' treatment based on antimicrobial susceptibility testing.

АНТИМИКРОБНА ОСЕТЛИВОСТ НА ИЗОЛАТИТЕ НА CAMPYLOBACTER *SPP*

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Вовед

Кампилобактериозите се најчесто самолимитирачки инфекции, а антибиотска терапија е потребна за тешки форми на ентероколити, ентероколити кај имунокомпромитирани пациенти и бактериемија предизвикана од *Campylobacter*. Многу често акутниот ентероколит предизвикан од *Campylobacter* се лекува емпириски со флуорокинолони и макролиди. Но, во последната деценија се зголемува бројот на изолати резистентни кон овие антибиотици. Целта на студијата е да се одреди бројот на изолати на *Campylobacter* кај хоспитализирани и амбулантски третирани пациенти и нивната антимикробна осетливост.

Материјал и методи

Во студијата се вклучени вкупно 3820 примероци од фецес од пациенти хоспитализирани на Клиниката за педијатрија, Клиниката за инфективни болести и фебрилни состојби, како и од амбулантски пациенти. Изолацијата и идентификацијата на патогените ентеробактерии, вклучувајќи го и *Campylobacter* беше направена со примена на стандардни микробиолошки методи. Осетливоста на изолатите на *Campylobacter* кон цефтриаксон, амоксицилин-клавуланска киселина, еритромицин, ципрофлоксацин, тетрациклин и гентамицин беше одредена со диск дифузионен метод. Одредувањето на минималната инхибиторна концентрација (МИК) беше направено кај сите изолати на *Campylobacter* за еритромицин и ципрофлоксацин. Интерпретацијата на резултатите беше според EUCAST критериумите. *Campylobacter jejuni* (ATCC 700819) и *E. coli* (ATCC 25922) беа употребени како тест соеви. X^2 тест и Fisher-ов егзактен тест беа применети за тестирање на разликите помеѓу пропорциите, при што вредноста на $p < 0.05$ означуваше статистичка сигнификантност.

Резултати

Стапката на изолација на *Campylobacter* во 2016 беше 2.20, а во 2017 година се зголеми на 2.90. Осумдесет и пет од 97 (87.7%) беа изолати на *C. jejuni*, а 12/97 (12.3%) на *C. coli.* Резистенцијата на изолатите на *Campylobacter* беше следна: 52.57% од изолатите беа резистентни на цефтриаксон, 35.06% на ципрофлоксацин, 32.99% на тетрациклин, 18.55% на амоксицилин-клавуланска киселина и 8.24% на гентамицин. Ниту еден изолат не беше резистентен на

еритромицин. Шеесет и три од 97 изолати (64.95%) беа осетливи на ципрофлоксацин со МИК помеѓу 0.064 и 0.125 μg/ml, а 34 изолати од 97 (35.05%) беа резистенти на ципрофлоксацин со МИК помеѓу 0.5 и > 32 μg/ml. Кај 15 од 34 (44.11%), 6/34 (17.64%) и 13/34 (38.23%) МІК изнесуваше > 32 μg/ml, 3 μg/ml и 1 μg/ml, последователно. Од вкупно 85 изолати на *C. jejuni* и 12 на *C. coli*, 26 од 85 (30.58%) изолати на *C. jejuni* и 6 од 12 (50.0%) на *C. coli* продуцираа бета-лактамази со проширен спектар (ESBL).

Заклучок

Постојаното зголемување на стапката на изолација на *Campylobacter* при гастроентерит укажува на потребата за подобрување на неговата лабораториска потврда. Еритромицинот останува најефективен антибиотик за третман на ентероколит предизвикан од *Campylobacter* кај нашите пациенти. Бидејќи високиот процент на изолати на *Campylobacter* покажа резистенција на ципрофлоксацин и цефтриаксон, многу е важно третманот на пациентите да се спроведе врз основа на тестирање на антимикробна осетливост.

AR4 RETROSPECTIVE EVALUATION OF ANTIBIOTIC RESISTANCE IN *ESCHERICHIA COLI* AND *KLEBSIELLA* ISOLATES IN HOSPITALIZED PATIENTS: A 7-YEAR ANALYSIS

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Introduction

E.coli and *Klebsiella spp.* are frequent causes of healthcare associated infections. Nevertheless, these pathogens express increasing resistance to antibiotics and their infections become difficult to treat. Resistance rates vary regionally and surveillance is recommended for antimicrobial stewardship and epidemiological purposes.

Objectives

In this study, we aimed to determine the antibiotic susceptibility profiles of *E.coli* and *Klebsiella spp*. isolated from hospitalized patients retrospectively in 7 year period.

Materials, Methods

Klebsiella spp. and *E.coli* isolates obtained from clinical samples between 2011-2017 were included (Table 1). Conventional methods

and automated systems (VITEK 2.0, bioMerieux, France; and Phoenix, Becton Dickinson, USA) were used for identification and antibiotic susceptibility determination.

Table 1. Specimen distribution for *Klebsiella spp.* and *E.coli* isolates

Bacteria	Urine	Blood	Tissue-abscess	Other	TOTAL
Klebsiella spp.	742	486	216	234	1678
E.coli	1815	436	432	215	2898

Results and Conclusion

Total of 1678 *Klebsiella spp.* and 2898 *E.coli* isolated from clinical samples of hospitalized patients in 7 year period. The antibiotic susceptibility profiles including extended spectrum Beta-lactamase (ESBL) production in *Klebsiella spp.* and *E.coli* isolates are shown in Table 2.

Table 2. Antibiotics susceptibility profiles in *Klebsiella spp.* and *E.coli* isolates between 2011-2017.

Klebsiella spp.	**2011 (n:204)**	**2012(n:210)**	**2013 (n:240)**	**2014 (n:316)**	**2015(n:257)**	**2016 (n:290)**	**2017 (n:161)**
ESBL(+)*	36% (n:74)	47% (n:98)	53% (n:127)	42% (n:135)	40% (n:102)	47% (n:61)	37% (n:60)
CR**	0	0	4% (n:11)	17% (n:54)	13% (n:33)	28% (n:81)	33% (n:53)
Imipenem	100% (n:204)	100%(n:210)	96% (n:231)	83% (n:262)	87% (n:213)	72% (n:209)	67% (n:108)
Meropenem	100% (n:204)	100%(n:210)	96% (n:231)	83% (n:262)	87% (n:213)	72% (n:209)	67% (n:108)
Ertapenem	100% (n:204)	90% (n:189)	77% (n:185)	59% (n:187)	62% (n:159)	48% (n:140)	40% (n:65)
Pip-Tazobactam	80% (n:163)	74% (n:156)	55% (n:132)	53% (n:167)	59% (n:152)	38% (n:111)	30% (n:48)
Amikacin	83% (n:169)	86% (n:181)	92% (n:221)	98% (n:310)	97% (n:249)	78% (n:116)	55% (n:88)
Gentamicin	48% (n:98)	56% (n:118)	60% (n:144)	55% (n:174)	55% (n:141)	42% (n:122)	40% (n:64)
Ciprofloxacin	65% (n:133)	58% (n:122)	52% (n:125)	45% (n:142)	40% (n:103)	30% (n:86)	28% (n:45)
Ceftazidime	57% (n:116)	50% (n:105)	28% (n:67)	37% (n:117)	29% (n:75)	22% (n:65)	20% (n:33)
Cefepime	57% (n:116)	48% (n:101)	31% (n:75)	45% (n:142)	30% (n:77)	28% (n:80)	22% (n:36)
E.coli	**2011(n:567)**	**2012(n:438)**	**2013 (n:416)**	**2014 (n:449)**	**2015(n:410)**	**2016 (n:417)**	**2017 (n:201)**
ESBL(+)*	38% (n:213)	49% (n:215)	47% (n:196)	55% (n:247)	58% (n:236)	62% (n:257)	64% (n:128)
Imipenem	100%(n:567)	100%(n:438)	100% (n:416)	100% (n:449)	100%(n:410)	100% (n:417)	100% (n:201)
Meropenem	100%(n:567)	100%(n:438)	100% (n:416)	100% (n:449)	100%(n:410)	100% (n:417)	100% (n:201)
Ertapenem	100%(n:567)	90% (n:394)	97% (n:404)	92% (n:413)	88% (n:361)	90% (n:375)	88% (n:177)
Pip-Tazobactam	86% (n:488)	88% (n:385)	85% (n:354)	76% (n:341)	76% (n:312)	75% (n:313)	77% (n:155)
Amikacin	84% (n:476)	90% (n:394)	95% (n:395)	98% (n:440)	98% (n:401)	98% (n:409)	99% (n:198)
Gentamicin	54% (n:306)	60% (n:263)	68% (n:283)	63% (n:283)	72% (n:295)	62% (n:259)	75% (n:151)
Ciprofloxacin	56% (n:317)	52% (n:228)	54% (n:225)	52% (n:234)	49% (n:201)	50% (n:208)	44% (n:89)
Ceftazidime	61% (n:346)	46% (n:202)	50% (n:208)	55% (n:247)	50% (n:205)	40% (n:167)	38% (n:76)
Cefepime	62% (n:352)	48% (n:210)	50% (n:208)	52% (n:234)	42% (n:172)	39% (n:163)	37% (n:74)

*Extended-spectrum Beta-lactamase; **Carbapenem-resistant *Klebsiella spp.*

Klebsiella spp. isolates exhibited a decreasing susceptibility to all tested antibiotics in 7 year study period. The rate of extended-spectrum Beta-lactamase positive isolates remained stable (around 50%); however carbapenem resistance rates are increased over time.

E.coli isolates remained susceptible to carbapenems but,

extended-spectrum Beta-lactamase producing isolates are increased to 64% in 7 year period.

The antibiotic susceptibility rates of Enterobacteriaceae are rapidly decreasing globally. Therefore, surveillance studies are important to establish antimicrobial stewardship programs and patient management.

AR5 CARBAPENEM RESISTANCE RATES OF ENTEROBACTERIACEAE ISOLATED FROM PEDIATRIC URINARY TRACT INFECTIONS

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Introduction
Enterobacteriaceae are the major cause of urinary tract infections (UTI) in infants and children. Early diagnosis and treatment of are important to preserve renal functions of the developing kidney. The increasing prevalence of ESBLs made carbapenems a drug of choice for treatment; but the emerge of carbapenem resistant Enterobacteriaceae is a menace to patients.

Objectives
We aimed to investigate the role of carbapenem resistant Enterobacteriaceae (CRE) in community associated urinary tract infections (UTI) of pediatric patients.

Material & Methods
Urine cultures of pediatric outpatients between January-December 2017 were evaluated. Bacterial identification was performed by MALDI-TOF MS (VitekMS, bioMérieux, France). Resistance to carbapenems was detected with automated system (VITEK-2 Compact, bioMérieux). Antimicrobial susceptibility interpreted by using EUCAST guidelines.

Results
A total of 1547 urine samples from pediatric outpatients were found to be positive for Enterobacteriaceae. Among the latter, *E.coli* (75.2%) was the most abundant pathogen of the family followed by *Klebsiella* spp. (14.4%), *Proteus* spp. (4.9%) and *Enterobacter* spp. (2.6%). Other members of the family were isolated in 3% of the samples. Ertapenem, imipenem and meropenem resistance was detected in 21 (1.4%), 13 (0.8%) and 8 (0.5%) isolates, respectively. Ten (0.6%) isolates were found to be non-susceptible to meropenem, while 49 (3.2%) isolates were found to be either intermediate or resistant to imipenem. The highest rate of carbapenem non-susceptible isolates (44.7%) was

found in *Proteus* spp. isolates: 7 resistant and 24 intermediate.

Conclusion
UTIs are among common childhood illnesses. Appropriate treatment is fundamental in order to avoid possible long term complications. Despite low rate of carbapenem resistance in community acquired infections, the number of isolates found intermediate-susceptible to carbapenems consists a great concern. It is important to formulate an antibiotic prescription policy leading to plan an effective antibiotherapy, prevent unnecessary or overuse of antibiotics and spread of antimicrobial resistance.

AR6 TOXIN GENES AND ANTIMICROBIAL RESISTANCE IN MRSA ISOLATED FROM INTENSIVE CARE UNIT

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INTRODUCTION
Methicillin-resistant Staphylococcus aureus (MRSA) is one of the leading causes of hospital-acquired infections worldwide. Antibiotic resistance and toxins contributing to bacterial virulence have a significant role in pathogenesis of MRSA infections, which cause high morbidity and mortality in ICU patients.

OBJECTIVES
In this study, we aimed to determine the toxigenic properties and antibiotic susceptibility profile of MRSA isolated from ICU patients during 2016-2017.

MATERIAL, METHODS
A total of 36 MRSA isolated from ICU patients were included. Identification was performed by MALDI-TOF MS (Vitek MS, bioMérieux, France) and antibiotic susceptibility was determined by using an automated system (VITEK 2 Compact, bioMérieux). We used PCR to detect the genes encoding for Panton-Valentine leukocidin (PVL, *luk*S / *F*), toxic shock toxin-1 (*tst*) and enterotoxins (*sea, seb, sec, sed, see*).

RESULTS
Study isolates obtained from following clinical samples; DTA (n = 21), blood (n = 9), wound swab (n = 4) and other samples (n = 2). A total of 30 isolates (30/36; 83.3%) were positive for one or more toxin genes. The distribution of the toxin genes is shown below Table. The staphylococcal enterotoxin D and E genes (*sed* and *see*) could not be detected in any isolate. All isolates were sensitive to linezolid, vancomycin, tigecycline and daptomycin. Resistance rates against

other tested antibiotics ranged from 13.9% to 52.8%.

Table. Toxin gene profiles of *S.aureus* isolates

Toxin profile	Isolate N (%)
sea	1 (2,8)
sea, PVL	7 (19,4)
sea, PVL, *tst*	2 (5,6)
seb, PVL	1 (2,8)
sec, PVL	1 (2,8)
PVL	14 (38,9)
PVL, *tst*	2 (5,6)
tst	2 (5,6)
sed	0 (0,0)
see	0 (0,0)
TOTAL	30 (83.3)

CONCLUSION

In our study, toxin production rate in MRSA isolates from ICU patients was found to be as high as 83.3%. Among the toxin-positive isolates, Panton-Valentine leukocidin carriers have a significant share (75%). Antibiotic resistance rates are especially high in isolates carrying Staphylococcal enterotoxin A (*sea*). In addition to toxin production capability and antibiotic susceptibility profiles, identification of the clonal relationship of these isolates and monitoring of spread in the hospital environment would be beneficial in terms of infection control.

AR7 t030 WAS THE MOST COMMON SPA TYPE AMONG "NON-TYPEABLE" METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS ISOLATES IN TURKEY

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Introduction
The results of a multicenter study for spa typing of 488 MRSA from 12 centers showed that the most common spa types of MRSA isolates in Turkey was t030 (Bozdogan 2013).

Objectives
During the study it was found that a total of 44 isolates were non typeable because with the primers used, spa-1113f and spa-1514r, there was no amplicon. The aim of the present study was to determine the spa types of the non typeable isolates.

Material
Among 44 non typeable isolates 27 MRSA were available and were included to present study. All isolates were negative for spa with the classical primers used. Primers used for amplification were spa-208f attcgtaaactaggtgtagg (208-227), spa-849f gctttctatgaaatcttac (849-867), spa-1739r ccagctaataacgctgcac (1739-1720).

Methods
PCR was done at 95C for 30 seconds, 55C of hybridisation for 30 seconds and elongation for 60 seconds at 72C for 35 cycles.

Results and Conclusion

The results of the present study showed that from 27 available isolates a fragment was amplified using primers spa-849f and spa-1739r for 21 isolates. For the remaining 6 isolates PCR was negative with these primers but positive with primers spa-208f and spa-1739r. The sequence analysis of 27 fragments amplified were analyzed and the results showed that of 27 isolates 17 were t030 and 10 were t359. The results of the present study showed that as among typeable MRSA isolates, among non typeable isolates the most common spa typa was t030. A modified primers should be designed to amplify from all *S. aureus* isolates.

Key words: MRSA, *spa* typing, non-typeable *spa*

AR8 INVESTIGATION OF THE CHANGE IN ANTIBIOTIC SUSCEPTIBILITY OF CLINICAL STREPTOCOCCUS AGALACTIAE ISOLATES OVER YEARS

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INTRODUCTION
Group B Streptoccoccus (GBS) is a leading pathogen in newborns, infants and immunocompromised adults. GBS are penicillin susceptible and this drug should be the first option for treatment. For patients with penicillin hypersensitivity, erythromycin or clindamycin is recommended for antibiotherapy.

OBJECTIVES
In the present study, we determined the change in antibiotic susceptibility of GBS isolates over the last 15 years.

MATERIAL, METHODS
Study was performed on 90 GBS isolated in 2003-2007 period and 70 GBS isolated between 2015-2017. The first group isolates were obtained from Marmara University Hospital and second group isolates were from S.B.H.S.U Okmeydani Hospital. All isolates were identified by using Gram staining, colony morphology and hemolysis on 5% sheep blood agar, catalase test and latex agglutination assay for Streptococcal grouping. CLSI and EUCAST criteria was used to evaluate antibiotic susceptibility of the first period and the second period isolates, respectively.

RESULTS
Penicillin resistance was not detected in any isolate. GBS isolated

between 2003-2007 exhibited a penicillin MIC range between 0.016-0.12 mg/L, 0.047 mg/L MIC50 and 0.094 mg/L MIK90 values. Erythromycin, clindamycin, and tetracycline resistance rates were 19%,
16% and 94%, respectively.
GBS isolated between 2015-2017 had a penicillin MIC range of 0.047-0.125 mg/L, 0.094 mg/L MIC50 and 0.125 mg/L MIC90 values. The resistance rates for erythromycin, clindamycin, and tetracycline were 34%, 13%, and 93%, respectively.
The rise in penicillin MICs and the increase in the rate of erythromycin resistant isolates over time were statistically significant ($p < 0.001$, $p < 0.05$, respectively).

CONCLUSION
Although penicillin resistance has not been detected in any GBS, rising MIC values are concerned. Since erythromycin resistance is significantly increased over the years, these antibiotics should be kept for patients with beta-lactam hypersensitivity. The rates of clindamycin and tetracycline sensitive GBS isolates remain stable between study periods.

AR9 SUSCEPTIBILITY RATES OF *MYCOBACTERIUM TUBERCULOSIS COMPLEX* ISOLATES

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Introduction
Tuberculosis remains an important public health problem all over the world. The most important obstacle in the treatment of tuberculosis is increased drug resistance. Regional and global drug resistance data should be known to control the spread of the disease. In this retrospective study, susceptibility data to primary drugs were analysed in *Mycobacterium tuberculosis complex* (MTC) strains isolated in our hospital.

Methods
Between January 2011 and September 2017, 15.172 samples were cultivated in our laboratory. BACTEC MGIT 960 (Becton Dickinson, USA) system was used for isolation and susceptibility testing of the bacteria according to the manufacturer's recommendations.
The strains evaluated as MTC by an immunochromatic test (Mp4 64) and antibiotic susceptibilities were investigated against streptomycin (SM) (2.0 µg/ml), isoniazid (INH) (0.1 µg/ml), rifampin (RF) (2.0 µg /ml)

and ethambutol (ETM) (2.5 μg/ml).

Results

Mycobacteria were detected in 426 of the samples and 355 (2.3%) of the isolates were detected as MTC, whereas 71 (0.46%) as non-tuberculous mycobacteria. Single isolate per patient was analysed, therefore 251 MTC strains were included. 159 (63.3%) isolates were from the respiratory system (sputum, bronkoalveolar lavage, tracheal aspirate, pleural fluid) and 92 (36.7%) were from the non-respiratory samples (urine, tissue-abscess, sterile body fluids, gastric aspirates). While 181 (72%) strains were susceptible to all tested drugs, 70 strains exhibited resistance to one or more drugs. Resistance rates were determined as 20%, 9.6%, 8.2% and 5.2% for INH, SM, ETM and RIF respectively (Table 1). The number of multidrug-resistant MTC was 10 (4%).

Conclusions

Drug resistance threatens progress made in TB care and control worldwide. Drug resistance arises due to improper use of antibiotics and a big challenge in areas with weak TB control. .Anti tuberculosis drug resistance screening should be done regularly. Continuous follow-up of these obtained data will greatly contribute to the implementation of a successful tuberculosis control program.

Table 1: Resistance rates of MTC isolates

	RESISTANT ANTIBIOTICS	**NUMBER(%)**
Single drug resistance	INH RIF SM ETM	29 (%11.5) 4 (%1.5) 6 (%2.4) 8 (%3)
Two drug resistance	INH+RIF INH+SM INH+ETM	2 (%0.7) 7 (%2.7) 2 (%0.7)
Three drug resistance	INH+SM+ETM	4 (%1.5)
Four drug resistance	INH+RIF+SM+ETM	8 (%3)
TOTAL		251

INH: isoniazid RIF: rifampicin SM: streptomycine ETM: ethambutol

AR10 ANTIBIOTIC SUSCEPTIBILITY OF *HELICOBACTER PYLORI* ISOLATES FROM BIOPTIC MATERIAL

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Helicobacter pylori is gram-negative bacterium often present in oral cavity and gastric mucosa. It can cause chronic gastritis, gastric and duodenal ulcers, gastric cancer and mucosa-associated lymphoid tissue lymphoma. Treatment is recommended in all symptomatic patients. Various consensus groups suggest treatment with two antimicrobial agents (clarithromycin and amoxicillin or metronidazole) and a proton pump inhibitor. Unfortunately, antimicrobial resistance in *H.pylori* is a growing problem leading consequently to therapeutic failure.

This study was conducted to evaluate the prevalence of primary antibiotic resistance to commonly used antimicrobial agents in *H. pylori* isolates from our laboratory.

Antral biopsy specimens were homogenized and bacteria were grown in brain heart infusion agar with 10% sheep blood and incubated at 37°C in 5% O_2, 10% CO_2, and 85% N_2 for 5 days. *H. pylori* isolates were identified based on colony morphology, Gram staining results, and positive reactions for oxidase, catalase, and urease. Isolated strains were stored at -80°C in brain heart infusion broth containing 10% glycerol and 10% horse serum. Susceptibility of *H.pylori* isolates to Amoxicillin, Doxycycline, Azithromycin, Clindamycin, Ciprofloxacin, levofloxacin, Metronidazole, Erythromycin and Clarithromycin was tested by disk diffusion method.

In the period from 2015. to 2017. totally 114 bioptic materials were analyzed. After cultivation during five days *H.pylori* was isolated from 52 specimens. The resistance to amoxicillin was detected in 9,6% of isolates, which is much higher than average results from European countries (0 – 2%). Very high percentage of resistance was obtained, also for metronidazole (57.7%). Results for Erythromycin and Clarithromycin are in the same range as results from most European countries. Interestingly, Ciprofloxacin and levofloxacin had rather decreased percentage of resistance: 7.7% and 1.9% respectively. Low percentage was also detected for doxycycline (1,9%), while azithromycin and Clindamycin had 11.5 and 34.6 percentage of resistant isolates. Antibiotic resistance of *H.pylori* is growing problem and most probably the main cause of therapeutic failure in patients suffering from chronic gastritis and ulcus diseases. That is why it should be followed and controlled for mechanisms and routes of spreading.

AR11 BACTERIAL ETIOLOGY AND ANTIBIOTIC RESISTANCE PATTERNS AT UNIVERSITY HOSPITAL "SHEFQET NDROQI" IN TIRANA, ALBANIA, DURING 2017

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Introductions: Antimicrobial resistance (AMR) is a global threat. Resistant infections may lead to fatal outcomes especially in immune-compromised patients or patients with underlying chronic conditions. It is important to timely detect and treat these infections as well as to prevent and control spread of dangerous pathogens in the hospital environment.

Objective: The aim of this study is to give an overview on bacterial etiology and resistance patterns of clinical strains isolated during 2017.

Methods and materials: This is a retrospective study on clinical strains isolated at the University Hospital "Shefqet Ndroqi" from January to December 2017. AST was performed after EUCAST disk diffusion methodology. Interpretation was performed by EUCAST Breakpoint Tables v 7.1.

Results: We collected data from ASTs of 538 clinical isolates (179 Staphylococcus spp (33%), 126 Enterobacteriaceae (23%), 86 Pseudomonas spp.(16%), 82 Streptococcus spp.(15%), 31 Enterococcus spp.(5,5%), 31 *Acinetobacter baumannii* (5,5%), 2 *Stenotrophomonas maltophilia* (0,37%) and 1 *Burkholderia cepacia*(0,18%)).

Among Enterobacteriaceae the most common isolates were *Escherichia coli* 55 (45%) and *Klebsiella pneumoniae* 28 (23%). *Escherichia coli* resistance rates were higher for fluorquinolones: ciprofloxacin (29,09%) levofloxacin (21,82%) and norfloxacin (21,82%) and lower for carbapenems: meropenem (0%). *Klebsiella pneumoniae* resistance rates were higher for piperacillin-tazobactam (50%) and cefalosporins: cefepime (42,86%), ceftriaxone (42,86%), ceftazidime (42,86%) and cefuroxime (39,29%). High rates were also observed for gentamycin (39,29%) and ciprofloxacin (35,71%). Lower resistance rates were observed for meropenem (3,57%) and imipenem (3,57%). *Acinetobacter baumannii* showed the following resistance patterns: meropenem (75%) imipenem (66,67%), gentamycin (65%) amikacin (62,5%), ciprofloxacin (60%), levofloxacin (36,36%), tobramycin (36,36%), trimethoprim-sulfamethoxazole (30,77%), tigecyclin (30%), netilmycin (0%). Regarding *Staphylococcus aureus*, we found 52,94% of all isolates to be methicillin resistant. Same proportion of methicillin resistance was also observed among other coagulase negative staphylococci.

Conclusions: Where possible use of antibiotics should be guided by laboratory results of AST.
The rapid detection of resistant isolates is critical to effective patient management.

AR12 PREVALENCE AND ANTIMICROBIAL RESISTANCE PATTERNS OF EXTENDED-SPECTRUM ß-LACTAMASE-PRODUCING ESCHERICHIA COLI IN BITOLA, 2017: A DESCRIPTIVE STUDY OF REPORTED ISOLATES

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Introduction
Extended-spectrum ß-lactamase-producing E.coli is an emerging public health concern due to increased prevalence and increased antimicrobial resistance. These infections caused by E. Coli ESBL have resulted in poor outcomes longer hospital stays, and greater hospital expenses.

Objectives
The objective of this study was to determine the prevalence and antimicrobial resistance of E. coli ESBL isolates in Bitola in 2017.

Materials and methods
Descriptive study of microbiological isolates and antimicrobial resistance reported in the Center for Public Health Bitola, for the region of Bitola for the period from January 1, 2017 to December 31, 2017.

Results
From all reported microbiological isolates (429), 76 (17.7%) were Escherichia coli ESBL. E. coli ESBL was most commonly,isolated from urine (88.3%), in patients aged 2- 86 years (mean age 57.9, SD 23.7). Most of the isolates were resistant to ampicillin, amoxicillin, cefuroxime and cefalexin, and most of the isolates were sensitive to meropenem, imipenem and ertapenem. All isolates (100%) showed resistance to more than three antibiotics.

Conclusion
Our result conclude that there are a significant nummber of E. coli ESBL isolates reported. It is higly recommended that antibiotic prescription should be monitored according to the guidelines. Antibiotic consumation should be monitored both in helathcare facilites as well as in community. The role of infection prevention and control is crucial in all healthcare facilities to decrise the occurence of antibiotic resistence.

