UMS '23
SERIES

SERBIAN SOCIETY FOR MICROBIOLOGY

30 MARCH - **01** APRIL / MONA PLAZA Belgrade

EMERGING INFECTIOUS DISEASES:
Are we ready for new evolutionary challenges?

CONGRESS
PROGRAM
AND
E-ABSTRACT
BOOK

UMS SERIES

30 MARCH
01 APRIL

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Esteemed colleagues, dear friends,

It is a great pleasure to greet you on the Congress

"EMERGING INFECTIOUS DISEASES: Are we ready for new evolutionary challenges?" of the Serbian Society of Microbiology UMS 2023 SERIES.

from 30th March to 1st April 2023 at the MONA PLAZA hotel in Belgrade.

After the great success of the FEMS Microbiology Conference Belgrade 2022, the Serbian Society for Microbiology (UMS) is announcing the start of the UMS Series 2023 – a succession of congresses and symposia regarding current global topics and challenges in the field of microbiology for this year.

The COV1D-19 pandemic has shed new light on the importance of advanced DNA technologies and the knowledge of pathogen evolution in the fight against emerging infectious diseases. Gaining the upper hand in this fight is achievable only with the continuous cooperation of microbiologists, clinicians, epidemiologists, biologists, bioinformaticians, representatives of public health and state policy makers.

These new global diagnostic and therapeutic challenges, extremely important in the third decade of the 21st century, will be addressed at the Congress from different sides. This will present an opportunity for microbiologists, epidemiologists and clinicians to recognize the importance of multidisciplinary approach to the prevention of epidemics, their timely detection and the prediction of potential epidemic events.

The Congress's main program sessions will present current topics at the global, regional and national level, while panel discussions will offer listeners the opportunity to exchange knowledge and experience with leading national and foreign experts in the fields of microbiology, molecular biology, infectology, epidemiology, pulmology, gastroenterology and pediatrics.

We believe that this Congress will present an important next step in strengthening and expanding cooperation at the national and regional levels, thus improving the surveillance of emerging pathogens in Serbia through the implementation of new analytical strategies.

Over sixty international, regional and national experts are with us and will share their experience and expertise with all of us.. The Congress program has been accredited by the Health Council of Serbia as an international congress with the maximum number of CME points, yet another proof of its quality and appropriateness.

We are grateful to all of you who showed large interest and joined the Congress in such large numbers. We believe that attractive topics, high-quality program, dynamic presentation formats and excellent lecturers will prove your choice right. We are certain that you share our belief in the new opportunities ahead of us, aiming towards enhanced professional exchange & development in the SEE region and beyond.

Indeed, we are glad to host you in a pleasant atmosphere of Belgrade beginning April, to benefit from the attractive and dynamic program, exchange knowledge, and, equally important, to refresh existing and establish new contacts with colleagues and friends, while enjoying our hospitality and cherish the moment in one of the best partying cities of Europe.

You are most welcome!!!

Assistant Professor
Irena Aranđelović
President of the
Organization Committee

Professor Lazar Ranin
President of the Serbian
Society for Microbiology

Professor
Aleksandra Knežević
President
of the Scientific Committee

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Dr. **Tijana Štajner** — *Serbia*

UMS
SERIES

23

30 MARCH

01 APRIL

SERBIAN SOCIETY FOR MICROBIOLOGY

CONGRESS
PROGRAM

DAN 1 ČETVRTAK 30. Mart 2023 / THURSDAY 30th March 2023 DAY 1

08:00-08:45 REGISTRACIJA UČESNIKA / PARTICIPANT REGISTRATION

08:45-09:00 **POZDRAVNA REČ ORGANIZATORA I OTVARANJE KONGRESA**

OPENING CEREMONY

9:00 - 10:00

SESIJA / SESSION

- **Savremeni pristup u otkrivanju, praćenju i prevenciji bolesti izazvanih pretećim patogenima**
- **Cutting-edge approaches to detection, monitoring & prevention of emerging infectious diseases**

Moderators: **Maja Stanojević, Aleksandra Knežević**

Maja Stanojević / *Serbia*

- Značaj primene molekularno-bioinformatičkih analiza u otkrivanju, praćenju i predviđanju bolesti izazvanih pretećim patogenima
- The importance of the application of molecular-bioinformatics analyses in the detection, monitoring and prediction of emerging infectious diseases

Aleksandra Knežević / *Serbia*

- Kako odabrati DNK tehnologiju: direktno sekvenciranje vs. sekvenciranje nove generacije
- How to choose DNA technology: direct sequencing vs. next generation sequencing

Marina Šiljić / *Serbia*

- Upoznavanje evolucije patogena kroz filogenetske i filodinamičke analize
- Understanding the evolution of pathogens through phylogenetic and phylodynamic analyses

Valentina Ćirković / *Serbia*

- Putevi širenja patogena: filogeografske analize
- Routes of pathogens spread: phytogeographic analyses

10:00-10:10 **LABENA** / KOMPANIJSKO PREDAVANJE

Sensitivity and resistance of Gram negative pathogens, the view from green and red corner. / **Gorkem Yaman**

Labena

10:10-10:25 **Pauza za kafu / Coffee break**

10:25 - 12:20

SESIJA / SESSION

- **Preteće mikobakterijske infekcije**
- **Emerging mycobacterial infections**

Moderators: **Lidija Ristić, Irena Aranđelović**

Paolo Miotto / *Italy*

- Genomika u dijagnostici antimikrobne rezistencije u tuberkulozi
- Genomics in the diagnosis of drug resistance in TB

Matthias Merker / *Germany*

- Transkontinentalno širenje i evolucija multirezistentnih sojeva *Mycobacterium tuberculosis* – implikacije za kliničku dijagnostiku i javno zdravlje
- Transcontinental spread and evolution of multidrug resistant *Mycobacterium tuberculosis strains* – implications for clinical diagnostics and public health

Lidija Ristić / *Serbia*

- Režimi lečenja MDR TB - izazovi sadašnjosti, nada budućnosti
- Regimens to treat MDR TB – today's challenge and hope for the future

Maja Stošić / *Serbia*

- Implementacija nacionalnog TB programa – izazovi u post-COVID eri
- National TB program implementation – challenges in post COVID era

Irena Aranđelović / *Serbia*

- 15 godina molekularne dijagnostike TB i mikobakterioza u Srbiji
- 15 years of molecular diagnostics of TB and mycobacterioses in Serbia

Ljiljana Žmak / *Croatia*

- Mikobakterioze: zanemareni javno-zdravstveni problem
- Mycobacterioses: a neglected public health problem

Ivana Dakić / *Serbia*

- Netuberkulozne mikobakterije kao produktori biofilma
- Nontuberculous mycobacteria as biofilm producers

SESIJA / SESSION

- **Virusne zoonoze**
- **Viral zoonoses**

Moderators: **Goran Stevanović, Marko Janković**

12:20 - 13:55

Marko Janković / *Serbia*

- Virusne zoonoze – poznati patogeni i nove pretnje
- Viral zoonoses – known pathogens and new threats

Anna Papa-Konidari / *Greece*

- Primena sekvenciranja nove generacije u evolutivnim analizama virusa Zapadnog Nila
- Next generation sequencing for the evolutionary studies of West Nile virus

Valentina Ćirković / *Serbia*

- Endemska populaciona dinamika virusa Zapadnog Nila u komarcima sa teritorije Srbije
- Endemic population dynamics of West Nile virus in mosquitoes from theterritory of Serbia

Jelena Protić / *Serbia*

- Hantavirusi na Balkanu: da li nas čekaju nove epidemije?
- Hantaviruses in the Balkans: can we expect new outbreaks?

Goran Stevanović / *Serbia*

- Epidemija majmunskih boginja: nova lica poznatog patogena
- The monkeypox outbreak: new faces of a known pathogen

Jasmina Poluga / *Serbia*

- Neuroinfektivni agensi: da li se nešto promenilo?
- Neuroinfectious agents: has anything changed?

14:00-15:00 **Ručak / Lunch**

SESIJA / SESSION

- ***Helicobacter pylori infekcija* i infekcije koje se prenose hranom**
- ***Helicobacter pylori infection* and foodborne infections**

Moderators: **Marina Milenković, Dušan Kekić**

15:00 - 16:30

Aleksandra Sokić-Milutinović / *Serbia*

- *Helicobacter pylori:* klinički značaj
- *Helicobacter pylori:* clinical importance

Tomica Milosavljević / *Serbia*

- Smernice za lečenje *Helicobacter pylori* infekcije
- Treatment guidelines for *Helicobacter pylori* infection

Vladimir Milivojević / *Serbia*

- Antimikrobna rezistencija *Helicobacter pylori*
- Antimicrobial resistance of *Helicobacter pylori*

Dušan Kekić / *Serbia*

- *Helicobacter pylori* infekcija u Srbiji: molekularna dijagnostika u rutinskoj praksi
- *Helicobacter pylori* infection in Serbia: molecular diagnostics in routine practice

Marina Milenković / *Serbia*

- Inovativne dijagnostičke metode za *Helicobacter pylori* infekciju
- Innovative diagnostic methods for *Helicobacter pylori* infection

Igor Kljujev / *Serbia*

- Patogeni koji se prenose hranom - načini širenja i njihova detekcija primenom molekularnih metoda
- Foodborne pathogens - ways of spreading and their detection by molecular methods

16:30-16:45 **Pauza za kafu / Coffee break**

16:45-17:45

Panel diskusija Panel discussion

- **Implementacija molekularno-bioinformatičkog pristupa u rutinski rad mikrobiološke laboratorije**
- **Implementation of the molecular-bioinformatics approach to routine microbiological diagnostics**

Učesnici / Participants: **Paolo Miotto, Matthias Merker, Ljiljana Žmak, Anna Papa-Konidari, Maja Stanojević, Aleksandra Knežević**

KRATKE ORALNE PREZENTACIJE SHORT ORAL PRESENTATIONS

Moderators: **Ivana Dakić, Marko Janković**

18:00 - 19:00

Margo Diricks / *Germany*

- Genomic surveillance of the emerging pathogen *Mycobacterium abscessus*

Ankica Vujović / *Serbia*

- Changing profile of intrahospital infections during the COVID-19 pandemic in Intensive Care Units - are we in danger of entering the pre-antibiotic era

Milica Šarčević / *B&H*

- Antimicrobial sensitivity and intrahospital transmission dynamics of *Klebsiella spp.* isolates during a one-year period

Anna Lyapina / *Russia*

- Immune response induced by the inactivated *C. psittaci* strain in mouse model

Artem Rozhin / *Russia*

- Synergistic effect of inhibiting bacterial growth when using an antibiotic and silver nanoparticles

Elena Krstevska-Kelepurovska / *North Macedonia*

- Change of antimicrobial resistance of *Streptococcus* agalactiae to erythromycin and clindamycin over a 10 years southwestern region of Republic of North Macedonia

Emel Mataraci Kala / *Turkey*

- *In vitro* activities of eugenol+ciprofloxacin combination against carbapenem-resistant Enterobacterales (CRE) strains Isolated from urinary tract infections

Mayram Hacioglu / *Turkey*

- Effects of CSA-44 and CSA-192 in combination with linezolid against *Enterococcus spp.*

Sanja Matić / *Serbia*

- The protective effect of IFNL3 and IFNL4 gene polymorphisms on development of COVID-19 pneumonia in female patients

Teodora Kukrić / *Serbia*

- *IN VITRO* ANTIFUNGAL ACTIVITY OF HYDROPHILIC CHOLINE CHLORIDE-BASED NATURAL DEEP EUTECTIC SOLVENTS

Damla Damar Celik / *Turkey*

- In vitro activities of ceftazidime/avibactam alone or in combination with antibiotics against multidrug-resistant *Stenotrophomonas maltophilia* isolates

Maria Pavlova / *Bulgaria*

- Epidemiological and clinical-laboratory characteristics of the covid 19 infection in Bulgaria during the spring-summer season of the 2022

DAN 2 PETAK 31. Mart 2023 / FRIDAY 31th March 2023 DAY 2

9:00 - 10:35

SESIJA / SESSION

- **Invazivne gljivične infekcije**
- **Invasive fungal infections**

Moderators: **Ivana Čolović-Čalovski, Aleksandra Barać**

Paul Verweij / *Netherlands*

- Kako se suočiti sa rezistencijom na antimikotike kao pretnjom za javno zdravlje?
- Tackling the emerging threat of antifungal resistance to human health

Aleksandra Barać / *Serbia*

- Multidisciplinarni pristup u prevenciji, dijagnostici i lečenju invazivnih gljivičnih infekcija
- A multidisciplinary approach in the prevention, diagnosis and treatment of invasive fungal infections

Sanja Mitrović / *Serbia*

- Aktuelni pristup u ispitivanju osetljivosti gljiva na antimikotike: referentne metode i komercijalne tehnike
- Current approach in antifungal susceptibility testing: reference methods and commercial techniques

Ivana Čolović-Čalovski / *Serbia*

- Multirezistentne preteće kvasnice kao dijagnostički izazov: *Candida auris*
- Multidrug-resistant yeasts as a diagnostic challenge: *Candida auris*

Borko Gobeljić / *Serbia*

- Invazivne gljivične infekcije kod dece sa transplantiranim matičnim ćelijama hematopoeze: klinička slika i terapijske mogućnosti
- Invasive fungal infections in children undergoing hematopoietic stem cell transplantation: clinical presentation and therapeutic options

Stefan Mijatović / *Serbia*

- Značaj primene multipleks PCR metode u dijagnostici invazivne kandidijaze
- The importance of applying the multiplex PCR method in the diagnosis of invasive candidiasis

10:35-10:50 **Pauza za kafu / Coffee break**

SESIJA / SESSION
- **Bolničke infekcije**
- **Healthcare-associated infections**

Moderators: **Vesna Mioljević, Ina Gajić**

10:50 - 12:30

Arjana Tambić-Andrašević / Croatia
- Kako se nositi sa problemom rezistencije na antibiotike u 21. veku?
- How to overcome the problem of antimicrobial resistance in the 21st century?

Snežana Jovanović / *Serbia*
- Da li smo spremni za izazove antimikrobne rezistencije?
- Are we ready for the challenges of antimicrobial resistance?

Vesna Mioljević / *Serbia*
- Značaj multidisciplinarnog pristupa u borbi protiv antimikrobne rezistencije
- The importance of a multidisciplinary approach in the fight against antimicrobial resistance

Ana Kaftandžieva / *North Macedonia*
- Karbapenem rezistentni sojevi *Klebsiella pneumoniae* – petogodišnja studija
- Carbapenem resistant *Klebsiella pneumoniae* – a five-year study

Ina Gajić / *Serbia*
- Molekularna epidemiologija infekcija izazvanih multirezistentnim sojevima *Pseudomonas aeruginosa* u Srbiji
- Molecular epidemiology of infections caused by multiresistant strains of *Pseudomonas aeruginosa* in Serbia

Zoran Todorović / *Serbia*
- Upravljanje antibioticima u bolnici u eri rastuće rezistencije: između kolektivne odgovornosti i istinske saradnje
- Hospital antibiotic stewardship in the era of increasing resistance: between collective responsibility and true collaboration

12:30-12:45

PFIZER / KOMPANIJSKO PREDAVANJE
Gljivične infekcije, koliko su retke i kako ih dijagnostifikujemo i lečimo?
/ **Eleonora Dubljanin**, *Serbia*

12:45-13:05

BIOMERIEUX / KOMPANIJSKO PREDAVANJE
Diagnostic stewardship: peer reviewed evidence to support the selection of the appropriate test for each patient / **Barrios Abraham Florencia**

13:05 - 13:50

SESIJA / SESSION

- **Streptokokne infekcije**
- **Streptococcal infections**

Moderator: **Nataša Opavski**

Nevena Jovičić / *Serbia*

- Izazovi u dijagnostici pneumokoknih pneumonija kod dece
- Challenges in the diagnosis of pneumococcal paediatric pneumonia

Uroš Karić / *Serbia*

- Različite kliničke prezentacije pneumokoknih meningitisa
- Different clinical presentations of pneumococcal meningitis

Nataša Opavski / *Serbia*

- Izmene u distribuciji invazivnih serotipova pneumokoka u Srbiji
- Changes in distribution of invasive pneumococcal disease serotypes in Serbia

13:50-14:05

PFIZER / INDUSTRY SESSION

Distribucija serotipova koji izazivaju pneumokoknu invazivnu bolest kod dece i značaj obuhvata serotipova konjugovanom vakcinom / **Nataša Opavski**, *Serbia*

Pfizer

14:00-15:00 Ručak / Lunch

15:00 - 17:00

SATELITSKI SIMPOZIJUM / SATELLITE SYMPOSIUM

- “Zajedno u borbi protiv antimikrobne rezistencije”
- “Fighting antimicrobial resistance: united we stand”

Moderator: **Irena Arandjelović**

Ana Kaftandžieva / *Medicinski fakultet, Univerzitet u Skoplju*

Snežana Jovanović / *Služba za mikrobiologiju, UKCS*

Janko Samardžić / *Institut za farmakologiju, kliničku farmakologiju i toksikologiju, MFUB*

Ana Stojanović / *KBC “Bežanijska kosa”*

Dragana Melentijević / *Dom zdravlja “Dr Simo Milošević” Čukarica*

Predrag Veljković / *Specijalna bolnica “Dr Žutić”*

Vesna Kovačević Jovanović / *Zdravstveni sistem Medigroup*

17:00-17:15 Pauza za kafu / Coffee break

17:15-18:15

Panel diskusija Panel discussion

- **Značaj saradnje mikrobiologa i kliničara za uvođenje molekularno-bioinformatičkog pristupa u programe nadzora nad pretećim patogenima**
- **The importance of cooperation between microbiologist and clinician for the introduction of the molecular bioinformatics approach in surveillance programs for emerging pathogens**

Učesnici / Participants: **Paul Verweij, Arjana Tambić-Andrašević, Zoran Todorović, Aleksandra Barać, Borko Gobeljić, Nataša Opavski**

KRATKE POSTER PREZENTACIJE SHORT POSTER PRESENTATIONS

Moderators: **prof. dr Ivana Čolović-Čalovski, asist. dr Stefan Mijatović**

18:15 - 19:15

Ljiljana Božić / *B&H*

- Immune response induced by different types of vaccines and SARS-CoV-2 infection in healthcare workers of the University Clinical Center, the Republic of Srpska

Ivan Pavlović / *Serbia*

- The role of dogs in the contamination of public surfaces in Belgrade with *Giardia intestinalis*

Mario Sviben / *Croatia*

- *Cryptosporidium spp.* and *Giardia spp.* in raw water used for water supply in Croatia

Radoslava Savić-Radovanović / *Serbia*

- The risk of *Listeria monocytogenes* occurrence in sushi

Elena Krstevska / *North Macedonia*

- Detection of respiratory viruses by Real-time polymerase chain reaction (PCR) in patients with acute respiratory infections in southwestern region of Republic of North Macedonia

Elvira Rozhina / *Russia*

- Biogenic silver nanoparticles: cytotoxic effect

KRATKE POSTER PREZENTACIJE SHORT POSTER PRESENTATIONS

Moderators: **prof. dr Ivana Čolović-Čalovski, asist. dr Stefan Mijatović**

18:15

-

19:15

Jasmina Nikolovska / *North Macedonia*

- Most common bacterial isolates from wound specimens

Sanja Jakovac / *B&H*

- *Klebsiella pneumoniae* resistant to carbapenems in the University Clinical Hospital Mostar

Vuk Marušić / *Serbia*

- Microbiological profile of surgical site infections after hip and knee arthroplasty in a tertiary surgical hospital

Nevena Milosavljević-Labus / *Serbia*

- Fluoroquinolone resistance rates of *Escherichia coli* isolates from outpatients urine samples in Medigroup system

Jasna Kureljušić / *Serbia*

- Listeriosis – zoonotic aspect

Amir Mešić / *B&H*

- *Helicobacter pylori* infection

Martina Jug / *Serbia*

- Monkeypox presenting as phimosis: a case report

Nataša Krtolina Banjac / *B&H*

- Effects of the COVID-19 pandemic on outpatient and in-hospital use of antibiotics and antimicrobial resistance rate

Dragica Đurđević-Milošević / *Serbia*

- Opportunistic Pathogen *Pseudomonas aeroginosa* – Determination of limit of Detection of PH. EUR.10th Method

Gordana Petrović / *Serbia*

- A Case Report of a Pediatric Patient with Atypical Form of Chickenpox

Gordana Petrović / *Serbia*

- Systemic form of cat scratch disease - differential diagnosis of prolonged fever in immunocompetent children

DAN 3 SUBOTA 01. april 2023 / SATURDAY 01st April 2023 DAY 3

9:30 - 10:50

SESIJA / SESSION
• Preteće parazitoze i vektorske bolesti
• Emerging parasitic infections and vector-borne diseases
Moderators: **Tijana Štajner, Snežana Tomanović**

Barbara Šoba Šparl / *Slovenia*
• Kolaborativni pristup u detekciji autohtone *Dirofilaria repens* u Sloveniji
• The collaborative approach to detection of autochthonous *Dirofilaria repens* in Slovenia

Tijana Štajner / *Serbia*
• Rizik od infekcije parazitom *Toxoplasma gondii* nakon transplantacije: rezultati prospektivne kohortne studije Nacionalne referentne laboratorije
• Transplantation-related risk of *Toxoplasma gondii* infection: the National Reference Laboratory prospective cohort study results

Zorica Dakić / *Serbia*
• Importovana malarija u Srbiji: nekad i sad
• Imported malaria in Serbia: then and now

Snežana Tomanović / *Serbia*
• *Borrelia miyamotoi, Neoerlichia micurensis i Babesia microti* - retki zoonotski patogeni prenošeni krpeljima u Srbiji
• *Borrelia miyamotoi, Neoerlichia micurensis and Babesia microti* - emerging zoonotic tick-borne pathogens in Serbia

Gorana Veinović / *Serbia*
• *Borrelia lusitaniae i Borrelia valaisiana* – potencijal za uzrokovanje lajmske borelioze u Srbiji
• *Borrelia lusitaniae and Borrelia valaisiana* – potential for causing Lyme borreliosis in Serbia

10:50-11:05 **Pauza za kafu / Coffee break**

11:05 - 12:20

SESIJA / Vakcine SESSION / Vaccines
Moderators: **Dragana Vuković, Eleonora Dubljanin**

Miloš Marković / *Serbia*
• RNK vakcine - novo oružje za kontrolu pretećih infektivnih bolesti
• RNA vaccines - a novel weapon to control emerging infectious diseases

Olivera Lijeskić / *Serbia*
• Antitela specifična za SARS-CoV-2 nakon iRNK vakcine kao treće doze: homologi i heterologi pristup revakcinaciji
• SARS-CoV-2 specific antibody response after an mRNA vaccine as the third dose: homologous versus heterologous boost

11:05 - 12:20

Eleonora Dubljanin / *Serbia*
- Da li je vakcina ključni faktor u borbi protiv malarije?
- Is vaccine a game changer in the fight against malaria?

Dragana Vuković / *Serbia*
- Vakcine protiv tuberkuloze: zašto množina?
- Vaccines against TB: why plural?

Ljiljana Pavlović / *Serbia*
- Da li je difterija zaboravljena bolest?
- Diphtheria: forgotten disease

SESIJA / Antimikrobna terapija
SESSION / Antimicrobial therapy

Moderators: **Aleksandra Šmitran, Jelena Srbljanović**

12:20 - 13:35

Alexandra Nascutiu / *Romania*
- Antimikrobna rezistencija i etička pitanja
- Approach to the ethical issues raised by antimicrobial resistance

Nijaz Tihić / *B&H*
- Nadzor nad antimikrobnom rezistencijom u Bosni i Hercegovini
- Surveillance of antimicrobial resistance in Bosnia and Herzegovina

Jelena Srbljanović / *Serbia*
- Eksperimentalna terapija malarije – novi vidici
- Experimental treatment of malaria – newperspectives

Aleksandra Šmitran / *RS, B&H*
- Potencijal nanočestica i nanokompozita u terapiji infekcija izazvanih multirezistentnim bakterijama
- The potential of nanoparticles and nanocomposites in the therapy of infections caused by multidrug-resistant bacteria

13:35-13:50

PFIZER / KOMPANIJSKO PREDAVANJE
Antimikrobna rezistencija Gram negativnih bakterija, tiha pandemija
Ili oluja u najavi? / **Snežana Jovanović**, *Serbia*

Pfizer

14:00-15:00

Panel diskusija Panel discussion
Terapijske mogućnosti i prevencija pretećih infektivnih bolesti
Treatment options and prevention of emerging infectious diseases
Učesnici / Participants: Alexandra Nascutiu, Miloš Marković, Nijaz Tihić, Tijana Štajner

15.00-16.00

KOKTEL I CEREMONIJA ZATVARANJA
FAREWELL RECEPTION AND CLOSING CEREMONY

UMS SERIES 23

SERBIAN SOCIETY FOR MICROBIOLOGY

30 MARCH

01 APRIL

CONGRESS ABSTRACTS

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/ SAVREMENI PRISTUP U OTKRIVANJU, PRAĆENJU I PREVENCIJI BOLESTI IZAZVANIH PRETEĆIM PATOGENIMA

/ CUTTING-EDGE APPROACHES TO DETECTION, MONITORING& PREVENTION OF EMERGING INFECTIOUS DISEASES

Primena filogenetske analize u praćenju i predviđanju pojave epidemija

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Filogenetska analiza je disciplina koja se bavi istraživanjem i rekonstrukcijom evolucione istorije ispitivane populacije/skupa jedinki – taksona, i danas se prevashodno zasniva na analizi DNK i aminokiselinskih sekvenci. Grafički prikaz evolucionih odnosa između taksona (organizama/gena) predstavlja filogenetsko stablo.

Preduslov za filogenetsku analizu je poznavanje sekvence gena/genoma ispitivanih organizama, te je prvi korak izvođenje i tumačenje reakcije sekvenciranja. Rekonstrukcija evolutivne istorije, vremenske i prostorne difuzije patogena može da omogući praćenje transmisione dinamike patogena i epidemisjkog trenda. Premda su osnove i metode filogenetske analize veoma napredovale tokom poslednjih par decenija, sa kovid-19 pandemijom, uzrokovanom novim koronavirusom, SARS-CoV-2, napredne tehnike DNK sekvenciranja i prateća bioinformatička infrastruktura i praksa, po prvi put su u „relanom vremenu“ korišćene za praćenje tekuće epidemije, otvarajući put novim principima molekularnog nadzora i praćenja epidemija

Phylogenetic analysis in epidemic monitoring and surveillance

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Phylogeny is a discipline that explores and reconstructs evolutionary history of the studied population/group (taxa). Nowadays, the analytical approach is mostly based on the study of DNA and amino-acid sequences. Phylogenetic tree represents graphical representation of evolutionary relationships. Availability of sequence data is a precondition for phylogenetic analysis, hence, generating and interpreting sequence data is the first step.

Reconstruction of pathogens' evolutionary history and diffusion in time and space, may provide insight into transmission dynamics and epidemic trend. Methods and application of phylogenetic analysis have largely improved in the last decades, however, it is with the pandemic of covid-19, caused by the novel SARS-CoV-2 virus, that advanced DNA sequencing techniques and relevant bioinformatic infrastructure and practice have for the first time been used in „real time" for epidemic monitoring, paving the way for novel approach to genomic surveillance.

How to choose DNA technology: direct sequencing vs. next generation sequencing

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DNA sequencing is a process which includes the use of different biochemical methods for determining precise order of the nucleotides in a DNA macromolecule. Over the last five decades, researchers have been developing methods and technologies of DNA sequencing that have made considerable progress and have had a great impact in numerous research fields, such as genome research, clinical disease research and drug development.

Today, two main types or three generations of sequencing technologies are in use: Sanger direct sequencing (the first generation) and next generation sequencing – NGS (the second and third generation). In planning and pursuance of research, the selection of DNA sequencing technology is challenging and sometimes very difficult. Criteria for choosing a sequencing method are many and depends on the research questions (mutational analysis, detection and characterization of pathogens, identification of epigenetic modifications, metagenomics, transcriptomics, etc.). The most common criteria include read length, accuracy requirements, throughput, sequencing time, number of samples and cost, but all of which tend to influence each other. It has been suggested that direct sequencing would be more appropriate for the analysis of smaller DNA target (up to 1000bp), a small set of genes (one or two genes) and small sample size (less than 1000 samples). In these types of analysis, NGS is more expensive and with complex workflow than Sanger sequencing. NGS is a good choice for whole genome sequencing, whole exome sequencing, target sequencing of large panels of genes, detecting rare variants, and discovery of novel variants. In addition, the use of NGS in epigenome and metagenome sequencing is dramatically increasing. The third generation of sequencing platforms enables direct RNA sequencing and the analysis of transcriptome. In some research, the use of two sequencing methods can be considered. For example, Sanger sequencing can be used as a confirmation method to verify variants detected by NGS. Therefore, a consideration of possibilities and limitations of different sequencing platforms plays important role in a decision point for the selection of appropriate sequencing method in research.

Kako odabrati DNK tehnologiju: direktno sekvenciranje vs. sekvenciranje nove generacije

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Proces DNK sekvenciranja podrazumeva upotrebu različitih biohemijskih metoda za određivanje preciznog redosleda nukleotida u DNK makromolekulu. Tokom poslednjih pet decenija, istraživači su razvijali metode i tehnologije DNK sekvenciranja koje su dovele do značajnog napredka što je imalo veliki uticaj na brojne istraživačke oblasti, kao što su istraživanje genoma, klinička istraživanja bolesti i razvoj lekova. Danas su u upotrebi dva glavna tipa ili tri generacije tehnologija sekvenciranja: Sanger direktno sekvenciranje (prva generacija) i sekvenciranje nove generacije – NGS (druga i treća generacija).

U planiranju i sprovođenju istraživanja, izbor tehnologije DNK sekvenciranja je izazovan i ponekad veoma težak. Kriterijumi za izbor metode sekvenciranja su brojni i zavise od istraživačkih pitanja (mutaciona analiza, detekcija i karakterizacija patogena, identifikacija epigenetskih modifikacija, metagenomika, transkriptomika, itd.). Najčešći kriterijumi uključuju dužinu čitanja odnosno veličinu ciljnog DNK fragmenta, nivo preciznosti, obrt, dužina trajanja procesa sekvenciranja, broj uzoraka koji se obrađuje i cenu, pri čemu svi navedeni kriterijumi imaju tendenciju da utiču jedni na druge. Smatra se da je Sanger sekvenciranje prikladnije za analizu manjih DNK ciljnih fragmenata (do 1000 bp), malog broja gena (jedan ili dva gena) i manjeg broja uzoraka (manje od 1000 uzoraka). U ovim vrstama analiza, NGS je skuplji i sa složenijim tokom rada od direktnog sekvenciranja. NGS je dobar izbor za sekvenciranje celog genoma, sekvenciranje celog egzoma, ciljano sekvenciranje velikih panela gena, otkrivanje retkih varijanti i otkrivanje novih varijanti. Pored toga, upotreba NGS-a u sekvenciranju epigenoma i metagenoma dramatično raste. Treća generacija platformi za sekvenciranje omogućava direktno sekvenciranje RNK i analizu transkriptoma. U nekim istraživanjima može se razmotriti upotreba dve metode sekvenciranja. Na primer, Sanger sekvenciranje se može koristiti kao metod potvrde za verifikaciju varijanti koje su dokazane NGS metodom. Stoga, razmatranje mogućnosti i ograničenja različitih platformi sekvenciranja je veoma važno u donošenju odluke za izbor odgovarajuće metode sekvenciranja u istraživanju.

Upoznavanje evolucije patogena kroz filogenetske i filodinamičke analize

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Preteće infektivne bolesti izazvane brzo evoluirajućim RNK virusima predstavljaju značajnu pretnju globalnom zdravlju, uključujući i potencijal za nastanak pandemija. Jedna od najznačajnijih karakteristika RNK virusa je njihova izrazito visoka stopa mutacija koja se dešava značajno učestalije nego u organizmima koji imaju DNK kao genetički materijal. Kao posledica toga, RNK virusi često nose značajne genetske varijacije unutar domaćina što ih čini idealnim organizmima za filogenetska istraživanja, pružajući optimalan filogenetski signal za evolucionu analizu, uključujući i onu zasnovanu na pojedinačnim genima.

Filogenetska analiza zauzima centralno mesto u proučavanju evolucije virusa. Sve veći broj filogenetskih tehnika je razvijen za tumačenje genomskih podataka iz virusnih patogena, posebno u kombinaciji sa metapodacima kao što su vreme uzorkovanja i geografska lokacija. Transmisija virusa sa jednog domaćina na drugi ostavlja molekularne „otiske" u genomu patogena koji omogućavaju istraživanje porekla i evolucije virusa, kao i potencijal za širenje epidemije. Filodinamička analiza danas predstavlja nezamenjiv deo moderne molekularne epidemiologije, pomoću koje se otkriva evoluciona (filogenetska) istorija virusa i poreklo, kao i vreme transmisije virusa između vrsta, ali i obrasci širenja virusa na prostornoj skali, što, takođe, pomaže da se identifikuju faktori životne sredine povezani sa pojavom ovih patogena. Korišćenjem ovih metoda, sada je moguće otkriti ključne aspekte nastanka, evolucije i širenja RNK virusa od nivoa pojedinačnih domaćina do globalnih metapopulacija.

U našem istraživanju koristili smo napredne filogenetske alate, uključujući softverske pakete Beast i Beast 2, kao i MrBayes i R softver, sa ciljem da istražimo evolucionu dinamiku pretećih brzo evoluirajućih RNK virusa. Istraživanje smo posebno fokusirali na virus Zapadnog Nila (VZN) i Usutu virus (USUV), koje smo molekularno identifikovali kod komaraca roda Culex sp, izlovljenih na teritoriji Srbije. Naše istraživanje dovelo je do otkrića genetske sličnosti lokalnih izolata sa određenim stranim izolatima, puteva migracije ovih virusa na globalnom nivou u prošlosti, otkrića novih interkontinentalnih migracionih događaja, kao i potencijal za dalje širenje epidemije, čime ukazali na potrebu za povećanim molekularnim nadzorom ovih patogena.

Understanding the evolution of pathogens through phylogenetic and phylodynamic analyses

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The emergence of rapidly evolving RNA viruses pose a significant threat to global health, including the potential of major pandemics. One of the most prominent features of all RNA viruses is their high mutation rate that often occurs several orders of magnitude more rapidly than it does in DNA-based organisms. As a consequence, RNA viruses often harbor extensive intra-host genetic variation which makes them ideal organisms for phylogenetic research providing sufficient phylogenetic resolution for evolutionary analysis, including that based on single genes.

Phylogenetic analysis sits at the heart of studies of viral evolution. A growing number of phylogenetic tools have been proposed to elucidate genomic data from viral pathogens, particularly in combination with metadata such as sampling time and geographic location. Those wishing to trace the origin and spread of virus epidemics have benefited from the fact that the process of host-to-host transmission often leaves a molecular footprint in the sequences of viruses. Phylodynamics has become a central pillar of modern molecular epidemiology that could revealed the evolutionary (phylogenetic) history of viral genomic sequences and their origins, timing of cross-species transmission events of many important emerging viruses, and could helped identify the patterns and determinants of the spread of these viruses on a spatial scale, which in turn could help to identify the environmental factors associated with an increased risk of emergence. Through the use of these methods, it is now possible to reveal key aspects of the emergence, evolution, and spread of RNA viruses from the scale of individual hosts to global metapopulations.

In our research we used advanced phylogenetic tools, including Beast and Beast 2 software packages, MrBayes and R software, aimed to investigate evolutionary dynamics of emerging highly variable RNA viruses. In particular, we focused our research on West Nile Virus (WNV) and Usutu virus (USUV) identified in mosquitoes; Culex sp, captured in the territory of Serbia. We suggested genetic relation of local isolates to the foreign ones, described past migration routes on the global scale, revealed two newly described intercontinental migration events and potential for further spread of epidemic, drawing attention to the need for upscaling surveillance of these pathogens.

Putevi širenja patogena: filogeografske analize

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Filogeografija podrazumeva prostorno-vremensku rekonstrukciju evolutivne istorije različitih patogena u cilju predviđanja pojave zaraznih bolesti, detekcijom ključnih vrsta domaćina i geografskih područija sa kojih se patogeni šire, kao i potencijalni uticaj kretanja prirodnih rezervoara u prirodi na dalje širenje zoonotskih bolesti. Filogeografska analiza može se raditi primenom dva različita pristupa: filogeografija sa diskretnim lokacijama i filogeografija u kontinuiranom prostoru.

Za rekonstrukciju prostorno-vremenske evolutivne istorije koristi se Bajesova metoda implementirana u BEAST softverski paket. Pored odgovarajućeg evolutivnog modela, izabranog na osnovu vrednosti AIC kriterijuma u jModelTest programu, neophodno je kalibrisati i ostale parametre u BEAST softveru (molekularni sat, demografski model) u cilju dobijanja što pouzdanijeg modela za analizu distribucije patogena.

U studiju smo uključili 275 sekvenci NS5 gena linije 2 Virusa Zapadnog Nila (VZN), izolovanih sa različitih geografskih lokacija u cilju analize prostorno-vremenskog širenja pomenutog patogena. Geografska lokacija zajedno sa godinom izolacije su bile dostupne u NCBI (https://www.ncbi.nlm.nih.gov/nucleotide/) bazi podataka. Filogeografska analiza je rađena u BEAST v1.10.4 softverskom paketu primenom relaksiranog molekularnog sata i Gaussian Markov random field (GMRF) Skyride modela. Za procenu konvergencije Markovljevog lanaca Monte Karlo (MCMC) i za utvrđivanje efektivne veličine uzorka za svaki od parametara (≥200), korišćen je program TRACER v1.6. Filogenetsko stablo konstruisano primenom Bajesove metode, analizirano je u programu FigTree v1.4.4.
Vremenski kalibrisano filogenetsko stablo NS5 gena VZN-a je pokazalo da se većina sekvenci iz Srbije grupiše zajedno sa sekvencama iz Grčke, formirajući jedan veliki monofiletski klaster. Analizom molekularnog sata je utvrđeno da je VZN-a ušao u Srbiju 2002. godine. Filogeografska analiza je ukazala da je VZN najverovatnije u Srbiju ušao iz Mađarske i da je nastavio lokalno da se širi.
Rezultati naše studije su pokazali da je u Srbiji linija 2 VZN-a bila u cirkulaciji čitavu deceniju pre prve epidemije 2012. godine, i time potvrdili značaj molekularno-bioinformatičkog pristupa u sagledavanju evolutivne istorije i puteva širenja ovog patogena.

Routes of pathogens spread: phytogeographic analyses

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Phylogeographic analysis reconstruction of evolutionary history of different pathogens aimed to predict of emergence of infectious diseases by detecting the key host species and the geographic areas from which pathogens spread and prediction of the impact of movement of natural reservoirs on the spread of viral diseases. Spatiotemporal reconstructions can be assessed by two different phylogeographic approaches; phylogeographic inference in discrete space and phylogeography in continuous space and time. The Bayesian method implemented in the BEAST software package is used to reconstruct the spatio-temporal evolutionary history. In addition to the appropriate evolutionary model, chosen based on the value of the AIC criteria in the jModelTest program, it is necessary to calibrate other parameters in the BEAST software (molecular clock, demographic model) in order to obtain the most reliable model for distribution analysis.

In the present study we included 319 partial NS5 segment sequences of WNV, isolated from different geographic areas with the aim to explore spatio-temporal dispersal out of Africa. Sampling time and trapping localities for all isolates were available in NCBI (https://www.ncbi.nlm.nih.gov/nucleotide/) public databases at the time of the study. Phylogeographic analysis was done in BEAST v1.10.4 software package using relaxed molecular clock i Gaussian Markov random field (GMRF) Skyride model. The convergence of parameters was assessed through the ESS>200 checked using Tracer v1.6. Phylogenetic tree was visualized using FigTree software v1.4.4.

Bayesian clade credibility (MCC) tree of the NS5 gene showed that majority of Serbian isolates was grouped in a single large clade together with sequences from Greece and Romania, thus forming large monophyletic clade. Molecular clock analysis estimated the time of the most recent common ancestor (tMRCA) of the local WNV epidemic in Serbia to around 2002. Phylogeographic analysis implied introduction of WNV-2 to Serbia from Hungary and further local spread.
Our results evidence presence and circulation of WNV-2 in Serbia a decade prior to the first evidenced outbreak in 2012, and demonstrate the usefulness of bioinformatic methods providing a comprehensive view of the viral evolutionary history.

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SESIJA / SESSION

/ PRETEĆE MIKOBAKTERIJSKE INFEKCIJE

/ EMERGING MYCOBACTERIAL INFECTIONS

Genomics in the diagnosis of drug resistance in TB

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Interpreting next generation sequencing data represents one of the main challenges in the uptake of this technology in routine laboratory diagnosis. In 2021 the WHO released the first catalogue of mutations and their association with drug resistance for interpreting genetic variants in M. tuberculosis complex.

A genotype-phenotype association study based on (i) phenotypic antimicrobial susceptibility testing (AST) results derived with WHO-endorsed methods, (ii) standardized WGS raw sequence data, and bioinformatics pipeline, and (iii) standardized, validated methodological approach for identifying variants associated with resistance phenotypes, generating statistics on the strength of the associations and confidence grading-associated variants was used to identify mutations associated with drug resistance for 13 anti-TB drugs.

The dataset included WGS and phenotypic AST for 38.215 clinical isolates from 41 countries.
1.149/15.667 (7.3%) mutations have been classified associated with drug resistance, whereas 107/15.667 (0.7%) were found to be not relevant for drug resistance. For rifampicin, isoniazid, ethambutol, fluoroquinolones, and streptomycin the sensitivity of the catalogue was >80%. The specificity was found >95% for all the drugs except for ethionamide (91.4%), moxifloxacin (91.6%), and ethambutol (93.3%). Only two mutations have been classified as marker of resistance for bedaquiline, delamanid, clofazimine and linezolid. This is mainly due to the low prevalence of phenotypic resistance for these drugs in the dataset.
The first catalogue of genetic markers of drug resistance in M. tuberculosis has been approved by the WHO and provides a global standard for the interpretation of mutations, thus encouraging the uptake and development of molecular AST for TB.

Transcontinental spread and evolution of multidrug resistant Mycobacterium tuberculosis strains - implications for clinical diagnostics and public health

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Transmission and evolution of multidrug resistant (MDR) Mycobacterium tuberculosis complex (Mtbc) strains are jeopardizing public health efforts to end the global tuberculosis epidemic. Particularly MDR clades belonging to Mtbc lineage 2 are extremely successful in multiple settings in Central Asia, the Russian Federation, and Eastern Europe.

The past years, we employed comparative genomics, and Bayesian statistics of different MDR outbreaks to trace the epidemic spread and evolutionary trajectories over time.

We found that the largest outbreak clades likely emerged in the 1960s and underwent successive epidemic expansion events. Starting from an isoniazid and streptomycin resistant ancestor, subsequent population expansions were accompanied by the evolution of resistance against up to 11 different antibiotics, and compensatory mechanisms which were associated with a higher epidemic success. Furthermore, many outbreak strains harbored already a hypervirulence-associated ppe38 gene locus, and mutation-mediated drug tolerance was detected.
We showed that MDR Mtbc lineage 2 clades have evolved an outstanding genetic background over the last decades which is an important factor for their long lasting, continent-wide transmission success. The ongoing expansion of these MDR strains is a challenge for clinical diagnostics, and the successful implementation of new MDR-TB drug regimens

Regimens to treat MDR TB – today's challenge and hope for the future

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Tuberculosis remains a threat to global public health and is one of the leading infectious causes of death globally. In 2020, an estimated 10 million people developed TB and 1.5 million died from the disease. Owing to the impact of the coronavirus disease (COVID-19) pandemic, TB incidence could increase globally in 2022 and 2023. In the latest data (from 2019), only one in three cases MDR TB were reported to have been treated. In recent years, significant progress in the availability of improved diagnostics and more effective medicines has led to earlier detection and higher treatment success rates among patients with MDR-TB. WHO drug-resistant TB guidelines have been introduced new definitions - pre-XDR-TB (TB caused by MDR/RR-TB strains also resistant to later-generation fluoroquinolones) and a change in the definition of XDR-TB (TB caused by M. tuberculosis strains fulfilling the definition of MDR/RRTB, resistant to later-generation fluoroquinolones and at least one additional Group A drug, bedaquiline or linezolid).

Three regimens for MDR TB are now available, lasting from 6 to 18 months :

1) The 6-month BPaLM regimen (bedaquiline [BDQ], pretomanid [Pa], linezolid [LZD] 600 mg and moxifloxacin [MFX]) used in programmatic conditions, thus replacing the previous shorter (9-month) or longer (>18 months) regimens in MDR TB patients aged ≥15 years. No prior exposure to the three drugs for ≥30 days is a precondition for its use. This regimen can be used as BPaL, without MFX in pre-XDR-TB patients when resistance to fluoroquinolones (FQs) is documented. Drug susceptibility testing (DST) to FQs is therefore strongly encouraged.

Regimens to treat MDR TB – today's challenge and hope for the future

2) The 9-month, all-oral, BDQ-containing regimens are preferred over the longer (>18 months) regimen in all patients (adults and children) affected by MDR TB, with a) no previous exposure to second-line treatment (including BDQ); b) no FQ resistance; c) no extensive pulmonary TB disease; d) severe extrapulmonary TB. In these regimens, 2 months of linezolid (at the daily dose of 600 mg) can replace 4 months of ethionamide. Rapid DST to rule out FQ resistance is considered necessary to reduce failure and resistance.

3) The regimen previously known as the longer regimen (≥18 months), which is individualised and based on the drug grouping (A, B, C) according to the 2020 WHO guidelines, is still considered necessary for specific categories of patients, for example, a) those with extensive forms of drug-resistant-TB (e.g., XDR-TB); b) those who are not eligible for the regimens described above, or c) those who have previously failed shorter treatment regimens.

Režimi lečenja MDR TB - izazovi sadašnjosti, nada budućnosti

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Tuberkuloza je i dalje pretnja globalnom zdravlju i jedan je od vodećih infektivnih uzroka smrti u svetu. Procenjuje se da je 2020.g.oko 10 miliona ljudi obolelo od tuberkuloze a 1,5 miliona je umrlo. Smatra se da je zbog pandemije Covid 19 incidenca tuberkuloze 2022 i 2023 godine i veća. Svake godine se registruje oko pola miliona obolelih od MDR tuberkuloze, ali se prema najnovijim podacima iz 2019 samo jedan od tri obolela leči. Poslednjih godina se ipak beleži značajan napredak u dostupnosti poboljšane dijagnostike i efikasniji terapijski režimi koji rezultiraju ranijem otkrivanju obolelih i većoj stopi uspešnog lečenja kod obolelih od MDR TB. Smernice SZO za TB su uvele nove definicije: pre XDR TB (TB uzrokovana MDR/RR TB koje su rezistentne na fluorohinolone novije generacije, a XDR TB je redefinisana kao MDR/RR TB uzrokovana bacilima rezistentnim na fluorohinolone novije generacije i najmanje jedan dodatni lek grupe A bedaquilin ili linezolid.

Sada postoje preporuke za primenu sledeća tri režima za lečenje MDR TB koji taju od 6 do 18 meseci :

1) Šestomesečni BPaLM režim (Bedaquilin B, pretonamid Pa, linezlid 600 mg L i moxifloxacin M koji se koristi kao zamena prethodnog kraćeg (9 meseci) režima ali i dužeg (18 meseci) režima lečenja kod pacijenata starijih od 15 godina i koji su bilo koji od ova 4 leka koristili kraće od 30 dana. Ukoliko se utvrdi rezistencija na hinolone ovaj šestomesečni režim može se sprovoditi kao BPaL režim. Stoga SZO snažno preporučuje utvrđivanje rezistencije na hinolone iz primoizolata sputuma.

2) Devetomesečni režim samo oralnim lekovima sa bedaquilinom su poželjniji u odnosu na stare režime do 18 meseci sa injekcionim lekovima kod obolelih koji prethodno nisu koristili lekove druge linije i nije dokazana rezistencija na hinolone, koji su bez ekstenzivne plućne i teške ekstrapulmonalne tuberkuloze. U ovim režimima se primena 2 meseca linezolida u dozi od 600 mg, ukoliko uzrokuje intenzivne neželjene efekte, može zameniti primenom 4 meseca etionamida. Neophodni su brzi testovi senzitivnosti pre započinjanja terapije da bi se isključila rezistencija na hinolone i smanjila stopa neuspešnog lečenja.

3) Režimi koji su poznati kao duži režimi, koji su individualizovani i zasnovani na grupisanju lekova na A, B, C i D grupu u skladu sa smernicama SZO iz 2020 i dalje se smatraju neophodnim za određene kategorije pacijenata kao što su oni sa XDR TB, i one koji ne ispunjavaju uslove sa gore opisane režime ili su prethodno neuspešno lečeni kraćim režimima lečenja.

Izazovi u sprovođenju programa prevencije i kontrole tuberkuloze u post COVID-19 eri

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Pored dugogodišnjih globalnih napora na eliminaciji tuberkuloze (TB) kao javno-zdravstvenog problema, i dalje 10 miliona slučajeva oboleva i 1,3 miliona umire od ove bolesti. Najveći uticaj na progres u kontroli tuberkuloze u svetu unazad tri godine imala je epidemija COVID-19 zbog brzog širenja infekcije virusom SARS CoV-2, težine kliničke slike i visoke stope mortaliteta.
Program prevencije i kontrole tuberkuloze TB u Republici Srbiji sprovodi se u okviru Programa zaštite stanovništva od zaraznih bolesti. U periodu od 2005. do 2015. godine programska realizacija je bila pomognuta sredstvim donacije Globalnog fonda za borbu protiv SIDE, tuberkuloze i malarije a aktivnosti usklađene sa tada najsavremenijim strategijama Svetske zdravstvene organizacije (SZO). U tom periodu, značajno su unapredjeni kapaciteti sistema zdravstvene zaštite što je rezultiralo smanjenjem notifikacione stope tuberkuloze sa 32/100.000 u 2005. na 13/100.000 u 2015. godini i održavanjem trenda smanjenja.

Uticaj COVID-19 na dijagnostiku i lečenje tuberkuloze je u našoj zemlji je veliki, jer su zdravstveni radnici koji su nosioci aktivnosti u oblasti kontrole tuberkuloze bili raspoređeni na zbrinjavanje COVID-19. Pored toga, izvršena je prenamena prostornih kapaciteta sa dijagnostike i lečenja tuberkuloze na COVID-19, što je dovelo do manjeg broja dijagnostikovanih slučajeva TB o čemu govore podaci. Tako je vrednost notifikacione stope u 2021. godini za 16% bila niža od vrednosti u 2020. godini, dok je za 65,6% bila niža u odnosu na 2019. godinu. Imajući u vidu da je unazad 15 godina prosečan godišnji pad notifikacione stope tuberkuloze bio 5–10%, ovaj podatak može ukazivati na smanjenu dijagnostiku i nekompletno prijavljivanje. U isto vreme, procenat direktno pozitivnih plućnih oblika TB među svim plućnim oblicima je bio 65% u 2020. i 2021. godini, dok do 2019. nije prelazio 60%. Ovo je pokazatelj kapaciteta programa za rano otkrivanje i njegova vrednost ne bi trebalo da prelazi 45%. Prethodni podaci ukazuju na to da su pacijenti otkrivani u kasnoj fazi kada je verovatnoća pojave teških oblika sa lošijom prognozom veća što se odražava na trasmisiju bolesti.
Sa ciljem poboljšanja učinka programa i sprečavanja preokreta epidemiološkog trenda u post COVID eri, neophodni su konsolidacija i unapređenje kapaciteta za dijagnostiku i lečenje TB.

Ključne reči: tuberkuloza, COVID-19, prevencija, kontrola

15 GODINA MOLEKULARNE DIJAGNOSTIKE TUBERKULOZE I MIKOBAKTERIOZA U SRBIJI

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U skladu sa preporukama Svetske Zdravstvene Organizacije, molekularni testovi zasnovani na principu reverzne hibridizacije PCR produkta za nitroceluloznu traku sa imobilisanim probama za različite regione genoma Mycobacterium tuberculosis i netuberkuloznih mikobakterija (NTM), primenjuju se u Nacionalnoj Referentnoj Laboratoriji (NRL) od 2008. godine. U periodu 2008-2023. godina, primenom GenoType MTBC testa, 2684 (99,7%) mikobakterijska izolata identifikovana su kao M. tuberculosis, dok 7 (0,3%) izolata pripada vrsti M. bovis BCG.

Od 1446 izolata analiziranih primenom GenoType MTBDRplus testa, 902 (62,4%) su osetljiva na rifampicin (RIF) i izoniazid (INH), 288 (19,9%) je multirezistentno (MDR), 134 (9,3%) izolata su rezistentna na INH, a 117 (8,1%) je rezistentno na RIF. Najveći broj izolata rezistentnih na RIF ima mutaciju rpoB S531L (228; 85,1%), dok su najčešće mutacije povezane sa rezistencijom na INH katG S315T (22; 95,7%) i inhA C15T (60; 96,8%).

Od 2829 izolata analiziranih primenom GenoType CM testa, 1724 (60,9%) pripada NTM. Od ukupno 11 identifikovanih vrsta NTM, najčešća je M. xenopi (216; 19%), dok 322 (28,3%) izolata nisu mogla biti identifikovana do nivoa vrste. Od 1138 pacijanata iz čijih respirstornih uzoraka su izolovane NTM, 302 (26,5%) ispunjava ATS/IDSA kriterijume za NTM respiratornu bolest, pri čemu je najčešći uzročnik M. xenopi (85; 30,4%). Primenom GenoType NTM-DR testa analizirano je ukupno 25 izolata i svi su osetljivi na aminoglikozide. Na makrolide je rezistentno svih 6 (100%) izolata M. abscessus subsp. abscessus, 2 (16,7%) izolata M. intracellulare i 2 (28,6%) M. avium. Sekvenciranje celog genoma se u NRL obavlja od 2014. godine u saradnji sa Supranacionalnom Referentnom Laboratorijom u Borstelu, Nemačka, isključivo u istraživačke svrhe. Sekvencirano je ukupno 110 MDR sojeva M. tuberculosis izolovanih u periodu 2008-2022. godina, a najveći broj izolata pripada genotipu TUR (39; 35,5%), dok se preko 70% izolata TUR genotipa nalazi u jednom lancu transmisije. TUR sojevi vode poreklo iz bolnice za lečenje psihijatrijskih bolesnika sa respiratornim bolestima u Beloj Crkvi, odakle se ovi sojevi preko 20 godina šire na ostale delove Srbije. U skladu sa tim, neophodan je kontinuirani monitoring transmisije MDR TB u opštoj populaciji, što zahteva neodložnu implementaciju savremenih molekularno-bioinformatičkih metoda u rutinski rad NRL u Srbiji.

15 YEARS OF MOLECULAR DIAGNOSTICS OF TB AND MYCOBACTERIOSES IN SERBIA

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The World Health Organization (WHO)-endorsed line-probe assays, based on the reverse hybridization of a PCR product to a nitrocellulose strip with immobilized probes, have been introduced into the algorithm of laboratory diagnostics of tuberculosis (TB) and mycobacterioses in the National Reference Laboratory (NRL) in 2008.

During the period 2008-2023, we identified 2684 (99.7%) mycobacterial isolates as M. tuberculosis, and 7 (0.3%) as M. bovis BCG by using the GenoType MTBC test. Of the 1446 isolates analyzed by the GenoType MTBDRplus test, 902 (62.4%) were susceptible to rifampicin (RIF) and isoniazid (INH), 288 (19.9%) were multiresistant (MDR), 134 (9.3%) were resistant to INH, while 117 (8.1%) were resistant to RIF. The most frequent mutation associated with resistance to RIF was rpoB S531L (228; 85.1%), while resistance to INH was predominantly mediated by mutations katG S315T (22; 95,7%), and inhA C15T (60; 96.8%). Of the 2829 isolates analyzed using the GenoType CM test, 1724 (60.9%) belonged to NTM.

Out of 11 identified NTM species, M. xenopi was the most common (216; 19%), while 322 (28.3%) isolates could not be identified to the species level. Of the 1138 patients with NTM isolates in respiratory samples, 302 (26.5%) met the ATS/IDSA criteria for NTM respiratory disease, while the most common causative agent was M. xenopi (85; 30.4%). Using the GenoType NTM-DR test, a total of 25 isolates were analyzed and all were susceptible to aminoglycosides. All 6 (100%) isolates of M. abscessus subsp. abscessus, 2 (16.7%) M. intracellulare and 2 (28.6%) M. avium isolates were resistant to macrolides. To date, Whole Genome Sequencing was performed for 110 MDR M. tuberculosis strains isolated from 2008 to 2023 in collaboration with the Supranational Reference Laboratory in Borstel, Germany, for research use only. The most predominant genotype is TUR (39; 35.5%), while over 70% of the TUR isolates were found in a single transmission chain. TUR strains originate from the hospital for the treatment of psychiatric patients with respiratory diseases in Bela Crkva, from where they have been spreading to other regions of Serbia for over 20 years. Therefore, continuous monitoring of MDR TB transmission in the general population is necessary, which requires the immediate implementation of molecular-bioinformatics methods in the routine work of NRL in Serbia.

Mikobakterioze: zanemareni javno-zdravstveni problem

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Netuberkulozne mikobakterije (NTM) čine sve vrste unutar roda Mycobacterium, osim članova MTBC kompleksa i M. leprae. Dinamika otkrivanja je značajna te se u prosjeku godišnje otkriju tri nove vrste. Od gotovo 200 trenutno poznatih vrsta, tridesetak uzrokuje bolest u ljudi, a u Hrvatskoj se najčešće susreću M. avium, M. intracellulare, M. xenopi, M. fortuitum, i M. gordonae. Tipični su mikroorganizmi okoliša, posebno zemlje i vode te se za razliku od M. tuberculosis ne prenose s čovjeka na čovjeka. Ova je grupa bakterija otkrivena pedesetih godina prošlog stoljeća te su većinom smatrane kontaminantima ili uzročnicima bolesti kod imunokompromitiranih osoba. Plućna NTM bolest je najčešći oblik NTM infekcije, a za samu dijagnozu nije dovoljan mikrobiološki izolat već je potrebno ispuniti mikrobiološki, radiološki i klinički kriterij. Značajnost NTM često ostaje u sjeni poznatijeg uzročnika u ovom rodu M. tuberculosis, osobito u zemljama koje još imaju visoku incidenciju tuberkuloze. Međutim, recentni podaci pokazuju porast mikobakterioza ne samo kod osoba sa predležečim bolestima, već i u zdravih osoba. Teško je odrediti stvarnu incidenciju ovih infekcija jer se one sustavno ne prijavljuju te ne postoji praćenje zaraze na nacionalnoj razini. S obzirom na starenje stanovništva te sve duži život osoba koje imaju komorbiditete koji su povezani s mikobakteriozama, za očekivati je kako će značajnost ovih patogena još i više rasti u narednom razdoblju. Nažalost, kliničari nisu dovoljno upoznati sa ovim patogenima te nedostaju jasne smjernice za liječenje većine vrsta unutar roda.

Godišnje u Hrvatskoj otkrijemo između 200-450 izolata, međutim, broj bolesnika je znatno manji s obzirom da ove bakterije nisu obligatni patogeni. Posljednjih 15 godina u Hrvatskoj udio NTM kod svih izoliranih mikobakterija raste, od 5% 2004. godine do više od 20% 2021. godine. Također raste i broj pacijenata kod kojih su izolirana dva ili više izolata te smo u 2021. godini imali 51 bolesnika s ispunjenim mikrobiološkim kriterijima za mikobakteriozu, među kojima predominiraju infekcije s M. xenopi, M.avium i M. intracellulare. Kod većine bolesnika radi se o plućnoj zahvaćenosti, međutim opisane su i kožne manifestacije te diseminacija sa smrtnim ishodom.

Mycobacterioses: a neglected public health problem

Nontuberculous mycobacteria (NTM) comprise all species within the genus Mycobacterium, except members of the MTBC complex and M. leprae. The dynamics of discovery is significant, and on average three new species are discovered annually. Out of almost 200 currently known species, about 30 cause disease in humans, and in Croatia the most common are M. avium, M. intracellulare, M. xenopi, M. fortuitum, and M. gordonae. These are typically environmental microorganisms, found in soil and water, and unlike M. tuberculosis, are not transmitted from person to person. This group of bacteria was discovered in the 1950s, and they were mostly considered contaminants or disease-causing agents in immunocompromised people. Pulmonary NTM disease is the most common form of NTM infection, and for the diagnosis itself, a microbiological isolate is not sufficient, but it is necessary to fulfill microbiological, radiological and clinical criteria. The significance of NTM often remains in the shadow of the more well-known causative agent in this genus, M. tuberculosis, especially in countries that still have a high incidence of tuberculosis. However, recent data show an increase in the number of mycobacteriosis not only in people with underlying diseases, but also in healthy people. It is difficult to determine the actual incidence of these infections because they are not systematically reported and there is no monitoring of infection at the national level. Considering the aging of the population and the longer lives of people who have comorbidities associated with mycobacteriosis, it is to be expected that the significance of these pathogens will grow even more in the coming period. Unfortunately, clinicians are not sufficiently familiar with these pathogens and clear guidelines for the treatment of most species within the genus are lacking.