ПРЕВАЛЕНЦИЈА И АНТИМИКРОБНА РЕЗИСТЕНЦИЈА НА ESCHERICHIA COLI ESBL ВО БИТОЛА, 2017: ДЕСКРИПТИВНА СТУДИЈА НА ПРИЈАВЕНИ ИЗОЛАТИ

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Вовед
Инфекциите предизвикани од Escherichia coli ESBL претставуваат важен проблем во јавното здравство пред се поради зголемена преваленција и зголемување на резистенцијата кон антимикробните средства. Овие инфекции најчесто резултираат со незадоволувачки исход на болеста, подолготрајна хоспитализација, а со тоа и зголемување на трошоците за болничка нега и терапија.

Цел
Целта на овој труд беше да се прикажи преваленцијата и антимикробната резистенција на Escherichia coli ESBL во Битола за 2017 година.

Материја и методи
Дескриптивна студија на обработени пријави за изолиран- докажан причинител на заразна болест и резистенција на антибиотици пријавени во Центар за јавно здравје Битола, за регионот на Битола во 2017 година во периодот од 01.01.2017-31.12.2017.

Резултати
Од вкупно 429 пријавени микробиолошки изолати, 76 (17,7%) припаѓаат на Escherichia coli ESBL. Најчесто бактеријата е изолирана од урина (88,3%), кај пациенти на возраст од 2 до 86 годишна возраст (средна возраст 57,9, SD 23,7). Најчесто изолатите се резистентни на ampicillin, amoxicillin, cefuroxime и cefalexin, а најчесто сензитивни се на meropenem, imipenem и ertapenem. Сите изолати (100%) покажуваат резистенција кон повеќе од три антибиотици.

Заклучок
Поради значаен број на пријавени микробиолошки изолати со Escherichia coli ESBL, мора да се зајакне контролата на употребата на антибиотици во болниците и во останатите здравствени установи на секое ниво. Значењето на контролата и превенцијата на инфекциите е есенцијално за здравствениот систем, а со тоа и за намалување на антибиотската резистенција.

AR13 RESISTANCE RATES OF *STAPHYLOCOCCUS* SPP. ISOLATED FROM WOUND AND NASAL SAMPLES AGAINST MUPIROCIN AND OTHER ANTIBIOTICS

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Introduction
Staphylococci are amongst the most significant human pathogens that cause infectious diseases ranging from mild skin infections to potentially fatal health care associated infections. Traditional treatment options were ß-lactam antibiotics, but with the occurrence of methicillin-resistance, treatment options have become very narrow.

Objectives
The aims of this study are; to determine mupirocin and other antibiotic susceptibilities of *Staphylococcus* spp. isolated from nose and wound specimens and to guide to empirical treatment options.

Materials and methods
102 staphylococci isolated from nose and wound specimens were included in this study. Clinical findings of the patients and Q scoring were considered in determination of the causative agents. Identification of isolates was performed using conventional methods and Phoenix (BD, USA) automated system. Kirby-Bauer disk diffusion method (5 and 200 µg discs for mupirocin) and Phoenix automated system were used in detection of antimicrobial susceptibilities. Results were interpreted according to EUCAST 2017 criteria. Inhibition zone of ≥14 mm was considered as sensitive for mupirocin.

Results and conclusion
67 of isolates were *S. aureus;* 49 (48%), methicillin sensitive *S. aureus,* 18 (18%), methicillin resistant *S. aureus*. 35 of isolates were coagulase negative staphylococci (CNS); 25 (25%), were methicillin resistant CNS, 10 (10%), methicillin sensitive CNS. Mupirocin and other antimicrobial resistance rates are shown in Table 1. Seven CNS isolates were identified as resistant to mupirocin, and no difference was detected between 5 and 200 µg antibiotic discs in terms of detecting resistance. In our study resistance to mupirocin has been found to be low but mupirocin resistance is increasingly being reported in many parts of the world. In order to reduce resistance development; it is important to identify common infectious agents and their antimicrobial susceptibilities and to select the antimicrobial agent to be used

according to culture and antimicrobial sensitivity test results.

Key words: *Staphylococcus* spp., mupirocin, antimicrobial resistance

Table. Antimicrobial resistance rates of *Staphylococcus* spp.

	MRSA* (n=18) (%)	MSSA* (n=49) (%)	MRCNS* (n=25) (%)	**MSCNS* (n=10) (%)**
Penicillin	100	98	100	100
Erythromycin	61	35	60	40
Clindamycin	33	2	24	0
Trimethoprim sulfamethoxazole	11	2	4	0
Vancomycin	0	0	0	0
Teicoplanin	0	0	0	0
Linezolid	0	0	0	0
Tetracycline	17	24	20	0
Ciprofloxacin	22	24	16	0
Gentamicin	6	20	32	0
Mupirocin 5 µg	0	0	24	10
Mupirocin 200 µg	0	0	24	10

* MSSA: Methicillin sensitive *S. aureus*, MRSA: Methicillin resistant *S.aureus*, MRCNS: Methicillin resistant coagulase negative staphylococci, MSCNS: Methicillin sensitive coagulase negative staphylococci

AR14 INVESTIGATION OF CARBAPENEMASES IN CARBAPENEM RESISTANT ENTEROBACTERIACEAE ISOLATES WITH VARIOUS METHODS

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Introduction
Carbapenem resistance is also common in our country as it is in the whole world. Dissemination of carbapenem resistant isolates, increases morbidity and mortality. Although investigation of resistance mechanisms does not change the outcome of antibiotic susceptibility, it is suggested for infection control and surveillance studies.

Objective
The aims of this study were; to investigate presence of carbapenemases

in carbapenem resistant Enterobacteriaceae by multiplex polymerase chain reaction (PCR), carbapenem inactivation method (CIM) and modified Carba NP tests, to determine a suitable test for practical application and to obtain epidemiological data of our hospital.

Materials and Methods
81 of the Enterobacteriaceae, isolated from samples sent to our laboratory, and is resistant to at least one of the carbapenems were included in the study. All isolates were investigated with CIM and modified Carba NP tests described before.
Presence of OXA-48, NDM, KPC, VIM, IMP, SIM, GIM, AIM, DIM, BIC, SPM genes were investigated with multiplex PCR.

Results
Klebsiella pneumoniae was the most common isolate (72,8%). Sixty four isolates (79%) were detected to harbour at least one of the carbapenemase genes investigated. Most common carbapenemase gene detected was solely OXA-48 (59.3%). Isolates included, and enzymes detected in the study are shown in Table. Results of Carba NP and CIM tests were 100% compatible with each other. Carba NP and CIM test were positive in 57 (87.6%) of 65 isolates harbouring one of the carbapenemases. Sensitivity of Carba NP and CIM test was 87.6%, specificity was 81%.

Conclusion
Although gold standard for detection of carbapenemases is molecular methods, phenotypic Carba NP and CIM tests are cheap and easily applicable tests in every laboratory, even though they have low sensitivity in detecting OXA-48. A significant advantage of Carba NP over CIM test is its speed.

Key words: Carba NP, CIM test, multiplex PCR

Table. Distribution of carbapenemase genes among *Enterobacteriaceae* involved in the study

Isolate	OXA-48	VIM	NDM	OXA-48+VIM
K. pneumoniae n=59	37 (63%)	4 (6,7%)	2 (3,3%)	8 (13,5%)
E. coli n=16	7 (43,7%)	-	-	2 (12,5%)
E. cloacae n=3	2 (66,7%)	-	-	-
K. oxytoca n=2	2 (100%)	-	-	-
E. aerogenes n=1	-	-	-	-

AR15 ANTIMICROBIAL SUSCEPTIBILITY PROFILE AND CUMULATIVE ANTIBIOGRAM OF URINARY E.COLI ISOLATES TO GUIDE EMPIRICAL TREATMENT IN OLDER PATIENTS

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Introduction: Urinary tract infections are one of the most prevalant bacterial infections in older patients, usually causing bacteriemia or sepsis. Rapid diagnosis and treatment is very important and empirical treatment is often prescribed without the urine culture results are available. According to treatment guidelines, empirical treatment choices should be based on susceptibility data.

Objectives: In this study, we aimed to evaluate the antibiotic susceptibility profile and cumulative antibiogram results of urinary *E.coli* isolates in older patients.

Material, Methods: Urinary *E.coli* isolates from patients ≥65 years of age submitted to Marmara University Hospital Microbiology Laboratory between January and December 2017 were included in the study. Isolates were identified by Vitek MS (BioMerieux,France) and antibiotic susceptibilities were performed by Vitek2 system (Biomériux,France). Cumulative antibiotic results were analyzed according to the susceptibility results

Results and Conclusion: A total of 395 *E.coli* isolates were included in our study. Of these 88 (22,3%) were from inpatients, whereas 307 (77,7%) from outpatient urinary isolates. As seen in table antibiotic susceptibility rates were detected highest in fosfomycin and nitrofurantoin among oral antibiotics whereas lower susceptibility rates were detected in all beta lactams including cefalosporins. Among oral antibiotics, fosfomycin and nitrofurantoin showed significantly highest activity with the susceptibility rates over 97% for treating both inpatient and outpatient urinary tract infections by *E.coli*. According to our data, amikacin and carbapenems seem to be the few parenteral options for *E.coli* isolates in older patients.

Table. Antibiotic susceptibility of urinary *E.coli* isolates in elderly patients.

ANTIBIOTICS	**Susceptibility%**		
	INPATIENT	OUTPATIENT	TOTAL
	n.88	n:307	n:395
Ampicilin	25	35,2	32,9
Amoxicillin-clavulanate	35,2	49,8	46,6
Piperacillin/tazobactam	61,4	73,6	70,9
Cefuroxime	37,5	54,4	50,6
Cefuroxime axetil	37,5	54,7	50,9
Cefixime	46,6	60,3	57,2
Ceftazidime	48,7	62,9	59,7
Ceftriaxone	48,7	61,6	59
Ertapenem	98,9	98	98,2
İmipenem	100	98,7	99
Meropenem	98,9	99	99
Amikacin	86,4	90,5	89,6
Gentamicin	79,4	76,5	77,2
Ciprofloxacin	41	47,9	46,3
Fosfomicin	97,7	99	98,7
Nitrofurantoin	98,9	97,4	97,7
Trimetoprim sulphametaxazole	55,7	59	58,2

AR16 ANTIMICROBIAL SUSCEPTIBILITY PATTERNS IN *HAEMOPHILUS INFLUENZAE* STRAINS ISOLATED IN MACEDONIA

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Background: *Haemophilus influenzae* (Hi) is a major causative factor of respiratory and otolaryngology infections, especially community-acquired pneumonia in elderly persons and otitis media and sinusitis in children. There has been a recent world-wide increasing incidence of resistance to aminopenicillins (induced not only by enzyme mechanisms but also by a change of their target) and to other antibiotics such as tetracyclines, chloramphenicol, trimethoprim/sulfamethoxazole, and fluoroquinolones, commonly used to treat Haemophilus infections.

Aim: The objective of this study was to determine antibiotic susceptibility of *Haemophilus influenzae* strains isolated from respiratory tract,

specifying the mechanisms of beta- lactam resistance.

Material: This stuady, which take place from January to December, 2017, made use of 144 *Haemophilus influenzae* strains isolated from tracheal aspirate, sputum, eye and ear swab at Microbiology laboratory at Center for public health Bitola,

Method: *Haemophilus influenzae* strains were isolated on Haemophilus Chocolate 2 agar, Biomerieux and were confirmed on VITEC 2, NH cards. Susceptibilities of Hi to ampicillin, amoxicillin-clavulanic acid, trimethoprim-sulfamethoxazole, chloramphenicol, tetracycline and ciprofloxacin were determined using Kirby- Bauer method according EUCAST critera. Mechanisms of beta- lactam resistance were determined by E-test for ampicillin and amoxicillin-clavulanic acid and Nitrocefin disk,Mast Group, Ltd., Marseyside.

Results: The isolates were found to be antibiotic nonsusceptible in the following order: trimethoprim-sulfamethoxazole (32,9%), ampicillin (24,8%), ciprofloxacin (6.8%), amoxicillin-clavulanic acid (6.1%), and we didn't detect any isolate that was resistant to chloramphenicol and tetracycline. The prevalences of each resistance class to beta-lactams were 75,2% for -lactamase-negative ampicillin-susceptible (BLNAS) strains; 4,8% for the beta-lactamase-negative ampicillin-resistant (BLNAR) strains; and 1,5% for beta-lactamase-positive amoxicillin-clavulanateresistant (BLPACR) strains, which showed both resistance mechanisms.

Conclusion: In summary, the susceptibility testing of *Haemophilus* influenzae strains, showed the increasing of resistance to tested antibiotics. Therefore, continued monitoring of the susceptibility trends will be needed to guide the appropriate antimicrobial chemotherapy.

АНТИМИКРОБНА ОСЕТЛИВОСТ КАЈ СОЕВИ НА *HAEMOPHILUS INFLUENZAE* ИЗОЛИРАНИ ВО МАКЕДОНИЈА

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Вовед: *Haemophilus influenzae* (Hi) е главен предизвикувач на респираторни и отоларинголошки инфекции, особено на пневмонија стекната во заедницата кај постари лица и отитис медиа и синузитис кај деца. Постојат податоци за зголемена инциденца на на резистентни соеви на *Haemophilus influenzae* кон аминопеницилини, кон тетрациклини, хлорамфеникол, триметоприм / сулфаметоксазол и флуорохинолони. Антибиотици кои најчесто се користат за во терапија овие инфекции.

Цел: Целта на оваа студија беше да се утврди антимикробната

осетливост кон ампицилин, амоксицилин-клавуланска киселина, триметоприм-сулфаметоксазол, хлорамфеникол, тетрациклин и ципрофлоксацин кај соевите на *Haemophilus influenzae*, специфицирајќи ги фенотипските механизми за резистенција кон бета-лактамските антибиотици.

Материјал: Во оваа студија , која се одвиваше од јануари до декември 2017 година, беа искористени 144 соеви на *Haemophilus influenzae* изолирани од трахеални аспирати, спутум, брисеви од око и уво во микробиолошката лабораторија при Центарот за јавно здравје Битола.

Метод: Соевите на *Haemophilus influenzae* беа изолирани на Haemophilus Chocolate 2 агар, Biomerieux и беа потврдени на VITEC 2, NH картички. Осетливоста кон испитуваните антибиотици беше тестирана со користење на Kirby- Bauer -метод според EUCAST критериуми. Фенотипски механизми на резистенција кон беталактамски антибиотици беа детектирана со Е-тест за ампицилин и амоксицилин-клавуланска киселина и Nitrocefin disk,Mast Group, Ltd., Marseyside.

Резултати: При тестирањето на атимикробната осетливост, процентот на резистенција кај соевите на *Haemophilus influenzae* беше детектиран по следниов редослед: триметоприм-сулфаметоксазол (32,9%), ампицилин (24,8%), ципрофлоксацин (6,8%), амоксицилин-клавуланска киселина (6,1%), не беше детектиран ниту еден изолат резистентен кон хлорамфеникол и тетрациклин. Преваленците на секоја класа на резистенцијата кон бета-лактамските антибиотици беше: 75,2% за соевите осетливи на ампицилин, бета- лактамаза негативни (BLNAS); 4,8% за соеви-резистентни кон ампицилин, бета- лактамаза-негативни (BLNAR); и 1,5% за бета -лактамаза-позитивни амоксицилин-клавуланска киселина резистентни (BLPACR), кои ги поседуваат и двата механизми на резистенција.

Заклучок: Земајќи го во предвид трендот на покачување на антимикробната резистенција, се препорачува континуирано тестирање и следење на механизмите на резистенција кај соевите на *Haemophilus influenzae* со цел ординирање на соодветна антимикробна хемотерапија.

СЕСИЈА 5/SESSION 5
ЛАБОРАТОРИСКИ МЕТОДИ ВО МИКРОБИОЛОГИЈАТА/ LABORATORY METHODS IN MICROBIOLOGY

M1 MICROBIOLOGICAL DIAGNOSTICS IN THE REPUBLIC OF MACEDONIA - WE WANT CLOSER TO THE EUROPEAN UNION

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Microbiological diagnosis has existed in R. Macedonia for more than 70 years. Over 100 specialist microbiologists, educated in the Republic of Macedonia and beyond, have continuously been upgraded theoretically and practically in order to be in a trend with the new achievements in diagnostics. The European Center for Disease Control coordinates work in EU countries, and in 2013 it showed interest to include us, aspirant countries for the EU, in particular Western Balkan countries. One of the tools for harmonizing microbiology is the questionnaire intended for EU countries, called EULabCap. For aspiring countries this year the questionnaire was modified and received the name ENLabCap. Responding to the ENLabCap questionnaire, I came to the conclusion that it is very good to see what are the new trends in the EU in microbiology, where we need to work more, what to do more in everyday work.

Major deficiencies in microbiological diagnosis in the Republic of Macedonia include problems with equipment shortages (biosafety facilities), molecular methods (Whole genome sequencing), serological and molecular typing of micro-organisms of particular interest in the EU (O-serogrouping of STEC / VTEC, MLVA genotyping of *Salmonella enterica* serotypes *Typhimurium* and *Enteritidis*, genotyping of resistant mycobacteria with MIRU_VNTR method, typing invasive meningococci with complete serotyping and sequencing of genes (MLST method, porA and fetA), identification of the type of ESBL, HIV genotyping and sequencing on antiretroviral targets, susceptibility of human influenza virus to neuraminidase inhibitors, *Legionella* cultivation to confirm cases of legionnaires disease, genotyping of Hepatitis A virus). Case definition guidelines are not used in the Republic of Macedonia (the latest version of the EU's Case Confirmation from 2012), which are translated into Macedonian. R. Macedonia has no

system for nominating reference microbiological laboratories and its functioning is not regulated, especially in terms of financing. The questionnaires require statistical data on the number of hospital beds-days and specific tests carried out on 1000 hospital beds-days, for which there is no calculated data in our country, such as blood cultures, tests for *Clostridium difficile*. We have no official data available on new HIV cases under 14 years of age with an initial CD4 count of 350 cells / μl. The participation on expert meetings in European networks for certain diseases (for example, ERLTB-Net tuberculosis, Euro-GASP gene resistance), as well as continued participation in external quality control (EQA) networks are not our practice, because of financial and personal reasons. Guidelines for the diagnosis, treatment and monitoring of many diseases of particular interest to the modern world has been prepared in the Republic of Macadonia, but we have no control over the implementation of these guidelines, for example for *Clostridium difficile*, resistance of *Enterobacteriaceae* to carbopenems, reporting of antimicrobial resistance of human *Salmonella* and *Campylobacter* according EUCAST protocols is not adequate or absent. We do not have a national system for collecting and reporting / monitoring infections with *Chlamydia trachomatis* and some other microorganisms. Reporting of diseases, isolated / proven microorganisms from microbiological laboratories and in part the antimicrobial resistance is laborious and time-consuming, primarily because of paper based copies, and not with an electronic system.

There are numerous achievements which are welcome: EQA result of the National Tuberculosis Reference Laboratory, the PCR method for the laboratory confirmation of *Bordetella pertussis*, the developed national guidelines for the investigation of colistin susceptibility and colistin acquired resistance of carbapenem-resistant enterobacteria as well as resistance mechanisms, and a more other quality work done at the highest level.

МИКРОБИОЛОШКА ДИЈАГНОСТИКА ВО Р.МАКЕДОНИЈА – САКАМЕ ПОБЛИСКУ ДО ЕВРОПСКАТА УНИЈА

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Микробилошка дијагностика во Р. Македонија постои веќе 70-тина години. Над 100 специјалисти микробиолози, едуцирани во РМ и надвор од неа, континуирано се надградуваа теоретски и практично, со цел да бидат во тренд со новите достигнувања во дијагностиката. Европскиот центар за контрола на болести ја

координира работата во земјите на ЕУ, но во 2013 година покажа интерес да не вклучи и нас, земјите аспиранти за ЕУ, пред се земји на Западен Балкан. Една од алатките за хармонизирање на микробиологијата е прашалникот наменет за земјите на ЕУ, наречен EULabCap. За земјите аспиранти оваа година прашалникот беше модифициран и доби име ENLabCap. Одговарајќи го ENLabCap прашалникот дојдов до заклучок дека е многу умесно да согледаме кои се новите трендови во ЕУ во микробиологијата, каде треба повеќе да поработиме, што да воведеме во секојдневната работа.

Поважни недостатоци во микробиолошката дијагностика во Р. Македонија се проблемите со недостаток на опрема (услови за безбедна лабораториска работа), молекуларни методи (Whole genome sequencing), серолошка и молекуларна типизација на микроорганизми од посебен интерес во ЕУ (О-серогрупирање на STEC/VTEC, MLVA генотипизација на *Salmonella enterica* serotype *Typhimurium* и *Enteritidis*, генотипизација на резистентни микобактерии со MIRU_VNTR метод, типизација на инвазивни менингококи со серогрупирање и секвенционирање на гени (MLST, рогА и fetA), идентификација на типот на ЕСБЛ, генотипизација на HIV со секвенционирање на антиретровирусни таргети, осетливост на Human influenza virus на инхибитори на неураминидаза, култивација на легионела за потврда на случаи на легионерска болест, генотипизација на Hepatitis A вирус). Во Р. Македонија не се употребуваат комплетно упатствата за дефиниција на случај (последна верзија за Case confirmation во ЕУ од 2012 година), кои се преведени на македонски. Р. Македонија нема систем за номинирање на референтни микробиолошки лаборатории и не е регулирано нивното функционирање, особено од аспект на финансирање. Прашалниците бараат статистички податоци за број на зафатени болнички легла - hospital bed-days и извршени специфични тестирања на 1000 hospital bed-days, како на пример изработени хемокултури, тестирања за *Clostridium difficile,* за кои во нашата земја нема пресметани официјални податоци. Немаме официјално достапни податоци за нови HIV случаи под 14 годишна возраст со иницијален број на CD4 под 350 клетки/μl. Учеството на стручни состаноци на европски мрежи за поединечни болести (на пример туберкулоза ERLTB-Net, за резистенција на гонококи Euro-GASP), како и континуирано учество во мрежите за надворешна контрола на квалитетот на тестирањата (EQA) не се наша пракса, од финансиски и од лични причини. Во Р. Макодонија има упатства за дијагноза, терапија и следење на повеќе болести кои се од посебен интерес за современиот свет, но немаме контрола над имплементацијата на тие упатства, како на пример за *Clostridium difficile*, резистенција на ентеробактерии кон карбапенеми, не соодветно или отсутно е пријавување на антимикробна резистенција на хумани салмонели и кампилобактер. Немаме национален систем за собирање и пријавување/следење на

инфекции со *Chlamydia trachomatis* и уште некои микроорганизми. Пријавувањата на болестите, на изолтатите/докажаните микроорганизми од микробиолошките лаборатории и делумно на антимикробната резистенција е макотрпно и одзема многу време, пред се заради пријави во печатена а не во електронска форма.

За поздравување се: EQA резултатот на Националната референтна лабораторија за туберкулоза, воведената ПЦР метода за лабораториска потврда на *Bordetella pertussis,* изработените национални упатства за испитување на осетливост на колистин и стекната резистенција кон колистин на карбапенем-резистентни ентеробактерии, како и докажување на механизмите на резистенција, и низа дгуги квалитетно извршени работи, на највисоко ниво.

M2 ALL THE TASKS OF CLINICAL MICROBIOLOGY LABORATORY

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Clinical Hospital Acibadem Sistina

Clinical laboratories play a vital part in health care. Microbiology laboratory is an integral part in the process of managing patients with various infectious diseases.

The laboratory has two main functions in the hospital: 1) to diagnose infection in a patient as an individual and 2) to support the hospital system of healthcare-associated infection prevention and control. In order to provide the main tasks it is important to identify causative agents of infectious diseases and to determine antimicrobial susceptibility profiles. When the etiological diagnosis of infection is rapid and accurate patient management is in proper time and way, the spreading of microorganisms is prevented in shorter time and eradication of the infecting microorganisms has more success.

Clinical microbiology lab has a duty of quality assurance procedures as well as external quality control of main procedures performed. Laboratory accreditation is a process that assures the use of appropriate standard operating procedures that cover all the aspects of laboratory work. The system is essential to prove the quality of the results.

Laboratory has to set criteria for proper specimen collection, transport and rejection. All specimens sent to the microbiology laboratory have to be taken from appropriate sites with proper techniques from trained personnel, collected on right time, in a quantity that will ensure good workout in the lab. Microbiology laboratory staff should educate clinical staff in order to ensure good specimen collection and transport.

Microbiological diagnostic tests chosen to be performed in the clinical

microbiology lab should be fast, accurate, with high sensitivity and specificity. The laboratory sets clear rules for the interpretative reporting.

Communication between the lab and clinicians is a critical point of patient care because it improves the efficient use of laboratory results by providing clinically relevant advice and guidance. Microbiology lab can choose to act as an early warning system using both verbal and written reporting to the clinician responsible for the patient care.

Efficient, close communication, collaboration and cooperation between microbiology laboratory, clinician and infection control personnel can fulfill the duties of successful patient care and healthcare-associated infection prevention and control.

СИТЕ ЗАДАЧИ НА ЛАБОРАТОРИЈА ЗА КЛИНИЧКА МИКРОБИОЛОГИЈА

Б. Ќурчиќ, К. Кубелка-Сабит, В. Филипче

Клиничка болница Аџибадем Систина

Клиничката лабораторија има витална улога во здравствената нега. Микробиолошката лабораторија е интегрален дел во процесот на менаџирање на пациентите со различни инфективни заболувања.

Лабораторијата има две главни функции во болницата: 1) да ја дијагностицира инфекцијата кај пациентот како индивидуа и 2) да го пддржи болничкиот систем за превенција и контрола на интрахоспитални инфекции. За да ги обавува овие две функции важно е да ги идентификува предизвикувачите на инфекциите и да ги одреди профилите на осетливост кон антимикробни средства. Кога етиолошката дијагноза е брза и точна третманот на пациентот е навремен и соодветен, ширењето на микроорганизмите се пречува за покусо време и ерадикацијата на инфективните микроорганизми има поголем успех.

Лабораторијата за клиничка микробиологија има задача да воведе процедури кои гарантираат квалитет и екстерни контроли на главните прцедури кои се изведуваат. Акредитација на лабораторијата е процес кој овозможува употреба на стандардни оперативни процедури кои го опфаќаат секој аспект од активностите на работата. Системот е неопходен да се гарантира квалитет на резултатите.

Лабораторијата мора да постави критериуми за правилно земање на примероци, нивен транспорт и отфрлање. Сите примероци кои се праќаат во лабораторијата треба да се земени од соодветно место со соодветни техники и од обучен персонал, земени во правилно време и во количина која ќе овозможи квалитетна обработка. Вработените во лабораторијата за клиничка микробиологија треба да го едуцираат болничкиот персонал за правилно земање и транспорт

на примероците.

Микробиолошките дијагностички тестови кои се избрани за изведување во лабораторијата треба да се брзи, точни, со висока сензитивност и специфичност. Лабораторија треба да сетира јасни правила за интерпретативно известување.

Комуникацијата меѓу лабораторијата и клиничарите е критична точка во негата на пациентот бидејќи ја подобрува ефикасната употреба на лабораториските резултати со обезбедување на клинички релевантни совети и насоки. Микробиолошката лабораторија може да одлучи да биде дел од системот за известување на панични критични вреднсти и наоди употребувајќи исторемено вербални и пишани известувања на докторот одговорен за пациентот.

Ефикасна, блиска комуникација и соработка на микробиолошката лабораторија со клиничарите и персоналот одговорен за превенција и контрола на интрахоспитални инфекции е единствен начин кој овозможува успешна нега на пациентот и стабилен процес на превенција и контрола на интрахоспитални инфекции.