Annually in Croatia, we detect between 200-450 isolates, however, the number of patients is significantly lower considering that these bacteria are not obligate pathogens. Over the last 15 years, the share of NTM among all isolated mycobacteria has been increasing, from 5% in 2004 to more than 20% in 2021. The number of patients in whom two or more isolates were isolated is also increasing, and in 2021 we had 51 patients with fulfilled microbiological criteria for mycobacteriosis, among which predominate infections with M. xenopi, M.avium and M. intracellulare. In most patients, there is pulmonary involvement, however, skin manifestations and dissemination with a fatal outcome have also been described.

Netuberkulozne mikobakterije kao produktori biofilma

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Netuberkulozne mikobakterije (NTM) čine veliku, heterogenu grupu koja obuhvata više od 200 vrsta roda Mycobacterium, koje pod određenim okolnostima izazivaju infekcije čoveka, a najčešća manifestacija je bolest pluća. NTM su široko rasprostranjene u prirodi gde naseljavaju različite eko-sisteme, od kojih veliki broj dele sa čovekom. Mikobakterije pokazuju izrazitu sklonost ka formiranju biofilma na različitim površinama, a biofilm predstavlja problem od velikog značaja za javno zdravlje, zbog toga što omogućava perzistenciju bakterija u nepovoljnim uslovima čineći ih manje osetljivim na faktore okruženja, uključujući dezinficijense i antibiotike.

Voda, kako u prirodi tako i u sistemu vodovoda, predstavlja najznačajniji rezervoar NTM, a putem vode za piće NTM se distribuiraju i dospevaju do čoveka. Rezultati brojnih istraživanja doveli su do saznanja da neke vrste NTM redovno kolonizuju sistem vodovoda i da sojevi izolovani iz uzoraka vode poreklom iz domaćinstava obolelih od NTM plućne bolesti imaju isti genotip kao i klinički izolati. Biofilm mikobakterija je dokazan u plućima pacijenata sa infekcijom vrstom Mycobacterium abscessus, ali i na površini veštačkih biomaterijala, a NTM su izolovane kao izazivači infekcija posredovanih medicinskim implantatima, kao što su infekcije krvi posredovane kateterima, infekcije posredovane veštačkim zglobovima, endokarditis kod veštačkih srčanih zalistaka i dr. Tokom poslednje decenije, M. chimaera, spororastuća vrsta iz M. avium kompleksa, opisana je kao izazivač invazivnih infekcija kod pacijenata na odeljenjima kardiohirurgije. Iako je broj opisanih slučajeva još uvek relativno mali, NTM kao izazivači ovakvih infekcija predstavljaju preteći javnozdravstveni problem, jer bakterije unutar biofilma pokazuju povećanu rezistenciju i sposobnost perzistencije u bolničkom okruženju, dok je terapija teška i sa neizvesnim ishodom. Zbog svega navedenog, kao i zbog sve učestalije primene invazivnih metoda u dijagnostici i terapiji bolesti, neophodna su detaljnija istraživanja biofilma mikobakterija u cilju razvoja novih terapijskih strategija i procedura za dezinfekciju i eliminaciju biofilma, jer se porast učestalosti ovih NTM infekcija može očekivati u budućnosti.

Nontuberculous Mycobacteria as Biofilm Producers

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Nontuberculous mycobacteria (NTM) comprise a large, heterogeneous collection of more than 200 Mycobacterium species. NTM are opportunistic pathogens, wide-spread in nature that can cause various infections in humans, most frequently pulmonary. NTM have been recovered from a wide variety of environmental habitats which they share with humans. In recent years, NTM have taken on new importance regarding their natural ability to assemble biofilms on different surfaces, while serious concerns have arisen about microbial biofilms because they can withstand a wide range of environmental stressors, including antibiotics and disinfectants, enabling bacterial persistence in harsh conditions. NTM biofilms are particularly found to be formed across water systems, both natural and human-engineered, reaching humans mainly through building plumbing.

Certain NTM species have long been known to colonize plumbing, and water in patients' households has been documented as a source of NTM infection. Mycobacterial biofilms have been described in the lungs of patients with NTM pulmonary disease, and in different medical device-associated infections, such as catheter-related bacteremia, prosthetic joint infections, prosthetic valve endocarditis, and others. Recently, Mycobacterium chimaera, a slow-growing species belonging to the M. avium complex, has emerged as an important nosocomial pathogen associated with cases of sterile site infection in patients after cardiac surgery throughout the world. Although few in number, NTM as the etiological agents of these clinical manifestations represent a great concern for public health, due to their ability to persist within biofilms, both in environmental and clinical settings, and increased resistance against antimicrobial agents provided by the biofilm, making the treatment of NTM infections particularly challenging. Therefore, with the increasing use of invasive diagnostic and treatment procedures, we need better understanding of NTM biofilms, improved disinfection processes and development of novel antibiofilm strategies, as an increase of these reports could be expected in the foreseeable future.

SESIJA / SESSION

/ VIRUSNE ZOONOZE

/ VIRAL ZOONOSES

Virusne zoonoze - poznati patogeni i nove pretnje

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Zoonoze, bolesti koje se mogu preneti sa životinja na ljude, predstavljale su stalnu opasnost tokom istorije. Jedna od najranijih zabeleženih zoonoza je bubonska kuga, koja je opustošila ljudsku populaciju u srednjem veku. U današnje vreme, virusne zoonoze predstavljaju značajan rizik za javno zdravlje. SARS-CoV-2 je izazvao globalnu pandemiju, naglašavajući potrebu za efikasnijom prevencijom i bržem reagovanju na pojavu virusnih zoonotskih oboljenja.

U budućnosti, virusne zoonoze će verovatno ostati goruće pitanje kako se ljudska populacija i dalje bude širila i zadirala u različita prirodna staništa, povećavajući rizik od izloženosti novim patogenima. Klimatske promene i drugi faktori životne sredine takođe mogu igrati ulogu u nastanku i širenju ovih bolesti. Međutim, napredak nauke i tehnologije mogu pomoći da bolje razumemo opasnosti od virusnih zoonoza i da se sa njima uspešnije borimo.

Dosadašnje iskustvo sa ovim patogenima naglašava važnost saradnje između lekara, veterinara, informatičara i rukovodilaca zdravstvene politike, a kako bi se predvideli, sprečili ili ublažili novi zoonotski izazovi. Ovo predavanje će biti putovanje kroz prošlost, sadašnjost i budućnost ovih virusnih pretnji.

Viral zoonoses – known pathogens and new threats

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Zoonoses, diseases that can be transmitted from animals to humans, have been a persistent hazard throughout human history. One of the earliest recorded zoonotic diseases is the bubonic plague, which devastated populations in the Middle Ages. In the present day, viral zoonoses continue to pose a significant public health risk. COVID-19, caused by the SARS-CoV-2 virus, has caused a global pandemic, highlighting the need for greater vigilance and preparedness in preventing and responding to viral zoonotic diseases.

Looking to the future, viral zoonoses are likely to remain a pressing concern as human populations expand and encroach on natural habitats, increasing the risk of exposure to novel pathogens. Climate change and other environmental factors may also play a role in the emergence and spread of zoonotic diseases. However, there is hope that advances in science and technology can help us better understand and manage viral zoonotic perils. Our experience with these pathogens highlights the importance of ongoing watchfulness and collaboration between human and animal health professionals to prevent and respond to these complex and evolving threats. This lecture will be a casual voyage through the past, present, and future of these not-so-casual viral threats.

Endemska populaciona dinamika virusa Zapadnog Nila u komarcima sa teritorije Srbije

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Prvi slučajevi infekcije virusom Zapadnog Nila (VZN) u Srbiji zabeleženi su 2012. godine, dok je 2018., prema izveštaju Evropskog centra za kontrolu i prevenciju bolesti (ECDC), Srbija prijavila najveći broj obolelih (385) od svih zemalja jugoistočne Evrope. Cilj ove studije je bio da se genetički okarakterišu sojevi VZN-a, koji cirkulišu u Srbiji.

U studiji je ispitano 200 pulova komaraca, skupljenih u Beogradu i široj okolini, u okviru redovnog nadzora koji sprovodi Zavod za biocide i medicinsku ekologiju u Beogradu u periodu od 2018-2020. Prisustvo VZN-a utvrđeno je reakcijom lančanog umnožavanja (engl. polymerase chain reaction – PCR), za detekciju NS5 gena, sa primenom Sangerovog DNK sekvenciranja dobijenih PCR produkata i njihovom preliminarnom identifikacijom primenom BLAST alata u NCBI bazi podataka.

Konstrukcija filogenetskog stabla rađena je primenom metode Bajesove statistike implementirane u MrBayes softverski paket, uz odgovarajući evolutivni model koji je izabran u jModelTest softveru. Iz 200 analiziranih pulova komaraca, koji su skupljeni u periodu od 2018-2020, dobili smo 45 sekvenci NS5 gena VZN-a. Rezultati studije su pokazali da je 22.5% (45/200) pulova komarca bilo pozitivno na prisustvo VZN-a. Najveći procenat pozitivnih pulova je iz 2018. godine i iznosi 57.8% (26/45), zatim iz 2019 26.7% (12/45) i iz 2020 15.5% (7/45). Identifikacija dobijenih sekvenci pokazala je da je u Srbiji trenutno u cirkulaciji samo linija 2 VZN-a. Dalja analiza je potvrdila da su sojevi VZN-a iz Srbije najsličniji izolatima iz Grčke. Dodatno, u jednom pulu komaraca, uzorkovanom u okolini Šapca 2019. godine, identifikovano je prisustvo Usutu virusa.

Dobijeni rezultati su potvrdili da u Srbiji cirkuliše linija 2 VZN-a kao i da postoji potreba za kontinuiranim molekularno-bioinformatički nadzorom nad ovim i sličnim zoonotskim virusima.

Endemic population dynamics of West Nile virus in mosquitoes from the territory of Serbia

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The first cases of West Nile virus (WNV) infection in Serbia recorded in 2012, while in 2018, according to the report of the European Centre for Disease Control and Prevention (ECDC), Serbia reported the highest number of cases (385) in the Southeast Europe. The aim of this study was to genetically characterize strains of WNV circulating in Serbia.

The present study included 200 pools of mosquitoes, collected in Belgrade and the surrounding area by the Institute for Biocides and Medical Ecology in Belgrade in the period from 2018-2020, as part of regular surveillance. The presence of WNV was detected by Nested-PCR (Polymerase chain reaction) method together with specific primers for detection of partial NS5 gene. All obtained specific PCR products were directly sequenced in both directions. Preliminary sequence identification was done by BLAST tool (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Bayesian method was employed to infer the evolutionary relationship of studied sequence dataset, using MrBayes software with the best-fitting nucleotide substitution model for the final sequence dataset, as selected by jModelTest 2.

In total, 45 partial NS5 gene WNV sequences were obtained from mosquito pools collected in Serbia between 2018 and 2020. The results obtained in this study showed that 22.5% (45/200) of mosquito pools were positive for the presence of WNV. The highest percentage of positive pools was detected in 2018 (57.8% (26/45)), followed by 2019 (26.7% (12/45)) and 2020 (15.5% (7/45)). Phylogenetic analysis conformed that only linage 2 of WNV is circulating in Serbia. Majority of Serbian isolates clustered with Greek strains, thus forming the Balkan cluster. Of note, in one mosquito pool sampled in the Western Serbia in 2019, we detected the presence of Usutu virus.

Results obtained in the present study confirmed the presence of WNV linage 2 in Serbia and the necessity for continuous molecular-bioinformatic monitoring of zoonotic viruses.

Hantavirusi su prepoznati kao jedan od aktuelnih pretećih patogena na Balkanu.

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Na Evroazijskom tlu hantavirusi (dominantno DOBV i PUUV) dovode do hemoragične groznice sa bubrežnim sindromom (HGBS), koja može da ima vrlo ozbiljnu kliničku prezentaciju. Bolest ima endemski karakter, sa sporadičnim i epidemijskim potencijalom, a slučajevi oboljevanja beleže se svake godine. Prosečni godišnji broj obolelih je oko 100. Humane infekcije najčešće nastaju izlaganjem aerosolu koji je kontaminiran izlučevinama inficiranih glodara. Specifična antivirusna terapija ne postoji.

Ekologija hantavirusa na Balkanskom poluostrvu je složena, što je posledica specifičnog geografskog položaja balkanskih država. Prisustvo ovog virusa na Balkanskom poluostrvu registrovano je kod četiri roda glodara: Apodemus, Myodes, Microtus and Rattus. Najčešći nosilac hantavirusa na Balkanu je glodar A. Flavicollis (domaćin Dobrava/Belgrade hantavirusa), koji naseljava guste listopadne šume karakteristične za naše područje, koje mu obezbeđuju veliki diverzitet biljnih vrsta i bogatu ishranu. Različiti faktori mogu da dovedu do boljeg opstanka virusa van domaćina kao što je temperatura, nivo UV zračenja, pH i vlažnost zemljišta. Klimatske promene na kompleksne načine utiču na prevalencu virusa kod glodara. Poslednja veća epidemija, u svim zemljama Balkanskog poluostrva, registrovana je tokom 2014. godine i povezana je sa intenzivnim poplavama koje su zadesile čitav region.

Ljudsko ponašanje povećava rizik od izlaganja izlučevinama, doprinoseći većoj incidenci HGBS. Zanimanje je dominantni faktor rizika, pa se tako oboljevanje najčešće registruje tokom letnjih meseci, uglavnom kod odraslih muškaraca (zemljoradnici, lovci, drvoseče, šumari, vojnici).

Balkan predstavlja područje sa potrebom za hantavirusnom vakcinom, a u Evropi ne postoji registrovana vakcina. Zbog toga, prevencija infekcije zasniva se na svesti i sprovođenju ličnih mera da bi se izbegla inhalacija kontaminiranih izlučevina, na edukaciji o pravilnom čišćenju i dezinfekciji površina koje sadrže izlučevine glodara, kao i kontroli brojnosti populacije glodara. Sve ove aktivnosti zahtevaju multidisciplinaran, odnosno interdisciplinaran pristup problemu hantavirusa na Balkanu.

Hantaviruses are recognized as one of the current emerging pathogens in the Balkans.

Jelena Protić[1]

On Eurasian soil, hantaviruses (dominantly DOBV and PUUV serotypes) cause hemorrhagic fever with renal syndrome (HFRS), which can have a very serious clinical presentation. The disease is endemic, with sporadic and epidemic potential, and cases are recorded every year. The average number of patients annually is around 100. Human infections are most often caused by exposure to an aerosol contaminated with the excrement of infected rodents. There is no specific antiviral therapy. The ecology of hantaviruses on the Balkan Peninsula is complex, which is a consequence of the specific geographical position of the Balkan countries. The presence of this virus on the Balkan Peninsula has been registered in four genera of rodents: Apodemus, Myodes, Microtus and Rattus.

The most common carrier of hantavirus in the Balkans is the rodent A. Flavicollis (the host of Dobrava/Belgrade hantavirus), which inhabits the dense deciduous forests characteristic of our region, which provide it with a great diversity of plant species and a rich diet. Various factors can lead to better survival of the virus outside the host, such as temperature, level of UV radiation, pH and soil moisture. Climate change affects the prevalence of viruses in rodents in complex ways. The last major epidemic, in all countries of the Balkan Peninsula, was registered in 2014 and was associated with intense floods that affected the entire region.

Human behavior increases the risk of excrement exposure, contributing to a higher incidence of HFRS. Occupation is the dominant risk factor, so the disease is most often registered during summer months, mainly among adult men (farmers, hunters, loggers, foresters, soldiers).

The Balkans represents an area with a need for hantavirus vaccine, and there is no registered vaccine in Europe. Therefore, prevention is based on awareness and implementation of personal measures to avoid inhalation of contaminated excreta, education on proper cleaning and disinfection of surfaces containing rodent excrement, as well as control of rodent populations. All these activities require a multidisciplinary, or rather, interdisciplinary approach to the Hantavirus problem in the Balkans.

NEUROINFEKTIVNI AGENSI: DA LI SE NEŠTO PROMENILO?

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Uvod

Infektivne bolesti utiču na nervni sistem miliona ljudi širom sveta. Sa otkrićem novih patogena, porastom upotrebe imunosupresivne terapije, poboljšanom dijagnostikom i napretkom u infektivnoj i autoimunoj neurologiji, oblast neuroinfektivnih bolesti se brzo razvija, a broj novih i ponovno pojavljivanje već poznatih neuroinfektivnih agenasa je u stalnom porastu. Različiti faktori, kao što su porast stanovništva, veća učestalost međunarodnih putovanja, doprineli su širenju patogena u ranije neendemske regione.

Diskusija

Tokom poslednje dve decenije, identifikovano je nekoliko novih organizama koji izazivaju neuroinfekcije, a registrovan je i neurotropni potencijal mnogih drugih poznatih patogena. Znatan broj neuroinfekcija se nedovoljno prijavljuje i dijagnostikuje, te se smatra da se i u tercijarnim centrima razvijenih zemalja, kod značajnog broja takvih pacijenata ne ustanovi etiološka dijagnoza. Razumevanje neuroloških komplikacija prethodnih pandemija i patofizioloških mehanizama koji leže u njihovoj osnovi, mogu nam pomoći kada su u pitanju mnogi neuroinfektivni agensi, potencijalni izazivači novih epidemija i pandemija. Svetska zdravstvena organizacija (SZO) na globalnom nivou prati širenje bolesti sa epidemijskim ili pandemijskim potencijalom, pri čemu većina ovih bolesti ima neurološke manifestacije.

Aktuelna pandemija COVID-19 pokazala je da patogeni sa potencijalom da se brzo i lako šire mogu uticati i na nervni sistem.

Zaključak

Učešće neuroinfektivnih agenasa u pandemijama i epidemijama je od velikog značaja jer može izazvati razorne posledice među pogođenom populacijom. Bez obzira na vrstu patogena, brze i agresivne mere za kontrolu širenja ovih agenasa su najvažniji faktori u smanjenju ukupnog morbiditeta i mortaliteta koje oni mogu da izazovu.

NEUROINFECTIOUS AGENTS: HAS ANYTHING CHANGED?

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Background

Infectious diseases affect the nervous system of millions of people worldwide. With the discovery of new pathogens, increased use of immunosuppressive therapy, improved diagnostics, and advances in infectious and autoimmune neurology, the field of neuroinfectious diseases is rapidly evolving, and the number of new and re-emerging neuroinfectious agents is constantly increasing. Various factors, such as population growth, greater frequency of international travel, have contributed to the spread of pathogens to previously non-endemic regions.

Discussion

Over the past two decades, several new organisms that cause neuroinfections have been identified, and the neurotropic potential of many other known pathogens has been registered.

A significant number of neuroinfections are under-reported and under-diagnosed, and it is considered that even in tertiary centers of developed countries, a significant number of such patients do not receive an etiological diagnosis. Understanding the neurological complications of previous pandemics and the pathophysiological mechanisms underlying them, can help us when it comes to many neuroinfectious agents, potential challengers of new epidemics and pandemics. The World Health Organization (WHO) monitors the global spread of diseases with epidemic or pandemic potential, with most of these diseases having neurological manifestations. The current COVID-19 pandemic has shown that pathogens with the potential to spread rapidly and easily can also affect the nervous system.

Conclusion

The involvement of neuroinfectious agents in pandemics and epidemics is of great importance because it can cause devastating consequences among the affected population. Regardless of the type of pathogen, prompt and aggressive measures to control the spread of these agents are the most important factors in reducing the overall morbidity and mortality they can cause.

SESIJA / SESSION

/ HELICOBACTER PYLORI INFEKCIJA I INFEKCIJE KOJE SE PRENOSE HRANOM
/ HELICOBACTER PYLORI INFECTION AND FOODBORNE INFECTIONS

Klinički značaj Helicobacter pylori infekcije

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Helicobacter pylori (Hp) infekcija prisutna je kod više od polovine celokupne svetske populacije. Kod svih inficiranih Hp izaziva gastritis nevezano za prisustvo simptoma. Kod većine pacijenata Hp infekcija je asimptomatska i karakteriše je hronični superficijalni gastritis. Samo kod nekih pacijenata infekcija će dovesti do nastanka ulkusne bolesti želuca ili duodenuma (približno 25% pacijenata), dok se adenokarcinom i limfom želuca retko dijagnostikuje, kod 1% svih pacijenata sa Hp infekcijom. Mastriht VI konsenzus postulira da je Hp patogena bakterija, a da je Hp gastritis infektivna bolest. Kod pacijenta sa dispepsijom obavezno je testiranje na Hp. Povezanost ulkusne bolesti i Hp infekcije dokazana je pre više od 3 decenije. Najveći broj duodenalnih i gotovo dve trećine ulkusa želuca povezani su sa prisustvom Hp infekcije.

Višedecenijska sistematska primena eradikacione terapije za Hp u indikaciji ulkusne bolesti smanjila je učestalost nekomplikovane ulkusne bolesti. Eradikacija Hp infekcije indikovana je kod pacijenata sa dispesijom, gastritisom, ulkusom želuca i/ili duodenuma, anamnezom o ranije dijagnostikovanom ulkusu, kao i kod onih koji koriste ulcerogene lekove. Hp je karcinogen prvog reda u nastanku adenokarcinoma želuca, koji se godišnje se dijagnostikuje kod više od milion novih pacijenata.
Dugotrajna Hp infekcija pokreće inflamatornu kaskadu koja preko atrofije, intestinalne metaplazije i displazije dovodi do nastanka karcinoma želuca. MALT limfom želuca posledica je hronične antigene stimulacije koju izaziva Hp u sluznici želuca. MALT limfom je niskog stepena maligniteta i eradikacija Hp je prva terapijska linija za lokalizovani MALT limfom želuca.

Klinički značaj Helicobacter pylori infekcije

Postoje i ekstragastrične manifestacije Hp infekcije, pa je eradikacija po Mastriht VI konsenzusu, preporučena za pacijente sa sideropenijskom anemijom nejasnog uzroka, idiopatskom trombocitopenijskom purpurom i deficitom vitamina B12. Iako je prisustvo Hp infekcije povezivano sa različitim bolestima za sada nema definitvnog zaključka o uzročno-posledičnoj vezi Hp infekcije i oftalmoloških, dermatoloških i neuroloških bolesti, diabetes melitusa i metaboličkog sindroma.

U poslednje vreme opisuje se i protektivni efekat Hp infekcije vezan za inverzni odnos prisustva Hp i alergijskih bolesti. Autori koji podržavaju ovu hipotezu smatraju da kod dece i mladih osoba prisustvo Hp utiče na adaptivni imuni odgovor promovišići toleranciju imunog sistema da bi održao infekciju, ali time istovremeno inhibira auto agresivne odgovore T ćelija.

Helicobacter pylori: clinical importance

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It is estimated that half of whole human population harbors Helicobacter pylori (Hp) infection. In all infected individuals Hp leads to gastritis irrespective of presence of symptoms. In majority of patients Hp infection is asymptomatic, while in some Hp leads to gastric or duodenal ulcers (25% of allinfected individuals). Gastric cancer and gastric MALT lymphoma are diagnsed in 1% of all infected individuals. Maastricht VI consensus recognised that Hp is a gastric pathogen and Hp gastritis is infectious disease.

In dzspepstic patients testing for Hp is mandatorz. Causal relationship of Hp infection with peptic ulcer disease was established more than 30 years ago. Majority of duodenal and two thirds of gastric ulcers are Hp related. Decades of eradication therapy in Hp positive patients with peptic ulcer lead to decreased incidence of uncomplicated peptic ulcer disease. Eradication therapy, if Hp infection is diagnosed is mandatory in patients with dyspepsia, peptic ulcer disease and patients using ulcerogenic drugs for other diseases. Hp is a first grade carcinogen in the pathogenesis of gastric adenocarcinoma that is yearly diagnosed in more than 1 000 000 patients. Long term Hp infection starts and promotes inflammatory cascade that trough atrophy, intestinal metaplasia and dysplasia leads to gastric cancer. Gastric MALT lymphoma arises as a consequence of Hp induced chronic antigen stimulation in gastric mucosa. MALT is a low grade lymphoma and Hp eradication is 1st line therapy for localized disease. Extragastric manifestations of Hp infection according to Maastricht consensus include iron deficiency anemia of unknown etiology, idiopathic tromocytopenic purpura and B12 deficiency.

All the other implicated extragastric manifestations including dermatological,neurological, ophtalmological disease, metablic syndrome and diabetes still lack evidence on causal relationship with Hp. Lately, there are reports suggesting protective effect of Hp in children and young adults. Authors supporting this hypothesis speculate that presence of Hp influences adaptive immune response and promotes immune tolerance in order to enable persistence of Hp but also inhibits autoagressive T cell mediated responses.

Helicobacter pylori infekcija u Srbiji: molekularna dijagnostika u rutinskoj praksi

Uvod: Sve češća rezistencija Helicobacter pylori na antibiotike je jedan od glavnih razloga neuspešne eradikacije. Tačkaste mutacije odgovarajućih gena su najčešće determinante rezistencije H. pylori na klaritromicin i fluorohinolone.

Cilj rada: Cilj rada je bio da se predstave rezultati molekularne dijagnostike H. pylori, odnosno genetička osnova rezistencije na klaritromicin i fluorohinolone.

Materijal i metode: U studiju je uključeno 176 pacijenata koji su u periodu od 2018. do 2023. godine podvrgnuti gornjoj gastrointestinalnoj endoskopiji radi dobijanja isečaka sluznice želuca. Bioptati su analizirani primenom molekularnog testa (GenoType HelicoDR, Hain Lifescience) kojim su detektovani H. pylori i najčešće mutacija odgovorne za rezistenciju na klaritromicin (23S rRNA gen) i fluorohinolone (gyrA).

Rezultati: Rezistencija H. pylori na klaritromicin je detektovana kod 58,5% sojeva, a rezistencije na fluorohinolone kod 53,9% sojeva, pri čemu je primarna rezistencija na klaritromicin bila 47,2%, a na fluorohinolone 39,6%. Udružena rezistencija na ove antibiotike iznosila je 39,8%. Najčešća mutacija koja kodira rezistenciju H. pylori na klaritromicin je bila A2147G (78,6%), dok je mutacija gena gyrA87 bila najčešća determinanta rezistencije na fluorohinolone (27,4%). Kod 7,8% sojeva rezistentnih na klaritromicin i 16,8% rezistentnih na fluorohinolone, detektovane su istovremeno >1 mutacije za njihovu rezistenciju. Mešani genotipovi su uočeni kod ukupno 14,7% sojeva.

Zaključak: Visoka rezistencija H. pylori na klaritromicin zahteva reviziju nacionalnih empirijskih vodiča za eradikaciju H. pylori i uspostavljanje nadzora nad ovom bakterijom. Protokoli za eradikaciju zasnovani na rezultatima molekularne dijagnostike daju visok procenat izlečenja.

Ključne reči: Helicobacter pylori, GenoType HelicoDR, rezistencija na antibiotike, 23S rRNA gen, gyrA gen

Helicobacter pylori infection in Serbia: molecular diagnostics in routine practice

Introduction: Increasing resistance of Helicobacter pylori to antibiotics is one of the main reasons for unsuccessful eradication. Point mutations of the corresponding genes are determinants of H. pylori resistance to clarithromycin and fluoroquinolones.

Aim: The aim was to present the results of molecular diagnostics of H. pylori, and the genetic basis of resistance to clarithromycin and fluoroquinolones.

Material and methods: The study included 176 patients who underwent upper gastrointestinal endoscopy in the period from 2018 to 2023 to obtain sections of the gastric mucosa. Biopsies were analysed using a molecular test (GenoType HelicoDR, Hain Lifescience) which detected H. pylori and the most common mutation responsible for resistance to clarithromycin (23S rRNA gene) and fluoroquinolones (gyrA).

Results: H. pylori resistance to clarithromycin was detected in 58.5% of strains, and resistance to fluoroquinolones in 53.9% of strains, whereby primary resistance to clarithromycin was 47.2% and to fluoroquinolones 39.6%. Dual resistance to these antibiotics was detected in 39.8% of isolates. The most common mutation encoding H. pylori resistance to clarithromycin was A2147G (78.6%), while the gyrA87 gene mutation was the most common determinant of fluoroquinolone resistance (27.4%). In 7.8% of strains resistant to clarithromycin and 16.8% resistant to fluoroquinolones, simultaneously >1 mutation for their resistance was detected. Mixed genotypes were observed in a total of 14.7% of strains.

Conclusion: The high resistance of H. pylori to clarithromycin requires a revision of the national empirical guidelines for the eradication of H. pylori and the establishment of surveillance of this bacterium. Eradication protocols based on molecular diagnostic results yield a high cure rate.

Keywords: Helicobacter pylori, GenoType HelicoDR, antibiotic resistance, 23S rRNA gene, gyrA gene

Innovative diagnostic methods for Helicobacter pylori infection

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Helicobacter pylori is Gram-negative, spiral shaped, catalase and oxidase positive bacterium that colonizes the human gastric mucosa. About 30 years ago, H. pylori was recognized and classified as Group 1 carcinogen associated with gastric adenocarcinoma, and gastric mucosa-associated lymphoid tissue (MALT) lymphoma.

Today, over 60% of the world's population is colonized with H. pylori. Notably, multiple virulence factors contribute to colonization of the human stomach, with this Gram-negative bacterium, resulting in changes in gastric acid production, chronic inflammation and tissue damage. Rapid diagnostic tools and early treatment onset have been strongly related to eradication and prevention of the development of H. pylori-related diseases. Extensive research has led to development of numerous diagnostic methods/tests, detecting antigens in the stool and serology. Invasive methods involve endoscopy, histological evaluation, rapid urea test, culture and PCR. In recent years, nano (bio) sensors have emerged as promising diagnostic approach, showing numerous advantages such as small volume of samples, simple and fast procedure, cost-effectiveness and portable and miniaturized instruments.

Nano (bio) sensors are highly sensitive and selective and focused on molecular targets, such as synthetic functional DNA molecules: DNA aptamers and DNAzymes obtained by in vitro selection or SELEX (Systematic Evolution of Ligands by Exponential Enrichment) for real-time analysis. The development of point-of-care and lab-on-a-chip devices offers important advantages in terms of improved speed of detection, portability, low cost and integration of smart instruments.

FOODBORNE PATHOGENS – WAYS OF SPREADING AND THEIR DETECTION BY MOLECULAR METHODS

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According to the World Health Organization, there are hundreds of millions of people suffering from diseases caused by contaminated food. The foodborne diarrheal disease is the most common illnesses world-wide (2.2-4 million cases per year). A large number of outbreaks are associated with fresh products like sprouts, leafy greens, tomatoes, melons, berries, fresh herbs, green onions as well as fresh-cut vegetables. These outbreaks are caused by E. coli, Salmonella spp., Listeria monocytogenes, etc. The contamination vegetables by human pathogens is important food safety risk and these pathogens are able to contaminate fresh vegetables at any point of the production chain.

The human pathogenic bacteria (E. coli, Salmonella spp), originated from irrigation water, can contaminate vegetables (carrot, parsley, celery, cabbage, spring onion, tomato, pepper and cucumber) and it is the most common source. The monitoring of contamination vegetables with pathogen bacteria was studied using: Gfp-transformation, Fluorescence In Situ Hybridization (FISH), 3D confocal laser scanning microscopy (CLSM), PCR.

The tomato, pepper, and cabbage, grown in soil with 372 MPN/g coliforms, were positive on Salmonella sp. Escherichia coli, Pseudomonas aeruginosa. E. coli was detected inside lettuce leaves (5.0×102 CFU/g) at plants which were irrigated with contaminated water. Also, Salmonella typhimurium was noticed at lettuce, tomato, celery, sweet corn roots (105 cells/mm3 absolutely dry root). The 25% tested vegetable samples (tomato, paper, cabbage, cucumber, carrot, parsley) were positive on Listeria spp. and Listeria monocytogenes has the ability for colonization of carrot, parsley, celery, sweet corn, lettuce, spinach root in very high number (105 cells/mm3 absolutely dry root).

FOODBORNE PATHOGENS – WAYS OF SPREADING AND THEIR DETECTION BY MOLECULAR METHODS

The Pseudomonas aeruginosa was detected inside vegetable stems, leaves and roots of leafy vegetables in number 104–106 cells/mm3 of absolutely dry root. It shows that these plants can cause infection if eaten in raw.

The pathogens were detected inside plants, although these plants were not in direct contact with contaminated irrigation water. This suggests that pathogen could be transported though the vascular system of the plant to edible parts of plants and caused infection of humans.

Key words: foodborne pathogen, E. coli, Salmonella typhimurium, Listeria monocytogenes, fresh vegetables, FISH, CLSM.

PATOGENI U HRANI – NAČINI ŠIRENJA I NJIHOVA DETEKCIJA MOLEKULARNIM METODAMA

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Prema podacima Svetske zdravstvene organizacije, stotine miliona ljudi pate od bolesti izazvanih kontaminiranom hranom. Dijareja, uzrokovana kontaminiranom hranom, je najčešća bolest širom sveta (2,2-4 miliona slučajeva godišnje). Veliki broj epidemija povezan je sa svežim proizvodima kao što su klice, lisnato povrće, paradajz, dinje, bobičasto voće, sveže začinsko bilje, mladi luk kao i sveže rezano povrće. Ove epidemije izazivaju E. coli, Salmonella spp., Listeria monocytogenes itd. Kontaminacija povrća patogenima predstavlja veliki rizik za zdravstvenu bezbednost hrane i ovi patogeni mogu kontaminirati sveže povrće u bilo kom delu proizvodnog lanca. Patogene bakterije (E. coli, Salmonella spp), poreklom iz vode za navodnjavanje, mogu kontaminirati povrće (šargarepa, peršun, celer, kupus, mladi luk, paradajz, paprika i krastavac) i to je najčešći izvor kontaminacije.

Kontaminacija povrća patogenim bakterijama proučavana je korišćenjem metoda: Gfp-transformacija, Fluorescence In Situ Hybridization (FISH), 3D konfokal laser skenirajuća mikroskopija (CLSM), PCR. Paradajz, paprika i kupus, gajani na zemljištu gde je detektovano 372 MPN/g koliforma, bili su pozitivni na prisustvo Salmonella sp. Escherichia coli, Pseudomonas aeruginosa. E. coli je detektovana unutar listova zelene salate (5,0×102 CFU/g) kod biljka navodnjavanih kontaminiranom vodom. Takođe, Salmonella typhimurium je detektovana u zelenoj salati, paradajzu, celeru, korenu kukuruza šećerca (105 ćelija/mm3 apsolutno suvog korena).

Ukupno 25% testiranih uzoraka povrća (paradajz, paprika, kupus, krastavac, šargarepa, peršun) bili su pozitivni na prisustvo Listeria spp., a Listeria monocytogenes ima sposobnost da kolonizuje koren šargarepe, peršuna, celera, kukuruza šećerca, zelena salata, spanaća u velikom broju (105 ćelija/mm3 apsolutno suvog korena).

PATOGENI U HRANI – NAČINI ŠIRENJA I NJIHOVA DETEKCIJA MOLEKULARNIM METODAMA

Pseudomonas aeruginosa je detektovan unutar stabla, listova i korena lisnatog povrća u broju 104–106 ćelija/mm3 apsolutno suvog korena. To ukazuje da ove biljke mogu izazvati infekciju ukoliko se konzumiraju sirove.

Patogeni su detektovani unutar biljaka, iako ove biljke nisu bile u direktnom kontaktu sa kontaminiranom vodom za navodnjavanje. Ovo sugeriše da patogene bakterije mogu biti transportovane kroz vaskularni sistem biljke do jestivih biljnih delova i izazvati infekciju ljudi.

Ključne reči: patogeni koji se prenosi hranom, E. coli, Salmonella typhimurium, Listeria monocytogenes, sveže povrće, FISH, CLSM.

SESIJA / SESSION

/ INVAZIVNE GLJIVIČNE INFEKCIJE

/ INVASIVE FUNGAL INFECTIONS

Multidisciplinarni pristup u prevenciji, dijagnostici i lečenju invazivnih gljivičnih infekcija

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Gljive su postale jedan od vodećih uzroka infektivnih bolesti poslednjih decenija kod pacijenata na imunosupresivnoj terapiji, nakon transplantacije alogenim matičnim ćelijama, nakon transplantacije čvrstih organa i kod pacijenata zaraženih virusom humane imunodeficijencije. S obzirom da su gljive široko rasprostranjene u vazduhu i životnom prostoru gljive obično izazivaju gljivične infekcije (GI) gornjih i donjih respiratornih puteva (RT) koje dovode do akutnih i hroničnih infekcija kao što je plućna aspergiloza koja se javlja kod teško obolelih od gripa i aspergiloza pluća kod pacijenata sa preležanom infekcijom COVID-19.

Gljive su prisutne u prašini i vazduhu bolničkih soba, uređajima za klimatizaciju i vlažnim zidovima, pogotovo u kupatilu, i na taj način predstavljaju važan rezervoar zaraze. Izloženost ljudi sporama gljiva u vazduhu je neizbežna, a udisanje spora gljiva je jedan od „okidača" za razvoj imunopatoloških procesa u RT koji mogu da se eksprimiraju kao alergijska reakcija ili infekcija. Pravovremena i tačna dijagnoza gljivične infekcije je ključna za rano započinjanje terapije, sprečavanje razvoja invazivne gljivične infekcije (IGI) i povećanje stope preživljavanja.

Multidisciplinarni pristup u prevenciji, dijagnostici i lečenju invazivnih gljivičnih infekcija

U svakoj tercijarnoj zdravstvenoj ustanovi u kojoj se leče ovakvi pacijenti postoji ogromna potreba za timskim radom eksperata u oblasti lečenja gljivičnih infekcija (klinički mikolog), dijagnostike gljivičnih infekcija (medicinski mikolog), imunologa, hematologa, pulmologa, rinologa i kliničkog farmakologa.

Pacijentima sa infekcijama koje su kompleksne za dijagnostiku i lečenje, kao što su IGI ili plućna aspergiloza, treba pružiti individualni pristup i sve odluke donositi na osnovu kombinovanja iskustava i znanja različitih stručnjaka a u skladu sa kliničkom slikom, laboratorijskim i mikrobiološkim nalazom takvog pacijenta. Postoji velika potreba za formiranjem multidisciplinarnog tima za racionalnu upotrebu antigljivičnih lekova u svakoj tercijarnoj zdravstvenoj ustanovi. Kombinovanjem komplementarne ekspertize, korišćenjem sindividualnog pristupa i pružanjem ekspertskih saveta o dijagnostici bradi i antigljivičnom lečenju, multidisciplinarni mikološki tim može imati krucijalni značaj u lečenju i smanjenju mortaliteta pacijenata oboelih od IGI.

A multidisciplinary approach in the prevention, diagnosis and treatment of invasive fungal infections

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Fungi become one of the leading causes of infectious disease in recent decades in those using immunosuppressive medication, allogeneic stem cell, solid organ transplant recipients, and those with human immunodeficiency virus infection. Given fungi are widespread in the air and in the living space, fungi commonly cause fungal infections (FI) of the upper and lower respiratory tract (RT) which lead to acute and chronic infection such as pulmonary aspergillosis occurring in severely ill influenza patients, and COVID-19-associated pulmonary aspergillosis.

Fungi are present in dust and air of hospital rooms, air conditioning and damp walls bathrooms, represent an important reservoir of infection. Human exposure to indoor and outdoor fungal spores in the air is inevitable and the inhalation of fungal spores is one of the “trigger” for the development of immunopathological processes in the RT, which can be in the form of allergic reactions or serious infection.

Timely and accurately diagnosis of fungal infection is crucial for timely initiation of the therapy; prevent the development of invasive fungal infection (IFI) and improving the survival rate. In each tertiary medical center there is a need for expertise medical doctor on fungal pathogens (clinical mycologist), expert in fungal diagnostic (medical mycologist), expert in immunotherapy (immunologist), haematologist, pulmologist, rhinologist and clinical pharmacologist. Patients with complex and problematic diseases for diagnosis and treatment such as IFI or pulmonary aspergillosis are should be observed individually and all decisions should be made after combining experiences and knowledge of different experts. This should be set up as multidisciplinary team for antifungal stewardship in each tertiary care hospital. By combining complementary expertise, employing a structured approach and providing comprehensive advice on diagnostic work-up and antifungal treatment, the multidisciplinary mycology team could have a significant impact on treatment and morbidity of patients with IFIs.

Aktuelni pristup u ispitivanju osetljivosti gljiva na antimikotike: referentne metode i komercijalne tehnike

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Učestalost invazivnih mikoza poslednjih decenija značajno raste, što je u skladu sa kontinuiranim porastom broja imunosuprimiranih pacijenata. Ovo je dovelo do povećane upotrebe sistemskih antimikotika (AM) preporučenih za prevenciju i lečenje. Došlo je i do razvoja rezistencije i multi-rezistencije gljiva na AM, što ugrožava efikasnost lečenja. Zato je ispitivanje osetljivosti gljiva na antimikotike (AFST) dobilo veliki značaj u izboru optimalne terapije.

Aktuelne AFST metode, uključujući referentne i komercijalne, zasnivaju se na inhibiciji rasta gljiva u prisustvu AM, u tečnom ili čvrstom medijumu. Referentne metode Instituta za kliničke i laboratorijske standarde (CLSI) i Evropskog komiteta za ispitivanje osetljivosti na antimikrobne lekove (EUCAST) su bujon mikrodilucione metode i one omogućavaju određivanje minimalnih inhibitornih koncentracija (MIC). CLSI i EUCAST su osnovane da bi izvršile standardizaciju AFST-a i utvrdile granične vrednosti osetljivosti, tj. kliničke granične vrednosti (CBP) i epidemiološke granične vrednosti (ECOFF), koje imaju i direktnu kliničku primenu. Takođe, CLSI je razvio i disk-difuzioni i gradijent-difuzioni metod (E-test) kao standardizovane za kvasnice i plesni. EUCAST je nedavno razvio standard za određivanje rezistencije aspergilusa na azole kao skrining, korišćenjem agar ploče sa četiri bunarčića. Pored referentnih, postoje i komercijalno dostupne metode za ispitivanje osetljivosti (Sensititre YeastOne, Vitek2, ATB Fungus 3, Neo-Sensitabs itd.).

Trenutno je u razvoju nekoliko inovativnih metoda za ispitivanje osetljivosti gljiva na AM. Tehnike zasnovane na MALDI-TOF-u, protočnoj citometriji i kompjuterizovanom imidžingu, obećavaju, ali im je i dalje potrebna opsežna klinička validacija. Dokazivanje mutacija gena povezanih sa rezistencijom na AM molekularnim tehnikama je važna alternativa standardnom AFST-u. Ovo se već koristi u rutinskim mikrobiološkim laboratorijama za detekciju sojeva aspergilusa rezistentnih na azole, iz kulture i direktno iz uzorka. Međutim, molekularna detekcija je i dalje ograničena samo na dokazivanje poznatih mutacija. Razvojem sekvenciranja i pristupom celom genomu gljivične ćelije ovo može da se prevaziđe. Do tada, standardne AFST tehnike su neophodne u rutinskom radu i izboru optimalne terapije i treba da budu lake za korišćenje, ponovljive, jeftine, brze i tačne.

Current approach in antifungal susceptibility testing: reference methods and commercial techniques

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The number of invasive fungal infections observed in the last decades has risen in line with the continually growing number of immunosuppressed patients. This has led to an increased use of systemic antifungals recommended for prevention or treatment. Antifungal resistance and multiresistance are developed compromising treatment efficiency.

Antifungal susceptibility testing (AFST) is therefore of increasing importance for managing patients and adapting therapy. Current AFST methods, including reference and commercial types, are based on growth inhibition in the presence of an antifungal, in liquid or solid media. The Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility testing (EUCAST) reference methods are broth microdilutions allowing the determination of the minimal inhibitory concentrations (MIC). CLSI and EUCAST are formed to standardize technical aspects of AFST and to develop breakpoint, clinical breakpoints (CBP) and epidemiological cut-off values (ECOFF), with direct clinical application. Also, the CLSI has developed disk diffusion and gradient diffusion method (E-test) as standardized protocols for yeast and molds. EUCAST has recently developed a standard for the determination of azole resistance in Aspergillus isolates using a four-well agar screening plate.

In addition to the reference methods, there are commercially available products for antifungal susceptibility testing (SensititreYeastOne, Vitek2, ATB Fungus 3, Neo-Sensitabs and other).

Several innovative methods are currently under development to improve AFST. Techniques based on MALDI-TOF, flow cytometry and computed imaging, are promising, but still need extensive clinical validation.

Current approach in antifungal susceptibility testing: reference methods and commercial techniques

Molecular detection of mutations associated with antifungal resistance is an important alternative to standard AFST. It is already used in routine microbiology labs for detection of azole resistance in Aspergillus isolates and directly from samples.

But, molecular detection is still restricted to known mutations. The development of Next Generation Sequencing (NGS) and whole-genome approaches may overcome this limitation. While promising approaches are under development, AFST techniques are necessary for routine practice and optimal patient management and should be user-friendly, reproducible, low-cost, fast and accurate.

Multirezistentne preteće kvasnice kao dijagnostički izazov: *Candida auris*

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Candida auris je sve značajnija vrsta kvasnica koja izaziva različite bolesti, a pre svega bolničke infekcije i pokazuje smanjenu osetljivost na antimikotike. Globalna epidemiologija još uvek nije definisana, pre svega zbog izazova u identifikaciji C. auris konvencionalnim laboratorijskim metodama. Filogenetskom analizom identifikovano je 5 različitih klada C. auris (Južna Azija, Istočna Azija, Južna Afrika, Južna Amerika, Iran). Važnu ulogu u patogenezi ima produkcija fosfolipaza i proteinaza, kao i specifičan rast u "suvom" biofilmu.

Faktori rizika za nastanak C. auris infekcije su hronične bolesti (dijabetes melitus, bolest bubrega), abdominalne i vaskularne operacije, urinarni i centralni venski kateteri, parenteralna ishrana, dužina boravka u jedinicama intenzivne nege, hemoterapija itd. Kliničke manifestacije uključuju sistemske infekcije, infekcije urinarnog trakta, srednjeg uha, kostiju, infekcije rana, apscese kože, miokarditise i meningitise. Identifikacija C. auris je ključna za započinjanje adekvatnog lečenja i kontrole bolničkih epidemija. Ova kvasnica se kultiviše standardnim metodama na Sabouraud dekstroznom agaru, s tim što toleriše temperature do 42°C. Na hromogenim podlogama se uočavaju bele, ružičaste ili ljubičaste kolonije. Kada se koriste biohemijske metode, može doći do lažno negativog nalaza, zato što se C. auris ne nalazi u većini baza podataka i može biti identifikovana kao neka druga kvasnica, najčešće Candida haemuloni. Praksa pokazuje da je MALDI-TOF najpouzdanija metoda za identifikaciju C. auris, gde se ova gljiva nalazi u bazi podataka. Kada je reč o molekularnoj dijagnostici, za sada su razvijena dva komercijalna PCR testa.

Mehanizmi rezistencije C. auris su slični mehanizmima drugih vrsta Candida. Ova vrsta pokazuje rezistenciju na flukonazol, kao i povišene vrednosti MIC za sve tri glavne klase antifungalnih lekova, tj. azole, poliene i ehinokandine. Iako za sada ne postoje zvanične preporuke i vodiči, kao terapija izbora u slučaju dokazane ili verovatne invazivne C. auris infekcije, preporučuju se ehinokandini, sa predloženim kliničkim graničnim vrednostima od 2 µg /ml za kaspofungin, 4 µg /ml za anidulafungin i 4 µg /ml za mikafungin.

Multidrug-resistant yeasts as a diagnostic challenge: *Candida auris*

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Candida auris is an emerging multi-drug resistant fungal pathogen responsible for nosocomial infections. The global epidemiology is not well known, mostly because is challenging to identify C. auris by conventional laboratory methods.

Phylogenetic analysis identified a strong phylogeographic structure comprising 5 distinct C. auris clades (South Asia, East Asia, South Africa, South America, Iran). Production of phospholipases and proteinases in a strain-dependent manner play important role in pathogenesis.

Risk factors for C. auris infections are chronic illnesses (diabetes mellitus, kidney disease), abdominal and vascular surgeries, urinary and central venous catheters, parenteral nutrition, length of ICU stay, chemotherapy, etc. The important and frequent clinical manifestations of C. auris infection include systemic infections in the bloodstream, infection in the urinary tract, middle-ear, bone, infections at surgical sites, skin abscesses, myocarditis, and meningitis. Identification of C. auris is crucial to initiate adequate treatment and contain hospital outbreaks. Colonies can be easily obtained on Sabouraud dextrose agar, where C. auris tolerate temperature up to 42°C. On chromogenic media colonies appear white, pink or purple. When using biochemical methods, it can be misidentified because C. auris is not referenced in most of the databases of former handbooks, which lead to false negative results and misidentification with other yeasts, notably Candida haemuloni clade. Definitive identification of C. auris species can be achieved by mass spectrometry MALDI-TOF with an up-to-date spectra database. For now two commercial PCR based tests are developed.

One of the reasons why the emergence of C. auris has been so alarming is the potential of this species to harbor or develop multidrug resistance. It is believed that the already established mechanisms of drug resistance in other Candida species might be in action in the multi-drug resistant C. auris. Almost all C. auris strains exhibit in vitro resistance to fluconasole. Some C. auris strains exhibit elevated MIC for all three major classes of antifungal drugs i.e. azoles, polyenes and echinocandins. As a first therapy line in the case of proven or probable C. auris invasive infection, echinocandines are suggesting, with proposed clinical breakpoints of 2, 4, and 4 µg/ml for caspofungin, anidulafungin and micafungin.

Invazivne gljivične infekcije kod dece, nakon transplantacije matičnih ćelija hematopoeze: klinička slika i terapijske mogućnosti

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Transplantacija matičnih ćelija hematopoeze (TMĆH) invazivna je procedura u lečenju malignih i nemalignih bolesti čijoj invazivnosti doprinose primene visokih doza citotoksičnih lekova, zračenje, posledično oštećenje tkiva i organa i razvoj teške, jatrogene aplazije koštane srži - imunokompromitovanosti, kao i drugih poremećaja proisteklih iz ovih faktora.

Infektivne komplikacije među najčešćima su u toku i posle TMĆH. To su obično oportunističke bakterijske i virusne infekcije, ali i gljivične infekcije koje kod teško imunokompromitovanih pacijenata mogu dovesti i do invazivnih, po život opasnih infekcija (IGI).

Klinička slika IGI širokog je spektra i obuhvata invaziju i poremećaje funckije pojedinačnih organa, ali i sistemske bolesti. U zavisnosti od zahvaćenog organa razvijaju se i simptomi – febrilnost, disfunkcija zahvaćenog organa (dispneja, desaturacija, povećanje enzima jetre, lokalna destrukcija tkiva, bol, otok).

Dijagnostika obuhvata postavljanje kliničke sumnje, radiografske pretrage kao i laboratorijska i mikrobiološka ispitivanja. Standardne mikrobiološke tehnike (kultivacija i identifikacija) zlatni su standard dijagnostike, dok se novije tehnike – biomarkeri (manan, galaktomanan) kao i molekularne tehnike (PCR) sve više koriste.

Terapija IGI obuhvata profilaktičku i terapijsku primenu antimikotika. Profilaksa je indikovana kod svih pacijenata sa visokim rizikom za razvoj IGI (incidenca IGI veća od 10% - AML, rekurentna ALL, stanje TMĆH) ali se i lokalni epidemiološki podaci moraju uzeti u obzir. Najčešće se u profilaksi koristi flukonazol, ali se preporučuje pretransplantaciono budući da deluje samo na kvasnice, dok se posttransplantaciono, kao i kod pacijenata sa visokim rizikom za IGI izazvane plesnima preporučuje

Invazivne gljivične infekcije kod dece, nakon transplantacije matičnih ćelija hematopoeze: klinička slika i terapijske mogućnosti

Terapija IGI obuhvata sistemsku primenu antimikotika i to kod invazivne aspergiloze, na prvom mestu, vorikonazola i lipozomalnog amfotericina B, dok kod invazivne kandidijaze prvi nivo preporuke čine kaspofungin, lipozomalni amfotericin B, mikafungin i vorikonazol. Drugi terapijski modaliteti uključuju operativno uklanjanje zahvaćenog tkiva (npr. mukormikoza), infuzije granulocita i sl. Cilj uspešne terapije je pravovremena stratifikacija pacijenata u grupe rizika, adekvatna profilaktička terapija, brza i efikasna dijagnostika i terapija, kao i kontrola komorbiditeta.

Invasive fungal infections in children following hematopoietic stem cell transplantation: clinical presentation and therapy

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Hematopoietic stem cell transplantation is a invasive procedure in the treatment of malignant and non-malignant diseases, due to use of high doses of cytotoxic drugs and radiation and consequential tissues and organ damage, leading to severe immunocompromised condition, as well as other disorders.

Infectious complications are among the most common ones during and after HSCT. Those are usually opportunistic bacterial and viral infections, but also fungal infections that can arise to invasive, life threatening, infections in heavily immunocompromised patients (IFI).
The clinical presentation of the IFI is of wide spectrum and includes invasion and disorders of individual organs, but also systemic disease. Depending on the affected organ, symptoms arise: fever, dyspnea, desaturation, increase of liver enzymes, local tissue destruction, pain, oedema.
Diagnostics include clinical suspicion, radiographic findings as well as laboratory and microbiological tests. Standard microbiological techniques (cultivation and identification) are golden standard of diagnostics, while newer techniques - biomarkers (mannan, galactomannan) as well as molecular techniques (PCR) are increasingly used.

IFI therapy includes prophylactic and therapeutic use of antimicotics. Prophylaxis is indicated in all high-risk patients (incidence of IFI greater than 10% - AML, recurrent All, state of HSCT), but local epidemiological data must also be considered. Most often prophylactic antimycotic used is fluconazole, but it’s use is recommended in the pretransplant period, as it only affects yeasts, while in post HSCT prophylaxis a drug of choice is posaconasole, due to its effect to molds as well as yeasts.
IFI therapy includes systemic use of antimicotics, in case of aspergillosis, in the first place, voriconazole and liposomal amphotericin B, while in cases of invasive candidiasis caspofungin, liposomal amphotericin B, micafungin and voriconazole. Other therapeutic modalities include surgical removal of affected tissue (e.g. mucormycosis), granulocyte infusions, etc.

Značaj primene multipleks PCR metode u dijagnostici invazivne kandidijaze

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Kao članovi mikrobiote kože i sluzokoža, gljive roda Candida mogu predstavljati invazivne patogene kod pacijenata pod rizikom, pre svega kod hematoonkoloških, transplantiranih i pacijenata u jedinicama intenzivnog lečenja. EORTC/MSG navode da je za potvrdu dokazane invazivne gljivične infekcije neophodna mikrobiološka i/ili histopatološka dijagnostička metoda. Pozitivna hemokultura je i dalje referentni standard za mikrobiološku potvrdu infekcija krvi/invazivnih infekcija izazvanih gljivama roda Candida, uprkos njenoj niskoj osetljivosti (otprilike 50%) i dužem trajanju procesa izolacije/identifikacije (3-5 dana). Nasuprot tome, testovi za detekciju 1,3-β-D-glukana (BDG), manana i anti-Candida/manan antitela omogućavaju dobijanje rezultata u kraćem vremenskom periodu, ali problem su njihova varijabilna senzitivnost i specifičnost kod različitih grupa pacijenata. Značajno je da ovi serološki testovi ne omogućavaju razlikovanje vrsta unutar roda Candida, što je veliki nedostatak u pogledu izbora terapije. Uočena je i niža osetljivost BDG i manan testova u dijagnostici invazivne infekcije izazvane vrstom C. parapsilosis.

Molekularni testovi kao što je PCR imaju potencijal da prevaziđu navedena ograničenja bržim otkrivanjem i identifikacijom vrste Candida, omogućavajući na taj način primenu rane, ciljane i efikasne terapije. Većina današnjih singlepleks i multipleks real-time PCR testova uglavnom su proizvod in-house protokola. Međutim, postoji i nekoliko komercijalno dostupnih multipleks real-time PCR testova prvenstveno dizajniranih da razlikuju flukonazol rezistentne vrste C. krusei i C. glabrata od drugih vrsta Candida. Nažalost, postoji nekoliko razloga zbog kojih PCR nije naširoko prihvaćen u dijagnostici invazivne kandidijaze: nedefinisan izbor optimalanog uzorka (puna krv, serum ili plazma); mali broj ćelija kvasnica u krvi, velike količine pozadinske DNK i koekstrahovanih supstanci koje ometaju ili inhibiraju PCR reakciju. Iz navedenih razloga dijagnostičke performanse dobijene nakon izvođenja PCR reakcija uveliko variraju u različitim studijama: senzitivnost se kreće u opsegu od 33% do 100% i specifičnost od 54% do 94%. Trenutne smernice za dijagnozu invazivne kandidijaze prepoznaju potencijal molekularne dijagnostike, ali postoje problemi zbog nedostatka uporedivih podataka i standardizacije testova.

Ključne reči: Candida, invazivna kandidijaza, multipleks real-time PCR

The importance of applying the multiplex PCR method in the diagnosis of invasive candidiasis

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As mucosal and skin microbiota, yeasts of the genus Candida can turn to invasive pathogens in vulnerable groups such as hemato-oncological, transplant and intensive care patients. EORTC/MSG states that, for proven diagnosis of invasive fungal infection, microbiological and/or histopathological diagnostic method is required. Thus, blood cultures are still the reference standard for the microbiological diagnosis of Candida bloodstream/invasive infections despite its low sensitivity (approximately 50%) and long turnaround times (3–5 days). In contrast, β-D-glucan (BDG), mannan and anti-Candida/manan detection assays promise more rapid results, but with variable sensitivity and specificity in different patient groups. Considerably, these serologic assays do not allow discrimination between different Candida species, which is a major drawback regarding therapy management. Lower sensitivity of BDG and mannan assays in the detection of C. parapsilosis invasive infection has been observed.

Molecular tests such as PCR are capable of overcoming these limitations allowing the rapid Candida detection and identification, providing the potential to administer early, effective species oriented therapy. The majority of singleplex or multiplex real-time PCR assays is mainly done using in-house protocols. However, there are a few commercially available multiplex real-time PCR assays primarily designed to distinguish fluconazole-resistant C. krusei and C. glabrata from other Candida species. Nevertheless, there are several reasons why Candida PCR testing has not gained widespread acceptance in diagnosis of invasive candidiasis: optimal specimen choice (whole blood, serum or plasma) remains unclear; low pathogen loads, high amounts of background DNA, and co-extracted substances perturbing or even inhibiting PCR reactions. These facts is exemplified by the diagnostic parameters established PCR assays, which varies broadly from 33% to 100% sensitivity and 54% to 94% specificity, depending on study. Based on all of the above current guidelines recognize the potential of molecular diagnostics but are concerned with the lack of comparable data and assay standardization.

Keywords: Candida, invasive candidiasis, multiplex real-time PCR

SESIJA / SESSION

/ BOLNIČKE INFEKCIJE
/ HEALTHCARE-ASSOCIATED INFECTIONS

Da li smo spremni za izazove antimikrobne rezistencije?

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UVOD: Rezistencija bakterija na antibiotike se razvija relativno brzo, prenosiva je. Može biti urođena ili stečena, odnosno može nastati u toku terapije. Infekcije uzrokovane mikroorganizmima otpornim na većinu ili sve dostupne antibiotike, su u porastu u posljednjih deset godina i predstavljaju veliki terapijski problem kod nas i u svetu. Prisustvo multirezistentnih mikroorganizama (MDR), ekstenzivno rezistentnih (XDR) i panrezistentnih mikroorganizama (PDR) u bolničkoj sredini, uz postojanje faktora rizika za njihovo prenošenje, omogućava njihovo širenje. Približno 10% hospitalizacija se komplikuje bolničkim infekcijama, naročito na odeljenjima intenzivne nege.

CILJ RADA: Prikazati rezultate tima za upravljanje antibioticima UKCS. Utvrditi značaj i ulogu mikrobiološke laboratorije u kontroli i praćenju profila rezistencije izolata pacijenata lečenih u UKCS.

METODOLOGIJA: Retrospektivna analiza rezultata tima za upravljanje antibioticima (ASP tim).

REZULTATI: Upravljanje antibioticima je set usklađenih intervencija koje imaju za cilj unapređenje i praćenje pravilne upotrebe antibiotika putem promocije odabira optimalnog leka, njegove doze, trajanja terapije i puta primene. Multidisciplinarni ASP tim UKCS čine infektolog, klinički farmaceut, mikrobiolog, epidemiolog i ordinirajući lekari.

Da li smo spremni za izazove antimikrobne rezistencije?

Brza i tačna dijagnoza, primena senzitivnih testova, primena EUCAST standarda i najnovijih preporuka o prvom izboru i rezervnim antibioticima pri ispitivanju osetljivosti na antibiotike, selektivno izveštavanje, monitoring, prepoznavanje i praćenje novih trendova rezistencije, kontrola upotrebe antibiotika, imaju značaj terapiji i u otkrivanju rezistentnih mikroorganizama. Mikrobiološki rezultati omogućavaju donošenje preporuka za upotrebu antibiotika, imaju značajni uticaj na smanjenje postojeće i razvoj nove rezistencije.