M3 THE ROLE OF MICROBIOLOGICAL DIAGNOSTICS IN FEVER OF UNKNOWN ORIGIN

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Introduction: Fever of unknown origin (FUO) is a clinical entity that comprises over 200 possible etiologic causes. Even today, same as decades ago, FUO continues to be a frustrating condition both for the patients and the physicians.

Objective: to assess the meaning of microbiological investigations in the definition of FUO and to evaluate their role during the diagnostic work-up in patients with FUO.

Patients and methods: Retrospectively were analyzed medical histories of 79 immunocompetent patients older than 14 years with classical FUO. The patients were managed at the University hospital for infectious diseases and febrile conditions in Skopje during the period 2012 to 2015. FUO was defined as: (a) axillary fever of 37.50C or more on several occasions with laboratory parameters of inflammation; (b) fever duration of at least 21 days; and (c) failure to reach an etiological diagnosis after the initial diagnostic work-up comprised of detailed history and physical examination, routine hematological and biochemical analysis, basic microbiological examinations, chest x-ray, abdominal ultrasound and EKG. The diagnostic evaluation in each patient with FUO was individualized

without algorithmic utilization. This evaluation included a wide spectrum of biochemical, hematological, microbiological analyses, imaging techniques, and other invasive and non-invasive procedures, always having in mind the epidemiological situation and eventual presence of potential diagnostic clues.

Results: During the initial evaluation all 79 patients with FUO had negative blood cultures (at lest two), urine culture, Rose Bengal and anti HIV test and sputum for Mycobacterium tuberculosis. Infections were the cause in 25 (32%), non-infective inflammatory disorders in 17 (21%), neoplasm in 14 (18%), miscellaneous in nine (11%) of the patients. Fourteen of the patients (18%) remained without etiological diagnosis. The diagnosis was discovered with (i) imaging techniques; (ii) microbiological investigations; (iii) clinical course and empiric therapy response; (iv) biochemical, hematological and immunological tests; (v) biopsies and histology; and (vi) endoscopic examination in 20 (31%), 13 (20%), 12 (18%), 10 (15%), nine (14%) and one (2%) respectively, among the 65 patients with FUO that had final diagnosis. Microbiological survey helped to find the cause for FUO in 13 out of 25 patients with infectious disease. In seven patients helpful were serological tests (visceral leishmaniasis in five, cytomegalovirus disease in two), cultural examinations in 4 (pyelonephritis, urosepsis, subacute endocarditis, and lung tuberculosis one patient each), skin test (tuberculosis) and direct smear of sternal aspiration (visceral leishmaniasis) both in one patient.

Conclusion: Some of the microbiological tests have their role in defining the criteria for FUO as a part of the initial diagnostic protocol. Also, these investigations are helpful in establishment of the etiological diagnosis in patients with FUO caused by infection. The introduction of molecular and more sophisticated microbiological techniques could be of important diagnostic help in significant part of patients with FUO that remain undiagnosed nowadays.

УЛОГА НА МИКРОБИОЛОШКАТА ДИЈАГНОСТИКА КАЈ ПРОЛОНГИРАНИТЕ ФЕБРИЛНИ СОСТОЈБИ

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Вовед: Пролонгираните фебрилни состојби (ПФС) се клинички ентитет кој се должи на преку 200 различни етиолошки фактори. И денес како и децении претходно, тие претставуваат фрустрирачки фактор и за пациентите и за лекарите.

Цел: Да се согледа улогата на микробиолошките иследувања во дефинирање на ПФС и да се процени нивното дијагностичкото значење кај пациените со ПФС.

Пациенти и методи: Ретроспективно беа анализирани медицинските истории на 79 имунокомпетентни пациенти постари од 14 години со класична ПФС. Пациентите беа лекувани на Универзитетската клиника за инфективни болести и фебрилни состојби во Скопје во периодот 2012 до 2015 година и ги исполнуваа следните критериуми: (а) аксиларна температура од најмалку 37.50Ц во неколку наврати заедно со лабораториски параметри за инфламација; (б) фебрилност во траење од најмалку 21 ден; и (в) неможност да се постави етиолошка дијагноза после иницијален дијагностички протокол. Иницијалниот дијагностички протокол беше сочинет од: детална анамнеза и физикален преглед, рутински хематолошки и биохемиски анализи, базични микробиолошки иследувања, радиографија на граден кош, ултразвук на абдомен и ЕКГ. Дијагностичкиот пристап кај секој пациент со ПФС беше индивидуализиран, без користење на алгоритми. Истиот вклучуваше широка палета на биохемиски, хематолошки, имунолошки, микробиолошки анализи, сликања и други инвазивни и неинвазивни процедури и во предвид ја земаше епидемиолошката ситуација и евентуалната појава на потенцијални дијагностички смерници.

Резултати: При иницијалната евалуација сите 79 пациенти со ПФС имаа негативни хемокултури (најмалку две), уринокултура, брза аглутинација за бруцелоза, анти ХИВ тест и спутум за Коховиот бацил. Инфекции беа причина за ПФС кај 25 (32%), неинфективни инфламаторни болести кај 17 (21%), малигни болести кај 14 (18%), разни состојби кај девет (11%) од пациентите. Кај 14 (18%) пациенти причина за ПФС не беше најдена. Дијагноза беше поставена со (i) техниките на сликање; (ii) микробиолошки иследувања; (iii) клиничкиот тек и одговор на емпириската терапија; (iv) биохемиски, хематолошки и имунолошки иследувања; (v) биопсии и патохистологија; и (vi) со ендоскопско иследување кај 20 (31%), 13 (20%), 12 (18%), 10 (15%), девет (14%) и еден (2%) респективно, меѓу 65 пациенти со ПФС со поставена етиолошка дијагноза. Микробиолошките иследувања помогнаа за откривање на причината на ПФС кај 13 од 25 пациенти со инфективна болест. Кај седум пациенти од помош беа серолошки иследувања (висцерална лајшманијаза кај пет, цитомегаловирусна болест кај двајца), култура кај четири (пиелонефритис, уросепса, субакутен ендокардитис и белодробна туберкулоза кај по еден пациент), кожен тест (туберкулоза) и директен препарат од стернален аспират (висцерална лајшманијаза) кај по еден пациент.

Заклучок: Некои микробиолошки постапки имаат улога во дефинирање на критериумите за ПФС и се составен дел на иницијалниот дијагностички протокол. Исто така, микробиолошките иследувања се од помош во поставување на етиолошка дијагноза кај пациенти со ПФС предизвикана од инфективни причинители. Воведување на молекуларни и други пософистицирани микробиолошки техники би можеле да бидат од дијагностичка помош кај дополнителен дел од пациентите со ПФС кои денес остануваат етиолошки непотврдени.

M4 DIAGNOSTIC POSSIBILITIES IN RESPIRATORY INFECTIONS

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Acute respiratory infections (ARIs) are the most common infections of the modern people, these are the most common diseases in the general population. Adults have infections 3-5 times a year and children up to 10 episodes. The most serious ARI disease is pneumonia and represents 1% of ARI.

The causes of respiratory infections are numerous bacteria, viruses, mycoplasmas, chlamydiae, rickettsiae, parasites and fungi. Some are more commonly some less common depending on the site of the disease, the age of the patient; some are associated with a certain predisposing condition to the patient, and some with a particular epidemiological situation.

Microbiological investigations are usually not recommended in primary care. In patients with mild clinical picture, routine microbiological testing is not performed. In patients with moderate severe and severe pneumonia, blood cultures and sputum should be taken and, optionally, pneumococcal and Legionella urine antigen tests.

Specific diagnostic tests include: bacteriological tests and diagnostic tests for the detection of atypical causes.

Proofing of microorganisms is often difficult even in hospital conditions when we are able to carry out all possible diagnostic tests and techniques in the most equipped laboratories.

Bacteriological tests can directly or indirectly prove bacterial agents in different samples from the patient. Bacteriologically examined: throat swab, sputum, blood, pleural punctate, bronchial aspirate, bronchoalveolar lavat (BAL), or lung sample obtained with bronchoscopy, transbronhaal and transthoracic aspiration puncture.

For the cytobacteriological examination of the sputum and adequate interpretation of the result of the finding, a quality sputum sample should be taken which should satisfy the following criteria: number of neutrophilic pilimorphonuclears> 25 in a field; number of epithelial cells <10 in the field; dominant flora during direct examination (colored in Gram); culture of monomorphic flora> 10^7 UI / ml. Productive cough, or sputum production, occurs in about 50% of patients with pneumonia.

Positive blood culture means an accurate and definitive aetiological diagnosis of pneumonia. However, bacteremia occurs in less than 10 to 30% of patients with bacterial pneumonia, and in clinical practice, positive blood cultures are obtained in about 5% of patients with CAP.

The routine diagnosis of atypical causes of pneumonia for clinical purposes is with serological confirmation of specific antibodies and highly specific and sensitive molecular techniques based on the detection of the structural nucleic acids of the causative agent, PCR, etc., which are at the threshold of clinical application, standardized and widely available.

At the Clinic for Infectious Diseases and Febrile Conditions - Skopje in the last three years, a total of 1122 sputum was processed, of which 431 (38.41%) were positive: *Streptococcus pneumoniae* - 2, *Haemophylus influenzae* - 14, *Pseudomonas aeruginosa* - 10, *Methicilin sensitive Staphylococcus aureus* - 1, Methicilin *resistant Staphylococcus aureus* - 4, *Klebsiella pneumoniae* - 3, *Klebsiella oxytoca* - 1, *Streptococcus pyogenes gr. A* - 20, *Aspergillus spp.*- 5, *Acinetobacter baumani complex* - 54, *Enterobacter aerogenes* - 1, *Enterobacter cloacae complex* - 2, *Proteus mirabilis* - 1, *Citrobacter casei* - 1 и *Candida albicans* - 310.

With multiplex polymerase chain reaction (PCR), the FilmArray respiratory tract for detecting the causes of respiratory tract infections, 51 nasopharyngeal swabs were followed. Of them, 23 (45,10%) received a positive finding: for *Influenza A* - 3, *Influenza B* - 5, *Parainfluenza* - 3, *Respiratory Syncytial Virus* - 1, *Human Rhinovirus/ Enterovirus* - 7 и *Bordetella pertussis* - 4.

When searching for an etiological diagnosis, we always start with simpler and easier tests, and then more complicated and invasive methods, taking into account the time for the result, the specificity and sensitivity of the method, the complications that could occur in the patient, the equipment of the patient laboratory and price. The interpretation of the results should be critical and always correlated with the clinical picture of the disease.

ДИЈАГНОСТИЧКИ МОЖНОСТИ КАЈ РЕСПИРАТОРНИТЕ ИНФЕКЦИИ

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Акутните респираторни инфекции (АРИ) се најчестите заболувања на современиот човек, тоа се најчестите заболувања во општата популација. Возрасните заболуваат 3 - 5 пати годишно, а децата и до 10 пати. Најсериозна болест од АРИ е пневмонијата и претставува 1% од АРИ.

Причинителите на респираторните инфекции се многубројни бактрии, вируси, микоплазми, хламидии, рикеции, паразити и габички. Некои се почести некои поретки зависно од местото на јавување на заболувањето, возраста на пациентот, некои се

асоцирани со одредена предиспонирачка состојба на пациентот, а некои со одредена епидемиолошка ситуација.

Микробиолошките проследувања обично не се препорачуваат во примарната здравствена заштита. Кај пациентите со лесна клиничка слика не се прават рутински микробиолошки проследувања. Кај пациентите со средно тешка и тешка пневмонија треба да се земе хемокултура и спутум и евентулно пневмококен и *Legionella* урина антиген тестови.

Специфичните дијагностички тестови опфаќаат: бактериолошки проследувања и дијагностички тестови за детекција на атипичните причинители.

Докажувањето на микроорганизмите честопати е тешко дури и во хоспитални услови кога сме во можност да ги спроведеме сите можни дијагностички тестови и техники во најсовремено опремени лаборатории.

Со бактериолошките проследувања може директно или индиректно да се докажат бактериските причинители во различни примероци од болниот. Бактериолошки се проследуваат: брис од грло, искашлок, крв, плеврален пункатат, бронхален аспират, бронхоалвеоларен лават (БАЛ), или белодробен примерок добиен со бронхоскопија, трансбронхална и трансторакална аспирациска пункција.

За цитобактериолошко испитување на спутумот и адекватно толкување на резултатот од наодот треба да се земе квалитетен примерокот од искашлок кој треба да ги задоволува следниве критериуми: број на неутрофилни пилиморфонуклеари > од 25 во поле; број на епителни клетки < од 10 во поле; доминантна флора при директен преглед (обоена по Gram); култура на мономорфна флора > 10^7 UI/ml. Продуктивна кашлица, односно продукција на спутум има само кај околу 50% од пациентите со пневмонија.

Позитивната хемокултура значи точна и дефинитивна етиолошка дијагноза кај пневмонијата. Меѓутоа, бактериемија настанува кај помалку од 10 до 30% од пациентите со бактериска пневмонија, а во клиничката практика позитивна хемокултура се добива само кај околу 5% од пациентите со CAP.

Рутинската дијагноза на атипичните причинители на пневмонии за клинички цели е со серолошка потврда на специфичните антитела и високоспецифични и сензитивни молекуларни техники, базирани на детекција на структурните нуклеински киселини на причинителот, PCR и др., кои се на прагот на клиничката примена, стандардизирани и широко достапни.

На Клиниката за инфективни болести и фебрилни состојби- Скопје во последниве три години обработени се вкупно 1122 спутуми од кои позитивни биле 431 (38,41%) и тоа: *Streptococcus pneumoniae* - 2, *Haemophylus influenzae* - 14, *Pseudomonas aeruginosa* - 10,

Methicilin sensitive Staphylococcus aureus - 1, Methicilin *resistant Staphylococcus aureus* - 4, *Klebsiella pneumoniae* - 3, *Klebsiella oxytoca* - 1, *Streptococcus pyogenes gr. A* - 20, *Aspergillus spp.*- 5, *Acinetobacter baumani complex* - 54, *Enterobacter aerogenes* - 1, *Enterobacter cloacae complex* - 2, *Proteus mirabilis* - 1, *Citrobacter casei* - 1 и *Candida albicans* - 310.

Со мултоплекс полимеразно верижната реакција (PCR), FilmArray респираторниот панел за детекција на причинителите на инфекции на респираторен тракт, проследени се 51 назофарингеален брис. Од нив кај 23 (45,10%) е добиен позитивен наод и тоа за *Influenza A* - 3, *Influenza B* - 5, *Parainfluenza* - 3, *Respiratory Syncytial Virus* - 1, *Human Rhinovirus/Enterovirus* - 7 и *Bordetella pertussis* - 4.

При трагање кон етиолошката дијагноза секогаш започнуваме со поедноставните и полесни за изведување тестови, а потоа покомплицираните и инвазивни методи, водејќи сметка за времето за кое би се добил резултатот, специфичноста и сензитивноста на методата, компликациите кои би можеле да настанат кај пациентот, опременоста на лабораторијата и цената. Толкувањето на резултатите треба да е критичко и секогаш во корелација со клиничката слика на заболувањето.

М5 УПОТРЕБА НА ИНДИКАТОРИТЕ ЗА КВАЛИТЕТ ВО ПРЕ-АНАЛИТИЧКА ФАЗА КАКО УСЛОВ ЗА УНАПРЕДУВАЊЕ НА ЕФИКАСНОСТА ВО МИКРОБИОЛОШКАТА ЛАБОРАТОРИСКАТА ПРАКСА

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Вовед: Основа за обезбедување на современа здравствена заштита е постоењето на ефикасни лабораториски услуги. Напредокот во технологијата ја унапредува и подобрува и лабораториската дијагностика, но сепак до денес грешките сеуште постојат и затоа е потребна евиденција на индикатори за квалитет (лабораториски грешки). Индикаторите според времето на настанување се класифицирани како: пре-аналитички, аналитички и пост-аналитички.

Цел: Да се идентификуваат и евалуира фреквенцијата на индикаторите за квалитет во пре-аналитичка фаза во нашата микробиолошка лабораторија

Метод: Анализиран е период од 5 години во кој во лабораторијата се следени индикаторите за квалитет во сите фази на работењето. Во пре-аналитичката фаза, покрај останатите, се анализирани и статистички обработени и следниве параметри: одбивање на примерок (со назнака за причината на одбивање), примерок

примен во несоодветен сад, недоволен волумен, по несоодветна подготовка на пациентот, несоодветно транспортиран (време, температура, транспортен медиум) и непотполно или несоодветно побарување на анализа од лекар.

Резултати: Според листата на индикатори за квалитет во испитаниот период во микробиолошката лабораторија најчести грешки се евидентирани во делот на одбивање на примерок и непотполно побарување на анализа од лекар со застапеност од 0,02%, потоа несоодветна подготовка на пациент 0,01%, а застапеноста на останатите индикатори е под 0,01%. Ако се направи споредба на податоците од анализираниот период, евидентно е сигнификантно намалување на процентот со секоја година од имплементацијата на стандардот MKS EN ISO 15189:2013.

Заклучок: Водењето на добра евиденција на грешките е услов за успешно менаџирање на секоја лабораторија. Ова подразбира јасно дефинирање на сите процеси и фази врз основа на што се анализираат индикаторите за квалитет. По нивна обработка се носат и превентивни мерки чија цел е намалување и елиминација на што е можно повеќе грешки, а со тоа и сеопфатна и веродостојна современа лабораториска пракса.

СЕСИЈА 6/SESSION 6
БИОТЕХНОЛОГИЈА, ЕКОЛОШКА МИКРОБИОЛОГИЈА/ BIOTECHNOLOGY, ENVIROMENTAL MICROBIOLOGY

BE1 *BACILLUS* PROTEIN SECRETION: FROM THE CRADLE TO THE GRAVE

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Bacillus subtilis is one of the most versatile and widely exploited industrial microorganisms, used for the production of a variety of industrially important products including enzymes, vitamins, amino acids, antifungal and antibacterial peptides, and surface-active agents such as surfactin. Analysis of this bacterium over more than 60 years has revealed detailed knowledge of its biochemistry, physiology and genetics, making it one of the most amenable host bacteria for use as a synthetic biology/industrial biotechnology processes.

B. subtilis efficiently secretes native proteins and those from related bacteria at concentrations in excess of 20 g/L. However, yields of heterologous proteins, such as therapeutic proteins, are much more variable (μu – mg/L). With a view to addressing the issue of why *Bacillus* species are generally poor secretors of heterologous proteins, we have systematically studied the protein secretion pathway from the moment of "birth" of a new protein to translocation to its final location. In so doing, we have identified, and attempted to overcome, various bottlenecks in this pathway. In particular, we have attempted to identify the characteristics that distinguish native proteins from foreign proteins. In part, the answer lies in the relationship between the rate of folding and final structure of native as compared with heterologous proteins, and their subsequent interactions with what we refer to as the cell's "Quality Control Machinery". This talk will review our current understanding of the *Bacillus* protein secretion pathway and discuss stages in the pathway that are still poorly understood.

BE2 *BIOSYNTHESIS OF SILVER NANOPARTICLES BY CULTURE FREE SUPERNATANT OF STAPHYLOCOCCUS CITRUS FNS-BCC 61 AND ITS ANTIBACTERIAL ACTIVITY*

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Introduction: Nanoscience and nanotechnology has attracted a great interest over the last few years due to its potential impact on many scientific areas such as energy, medicine, pharmaceutical industries, electronics, and space industries. Green synthesis has a potential to develop simple, attractive, costeffective and eco-friendly methods for production of potent antibacterial silver nanoparticles (Ag-NPs).

Aim: This study was aimed to explore the nanoparticle synthesizing properties of a *Staphylococcus citrus* FNS-BCC 61 from The Collection of Microorganisms of Department of Microbiology and Microbial Biotechnology, Institute of Biology, Faculty of Natural Sciences and Mathematics.

Material and methods: The bacterium *Staphylococcus citrus* FNS-BCC 61 was inoculated in sterile Mueller-Hinton Broth. The inoculated broth was incubated aerobically at 37°C for 24 hrs on 220 rpm. After incubation, the medium was centrifuged at 5000 rpm for 20 minutes and the supernatant obtained were labeled as culture free supernatant (CFS) and used for biosynthesis of silver nanoparticles. 10 ml of the CFS was added to 5 ml of 1 mM aqueous solution of silver nitrate ($AgNO_3$) prepared freshly in deionized water under stirring conditions and the mixture was incubated 30^{0}C within 24 hours in a dark place. The antibacterial activity of the biosynthesized nanoparticles was done using agar well diffusion method and broth method. Some selected bacteria (*Listeria monocytogenes*, *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027 and *Salmonella enteridis*) were used as indicator organisms.

Results: The bacterium was found to have the ability to form extracellular silver nanoparticles at 30^{0}C within 24 hours. Formation of yellowish brown colour indicates the formation of silver nanoparticles. Spectrophotometric analysis of the bio-reduced silver ion (Ag+) to silver nanoparticles (Ag^0) was determined using UV-Visible spectrophotometer which showed a peak at 430 nm. The results showed that Gram positive and Gram negative bacteria are susceptible to this antibacterial agent.

Conclusion: This study provides evidence for a cheap and effective method for synthesizing potent antibacterial Ag-NPs and demonstrates their effectiveness against different bacteria.

Keywords: silver nanoparticle; biosynthesis; bacteria; agar well diffusion method; broth method

БИОСИНТЕЗА НА СРЕБРЕНИ НАНОЧЕСТИЧКИ СО ПОМОШ НА СУПЕРНАТАНТ ОД STAPHYLOCOCCUS CITRUS *FNS-BCC 61 И НЕГОВА АНТИБАКТЕРИСКА АКТИВНОСТ*

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Вовед: Нанонауката и нанотехнологијата привлекуваат голем интерес последниве неколку години како резултат на нивното позитивно влијание во многу научни подрачја како што се енергијата, медицината, фармацевтската индустрија, итн. Микробната синтеза поседува потенцијал да развие едноставна, атрактивна, нискобуџетна и еколошки поволна матода за производство на моќни антибактериски сребрени наночестички (Ag-NPs).

Цел: Ова истражување има за цел да ја испита моќта на синтетизирање на наночестички на бактеријата *Staphylococcus citrus* FNS-BCC 61, која е дел од Колекцијата на Микроорганизми на Одделот за микробиологија и микробна биотехнологија, Институт за биологија, Природно-математички факултет, Универзитет "Св. Кирил и Методиј".

Материјал и методи: Бактеријата *Staphylococcus citrus* FNS-BCC 61 се инокулираше во стерилен Mueller-Hinton бујон и се инкубираше аеробно на 37°C/ 24 hrs на 220 rpm. По инкубацијата медиумот беше центрифугиран на 5000 rpm/ 20 minutes и добиениот супернатант се користеше понатаму за биосинтеза на сребрени наночестички. 10 ml од супернатантот се додаде во 5 ml 1 mM воден раствор на $AgNO_3$ и мешавината се инкубираше на 30^0C/ 24 hours на темно. Антибактериската активност на биосинтетизираните наночестички се испитуваше со дифузионен и дилуционен метод. Како тест микроорганизми се користеа *Listeria monocytogenes*, *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027 и *Salmonella enteridis*.

Резултати: Беше најдено дека бактеријата има способност да формира екстрацелуларни сребрени наночестички на 30^0C за време од 24 часа. Формирањето на жолтеникаво-кафено обојување индицира на формирање на сребрени наночестички. Спектрофотометриската анализа на био-редуцираното сребро (Ag+) до сребрени наночестички (Ag^0) се одредуваше преку UV-Visible спектрофотометар, кој покажа пик на 430 nm. Резултатите покажуваат дека Gram позитивните и Gram негативните бактерии се чувствителни на овој антибактериски агенс.

Заклучок: Ова истражување покажува постоење на евтин и ефективен метод за синтеза на моќни антибактериски сребрени наночестички.

Клучни зборови: сребрени наночестички; биосинтеза; бактерии; дифузионен тест; дилуционен тест

BE3 *AEROBIC GRANULAR MICROORGANISMS - A NEW TECHNOLOGY IN INDUSTRIAL WASTEWATER TREATMENT*

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Introduction: The wastewater from the coke plant Dunaferr, Dunaújváros, R. Hungary, is consisted of heterogeneous mixture of many toxic compounds such as ammonia, cyanide, thiocyanates, phenols, mono and polycyclic nitrogen compounds and polycyclic aromatic hydrocarbons. Such composition of the wastewater represents a big limiting factor in the selection of appropriate technology for biological treatment. In order to improve the existing technology for treating industrial wastewater in the wastewater treatment plant in Dunaferr, usage of the available bioremediation and inoculation processes with specific granulated populations of bacteria is required.

The aim of this work was to precisely define a new biological process to treat the industrial wastewater from the coke plant Dunaferr by using aerobic granular microorganisms. Aerobic granular microbial biomass was prepared composed of 32 isolates. Using 16s rDNA sequencing methods, all isolates were determined to a taxonomic unit, strain.

Material and methods: The biological tests and multiplication of microbial biomass were conducted in a laboratory bioreactor with volume of 2 L, semi-industrial digester with volume of 1 m3 and industrial propagator of 660 m3. 5900 kg granulated microbial biomass were produced in the industrial propagator and were injected in three newly built bioreactors with a total volume of 5100 m3. Under optimal kinetic conditions of the technological process, of the wastewater treatment plant: temperature of 19,8-27,5oC, air flow of 1,3-1,7 Lmin-1, pH = 6,9-7,8, the COD value is reduced from 5000 mg L-1 to 430 mg L-1, the concentration of S-CN value was reduced from 1044 mg L-1 to 2,9 mg L-1, the concentration of phenols was reduced

from 928 mg L-1 to 0,1 mg L-1 and the concentration of N-NH4 value was reduced from 349 mg L-1 to 12 mg L-1.

Results confirm the superiority of the treatment of industrial wastewater using advanced wastewater treatment technology enriched with granulated microorganisms compared to classic technologies that are still based on continuous input and output of waste water treated with native active sludge.

Keywords: wastewater treatment, granulated microbial biomass, nitrification and denitrification

BE4 *BIOLOGICAL TREATMENT OF PETROCHEMICAL WASTEWATER BY APPLYING NATIVE AND GRANULATED MICROORGANISMS*

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Introduction: There are several strategies for managing wastewater in the petrochemical industry which is one of the predominant pollutants, including procedures that contribute to reducing the amount of used water for the needs of the industry. Among the all process, biological treatment is one of the most effective, environmentally friendly and energy-saving method for removing pollutants of petrochemical wastewater.

Aim: Several parameters in the influents and effluents were monitored, as well as a comparative analysis between the native active sludge from the wastewater and granulated microorganisms. Putting the emphasis on phenol as the main carcinogen, mutagen and teratogenic pollutant of this type of wastewater.

Material and methods: The formation of aerobic granules takes place in a predetermined medium containing acetate as the primary source of carbon and (NH4) 2 SO4 as a source of nitrogen. In addition, proper adaptation of native isolates (bacteria and yeasts) and their selection was carried out. Analyzes were performed by supplementing 50, 100, 200, 300 mg * L-1, in MSM and 24 h agitation at 150 rpm, 30oC.

Results: PH, biomass, COD, oil materials, NH4, NO3, chlorides, Sulphates, phosphates and tolerance to phenol were analyzed before and after 24 h aeration of the refinery waste water in the bioreactor.

COD reduction from 360 mg / L to 60 mg / L, chloride from 102 mg / L to 76.0 mg / L, nitrates (6.9 mg / L - 1.0 mg / L), without major changes.