ZAKLJUČAK: Mikrobiološka laboratorija je važan, integralni deo ASP tima. Brzi razvoj mikrobioloških tehnika i testova zahteva prisustvo mikrobiologa u timu, stalnu komunikaciju, saradnju sa kliničarima.

KLJUČNE REČI: MDR, antimikrobni lekovi, rezistencija bakterija, tim za upravljanje antibioticima

Are we ready for the challenges of antimicrobial resistance?

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Background: Bacterial resistance to antibiotics is developed relatively fast, may be transferred and formed in the course of treatment. Infections caused by microorganisms resistant to most or all available antibiotics are increasing in the past decade and represent a major therapeutic, multidisciplinary problem. The presence of MDR, XDR and PDR microorganisms in the hospital environment, with risk factors for transmission, enabling their expansion. Approximately 10% of hospitalizations complicated by nosocomial infections, especially in the intensive care unit.

OBJECTIVE: To show UCCS Antimicrobial Stewardship Team (ASP team) results. To determine the significance and role of the microbiological laboratory in the control and monitoring of the resistance profile of isolates from patients treated in UCCS.

Methods: A retrospective analysis of the ASP Team results.

Results: Antibiotic Stewardship is a set of coordinated interventions aimed at improving and monitoring the correct use of antibiotics by promoting the selection of the optimal drug, its dose, duration of therapy and route of administration. The multidisciplinary ASP team of the UCCS consists of an infectious disease specialist, a clinical pharmacist, a microbiologist, an epidemiologist and prescribing physicians. Fast and accurate diagnosis, the application of sensitive tests, the application of EUCAST standards and the latest recommendations on first-choice and backup antibiotics when testing antibiotic sensitivity, selective reporting, monitoring, recognition and monitoring of new resistance trends, control of antibiotic use, are important for therapy and in the detection of resistant microorganisms. Microbiological results have a significant impact on the reduction of existing and the development of new resistance.

Conclusions: The microbiology laboratory is an integral part of the ASP team. The rapid development of microbiological techniques and tests requires the presence of microbiologists in the team, constant communication and cooperation with clinicians.

KEY WORDS: MDR, antibiotics, bacterial resistance, Antibiotic Stewardship Team

Značaj multidisciplinarnog pristupa u borbi protiv antimikrobne rezistencije

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Uvod

Antimikrobna rezistencija jedan je najvažnijih problema u zemljama širom sveta i veliki je javno zdravstveni problem. Potreban je detaljan i sveobuhvatan plan za borbu protiv otpornosti na antimikrobne lekove. Uloženo je mnogo napora na međunarodnom nivou da se reši ovaj globalni zdravstveni problem, ali ostaje mnogo posla. Prema podacima Centra za kontrolu i prevenciju bolesti (SDS) iz 2019 godine, svake godine registruje se 2,8 miliona infekcija i 32.000 smrtnih slučajeva izazvanih uzročnicima otpornih na antimikrobne lekove.

Pored toga, Svetska zdravstvena organizacija (SZO) je odobrila smernice za praćenje i procenu rastuće antimikrobne rezistencije. Evropski centar za prevenciju i kontrolu bolesti (ECDC) procenjuje da se skoro 9 miliona bolničkih infekcija (ili infekcija povezanih sa zdravstvenom negom) javlja svake godine samo u evropskim bolnicama, pri čemu je 1/3 ovih infekcija uzrokovano uzročnicima otpornim na antimikrobne lekove

Cilj

Cilj rada je ukazati na značaj multidisciplinarnog pristupa u borbi protiv antimikrobne rezistencije

Metodologija

World Health Organization Antimicrobial Resistance Division https://www.who.int/antimicrobial-resistance/en/; CDC Training on Antibiotic Stewardship; Antimicrobial stewardship interventions: a practical guide (WHO Regional Office for Europe, 2021)

Značaj multidisciplinarnog pristupa u borbi protiv antimikrobne rezistencije

Rezultati

Zbog značaja problema otpornosti uzročnika na antimikrobne lekove neophodan je multidisciplinarni pristup rešavanja problema AMR, u kome učestvuju lekari različitih specijalnosti (infektolog, anesteziolog, hirurg, mikrobiolog, epidemiolog, klinički farmalokog...). Antimikrobni programi upravljanja (ASP) su instrumentalni i ključni u borbi protiv antimikrobnih otpornosti. ASP je efikasan borbi protiv rastuće otpornosti uzročnika na antimikrobne lekove a sve u cilju smanjenja antimikrobne rezistencije, smanjenjem infekcija povezanih sa zdravstvenom negom i smanjenjem troškova lečenja, sa naglaskom multidiciplinarnog pristupa u rešavanju ovog velikog globlnog javno zdravstvenog problema.

Zaključak

Multidiciplinarni pristup u rešavanju rastuće rezistencije na antimikrobne lekove od velikog je značaja u rešavanju ovog velikog globalnog javno zdravstvenog problema.

Ključne reči

antimikrobna rezistencija, upravljalje antibioticima, multidisciplinarni pristup.

The importance of a multidisciplinary approach in the fight against antimicrobial resistance

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Introduction: Globally, antimicrobial resistance has emerged as a significant threat. A comprehensive plan is required to combat antimicrobial resistance. There have been national and international efforts to address this global health problem, but much work remains. The Centers for Disease Control and Prevention (CDC) reported in 2019 that 2.8 million infections and 32,000 deaths due to resistant pathogens occur each year.

Additionally, the World Health Organization (WHO) has endorsed guidance to monitor and evaluate rising antimicrobial resistance and delegated responsibilities for country governments, the One Health Tripartite mission, and other global partners. The European Center for Disease Prevention and Control (ECDC) estimates that almost 9 million nosocomial infections (or healthcare-associated infections) occur each year in European hospitals alone, with 1/3 1/3 of these infections being caused by bacteria with some level of AMR.

Objective: The aim of the paper is to point out the importance of a multidisciplinary approach in the fight against antimicrobial resistance.

Methodology: World Health Organization Antimicrobial Resistance Division https://www.who.int/antimicrobial-resistance/en/; CDC Training on Antibiotic Stewardship; Antimicrobial stewardship interventions: a practical guide (WHO Regional Office for Europe, 2021)

Results: To highlight the urgent threats posed by antimicrobial resistance, the CDC has produced a report (updated in 2019) to characterize the significant issues related to antimicrobial resistance as well as threat level designations for resistant pathogens. Due to the importance of this problem, a multidisciplinary approach to solving the problem of AMR is necessary, in which doctors of various specialties participate (infectologist, anesthesiologist, surgeon, microbiologist, epidemiologist, clinical pharmacologist...).

The importance of a multidisciplinary approach in the fight against antimicrobial resistance

Antimicrobial stewardship programs (ASPs) are instrumental and pivotal in the fight against antimicrobial resistance. ASPs are effective at providing high-value care by reducing unnecessary antimicrobials, reducing rates of antimicrobial resistance, reducing healthcare-associated infections, and reducing costs.

Conclusion: Implementation of educational interventions for trainees encompassing these key areas along with training on policy and leadership development is critical to enable sustainability of these efforts to fight back against antimicrobial resistance.

Key words: antimicrobial resistance, Antimicrobial stewardship programs, multidisciplinary approach in Antimicrobial stewardship programs.

Carbapenem resistant Klebsiella pneumoniae – a five-year study

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The aim of the study was to determine the prevalence, susceptibility, and the type of carbapenemases of carbapenem-resistant K.pneumoniae (CRKp).

During a five-year-period (2017-2021) all samples (urine, wound, blood, tubus/canila, sputum, tracheal aspirates) obtained from the hospitalized patients were analyzed at the Institute of Microbiology and Parasitology, Faculty of Medicine, Skopje. Determination of the susceptibility was done by disk diffusion method, Vitek 2 and E-tests. CIM method and carbapenemase set (Mast Diagnostic) were performed for detection of carbapenemase production to all strains that showed smaller inhibition zones to carbapenems according EUCAST. Easyplex SuperBug CRE (Amplex Biosystems, Germany) was performed for detection of beta-lactamase genes.

Out of the total number of Klebsiella pneumoniae – Kp, carbapenem resistance was detected in 18-22% of the strains. In 90 randomly selected isolates with positive phenotypic tests for carbapenemase detection, metallo-beta lactamases encoding genes were detected, such as: NDM (89.5%), OXA-48 (9.2%), VIM (1.3%). Between 90-100% of Kp strains were resistant to 9 tested beta-lactams and ciprofloxacin. The resistance to meropenem and to cotrimoxazole was between 80-89%, to imipenem 50-80%, to gentamicin 40-80% and to amikacin 30-50%. Increased MICs to colistin were detected in 12,5-18% of all isolates in a tested period.

The percentage of CRKp isolates is increasing and it is the right time to perform all strategies to control their spread.

Key words: Carbapenem-resistance, Klebsiella pneumoniae, antibiotics

Molekularna epidemiologija multirezistentnih sojeva Pseudomonas aeruginosa u Srbiji

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Uvod: Pseudomonas aeruginosa je značajan bakterijaki patogen koji izaziva spektar vanbolničkih i bolničkih infekcija. Svetska zdravstvena organizacija je proglasila karbapenem rezistentni P. aeruginosa (CRPA) za jedan od tri kritična patogena. Ciljevi prve nacionalne multicentrične studije sprovedene u Srbiji su bili sledeći: procena učestalosti i mehanizama rezistencije kliničkih izolata P. aeruginosa na antibiotike, sagledavanje genetičkog konteksta gena koji kodiraju metalo-beta-laktamaze (MBL) i dobijanje uvida u genetičku srodnost MBL-pozitivnih izolata.

Materijal i metode: U periodu od 2018. do 2021. godine, od ispitivanih 5334 kliničkih uzoraka iz različitih bolnica širom Srbije, P. aeruginosa je izolovan iz 320 (6%). CRPA izolati su testirani na prisustvo blaCTX-M-2, blaPER, blaTEM, blaSHV, blaVEB i blaGES,, dok su MBL-pozitivni izolati ispitivani na gene blaVIM, blaIMP i blaNDM. MLST (engl. Multilocus sequence typing) genotipizacija je urađena kod svih MBL pozitivnih P. aeruginosa. Takođe, izvršena je filogenetska analiza četri izolata koji su nosioci blaNDM i 161 genoma sa istim tipom sekvence (ST) koji su preuzeti iz NCBI baze.

Rezultati: Većina P. aeruginosa je izolovana iz uzoraka donjeg respiratornog trakta (n=120; 37.5%) i rana (n=108; 33.75%). CRPA izolati su činili 43.1% (n=138) izolovanih P. aeruginosa, a 31 od njih je bio blaNDM-1-pozitivan (22.5%). Prevalencija rezistencije na kolistin je iznosila 0,3%. MLST tipizacijom je utvrđeno da su najčešći ST P. aeruginosa bili ST235 (n=25) i ST654 (n=6), čija je rasprostranjenost uglavnom ograničena na Srbiju. Na osnovu distribucije gena koji kodiraju beta-laktamaze utvrđeno je da su izolati klonski diseminovani uz moguće rekombinacije: ST235/blaNDM-1, ST235/blaNDM-1/blaPER-1, ST654/blaNDM-1, ST654/blaNDM-1/blaPER-1, and ST654/blaNDM-1/blaGES-5.

Zaključak: U Srbiji su po prvi put detektovani visoko rizični klonovi P. aeruginosa ST235 i ST654 koji predstavljaju značajne vektore za MBL i ESBL. Cirkulisanje ovih multirezistentnih klonova u našoj zemlji je zabrinjavajuće.

Ključne reči: karbapenem rezistentan P. aeruginosa, metalo-beta laktamaze, MLST, blaNDM-1

Molecular epidemiology of multidrug-resistant isolates of Pseudomonas aeruginosa in Serbia

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Introduction: Pseudomonas aeruginosa is an important pathogen involved in a variety of community and hospital-acquired infections. World Health Organisation designated Carbapenem-resistant P. aeruginosa (CRPA) as one of three critical priority pathogens. The aims of the first Serbian nationwide multicentre study were to provide data on antimicrobial susceptibility, underlying resistance mechanisms, genetic context of metallo-β-lactamase (MBL) genes, and clonal relationships between clinical isolates of P. aeruginosa carrying MBL genes.

Material and methods: Overall, P. aeruginosa was detected in 320 out of 5334 (6%) of isolates that were collected between 2018 and 2021 from patients admitted to various hospitals throughout Serbia. Whereas CRPA isolates were screened for the presence of the blaCTX-M-2, blaPER, blaTEM, blaSHV, blaVEB, and blaGES, MBL-positive isolates were examined for the existence of the blaVIM, blaIMP, and blaNDM genes. Multilocus sequence typing (MLST) was carried out for P. aeruginosa-producing MBL. In addition, four genomes of P. aeruginosa harbouring the blaNDM gene together with 161 previously published genomes of the same STs, available in the NCBI Pathogen Detection database were subjected to phylogenomic analyses.

Results: The majority of the isolated P. aeruginosa were recovered from the lower respiratory tract (n=120; 37.5%) and wound specimens (n=108; 33.75%). CRPA isolates accounted for 43.1% (n=138) of the tested isolates, 31 out of them being blaNDM-1-positive (22.5%). The prevalence of colistin resistance was 0.3%. MLST analysis revealed the occurrence of ST235 (n=25) and ST654 (n=6), mostly confined to Serbia. The distribution of the beta-lactamase-encoding genes in these isolates suggested clonal dissemination and possible recombination: ST235/blaNDM-1, ST235/blaNDM-1/blaPER-1, ST654/blaNDM-1, ST654/blaNDM-1/blaPER-1, and ST654/blaNDM-1/blaGES-5.

Conclusion: High-risk clones ST235 and ST654 identified for the first time in Serbia, are important vectors of acquired MBL and ESBL and their associated multidrug resistance phenotypes represent a cause for considerable concern.

Keywords: Carbapenem-resistant P. aeruginosa, of metallo-β-lactamase, MLST, blaNDM-1

Hospital antibiotic stewardship in the era of increasing resistance: between collective responsibility and true collaboration

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More than ten years ago, Isabelle Baker wrote about overcoming the "bystander effect" concerning collective responsibility. Namely, she emphasized whether collectives can be "morally responsible" like individuals. Antibiotic stewardship first appeared as a term on Pubmed in 1996, and in 2005 there were ten such results per year, in 2008 - 50 and 2011 - 100. Finally, leading organizations regularly issue recommendations for the sparing use of antibiotics (the latest edition of ECDC guide 2017, CDC - 2019, and WHO-2022). Finally, a similar guide was published in the Republic of Serbia at the end of 2018. All such recommendations clearly define the responsibility of individuals in healthcare institutions to slow the development of antimicrobial resistance and overuse of antibiotics. Of course, guidelines for the use of antibiotics in hospitals are only part of the overall combat for the sparing use of antibiotics, including the outpatient and veterinary sectors. Despite that, we are witnessing the growing resistance of bacteria to antibiotics and numerous examples of irrational use. The COVID-19 pandemic has only exacerbated the problem, raising whether hospital antibiotic stewardship only relies on collective responsibility.

The concept of medication therapy management (MTM) has been developing in parallel for the last twenty years, primarily in the pharmaceutical sector. Hospital MTM has mechanisms for effective control of antibiotic management; however, many elements need to be improved in clinical practice (e.g., issuing reserve antibiotics for 'emergencies' without following the adopted procedure, errors in prescribing antimicrobial drugs, unnecessarily long administration of antibiotics, delay in issuing microbiological results or prescribing antibiotics solely based on growth biomarker values). Strengthening individual responsibility, better education, and improving hospital MTM systems can help reduce bacterial resistance. However, only comprehensive action that includes both hospital and non-hospital health facilities, that is, the humane and veterinary sectors, can bring sustainable progress.

Upravljanje antibioticima u bolnici u eri rastuće rezistencije: između kolektivne odgovornosti i istinske saradnje

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Pre više od deset godina, Izabel Bejker je pisala o prevazilaženju „efekta posmatrača" u odnosu na kolektivnu odgovornost. Naime, ona je naglasila pitanje da li kolektivi mogu biti 'moralno odgovorni' kao i pojedinci. Upravljanje antibioticima se prvi put pojavilo kao termin na Pubmed-u 1996. godine, a 2005. godine bilo je deset takvih rezultata godišnje, 2008. - 50, a 2011.- 100. Konačno, vodeće organizacije redovno izdaju preporuke za štedljivu upotrebu antibiotika (poslednje izdanje ECDC vodiča 2017, CDC - 2019. i SZO.-2022). Konačno, sličan vodič je objavljen i u Republici Srbiji krajem 2018.

Sve takve preporuke jasno definišu odgovornost pojedinih aktera u zdravstvenim ustanovama za usporavanje razvoja antimikrobne rezistencije i prekomerne upotrebe antibiotika. Naravno, uputstva za upotrebu antibiotika u bolnicama su samo deo ukupne borbe za štedljivu upotrebu antibiotika, koja uključuje i vanbolnički sektor, kao i veterinu. Bez obzira na sve, svedoci smo da raste rezistencije bakterija na antibiotike, kao i brojnih primera neracionalne upotrebe. Pandemija COVID-19 samo je pogoršala problem, postavljajući pitanje da li bolničko upravljanje antibioticima počiva samo na kolektivnoj odgovornosti.

Koncept upravljanja lekovima (Medication Therapy Management, MTM) se paralelno razvijao poslednjih dvadeset godina, pre svega u farmaceutskom sektoru. Bolnički MTM ima mehanizme za efikasnu kontrolu upravljanja antibioticima; međutim, u kliničkoj praksi potrebno je usavršiti mnoge elemente (npr. izdavanje rezervnih antibiotika za 'hitne slučajeve' bez poštovanja usvojene procedure, greške u propisivanju antimikrobnih lekova, nepotrebno dugo davanje antibiotika, kašnjenje u izdavanju mikrobioloških rezultata ili propisivanje antibiotika isključivo na osnovu na porasta vrednosti biomarkera). Jačanje individualne odgovornosti, bolja edukacija i unapređenje MTM sistema u bolnicama mogu pomoći u smanjenju otpornosti bakterija. Međutim, samo sveobuhvatna akcija koja uključuje i bolničke i vanbolničke zdravstvene ustanove, odnosno i humani i veterinarski sektor, može doneti održiv napredak.

/ STREPTOKOKNE INFEKCIJE
/ STREPTOCOCCAL INFECTIONS

Različite kliničke prezentacije pneumokoknih meningitisa

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S. pneumoniae je najznačajniji uzročnik bakterijskog meningitisa u razvijenim zemljama. Naročito je zabrinjavajuća činjenica da je smrtnost od ove infekcije oko 10% uprkos dostupnosti sofistiricanih mera lečenja. Uzevši u obzir permanentnu koevoluciju ovog patogena sa čovekom, kao i izmenjen profil potencijalnih domaćina gde je sve više starih i sekundarno imunokompromitoanih pacijenata, postavlja se pitanje da li se pneumokokni meningitis promenio u kliničkom kontekstu. Cilj ove studije je da ustanovimo kako se pneumokokni meningitis aktuelno prezentuje i leči u skladu sa principima medicine zasnovane na dokazima.

Takođe, pregldom stručne literature, pokušaćemo da identifikujemo relevantne trendove u manifestovanju, toku, lečenju i ishodu ove bolesti. U ovoj retrospektivnoj kohortnoj studiji prikupili smo podatke o svim pacijentima lečenim u Klinici za infektivne i tropske bolesti, UKCS zbog meningitisa izazvanog S. pneumoniae od 15. marta 2014. do 15. marta 2023. godine. Koristeći metode deskriptivne statistike, predstavićemo demografske karakteristike i podatke o predisponirajućim stanjima pacijenata u ovoj kohorti. Takođe ćemo opisati tok, manifestacije, kao i ishode ove bolesti. Pregledom literature dostupne pretragom preko PubMed platforme ispitaćemo potencijalne promene u globalnim epidmiološkim i kliničkim karakteristikama poredeći radove publikovane na temu pneumokoknog meningitisa tokom 1980-ih sa onima publikovanim od 15. marta 2014. do 15. marta 2023.

Konačno, osvrnućemo se aktuelnosti i nedoumice u lečenju pacijenata sa pneumokoknim menigitisom. Uprkos nesumnjivom kliničkom značaju i velikom naporu uloženom u poboljšanje našeg razumevanja S. pneumoniae meningitisa i posledičnim unapređenjem zbrinjavanja ovakvih pacijenata, još uvek postoje nejasnoće koje potencijalno utiču na ishode lečenja.

Different clinical presentations of pneumococcal meningitis

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S. pneumoniae is the single most important causative agent of bacterial meningitis in developed countries. Particularly worrisome is the fact that the mortality associated with this infection remains approximately 10% in spite of sophisticated treatment modalities. Considering the perpetual coevolution of this pathogen with humans as well as the ever-changing profiles of potential hosts with an increasingly aging population and a sharp rise in the number of secondarily immunosuppressed patients, one must consider whether pneumococcal meningitis has changed in a clinically relevant context. The goal of this study is to determine the presenting features of pneumococcal meningitis and how it is treated in keeping with the principles of evidence-based medicine.

We will proceed to a literature review in an attempt to identify relevant trends in the presentation, course, management and prognosis of this disease. In this retrospective cohort study we compiled data on all patients treated for pneumococcal meningitis at the University Clinic for Infectious and Tropical Diseases, University Clinical Center of Serbia form March 15th 2014 to March 15th 2023.

Utilizing the methods of descriptive statistics we will present data on the demographic characteristics and predisposing conditions in our cohort. We will also describe the course, clinical manifestations and the outcomes of this disease. Conducting a comprehensive search of the scientific literature using the PubMed platform we will attempt to evaluate the potential changes in the epidemiologic and clinical characteristics on a global scale through a comparison of the data published on pneumococcal meningitis in the 1980s with that published from March 15th 2014 to March 15th 2023. Finally, current developments and lingering uncertainties concerning pneumococcal meningitis management will be discussed. Despite the unquestionable clinical significance and the considerable efforts invested into the betterment of our understanding of S. pneumoniae meningitis, remaining uncertainties continue to influence treatment outcomes.

Changes in invasive pneumococcal disease serotypes in Serbia

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Streptococcus pneumoniae is the most common bacterial cause of acute otitis media in children, pneumonia, sepsis, and meningitis in all age groups. Of the total 100 known pneumococcal serotypes, some are recognized to be common causative agents of invasive pneumococcal disease (IPD) - bacterial pneumonia, sepsis, and meningitis. Pneumococcal conjugate vaccines (PCVs) - PCV10 and PCV13 - have been identified in the Serbian National Immunization Program (NIP) as as mandatory for all children under two years, with PCV10 is free. Since 2022, it has been replaced by PCV13. Since IPD surveillance in our country is done on a voluntary laboratory basis, the incidence of IPD is underestimated.

During the 13-year period, the Serbian Reference Laboratory for Streptococci performed serotyping of 705 invasive pneumococcal isolates. Serotype 3 dominates in the adult population, while serotypes 6A, 6B, 7F, 14, 18C, 19F, 19A, and 23F are evenly distributed across all age groups. Serotypes 4, 8, 9N, and 9V are found almost exclusively in adults. In the post-PCV10 period, so-called “post PCV-10”serotypes 3, 6A, and 19A are on the rise in all age groups, while 14, 19F, and 18C are on the decline. In the pediatric population, an increase in serotype 3 was observed more frequently in the 2 ≤5 age group and 19A in the ≤ 2 age group.

The vaccination coverage rates of PCV10 and PCV13 in the pediatric population ≤ 2 years of age are 40.8% and 73.5%, respectively. Resistance to penicillin and macrolides in IPD isolates is high (27% and 40%, respectively). It can be concluded that the introduction of PCV10 in the Serbian NIP has led to a significant reduction of some common PCV pediatric serotypes (14, 18C and 19F), but also to an exceptional increase in serotypes 3 and 19A (PCV13-non PCV10). PCV13 serotype coverage is statistically significantly higher than with PCV10 in our country.

Izmene u distribuciji invazivnih serotipova pneumokoka u Srbiji

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Streptococcus pneumoniae je najčešći bakterijski uzročnik akutne upale srednjeg uha kod dece, kao i pneumonije, sepse i meningitisa u svim starosnim grupama. Od ukupno 100 poznatih serotipova pneumokoka, neki su identifikovani kao najčešći uzročnici invazivne pneumokokne bolesti, engl. invasive pneumococcal disease (IPD) - bakterijemijske pneumonije, sepse i meningitisa.

Konjugovane pneumokokne vakcine, engl. pneumococcal conjugate vaccines (PCVs) - PCV10 i PCV13 se nalaze u Nacionalnom programu imunizacije Srbije (NIP) kao obavezne za svu decu mlađu od dve godine, a PCV10 je besplatna. Od 2022. godine je zamenjena PCV13. Budući da je nadzor nad IPD u našoj zemlji laboratorijski i na dobrovoljnoj osnovi, incidencija IPD je potcenjena. U periodu od 13 godina u Referentnoj laboratoriji za streptokok je serotipizirano 705 invazivnih izolata pneumokoka. U populaciji odraslih dominira serotip 3, dok su serotipovi 6A, 6B, 7F, 14, 18C, 19F, 19A i 23F ravnomerno raspoređeni u svim starosnim grupama. Serotipovi 4, 8, 9N i 9V se nalaze skoro isključivo kod odraslih.

U periodu posle početka primene PCV10, takozvani “post PCV-10” serotipovi - 3, 6A i 19A su u porastu u svim starosnim grupama, dok su 14, 19F i 18C u padu. U pedijatrijskoj populaciji, povećanje serotipa 3 je češće primećeno u starosnoj grupi dece od >2 do ≤5 godina, a 19A u starosnoj grupi mlađih od 2 godine. Stope pokrivenosti serotipova vakcinama u pedijatrijskoj populaciji ≤ 2 godine su 40,8% za PCV10 i 73,5% za PCV13. Rezistencija pneumokoka izazivača IPD je visoka - na penicilin je 27%, a na makrolide 40%. Može se zaključiti da je uvođenje PCV10 u NIP Srbije dovelo do značajnog smanjenja nekih čestih vakcinalnih pedijatrijskih serotipova (14, 18C i 19F), ali i do izuzetnog povećanja serotipova 3 i 19A (PCV13-non PCV10). U našoj zemlji je obuhvat cirkulišućih serotipova PCV13 statistički značajno veća u odnosu na PCV10.

The collaborative approach to detection of autochthonous Dirofilaria repens in Slovenia

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The infection with Dirofilaria repens has been historically endemic in Mediterranean countries. However, in the last two decades it has spread to the rest of Europe, where it is considered an emerging infection. Canids act as a reservoir for the parasite, while mosquitoes serve as vectors. Humans can also become infected, but are considered a dead-end host. Several cases of D. repens infection in humans have been reported in Slovenia. Due to the proximity to neighbouring Croatia and Italy, both endemic for dirofilariasis, it is difficult to determine whether the cases are autochthonous or not. A collaborative approach was taken by microbiologists, veterinarians and zoologists to clarify the possibility of autochthonous transmission of D. repens dirofilariasis in Slovenia.

The collaborative approach to detection of autochthonous Dirofilaria repens in Slovenia

A statistically representative number of 465 dogs older than one year and born in Slovenia were recruited between April and October 2018. Epidemiological data were collected and blood samples were taken. Real-time PCR was performed to detect D. repens DNA. Mosquitoes were collected and identified between 2021 and 2022 as part of a vector and vector-borne diseases monitoring project. All adult mosquitoes were grouped into primary pools (up to 40 per vial) according to species and sex. In secondary pools, 10 nucleic acid isolates from the primary pools per vial were grouped by capture date and location. For molecular detection and identification of filarioid species, a real-time PCR approach was used, followed by conventional PCR and sequencing.

Three out of 465 (0.64 %) dogs tested positive for D. repens. Two of them had never travelled outside the country suggesting autochthonous infection. Dirofilaria repens was detected in four Aedes sp. pools, one Coquillettidia sp. pool, and one Anopheles sp. pool. The detection of D. repens in mosquito vectors indicates that the causative agent of subcutaneous dirofilariasis is present in Slovenia.

The study was supported by the Slovenian Research Agency (Grant Nos. J3-8197, V3-1903, P3-0083, P4-0053 and P4-0092), the Ministry of Health and Ministry of the Environment and Spatial Planning (Grant No. V3-1903).

Rizik od infekcije parazitom Toxoplasma gondii nakon transplantacije: rezultati prospektivne kohortne studije Nacionalne referentne laboratorije

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Toksoplazmoza je česta ali kod pacijenata lečenih transplantacijom uglavnom zanemarena i pogrešno dijagnostikovana oportunistička infekcija koja može ugroziti engraftment ali može i evoluirati u životno ugrožavajuću diseminovanu infekciju. Nakon transplantacije, infekcija parazitom Toxoplasma gondii se može razviti kao reaktivacija hronične infekcije ili može biti preneta graftom.

Naša osmogodišnja prospektivna studija bila je usmerena na dijagnostiku i monitoring toksoplazmatske infekcije (TI) kod primalaca matičnih ćelija hematopoeze (haematopoietic stem cell transplant, HSCT) u centru koji primenjuje protokol uzdržavanja od profilakse do engraftmenta, i kod primalaca transplantata srca (heart transplant, HT) koji su na kontinuiranoj profilaksi trimetoprim-sulfametoksazolom (TMP-SMX).

Cilj nam je bio utvrđivanje incidence TI u ova dva vrlo različita transplantaciona režima, i to pre nego što evoluira u klinički manifestnu, potencijalno fatalnu bolest (Toxoplasma disease, TD). Pre-transplantacioni serološki i qPCR skrining u post-transplantacionom toku zamenjen je redovnim qPCR monitoringom iz uzoraka periferne krvi (peripheral blood, PB) usmerenim na Toxoplasma 529 bp gen.

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Kod primalaca HSCT, qPCR je rađen jednom nedeljno dok je kod primalaca HT qPCR rađen jednom mesečno prva dva meseca post-HT i potom jednom godišnje. TI je dijagnostikovana na bazi pozitivnog PCR rezultata iz bar jednog uzorka PB. TI je dijagnostikovana kod 21/104 (20.2%) primalaca HSCT, prevashodno nakon alogene (19/75) i retko nakon autologne HSCT (2/29). Više od 50% slučajeva TI dijagnostikovano je tokom prvog meseca post-HSCT, pre engraftmenta odnosno tokom uzdržavanja od profilakse. Sa druge strane, TI je dijagnostikovana kod 3/37 (8.1%) primalaca HT. Uprkos primeni TMP-SMX, qPCR je postao pozitivan godinu dana posle HT kod dva i dve godine post-HSCT kod trećeg pacijenta. Infekcija je bila preneta graftom kod 2/3 (seronegativni) a reaktivirana kod 1/3 primalaca HT (seropozitivni primalac HT poreklom od seropozitivnog donora).