Conclusion: The tolerance and viability of native and granulated biomass at high concentrations of phenol, promises potential for their utilization for purification of wastewater in the petrochemical industry.

Key words: Granulated microorganisms, Petroleum wastewater, phenol.

БИОЛОШКИ ТРЕТМАН НА ОТПАДНИ ВОДИ ОД ПЕТРОХЕМИСКИ ИНДУСТРИЈА СО ПРИМЕНА НА НАТИВНИ И ГРАНУЛИРАНИ МИКРООРГАНИЗМИ

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Вовед: Постојат повеќе стратегии за менаџирање на отпадните води во петрохемиската индустрија, како една од предоминантните загадувачи, вклучувајќи планирани постапки кои придонесуваат кон редуцирање на количината на искористена вода за потребите на индустријата. Меѓу другите, биолошкиот третман е еден од најефективните, еколошки и енергетско-заштедувачки процес за отстранување на хемиските полутанти во отпадните води од оваа индустрија.

Цел: Вршено е следење на повеќе биолошки и хемиски параметри во инфлуентот и ефлуентот, како и компаративна анализа помеѓу нативната активна тиња од отпадната вода и гранулираните микроорганизми, ставајќи го акцентот на фенолот, како главен канцероген, мутаген и тератоген полутант од овој вид на отпадни води. При тоа, вршена е и соодветна адаптација на нативните изолати (бактерии и кваци) и нивна селекција.

Материјал и методи: Анализите се вршени со суплементирање на 50, 100, 200, 300 mg*L-1, во MSM и 24 h агитација на 150 BM, 30 oC. Формирањето на аеробните гранули се одвива во претходно определен медиум, кој содржи ацетат како примарен извор на јаглерод и (NH4)2 SO4 како извор на азот. Следени се промените на pH, биомаса, ХПК, масни материи, NH4 + , NO3-, хлориди, сулфати, фосфати и толеранција кон фенол, пред и по 24 h аерација на рафинериска отпадна вода во биореактор

SESION 6

Резултати: Забележано е намалување на ХПК од 360 mg/L до 60 mg/L, хлориди од 102 mg/L до 76.0 mg/L, нитрати (6.9 mg/L – 1.0 mg/L) , без поголема промена на биомаса.

Заклучок: Толеранцијата и вијабилноста на нативната и гранулираната биомаса на високи концентрации на фенол, ветува потенцијал за нивно искористување за прочистувањето на отпадните води во петрохемиската индустрија.

BE5 *CHARACTERIZATION AND ARSENIC-TOLERANCE POTENTIAL OF* HALOMONAS *SP. FROM VAN LAKE, TURKEY*

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Introduction: Increasing global warming and so arsenic contaminated resources are steadily increasing and as a consequence of this, the size of the threats over public health grows; the characterization of arsenic-resistant bacteria and the determination of arsenic tolerance in bacteria with the aim to use in bioremediation processes have started to become essential.

Objectives: In this study, it is aimed the isolation and characterization of *Halomonas* strains from Van Lake, the world's largest soda lake, and also to determine arsenate [As(V)] and arsenite [As(III)] resistance of them both at cultural and molecular level.

Material and Method: Sediment sampling from 7.5 metres was performed from Van Lake. Total arsenic amount of samples was determined by using ICP-MS. Some particular phenotypic characteristics of isolates were determined, their 16S rRNA gene regions were reproduced with universal primers. For determination of arsenic metabolizing pathways; PCR assays were performed with specific primer sets for *ars*C, *ars*B and *arr*B gene regions. Also, microplate technique was used to reveal susceptibility profiles of arsenic-resistant bacteria against As(V) and As(III).

Results: It was determined that sediment samples of Van Lake has contained 26.070 ppb arsenic in autumn and 9370 ppb in winter. Also, it was revealed that strains diagnosed as *Halomonas* sp. and strains are Gr(-) and bacilli with capsule and endospore; commonly produce extracellular lecithinase enzyme; assimilate glucose, mannose and galactose; show better growth at 30°C and pH 9.5 - 10.5; prefer more for growth 1-2% NaCl concentration; have MIC value for As(V) and As(III), 80 - >320 mM and 8- >32 mM, respectively and have the gene region *ars*C.

Conclusion: As is a growing environmental problem worldwide and as a result of accumulation in the human body, resistance to various antimicrobial agents as well as increases in cancer incidence increases. So, isolation and characterization of As-resistant bacteria from different sources and at the same time determination of arsenic metabolism and tolerance potential for bioremediation from contaminating areas is of importance.

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Keywords: *Halomonas* sp., Van Lake, arsenic tolerance, arsenate, arsenite.

BE6 ATMOSPHERIC PRESSURE PLASMA DECONTAMINATION OF MEDICALLY IMPORTANT BIOFILMS

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Introduction

Biofilms are microbial communities attached to a surface and embedded in a matrix composed of exopolysaccharides together with proteins and excreted nucleic acids. In natural and manmade environments, bacterial biofilms are ubiquitous. They are present almost everywhere and impact all aspects ion our life, in many cases their presence leads to a disease, implant colonisation, product contamination, product contamination, biofouling, industrial equipment damage,... Biofilms formation begins with planktonic bacteria that first reversibly absorb and then irreversibly attach to a surface, divide and attract other cells to attach to the bacteria already attached to a surface. Single- species biofilms are rarely present in the environment. Mostly, biofilms are composed of various bacterial species. Biofilms have a complex architecture, with physiologically organised bacterial microcolonies. It is the structure and organisation of the biofilm that conveys numerous advantages over unprotected planktonic cells, providing protection against immune system defence and the diffusion of antibiotics. The resistance against antimicrobial agents in a biofilm can be up to 1000 times higher compared to planktonic cells.

Objectives

Conventional methods to control bacteria by chemical, physical and biological ways are usually inefficient in the case of biofilms. For this reason, there is an urge to establish new strategies for inactivation of established

biofilms. In this contribution we present the challenges which should be considered before using plasma technology as a new approach for the elimination of bacterial biofilms.

Materials and methods

Surface barrier discharge (SBD) air plasma was used in our study. This plasma configuration is situated remotely from the sample being treated, consequently, reactive plasma species, such as O and OH do not reach the bacteria. The antimicrobial effect of the SBD is attributed to longer lived plasma generated species, such as O3 and NO. By varying the plasma generation parameters, such as discharge power, we detail how the long-lived reactive plasma chemistry can be manipulated to enhance bacteria kill. Finally, the influence of the plasma chemistry on the inactivation of different bacteria species in a mixed species biofilm was considered. Single-species biofilms of *Pseudomonas aeruginosa* and *Staphylococcus aureus* were used as models for G- and G+ species bacteria. Mixed-species biofilms were composed of *S. aureus*, *P. aeruginosa*, *K. pneumoniae* and. *E. faecalis*.

Results

The exposure of single-species biofilms showed that sensitivity of the two species to inactivation was different with respect to the discharge regime and each other. *P. aeruginosa* biofilms were significantly more susceptible to the N_xO_y-dominated conditions than *S. aureus*. The susceptibility of *P. aeruginosa* and *S. aureus* was found to be different within the multispecies communities.

Conclusions

The study provides strong evidence that defined configurations of atmospheric pressure air plasmas can achieve rapid and highly effective bacterial kill in complex models. It starts to define some of the key research questions in terms of optimising plasma discharges to generate more RNS species. It also identifies gaps in or understanding of the architecture of co-cultured biofilms and the cross-dependency of individual species within it.

BE7 DESIGN OF ADVANCED ANTIBACTERIAL COATINGS WITH PLASMA-ASSISTED DEPOSITION

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The engineering of new materials with antibacterial properties receives a lot of interest from the industrial, medical, and healthcare sectors. The biomedical requirements of a material in terms of physical, chemical and mechanical properties results in a shift in a technology from embedding antibacterial agents in the matrix structure of composites to the engineering of surfaces possessing

high antibacterial activity. The foremost strategy is considered to consist of deposition of a thin layer of antibacterial coating on the top surface of materials, such as non-woven fabrics (bandages, wound textile, medical masks) so that only the surface of the material will change and bulk properties are not affected. Such processes preserve all the properties of the bulk material as only a thin layer of 10 - 500 nm is deposited. A general trend in this research area is to develop coatings with advanced properties of low toxicity, high antibacterial efficiency, highly controllable release of the antibacterial agent even through external triggering the coatings performance. Some of the most promising future strategies are presented in Figure 1.

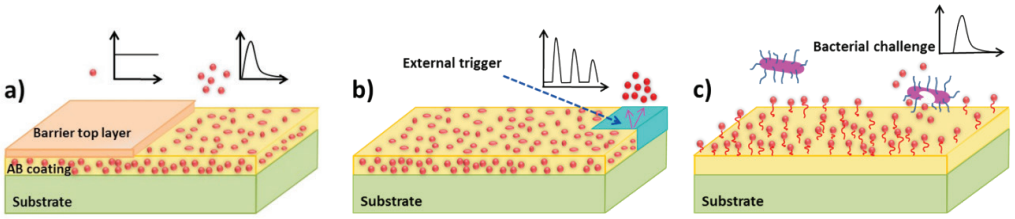

Figure 1. Advanced antibacterial coating strategies: a) use of the barrier layer for control of the drug release from long lasting release kinetics to peak release kinetics; b) external triggering the release by pH, temperature, magnetic or light field; c) use of green coatings with antibacterial agent targeting specific bacteria

A promising approach for the deposition of antibacterial coatings on an industrial scale is plasma assisted polymerization. Thin films composed of silver nanoparticles in a polymer matrix can be deposited by combining plasma polymerization and silver nanoparticles. The content of silver ranges from a very small percentage to as large as 29%. Since the expensive low pressure plasma deposition system has the limitation of low deposition rate we moved from sub-atmospheric to atmospheric pressure processing established in frame of H2020 project M-Era.net PlasmaTex. As an alternative to antibiotic loaded coatings and green coatings, a considerable attention was paid to antibacterial coatings with incorporated nano-particles of metals and metal oxides. Lastly, the developed antibacterial coatings are in last phases tested not only for antibacterial activities, but also for cytotoxicity and genotoxicity as well as wearing resistance. All this aspects in design of advanced coatings will be presented in the talk.

BE8 PLASMA TECHNOLOGY FOR DECONTAMINATION OF MYCOTOXINS

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Introduction
Mycotoxins are toxic secondary metabolites produced by food contaminating fungal species [1]. They represent a growing problem due to their increase in food contamination. Massive food production, incorrect handling of the food during harvest time, transport and storage and climate change contribute the most to the undesirable growth of mycotoxin producing fungi. Although many preventive measures are carried out, the trend of mycotoxin contamination is still growing [2]. Therefore, the development of the new and more effective method is necessary.

Objectives
Plasma technology is already successfully applied as a new food processing approach for decontamination of food pathogens and other harmful agents. Fundamentally, plasmas are dissociated and ionized gases [3]. As a mixture of highly reactive particles, it represents a very promising, low-cost and eco-friendly method for decontamination of mycotoxins with negligible effect on the quality of treated food products. For this reason, our aim was to set the right parameters for mycotoxin decontamination plasma system.

Methods and materials
Aflatoxin B1 (AFB1) was used as a model mycotoxin. Various types of atmospheric pressure plasma set-ups were applied along with different discharge parameters. Moreover, different distances from plasma generation point to treated substrate and exposure times were tested as well. The AFs were treated in both - liquids or dried surfaces. After the exposure, the degradation efficiency was monitored by liquid chromatography-mass spectrometry (LC-MS).

Results and conclusion
The LC-MS analysis showed a down-going trend of AFB1 concentrations at both liquid and solid surface treatments, which was proportional to increasing exposure time. The lowest concentration values were observed at the longest treatment times. As demonstrated,

atmospheric pressure plasma can be applied as a new non-thermal food processing method for mycotoxin decontamination. However, little is known about the nature of degradation products. Thus, future work should be focused on the dynamics of mycotoxin degradation.

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BE9 EFFECT OF SECONDARY METABOLITES OF *LACTOBACILLUS* SPECIES ISOLATED FROM VAGINAL CULTURES ON THE PROLIFERATION OF HCT 116 COLON CANCER CELLS

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Introduction: Colon cancer is the second leading cause of cancer-related deaths in the United States. New therapeutic strategies are needed in the treatment of this disease.

Objectives: In this study, we aimed to investigate the antiproliferative effects of secondary metabolites of five different *Lactobacillus* species isolated from vaginal cultures on HCT 116 colon cancer cells and to perform HPLC analyzes.

Materials and Methods: For this purpose, HCT 116 colon cancer cells were treated with different doses of secondary metabolites of five *Lactobacillus* species (*L. gasseri*-1, *L. saerimneri*-1, *L. crispatus*-1, *L. gasseri*-2, *L. crispatus*-2) at %0.83-%16.6 concentration range for 24 h. After incubation, MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) assay was performed. Survival (%) was calculated relative to control.

HPLC analysis of secondary metabolites of *Lactobacillus* species was

carried out on Agilent 1100 series. Separation was carried out on Inertsil ODS-3 (150 length x 4.6 mm i.d., 5 µm). Elution was performed with a mobile phase as % 0.025 phosphoric acid solution. The flow rate was set at 0.75 mL/min, injection volume was 5 µL and temperature of the column was maintained at 25°C. Compounds were detected at a wavelength of 210 nm.

Results: HPLC analysis of isolates showed secondary metabolites contain acetic acid, lactic acid and hydrogen peroxide at various concentrations. We found that *L. crispatus*-1, and *L. crispatus*-2 inhibited the cell proliferation significantly ($p<0.001$) and dose dependently at %0.83- %16.6 dose range and *L. gasseri*-1, *L. saerimneri*-1, *L. gasseri*-2 didn't have any effect at %0.83 concentration, but they decreased cell viability significantly and dose dependently between %4.16 and %16.6 dose range.

Conclusion: As a conclusion, secondary metabolites of all tested vaginal *Lactobacillus* species decreased cell viability of HCT 116 colon cancer cells. This antiproliferative effect can be due to the content of these secondary metabolites. These species can be used as potential agents in colon cancer treatment.

BE10 ISOLATION AND CHARACTERISATION OF WILD YEASTS OF WINERY INTEREST

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Introduction: Wine consumption has been increasing steadily worldwide not only due to its good health function but as a cultural preference. The increasing economic interest in the sector of wine has stimulated a renewed interest in microbial resource management. Wild starter cultures for wine production need to be selected in order to produce quality and unique wine.

Aim: Wild yeasts on the surface of various grapes were surveyed to obtain yeast strains suitable for fermenting a novel, highly specific, domestic wine with unique enological characteristics. Non-Saccharomyces strains were also isolated and examined.

Material and methods: The yeasts were isolated from Stanushina grapes, grape juice and different phases of the wine fermentation processes. Wild yeasts were isolated on plates of an agar-solidified

YPD medium (1% yeast extract, 2% peptone, 2% dextrose, pH 4.5) containing 50 μg/mL chloramphenicol. The isolated strain of yeast was maintained on YPD slopes. The yeast isolates were examined for their resistance to alcohol, osmotic pressure, temperature, S02, production of CO2, and flocculation.

Results: A diversity of 6 yeast strains with tolerance to high alcohol and osmotic pressure was isolated. Among the isolates studied, 100% showed to be resistant to osmotic stress with sucrose, 12% showed alcohol tolerance up to 18%, 97% did not grown at temperature of 45 oC. 30% of isolates showed to be resistant to 150 mg/L SO2. All isolates produce high amount of CO2.

Conclusion: This study has shown that wild yeasts have highly potential in production of authentic, regional-specific wines as well as organic, natural wines and wines with lower alcohol concentration. These isolates could contribute for the improvement of the wine quality and also could be used to create an identity for the wine produced in local regions.

Key words: wild yeasts, wine production, authentic wines.

ИЗОЛАЦИЈА И КАРАКТЕРИЗАЦИЈА НА ДИВИ КВАСЦИ ОД ИНТЕРЕС НА ВИНАРСТВОТО

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Вовед: Конзумацијата на вино на светско ниво се зголемува стабилно, не само поради здравствените функции, туку и како културна преференца. Зголемениот економски интерес во винарскиот сектор, стимулира обновен интерес за менаџирање на микробните извори. Стартер-културите од дивите квасци за производство на вино треба да бидат селектирани, со цел производство на квалитетно и уникатно вино.

Цел: Диви квасци, од површината на различни гроздови, беа анализирани, за да се пронајдат соеви на квасци способни за ферментација на нови, високо-специфични вина со уникатни енолошки карактеристики. Не-сахаромицетни соеви беа исто така изолирани и проучувани.

Материјали и методи: Квасците беа изолирани од гроздови од Станушина, неговата шира и различни фази од процесот на

ферментација на виното. Дивите квасци беа изолирани на YPD - агар медиум (1% квасен екстракт, 2% пептон, 2% декстроза, pH 4.5) кој содржи 50 µg/mL хлорамфеникол. Изолираните соеви на квасци беа конзервирани на YPD кос-агар. Истите беа испитувани за нивната толеранција на алкохол, осмотски притисок, температура, SO2, продукција на CO2 и флокулација.

Резултати: Беше изолиран диверзитет од 6 соеви на квасци толерантни на висока содржина на алкохол и осмотски стрес. Помеѓу изолатите 100% покажаа резистентност на осмотски стрес со сахароза, 12% покажаа толеранција на алкохол до 18%, 97% не израснаа на температура од of 45 oC. 30% од изолатите покажаа резистенција на 150 mg/L SO2. Сите изолати продуцираа висока количина на CO2..

Заклучок: Оваа студија покажува висок потенцијал на дивите квасци за производство на автентични, регионо-специфични, како и органски, природни вина и вина со намалена концентрација на алкохол. Овие изолати можат да допринесат за подобрување на квалитетот на виното и можат да се користат за унапредување на винскиот идентитет во локалните региони.

Клучни зборови: диви квасци, производство на вино, автентични вина.

BE11 EFFECT OF ARSENATE AND ARSENITE ON THE GROWTH OF ARSENIC-RESISTANT HALOMONAS CAMPISALIS FROM VAN LAKE, TURKEY

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Introduction: When the concentration of dissolved metal ions in the primordial oceans is considerably higher than today, life is present before the atmospheric oxygenation begins. However, species that have not been able to detoxify toxic As can not survive on these conditions. Because Arsenic (As) is the most common environmental toxins classified as Group I human carsinogen and toxic to all life forms including microorganisms.

Objectives: In this study, it was aimed to make molecular identification of the As-resistant isolate from Van Lake, the world's largest soda lake, to calculate the generation time by creating the growth curve under normal growth conditions and to determine the effects of arsenate [As (V)] and arsenite [As (III)] on the bacterial growth by creating growth curve.

Material and Method: The 16S rRNA gene region of the isolate was amplified using universal primers and sequence analyzes were performed. The pathway for As metabolism of strain was identified by using spesific primers of *ars*C, *ars*B and *arr*B gene regions. Using Luria Berthani (LB) medium with a pH 9.5, the growth curve covering the 58 hour period was subtracted based on the spectrophotometric method. In the same media containing 10 mM As(V) and 1 mM As(III), the growth curves were created under the same conditions and the generation times were calculated.

Results: Strain was identified as *Halomonas campisalis* with 99% 16S rRNA gene region similarity. As a result of molecular analysis, it was determined that it has the *ars*C gene region. It was determined that the generation time in LB was 42 minutes and in As(III) containing medium was 77 minutes and As (V) was 42 minutes.

Conclusion: As(III) is more toxic than As(V) and in the result of the study the elongation of the generation time was detected in this medium as expected. In the medium containing As(V), the unchange in the generation time was considered as a consequence of the adaptation process. For the bioremediation processes to be used in coping with environmental problems arising from arsenic pollution, the effects of As(V) and As(III) on the growth of As-resistant bacteria should be determined.

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Keywords: *Halomonas campisalis*, arsenate, arsenite, Van Lake.

BE12 TRENDS OF BATHING WATER QUALITY IN VELIPOJA, SHËNGJIN, DURRËS, GJIRI I LALËZIT, KAVAJË, DIVJAKË, SEMAN, VLORA, PALASË, DHËRMI, HIMARË, QEPARO, BORSH AND SARANDA COASTALS DURING 2013-2017

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INTRODUCTION: Recreational water qualities are highly vulnerable to microbial pollution from municipal sewage, industrial effluents as well as agriculture run-off and river discharges. Fecal contamination not only impairs water quality but also potentiates human health risks.

OBJECTIVES: The aim of our study was to see the 5 years trend of microbiological quality of bathing waters in Albania.

MATERIAL and METHODS: Every year we collect 900 samples, which are to be taken 30 centimeters below the water's surface and in water that is at least one meter deep. Assessment of bacterial load of the coastal waters was done 9 times for every point, for the *Escherichia Coli* and *Intestinal Enterococci,* according to the methods ISO 7899-1 and ISO 9308-3.

Bathing water assessment is to be classified according to the categories indicated in the Directive 2006/7/EC and reccomandations of WHO/UNEP-2010.

RESULTS*:* Microbial Water Quality Assessment Category (cfu/100 ml water) done in 100 monitoring point were:

During **2013** Excellent quality 42%, Sufficient quality 11%, Good quality 4%, Poor quality 42%. During **2014** Excellent quality 30%, Sufficient quality 11%, Good quality 10%, Poor quality 49%. During **2015** Excellent quality 53%, Sufficient quality 23%, Good quality 9%, Poor quality 15 %. During **2016** Excellent quality 39%, Sufficient quality 14%, Good quality 4%, Poor quality 43%. and during **2017** Excellent quality 74.5%, Sufficient quality 15.7%, Good quality 6.9%, Poor quality 2.9%.

CONCLUSION: The number of monitoring point with Excellent quality was increased for year 2017 compared to years 2013-2016.

Good quality category was increased for 2017 compared to 2013-2015 and decreased compared 2016.

Sufficent quality was increased for 2017 compared to 2013 and 2015; and decreased compared to 2014 and 2016.

Poor quality was decreased for 2017 compare to 2013 -2016.

Microbial quality of recreative bathing waters in Albania have an sinjificant increasing of the quality.

СЕСИЈА 7/SESSION 7
МИКРОБИОЛОГИЈА И БЕЗБЕДНОСТ НА ХРАНА/ MICROBIOLOGY AND FOOD SAFETY

FM1 CLIMATE CHANGES AND FOODBORNE PATHOGENS

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Foodborne zoonoses are a significant and widespread public health threat – annually more than 315,000 human cases are confirmed and reported in the EU, but the real number is likely to be much higher. The most frequent bacterial pathogens in the food chain belong to the genera Campylobacter (229,213 human cases for 2015), Salmonella (94,625 human cases in 2015), Yersinia (7,202 human cases or 2015) and the species Escherichia coli (5,910 human cases in 2015) and Listeria monocytogenes (2,206 human cases in 2015). Many of those survive and proliferate both in the environment and in foods of plant and animal origin, regardless of storage conditions (cold temperature, vacuum packs, salt concentrations, etc.). Climate changes and antimicrobial resistance are the most important among the complex groups of risk factors and their interactions leading to the emergence of surprisingly severe foodborne infections in humans, as it was the E. coli infection in 2011 in Germany. The last EARS-Net report on antibiotic resistance highlights an especially worrying situation with regard to Gram (-) bacteria, wherein one of the most emerging threats is the significantly increasing resistance to life saving antibiotics in E. coli and other foodborne pathogens. Additional microbiological data will reveal the importance and potential risk of zoonotic foodborne infections depending on the temperature of cultivation. Standard and modern microbiological methods and approaches will contribute to effective monitoring and evaluation of foodstuff and will complement the overall strategy to ensuring food safety and protection the consumer's health.

FM2 MOLECULAR CHARACTERIZATION OF FOODBORNE PATHOGENS

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The ability to accurately distinguish strains of foodborne pathogens is crucial for efficient epidemiological and surveillance analysis, studying microbial population structure and developing improved public health control strategies. To accomplish such goals, several molecular typing methods have been proposed that can identify disease outbreaks isolates. Monitoring of PFGE profiles makes it possible to assess the molecular diversity and circulation of food pathogens within the food chain. Finding a PFGE profile of a strain isolated from food that matches a human strain profile does not necessary imply that this food is the source of the contamination. Pulsed-field gel electrophoresis (PFGE) is considered as the "gold standard" method for Listeria monocytogenes and other epidemiological investigations. There other ultimate molecular methods such as multi locus sequence typing (MLST) and whole genome sequencing (WGS), with great promise for typing in the future, they are currently not widely available and accepted. For the food industry, tracking of major foodborne pathogens can give scientific evidence about where food poisoning bacteria are entering a process, where cross contamination may be occurring, whether a particular strain is endemic and/or persistent in a factory environment and, most importantly, where controls should be directed. All gathered epidemiological information in the food chain such as timeline, product description, food processing and sampling stage should be crossed with PFGE, MLST or WGS typing data. This is necessary to conclude a relationship between different contaminations events. The aim is therefore to collect as much scientific evidence to help authorities to decide whether to withdraw a product from the market or to locate the source of foodborne outbreak. This demonstrates that establishing and sharing molecular typing data is the best way to link cases from one source to another, from one country to another, thereby enabling the identification of a potentially common source of a national or multinational outbreak. Nevertheless, detecting a human strain profile in food should improve the rapidity and precision of outbreak detection. Therefor European Food Safety Authority (EFSA) collects information on food and animals as well as food-borne outbreaks from the Member States (MSs) and publishing annual report. That is why EFSA has created a Task Force on Zoonoses Data Collection, with participants from all

the MSs, as well as the DG SANCO and the European Centre for Disease Prevention and Control. The ECDC coordinates a network of national public health laboratories (NPHLs), in charge of typing food pathogens strains isolated from national clinical cases. The ECDC has developed the European molecular surveillance database with the objective to share, in real time, the molecular epidemiological information and PFGE and WGS data on strains of Lm, E.coli, Salmonella isolated from human. All this tools and networking will enable locating the source of pathogen microorganism in food chain and their successful eradication.

FM3 ANTIBIOTIC RESISTANCE AND *SPA* TYPES OF *STAPHYLOCOCCUS AUREUS* STRAINS ISOLATED FROM FOOD WORKERS IN FOOD BUSINESSES AND HOSPITAL KITCHEN IN CANAKKALE, TURKEY

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Staphylococcus aureus is an important organism in public health, as a cause of nosocomial infections and food poisoning incidents. The aim of this study was to determine antibiotic resistance profiles and *spa* types of *Staphylococcus aureus* strains isolated from food workers in Canakkale, Turkey. *215 S. aureus* isolates were collected from hand and nasal swabs of 300 food workers working in 17 food businesses and 9 hospital kitchens.) Antimicrobial resistance was detected by the disk diffusion method (cefoxitin amoxicillin/clavulanic acid, cefazolin, cefoperazone, cefotaxime, ceftazidime, cefaclor, imipenem, meropenem, gentamicin, erythromycin, tetracycline, ciprofloxacin, chloramphenicol, rifampin, and linezolid). The *mec*A genes detected by PCR. One *S. aureus* isolate obtained from each carrier was typed by staphylococcal protein A (*spa*) typing method. Staphylococcal cassette chromosome *mec* (SCC*mec)* and multilocus sequence typing (MLST) of MRSA were performed by sequencing method. Erythromycin and tetracycline resistance rates among the 215 *S. aureus* isolates were found to be 8.8 and 6.9 %, respectively. Only three (1.4 %) isolates were identified as methicillin-resistant *S. aureus* (MRSA). These results indicate that the risk of MRSA being transferred from food businesses and hospital kitchens is not high.Sixty *spa* types were identified among the 121 MSSA isolates, the most common was t084 (9%). A novel

spa type was determined and added to the Ridom SpaServer database as t14963. The MLST types of MRSA strains identified as *spa* types t786 and t223 (n=2) were ST88 and ST22 (n=2). All MRSA were determined to be SCC*mec* type IVa. *spa* typing can be performed to predict transmission route of *S. aureus*. t786, ST88, SCCmec IVa MRSA strain was identified for the first time in Turkey.