Naši rezultati potvrđuju da je sistemski qPCR monitoring iz uzoraka PB dragocen u dijagnostici TI ne samo kod primalaca HSCT već i kod primalaca solidnih organa, posebno nakon HT. Učestalost qPCR monitoringa se mora adaptirati shodno specifičnostima transplantacionog protokola, pre svega primeni profilakse ali i osnovnoj dijagnozi, na način koji omogućava pravovremenu primenu specifične terapije u svakom slučaju TI.

Izvori finansiranja: Ovo istraživanje je bilo podržano grantovima Ministarstva prosvete, nauke i tehnološkog razvoja Srbije (Br. ugovora: 451-03-9/2021-14/200015 i 451-03-68/2022-14/200015) dodeljenim Institutu za medicinska istraživanja, Univerzitet u Beogradu, Srbija.

Transplantation-related risk of Toxoplasma gondii infection: the National Reference Laboratory prospective cohort study results

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Toxoplasmosis is a common but often neglected and misdiagnosed opportunistic infection in transplant recipients, which can not only compromise the engraftment, but also evolve into life-threatening disseminated infection. Post-transplantation, Toxoplasma gondii infection can develop as a reactivation of chronic infection or could be graft-transmitted. We conducted an eight-year-long prospective study on the diagnosis and monitoring of Toxoplasma infection (TI) in haematopoietic stem cell transplant (HSCT) recipients in a setting that withholds prophylaxis until engraftment, and in heart transplant (HT) recipients on continuous trimethoprim-sulfamethoxazole (TMP-SMX) prophylaxis.

The objective was to determine the incidence of TI before it evolves into clinical, potentially fatal Toxoplasma disease (TD), in these two very different transplantation settings. Pre-transplantation serological and qPCR screening was followed by post-transplantation peripheral blood (PB)-based qPCR monitoring targeting the Toxoplasma 529 bp gene. In HSCT recipients, qPCR was performed weekly while in HT recipients, qPCR was performed monthly for two months post-HT and then yearly. TI was diagnosed based on a positive PCR result in at least one PB sample.

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TI was diagnosed in 21/104 (20.2%) HSCT recipients, predominantly after allogeneic (19/75) and rarely after autologous HSCT (2/29). Over 50% of TI cases were diagnosed during the first month post-HSCT, while awaiting engraftment without prophylaxis. On the other hand, TI was diagnosed in 3/37 (8.1%) HT recipients. Regardless of the TMP-SMX prophylaxis, qPCR became positive one year after HT in two and two years post-HSCT in third patient. Infection was graft-transmitted in 2/3 (seronegative) and reactivated in 1/3 OHT (seropositive recipient of a seropositive donor's heart transplant).

The presented results show that systematic PB-based qPCR monitoring is a valuable resource for the diagnosis of TI not only in HSCT but also in solid organ recipients, especially after HT. Frequency of qPCR monitoring should be adjusted according to the specificity of the transplantation setting, especially in terms of prophylaxis but also an underlying diagnosis, in a manner allowing for prompt introduction of specific treatment in each case of TI.

Funding source: This study was supported by the Ministry of Education, Science and Technological Development of Serbia, through grants (contract No. 451-03-9/2021-14/200015 and 451-03-68/2022-14/200015) to the Institute for Medical Research, University of Belgrade, Serbia.

Borrelia lusitaniae i Borrelia valaisiana – potencijal za uzrokovanje lajmske borelioze u Srbiji

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Kompleks Borrelia burgdorferi sensu lato trenutno obuhvata dvadest i jednu opisanu vrstu borelija a karakteriše ih značajna varijabilnost u pogledu geografske distribucije kao i specifičnosti na relaciji domaćini rezervoari- kompetentni vektor. Poznato je da samo određeni sojevi vrsta borelija izazivaju bolest kod ljudi. U Severnoj Americi, lajmsku boreliozu uzrokuje samo jedna vrsta borelija-Borrelia burgdorferi sensu stricto (s.s.), dok u Evropi infekciju kod ljudi može uzrokovati nekoliko različitih vrsta borelija-Borrelia afzelii, Borrelia garinii, Borrelia bavariensis, Borrelia spielmanii i Borrelia burgdorferi s.s.

Vrste Borrelia valaisiana, Borrelia lusitaniae i Borrelia bissettii identifikovane su samo u pojedinačnim slučajevima i prepoznate su kao potencijalni patogeni. Različite vrste borelija povezane su sa različitim kliničkim manifestacijama lajmske borelioze; B. afzelii obično ostaje lokalizovana u koži, B. garinii i B. bavariensis se obično povezuju s poremećajima nervnog sistema, dok se B. burgdorferi s.s. često povezuje sa razvojem artritisa. Iako postoje registrovani slučajevi lajmske borelioze u Srbiji, ne postoje podaci o tome koje vrste borelija izazivaju određene kliničke manifestacije lajmske borelioze.

Region Srbije karakteriše visoka rasprostranjenost i raznovrsnost vrsta borelija u krpeljima i njihovim domaćinima. Istraživanja prisustva borelija u krpeljima kao i mikrobiološka izolacija sojeva borelija iz krpelja u Srbiji, ukazala su na dominaciju vrste B. lusitaniae, slede vrste B. afzelii, B. bavariensis, B. garinii, B. valaisiana i B. burgdorferi s.s. Takođe, B. lusitaniae je detektovana u krpeljima sakupljenim sa guštera i u uzorcima slezine lisica, dok je B. valaisiana detektovana u krpeljima sakupljenim sa šakala i ptica. Ispitivanje in vitro osetljivosti lokalnih sojeva borelija (izolovanih iz krpelja vrste Ixodes ricinus sakupljenih sa vegetacije) na ljudski serum, pokazalo je da su sojevi B. valaisiana otporni na komplement, dok su određeni sojevi B. lusitaniae (u prisustvu komplementa) podjednako pokretljvi kao i jedini postojeći soj B. lusitaniae izolovan iz hronične kožne lezije, poreklom iz Portugala. Iako su lokalni sojevi borelija osetljivi na antibiotike in vitro, ostaje nepoznato koje kliničke manifestacije lajmske borelioze mogu izazvati vrste B. valaisiana i B. lusitaniae. Potrebno je da se razjasne mehanizami rezistencije sojeva B. valaisiana i B. lusitaniae (koji cirkulišu u ovoj oblasti) u prisustvu komplementa.

Borrelia lusitaniae and Borrelia valaisiana – potential for causing Lyme borreliosis in Serbia

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The twenty-one described Borrelia species with considerable variability in host and vector associations, and geographical distribution have been classified to the Borrelia burgdorferi sensu lato complex. Only a subset of Borrelia species is known to cause human illness. In North America, Lyme borreliosis (LB) is predominantly caused by the Borrelia burgdorferi sensu stricto (s.s.), while in Europe, the overall diversity of Borrelia species is higher and LB can be caused by Borrelia afzelii, Borrelia garinii, Borrelia bavariensis, Borrelia spielmanii, and Borrelia burgdorferi s.s., while Borrelia valaisiana, Borrelia lusitaniae and Borrelia bissettii have been identified in single cases only and are recognized as potential pathogens. Different Borrelia species are involved in distinct clinical manifestations of LB; B. afzelii usually remains localized in the skin, B. garinii and B. bavariensis are usually associated with nervous system disorders, while B. burgdorferi s.s. is commonly associated with development of the arthritis.

Although there are registered cases of LB in Serbia, there is no data on which Borrelia species cause certain clinical manifestations of LB. Region of Serbia is characterized by high prevalence and diversity of Borrelia species in ticks and their vertebrate hosts. The studies on the presence and isolation of Borrelia from ticks from Serbia pointed to the domination of B. lusitaniae followed by B. afzelii, B. bavariensis, B. garinii, B. valaisiana and B. burgdorferi s.s. Also, B. lusitaniae was detected in ticks collected from lizards and in spleen samples collected from red foxes, while B. valaisiana was detected in ticks collected from golden jackals and birds. In vitro susceptibility of local Borrelia strains isolated from ticks to human complement, showed B. valaisiana strains resisted complement-mediated killing, while certain B. lusitaniae strains (in the presence of complement) were equally motile as the only existing one B. lusitaniae human isolate from Portugal. Although local Borrelia strains are susceptible to antibiotics in vitro, it remains unknown what clinical manifestations of LB can be caused by B. valaisiana and B. lusitaniae. It is needed to elucidate the mechanism behind B. valaisiana and B. lusitaniae strains (circulating in the area) survival in the presence of human complement.

SESIJA / SESSION

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RNK vakcine – novo oružje za kontrolu pretećih infektivnih bolesti

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U slučaju izbijanja velikih epidemija izazvanih novim patogenima ili izmenjenim varijantama postojećih patogena, vakcine koje su zasnovane na tradicionalnim platformama sa atenuisanim ili inaktiviranim infektivnim agensima, odnosno njihovim strukturnim komponentama i produktima, često nisu pogodne za brzu proizvodnju i masovnu primenu, jer zahtevaju kultivaciju patogena, visok stepen bezbednosti i dugotrajan i skup razvoj.

S druge strane, inovativne vakcine koje sadrže informacije o antigenima određenog patogena u obliku nukleinske kiseline, a posebno vakcine sa informacionom ribonukleinskom kiselinom (iRNK), predstavljaju obećavajuću alternativu konvencionalnim vakcinama zbog svoje visoke imunogenosti i efikasnosti, jednostavne i bezbedne primene, mogućnosti uključivanja većeg broja patogena u njihov sastav i potencijala za njihov brz razvoj i jeftinu proizvodnju.

Tokom tekuće pandemije COVID-19, u izuzetno kratkom i do sada nezabeležnom vremenskom periodu razvijene su i primenjene dve efikasne RNK vakcine protiv SARS-CoV-2, i njhova široka primena je spasla milione života i pomogla u povratku u normalnost u mnogim zemljama širom sveta.

RNK vakcine – novo oružje za kontrolu pretećih infektivnih bolesti

Iako je danas jasno da RNK vakcine mogu brzo i bezbedno da zaštite ljude od teškog COVID-19, potrebna su dodatna istraživanja sa ciljem da bi se optimizovala struktura i administracija RNK vakcina radi njihove primene u profilaksi drugih infektivnih bolesti. I zaista, veći broj RNK vakcina protiv gripa, besnila, zika virusa, citomegalovirusa (CMV), respiratornog sincicijalnog virusa (RSV), virusa humane imunodeficijencije (HIV) i drugih bolesti, odnosno patogena su u razvoju i trenutno se nalaze u različitim fazama kliničkih ispitivanja.

Štaviše, zahvaljujući sveobuhvatnosti tehnologije iRNK, RNK vakcine imaju izuzetan potencijal i u terapiji mnogih neinfektivnih bolesti, od različitih oblika malignih tumora pa do urođenih genetskih poremećaja. Na ovom predavanju biće opisana tehnologija koje se nalazi u osnovi RNK vakcina. Pored toga, biće dat pregled RNK vakcina protiv različitih infektivnih agenasa koje se nalaze u razvoju i razmotrena ključna pitanja za dalji razvoj i primenu ove revolucionarne platforme za vakcine u budućnosti.

RNA vaccines – a novel weapon to control emerging infectious diseases

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In the event of large outbreaks of newly emerged pathogens or altered variants of existing pathogens, traditional vaccine platforms based on attenuated or inactivated infectious agents or their structural components and products may not always be suitable or even feasible for implementation at a large scale, due to necessity of cultivation and propagation of pathogens, requirement of a high level of safety and long and costly development process . On the other hand, innovative vaccines that carry information for the antigen of selected pathogen in the form of nucleic acid, and messenger ribonucleic acid (mRNA) vaccines in particular, represent a promising alternative to conventional vaccine approaches because of their high immunogenicity and effectiveness, safe administration, possibility of multiplexing, capacity for rapid development and potential for low-cost manufacture.

During the ongoing COVID-19 pandemic, two effective mRNA vaccines against SARS-CoV-2 were developed and rolled out at an unprecedented speed, saving millions of lives and helping rebuild societies worldwide. Although it is now clear that mRNA vaccines can rapidly and safely protect people from severe SARS-CoV-2 infections, additional research is required to optimize mRNA design, intracellular delivery and applications beyond COVID-19 prophylaxis. Indeed, a number of mRNA vaccines in development against influenza, rabies, zika virus, cytomegalovirus (CMV), respiratory syncytial virus (RSV), human immunodeficiency virus (HIV) and other diseases and pathogens are currently in various stages of clinical studies.

Furthermore, owing to its versatility, the mRNA platform show incredible potential for the development of therapeutic drugs for many different non-infectious diseases, ranging from cancers to various congenital genetic disorders. In this lecture, the technologies that underlie mRNA vaccines will be described. In addition, an overview of the pipeline of mRNA vaccines against various infectious agents will be given and key questions for the future application of this breakthrough vaccine platform will be discussed.

Antitela specifična za SARS-CoV-2 nakon iRNK vakcine kao treće doze: homologi i heterologi pristup revakcinaciji

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Implementacija treće doze vakcine protiv SARS-CoV-2 u preporuke širom sveta otvorila je polje istraživanja heterologog pristupa revakcinaciji, odnosno kombinacije primarne serije vakcine i treće doze različite vakcinalne platforme. Iako je literatura bogata radovima na temu heterologog pristupa, imunogenost i trajanje humoralnog imunskog odgovora nakon kombinacije inaktivisane BBIBP-CorV i iRNK vakcine nisu dovoljno istraženi. Stoga, cilj ove studije bio je ispitivanje razlike u imunogenosti i dugotrajnosti humoralnog imunskog odgovora u okviru perioda od šest meseci nakon treće doze kod homologog (tri doze BNT162b2) i heterologog (BBIBP-CorV/BNT162b2) pristupa revakcinaciji tokom Omikron talasa u Srbiji. U studiju je uključen 91 ispitanik, od kojih se 55 odlučilo za homologi a 36 za heterologi pristup.

Serumi ispitanika analizirani su u četiri vremenske tačke: šest meseci nakon prve doze, a zatim tri nedelje, tri meseca i šest meseci nakon treće doze. IgG antitela specifična za receptor-vezujući domen "šiljastog" (eng. spike) proteina detektovana su BioMerieux VIDAS SARS-CoV-2 IgG testom. Tri nedelje nakon treće doze, oba pristupa revakcinaciji dovela su do značajnog porasta u koncentraciji antitela ($p<0.0001$). Štaviše, ispitanici koji su se opredelili za heterologu kombinaciju imali su statistički značajno više koncentracije antitela od homologe grupe, u kontrolnim vremenskim tačkama na tri nedelje i tri meseca nakon treće doze ($p=0.025$, $p=0.0006$). Međutim, značajan pad humoralnog imunskog odgovora zapažen je tokom vremena kod oba pristupa. Većina infekcija nakon vakcinacije registrovana je u periodu između tri i šest meseci nakon treće doze ($n=22$), a ukupna incidencija ovih infekcija za posmatrani period iznosila je 36.36% (20/55) nakon homologog i 16.67% (6/36) nakon heterologog pristupa.

Antitela specifična za SARS-CoV-2 nakon iRNK vakcine kao treće doze: homologi i heterologi pristup revakcinaciji

Međutim, ispitanici sa potvrđenom infekcijom nakon vakcinacije nisu imali pneumoniju niti su bili hospitalizovani. Iako je heterologi pristup indukovao više koncentracije antitela, naši rezultati ukazuju da su i heterologi i homologi pristup indukovali potentan humoralni imunski odgovor i odgovarajuću zaštitu od hospitalizacije i smrtnog ishoda tokom Omikron talasa. Međutim, opadanje imunskog odgovora opaženo kod oba vakcinalna pristupa u periodu od šest meseci, kao i konstantna opasnost od pojave novih pretećih varijanti, ukazuje na potrebu preispitivanja trenutne vakcinalne strategije.

Izvori finansiranja: Ovo istraživanje je bilo podržano grantom Ministarstva prosvete, nauke i tehnološkog razvoja Srbije (Br. ugovora: 451-03-68/2022-14/200015), dodeljenim Institutu za medicinska istraživanja, Univerzitet u Beogradu, Srbija.

SARS-CoV-2 specific antibody response after an mRNA vaccine as the third dose: homologous versus heterologous boost

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Worldwide implementation of the third dose of vaccine against SARS-CoV-2 opened a new field of research concerning the heterologous boost i.e., the combination of the primary vaccine series and a different vaccinal platform for the third dose. Although literature is replete with studies of heterologous boosts, longevity and immunogenicity of the inactivated BBIBP-CorV and mRNA BNT162b2 combination remains under-explored. Thus, the aim of this study was to evaluate the differences in immunogenicity and longevity of the humoral immune response within six months after the third dose in both homologous (BNT162b2) and heterologous (BBIBP-CorV/BNT162b2) vaccination setting, and to assess the real-life data in the middle of the Omicron surge in Serbia.

A total of 91 individuals were included in this study, of which 55 received homologous and 36 heterologous boost. Serum samples were analyzed at four timepoints: six months after the first dose; three weeks, three months, and six months after the third dose. Specific IgG antibodies against the receptor-binding domain of the spike protein were detected using BioMerieux VIDAS SARS-CoV-2 IgG kit. Both groups showed a highly significant increase in antibody concentrations ($p<0.0001$) three weeks after the boost.

Furthermore, comparison per timepoint has shown that recipients of heterologous boost had significantly higher antibody concentrations than homologous group, at three weeks and three months after the boost ($p=0.025$, $p=0.0006$). However, a significant decline in antibody response over time was noted for both strategies. The majority of breakthrough infections were registered in the period between three and six months after the boost ($n=22$).

SARS-CoV-2 specific antibody response after an mRNA vaccine as the third dose: homologous versus heterologous boost

Furthermore, total incidence was estimated at 36.36% (20/55) for homologous group, and 16.67% (6/36) for heterologous group. Most importantly, none of the recipients of the third dose developed pneumonia during the breakthrough infection, and none were hospitalized. In conclusion, although heterologous approach resulted in higher antibody concentrations, our findings imply that both homologous and heterologous boost induce potent humoral immune response and adequate protection against hospitalization and death in the Omicron setting. However, waning immune response registered for both types of boosts within six months and constant threats of new emerging variants, calls for an update of vaccine strategy.

Funding source: This study was supported by the Ministry of Education, Science and Technological Development of Serbia, through grant to the Institute for Medical Research, University of Belgrade, Serbia (contract No. 451-03-68/2022-14/200015).

Da li je vakcina ključni faktor u borbi protiv malarije?

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Malarija je velika globalna pretnja po javno zdravlje, koja uzrokuje teško obolevanje i umiranje, posebno kod dece mlađe od 5 godina. Procenjuje se da malarija ubija više od 600.000 ljudi godišnje širom sveta i izaziva bolest kod još 200 miliona ljudi, pri čemu se većina smrtnih ishoda javlja među decom koja žive u podsaharskoj Africi. Skoro 85% smrtnih slučajeva od malarije na globalnom nivou dogodilo se u 19 zemalja afričkog regiona i Indiji. Sadašnje metode prevencije malarije i kontrole vektora, kao što je upotreba mreža tretiranih insekticidima, zaprašivanje zatvorenih prostora i primena preventivne terapije, uticale su na značajno smanjenje morbiditeta i mortaliteta od malarije tokom poslednje decenije. Takođe, u kontroli malarije neophodno je obezbediti i brzu laboratorijsku dijagnozu malarije putem mikroskopiranja direktnih preparata periferne krvi ili upotrebom brzih imunohromatografskih testova za detekciju antigena parazita kao i promptnu terapiju obolelih antimalaricima.

Međutim razvoj otpornosti komaraca na insekticide, promene u životnoj sredini, kao i razvoj otpornosti parazita na postojeće antimalarike uslovila je hitnu potrebu da se identifikuju dodatni alati za prevenciju i iskorenjivanje malarije. Svetska zdravstvena organizacija je 2021. preporučila RTS,S/AS01, prvu vakcinu protiv malarije na svetu, za decu u podsaharskoj Africi i u drugim regionima sa umerenom do visokom transmisijom malarije izazvane Plasmodium falciparum. RTS,S/AS01 je preeritrocitna rekombinantna subjedinična vakcina u kojoj je cirkumsporozoitni protein parazita vezan za površinski antigen hepatitis B virusa i predstavlja prvu generaciju vakcina protiv malarije. Nekoliko drugih vakcina protiv malarije je u različitim fazama razvoja, a preliminarni rezultati su obećavajući. Za razvoj visoko efikasne vakcine protiv malarije i postizanje trajne zaštite visokog nivoa potreban je dalji razvoj na selekciji antigena parazita, dizajnu imunogena, kao i na strategiji imunizacije. Vakcine sledeće generacije će biti od suštinskog značaja za postizanje viših i održivih nivoa zaštite od malarije optimizacijom i unapređivanjem postojeće preeritrocitne vakcine ili pak razvojem dodatnih strategija koje blokiraju transmisiju parazita.

Is vaccine a game changer in the fight against malaria?

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Malaria is a major global public health threat, causing severe illness and death, particularly in children under 5 years of age. Malaria is estimated to kill more than 600,000 people a year worldwide and cause illness in another 200 million people, with the majority of deaths occurring among children living in sub-Saharan Africa. Almost 85% of malaria deaths globally occurred in 19 countries in the African region and India.

Current malaria prevention and vector control methods, such as the use of insecticide-treated nets, indoor residual spraying, and the use of preventive therapy, have resulted in significant reductions in malaria morbidity and mortality over the past decade. Also, in the control of malaria, it is necessary to provide rapid laboratory diagnosis of malaria through microscopy of direct preparations of peripheral blood or the use of rapid immunochromatographic tests for the detection of parasite antigens, as well as prompt treatment of patients with antimalarial drugs. However, the development of mosquito resistance to insecticides, changes in the environment, as well as the development of parasite resistance to existing antimalarial drugs has caused an urgent need to identify additional tools for the prevention and eradication of malaria. In 2021, the World Health Organization recommended RTS,S/AS01, the world's first malaria vaccine, for children in sub-Saharan Africa and other regions with moderate to high transmission of Plasmodium falciparum malaria. RTS,S/AS01 is a pre-erythrocyte recombinant subunit vaccine in which the circumsporozoite protein of the parasite is linked to the surface antigen of the hepatitis B virus and represents the first generation of malaria vaccines.

Several other malaria vaccines are in various stages of development, and preliminary results are promising. Further development in parasite antigen selection, immunogen design, as well as immunization strategy is required to develop a highly effective malaria vaccine and to achieve a sustained high level of protection. Next-generation vaccines will be essential for achieving higher and sustainable levels of protection against malaria by optimizing and improving existing pre-erythrocytic vaccines or developing additional strategies that block parasite transmission.

Vakcine protiv tuberkuloze: zašto množina?

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Bacille Calmette-Guérin (BCG) vakcina je prvi put upotrebljena 1921. godine i danas je još uvek jedina odobrena vakcina protiv tuberkuloze (TB). Prema tome, "množina" u naslovu izražava istovremeno optimizam i nestrpljenje, jer uključuje i dugo očekivane nove TB vakcine. Uprkos masovnoj BCG imunizaciji na globalnom nivou, procenjuje se da oko 1,7 milijardi ljudi danas ima latentnu TB infekciju (LTBI), od kojih će 5-10% razviti aktivnu bolest. Samo tokom 2021. godine bilo je 10,6 miliona novoobolelih i 1,6 miliona umrlih od TB, što Mycobacterium tuberculosis svrstava kao drugog vodećeg pojedinačnog infektivnog uzroka smrti, iza SARS-CoV-2. Efektivnost BCG vakcinacije na rođenju u zaštiti od TB danas se procenjuje na 18%, pri čemu je zaštita značajna samo kod dece mlađe od pet godina. Jasno je da je vakcina koja će biti efektivna u svim uzrasnim grupama urgentno potrebna, uz poseban fokus na zaštitu od plućne TB kod adolescenata i odraslih, koji se smatraju ključnim za transmisiju.

Za nove vakcine potrebno je izvršiti procenu nivoa zaštite od infekcije i nivoa zaštite od nastanka plućne bolesti, nakon primarne infekcije ili nakon reaktivacije LTBI. Treća značajna ciljna indikacija za nove vakcine je terapijska primena, usmerena ka poboljšanju ishoda lečenja osoba sa već razvijenom aktivnom TB. Trenutno se u različitim fazama kliničkog razvoja nalazi 16 kandidata za nove TB vakcine. Platforme vakcina kandidata uključuju žive atenuisane, cele ćelijske inaktivisane, virusne vektorske i subjedinične proteinske vakcine sa adjuvansom, a 2022. godine najavljen je i prvi kadidat za iRNK TB vakcinu. Nove TB vakcine takođe se razlikuju i prema ciljnim indikacijama primene, od profilaktičkih do terapijskih. Primer ohrabrujućih postignutih rezultata je 49,7% zaštite od razvoja plućne TB kod odraslih HIV-seronegativnih osoba sa LTBI, koja je ostvarena primenom subjedinične M72/AS01E vakcine. Ipak, postojeća situacija sa kandidatima za nove TB vakcine ne razlikuje se suštinski od statusa koji smo imali prethodnih godina, odnosno nastavlja se spor trend razvoja. Trijumfalni uspeh u razvoju vakcina protiv oboljenja COVID-19 trebalo bi da bude putokaz i za razvoj novih TB vakcina. Neophodno je da se razvoj novih TB vakcina ubrza i odredi kao urgentan prioritet, kako bismo do kraja ove decenije ostvarili cilj dostupne nove efikasne vakcine protiv TB.

Vaccines against tuberculosis: why plural?

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The Bacille Calmette-Guérin (BCG) vaccine was first used in 1921, and remains the only licensed vaccine against tuberculosis (TB). Thus, the "plural" in the title was used with both optimism and impatience, as it includes the long-awaited new TB vaccines as well. Despite globally widespread BCG immunization, latent TB infection (LTBI) is present in approximately 1.7 billion people, of whom 5-10% will develop TB disease. In 2021 alone, an estimated 10.6 million people fell ill with TB and 1.6 million died from the disease, which makes Mycobacterium tuberculosis the world's leading cause of death from a single infectious agent next to SARS-CoV-2. The overall protection against TB provided by infant BCG vaccination has recently been estimated at 18%, with significant protection only in children younger than five years.

Clearly, a vaccine effective across all age groups is urgently needed, with a particular focus on prevention of pulmonary TB in adolescents and adults as the leading drivers of transmission. The vaccine should be evaluated for prevention of infection, and for ability to prevent pulmonary disease due to either primary infection or reactivation of LTBI. The third vaccine target indication is therapeutic application aimed at improving outcomes of drug treatment regimens for persons with TB disease. The TB vaccine pipeline currently includes 16 candidates under clinical development. The platforms employed are live attenuated, whole cell inactivated, viral vector and protein/adjuvant subunit vaccines, and the first TB vaccine candidate based on mRNA was announced in 2022. The candidates are also varied in intended use, from prophylactic to therapeutic applications. Encouraging results have been obtained, such as 49.7% protection against development of pulmonary TB disease among HIV-negative adults with LTBI conferred by the adjuvanted protein vaccine M72/AS01E. However, the current TB vaccine pipeline is not profoundly different from those in previous years, and progress in developing new vaccines continues to be slow. The remarkable speed at which COVID-19 vaccines progressed should serve as a benchmark for the TB field. We need to prioritize and accelerate the development of new TB vaccines, and achieve the goal of a highly efficacious vaccine against TB within this decade.

SESIJA / SESSION

/ ANTIMIKROBNA TERAPIJA

/ ANTIMICROBIAL THERAPY

Approach to the Ethical Issues Raised by Antimicrobial Resistance

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We owe to Sir Alexander Fleming the discovery of antibiotics, almost one hundred years ago. It was him as well who warned us about the moral responsibility we will have when patients will start dying because of infections with penicillin-resistant organisms.

Since then, much has been said about antimicrobial resistance from technical and medical points of view, but we should not forget that it also represents a distinct ethical issue.

Tackling antimicrobial resistance becomes more and more an ethical obligation. There are ethical issues in both action and inaction in this field. Responsibilities accountable for action to reduce or overcome antimicrobial resistance are both individual and institutional. Policy decisions should take into account ethical evaluations and these should have to find the right balance between patient's health, society health, fairness and freedom. Antimicrobial resistance impacts a multitude of persons, institutions, and societies, and thus the One Health approach should have to deal with important moral values.

Approach to the Ethical Issues Raised by Antimicrobial Resistance

This presentation will briefly review issues related to the ethics of drug prescription and of antimicrobial stewardship, of agricultural and farming practices, of drug development (with emphasis on both pharmaceutical companies and regulatory authorities), of resource allocation for new drug development, public awareness and media campaigns.

These ethical questions will bring forth concerns about the adequacy and suitability of the current ethical frameworks to address the slowly emerging disaster called antimicrobial resistance.

Experimental treatment of malaria – new perspectives

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With an estimated 247 million cases annually and 619.000 deaths (in 2021) malaria remains a major disease of the developing world and globally the most important parasitic disease. Because of widespread resistance to available antimalarials including chloroquine (CQ) and its derivatives, new drugs are urgently needed. Here we report on the antimalarial efficacy of new 4-aminoquinoline derivatives, with modifications at the linker and at the quinoline nucleus.

In vitro screening was performed by the lactate dehydrogenase assay, based on measurement of the plasmodial lactate dehydrogenase activity in both a CQ-sensitive (3D7) and a CQ-resistant (Dd2) strain of Plasmodium falciparum, with a CQ as a control. In vivo antimalarial activity was investigated in C57BL/6 mice infected with Plasmodium berghei ANKA strain by the modified Thompson test.

Compounds were first tested for toxicity. A total of 37 compounds were screened in vitro. Of the 22 that passed the first screening, 18 had IC50 values lower than CQ in the Dd2 strain while only one was efficient in the 3D7 strain. However, even 15 compounds showed in vivo activity, significantly ($P<0.05$) prolonging survival of treated vs. untreated mice. Among these, seven compounds afforded the survival of 20–100% of treated mice up to Day 31, with or without the detection of parasites in peripheral blood.

Most importantly, three of these, including ClAQ1, FClAQ1 and ClAQ8, afforded survival of 100% of animals, the first two at 80 and 160 mg/kg/day and the last only at 160 mg/kg/day.

Experimental treatment of malaria – new perspectives

Survival was associated with complete parasite clearance, as shown by both microscopy and qPCR. Of note, continuous monitoring of parasitemia allowed the observation of a potentially important phenomenon, that a number of compounds were able to confer resistance to cerebral malaria and afford a switch to hyperparasitaemia to mice prone to the neurological syndrome.