Keywords: Food workers, food handler, *Staphylococcus aureus*, antibiotic resistance, *spa* typing.

FM4 QUALITY PROVISION OF LABORATORY RESULTS IN THE LABORATORY FOR SANITARY MICROBIOLOGY

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Introduction
For several decades, laboratories worldwide have been using schemes of inter-laboratory comparative tests (PT - schemes) as an external quality control of laboratories.

Aim of research
The laboratory accreditation procedure in accordance with ISO / IEC 17025 standard (Procedure 5.9 of the standard) obliges the laboratory to have results from participation in PT - schemes as one of the key elements for ensuring the quality of the test results.

Material and methods
PT - schemes involving the laboratory for sanitary microbiology are organized by the Food Examination Performance Assessment Scheme (FEPAS) and LEAP (Laboratory Environmental Analysis Proficiency Schemes). The PT - scheme organizer delivers a sample (water or food) and identifies and enumerates the defined parameter *(Salmonella* spp., *Listeria monocytogenes*, *Enterobacteriaceae*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterococcu*s, etc.).
The choice of parameters is planning according to the needs of the laboratory and in which the matrix will be examined. The results of the examinations are sent to the PT scheme organizer, who is obliged to prepare a report on the results that the laboratories participated in. The results of the inter-laboratory examinations are based on the z-value that is calculated as the ratio of the x-score of individual laboratories, X-assigned value obtained in the reference laboratory and the standard deviation. The purpose of each laboratory is to obtain acceptable results on the test, i.e. $z \leq 2$.

In the period from 2009 - 2017, the laboratory participated in 21 inter - laboratory examination (PT - schemes). Of the total 14 food samples examined, the values of the z-score obtained in all trials were z<1 (for example: 0.2; -0.3; 0.8; -0.5; -0.8; -0.3; -.0.6; -0.2; 0.5; 0.3).
For qualitative trials, the result is expressed as satisfactory (S) and unsatisfactory. Also, these tests are considered satisfactory. Of the total 7 water samples tested, the results obtained were z <1 (example: 0.9; 0.5; -0.3; -0.3; 0.2; -0.7; 0.4).

Conclusion
The concept of quality assurance of laboratory results is the complementarity of internal control in the laboratory and external quality assessment, i.e. participation in inter - laboratory examinations (PT - schemes). The results of participation in these trials of our laboratory are a good indicator of the work quality with applied methods for food and water testing. The laboratory, with its competent test results, provides elimination of barriers in international trade, based on an international system of results recognition.

ОБЕЗБЕДУВАЊЕ НА КВАЛИТЕТОТ НА ЛАБОРАТОРИСКИТЕ РЕЗУЛТАТИ ВО ЛАБОРАТОРИЈАТА ЗА САНИТАРНА МИКРОБИОЛОГИЈА

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Вовед
Веќе неколку децении, лабораториите во светот користат шеми на меѓулабораториски споредбени испитувања (eng. Proficiency testing schemes - PT шеми) како екстерна контрола на квалитетот на работата на лабораториите.

Цел на истражување
Постапката на акредитација на лабораториите согласно стандардот ISO/IEC 17025 (Процедурата 5.9. од стандардот) задолжително бара лабораторијата да поседува резултати од учество во PT шеми како еден од клучните елементи за обезбедување на квалитет на резултатите од испитувањата.

Материјал и методи
PT шеми во кои учествува лабораторијата за санитарна микробиологија ги организира референтната лабораторија FEPAS (Food Examination Performance Assesment scheme) и LEAP (Laboratory Enviromental Analysis Proficiency schemes). Ораганизаторот на

PT шемите доставува примерок (вода или храна) и се прави идентификација и енумерација на дефинираниот параметар (*Salmonella* spp., *Listeria monocytogenes*, *Enterobacteriaceae*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterococcus* и др.). Изборот на параметри го планираме предходно според потребите на лабораторијата и во кој матрикс ќе се испитува. Резултатите од испитувањата се праќаат до организаторот на PT шемите кој има обврска да изготви извештај од резултатите коишто лабораториите учесници ги остариле. Резултатите од меѓулабораториските испитувања се темелат на z-вредноста која се пресметува како сооднос на x-резултат на поединечни лаборатории, X-доделена вредност добиена во референтната лабораторија и стандардната девијација. Целта на секоја лабораторија е да добие прифатливи резултати на испитувањето, односно z≤2.

Во периодот од 2009 - 2017 год. лабораторијата учествуваше во 21 меѓулабораториско испитување (PT - шеми). Од вкупно испитаните 14 примероци храна, вредностите на добиените резултати z - score во сите испитувања биле z<1 (пример: 0.2; -0.3; 0.8; -0.5; -0.8; -0.3; -.0.6; -0.2; 0.5; -0.3).

За квалитативни испитувања резултатот се изразува како задоволителен (S) и незадоволителен. Исто така, и овие тестирања се оценети како задоволителни. Од вкупно испитаните 7 примероци вода вредностите на добиените резултати биле z<1 (пример: 0.9; 0.5; -0.3; -0.3; 0.2; -0.7 ;0.4).

Заклучок

Концепт за обезбедување на квалитет на лабораториските резултати е комплементарност на интерната контрола во лабораторијата и надворешна оценка на квалитетот односно учеството во меѓулабораториски испитувања (PT - шеми). Резултатите од учеството во овие испитувања на нашата лабораторија се добар индикатор за квалитетот на работата со применетите методи за тестирање на храна и вода. Лабораторијата со своите компетентни резултати од испитувањата обезбедува отклонување на бариерите во меѓународната трговија, која се темели на меѓународен систем на признавање на резултатите.

СЕСИЈА 8/SESSION 8 АНТИМИКРОБНИ СРЕДСТВА/ ANTIMICROBIAL AGENTS

AA1 MICROBIOLOGICAL ASPECTS OF SOME AGENTS AND METHODS FOR ORAL PREVENTION IN SCHOOL CHILDREN

A. Dimkov

The fact that microorganisms are one of the most important factors in dental caries etiology poses the question regarding their elimination or their reduction to minimal values. There is today a huge number of agents and methods for oral health prevention. The new scientific and technological achievements in the field of prevention place each day at our disposal a large number of new products for oral health improvement and care. Some of them are intended for home use, while other are intended for professional use. The contemporary mechanical and chemotherapeutical approaches to orgal hygiene aim to change the oral microflora and to contribute to a healthy dental and periodontal tissue. The results of microbiological analyses regarding the reduction of microbiological flora, the cariogenic one above all, and consequently regarding the reduction of dental caries, by using antimicrobial agenst and procedures for bacteria removal, are presented in this paper. The analyses were carried out by comparing the quantitative presence of total salivary microbiological flora with the quantitative presence of *Strepotcoccus mutans* and *Lactobacillus species* by analyzing the saliva before and after the use of the agents and/or procedures, by establishing the degree of the differences in the reduction of the salivary cariogenic microorganisms between the chemical and mechanical agents/methods, as well as by comparing the antimicrobial effect of the used agents.

Key words: dental caries, antimicobial agents, *Strepotcoccus mutans, Lactobacillus species*

МИКРОБИОЛОШКИ АСПЕКТИ НА ОДРЕДЕНИ СРЕДСТВА И МЕТОДИ ЗА ОРАЛНА ПРЕВЕНТИВА КАЈ ШКОЛСКИ ДЕЦА

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Фактот дека микроорганизмите претставуваат еден од најбитните фактори во етиологијата на денталниот кариес, го наметнува прашањето на нивна елиминација или сведување на минимални вредности. Денес, бројот на средствата и методите за зачувување на оралното здравје е огромен. Новите научно-технолошки достигнувања во областа на превентивата секојдневно ни ставаат на располагање голем број нови производи за подобрување на оралното здравје и за негова нега. Дел од нив се наменети за домашна употреба, а дел се за професионална употреба. Современите механички и хемотерапевтски пристапи за оралната хигиена имаат за цел да ја променат оралната микрофлора и да придонесат за здраво дентално и периодонтално ткиво. Во овој труд ќе бидат прикажани резултатите од микробиолошките анализи за редукцијата на микробиолошката флора, пред с кариогената, а следствено на тоа и на денталниот кариес, со користење на антимикробни средтства и постапки за отстранување на бактериите. Анализите беа извршени преку споредба на квантитативната застапеност на вкупната саливарна микробиолошка флора и квантитативната застапеност на *Streptococcus mutans* и *Lactobacillus species* преку анализа на плунката пред и по употребата на средствата и/или постапките, преку одредување на степенот на разликите во редукцијата на саливарните кариогени микроорганизми меѓу хемиските и механичките средства/методи, како и преку компарација на антимикробниот ефект на употребените средства.

Клучни зборови: дентален кариес, антимикробни соединенија, *Strepotcoccus mutans, Lactobacillus species*

AA2 ФАЛСИФИКУВАНИ ЛЕКОВИ КАКО УСПЕШНО ДА СЕ БОРИМЕ ПРОТИВ ОВОЈ ГЛОБАЛЕН ПРЕДИЗВИК И ПРОБЛЕМ

И. Захариев, С. Захариева

Здравствениот систем, здравствените работници, фармацевтската индустрија во последните децении се соочуваат со голем предизвик, но и голем проблем со појавата на лекови кои не ги задоволуваат стандардите за ефикасност, безбедност и за квалитет. Се работи за појава за пуштање на лекови во промет ко спаѓаат на лекови во категоријата на фалсификувани лекови кои можат да направат голема штета по здравјето на пациентите на целиот здравствен систем но и на фармацевтската компанија

Фалсификувањето на лекови има долга историја но денеска во светски рамки ова појава добива во масовност. Според податоците на Светската здравствена организација дури 10% од лековите во развиените земји кои се наоѓаат во промет се фалсификувани лекови, додека кај неразвиените земји тој процент изнесува дури 30%. Лидери во производството на фалсификувани лекови според Светската здравствена организација се Индија 35%, Кина 29%, Нигерија 23%, Пакистан 13. Интересно е што Индија во своето национално законодавство забранува да се продаваат овие лекови во матичната земја, но не забранува извоз на лекови во трети земји.

Под фалсификувани лекови подразбираат сите лекови кои личат на оригиналните лекови при што намерно и лажно се со претставена содржина, состав и потекло заради стекнување на профит. Значајно е дека тие:

- **Постојат**
- **Се произведуваат во нестандардизирани услови**
- **Имаат недефинирано потекло**
- **Немаат контрола на квалитет**
- **Се земаат најчесто преку интернет 50%**
- **Ноќни клубови, преку пријатели**
- **Се купуваат во странство**

Во Република Македонија се откриени фалсификувани лекови за среќа дел од нив се откриени на самите гранични премини и не беа пуштени во промет додека дел се најдоа во здравствените установи и по откривањето истите се повлечени и се ставени надвор од употреба.

Најфрапантен случај е откриениот фалсификат од лекот Tetagam или серумAntitetanikum кој не беше откриен во Република Македонија но истиот беше во промет во Република Србија, Косово и Црна Гора. Над 3000 пациенти

биле третирани со тој фалсификат. Интересно е што во една ампула имало само вода за инјекции, а во другата Gentamicin. Последиците од користење и употреба на овие лекови можеме да ги групираме во четири групи и тоа:

1. НЕМАЊЕ ОДГОВОР ОД ТЕРАПИЈАТА
2. ПОЈАВА НА СЕРИОЗНИ НЕСАКАНИ РЕАКЦИИ
- Алергии
- Смрт поради директно токсично дејство и поради изостанок на тераписки одговор
- Кај антибиотиците појава на резистенција
3. ФИНАНСИСКА ШТЕТА ВРЗ ЗДРАВСТВЕНИОТ СИСТЕМ
4. ОГРОМНИ ШТЕТИ ТРАПИ И ФАРМАЦЕВТСКАТА ИНДУСТРИЈА

Светската здравствена организација но и националните агенции за лекови имаат голема одговорност во успешната борба против фалсификуваните лекови, затоа што загрозувањето на здравјето на населението како директна последица е примената на таквите лекови може да биде со големи последици, а да не заборавиме дека штетите кои ќе ги претрпи здравствениот систем се огромни.

АА3 CONSUMPTION OF ANTIBACTERIAL PRESCRIPTION MEDICINES ON THE BURDEN OF THE HEALTH INSURANCE FUND OF MACEDONIA

K. Hristova, B. Tushi, S. Zlatanovska

The Health Insurance Fund of Macedonia

Introduction: The Health Insurance Fund of Macedonia monitors and analyzes prescription medicines consumption that are issued on a prescription in pharmacies on the HIFM burden in primary health care (PHC). The result of the conducted analyzes provides a good basis and an opportunity to define the necessary new measures in order to achieve better rational and safe use of medicines. This should provide better effects in the patients' medical treatment and the efficient use of the resources in our health care system.

Purpose: Presentation of the results on consumption of antibacterial prescription medicines for systemic use that are issued on a prescription in pharmacies on the burden of the HIFM.

Method: The data based on reliable sources and parameters, have been analyzed also according to the WHO methodology based on the ATC international classification of drugs and their defined daily doses (DDD). Results: Antibacterial drugs for systemic use, which are issued on a prescription in pharmacies on HIFM burden, in 2017 year participate with 7.72% in all realized prescriptions and with 10,62% in the total

financial amount allocated for medicines in primary health care through the pharmacies. From all antibacterial medicines for systemic use, beta-lactams, penicillins and other beta-lactam antibacterial agents are the most commonly used. In addition, their use differs by cities on the territory of the Republic of Macedonia, depending on many factors.

Conclusion: Analyzes and monitoring on the medicines consumption in primary health care, on HIFM burden, by many parameters (diagnosis, number of realized recipes, amounts etc.), provide good insight into the structure of the data, which is a solid basis for further analysis and measures that tend to ensure better rational use of drugs, especially antibiotics, which should influence on the reduction of antibiotic resistance, to provide more efficient re/allocation of resources in the health system and, above all, to provide safe medical treatment and better health care for patients in the country.

ПОТРОШУВАЧКА НА АНТИБАКТЕРИСКИ ЛЕКОВИ НА РЕЦЕПТ НА ТОВАР НА ФОНДОТ ЗА ЗДРАВСТВЕНО ОСИГУРУВАЊЕ НА МАКЕДОНИЈА

К. Христова, Б. Туши, С. Златановска

Фонд за здравствено осигурување на Македонија

Вовед: Фондот за здравствено осигурување на Македонија ја следи и анализира потрошувачката на лекови кои се издаваат на рецепт во аптеките на товар на ФЗОМ во примарната здравствена заштита (ПЗЗ). Резултатите од спроведените анализи даваат добра основа и можност да се дефинираат и потребни нови мерки со цел да се постигне порационална и безбедна употреба на лековите, со што би се обезбедиле подобри ефекти во третманот на пациентите и поефикасно користење на ресурсите во нашиот здравствен систем.

Цел: Презентација на резултатите за употребата на антибактериските лекови за системска употреба кои се издаваат на рецепт во аптеките на товар на ФЗОМ во примарната здравствена заштита.

Метод: Податоците за лековите издадени во аптеките на товар на ФЗОМ се анализирани и според меѓународната методологија на Светската здравствена организација врз основа на АТЦ класификацијата на лекови и нивните дефинирани дневни дози (ДДД).

Резултати: Антибактериските лекови за системска употреба, кои се издадени во аптеките на рецепт на товар на ФЗОМ, во 2017 година учествуваат со 7,72% во вкупно реализираните рецепти во примарната здравствена заштита и со 10,62% во вкупните финансиски средства за лекови на рецепт. Од антибактериските лекови за системска употреба најчесто се користени бета-

лактамите, пеницилините и другите бета-лактамски антибактериски лекови. Покрај тоа, нивната употреба се разликува по градови на територијата на Република Македонија, која што зависи од многу фактори.

Заклучок: Анализите и следењето на потрошувачката на лекови во примарната здравствена заштита на товар на ФЗОМ по различни параметри (дијагноза, број на реализирани рецепти, износи и др.), обезбедува увид во структурата на податоците според реализираните рецепти за лекови на товар на ФЗОМ. Тоа е солидна основа за понатамошни анализи и мерки за обезбедување на порационална употреба на лековите, особено на антибиотиците, кои треба да влијаат на намалување на резистенцијата на антибиотици, подобра пре/распределба на ресурсите во здравствениот систем и пред сè, безбеден третман и подобра здравствена грижа за пациентите во земјата.

AA4 PROPHYLACTIC ANTIMICROBIAL PRESCRIBING IN ADULT INPATIENTS - DATA FROM THE GLOBAL POINT PREVALENCE SURVEY OF ANTIMICROBIAL CONSUMPTION AND RESISTANCE (GLOBAL -PPS 2015) IN SKOPJE

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Background: Antimicrobial prophylaxis is widely used in hospitals for preventing numerous infectious diseases. Development of antimicrobial resistance is one of the most dangerous consequences of extended use of antimicrobials. Point prevalence surveys are a good tool for gathering information regarding antimicrobial use in hospitals and setting targets for improved prescribing.

Methods: The standardized and validated protocol of Global PPS for antimicrobial consumption and resistance was used for collecting data

from 12 clinics with total 1005 beds in Skopje. The study was conducted from March to August 2015. Data of adult inpatients receiving antimicrobials for prophylactic purposes on the day of survey were analyzed.

Results: Out of 527 antimicrobials administered on the day of survey, 37.9% (n=200) were applied for prophylactic purposes in adult hospitalized patients; 12% (n=24) for medical prophylaxis-MP and 88% (n=176) for surgical prophylaxis- SP. Third - generation of cephalosporins, were the most frequently prescribed antimicrobials 65.5% (n=131), followed by fluoroquinolones 11%(n=22) and lincosamides 8.5% (n=17). Guidelines for empiric antimicrobial prescribing were mostly missing for both MP and SP (95.7 % and 99.4% respectively). All antimicrobials prescribed for SP were administered for > 1 day. The rate of reporting stop/review date for both MP and SP was high (100%).

Conclusions: The absence of guidelines, prolonged surgical prophylaxis and high use of the third –generation cephalosporines for surgical prevention which can lead to propagation of highly resistance bacteria, were identified targets for intervention.

Keywords: Point prevalence survey, antimicrobial consumption, adults, prophylaxis, Macedonia

AA5 ANTIBIOTICS, A PART OF THE HOSPITAL TREATMENT

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Introduction: Antibiotics are used in the treatment of infection, but also in the prophylaxis and blocking of complications of many invasive diagnostic and therapeutic procedures. The irrational use of antibiotics at each level leads to a large increase in resistance, therefore it is necessary to monitor them and to create their own national and local policies for their use, in order to serve us for a long time.

Objective: To determine the average antibiotic exposure of each hospital patient.

Material and results: Observing and displaying the consumption of antibiotics for a period of three years, using materials from the hospital pharmacy, and arranging according to international standards ATC - anatomic therapeutic classification and DDD-defined daily dose, in order to make a usable analysis that could be compared more widely. We do not have the financial data and therefore we cannot tell how much from „ the pie " the antibiotics have taken. For antibiotics like drugs that

take a limited time, we show consumption by number of hospitalized patients. From the work done, it can be seen that in the Clinical hospital - Bitola (CHB) in 2015 the DDD of antibiotics of hospitalized patients per year is 4.82 days, which means that everyone who was lying in the hospital received antibiotics for 4.82 days, a number which grows, and in the next 2016 year it will be 5.55 days, and accordingly in 2017 it will be redefined and will be 5.60 days.

Conclusion: Today, it is almost impossible to find a person who has never used an antibiotic. Modern medicine is trying to win the war against infections, but finding new antibiotics is a long and expensive process that has lasted for years, so their abuse will punish us all. The more we consume antibiotics, we lose them faster, so the sooner we become aware of that, we will have them longer.

АНТИБИОТИЦИТЕ, ДЕЛ ОД БОЛНИЧКОТО ЛЕКУВАЊЕ

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Вовед: Антибиотици се користат во терапија за лекување на инфекции, но и во профилакса и купирање на компликации на многу инвазивни дијагностички и терапеутски процедури. Нерационалната употреба на антибиотици на секое ниво води до голем пораст на резистенцијата, затоа е потребно нивно следење и креирање свои национални и локални политики за нивно користење, се со цел долго да не служат .

Цел: Одредување на просечна изложеност на антибиотици на секој болнички пациент.

Материјал и резултати : Пратење и прикажување на потрошувачката на антибиотици за период од три години, користејќи ги материјалите од болничката аптека и средувајќи ги по меѓународни стандарди АТК- анатомско тераписка класификација и ДДД- дефинираната дневна доза, како би се направила употреблива анализа која би можела да се споредува пошироко. Го немаме финансискиот податок и затоа не можаме да кажаме колкав дел од колачот земаат антибиотиците. За антибиотиците како лекови кои се земаат ограничено време, ја прикажуваме потрошувачката по број на хоспитализирани болни. Од сработеното се гледа дека во Клиничка болница Битола (КББ) во 2015година ДДД на антибиотици на хоспитализирани болни на годишно ниво е 4,82 дена, што значи да секој кој лежел во болницата примал антибиотик 4,82 дена, бројка која расте, и во наредната 2016 година изнесува 5,55 дена,

а во 2017година е со повторен раст и изнесува 5,60дена.

Заклучок: Денес е скоро неможно да се најде човек кој никогаш не користел антибиотик.

Модерната медицина се обидува да ја добие војната против инфекциите, меѓутоа пронаоѓањето на нови антибиотици е долг и скап процес и трае со години, па затоа нивна злоупотреба ќе не казни сите нас. Што повеќе ги трошиме антибиотиците, побргу ги губиме, колку побрзо станеме свесни, ќе ни останат подолго во употреба.

AA6 WHICH ANTIBIOTICS ARE MOST USED IN CLINICAL HOSPITAL BITOLA

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Introduction: Antibiotics are not food! The Republic of Macedonia is still in the group of countries with high consumption of antibiotics in relation to the northern European countries, as well as its unfavorable structure.

Objective: To establish the best-fitted antibiotic in the hospital, newly introduced, and to act to tailor its local policy for the use of antibiotics.

Material, results and discussion: A retrospective analysis of the consumption of antibacterial drugs in a three year period was carried out for the years 2015, 2016 and 2017. Data from the consumption of antibacterial drugs (J01A-J01X) of a hospital pharmacy with daily dose determination - DDD were used. From these analysis, the following results were obtained: The first place is J01D, the beta-lactam antibiotics cephalosporin by 63% in 2015, 66% in 2016, and 64% in 2017, of which about 90% belong to J01DD04, i.e. (CRO IIIgr) - ceftriaxone third generation. The second place for use is divided into three groups of antibiotics, approximately equally, and these are J01F, J01G, and J01M. Added to use in 2016 is MTZ, and in 2017 are CS, LZ. Unfortunately but forgotten for use are J01C, beta-lactam antibiotics and penicillin which represent 0.5%. Each institution should establish its own local antibiotic use policy and coordinate it at the national level.

Conclusion: Mediterranean countries are paying the tax on uncontrolled consumption of antibiotics for decades. Analyzing the use of antibiotics and their resistance leads to the creation of its own policy for which

antibiotic how much and where has to be used, and which has to be kept as a reserve. The differences in the consumption of antibiotics between us and the EU countries are scarce.

КОИ АНТИБИОТИЦИ НАЈМНОГУ СЕ КОРИСТАТ ВО КЛИНИЧКА БОЛНИЦА БИТОЛА

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Вовед: Антибиотиците не се храна! Република Македонија сеуште спаѓа во група на земји со голема потрошувачка на антибиотици, во однос со северноевропските земји, како и со неповолна структура на неа.

Цел: Да се утврди најпотрошуван антибиотик во болниците, ново воведен, и да се делува на кроење своја локална политика за употреба на антибиотици.

Материјал, резултати и дискусија: Спроведена е ретроспективна анализа на потрошувачка на антибактериски лекови во три годишен период, и тоа за 2015год, 2016год и 2017год. Користени се материјали од потрошувачка на антибактериски лекови (J01A-J01X) од болничка аптека со дефинирање на дневна доза – ДДД. Од анализата добиени се следните резултати: На прво место се J01D, бета-лактамските антибиотици цефалоспорините со 63% во 2015год, со 66% во 2016год, и со 64% во 2017год од кои околу 90% отпаѓаат на J01DD04, односно (CRO IIIgr)- цефтриаксон трето генерациски. Второ место по користење го делат три групи на антибиотици, горе долу подеднакво, а тоа се J01F, J01G, и J01M. Додадени во употреба во 2016год е MTZ, а во 2017год се CS, LZ. Жално но заборавени за употреба се J01C, бета-лактамски антибиотици, пеницилините кои се застапени 0,5%. Секоја институција треба да формира „своја локална политика на употреба на антибиотици и да се координира на национално ниво„.

Заклучок Медитеранските земји го плаќаат данокот на неконтролираната потрошувачка на антибиотиците со децении наназад. Анализа на користење на антибиотици и нивната резистенција води до креирање на своја политика за кој антибиотик колку и каде треба да се користи , а кој да се чува како резерва. Фрапантни се разликите во потрошувачка на антибиотици помеѓу нас и земјите во ЕУ.

AA7 POTENTIAL ANTIMICROBIAL ACTIVITY OF DIFFERENT HONEY TYPES FROM MACEDONIA

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Introduction: Honey has been identified as a potential alternative to the widespread use of antibiotics, which are of significant concern considering the emergence of resistant microorganisms.

Objectives: In this context, the potential antimicrobial activity of five types of local honey were screened for both their bactericidal/ bacteriostatic activities against different Gram positive and Gram negative bacteria.

Material and Methods: Five types of home made honey available in Macedonia were used in the study: *Acacia, Flower, Linden, Forest* and *Sunflower Honey*. Two different assays were performed to evaluate the antimicrobial potential of the honey samples: agar-well diffusion and broth microdilution method. Agar well diffusion was used to generate zones of inhibition against microorganisms while broth microdilutions were used to study the minimal inhibitory concentrations (MICs) and minimal bactericidal concentrations (MBCs). As a test microorganisms we used different bacteria, including four Gram negative bacteria (*E. coli* ATCC 8739, *Ps. aeruginosa* ATCC 9027, *S. typhimurium*, *S. enteridis*) and nine Gram positive bacteria (*B. subtilis* ATCC 6633, *B. pumillus* NCTC 8241, *Bacillus sp.*, *S. citrus*, *S. albus*, *S. aureus*, *M. luteus*, *L.monocytogenes* and *S.lutea*).

Results: All five honey samples were identified as having antimicrobial activity using the agar diffusion method. The microdilution technique presented greater sensitivity for the antimicrobial testing, since all honey samples showed activity. The results indicated that the effect was concentration and type of honey dependant. Most microorganisms showed similar growth inhibition patterns for all the five honeys tested, but some variations were detected. The minimal inhibitory concentration was in range of 6.25% - 12.5% for tested honey samples (MIC- values), and the minimal bactericidal concentration was in range of 12.5% - 25%.

Conclusions: Honey samples tested in this work showed antimicrobial activity against all tested microoganisms. The results reported herein highlight the potential of using honey to control bacterial growth. Antimicrobial agents from such natural sources present a potential affordable treatment of different infections caused by microorganisms.

Keywords: honey, minimal inhibitory concentrations, minimal bactericidal concentrations, agar-well diffusion method, broth microdilution method.

ПОТЕНЦИЈАЛНА АНТИМИКРОБНА АКТИВНОСТ НА РАЗЛИЧНИ ВИДОВИ МЕД ОД РЕПУБЛИКА МАКЕДОНИЈА

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Вовед: Медот претставува потенцијална алтернатива за широката употреба на антибиотиците, чија употреба станува алармантна поради сé поголемата појава на резистентни кон нив микроорганизми.

Цел: Поради овој факт, целта на трудот беше испитување на потенцијалната антимикробна активност на пет видови мед и одредување на нивната бактерицидна/ бактериостатска активност кон различни Грам позитивни и Грам негативни бактерии.

Материјал и методи: Во оваа студија се користеа пет различни видови мед од Македонија: багремов, цветен, липов, шумски и мед од сончоглед. За одредување на антимикробниот потенцијал се користеа две различни методи: агар дифузониот и микродилуционот метод. Со агар дифузониот метод се испитува и потврдува антимикробниот потенцијал, додека со микродилуционата метода се одредува минималната инхибиторна концентрација (МИК) и минималната бактерицидна концентрација (МБЦ) на примероците мед кон испитуваните бактерии. Како тест микроорганизми се користеа четири Грам негативни бактерии (*E. coli* ATCC 8739, *Ps. aeruginosa* ATCC 9027, *S. typhimurium, S. enteridis*) и девет Грам позитивни бактерии (*B. subtilis* ATCC 6633, *B. pumillus* NCTC 8241, *Bacillus sp., S. citrus, S. albus, S. aureus, M. luteus, L.monocytogenes* и *S.lutea*).

Резултати: Сите пет примероци мед покажаа антимикробна активност кон испитуваните бактерии. Микродилуционата метода претставува метода со поголема сензитивност за антимикробни тестирања. Минималната инхибиторна концентрација на медот кај испитуваните бактерии се движеше во опсег од 6.25% - 12.5%, додека минималната бактерицидна концентрација беше во опсег од 12.5% - 25%.

Заклучок: Примероците од мед тестирани во овој труд покажаа антимиктобна активност кон сите тестирани микроорганизми. Ова покажува дека антимикробни средства добиени од вакви природни извори може да се користат како потенцијални средства во третман на различни инфекции предизвикани од бакетрии.

Клучни зборови: мед, минимална инхибиторна концентрација, минимална бактерицидна концентрација, агар-дифузиона метода, микродилуциона метода

AA8 TESTING VIABILITY OF THE STRAIN LACTOBACILLUS RHAMNOSUS LB 64 DURING THE PRODUCTION OF A PROBIOTIC MEDICATION IN THE FORM OF TABLETS

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The strain *Lactobacillus rhamnosus* LB-64 is an active component of the probiotic medication Liobif, whose complete production takes place at the Institute Torlak. The production process consists of reviving the strain, its multiplication, separation of bacterial sediment, liofilization of sediment , homogenization of lyophilisate and tablet mass and tabletting.

The aim of our work was to test viability of the strain *Lactobacillus rhamnosus* LB-64 at each stage of the production of a probiotic medication in the form of tablets.

Material and methods: The strain is revived in the MRS broth (Difco Becton-Dickinson)and multiplies in the fermenter (The first spark), the bacterial residue is obtained by centrifugation (in Scarples Centrifuges), and lyophilized (liofilizator Usifroid), homogenizes in a mill (Erweka) and tablets on the tablet (Fette). The viability of the strain is monitored by the determination of the total number of living bacteria by decimal dilutions of samples in the tubes and their sowing on the MRS agar.

Results of work: By providing appropriate conditions for growth, the strain LB-64 remains viable at all stages of production. The total number of live bacteria in the fermentor inoculum and the growing culture is 10^{10}CFU/ml, in bacterial residue 10^{11} CFU/ml, in lyophilisate 10^{10}CFU/ml. Standardized production produces tablets with the total number of bacteria 10^{8}-10^{10}CFU/ tablets. **Conclusion:** Output results viability of the strain *Lactobacillus rhamnosus* LB-64in all stages of production of the probiotic preparation are tablets with the total number of living bacteria 10^{8}-10^{10} CFU/tablets according to the registration requirement.

Key words: *Lactobacillus rhamnosus, Determining the total number of live bacteria*, lyophilization

AA9 ASCENDANT OF LACTIC ACID BACTERIA *LACTOBACILLUS BREVIS* L62 OVER *SALMONELLA ENTERIC SEROTYPE TYPHIMURIUM* STR^R IN MIXED CULTURE

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INTRODUCTION: Almost every environmental niche harbours multi-species community with mixed bacterial cultures which coexist in different relationships. In laboratory conditions, bacterial interaction can be followed by cultivation of mixed bacterial cultures under hunger conditions during prolonged stationary phase with bacterial species of different age to distinguish their competitive potential. **Comparison and measurement of competitive growth in mixed bacterial cultures has great importance for microbial food safety.**

OBJECTIVES: To compare the competitive growth of one and six days old S. enterica serotype Typhimurium LT21 bacterial cultures and one-day old Lactobacillus brevis L62 bacterial culture, the cultures were mixed in ratios 1:1 and 1:10 (vol:vol) and cultivated at 37 °C. Considering the described phenomenon of constant protein synthesis in bacterial culture during stationary phase, the qualitative protein profile was measured.

MATERIAL: The bacterial strains used in this paper were: S. enterica serotype Typhimurium LT21 wild type and strains harbouring the resistance to streptomycin (Str^R), and Lactobacillus brevis L62 harbouring the resistance to nalidixic acid (Nal^R).

METHODS: In a typical growth advantage in stationary phase competition experiment, cells from a 6-day-old culture are inoculated as a numerical minority (1:10 vol/vol) into a young (1-day-old) culture or in ratios 1:1 (vol:vol). Proteomic analysis of cells was performed by purification and separation of the peptides by liquid chromatography. Mass spectra were analyzed on a MALDI TOF/TOF mass spectrometer (Autoflex Speed, Bruker Daltonics, Bremen, Germany).

RESULTS: Lactobacillus brevis L62 (1-day-old) cell cultures grown in a mixed culture with 1-day-old bacterial culture of Salmonella enteric serotype Typhimurium LT21 in ratio 1:1 or 10: 1 at 37 °C on a minimal substrate showed strong competitive growth.

S. enterica serotype Typhimurium LT21 (6-days-old) cell cultures grown in a mixed culture with 1-day-old cells of L. brevis L62 in ratio 1:10 at 37 °C on a minimal basis show abortive phenotype of growth advantage in stationary phase.

Proteomic analysis showed that proteins required for essential cellular processes are present in L. brevis L62 cells.

CONCLUSION: Bacterial cells of L. brevis L62 have shown a strong

competitive growth against 1-day and 6-day-old cells of S. enterica serotype Typhimurium and can be used to protect and remove contamination with antibiotic resistance pathogenic species of Enterobacteria. This is a manner by which human foodborne pathogen can be prevented and controled.

AA10 EVALUATION OF *IN VITRO* ANTIBACTERIAL ACTIVITY OF EXTRACTS FROM LIVERWORT PLANTS OBTAINED IN BLACK SEA REGION OF TURKEY

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INTRODUCTION

The ongoing search for antimicrobial activity showing plants has gained special importance in recent years due to the increasing rate of infections caused by antibioticresistant bacteria. The liverworts (Marchantiophyta) are a diverse group of land plants that usually colonize moist habitats with extremely variable conditions and classified in the plant phylum Bryophyta. Traditionally, these plants were used as a natural medicine in some parts of the world because of their antimicrobial activity.

OBJECTIVES

In this study, we aimed to evaluate the *in vitro* antimicrobial activity of 7 liverwort species collected from different locations in Black Sea region of Turkey.

MATERIAL, METHODS

Methanol and N-hexane extracts were obtained from the following plants: *Porellaplatyphylla*, *Metzgeriafurcata*, *Plagiochilaasplenioides*, *Frullariatamarisci*, *Radula lindbergiana*, *Conocephalum conium* and*Marchantiapolymorpha* with soxhlet extraction method.
Antimicrobial activity of the extracts were tested against *Staphylococcus aureus* (ATCC 29212), and *Escherichia coli* (ATCC 25922) by using broth microdilution method according to EUCAST. The tests were performed in triplicate for each microorganism.

RESULTS AND CONCLUSION

MICs and MBCs of liverwort plants' extracts for tested bacteria are shown in Table.

Table. Antimicrobial activity of the Liverwort plant extracts

	Staphylococcus aureus ATCC 29213				*Escherichia coli* ATCC 25922			
	Methanol extract		Hexane extract		Methanol extract		Hexane extract	
Liverwort species	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)
Porella platyphylla	1,25	>2,5	1,25	≥ 2,5	0,625	≥ 0,625	0,625	≥ 0,625
Metzgeria furcata	1,25	>2,5	1,25	≥ 2,5	0,625	≥ 0,625	0,625	≥ 0,625
Plagiochila asplenioides	0,625	>2,5	1,25	≥ 2,5	0,625	≥ 2,5	0,625	≥ 0,625
Frullaria tamarisci	1,25	>2,5	1,25	≥ 2,5	0,312	≥ 0,625	0,625	≥ 2,5
Radula lindbergiana	1,25	>2,5	1,25	≥ 2,5	0,625	≥ 0,625	0,625	≥ 0,625
Conocephalum conium	1,25	>2,5	1,25	≥ 2,5	0,625	≥ 0,625	0,625	≥ 0,625
Marchantia polymorpha	0,625	>2,5	1,25	>2,5	0,625	≥ 0,625	0,625	≥ 0,625

MIC: Minimum inhibitory concentration; MBC: Minimum bactericidal concentraton

Both methanol and hexane extracts from liverwort species have shown *in vitro* antibacterial activity against tested bacteria (Table). Hexane extracts were more active against to *E.coli* (MIC range 0.312-0.625 mg/mL) than methanol extracts and expressed promising MBCs for this microorganism. Further evaluation is needed to assess the antibacterial activity against other bacterial species and clinical isolates.

AA11 METHOD SUITABILITY TEST FOR DETERMINATION OF MICROBIOLOGICAL PURITY OF ACIKLOVIR CREAM 5%

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Introduction: Aciklovir cream 5% is an antiviral cream for topical use. For pharmaceutical topical forms, the parameters that should be quality controlled from microbiological aspect are Total Aerobic Microbial Count (TAMC), Total Combined Yeasts/Molds Count (TYMC) and test for *Staphylococcus aureus* and *Pseudomonas aeruginosa.*

Objective: The aim of this project is to validate the analytical method which will be used in the routine work for determination of the microbiological purity of Aciklovir cream 5%.

Materials: Standard equipment and accessories that are used in microbiological laboratory. The test microorganisms for this validation as well as the media used are recommended for the method suitability test.

Method: For determination of the antimicrobial activity and validation

of the parameters TAMC and TYMC, surface-spread method was used and direct inoculation for *S. aureus* and *P. aeruginosa*, according to cPhEur. When verifying the suitability of the plate-count method, a calculated mean from the results should give an acceptability factor not greater than 2.

Results: The working dilution 1 in 10 of Aciklovir cream 5% does not have antimicrobial activity against the used test microorganisms. Furthermore all the individual challenge tests, yielded positive results with the factor of acceptability within range. In the challenge tests for *S. aureus* and *P. aeruginosa*, with direct inoculation, there was good visible growth.

Conclusion: The method suitability test of the product revealed that the routine microbiological analysis should be performed with surface-spread method from 1 in 10 dilution for TAMC and TYMC and with the method of direct inoculation for the tests of *S. aureus* and *P. aeruginosa*. The acceptability factors never exceeded the value of 2.

СООДВЕТНОСТ НА ТЕСТОТ ЗА ОПРЕДЕЛУВАЊЕ МИКРОБИОЛОШКА ЧИСТОТА НА АЦИКЛОВИР КРЕМ 5%

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Вовед: Ацикловир крем 5% е антивирусен крем за надворешна дермална употреба. Фармацевтските креми се тестираат на параметрите за вкупен број аеробни микроорганизми (TAMC) и вкупен комбиниран број на квасци и мувли (TYMC), како и специфични тестови за *Staphilococcus aureus* и *Pseudomonas aeruginosa*.

Цел: Целта на овој проект е да се валидира аналитичката метода која што се користи за рутинска анализа на микробиолошката чистота на кремот.

Материјали: Стандардана лабораториска опрема и додатоци кои што се користат во микробиолошка лабораторија. Тест микроорганизмите користени за валидацијата се препорачани за испитување на соодветноста на методот.

Методи: За одредување на антимикробните својства и за валидација на аналитичката метода, се вршат тестови со разлевање на цврст хранлив медиум за TAMC и TYMC, како и методот на директна инокулација врз соодветниот течен хранлив медиум за *S. Aureus* и *P. Aeruginosa*. Кога се одредува соодветноста на методот, се пресметува факторот на прифатливост, кој не треба да биде поголем од 2.

Резултати: Во работна дилуција на продуктот 1 во 10, се докажа дека Ацикловир кремот 5% нема антимикробни својства против користените микроорганизми. Исто така индивидуалните тестови за соодветност на методот, дадоа позитивни резултати со фактор на прифатливост во рамките на лимитот. Како и тесовите за специфичните микроорганизми дадоа позитивни резултати со пораст во медиумите.

Заклучок: Тестот за соодветност на аналитичкиот метод за Ацикловир крем 5%, покажа дека рутинската микробиолошка анализа треба да биде изведена со разредувње во соодветен медиум 1 во 10 за TAMC и TYMC, како и директна инокулација со збогатување за тестовите за отсуство на *S. Aureus* and *P. Aeruginosa.* Сите фактори на прифатливост не надминуваат вредност поголема од 2.

AA12 VALIDATION OF METHOD FOR DETERMINATION OF MICROBIOLOGICAL PURITY OF VERAPAMIL RETARD TABLETS 240 mg

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Introduction: Verapamil retard tablets 240 mg with prolonged release are pharmaceutical solid dosage form from the group of calcium channel blockers with pharmacological effect on cardiovascular system. According to European Pharmacopoeia microbiological parameters which are examined for quality control of the pharmaceutical solid oral forms are: Total Aerobic Microbial Count (TAMC), Total combined Yeasts/Molds Count (TYMC) and parameter absence of *Escherichia coli.*

Objective: The aim of this study is to validate the analytical method which will be used in the routine control for determination of microbiological purity of Verapamil retard tablets 240 mg before releasing of the batch.

Materials: Standard microbiological equipment and materials were used as well as media and test microorganisms recommended by European Pharmacopoeia.

Method: Verapamil retard tablets 240 mg showed antimicrobial effect against the used test microorganisms in dilution 1 in 10. Because of that, method of choice for parameters TAMC and TYMC is surface spread method where peptone solution with neutralizers (Tween 80, Lecithin и Histidine hydrochloride) was used as diluent. According to the validation study, working dilution for parameter TAMC is 1 in

200, for parameter TYMC is 1 in 100 and the parameter absence of *Escherichia coli* was validated with method of direct inoculation of 10 ml from dilution 1 in 10 in 100 ml Trypcase Soy Broth.

Results: With comparison of the microbial growth in the groups with product compared with the control groups, the acceptability factor never exceeded 2 for all of the test microorganisms which is compatible with the recommendations of European Pharmacopoeia. In the challenge test with *Escherichia coli* in presence of product, the test microorganism *Escherichia coli* was detected.

Conclusion: In accordance to the results of the validation study suitable method for determination of microbiological purity of Verapamil retard tablets 240 mg was validated.

ВАЛИДАЦИЈА НА МЕТОДОТ ЗА ОПРЕДЕЛУВАЊЕ НА МИКРОБИОЛОШКА ЧИСТОТА НА ВЕРАПАМИЛ РЕТАРД ТАБЛЕТИ 240 mg

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Вовед: Верапамил таблети од 240 mg со продолжено ослободување претставуваат цврсти фармацевтски дозажни форми од групата блокатори на калциумови канали кои делуваат на кардиоваскуларниот систем. Според Европската фармакопеја, микробиолошки параметри кои се испитуваат во рамки на контролата на квалитетот на цврстите фармацевтски форми се: Вкупен број на аеробни микроорганизми (TAMC), Вкупен број на квасци и мувли (TYMC) и параметарот отсуство на *Escherichia coli*.

Цел: Целта на оваа студија е валидација на аналитички метод кој ќе се користи во рутинска контрола за определување на микробиолошката чистота на Верапамил ретард таблети 240 mg пред да се ослободи серијата од готовиот производ.

Материјали: Се користеше стандардна микробиолошка опрема и материјали, како и медиуми и тест микроорганизми препорачани од Европската фармакопеја.

Методи: Верапамил таблетите од 240 mg со продолжено ослободување имаат антимикробен ефект врз користените тест микроорганизми во дилуција 1 во 10. Според тоа за параметрите TAMC и TYMC избран е метод на броење на плоча со површинско распоредување, со користење на пуферен пептонски раствор со неутрализатори (Tween 80, Lecithin и Histidine hydrochloride). Согласно валидационата студија работна дилуција за параметарот TAMC е 1 во 200, за параметарот TYMC е 1 во 100, додека

параметарот отсуство на *Escherichia coli* се валидираше со методот на директна инокулација на 10 ml од дилуција 1 во 10 во 100 ml Trypcase Soy Broth .

Резултати: Со споредба на порастот на микроорганизмите во групите со продукт со контролните групи, факторот на прифатливост за сите тест микроганизми не е поголем од 2, со што се задоволуваат барањата на Европската фармакопеја. Во предизвик тестот со *Escherichia coli* во присуство на продукт се детектираше присуство на *Escherichia coli.*

Заклучок: Во согласност со резултатите од студијата се валидираше метод кој е соодветен за одредување на микробиолошката чистота на Верапамил ретард таблети 240 mg.

AA13 VALIDATION OF METHOD FOR EXAMINATION OF MICROBIOLOGICAL QUALITY OF MOXIFLOXACIN FILM-COATED TABLETS 400 mg-ALKALOID

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Introduction: *Moxifloxacin* is fluoroquinolone antibiotic with strong Gram-positive and good Gram-negative antimicrobial activity. It has methoxy group on carbon 8 that differentiates it chemically from other fluoroquinolones.

Objective: The objective of this study is validation of suitable method for examination of microbiological quality of Moxifloxacin film-coated tablets 400 mg. The parameters which are subject of validation are: TAMC (Total Aerobic Microbial Count), TYMC (Total combined yeast/moulds count) and test for *Escherichia coli*.

Material: The test microorganisms which were used as challenge organisms for the validation are from the American Type Culture Collection. All of the used solutions and culture media comply with the European Pharmacopoeia. 1 M solution of Magnesium chloride was used as an neutralization agent of the antimicrobial activity of Moxifloxacin film-coated tablets 400 mg.

Method: The method used for validation of TAMC and TYMC is membrane filtration from dilution 1 in 1000 with 1 M $MgCl_2$ applied in the working dilution and applied and absorbed on Trypcase Soy Agar for the parameter TAMC, and from dilution 1 in 100 for the parameter TYMC. The validation of the parameter *Escherichia coli* was performed with membrane filtration from dilution 1 in 10, after which the filters were transferred in Trypcase Soy Broth with a suitable amount of the

neutralizing agent. Rinsing of the filters with recommended solution was included for all of the parameters.

Results: From the obtained results of the microbial growth in the inoculated products compared with the control groups, the percentage of recovery is above 70 % for all of the microorganisms. With performed challenge test method, growth of *Escherichia coli* in the presence of the product was detected and confirmed with biochemical tests.

Conclusion: According to the results, a suitable method for determination of microbiological quality of Moxifloxacin film- coated tablets 400 mg was validated.

ВАЛИДАЦИЈА НА МЕТОД ЗА ИСПИТУВАЊЕ НА МИКРОБИОЛОШКИ КВАЛИТЕТ НА MOXIFLOXACIN ФИЛМ ОБЛОЖЕНИ ТАБЛЕТИ 400 mg-АЛКАЛОИД

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Вовед: Моксифлоксацинот претставува флуорохинолонски антибиотик со силна Грам-позитивна и добра Грам-негативна антимикробна активност. Од останатите флуорокинолони хемиски се разликува поради присуството на метокси (CHO-) група на осмиот јаглероден атом.

Цел: Развивање на соодветен метод за испитување на микробиолошкиот квалитет на Moxifloxacin филм обложени таблети 400 mg. Параметри кои се предмет на валидационата студија се: ТАМС (Вкупен број на аеробни микроорганизми), ТҮМС (Вкупен комбиниран број на квасци/мувли) и тестот за *Escherichia coli*.

Материјали: Тест микроорганизмите кои се користат како предизвик организми во валидацијата припаѓаат на Американската колекција на култури на соеви. Употребените раствори и медиуми се во согласност со барањата на Европската фармакопеја. Како неутрализирачки агенс на антимикробното дејство на Moxifloxacin филм обложените таблети 400 mg се примени 1 М раствор на магнезиум хлорид.

Метод: Валидација на параметрите ТАМС и ТҮМС беше изведена со метод на мембранска филтрација од дилуција 1 во 1000 со апликација на 1 М $MgCl_2$ во работната дилуција и аплицикација и ресорбција на Trypcase Soy Agar за параметарот ТАМС, и од дилуција 1 во 100 за параметарот ТҮМС. Тестот за *Escherichia coli* беше валидиран со метод на мембранска филтрација од дилуција 1 во 10, после што филтрите се трансферирани во Trypcase Soy Broth

со соодветно количество од неутрализирачкиот агенс. За сите параметри беше применето плакнење на филтрите со соодветен раствор.

Резултати: Процентот на "recovery" добиен како резултат на споредба на микробниот пораст во инокулираните продукти во споредба со контролните групи е поголем од 70 % за сите тест микроорганизми. Во предизвик тестот со *Escherichia coli* во присуство на продукт беше детектиран и потврден пораст на *Escherichia coli* со биохемиски тестови .

Заклучок: Во согласност со добиените резултати, се валидираше соодветен метод за испитување на микробиолошкиот квалитет на of Moxifloxacin film- coated tablets 400 mg-Alkaloid.

СЕСИЈА 9/SESSION 9
ПАРАЗИТОЛОГИЈА, МИКОЛОГИЈА, ЕНТОМОЛОГИЈА/ PARASITOLOGY, MYCOLOGY, ENTOMOLOGY

PME1 SHORT OVERVIEW OF PARASITIC ZOONOTIC DISEASES IN R.MACEDONIA AND ONE HEALTH APPROACH

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The One Health approach aims to bring together human and animal health, as well as the environmental factors. It plays a significant role in prevention and control of zoonoses. Utilizing One Health strategy for foodborne and vector-borne parasitic zoonotic diseases is the most effective approach in reducing human and animal exposure to parasites. Data revealing the presence and prevalence of parasitic zoonoses in animals are essential for analysis of their transmission and risk assessments. According to present data, several parasitic zoonoses are identified as being a major public health concern such as echinococcosis, leishmaniosis, toxoplasmosis and trichinellosis.

Cystic echinococcosis is endemic all over the country with prevalence of approximately 50% both in cattle and sheep and no significant difference of prevalence in different regions. The high prevalence recorded in young animals (38.5% in cattle within 24 months) suggests a high environment contamination with *Echinococcus* eggs and a consequent high exposure to the infection for animals and likely humans.

Canine leishmaniosis, caused by *Leishmania infantum*, is an endemic disease in R. Macedonia with 28.2% seroprevalence among the dog population. *Phlebotomus spp.* as the main vector of this disease is consisted of 8 different species and is present throughout the country. Thus, the risk of exposure for human and canine population is relatively high.

Toxoplasmosis prevalence is 33.26% in sheep and goats and 17%

among the sows which represent a risk of transmission in undercooked meat. The prevalence among cats as a main reservoir of this diseases is still missing.

Presence of *Trichinella spp.* in domestic pigs hasn't been reported in the slaughter houses in the past 15 years. Since the importance of wildlife as reservoirs of human diseases has also been widely recognized for most of the parasitic zoonoses, the presence of *Trichinella spp*. has been investigated among wild boars, red foxes, wolfs and bears. The highest prevalence (23.25%) had been found among red foxes which indicates the presence of active route of transmission among wildlife and consequently a risk for farmed pigs slaughtered for human consumption.

A continuous One Health approach is needed to integrate all data and to establish national epidemiological concept, surveillance, risk analyses, risk assessment and risk management programs.

PME2 TAENIASIS/NEUROCISTICERCOSIS (NCC) IN THE REPUBLIC OF MACEDONIA: IMPORTANCE AND GAPS

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Taenia solium and *Taenia saginata* are food-borne parasites (FBP) of public health importance. *T. solium*, the cause of neurocysticercosis (NCC), ranked first in the relative global importance ranking of 24 FBP made on the basis of a Multi Criteria decision analysis (MCDA) proposed by FAO/WHO. Although in 1993 *taeniasis/cysticercosis*were declared as potentially eradicable in Europe, the life cycle of *T. solium* is still active nowadays. For control and prevention of taeniasis (potential carriers of *T.solium*), it is crucial to have available proper diagnostic tools. Microscopic methods are the tools most frequently used for the

diagnosis of taeniasis.

The aims of our study were to prioritize *taeniasis*/NCC in the Republic of Macedonia (RM), to assess the quality (QA) of diagnostic tools used and to identify the gaps.

We ranked FBP in RM based on EURO-FBP criteria and weights, using the MCDA method. Prioritization of taeniasis/neurocysticercosis was performed by the evaluation of the reports from national surveillance systems and data obtained by a questionnaire sent through the Macedonian Microbiology Society (MMS) network. Data from gray literature and personal contacts with experts were also included. In addition, within the CYSTINET COST Action frame, a proficiency testing (PT) for the detection of *Taenia* spp. by microscopic methods in human stool samples used by laboratories in their routine procedures was carried out. The PT was organized according to the ISO/IEC 17043:2010 standard.

According to official data, there is no *T.solium* or NCC in RM. On contrary, the collected data reveal that there are sporadic autochthonous cases of NCC. Regarding *T. saginata*, the official incidence rate was lower than expected.

PME3 TINEA CAPITIS CAUSED BY TRICHOPHYTON – CASE REPORT

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Dermatophytes are fungi that are known for causing topical mycoses, also known as dermatomycoses or dermatophytoses. They are keratophylic microorganisms that show affinity towards skin, hair and nails. Their natural habitat is soil but they express their parasitic nature in humans and some animals. There are three types of dermatophytes that can cause an infection in humans. These three types cause only localized lesions (with or without presence of hair) and never dwell deeper in the human body; hence they never cause a systemic infection. Trichophyton is one of the most common culprits in the cause of dermatomycoses worldwide. The case report shows a young boy, at the age of 9, with a single squamous lesion on the top of the head, no larger than a coin and accompanied by total absence of hair. It is this lesion that we swabbed and the sample cultivated in a petri dish with Sabouraud medium for a total of 14 days, on two temperature levels (27 °C and 35 °C). What came out on the 10th day, macroscopically, are white, rough, cotton-like colonies. We prepped the colonies with lactophenol blue and then did a light microscopy where we got matchstick-like micronidiae, spread along the hyphae.

Furthermore, no signs of macronidiae were found. After our findings, we referred the patient to a dermatologist to receive appropriate treatment. By using the previously mentioned methodology we were unable to differentiate the type of Trichophyton that caused this lesion due to lack of specific growth mediums. This was the 1st registered case of dermatophytosis in our laboratory.