By comparing the antimalarial activity of this group of novel compounds, we found that even minor structural modifications substantially affect activity. The results of this extensive study are important, as they may guide future work involving structural modifications of aminoquinolines, and as a contribution to the knowledge in the field of malarial chemotherapy.

Funding source: This study was supported by the Ministry of Education, Science and Technological Development of Serbia, through grants (contract No. 451-03-68/2020-14/200015) to the Institute for Medical Research, University of Belgrade, Serbia.

Eksperimentalna terapija malarije – novi vidici

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Malarija ostaje globalno najznačajnija parazitska infekcija sa procenjenih 247 miliona slučajeva i 619.000 smrtnih slučajeva godišnje (2021.). Zbog široko rasprostranjene rezistencije na dostupne antimalarike, uključujući hlorokvin (CQ) i njegove derivate, hitno su potrebni novi lekovi.

U ovom istraživanju ispitana je potencijalna antimalarijska aktivnost 37 novosintetisanih aminohinolina sa hemijskim modifikacijama na aminohinolinskom jezgru i bočnom lancu. In vitro skrining aktivnosti jedinjenja vršen je kolorimetrijskim esejom laktat dehidrogenaze na dva soja Plasmodium falciparum, osetljivim (3D7) i rezistentnim (Dd2) na CQ, uz CQ kao pozitivnu kontrolu. Aktivnost u in vivo sistemu je ispitana na ženkama miševa soja C57Bl/6 inficiranim ANKA sojem Plasmodium berghei primenom modifikovanog Thompson-ovog testa. Ispitivanju aktivnosti jedinjenja prethodila je faza kliničkog praćenja zdravih životinja terapiranih eksperimentalnim jedinjenjima. Od 37 jedinjenja ispitanih u fazi in vitro skrininga, 22 koja su inhibirala ≥50% rast bar jednog od dva soja P. falciparum odabrana su za titraciju do IC50 vrednosti.

Prema soju rezistentnom na CQ, 18 jedinjenja se pokazalo aktivnijim od CQ, dok je među njima samo jedno jedinjenje bilo aktivnije i prema osetljivom soju. Čak 15 jedinjenja ispitanih u in vivo sistemu značajno je produžilo život inficiranim životinjama u odnosu na kontrolnu grupu ($P < 0.05$).

Među njima, sedam jedinjenja je omogućilo preživljavanje 20–100% tretiranih miševa do dana 31, sa ili bez nalaza parazita u perifernoj krvi. Posebno treba istaći tri jedinjenja koja su dovela do izlečenja svih tretiranih životinja, ClAQ1 i FClAQ1 (80 i 160 mg/kg/dan) i ClAQ8 (160 mg/kg/dan).

Eksperimentalna terapija malarije – novi vidici

Preživljavanje je bilo praćeno i kompletnim klirensom parazita što je dokazano mikroskopskim pregledom razmaza kao i qPCR analizom krvi i tkiva jetre preživelih životinja. Važno je pomenuti da je kontinuirano praćenje parazitemije svih tretiranih miševa omogućilo da se zapazi potencijalno značajan fenomen.

Naime, neka jedinjenja su omogućila da miševi postanu otporni na razvoj cerebralne malarije i uzrokovala da miševi skloni razvoju neurološkog sindroma tolerišu preživljavanje sa izuzetno velikim brojem parazita. Poređenjem antimalarijske aktivnosti novosintetisanih aminohinolina uočeno je da i male strukturne promene u velikoj meri menjaju aktivnost. Rezultati ovog opsežnog istraživanja su od značaja za buduća istraživanja strukturne modifikacije aminohinolina i doprinose proširenju znanja u oblasti hemioterapije malarije.

Izvori finansiranja: Ovo istraživanje je bilo podržano grantom Ministarstva prosvete, nauke i tehnološkog razvoja Srbije (Br. ugovora: 451-03-68/2020-14/200015) dodeljenim Institutu za medicinska istraživanja, Univerzitet u Beogradu, Srbija.

Potencijal nanočestica i nanokompozita u terapiji infekcija izazvanih multirezistentnim bakterijama

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Zahvaljujući nekontrolisanoj upotrebi antibiotika, izraženoj naročito tokom COVID-19 pandemije, možemo očekivati razbuktavanje multirezistencije i panrezistencije među bakterijama. Primarni cilj farmaceutske industrije i srodnih naučnih grana je pronalaženje i razvoj novih antimikrobnih terapeutika. Nanočestice i nanokompoziti metala predstavljaju obećavajuće antimikrobne supstance, koji zahtijevaju dodatne modifikacije zbog toksičnog dejstva samih metala, kao i ekonomskog efekta.

Nanočestice srebra su bile među prvim testiranim nanočesticama sa značajnim antimikrobnim efektom. Poslije srebrnih nanočestica se krenulo sa ispitivanjem velikog broja različitih metala (zlata, bakra, željeza, titanijuma), ali je kod većine bio problem ili nedovoljnog antimikrobnog efekta u netoksičnim koncentracijama ili cijena samih metala ili komplikovana metodologija sinteze. Akcenat se stavlja i na tzv. "zelenu hemiju", odnosno ekološki prihvatljivu sintezu prilikom koje se neće stvarati toksični produkti za spoljašnju sredinu. Najnovija istraživanja se odnose na nanočestice selena, elementa u tragovima u našem tijelu, koji ima prihvatljivu i metodu i cijenu sinteze. Naša ispitivanja sa nanočesticama selena obloženim sa katjonskim surfaktantima su pokazala inhibitorni efekat čak i na multirezistentne planktonske A. baumannii izolate, kao i na sprečavanje formiranja biofilma. U budućim istraživanjima planiramo da ispitamo njihovo dejstvo na zreli, formiran biofilm, kao njihovu i toksičnost u korištenim inhibitornim koncentracijama.

Nanokompoziti su multifazne strukture kod kojih je jedna faza (supstanca) u nanodimenzijama. Spadaju u relativno novije supstance i mogu imati izuzetno širok spektar upotrebe. Zahtijevaju posebne tehnološke procese, te zbog toga još uvijek nemaju široku primjenu u medicini. Naša iskustva su bazirana na nanokompozitima gline pirofilita sa nanočesticama srebra, koje su pokazale odlično antimikrobno i antiinflamatorno dejstvo. Aktivnost su pokazale samo na planktonske sojeve i to u koncentracijama koje su dozvoljene za spoljašnju upotrebu, a antibiofilm eksperimenti nisu dali očekivane rezultate. Neophodno je uraditi dodatne modifikacije u cilju dobijanja i antibiofilm efekta.

The potential of nanoparticles and nanocomposites in the therapy of infections caused by multidrug-resistant bacteria

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After the worldwide uncontrolled antibiotics usage during the COVID-19 pandemia, we can expect the outbreak of multi- resistance and pan-resistance among antibiotic-sensitive bacteria. The primary objective of the pharmaceutical industry is the discovery and development of new antimicrobial drugs. Metal-based nanoparticles and nanocomposites represent promising antimicrobial substances, which require additional modifications due to their toxic effect or the cost-effect of the production process.

Silver nanoparticles were among the first tested nanoparticles with a significant antimicrobial effect. After silver nanoparticles, different metal nanoparticles (gold, copper, iron, titanium) were investigated, but with several problems due to either insufficient antimicrobial effect at non-toxic concentrations, or the cost effect and complicated synthesis methodology. Nowadays, acceptable synthesis methods should follow "green chemistry" principles, i.e. ecologically acceptable synthesis, without production of toxic environmental products. The recent scientific researches investigated selenium nanoparticles. In human body, selenium is a trace element, and its nanoparticles can be produced with acceptable "green" synthesis and reasonable cost. Our studies with selenium nanoparticles coated with cationic surfactants showed an inhibitory effect even on multiresistant planktonic A. baumannii isolates, and also prevented biofilm formation. In future studies, we want to investigate the effect of selenium nanoparticles on the mature biofilm, as well as to analyze the toxicity of inhibitory concentration in vitro.

Nanocomposites are multiphase structures with at least one phase in nanoscale dimensions. They belong to newly developed substances with a very broad variety of potential applications. Their synthesis methods are very demanding and expensive. In our study we used nanocomposite pyrophyllite clay with silver nanoparticles, which have shown excellent antimicrobial and anti-inflammatory effects. Our nanocomposite showed activity only on planktonic multidrug strains, in concentrations that are allowed for external use in humans, while we need to make additional modifications for the antibiofilm effect.

SESIJA / SESSION

/ KRATKE ORALNE PREZENTACIJE

/ SHORT ORAL PRESENTATION

Approach to the Ethical Issues Raised by Antimicrobial Resistance

Moderators: **Ivana Dakić, Marko Janković**

Margo Diricks

Mycobacterium abscessus is a multidrug-resistant non-tuberculous mycobacterium that is increasingly being reported as the etiological agent of severe respiratory, skin and mucosal infections. Genomic surveillance is crucial to understand how this pathogen spreads, evolves and how we can control them. To facilitate this for *M. abscessus*, we established and validated a core genome multilocus sequence typing (cgMLST) scheme.

The scheme allows to classify new strains up to subspecies level and into known dominant circulating clonal complexes. In addition, it can be used for detailed outbreak analysis and to unravel new transmission routes. The standardized and stable scheme (i.e. fixed set of genes that are compared between strains), together with a harmonized expandable nomenclature (i.e. allele numbers) allows direct comparability of results by different laboratories and therefore facilitates prospective global Mab surveillance.

Promena profila intrahospitalnih infekcija tokom COVID-19 pandemije u jedinicama Intenzivnog lečenja – da li nam preti ulazak u preantibiotsku eru?

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Tokom COVID-19 pandemije primećena je povećana rezistencija bakterija na antibiotske lekove, a ono što posebno zabrinjava je podatak da je došlo do pojave sojeva otpornih na većinu rezervnih antibiotskih lekova. U odeljenju Intenzivne nege Klinike za infektivne i tropske bolesti, Univerzitetskog kliničkog centra Srbije sprovedena je dvogodišnja retrospektivna studija u periodu od aprila 2019. do aprila 2021.godine, u cilju procene uzročnika intrahospitalnih infekcija (HAI) i učestačosti antimikrobne rezistencije (AMR), pri čemu je pandemija COVID-19 usledila na polovini studije. Ukupno je bilo analizirano 611 bolesnika od kojih je kod 114 bio ispunjen kriterijum za dijagnozu barem jedne HAI. Trećina svih infekcija su bile polimikrobne. Potvrđene su ukupno 163 epizode HAI sa 226 izolata.

Kod pacijenata sa COVID-19, Acinetobacter spp. bio je dominantan uzročnik intrahospitalnih infekcija i češće je izolovan nego kod pacijenata koji nisu oboleli od COVID-19 (p = 0,001), potom Enterococcus spp., koagulaza-negativni Staphilococcus, Klebsiella spp. i Pseudomonas aeruginosa.

Najveća učestalost rezistencije zabeležena je na fluorohinolone (levofloksacin 97,4%, ciprofloksacin 96,4%, moksifloksacin 94,4%) i cefalosporine (cefotaksim 96,3%, cefaleksin 95,8%, ceftriakson 95,7%, ceftazidim 90,0%). Otpornost na trimetoprim-sulfametoksazol bila je 88,8%, dok je rezistencija veća od 80% takođe primećena za oba aminoglikozida (91,6% i 80,4% za gentamicin i amikacin). Sa izuzetkom amoksicilin-klavulanske kiseline (75,0%), učestalost rezistencije je bila veća od 80% kod svih ostalih penicilina.

Promena profila intrahospitalnih infekcija tokom COVID-19 pandemije u jedinicama Intenzivnog lečenja – da li nam preti ulazak u preantibiotsku eru?

Otpornost na karbapeneme je takođe bila visoka (85,4% za meropenem i 81,3% za imipenem). Otpornost na vankomicin (30,8%) i tigeciklin (28,6%) je niža u poređenju sa većinom lekova, dok je najniža bila za kolistin (7,1%) i linezolid (2,7%). Potom smo poredili učestačost rezistencije između COVID-19 pacijenata lečenih tokom 2020/2021.godine i ne-COVID-19 pacijenata, lečenih tokom 2019/2020.godine i pronašli statistički značajno veću učestalost u grupi COVID-19 pacijenata i to na ciprofloksacin (p = 0,04), imipenem (p<0,001), meropenem (p<0,001) i ertapenem (p = 0,03).

Može se zaključiti da je jedna od posledica COVID-19 pandemije promenjen profil uzročnika intrahospitalnih infekcija u odeljenjima intenzivne nege sa pratećom redukcijom terapijskih opcija čime nam preti pojava panrezistentnih bakterijskih sojeva, te je hitno potrebno uvođenje novih lekova za ovakve infekcije i rigorozan nadzor nad upotrebom antibotika kako ne bismo ušli u preantibiotksu eru.

Changing profile of intrahospital infections during the COVID-19 pandemic in Intensive Care Units - are we in danger of entering the pre-antibiotic era?

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An increase in antibiotic resistance has been noticed during COVID-19 pandemic and the most concerning thing is the appearance of bacteria resistant to the majority of the reserve antibiotics. Two year retrospective study was conducted at the Intensive Care Unit of The Clinic for Infectious and Tropical Diseases, University Clinical Center of Serbia, from April of 2019 to April of 2021.

The objective was to evaluate the causes of healthcare-associated infections (HAI) and the incidence of antimicrobial resistance (AMR). COVID-19 pandemic happened in the middle of this study. Number of patients who were analyzed during this study was 611 out of which 114 met criteria for HAI diagnosis. One third of all the infections were polymicrobial and 163 episodes of HAI were confirmed from 226 samples. In patients with COVID-19 the most frequent cause of HAI was Acinetobacter spp. which was isolated more often in these patients than in non-COVID-19 patients ($p=0.001$). Next the most frequent isolates were Enterococcus spp., Coagulase-negative Staphylococcus, Klibsiella spp. and Pseudomonas aeruginosa. The highest incidence of AMR was noted in fluoroquinolones (levofloxacin 97.4%, ciprofloxacin 96.4%, moxiflocacin 94.4%) and in cephalosporins (cefotaxime 96.3%, cefalexin 95.8%, ceftriaxone 95.7%, ceftazidime 90.0%).

Resistance to trimethoprim-sulfamethoxazole was 88.8% while resistance higher than 80% was noted in both aminoglycosides (91.6% and 80.4% for gentamicin and amikacin). With the exception of amoxicillin-clavulanic acid (75.0%), friquency of AMR was higher than 80% in all the other penicillins. Carbapenems resistance was also very high (85.4% for meropenem and 81.3% for imipenem).

Changing profile of intrahospital infections during the COVID-19 pandemic in Intensive Care Units - are we in danger of entering the pre-antibiotic era?

Resistance to vancomycin (30.8%) and tigecycline (28.6%) was lower in comparison to majoriry of medications, and the lowest resistance was found in colistin (7.1%) and linezolid (2.7%). Further we compared the results of AMR between COVID-19 patients who were treated during 2020 and 2021 and non-COVID patents who were treated during 2019 and 2020.

In COVID-19 patients we found significantly higher frequency of AMR to ciprofloxacin ($p=0.04$), imipenem ($p<0.001$), meropenem ($p<0.001$) and ertapenem ($p=0.03$). So, one of the consequences of COVID-19 pandemic is changed profile of causes of HAI in Intensive Care Units with reduction of therapy options which can cause the appearance of pandrug-resistant infections. The urgent introduction to new antibiotics is needed, with strict monitoring of antibiotic use so that we would not enter the pre-antibiotic era.

Antimicrobial sensitivity and intrahospital transmission dynamics of Klebsiella spp. isolates during a one-year period

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Background: Bacteria belonging to the genus Klebsiella frequently cause human nosocomial infections associated with high morbidity and mortality due to limited treatment options. Aim of this study was to determine antimicrobial sensitivity of Klebsiella spp. isolated among inpatients in Doboj General Hospital.

Methods: The isolates were recovered from patients admitted to Hospital "Sveti Luka" in Doboj, Republic of Srpska during a one year period (01.01.-31.12.2022). Antimicrobial effect of antibiotics (amoxicillin-clavulanic acid, ceftriaxone, cefotaxime, ceftazidime/avibactam, meropenem, imipenem, amikacin, ciprofloxacin, colistin, chloramphenicol, phosphomicin, and trimethoprim-sulfamethoxazole) was determined by disk-diffusion assay according to EUCAST.

Results: During one year period 4822 specimens obtained from inpatients were received in microbiological laboratory. Out of the total 1051 isolated pathogens, Klebsiella spp. was recovered from 284 (27%) samples. According to antimicrobial testing, all isolates were classified in three groups: group of isolates sensitive to more than 4 antibiotics (n=229, 81%), multidrug resistant or MDR (n=8, 3%) and extended drug resistant or XDR (n=47, 16%). Among sensitive group of isolates, sensitivity to antibiotics was as follows: amoxicillin-clavulanic acid (25%), ceftriaxone (52%), cefotaxime (52%), meropenem (100%), imipenem (90%), amikacin (90%), ciprofloxacin (42%), and trimethoprim-sulfamethoxazole (33%). Among XDR and MDR isolates, all isolates were carbapenem- resistant.

Antimicrobial sensitivity and intrahospital transmission dynamics of Klebsiella spp. isolates during a one-year period

Sensitivity to only one antibiotic (colistin or chloramphenicol or ceftazidime/avibactam) among MDR/XDR was noticed among 21 out of total 55 MDR/XDR isolates (3, 16 and 2 isolates, respectively). We detected 24 (8%) isolates resistant to colistin, but this needs to be aditionally confirmed with the broth microdilution method. Majority of MDR/XDR isolates (35/55) were recovered from specimens sent from surgery and intensive care units. Also, it is noteworthy that first MDR/XDR isolate was detected in April and second in June. All other strains were detected during the second half of the last year.

Conclusion: Although we detected a relatively small percentage of MDR/XDR Klebsiella spp. isolates, it is worrisome that the majority of MDR/XDR isolates was mainly detected during the last 6 months of 2022. Also, most of the XDR isolates resistant to colistin were sensitive only to one antibiotic (chloramphenicol or phosphomicin).

Antimikrobna osjetljivost i dinamika intrahospitalne transmisije bakterija iz roda Klebsiella tokom jednogodišnjeg perioda

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Uvod: Bakterije iz rod Klebsiella su veoma česti uzročnici humanih intrahospitalnih infekcija koje su udružene sa značajnim morbitidetom i mortalitetom zbog ograničenog izbora antimikrobne terapije.

Cilj ove studije je bio da se odredi antimikrobna osjetljivost i dinamika širenja Klebsiella spp. izolovanih kod ležećih pacijenata u dobojskoj bolnici.

Metode: Antimikrobna osjetljivost Ispitivana je disk difuzionom metodom prema EUCAST uputstvima kod bakterija iz roda Klebsiella izolovanih tokom jednogodišnjeg perioda (01.01.-31.12.2022) u bolnici "Sveti Luka", Doboj na sljedeće antibiotike: amoksicilin-klavulanska kiselina, ceftriakson, cefotaksim, ceftazidim/avibaktam, meropenem, imipenem, amikacin, ciprofloksacin, kolistin, hloramfenikol, fosfomicin i trimetoprim sulfametoksazol.

Rezultati: Tokom navedenog perioda u mikrobiološkoj laboratoriji su analizirana 4822 uzorka kod kojih je izolovan 1051 uzročnik. Klebsiella spp. je izolovana kod 284 (27%) pacijenta. Na osnovu antimikrobne osjetljivosti, svi izolati su klasifikovani u 3 grupe: izolati osjetljivi na više od 4 antibiotika (n=229, 81%), multirezistentni (MDR) izolati (n=8, 3%) i prošireno rezistetni (XDR) izolati (n=47, 16%). U grupi izolata osjetljvih na više od 4 antibiotika, antimikrobna osjetljivost je bila sljedeća: amoksicilin-klavulanska kiselina (25%), ceftriakson (52%), cefotaksim (52%), meropenem (100%), imipenem (90%), amikacin (90%), ciprofloksacin (42%), i trimetoprim sulfametoksazol. (33%). SVi MDR i XDR izolati su bili rezistentni na karbapeneme. Osjetljivost na samo 1 antibiotik u MDR/XDR grupama je uočena kod 21 od 55 izolata, i to na kolistin (n=3), hloramfenikol (n=16) i ceftazidim/avibaktam (n=2). Rezistencija na kolistin je detetkovana kod 24 (8%) izolata, ali je neophodno da se uradi potvrda bujon dilucionom metodom.

Antimikrobna osjetljivost i dinamika intrahospitalne transmisije bakterija iz roda Klebsiella tokom jednogodišnjeg perioda

Većina MDR/XDR bakterija je izolovana iz uzoraka koji su poslati sa hirurgije i jedinice intenzivne njege. Zanimljivo je napomenuti da je prvi MDR/XDR izolat detektovan u aprilu, drugi u junu, a svi ostali tokom druge polovine godine.

Zaključak: Iako smo dokazali relativno mali broj MDR/XDR izolata iz roda Klebsiella, zabrinjavajući je njihov porast tokom druge polovine 2022. godine. Većina XDR izolata rezistetnih na kolistin su bili osjetljivi samo na 1 antibiotik (hloramfenikol ili fosfomicin).

Immune response induced by the inactivated C. psittaci strain in mouse model

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Chlamydia infections caused by the representatives of Chlamydia family pathogens are the widespread bacterial diseases affecting humans, wild and domestic animals, and birds. Zoonotic chlamydia infections can be transmitted from animals to humans accentuating the necessity for development of effective measures to control the disease in animal population, primarily in livestock. Live whole-cell attenuated vaccines developed and used in Western countries for chlamydia prophylaxis in farm animals are considered as the best ones due to their ability to induce strong humoral and cellular immunity, while being not absolutely safe. In contrast, only killed (inactivated) whole cells vaccines are used for prevention of chlamydiosis for the safety reasons in Russian Federation.

This study aimed to investigate antigen-specific immune responses induced in mouse model by immunization with the candidate inactivated whole-cell vaccine based on the C. psittaci AMK-16 strain (AMK-16) isolated from the infected fetus of an aborted goat with chlamydia genital infection. Material&Methods. Outbred mice were immunized twice intraperitoneally with the inactivated suspension of C. psittaci AMK-16 bacteria mixed with complete/incomplete Freund's adjuvant, and the antibody and cellular immune responses were evaluated in immunoblot and lymphocyte proliferation assay using the homologous and wild type (wt) recombinant major outer membrane proteins (MOMPs), chemically derived outer membrane extract (C-antigen) and whole-cell bacterial lysate. Results. Strong humoral and cellular responses directed to the C-antigen, to the homologous and, notably, wt recombinant MOMPs were revealed in outbred mice immunized with the AMK-16.

Immune response induced by the inactivated C. psittaci strain in mouse model

These results indicate that the immune response induced by the inactivated C. psittaci AMK-16 could be protective against the infection caused by the wild type C. psittaci strains. Also, our data are in a good correlation with the recent report showed that the candidate killed whole-cell vaccine based on the C. psittaci AMK-16 strain provided a marked protection against both homologous lethal challenge and Chlamydia-associated abortions and stillbirths in small laboratory animal models (Evstifeev et al., 2020, 2021). This study was supported by the RSF project # 22-16-00165.

Synergistic effect of inhibiting bacterial growth when using an antibiotic and silver nanoparticles

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A significant problem with the use of antibiotics is the appearance of bacterial resistance (1). Many researchers claim pronounced bactericidal properties of silver nanoparticles (2). With the combined use of these substances, it would be possible not only to cope with the drug resistance of bacteria, but also to reduce the dosage of drugs, without decreasing their effectiveness.

In this work, the bacterium Pseudomonas putida was taken as a safe alternative to Pseudomonas aeruginosa. Biogenic silver nanoparticles synthesized with Sphagnum fallax moss extract were used. With the help of a disco-diffusion analysis, the most effective ciprofloxacin was selected from six antibiotics. The bacteria were cultured with nanoparticles and an antibiotic for 48 hours, and growth curves were constructed according to hourly optical density measurements.

The concentrations at which the minimal inhibitory effect is observed individually are 0.1 µg/ml of ciprofloxacin and 2.5 µg/ml of silver nanoparticles. At the same time, the combined use of these substances in such concentrations reduced the peak concentration of bacteria by 20% and increased their overall inhibition by more than 50%. This effect was also observed at higher concentrations.

With a separate application, complete inhibition began at 1.0 µg/ml of ciprofloxacin and 25 µg/ml of silver nanoparticles. The combined use of the antibiotic with nanoparticles made it possible to completely inhibit the growth of bacteria at concentrations of 0.2 µg/ml of the antibiotic and 10 µg/ml of nanoparticles, which is the result of a synergistic effect that reduced the dosage by 2.5-5 times.

Synergistic effect of inhibiting bacterial growth when using an antibiotic and silver nanoparticles

Given that the synergistic effect is shown on P. putida bacteria, this approach seems promising in the development of drugs based on silver nanoparticles and antibiotics potentially effective against ESCAPE bacteria.

Supported by the Russian Science Foundation, No. 21-74-10034.

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Change of antimicrobial resistance of Streptococcus agalactiae to erythromycin and clindamycin over a 10 years southwestern region of Republic of North Macedonia

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Introduction: Goup B Streptococcus (GBS) is a leading cause of meningitis and bacteriemia in newborns and also causes a variety of invasive diseases in pregnant women and in adult patients. Pregnant women should be tested for presence of GBS between 35-37 weeks of gestation. If they are carrying GBS during intrapartum period, it is recommended that they are treated with penicillin. Erythromycin and clindamycin are useful alternative therapy in penicillin-allergic patients, however, emergence of strains resistant to macrolides has is a growing concern.

The aim of this study was to compare the antibiotic susceptibility patterns of GBS strains to penicillin, erythromycin, clindamycin and vancomycin during two periods 2010-2013 and 2020-2022.

Materials and Methods: The samples were collected from vaginal and vulvo-rectal swabs from pregnant women as a part of the screening for colonization with GBS and from clinical samples at Microbiology department in Center for public health Bitola, between 2010-2013 and 2020-2022. Isolates GBS were identified using standard microbiological techniques. Antibiotic susceptibility testing was performed using the disk diffusion method according to EUCAST guidelines.

Results: A total of 794 GBS isolates were included in the study, 157 from 2010-2013 and 637 from 2020-2022. The results showed that all strains from both periods were susceptible to penicillin and vancomycin, Erythromycin and clindamycin resistance among GBS isolates has increased over past decade from 28,57% and 30,38% (consecutively) in 2010-2013 to 37,60% and 40,56% (consecutively) in 2020-2022.

Change of antimicrobial resistance of Streptococcus agalactiae to erythromycin and clindamycin over a 10 years southwestern region of Republic of North Macedonia

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Conclusion: The results of this study indicate that penicillin and ampicillin are still treatment of choice for intrapartum antibiotic prophylaxis and therapy for infections caused by GBS in Republic of North Macedonia. But there has been a significant increase in the resistance of GBS to erythromycin and clindamycin between 2010-2013 and 2020-2022. These findings suggest that continuous monitoring of antibiotic susceptibility patterns is essential for the proper treatment of GBS infections and the prevention of antibiotic resistance.

Key words: GBS, antibiotic susceptibility patterns, intrapartum antibiotic prophylaxis

In Vitro Activities of Eugenol+Ciprofloxacin Combination Against Carbapenem-Resistant Enterobacterales (CRE) Strains Isolated From Urinary Tract Infections

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Background: The challenge presented by the emergence of antibiotic resistance is increasingly significant. Difficulties in treating resistant microorganisms result in high morbidity and mortality. Treatment options for infections caused by these resistant microorganisms are reduced to a few antibiotics. Essential oils obtained from parts of plants such as leaves, fruits, bark and roots are promising natural compounds due to their broad antimicrobial properties. In recent years, studies reporting that some essential oils have synergistic effects when used together with antibiotics, frequently used to treat resistant microorganisms, have increased rapidly.

Objectives: This study aimed to investigate the in vitro effectiveness of the eugenol+ciprofloxacin combination against CRE isolates.

Methods: Thirty-five CRE strains were screened, and the MIC of eugenol and ciprofloxacin were determined by broth microdilution. The in vitro synergistic activities of eugenol with ciprofloxacin were determined by time-kill assays at 2xMIC, 1xMIC and 0.5xMIC ciprofloxacin concentration+100mg/L eugenol against four both K.pneumoniae and E.coli, respectively. Also, mutant prevention concentration (MPC) was evaluated.

Results: The MIC values for eugenol, ciprofloxacin and eugenol+ciprofloxacin against K.pneumoniae and E.coli were 1250, 4, 8 and 1250, 128, and 128 mg/L, respectively. At 2xMIC, the eugenol+ciprofloxacin combination showed synergistic activity against all the tested strains. The synergistic interactions of these antimicrobial agents were also performed with combinations of cefiderocol+colistin. No antagonism was observed. Also, the eugenol+ciprofloxacin combination showed lower MPC compared with ciprofloxacin alone.

Conclusions: Our findings present significant implications for using the eugenol combination against treating infections caused by CRE isolated from urinary tract infections.

Effects of CSA-44 and CSA-192 in combination with linezolid against Enterococcus spp.

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Although Enterococcus spp., which are a member of the normal flora of the human intestine, mouth, vagina, urethra and biliary tract, were considered to be bacteria with low virulence until recently, they have been increasingly detected as agents in serious hospital and community acquired infections in recent years. They can cause urinary tract infections, bloodstream infections, endocarditis, wound infections, pelvic infections, foreign body infections and meningitis.

Today, the most important feature of enterococcus is the increasing resistance rates to antibiotics, especially vancomycin-resistant enterococcus (VRE) have taken their place in the group of microorganisms whose importance are increasing worldwide. Attempts have been made to discover new antimicrobial agents that target new sites that can overcome resistance. The cationic steroid antibiotics (CSA) designed to mimic the activities of antimicrobial peptides, are a new class of antimicrobial agent.

In this study, in vitro combinations of the novel cationic steroid molecules, CSA-44 and CSA-192 and linezolid were investigated against fifty Enterococcus spp. (16 of them VRE) isolated from various clinical speciemens submitted to the Synevo Laboratories Ankara Central Laboratory in Turkey (2021-2022). The effects of CSA-44 and CSA-192 in combination with linezolid were assessed by using the microbroth checkerboard technique. With this method, synergy was defined as fractional inhibitory concentration (FIC) index of ≤0.5 and antagonism as a FIC index of ≥4.0. A FIC index of 0.5 but ≤4 was considered to be the result of indifference.

Effects of CSA-44 and CSA-192 in combination with linezolid against Enterococcus spp.

The minimum inhibitor concentration ranges (µg/ml) for CSA-44, CSA-192 and linezolid were found 1-16, 0.5-32 and 0.125-1, respectively. According to FIC index, synergistic interactions were only seen with CSA-192- linezolid combination against 3 Enterococcus sp., which one of them VRE. No antagonism was observed.