TINEA CAPITIS ПРЕДИЗВИКАНА ОД TRICHOPHYTON – ПРИКАЗ НА СЛУЧАЈ

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Дерматофити се габи кои предизвикуваат површински микози, уште наречени и дерматомикози или дерматофитози. Дерматофити се кератофилни микроорганизми кои покажуваат осебен афинитет за кожа, влакна и нокти. Средина на живеење им е земја, но паразитираат во животни и кај луѓе. Постојат три вида на дерматофити кои може да предизвикаат заболување кај човекот, но никогаш системски туку ексклузивно на локализирани лезии, на кожа со и без влакна. Trichphyton е еден од споменатите видови на дерматофити, а заболувањето кое го предизвикува е наречено трихофитија и преставува едно од најраспространетите микози во светот. Во нашиот случај станува збор за единечна сквамозна лезија на Tinea capitis кај 9 годишно момче (А.М.), локализирана во пределот на темето, со големина на монета и без присуство на здрави влакна. Примерокот за брис беше земен од самата промена и култивиран на Sabouraud хранителна подлога, на температура од 27 ° и 35 ° во тек на 14 дена. После 10 дена, израснатите колонии беа макроскопски и микроскопски анализирани. При макроскопскиот наод се забележаа рапави, памучести колонии, бели по боја. Микроскопски, по препарирање на колониите со lactophenol blue, беа забележени микроконидии во облик на кибрит, поставени по должината на хифите. Во истиот препарат не беа пронајдени макроконидии. По наодот, пациентот беше упатен на дерматолог за понатамошен третман. Според изгледот на колониите и микроскопскиот наод, не беше во можност да се диференцира видот на Trichophyton. Со цел да се диференцираат видот на Trichphyton потербни се други видови на хранителни подлоги. Ова е првиот регистриран случај на дерматофитоза во нашата лабораторија.

PME4 SURVEILLANCE OF INVASIVE MOSQUITOES IN THE REPUBLIC OF MACEDONIA IN 2016-2017

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R.Macedonia, due to global warming and climate change, with its geographical position and moderate climate faces the danger of possible occurrence of tropical and subtropical diseases Zika virus, the western Nile virus, Denga, Chikungunya, East horse encephalitis, Yellow fever, the virus " Cache Velleu "(CVV)," La Crosse "encephalitis (LACV), and the occurrence of diseases already eradicated in the Republic of Macedonia (Malaria), and transmitted through vectors (mosquitoes).

From the public health aspekt, surveillance of Aedes (Stegomyia) albopictus (Skuse 1894) (Diptera, fam.Culicidae) is necessary .The surveillance was carried out with the setting of the ovitraps. The first surveillance was in August 2016, and the surveys were placed on three different locations in Banja Bansko (near Strumica), Mrzenci (Gevgelija) and the Bogorodica border crossing (Macedonia-Greece). These ovitraps were exposed for two weeks over a month, near the lush flora, the presence of people and of course in an area where there is a water surface. Collected eggs from the ovitraps were returned into the Entomological laboratory at the CPH-Skopje and placed them in an entomological cage. At optimum humidity and temperature, mosquitoes have exploded. The determination was performed with morphological-taxonomic determination with binocular. Of the 50 mosquitoes that were collected, 23 were male Aedes albopictus, and 27 were Aedes albopictus women. These samples are verified by prof.dr. Dusan Petric from the Faculty of Agricultural Sciences in Novi Sad, and for the first time was shown the presence of a tiger mosquito in the Republic of Macedonia.

In June 2017, the same locations were repeated from 2016, followed by 4 new locations in the north-eastern region, and were placed in the bar "Jet" - (Stip), the termal bath "Kezhovica" (Stip), hotel Romantic- (Veles) and restaurant "Stop and Go"before border crossing (Macedonia-Kosovo). In the new locations no invasive mosquitoes were found, and we continued the supervision in August 2017 at the Tabanovce GP. , his presence has not yet been proven. The Asian tiger mosquitoes surveillace will continue in the future according to the plan and program by the expert team from the Entomological Laboratory, and this is necessary because they are vectors of infectious diseases and are of public health interes.

Key words: surveillance, Asian tiger mosquitoes, collection of eggs, Macedonia

НАДЗОР НА ИНВАЗИВНИТЕ КОМАРЦИ ВО РЕПУБЛИКА МАКЕДОНИЈА ВО 2016-2017г

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Р.Македонија поради глобалното затоплување и климатските промени, со својата географска положба и умерената клима се соочува со опасноста на евентуална појава на тропски и субтропски болести Zika вирус, вирусот на западен Нил, Denga, Chikungunya, Источно коњски енцефалитис, Жолта треска, вирусот "Cache Velleu"(CVV), "La Crosse" encephalitis (LACV) и др.) , како и појава на болести кои се веќе ерадицирани во Р.Македонија (Маларија), а се пренесуваат преку вектори (комарци).

Од јавно-здравствен аспект е неопходен надзор на Aedes (Stegomyia) albopictus (Skuse 1894) (Diptera, fam.Culicidae). Надзорот се вршеше со поставување на овитрапови.Прв надзор беше извршен во август 2016 година ,и овитраповите беа поставени на три различни локации , и тоа во Бања Банско (во близина на Струмица), Мрзенци (Гевгелија) и на Граничниот премин Богородица (Македонија-Грција). Овие овитрапови беа изложени по две недели во текот на еден месец, во близина на бујна флора, присуство на луѓе и секако во околина каде има водена површина. Собраните јајца од овитраповите ги враќавме во Ентомолошката лабораторија при ЦЈЗ-Скопје и беа постави во ентомолошки кафез. При оптимална влажност и температура, комарците еклодираа. Одредувањето на видот беше изведено со морфолошко-таксономска детерминација со бинокулар. Од вкупно 50 комарци кои беа собрани, 23 беа машки Aedes albopictus, а 27 се жени Aedes albopictus. Овие примероци се верифицирани од проф.др. Душан Петрич од Факултетот за Земјоделски науки во Нови Сад и за прв пат беше докажано присуство на тигрестиот комарец во Р.Македонија.

Во јуни 2017 , на истите локации од 2016г беа повторно поставени овитрапови, потоа беа опфатени 4 нови локации во северо-источниот регион и беа поставени овитрапови во кафе бар „Цет„ - (Штип), Бањата „Кежовица„ - (Штип), хотел Романтик- (Велес) и во ресторант „Стоп анд Гоу„ пред Граничниот премин Табановце (Македонија-Косово). На новите локации не беа најдени инвазивни комарци и затоа надзорот го продолживме во август 2017 на ГП Табановце. Но, повторно не беше докажано неговото присуство.Надзорот на тигрестиот комарец ќе продолжи и наредните години според планот и програмата на стручниот тим кој работи во Ентомолошката лабораторија, а тоа е неопходно бидејќи се вектори на заразни заболувања и се од јавно-здравствен .

Клучни зборови: надзор, азиски тигрест комарец, овитрапови, собирање на јајца, Македонија

PME5 FLUORESCENCE MICROSCOPY OF ASPERGILLUS IN BRONCHIAL BRUSHING SAMPLES – CASE REPORT

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Introduction. Aspergillus is an opportunistic fungus, usually seen in immunocompromised patients, where it affects lungs, upper airways, skin and brain. Clinical findings are usually nonspecific and the diagnosis is based on imaging investigations and various laboratory tests.

Objectives. We present a simple method for visualization of Aspergillus on standard cytological specimens.

Material and Methods. We received bronchial brushing samples from a patient with clinical history of bronchiectasiae and bilateral hilar shadows on performed CT scans. The smears after short fixation in ethanol were stained by a standard procedure with hematoxylin-eosin.

Results. The light microscopy showed presence of epithelial cells in three-dimensional arrangement highly suspicious for malignancy, among which necrotic material with mycelar structures were seen. The same slides, without any immunoreagent or fluorochrome, were examined under ultraviolet light on fluorescence microscope at wavelength of 450 to 490 nm. The fluorescence microscopy highlighted solitary hyphae and hyphae organized in mycelia. The hyphae showed dichotomous branching in two nearly equal branches at 45 degrees.

Synchronously performed fungal culture from the bronchial brushing material confirmed the presence of Aspergillus fumigatus.

Conclusion. The presented method is an accurate, simple, cheap, and, most importantly, rapid adjuvant method that does not require any special stainings for the purpose of visualization of fungal hyphae, based on autofluorescence originating from endogenous fluorophores and can be utilized in any pathology laboratory that is equipped with fluorescent microscope. This is particularly useful when only a limited number of slides with smears are available for analysis and of utmost importance knowing that early diagnosis is critical for successful treatment.

ФЛУОРЕСЦЕНТНА МИКРОСКОПИЈА НА ASPERGILLUS ВО ПРИМЕРОЦИ ЗА ЦИТОЛОШКА АНАЛИЗА ОД БРОНХ - ПРИКАЗ НА СЛУЧАЈ

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Вовед. Aspergillus е опортунистичка габа, која обично се сретнува кај имунокомпромитирани пациенти, каде што се засегнати белите дробови, горните дишни патишта, кожата и мозокот. Клиничките наоди обично се неспецифични и дијагнозата се базира на радиографски испитувања и разни лабораториски тестови.

Цели. Ви претставуваме едноставен метод за визуелизација на Aspergillus на стандардни цитолошки примероци.

Материјал и методи. Добиени се размаски од направен бронхијален „брашинг" од пациент со клиничка историја на бронхиектазии и билатерални хиларни сенки на направената компјутеризирана томографија. Размаските по кратка фиксација во етил алкохол беа обоени со стандардна процедура со хематоксилин-еозин.

Резултати. Светлосната микроскопија покажа присуство на епителни клетки во тродимензионален аранжман високо сомнителни за малигнитет, меѓу кои се гледа и некротичен материјал со мицеларни структури. Истите препарати, без имунореагенс или флуорохром, беа испитувани под ултравиолетова светлина на флуоресцентен микроскоп на бранова должина од 450 до 490 nm. Флуоресцентната микроскопија ги нагласи единечните хифи и хифи организирани во мицелиуми. Хифите покажаа дихотомно разгранување во две речиси еднакви гранки под агол од 45 степени.

Истовремено направената габична култура од бронхијалниот материјал го потврди присуството на Aspergillus fumigatus.

Заклучок. Презентираниот метод е точен, едноставен, ефтин и, што е најважно, брз адјувантен метод кој не бара посебни бои за визуелизација на габичните хифи, се базира на автофлуоресценција која потекнува од ендогени флуорофори и може да се користи во било која патолошка лабораторија која е опремена со флуоресцентен микроскоп. Ова е особено корисно кога е достапен само ограничен број препарати со размаски за анализа и е од особено голема важност знаејќи дека раната дијагноза е критична за успешен третман.

PME6 ABDOMINAL ECHINOCOCCOSIS WITH MULTIPLE BILATERAL OVARIAN ECHINOCOCCUS GRANULOSUS CYSTS (CASE REPORT)

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Echinococcus granulosus and *Taenia solium* are cestode parasites (tapeworms) that cause cysticercosis and hydatid infections. Both diseases are caused by larvae that develop after ingestion of tapeworm eggs. Hydatid disease is caused by ingestion of eggs of echinococcal species. For *Echinococcus granulosus* the definitive hosts are dogs, and sheep are the usual intermediate hosts. Humans are accidental intermediate hosts, infected by ingestion of food contaminated with eggs shed by dogs or foxes.

Aim: We present an illustrative biopsy case of multiple bilateral ovarian echinococcus cysts.

Method: A 41-year-old female admitted at University Clinic of Digestive Surgery with a clinical diagnosis for abdominal echinococcosis. Laparotomy disclosed 23 cysts at the left ovary and one cyst at the right. Two of the left ovary cysts where very large (14,5x11x8,5 cm, weight 620 g. and 13x10x9,5 cm, weight 529 g.). Smaller cysts measured between 1 to 3.5 cm. The right ovary cyst was 3 cm.

Results: Macroscopic examination of all cysts showed hydatid cyst inside with opalescent fluid and a fibrous pericyst outside. Histological examination revealed the wall of the

cyst was made up of an inner cellular germinative layer and an outer acellular laminated layer. The connective tissue covers the outer layer, there was a inflammatory reaction that produces a zone of fibroblasts, giant cells, and mononuclear and eosinophilic cells.

Around of same cysts was thin layer of compressed ovary tissue.

Conclusion: About two thirds of human *E. granulosus* cysts are found in the liver, 5% to 15% in the lung, and the rest in bones and brain or other organs. Ovary presentation is very rare.

PME7 SEROPREVALENCE OF HUMAN HYDATIDOSIS IN THE CITY OF NIS – SOUTHEASTERN SERBIA

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Introduction: Echinococcosis/hydatid disease (HD) is a zoonotic infection caused by adult forms or larvae of a cestode from the genus Echinococcus. In numerous countries worldwide it is still a public health problem, despite echinococcosis control and surveillance programs.

Aim: Our aim was to establish the significance of immunoblot testing in the diagnosis of echinococcosis in patients with symptoms and clinical signs of cystic echinococcosis (CE) in the Nis municipality in relation to the screening methods.

Material and methods: In the period 2011-2016, the following serological screening tests were done in 1102 patients with suspected CE: Indirect Fluorescent Antibody test, IFAT; Indirect Hemagglutination test, IHA; Enzyme Linked Immunosorbent Assay, ELISA; and in 56 patients, confirmatory immunoblot (Westem Blot, WB) tests were done to detect the antigens of IgG classes for *E. granulosus/E.multilocularis.*

Statistical analysis of the obtained results was done using the method of descriptive and quantitive analysis (SPSS 16.0). The comparison of frequency of attributive features was done by x^2 testing. The compatibility of these tests with WB test was done using the Cohen *Kappa* (K) test. *Kappa* coefficient was interpreted using the Landisa and Kocha scale. The values of $p<0.05$ were considered to be statistically important.

Results: In the period 2011-2016, the total of 1102 blood samples were examined, out of which 251 were positive (22,77%), and most of the positive results were obtained in 2012 (35,90%).

The study involved 113 patients aged 51.58±18.89 years on the average (min 3,00; max 82,00), 73 (64.60%) of female and 40 (35.40%) of male gender. Positive serology using the ELISA was found in 77 out of 106 examinees 106 (72,60%), while with IHA testing positive results were obtained in 52 out of 95 examinees (47,60%), and with IFT testing in 36 out of 45 examinees (79,50%). WB was done in all individuals from the studied population. With WB, positive findings were seen in 56 patients (49,60%).

Comparing the results of immunodiagnostic tests, a substantial agreement of WB with IHA tests was found (k=0,741, p<0,001).

Conclusion: The territory of the city of Niš represents an endemic area for echinococcosis, which requires better prevention and control of the disease. Improvement of the procedures in the diagnosis of this parasitosis is necessary for as early as possible disease detection and adequate follow-up of treatment outcomes.

Key words: cystic echinococcosis, immunodiagnosis, immunoblot, seroprevalence

PME8 SEROLOGICAL DIAGNOSIS OF INFECTION WITH ECHINOCOCCUS – OUR EXPERIENCE

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INTRODUCTION: Echinococcus, known from the time of Hippocrates, which the ancient Greeks called a "water bubble", can be a major medical problem in many countries, and somewhere there is an endemic character. Zoonosis caused by larvae of the canine tapeworm, for which a human can be a random transitional host and cause severe parasitic disease.

PURPOSE: The purpose of this study is to present the presence of positive findings for Echinococcus in our laboratory with serological diagnosis, through the present antibodies in the examined sera.

MATERIAL: As a test material, a serum of patients sent to our laboratory was used, with a reason to believe in the existence of Echinococcal cyst.

METHODS: Serums are made by the method of indirect haemagglutination ELI: X: A Echinococcus (ELITE MICROBIO - France). This working method is qualitative and quantitative, is also given the antibody titer up to 1: 2560.

RESULTS: Of the total 98 examined sera, in a period of 2016/2017 year, a positive finding was confirmed in 5 sera or 5,1% .In terms of age, there are patients from 5 to 70 years old, and in sex ratio has no difference between male and female gender. In relation to the above diagnosis, Dg is dominant. B67 – Echinoconstriction (54), K73-K80 (21) - symptomatic liver disease, and P10-undefined abdominal pain (12). The remaining patients are with general Dg. ZOO (11). Dg of our patients points to the suspicion of echinococcal cysts of the liver. The Atter titre ranges from 1: 320 to 1: 2560. There are patients who have maintained high levels of antibodies for years, as is the case of a child born in 2010. Which comes the first time in our laboratory in 2015, when antibody 1:2560 antibody titre has been established, which is still ongoing. What kind of therapy, medication or surgical, remains to determine the relations in every case.

CONCLUSION: In the diagnosis of certain diseases, the existence of the disease in our environment should be taken into account in order to be previously discovered, and thus appropriately treated with medical or surgical therapy.

СЕРОЛОШКА ДИЈАГНОСТИКА НА ИНФЕКЦИЈА СО ECHINOCOCCUS - НАШИ ИСКУСТВА

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ЈЗУ ЦЈЗ Прилеп, Р.Македонија

ВОВЕД: Echinococcus познат уште од времето на Hippocrates, кој старите Грци го нарекувале "воден меур", може да биде голем медицински проблем во повеќе земји, а некаде има и ендемски карактер. Зооноза предизвикана од ларвена фаза на кучешка тенија, за која човекот може да биде случаен преоден домаќин и да предизвика тешко паразитарно заболување.

ЦЕЛ : Цел на трудот е да го презентираме присуството на позитивните наоди за Echinococcus во нашата лабораторија со серолошка дијагностика,преку присутните антитела во испитуваните серуми.

МАТЕРИЈАЛ: Како материјал за испитување користени се серуми на пациени испратени во нашата лабораторија со сомнеж за постоење на Ехинококна циста.

МЕТОДИ: Серумите се работени со метода на индиректна хемаглутинација ELI.H.A Echinococcus (ELITech MICROBIO - Франција). Методата за работа е квалитативна и квантитативна , т. е го дава и титарот на антитела кој се движи до 1: 2560.

РЕЗУЛТАТИ: Од вкупно 98 испитани серуми,во период од 2016 и 2017 год., позитивен наод е потврден кај 5 серуми или5,1% Во однос на возраста застапени се пацинти од 5 до 70 години, а и во однос на полот нема некоја разлика помеѓу машки и женски пол. Во однос на упатната дијагноза, доминира Дг. B67 – Echinococcosa, (54 пациенти), K73-K80 – симптоматологија од страна на хепар (21), како и P10- т.е недефинирана болка во стомакот(12). Останатите пациенти се со општа Дг. Z00.(11) .Дг. на нашите пациенти упатува на сомневање за ехинококна циста на хепар. Титарот на Ат се движи од 1: 320 до 1: 2560. Има пациенти кај кои со години се одржува високо ниво на антитела, како што е случај со дете родено 2010 год. кое прв пат се јавува во нашата лабораторија 2015 год., кога е утврден титар на антитела 1 :2560, кој се одржува и денес.Каква терапија, медикаментозна или хируршка останува одговор на прашање во однос на секој случај.

ЗАКЛУЧОК: Во дијагностика на одредени заболувања треба да се има во предвид постоењето на Echinococcus во нашата средина, се со цел порано да биде откриен , а со тоа и соодветно лекуван, било со медикаментозна, или хируршка терапија.

PME9 PARASITES IN THE REPUBLIC OF MACEDONIA FOR A PERIOD FROM 2011 TO 2017 YEAR

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Introdaction: Parasitic diseases are significant health problem in the world, especially in developing countries. Some types of parasites cause disease with severe clinical picture and some cause death.

Objective: The objective of the paper is to show the presence of parasitosis in R. of Macedonia.

Materials and metodhs: As materials are used the applications for microbiological isolates for the period from 2011 to 2017 year. The data are made using standard statistical methods.

Results: The largest number of reported parasite isolates are: 105 in 2017 year, 103 in 2015 year and 90 in 2014 year. Of the total number of isolated parasites for the given period, the most common parasites are Ascaris lumbricoides (32%), Giardia Lamblia (24%) and Echinococcus granulosus(17%). Most of the parasites are isolated in Skopje (Plasmodium, Toxoplasma gondii, Giardia lamblia, Echinococcus granulosus and Leishmania) and Berovo Берово (Trichinela spiralis, Trichiuris trichiura, Ascaris lumbricoides).

Conclusion: the measures for the prevention of parasitoses are: maintaining the hygiene of hands and personal hygiene, washing vegetables and fruits well, using bacteriologically and chemically proper drinking water.

ПАРАЗИТОЗИ ВО Р. МАКЕДОНИЈА ЗА ПЕРИОД ОД 2011 ДО 2017 ГОДИНА

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Вовед: заболувањата предизвикани од паразити претставуваат значаен здравствен проблем во светот, посебно во земјите во развој. Некои видови на паразити предизвикуваат заболувања со тешка клиничка слика, а некои предизвикуваат смрт.

Цел на трудот е да се прикаже застапеноста на паразитозите во Р. Македонија.

Материјал и методи: како материјал се искористени пријавите

за микробиолошки изолати за период од 2011 до 2017 година. Податоците се изработени со стандардни статистички методи.

Резултати: Најголем број на пријавени изолати за паразити се: 105 во 2017 година, 103 во 2015 година и 90 во 2014 година. Од вкупниот број на изолирани паразити за дадениот период, најзастапени паразити се Ascaris lumbricoides (32%), Giardia Lamblia (24%) и Echinococcus granulosus(17%). Најголем број од паразитите се изолирани во Скопје (Plasmodium, Toxoplasma gondii, Giardia lamblia, Echinococcus granulosus и Leishmania) и Берово (Trichinela spiralis, Trichiuris trichiura, Ascaris lumbricoides).

Заклучок: Мерките за превенција на паразитозите се: одржување на хигиената на рацете и личната хигиена, добро да се мие зеленчукот и овошјето, да се користи бактериолошки и хемиски исправна вода за пиење.

PME10 THE EFFECTS OF RECENT MIGRATION WAVES ON THE IMPORTING MALARIA IN SERBIA

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Introduction: After malaria eradication in Serbia 1964, the dominant species was Plasmodium *falciparum* and dominant mode of importing malaria has always been by Serbians workers who working in malaria endemic areas, predominantly from Africa.

Goal: An overview of the effects of recent migration waves from malaria-endemic areas on trend of importing malaria in Serbia.

Method: Data of all malaria cases diagnosed at the Parasitological Laboratory, CCS (*Reference Laboratory* for Tropical Parasitic Diseases) in Belgrade from 2001 to 2017 were analyzed. Diagnosis of malaria was based on microscopic examinations of Giemsa-stained thick and thin blood smears in combination with rapid diagnostic tests.

Results: From 2001 to 2017, 252 cases of malaria were confirmed at the Parasitological Lab, CCS; 57% were due to *P. falciparum*. Until 2015 the most commonly isolated species was *P. falciparum.* After that as a consequence of recent migration waves, *P. vivax* has taken the primacy over *P. falciparum*. Since June 2015, 117 Afghan and Pakistan refugees were tested for malaria. 43 malaria cases have been reported in arrived Afghan and Pakistan refugees, all of them *P. vivax*. Some of

them had previous malaria attacks in origin countries and/or during migration route in Bulgaria, Greece or Turkey. All patients were young men with fever. Parasitemia was generally low about 0,2%; maximum 0,9%. Five relapses were occurred due to unavailability of primaquine in Serbia. The *prevalence* of *gametocyte* carriage was 79% (34/43), over half during summer.

Conclusion: After years of the dominance of *P. falciparum* among imported malaria species in Serbia, waves of migrants from malaria-endemic areas increase of imported *P. vivax* malaria. In prevention of reintroduction of *P. vivax* into this area, that containing residual population of former *Anopheles* vectors, efficient vector control is necessary as well as the screening of migrants.

Key words: migration waves, Serbia, imported malaria, *Plasmodium vivax*

PME11 PARASITIC INFECTION IN INFANT

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Introduction: Enterobiasis as a most spread intestinal parasitosis in children is caused by Enterobius (Oxyuris) vermikularis. They are small white worms, the males are 5 mm long and the females are 10 mm. The infection spreads by ingesting infectious pinworm eggs from dirty hands, toys or other objects. From the eggs emerge larvae which after two weeks differentiate sexually, mate and where after the male pinworms die. After the 25-th day of the infection, especially during the night, because of the relaxation of the external anal sphincter, females spread form the anus in the perianal region releasing more then 10000 eggs. They become mature after 5 to 6 hours. While releasing the eggs, sticky secretion containing formic acid causes the main symptom, the itching. The disease clinically manifests with light, moderate or severe sense of itching in the anal region, most manifested in the night, lasting few weeks and then disappearing.If the reinfections occur, the itching periods reappear in intervals of 3 weeks.

Aim: Presenting case of Enterobiosis in a female infant, 6 weeks old, infected by her mother.

Method: Macroscopic finding and microscopic native example

Discussion: This case report it's about 6 weeks old infant, who was acting upset lately and often crying. It didn't eat and sleep enough. One morning, the mother in the excrement of the infant, found white worms and took him to the hospital to take an exam. Microscopic native exemple was made in the Department of Microbiology in Struga. The diagnose was Enterobiasis. The exact same exam, was recommended for all of the members of that family, including the mother of the infant, where it was detected the same diagnose. The recommended therapy was Mebendazole suspension. This kind of therapy was used repetedly for two times in 20 days, because of the possibility od recidives. Also, normal hygiene habbits, were recommended to avoid the same or similar problems in the future.

ПРИКАЗ НА СЛУЧАЈ НА ПАРАЗИТАРНА ИНФЕКЦИЈА КАЈ ДОЕНЧЕ

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Вовед: Ентеробиозата како најраспространета цревна паразитоза кај децата е предизвикана од Enterobius(Oxyuris) vermikularis. Тоа се ситни бели црви од кои машките се долги 5мм а женските 10мм. Заразувањето настанува со проголтување на јајцата од паразитот преку нечисти раце , играчки и други предмети. Од јајцата се ослободуваат ларви кои после две седмици полово се диференцираат, се оплодуваат, после што машките умираат. После 25 тиот ден од заразувањето особено преку ноќта заради релаксирање на shincter ani externus, женките излегуваат од анусот и во перианалната регија испуштаат повеќе од 10 000 јајца кои за само 5-6 часа стануваат зрели (инвазивни) . При испуштањето на јајцата се одделува леплив секрет кој содржи мравја киселина која всушност е предизвикувач на главниот симптом – сврбежот. Заболувањето клинички се манифестира со лесен , умерен или силен неподнослив сврбеж , во анална регија , најизразен ноќе кој трае неколку недели последователно а потоа исчезнува. Ако се повторуваат последователни реинфекции , периодите со сврбеж се повторуваат во интервали од околу 3 седмици .

Цел: Да се прикаже случај на Enterobiasis кај женско доенче на возраст од 6 седмици кај кое заразувањето настанало од неговата мајка.

Метод: Макроскопски наод и микроскопски нативен препарат

Дискусија: Се работи за женско доенче на возраст од 6 седмици кое во последно време било доста вознемирено, плачливо , слабо цицало, и слабо спиело . Утрото во изметот на пелената мајката забележала ситни бели црви поради што се јави на преглед. Направен е нативен микроскопски препарат во ЦЈЗ Охрид, Микробиолошката лабораторија Струга со што е потврдена дијагнозата за Enterobiasis. Препорачано е, и направен е микробиолошки преглед на мајката како и на другите членови на семејството , при што е најден позитивен наод и кај мајката . Како терапија е ординирана Suspensio Mebendazole . Истата терапија се повтори после 20 дена како противрецидивантна терапија после што е направен микробиолошки преглед кој беше негативен . Истата постапка истовремено беше применета и кај мајката, на која истовремено и беше укажано и за хигиенските постапки при доење и нега на доенчето.

Заклучок: Ентеробиозата е најчеста цревна паразитоза кај децата во целиот свет. Таа брзо се шири во детските колективи и во семејното опкружување на детето болно со ентеробиаза. Важно е, да покрај заразеното дете, паразитолошки се прегледаат и сите членови во семејството и други лица од неговата најблиска околина, и ако се открие и друг заболен, и тој истовремено да се лекува. За понатамошно спречување на ентеробиозата треба да се спроведе здравствено воспитување : да се обучат децата и нивните родители за задолжително спроведување на хигиенски мерки.

СЕСИЈА 10/SESSION 10 ВИРУСОЛОГИЈА/ VIROLOGY

V1 PREVALENCE AND MOLECULAR EPIDEMIOLOGY OF WEST NILE VIRUS INFECTIONS IN CROATIA, 2017

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INTRODUCTION: West Nile virus (WNV) is one of the most widely distributed arthropod-borne virus. In the last decades, WNV infections are detected in many European countries. In Croatia, first clinical cases of WNV neuroinvasive disease was reported in 2012 and thereafter, cases were continuously notified. In addition to human cases, serologic evidence of WNV infection was also recorded in sentinel animals (horses, poultry).

OBJECTIVES: To analyze the prevalence of WNV in humans, horses, poultry and mosquitoes in Croatia in 2017.

MATERIAL: During 2017 transmission season, a total of 90 patients with neuroinvasive infection (meningitis/encephalitis), 172 asymptomatic persons, 560 horses, 1580 poultry and 1186 mosquitoes (*Culex pipiens* and *Aedes albopictus*) were tested for the presence of WNV RNA and/or WNV antibodies. In patients with neuroinvasive infection, cerebrospinal fluid (CSF), serum and urine samples were collected. In asymptomatic subjects and sentinel animals serum samples were collected.

METHODS: WNV IgM/IgG antibodies in human, horse and poultry sera were detected using a commercial ELISA. WNV RNA was detected in human and mosquito samples using real-time and nested RT-PCR. WNV positive human serum samples were confirmed using a virus neutralization test.

RESULTS AND CONCLUSION: Neuroinvasive WNV infection was confirmed in 8 (8.9%) patients by detection of WNV IgM and IgG antibodies of low avidity and/or WNV RNA in CSF and urine. Phylogenetic analysis of four detected strains showed circulation of WNV lineage 2. Four (2.3%) asymptomatic persons were found to be IgG seropositive to WNV. In one participant, recent WNV infection was documented by low IgG avidity. WNV IgG antibodies were detected in 69 (12.3%) sentinel horses and 165 (10.4%) poultry. No one of the tested mosquito pool was found to be WNV RNA positive. Our results confirm the importance of multidisciplinary "One health" approach in the surveillance of this emerging viral zoonosis.

V2 INVESTIGATION OF ANTI DELTA ANTIBODY PREVALENCE IN HBSAG POSITIVE PATIENTS FROM TURKEY

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Introduction

Hepatitis D virus (HDV) is a defective agent, dependent on hepatitis B virus (HBV) for its life cycle. HDV infection can not occur in the absence of HBV, but occurs as an acute coinfection with HBV or as a superinfection. Around 5% of the HBV surface antigen positive (HBsAg) patients worldwide are also infected by HDV, which refers to more than 15 million co-infected people.

Objective

In this study, we aimed to determine the presence of anti delta antibodies among HBsAg positive patients admitted to Marmara University Hospital.

Material & Methods

Between June 2014 and December 2017, non-duplicate collection of 2243 samples sent routinely to the serology laboratory for the presence of HBsAg investigation was included in the study. All the samples were screened for HBsAg presence with Architect system (Abbott, USA). HBsAg positives were tested for total HDV antibody and HBcIgM titres using micro ELISA (Dia Pro, Italy) and Architect system respectively.

Results
Among 2243 samples, 1422 (63,40%) were found to be HBsAg positive. Seventy one (4.99%) of HBsAg carrying patients were also anti-HDV positive. Additionally, Anti HBcIgM was not detected in any of the 71 samples. HBsAg and HDV positivity in numbers and ratio by the years are shown in Table 1.

Table 1. HBsAg positivity, HDV antibody positivity, among years

2014		2015	2016	2017	Total
HBsAg + (n)	151	382	414	475	1422
HDV + (n)	5	15	24	27	71
Ratio (%)	3,31	3,93	5,79	5,68	**4,99**

Conclusion
In this study, the mean anti HDV antibody prevalence was detected as 4,99%, in accordance with the literature. The noticeable rise of the positivity ratio from 3,31% to 5,68% by years seems to be similar with European data. This increase can be explained by the effect of the immigration in this time period.

V3 MEASLES OUTBREAK IN KOSOVO: EPIDEMIOLOGY AND LABORATORY DIAGNOSIS

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Introduction: Measles is highly contagious viral infection that primarily affects children in developing countries. In Kosovo there were no registered cases of measles since 2001. Vaccination coverage with MMR vaccine was between 92-94 % during the last two years.

Objective: The aim of this study was to investigate the epidemiological and laboratory pattern of measles outbreak in Kosovo.

Materials: cross-sectional study was carried out in the University Clinical Centre of Kosovo during 2017 analysing clinical, epidemiological and laboratory data. Laboratory testing was carried out at the Department for Microbiology within the National Institute of Public Health of Kosovo. Laboratory confirmation was performed using Elisa test (Nova Tech, Germany). Molecular tests took place at the WHO European Regional Reference Laboratory in Luxembourg.

Results : Between March and 31 December 2017, a total of 779 cases were investigated, of which 538 (69.1%) were laboratory confirmed and 278 patients (51.7%) were male. According to the vaccination status of 538 positive cases, 95.7% were unvaccinated. Most affected age group were age 1-2 years old with 112 cases (20.8%), followed by age 0-1 year with 83 cases (15.4%).

Based on ethnicity, the majority of patients (53.3%) were Roma population, followed by Albanians (41%). The city of Fushe Kosova reported the highest number of cases (n=191, 35.6%) followed by the capital city of Prishtina (120, 22.5%). The largest number was registered on October (n=253) and November (n=196).

Twenty eight cases occurred among healthcare workers, defined as any hospital or other healthcare staff having regular contact with patients. Genotypes B3, Dublin variant was identified and sequence was MVs/PrishtinaKosovo/41/17 and they are submitted to MeaNS.

Conclusions: In conclusion, the size of the outbreak emphasize measles immunity gaps among Roma population, which together with nosocomial transmission represents the challenges for public health system in Kosovo.

Key words : *Measles , outbreak, Elisa, Kosovo*

V4 КАРАКТЕРИЗАЦИЈА НА 2017/2018 ИНФЛУЕНЦА СЕЗОНАТА ВО РЕПУБЛИКА МАКЕДОНИЈА

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Инфлуенца вирусите се инфективни агенси кои припаѓаат на Orthomyxoviridae вирусната фамилија и предизвикуваат акутно заболување на дишните патишта. Целта на оваа студија е да се претстават карактеристиките на инфлуенца сезоната 2017/2018 во Република Македонија. Анализиравме вкупно 460 хумани материјали (брисеви од нос/грло), од кои 328 беа од амбулантски и болнички лекувани пациенти од сентинел надзорот (154-ИЛИ и 174-САРИ), а 132 примероци беа рутински испратени за тестирање во нашата лабораторија. Од вкупниот број на тестирани примероци 239 (52%) беа позитивни за присуство на вирусот на инфлуенца. Оваа сезона предоминираше инфлуенца В, на која отпаднаа 69.5% од тоталниот број инфлуенца позитивни примероци, додека инфлуенца А оваа година имаше намален интензитет со позитивитет кај 30.5% од случаите. Највисок број на тестирани

примероци, како и највисока стапка на детектирани позитивни случаи беше забележан во 6-тата недела од 2018 година, каде 80% од тестираните примероци беа позитивни. Во споредба со сезоната 2016/2017, каде доминираше инфлуенца A(H3N2), достигнувајќи фрекфенција од 96.77%, во овогодинешната сезона најфрекфентна беше инфлуенца B (Yamagata) со 88.6%. Исто така оваа сезона, од вкупниот број на детектирани инфлуенца А случаи најзастапен беше субтипот A(H1N1)pdm со 80.8%. Кон крајот на сезоната детектиравме 8 случаи (4.8%) на инфлуенца B (Victoria). Оваа сезона достигна висок интезитет на почетокот на 2018 година и беше забележана прилично динамична дистрибуција и циркулација на инфлуенца вирусите.

V5 ACTIVITY OF NOVEL TREATMENT SCHEME AGAINST COXSACKIEVIRUS B1 NEUROINFECTION IN MICE

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INTRODUCTION Coxsackieviruses (CVs) are relatively common human pathogens that are associated with numerous disease symptoms in many organ systems of the body. At present no therapies are available, mainly due to the development of drug resistance. In the last few years our team has developed an experimental alternative treatment strategy based on consecutive alternating application (CAA) of inhibitors with different mode of action. This work represents the antiviral activity of double and triple combinations of anti-enteroviral compounds applied via CAA course against Coxsackievirus B1 neuroinfection in mice.

OBJECTIVES Antiviral combination effects of double and triple combinations of tested compounds were examined through CAA treatment scheme in ICR newborn mice infected s.c. with 20 MLD50.

MATERIAL AND METHODS The antiviral activity of pleconaril, guanidine HCl, MDL-860 and oxoglaucine on neurotropic CVB1 was determined in in vivo experiments. Cumulative mortality (percentage), mean survival time (MST) (days) and weight (grams) of suckling mice were registered.

RESULTS AND CONCLUSION The CAA double and triple combinations of pleconaril with MDL-860, guanidine HCl and oxoglaucine demonstrated a marked activity in vivo. In double combinations the highest effect was observed when pleconaril was combined with oxoglaucine (MST was increased 4.4 days more than placebo). The results for triple combinations

indicate markedly improved efficacy of PGO and PMO - decreased mortality rate and lengthening of the MST.

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V6 COMBINED EFFECS OF NEWLY SYNTHESIZED DIARYL ETHERS AND SOME ENTEROVIRAL INHIBITORS AGAINST COXSACKIEVIRUS B1

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INTRODUCTION Coxsackievirus B (CV) infection is common in children and young adults. The current absence of effective vaccines for most viral infection and no clinical available antiviral drugs for the treatment of CVs highlight the urgency and significance of developing antiviral agents. We investigated the effects in cell culture based on combination of inhibitors with different mode of action and some new synthesized diethyl ethers.

OBJECTIVES Double combinations by newly synthesized diethyl ethers (derivatives of MDL-860) – SHIB13403 and SHIB13602 with pleconaril, guanidine hydrochloride, and oxoglaucine were tested in vitro on HEp-2 cells for their activity against Coxsackievirus B1 strain Connecticut-5.

MATERIAL AND METHODS Antiviral combination effects due to drug–drug interaction were examined by relying on the three-dimensional model developed by Prichard and Shipman (1990) by using the program MacSynergy™ II.

RESULTS AND CONCLUSION The combinations of SHIB13403 or SHIB13602 with pleconaril and oxoglaucine were synergistic with the exception of the additive effect with guanidine hydrochloride. The highest volume of synergy is observed when tested diethyl ethers was combined with pleconaril. The resistance occurring after monotherapy with a certain anti-enteroviral drugs makes it reasonable to focus interest on combined administration of antivirals. The results in this study show that the combination application of tested compounds has an antiviral effect in *in vitro* experiments with Coxsackievirus B1.

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V7 PARVOVIRUS B19 AND ANEMIC SYNDROME IN PREGNANCY

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INTRODUCTION

Pregnancy, as well as some aggravating factors, such as individual physiology or infectious agents, can lead to pathological changes and abnormal blood counts.

Aim: The study aims to determine the role of parvovirus B19 infection in the development of anemic syndrome during pregnancy.

Materials and Methods: For period February 2016 - February 2018 in the National Reference Laboratory Measles, Mumps, Rubella, NCIPD, 55 serum samples from pregnant women on age 16 to 44 years, with diagnosis anemia were tested. The patients had visited the Gynecological Hospitals in Sofia. ELISA (detected of specific IgM/IgG antibodies) and PCR (detected of specific genomic region) methods were used. Iron homeostasis was determined by CLIA, AAS, NEPH methods. An ELISA assay was used to determine the serum hepcidin level.

Results and discussion: Acute B19V infection with presence of specific B19V IgM antibodies was demonstrated in 3/55 (5.45%) pregnant women. The combination of laboratory and patient (information for contact with children infected with fever and rash syndrome) data suggested a possible etiological role of B19V in reducing serum iron levels during pregnancy. In 28/55 (50.91%) serum samples positive B19V IgG results were detected. The presence of B19V DNA in 10/55 (18.18%) of patients were found and in 7/55 (12.73%) the confirmation of B19V infection was based only by PCR analysis. This can be explained by a fast transient B19V IgM response or persistent viral replication.

Anemia was defined as iron-deficient anemia with a low serum hepidine levels - 2.54 ± 0.4 μg/l, compared to 25.9 ± 2.8 μg/l in control group pregnant women without anemia.

Conclusion: B19V is an infection agent with a significant role in the development of anemic syndrome during the pregnancy. It has the ability to blocking blood cell cycle, which resulting in mass destruction

of erythroid progenitor cells. Inclusion as a screened viral agent is important in early diagnosis and prevention of maternal health care.

Keywords: parvovirus B19, pregnancy, anemia, hepcidin

V8 DRIED BLOOD SPOTS AS A CLINICAL MATERIAL FOR LABORATORY DIAGNOSIS OF VACCINE-PREVENTABLE DISEASES

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Background: In recent years dried blood spots as clinical materials in virology have increasingly wider application. Their advantages are: (1) compared to conventional venipuncture, requires less blood volume, which is especially important in pediatrics and neonatology; (2) the procedure for blood collection is easy, inexpensive and noninvasive; (3) the risk of bacterial contamination or hemolysis is minimal; and (4) DBS can be maintained for a long time with almost no impact on the quality of the analysis.

Aim: This study aims to demonstrate by immunoenzyme techniques viral markers of measles, mumps, rubella and hepatitis B virus in dried blood spots.

Materials and Methods: The total 82 patients with two types of clinical material (serum samples and dried blood spots) were tested. The specimens were collected according to a research project funded by the National Science Fund, Bulgaria, Contract №DM 03/1,12.12.2016. Serological (indirect ELISA) methods for the detection of specific viral markers - IgM/IgG antibodies, HbsAg, anti-HBs and anti-HBc were used.

Results: In the present study, patients were divided into 11 age groups and the median age was 38,58 years. The majority of patients were under 30 years of age from capital Sofia. Only in one patient (serum samples and DBS) was confirmed acute measles infection by ELISA. All others have negative IgM results for measles, mumps and rubella in both types of clinical materials. Measles, mumps, rubella IgG seroprevalence in DBS was 83%, 90% and 76%, respectively. In combination immunoenzymatic testing of measles, mumps, and rubella IgM/IgG markers coincidence for both types of clinical materials were found in ≥80%. All serum samples and their DBS eluates were negative for HBsAg. A positive result for anti-HBc, have been demonstrated in two of the sera, but in their corresponding DBS have not been proven. Four positive anti-HBs results were detected

in serum samples, but the presence of this antibodies class was not detected in thier DBS. No significant differences in the results in terms of gender and age were found.

Conclusion: The optimizing of DBS technique as an alternative approach (non-invasive, inexpensive, not requiring trained staff and cold chain for transport and storage) of venipuncture in virology is very important in conducting seroepidemiological studies of vaccine-preventable diseases. Serum-based technology remains a major approach to the immunoenzymatic diagnosis of viral infections.

Key words: dried blood spots, ELISA, vaccine-preventable diseasesce Laboratory Measles, Mumps, Rubella, NCIPD, 55 serum sampl

V9 RETROSPECTIVE EVALUATION OF VIRAL HEPATITIS SEROLOGY IN PATIENTS WHO APPLY TO MARMARA UNIVERSITY EDUCATION AND RESEARCH HOSPITAL

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Aim
In this study, it was aimed to determine the effectiveness of national vaccination programs by analysis of seropositivity rates by age in sera sent to Marmara University Laboratory.

Material and Methods
HBsAg, antiHBs, antiHAV-IgG, antiHAV-IgM, antiHBC-IgM, antiHBC-IgG and antiHCV markers in sera sent to the laboratory between January 2016 and January 2017 were studied by microparticulate enzyme immunoassay(EIA) method.Results were evaluated retrospectively and data were entered into SPSS 11.0 program; number and percentage values, and standard deviations of the data were evaluated by chi-square test.

Results
Serology was anti-HBs positive in 9875(41.5%) of 23770 studied; 2240(9.42%) samples were positive for HBsAg. HBV-DNA positivity was detected in 76.9% of HBsAg-positive samples. Anti-HBc-IgG was negative in 95.2% of those born in 1998 and thereafter, which was 54.8% in patients born before 1998 ($p<0,05$).

Anti-HCV positive was found in 1,6% of the 32615 cases in which hepatitis C serology was performed.

Anti-HAV-IgG positivity was found in 70,02% of the 6622 samples for which hepatitis A serology was performed. Anti-HAV-IgG negativity

was detected in 23% of the births after the vaccination program. AntiHAV-IgG positivity was found in 97.79% of patients older than 40 and 40 years ; 56% in patients under 40 years of age.

Conclusion:
National vaccination program has been successful for HepatitisA and B. In 95.2% of the samples after the vaccination program, antibodies against hepatitis B virus were developed, whereas for hepatitisA this rate was 77%, indicating that vaccination did not apply to everyone or immunity did not develop.

Keywords: hepatitis, hepatitis serology, hepatitis seroprevelance

V10 TREATING ACUTE HEPATITIS B WITH ISOPRINOSINE

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Introduction: Hepatitis B infection can be with acute flow, to pass into chronic infection with further complications expected or to stay in a virus carrier state.

Aim: Presentation of a clinical case study of an acute hepatitis B infection and the effects of the Isoprinosine usage as a therapeutic drug choice.

Material and methods: Usage of the hospital history of a patient diagnosed with an acute hepatitis B and treated in the department of Infection diseases of General hospital in Veles. Patient's history has been taken into consideration after all the diagnostic tests were undertaken (clinical, epidemiological, laboratory and serological investigations).

Results: A 40year old patient presented with an infective syndrome – fever, nausea, vomit, pain in his muscles and wrists, fatigue, dark urine, intensive icterus on skin and visible mycosis. The inoculation moment is unknown also the risk behavior. The initial laboratory analysis (ALT 1292, AST 963, Tot. Bilirubin 186, Direct 73, Alkaline Phosphatase 128) three day later, an increase has been noticed (ALT 3173, AST 2854, Tot. Bilirubin 194, Direct 174, Alkaline Phosphatase 157) and a clinical worsening of the health condition (vomit and intensifying of the icterus) because of these signs, aside the already prescribed hepatoprotective therapy, Isoprinosine was added in therapeutic dosages. The virus markers aHBcIgM positive, HBsAg positive, aHAVIgM negative, aHCV negative. A regression in the control analysis 10 days later was presented (ALT 1616, AST 496, Tot Bilirubin 122, Direct 86, Alkaline Phosphatase 94 and echocardiograpgy of the abdomen – normal result) and a clinical stabilizing of the health condition. After

one month of hospital treatment, a preventive dosages of Isoprinosine were prescribed and stabilizing of the transaminase's activity with HBsAg negativization after six months period followed.

Conclusion: Aside the hepatoprotective therapy, the multivitamin therapy and the appropriate hygiene diet plan; the includment of Isoprinosine at the beginning of the treatment and its usage has lead to decreasing the intensity of the symptoms, shorten the sickness time, avoiding complications and a fast immune response.

ISOPRINOSINE ВО ТЕРАПИЈА НА АКУТЕН Б ХЕПАТИТИС – ПРИКАЗ НА СЛУЧАЈ

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Вовед: Инфекцијата со хепатитис Б вирус може да има акутен тек, да премине во хронична инфекција со натамошни очекувани компликации или да остане како вирусоносителство.

Цел: Приказ на случај со акутна Б хепатитис инфекција и ефектот од примена на таблети Isoprinosine во лекувањето на истата.

Материјал и методи: Користена е болничка историја на пациент со акутен Б хепатитис лекуван на инфективно одделение Велес, обработена клинички, епидемиолошки, лабораториско-биохемиски и серолошки.

Резултати: Пациент на 40годишна возраст со инфективен синдром – температура, гадење, повраќање, мускулно-зглобни болки, силна малаксаност, темна мокраќа, иктензивен иктер на кожа и видливи лигавици. Нема познат момент на инокулација ниту ризично однесување. Лабораториските анализи на прием (ALT 1292, AST 963, Tot. Bilirubin 186, direkten 73, Alkalnafosfataza 128) по три дена со нагол пораст (ALT 3173, AST 2854, Tot. Bilirubin 194, direkten 174, AlkalnaFosfataza 157) и клиничко влошување на состојбата (повраќање и интензивирање на иктер) поради што, покрај започнатата хепатопротективна терапија беше воведен и Isoprinosine во тераписки дози. Вирусни маркери : aHBcIgM позитивен, HBsAg позитивен, aHAVIgM негативен , aHCV негативен. Контролните анализи после 10 дена терапија веќе беа во регресија (ALT 1616, AST 496, Tot Bilirubin 122, direkten 86, AlkalnaFosfataza 94, Exo абдомен – уреден наод) и имаше клиничко стабилизирање. По еден месец болнички третман, лекувањето продолжи со превентивни дози Isoprinosine па со нормализирање на трансаминазната активност и контролните серолошки тестови по 6 месеци покажаа HBsAg негативизација.

Заклучок: Покрај хепатопротективната, поливитаминска терапија и соодветниот хигиено-диететски режим, вклучувањето на

Isoprinosine во почетокот на лекувањето и доволно долго придонесе за намалување на интензитетот на симптомите, скратување на текот на болеста, брз имунолошки одговор и избегнување на компликациите.

V11 СКРИНИНГ НА ВИРУСОТ НА ЗАПАДНО НИЛСКА ТРЕСКА- WNV ВО КОМАРЦИ ОД СКОПСКИОТ РЕГИОН

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Вовед WNV е арбовирус од Фамилија Flaviviridae. Резервоар и домаќин на вирусот се птиците, од каде со вектори- орнитофилни комарци се пренесува на вертебратите: коњи и луѓе. Векторите се најчесто од родот на Culex комплекс. Вирусот се реплицира во саливарните жлезди на комарецот и задржува во хемолимфата. WNV е присатен во сите земји во Европа, со големи епидемии во соседна Грција по делтата на р.Вардар. Кај луѓето инфекцијата во 80% од случаевите поминува асимптоматски, кај 20% се јавува треска, а кај 1% се јавува неуроинвазивна форма која индуцира леталитет.

Целта на трудот е да ја прикаже трансмисијата на вирусот во нашата средина и да ја покаже потребата за следење на оваа зооноза.

Материјал и методи Комарците ги собиравме во текот на Септември и Октомври месец 2015. Користени беа замки со атрактант CO_2 за адултни форми на комарци. Замките беа поставени на висина од 1,5 -2 м., на пет локации во околината на Скопје: Ново село 1 , Ново село 2, Орешани 1, Орешани 2 и Драчево 1.

Детерминација на комарците по пол и вид се вршеше со детерминатор за Медитеранските видови комарци. За работа се одвојуваа женките од видовите Aedes и Culex, бидејќи претставуваат вектори кои се хранат и од луѓето.

По 25 комарци во епендорф се мацерираа, за да се разбие хитинската обвивка и ослободи вирусот од хемолимфата. Од супернатантот се изолираше РНА. Се работеше РТ ПЦР за WNV со специфични прајмери и проби. Во текот на тестрањето се користеа две контроли, хуман серум позитивен на WNV и интерна контрола на вирусот.

Заклучок Трудот има за цел да ја покаже важноста за детекција на WNV кај векторите комарци во Р.Македонија, со тоа укажувајќи на ризикот од инфекција кај луѓе и коњи, како и за потребата за имплементирање на програма за надзор на овој вирус.

V12 CEPHEID S GENEXPERT SYSTEM IN GENOTYPING OF HUMAN PAPILLOMAVIRUSES

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Cervical cancer is preventable disease,but is among the leading causes of death ,most of the death cases occur in undeveloped countries. Cervical cancer can be caused by persistent infection with human papillomavirus (HPV) .During 2012 ,528.000 new cases recorded - 266.000 deaths.

Aim: To establish presence of HPV among the women with positive PAP test , to find the types of HPV among them.

Materials and methods: 93 women with positive PAP test. Swabs where taken, inoculated in buffer (PBS) , transported to laboratory. Cepheid s GeneXpert is polymerase chain reaction in real time, procedure takes 58 minutes. Software reports presence of single HPV 16,then a paired HPV types 18_45.Other HPV types are grouped in P3, P4 and P5. HPV types31,33,35,52 and 58 form P 3 group, then 51 and 59 - P 4, and 39,56,66 and 68 form P 5 . From 92 cervical swabs 75 where negative. Sixteen swabs where positive , and type 16 was found in 3 , type 18_45 was found in 3 , group P 3 in 7 , group P 4 in 3 materials and no materials positive for P 5 . One was positive for both type 16 and P 3.One material did not passed the system control.

Conclusions: Persistent HPV infection is a risk factor for pre cancer and cancer. Cytological screening is an excellent method to establish the early cervical lesions ,but it should be combined with HPV screening. GeneXpert system is reliable to work with, fast and simple to handle. Procedure takes only 1 hour, and all of the important HPV types are included.

Key words: Cervical cancer, HPV infection, genotyping

ТИПИЗАЦИЈА НА ХПВ СО GENEXPERT, НАШИ ИСКУСТВА

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Вовед: Ракот на грлото на матката (РГМ) е болест која може да се превенира,но сепак е меѓу водечките причини за смрт, посебно во земјите со низок стандард.Скоро сите случаи се причинети од онкогени типови на ХПВ вируси. Во 2012 година се забележани 528.000 нови случаи со 266.000 смртни случаи.

Цел: Да се испита присуството на ХПВ вирусите кај жени со промени после ПАП тест.Во исто време да се одреди кој типови од ХПВ вирусите се присутни кај нашата популација.

Материјали и методи: Испитани се 93 жени со позитивен цитолошки наод. Гинеколозите ги земаа брисевите,ги инокулираа во фосфатен пуфер(PBS) и беа донесени во микробиолошката лабораторија. Материјалите беа анализирани со GeneXpert системот на Cepheid. Полимераза верижна реакција во реално време, амплификацијата на генетскиот материјал, трае 58 минути. Системот дава резултат за присуство на тип 16, типовите 18_45, другите ризични типови на ХПВ се во 3 групи P 3, P 4 и P 5. Во P 3 спаѓаат 31, 33, 35, 52 и 58, во P 4 се 51 и 59, а во P 5 се 39, 56, 66 и 68. Од вкупно 92 испитани материјали 75 беа негативни. Позитивни беа вкупно шеснаесет од кои типот 16 кај 3 пациентки, типот 18_45 кај 3 пациентки, типовите од група P 3 беа кај 7 пациентки, групата P 4 е докажана кај 3 пациентки, а немаше позитивен наод за групата P 5. Еден материјал е позитивен за типот 16 и за P 3. Еден материјал не ја мина контролата на системот.

Заклучок: ХПВ инфекција е ризик фактор за пре-канцер и канцер. Цитолошкото испитување е одличен метод за дијагностика на почетните промени, но треба да се комбинира со докажување на присуство на ХПВ вирусите кај пациентите. GeneXpert е едноставен за работа, резултати за еден час, а со системот се докажуваат скоро сите битни типови на ХПВ.

Клучни зборови: РГМ, ХПВ инфекција, генотипизација

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