ACKNOWLEDGEMENTS

This work was supported by the Health Institutes of Turkey (TUSEB) [project number 2022ACİL-12093]

Protektivna uloga polimorfizama IFNL3 i IFNL4 gena u razvoju COVID-19 pneumonije kod žena

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Uvod: Interferoni (IFN) tip III, poznatiji kao IFN-λ, su glavni interferoni indukovani u epitelnim ćelijama respiratornog trakta, produkovani čak i pre IFN tipa I, čija je glavna uloga rano ograničavanje virusne infekcije u gornjim disajnim putevima. Značaj polimorfizama IFNL3 i IFNL4 gena prepoznat je u mnogim virusnim infekcijama.

Protektivna uloga polimorfizama IFNL3 i IFNL4 gena u razvoju COVID-19 pneumonije kod žena

Shodno tome, cilj ovog istraživanja je da se utvrdi uticaj polimorfizama gena za IFNL3 i IFNL4 na težinu COVID-19. Metode i pacijenti: Ova opservaciona studija obuhvatila je 178 pacijenata hospitalizovanih u Univerzitetskom kliničkom centru Kragujevac sa laboratorijski potvrđenom SARS-CoV-2 infekcijom. Demografski i klinički podaci na prijemu preuzeti su iz elektronske medicinske dokumentacije. Komercijalno dostupni TaqMan eseji su korišćeni za genotipizaciju polimorfizama pojedinačnih nukleotida na lokusima IFNL3 (rs8099917 i rs12980275) i IFNL4 (rs12979860 i rs368234815).

Rezultati: Veći odnos neutrofila i limfocita (N/L) i viši nivo LDH na prijemu bili su povezani sa teškim formama bolesti. Stariji pacijenti sa nižom koncentracijom albumina i većim brojem komorbiditeta (izražen Čarlsonovim indeksom komorbiditeta - CCI) na prijemu u hospital imali su veću verovatnoću za progresiju u teške forme bolesti. Sa druge strane, žene su imale manje šanse da razviju umereni COVID-19 u poređenju sa blagim slučajevima. Uočen je statistički značajan uticaj polimorfizama IFNL3 i IFNL4 gena na razvoj od blage do umerene kliničke slike COVID-19, dok umešanost u dalju progresiju bolesti nije zabeležena. Daljom analizom utvrđeno je da je uticaj polimorfizama IFNL3 i IFNL4 gena na razvoj pneumonije statistički značajano prisutan samo u populaciji žena.

Multivarijabilnom regresijom utvđeno je da žene koje su nosioci makar jednog minornog alela IFNL3 rs8099917 (TG+GG) ili rs12980275 (AG+GG) imaju od 94,2% do 96% manju verovatnoću za razvoj COVID-19 pneumonije, dok se taj rizik smanjuje i do 99,2% kod žena sa oba minorna alela IFNL4 rs368234815 (ΔG/ΔG). Istovremeno, veće vrednosti CCI i N/L su bili statistički značajni faktori rizika za razvoj COVID-19 pneumonije.

Zaključak: Minorni aleli testiranih polimorfizama IFNL3 i IFNL4 imaju protektivni uticaj na pojavu COVID-19 pneumonije samo kod žena, dok su postojeći komorbiditeti i disbalans neutrofila i limfocita indikatori progresije COVID-19.

The Protective Effect of IFNL3 and IFNL4 Gene Polymorphisms on Development of COVID-19 Pneumonia in Female Patients

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Introduction: Type III interferons (IFN), also known as IFN-λ, are the main interferons induced in respiratory epithelial cells and produced even before IFN type I, with the primary role of limiting viral infection in the upper respiratory tract.

The Protective Effect of IFNL3 and IFNL4 Gene Polymorphisms on Development of COVID-19 Pneumonia in Female Patients

The significance of IFNL3 and IFNL4 gene polymorphisms has been recognized in many viral infections. Therefore, the aim of this research is to determine the association of IFNL3 and IFNL4 gene polymorphisms with COVID-19 severity.

Methods and Patients: This observational study included 178 patients hospitalized at the University Clinical Center Kragujevac with laboratory-confirmed SARS-CoV-2 infection. Demographic and clinical data at admission were obtained from the electronic medical documentation. Commercially available TaqMan assays were used for genotyping single nucleotide polymorphisms at the IFNL3 (rs8099917 and rs12980275) and IFNL4 (rs12979860 and rs368234815) loci.

Results: Severe COVID-19 cases had higher neutrophil-to-lymphocyte ratio (N/L) and LDH levels at admission. Older patients with a lower albumin concentration and a higher number of comorbidities (expressed as Charlson Comorbidity Index - CCI) at admission had a higher likelihood of progressing to severe forms. On the other hand, women had lower odds of developing moderate COVID-19 compared to mild cases. A statistically significant impact of IFNL3 and IFNL4 gene polymorphisms on the development of mild to moderate clinical COVID-19 was observed, while involvement in further disease progression was not recorded. Further analysis showed that the association between IFNL3 and IFNL4 gene polymorphisms and the development of pneumonia was statistically significant only in the female population. Multivariable regression showed that women carriers of at least one minor allele of IFNL3 rs8099917 (TG+GG) or rs12980275 (AG+GG) have a 94.2% to 96% lower likelihood of developing COVID-19 pneumonia, while this risk is reduced to 99.2% in women with both minor alleles of IFNL4 rs368234815 (ΔG/ΔG). Higher CCI and N/L values were statistically significant risk factors for the development of COVID-19 pneumonia.

Conclusion: The minor alleles of the tested IFNL3 and IFNL4 polymorphisms have a protective effect on the development of COVID-19 pneumonia only in women, while the presence of preexisting comorbidities and an imbalance of neutrophils and lymphocytes are indicators of COVID-19 progression.

In vitro antifungalna aktivnost prirodnih hidrofilnih eutektičkih smeša na bazi holin hlorida

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Poslednje dve decenije u svetu je zabeležen porast broja kandidijaza – infekcija Candida vrstama kod ljudi pri čemu su sistemske kandidijaze (kandidemije) najteži oblik infekcija. Njihova učestalost je veća kod imunokompromitovanih osoba čiji su odbrambeni mehanizmi oslabljeni, kao i kod osoba kod kojih postoje drugi faktori rizika. Vrsta Candida albicans je vodeći fungalni intrahospitalni patogen, ali i normalni stanovnik mukoznih membrana čoveka.

Zahvaljujući faktorima virulencije ona aktivno učestvuje u patofiziologiji nastanka i napredovanja infekcija. Vrsta C. guillermondii ostvaruje znatno manju incidencu kandidemije, ali se pokazala kao invazivna vrsta koja brzo razvija rezistenciju prema antifungalnim lekovima. Usled invazivnosti, toksičnosti i pojave sve veće rezistencije Candida vrsta na postojeće antifungalne lekove proces intenzivnog otkrivanja novih antifungalnih agenasa još uvek traje.

U skladu sa principima očuvanja životne sredine i usled toksičnosti konvencionalnih antifungalnih agenasa danas se ispituju različite prirodne supstance uključujući i prirodne eutektičke smeše (NADES) koje imaju široku upotrebu u različitim tehnološkim procesima, a neispitane su biološke aktivnosti. Cilj rada je bio ispitivanje antifungalne aktivnosti 10 hidrofilnih NADES-a na bazi holin hlorida (ChCl:MalA, ChCl:LacA, ChCl:AscA, ChCl:Ur, ChCl:Gly, ChCl:Sor, ChCl:1,3PD, ChCl:1,2PD, ChCl:1,3BD, ChCl:1,4BD) pri tretmanima 1,25 – 100%. Ispitivane su C. albicans (ATCC 10231) i C. guillermondii (JR 23) poreklom iz kolekcije kultura Katedre za mikrobiologiju PMF UNS i dva humana klinička izolata (H1; H2) poreklom iz laboratorije Instituta za javno zdravlje Vojvodine Novi Sad, Srbija.

In vitro antifungalna aktivnost prirodnih hidrofilnih eutektičkih smeša na bazi holin hlorida

Primenom mikrodilucione metode određivana je minimalna inhibitorna koncentracija (MIC) uz pomoć binokularne lupe, kao i minimalna fungicidna koncentracija (MFC) prebrojavanjem kolonija na čvrstim podlogama CFU metodom. Prema dobijenim rezultatima svih deset NADES-a pokazalo je antifungalnu aktivnost i to pri tretmanima 1,25 – 40%. Najefektivniji su bili ChCl:MalA, ChCl:LacA, ChCl:AscA pri tretmanu 1,25% na H1. Preliminarno određivanje antifungalne aktivnosti NADES-a predstavlja prvi korak u otkrivanju novih antifungalnih agenasa i baza za dalja ispitivanja njihove biološke aktivnosti.

Ključne reči: Candida, kandidemije, NADES

In vitro activities of ceftazidime/avibactam alone or in combination with antibiotics against multidrug-resistant Stenotrophomonas maltophilia isolates

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Objectives: Stenotrophomonas maltophilia is an important drug-resistant opportunistic pathogen that can cause various infectious such as meningitis, endocarditis, acute respiratory tract infections and bacteraemia in humans. The high incidence of S. maltophilia infections is threatening for immunocompromised people. Besides, this pathogen can cause infections in the lungs of patients with cystic fibrosis. S. maltophilia is remarkably understudied compared with other Gram-negative bacteria. This study was aimed to investigate the antimicrobial activities of various antibiotics against Multi-drug resistant S maltopohilia strains isolated from intensive care units. Also, the synergistic effects of ceftazidime/avibactam with tigecycline, chloramphenicol, levofloxacin, colistin or amikacin were investigated with seven S. maltophilia strains presenting different susceptibility patterns.

Materials/methods: The antimicrobial activities of various antibiotics were studied against 37 strains of S. maltophilia. MICs were determined by microdilution method according to EUCAST. The in-vitro synergistic activities of ceftazidime/avibactam with antibiotic combinations were determined by time-kill assays at 1× MIC and 4× MIC against seven MDR S.maltophilia isolates.

Results: Based on MIC results, although, all isolates were found to be resistant to ceftazidime, the MIC50 and MIC90 for ceftazidime/avibactam were 16 µg/ml and 128 µg/ml and showed better effect compared with ceftazidime alone. At 4×MIC, the synergistic activities of ceftazidime/avibactam in combination with the tested antibiotics were observed against the tested strains. No antagonism was observed.

Conclusions: To our knowledge, this is the first study to investigate the of synergistic effects of ceftazidime/avibactam with antibiotics against S. maltophilia strains.

Epidemiological and clinical-laboratory characteristics of the covid 19 infection in Bulgaria during the spring-summer season of the 2022

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Unlike the flu, which in Bulgaria has a marked seasonality during the winter-spring season and usually, the epidemic waves of it have their highest peaks in the months of January and February, the covid 19 infection "attacks" the human population during the remaining period of time. The purpose of our study is to characterize the epidemiological, clinical and paraclinical changes in patients with covid 19 in the summer season of 2022 in patients in the West Bulgaria region.

Materials and methods: A retrospective analysis was carried out of 145 patients who had undergone treatment through the Department of Infectious Diseases of two major medical centers. Anamnestic, epidemiological, clinical, paraclinical and therapeutic data were used. Antigenic tests were carried out for Covid- 19, microbiological studies of throat secretions and imaging studies. A telephone survey was also conducted to draw conclusions about the long consequences of covid 19 infection. Results. The largest number of patients are urban residents ahead of those from villages. Only 6.2% of those studied were immunized, the rest were without a single dose of Covid-19. The largest share is in the age groups between 69 and 75 years. The most common symptoms were cough – at 90.8% fatigue at 64.6%, shortness of breath at 43.10% phlegm at 27.2%, appetite and sore throat at 26.2%.

Epidemiological and clinical-laboratory characteristics of the covid 19 infection in Bulgaria during the spring-summer season of the 2022

The remaining symptoms such as nausea, vomiting, diarrhoea, and muscle and joint pain are less common. From the conducted microbiological studies of throat secretion and sputum – pathogenic microorganisms are not isolated. Of the imaging methods, radiography of the lung and heart is found, and it was found that with pneumonia 76.9% as they have a respiratory failure of them are four, there are one exit letails, with bronchitis - is 1.5%, with pleural effusion - 3.1%. Without complications, the part of the lung is 13.8% of those hospitalized.

It was found that four of the patients had persistent fatigue, two had joint pain, and the other patients did not report complaints. We found no statistical relationships between haemoglobin and fatigue and leukocytes and pneumonia. Conclusion: Vaccine coverage is at a low rate in the risk groups such as old people. They remaining to be the most affected group in need of intensive medical care

Keywords: Covid-19, influenza, sore throat, no appetite, sputum, pneumonia.

The study was supported by Grant BG05M2OP001-1.002-0001-C04.

/ KRATKE POSTER PREZENTACIJE

/ SHORT POSTER PRESENTATIONS

Moderators: Ivana Čolović-Čalovski, Stefan Mijatović

Imunski odgovor izazvan različitim vrstama vakcina i SARS-CoV-2 infekcijom kod zdravstvenih radnika Univerzitetsko-kliničkog centra u Republici Srpskoj

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Uvod. U posljednje tri godine virus teškog respiratornog sindroma (engl. Severe Acute Respiratory Syndrome Coronavirus-2, SARS-CoV-2) je uzrokovao značajan morbiditet i mortalitet, te je prema dosadašnjim podacima prouzrokovao smrt kod preko 6 miliona ljudi, a potvrđeno je preko 600 miliona slučajeva infekcije širom svijeta. Većina vakcina koje su u upotrebi sadrže kao glavni antigen S protein na koji se produkuju anti-SARS-CoV-2 antitijela. Cilj ove studije je bio da se odredi titar anti-S IgG antitijela kod zdravstvenih radnika koji su potpuno vakcinisani sa nekom od vakcina, te da se rezultat uporedi sa titrom anti-S IgG antitijela ispitanika oporavljenih od COVID-19.

Materijal i metode. Analizirani su serumi zdravstvenih radnika bez pridruženih oboljenja koji su zaposleni na Univerzitetsko-kliničkom centru, Republika Srpska. U studiju je uključen 261 ispitanik,od kojih je 227 bilo vakcinisano, a 34 su bili ispitanici koji su se oporavili od COVID-19 (bez kliničkih znakova pneumonije). Zdravstveni radnici su vakcinisani istim preparatom neke od dostupnih vakcina (37 ispitanika vakcinisano sa Pfizer-BioNTech, 17 sa Sinopharm, 154 sa Sputnik V, 11 sa Sinovac i 8 sa Oxford/AstraZeneca vakcinom).

Imunski odgovor izazvan različitim vrstama vakcina i SARS-CoV-2 infekcijom kod zdravstvenih radnika Univerzitetsko-kliničkog centra u Republici Srpskoj

Kod vakcinisanih ispitanika uzorci seruma su prikupljeni 21. dan nakon prve doze, 60. dan od druge doze vakcine (odnosno 90. dan od prve doze vakcine) i 180. dan nakon druge doze vakcine. Kod rekonvalescenata titri antitijela su određeni 90 dana nakon potvrđene COVID-19. Serološko testiranje je urađeno primjenom komercijalnog ELISA seta prema uputstvu proizvođača.

Rezultati. Šezdeset dana nakon druge doze vakcine najviši titar antitijela (12 AU/mL) izmjeren je kod vakcinisanih sa Pfizer-BioNTech, zatim kod ispitanika vakcinisanih sa Sinopharm (9,3 AU/mL), Sputnik V (5,9 AU/mL), Sinovac (4,6 AU/mL), dok je kod vakcinisanih sa Oxford/AstraZeneca vakcinom izmjeren najniži titar (2,5 AU/mL). Stopa seropozitivnosti za mRNK vakcinu bila je 88,5%, za vektorske vakcine 86,2%, dok je za inaktivisane vakcine bila 71,4%. Poređenjem titra antitijela 90. dan od prve doze vakcine kod vakcinisanih ispitanika (7,06 AU/mL) sa titrom antitijela kod rekonvalescenata (5,76 AU/mL), utvrđeno je da nije bilo značajne razlike (p=0,08).

Zaključak. Veću stopu serokonverzije imali su ispitanici vakcinisani sa mRNK i vektorskim vakcinama u poređenju sa ispitanicama vakcinisanim sa inaktivisanim vakcinama, ili u poređenju sa rekonvalescentima.

THE ROLE OF DOGS IN THE CONTAMINATION OF PUBLIC SURFACES IN BELGRADE WITH GIARDIA INTESTINALIS

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Pets, especially dogs, play a significant role in the lives of modern people in urban environments. Alienation, stress, and other factors that burden the city man are often overcome psychologically by acquiring a dog that, with its affection, represents excellent psychotherapy. Unfortunately, in addition to this favorable influence, the presence of dogs in urban areas has its other side. In the urban environment, green areas and parks are the main places where children play and they represent resting places for city people, but they are also places where dog owners take their pets out. During their stay on these surfaces, they constantly contaminate them with their feces, which, in addition to the unpleasant smell, also present an epidemiological danger because dogs are carriers of many zoonotic parasites, including protozoa Giardia intestinals.

It is a zoonotic flagellate that occurs in the form of a vegetative form that is pear-shaped with 8 flagella or a cystic form that is egg-shaped. Giardia cysts survive well in the external environment and only a small number of cysts are needed to cause infection, either through contact with an infected animal (sniffing and coprophagia in dogs) or through contaminated food and water. After the cyst enters the digestive tract, the vegetative forms of giardia are released, which will remain on the surface of the intestinal villi, multiply and, after a certain time, become encysted. Parasites live in the duodenum and can also reach the liver and bile ducts. In dogs, giardiasis is accompanied by long-term diarrhea with extremely unpleasant-smelling excrement that contains slime and has the appearance and color of clay. In order to examine the infection of dogs and the pollution of public areas in Belgrade, in 2021, an examination of 74 soil samples from public areas and 210 samples of dog excrement collected from public areas and by veterinarians from clinics from dogs that had clinical symptoms indicating this parasitosis.

THE ROLE OF DOGS IN THE CONTAMINATION OF PUBLIC SURFACES IN BELGRADE WITH GIARDIA INTESTINALIS

Examinations of soil and feces were performed using the flotation method, while 82 samples of feces were examined in clinics with the IDEXX Snap Giardia test. During these inspections, 15.62% of the land sample was contaminated with giardia, and the inspection of the dogs revealed infection in 45.78%.

The presence of infected dogs (and people) contaminating public areas with their excrement creates the conditions for the spread of giardiasis and the permanent possibility of infection of dogs and people in urban areas.

ULOGA PASA U KONTAMINACIJI JAVNIH POVRŠINA BEOGRADA SA GIARDIA INTESTINALIS

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Životinje ljubimci, a posebno psi imaju značajnu ulogu u životu savremenih ljudi u gradskom okruženju. Otuđenost, stres, i ostali faktori koji opterećuju gradskog čoveka često se psihički prevazilaze nabavkom psa koji svojom privrženošću predstavlja odličnu psihoterapiju. Nažalost pored ovako povoljnog uticaja, prisustvo pasa u urbanim sredinama ima i svoju drugu stranu.

U urbanoj sredini zelene površine i parkovi su glavna mesta na kome se igraju deca i ona predstavljaju odmorišta gradskih ljudi, ali takođe su i mesta na kojima vlasnici pasa izvode svoje ljubimce. Tokom boravka na ovim površinama oni ih stalno kontaminiraju svojim fecesom, koji osim neprijatnog mirisa predstavlaj i epidemiološku opasnost jer su psi nosioci mnoštva zoonotski parazita uključujući protozoe Giardia intestinals.

To je zoonotska flagelata koja se javlja u vidu vegetativnog oblika koji je kruškolikog zgleda sa 8 bičića ili cističnog koji je jajastog oblika. Ciste giardija dobro opstaju u spoljašnjoj srediniu i potreban je samo mali broj cisti da uzrokuju infekciju, bilo kontaktom sa zaraženom životinjom (njuškanje i koprofagija kod pasa) ili zagađenom hranom i vodom. Nakon ulaska cisti u digestivni trakt, oslobađaju se vegetativni oblici giardia koji će se zadržavati na površini crevnih resica, razmnožiti se i posle određenog vremena, encistirati. Paraziti žive u duodenumu a mogu dospeti i u jetru i žucne puteve. Kod pasa je gardiaza praćena dugotrajnim diarejama sa izmetom izuzetno neprijatnog mirisa koji sadrži primese slizi i ima izgled i boju gline. Da bi se ispitala inficiranost pasa i zagađenost javnih površina u Beogradu je tokom 2021 izvršen pregled 74 uzorka zemljišta sa javnih površina i 210 uzoraka izmeta pasa prikupljenih sa javnih površina i od strane veterinara iz ambulanti od pasa koji su imali kliničke simptome koji su ukazivali na ovu parazitozu.

ULOGA PASA U KONTAMINACIJI JAVNIH POVRŠINA BEOGRADA SA GIARDIA INTESTINALIS

Pregledi zemljišta i fecesa su rađeni primenom flotacione metode dok je 82 uzorka fecesa pregledano u ambulantama i sa IDEXX Snap Giardia testom. Tokom ovih pregleda 15.62% uzorka zemeljišta je bilo kontaminirano giardijom a pregledom pasa infekcija je ustanovljena u 45.78%. Prisustvom inficiranih pasa (i ljudi) koji svojim izmetom kontaminiraju javne površine sticu se uslovi za širenje dardijaze i permanentnu mogućnost infekcije pasa i ljudi u urbanim sredinama.

Cryptosporidium spp. i Giardia spp. u neobrađenim (sirovim) vodama koje se koriste za potrebe vodoopskrbe u Hrvatskoj

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Uvod i ciljevi - Prema Direktivi (EU) 2020/2184 o kvaliteti vode namijenjene za ljudsku potrošnju, zdravstveno ispravna voda ne smije sadržavati parazite, mikroorganizme i tvari koje u određenim koncentracijama ili brojevima predstavljaju potencijalnu opasnost za ljudsko zdravlje. Budući da u Hrvatskoj do sada voda nije analizirana na prisutnost parazita, provedena je pilot studija na neobrađenim (sirovim) vodama koje se koriste za potrebe vodoopskrbe prema novouvedenoj metodi za detekciju i brojanje Giardia spp. i Cryptosporidium spp. baziranoj na USEPA Method 1623.1 i ISO 15553:2006.

Metoda - Uzorci sirove vode (25 L) filtrirali su se na licu mjesta kroz IDEXX Filta-Max filter module. Uzorci su nakon toga isprani pomoću uređaja Filta-Max xpress za eluiranje pod tlakom. Eluat se centrifugirao kako bi se istaložile (oo)ciste, a gornji sloj supernatanta je aspiriran. Uzorci su zatim pročišćeni tijekom imunomagnetske separacije te obojeni fluorescein izotiocijanatom (FITC) i 4',6-diamidino-2-fenilindolom (DAPI). Za identifikaciju (oo)cista koristio se fluorescentni mikroskop, a kvantitativna analiza provedena je brojanjem potvrđenih (oo)cista.

Rezultati - Tijekom tri mjeseca ukupno je prikupljeno 47 uzoraka sirovih voda s pet vodozahavta koji se korsite za potrebe vodoopskrbe. Sveukupno su četiri uzoraka (8,5 %) bila kontaminirana bar s jednim parazitom od interesa. Oociste Cryptosporidium pronađene su u sva četiri pozitivna uzoraka, dok su ciste Giardia pronađene u jednom uzorku sirove vode. Studija po prvi put potvrđuje prisutnost Cryptosporidium spp. i Giardia spp. u sirovim vodama za potrebe vodoopskrbe u Republici Hrvatskoj.

Cryptosporidium spp. i Giardia spp. u neobrađenim (sirovim) vodama koje se koriste za potrebe vodoopskrbe u Hrvatskoj

Priznanja - Studija je rađena u okviru projekta IRI 2 "WaterQ – digitalna transformacija praćenja kvalitete voda" koji je sufinanciran iz Europskog fonda za regionalni razvoj u sklopu Operativnog programa Konkurentnost i kohezija 2014.-2020. (KK.01.2.1.02.0229).

Ključne riječi: Cryptosporidium, Giardia, sirova voda, monitoring vode za ljudsku potrošnju

Rizik od nalaza Listeria monocytogenes u sushiju

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Kratak sadržaj - Sushi (jap. Su-sirće i Me/Shi- pirinač) je tradicionalna azijska hrana, koja potiče iz jugoistočne Azije, a preneta je u Japan pre oko 2000 godina. Priprema se od pirinča začinjenog sirćetom, solju, šećerom i ribe ili morskih plodova, koji su najčešće sirovi, a ponekad termički obrađeni. Spada u hranu spremnu za konzumiranje, što znači da se pre konzumiranja termički ne obrađuje, stoga postoje dileme, pa čak i strah kod konzumenata da li je ova hrana bezbedna, iako je dobar izvor hranljivih materija, pre svega proteina i niskog sadržaja masti i zasićenih masnih kiselina u odnosu na meso. Posebno osetljive grupe su YOPI (Young,Old, Pregnant, Immunodeficient). U literaturi su prisutni podaci o infekcijama Listeria monocytogenes nastalih posle konzumiranja ove vrste hrane, stoga smo za cilj rada postavili da ispitamo uzorke susjija u ugostiteljskim objektima u Beogradu.

Materijal je predstavljalo 40 uzoraka sushija, koji su prikupljeni tokom monitoringa 2021. i 2022.godine. Uzorci su uzeti aseptično u prometu (ugostiteljski objekti) na području Beograda. Zapakovani su u sterilne plastične posudice i u ručnom frižoderu pri temperaturi do 4 °C dostavljeni u laboratoriju gde je analiza započeta. Za dokazivanje L. monocyytogenes je koršćena standardna metoda, EN ISO 11290-2. Rezultati su pokazali da ni u jednom uzorku sushija nije dokazano prisustvo L. monocyytogenes, što ukazuje na dobru higijensku praksu i primenu HACCP sistemu u proizvodnji ove vrste hrane. U prilog tome govore i epidemiološki podaci da se L.monocytogenes ne navodi kao uzrok infekcija posle konzumiranja sushija na teritoriji grada Beograda.

Ključne reči: bezbednost, hrana, sushi, L. monocytogenes.

Napomena: Rad je podržan sredstvima Ministarstva prosvete, nauke i tehnološkog razvoja Republike Srbije (Ugovor broj 451-03-68/2022-14/200143).

The risk of Listeria monocytogenes occurrence in sushi

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Sushi (Japanese: Su-vinegar and Me/Shi-rice) is a traditional Asian food, which originates from Southeast Asia, and was brought to Japan about 2000 years ago. It is prepared from rice seasoned with vinegar, salt, sugar and fish or seafood, which are usually raw and sometimes heat processed. It belongs to ready-to-eat food, which means that it is not thermically processed before consumption. Therefore, dilemmas and even fear are present among consumers whether this food is safe, although it is a good source of nutrients, primarily protein and low fat content and saturated fatty acids compared to meat. Especially, sensitive groups are YOPI (Young, Old, Pregnant, Immunodeficient). Literature data about Listeria monocytogenes infections that occurred after consumption of sushi are present, therefore the aim of this research was to examine the samples of sushi in catering facilities in Belgrade.

The material represented 40 samples of sushi, which were collected during monitoring in 2021 and 2022. The samples were taken aseptically at retail market (catering facilities) in the area of Belgrade. They were packed in sterile plastic containers and delivered in a manual refrigerator at a temperature of up to 4 °C to the laboratory where the analysis were immediately started. The standard method EN ISO 11290-2 was used to detect the preence of L. monocytogenes. The obtained results showed that the presence of L. monocytogenes was not detected in any sample of sushi, which indicates good hygiene practices and the application of the HACCP system in the production of this type of food. In support to this, epidemiological data also show that L. monocytogenes is not cited as a cause of infections after consumption of sushi in the territory of the city of Belgrade.

Key words: safety, food, sushi, L. monocytogenes

Acknowledments: The research was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Contract number 451-03-68/2022-14/200143).

Change of antimicrobial resistance of Streptococcus agalactiae to erythromycin and clindamycin over a 10 years southwestern region of Republic of North Macedonia

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Introduction: Goup B Streptococcus (GBS) is a leading cause of meningitis and bacteriemia in newborns and also causes a variety of invasive diseases in pregnant women and in adult patients. Pregnant women should be tested for presence of GBS between 35-37 weeks of gestation. If they are carrying GBS during intrapartum period, it is recommended that they are treated with penicillin. Erythromycin and clindamycin are useful alternative therapy in penicillin-allergic patients, however, emergence of strains resistant to macrolides has is a growing concern.

The aim of this study was to compare the antibiotic susceptibility patterns of GBS strains to penicillin, erythromycin, clindamycin and vancomycin during two periods 2010-2013 and 2020-2022.
Materials and Methods: The samples were collected from vaginal and vulvo-rectal swabs from pregnant women as a part of the screening for colonization with GBS and from clinical samples at Microbiology department in Center for public health Bitola, between 2010-2013 and 2020-2022. Isolates GBS were identified using standard microbiological techniques. Antibiotic susceptibility testing was performed using the disk diffusion method according to EUCAST guidelines.

Results: A total of 794 GBS isolates were included in the study, 157 from 2010-2013 and 637 from 2020-2022. The results showed that all strains from both periods were susceptible to penicillin and vancomycin, Erythromycin and clindamycin resistance among GBS isolates has increased over past decade from 28,57% and 30,38% (consecutively) in 2010-2013 to 37,60% and 40,56% (consecutively) in 2020-2022.

Change of antimicrobial resistance of Streptococcus agalactiae to erythromycin and clindamycin over a 10 years southwestern region of Republic of North Macedonia

Conclusion: The results of this study indicate that penicillin and ampicillin are still treatment of choice for intrapartum antibiotic prophylaxis and therapy for infections caused by GBS in Republic of North Macedonia. But there has been a significant increase in the resistance of GBS to erythromycin and clindamycin between 2010-2013 and 2020-2022. These findings suggest that continuous monitoring of antibiotic susceptibility patterns is essential for the proper treatment of GBS infections and the prevention of antibiotic resistance.

Key words: GBS, antibiotic susceptibility patterns, intrapartum antibiotic prophylaxis

Biogenic silver nanoparticles: cytotoxic effect

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The search for fundamentally new antimicrobial agents with a lower probability of leading to the development of bacterial resistance is relevant. One of the most promising directions for solving this problem is the use of silver nanoparticles, because of a wide range of their actions against gram-positive and gram-negative bacteria, as well as a much lower ability of microbes to develop resistance to silver nanoparticles, in comparison with antibiotics. Plant extracts are preferred over other biological sources for the synthesis of nanomaterials due to their availability and wide range of reducing metabolites. The rate of biological synthesis using plant parts is higher than in methods with microorganisms, and the resulting nanomaterials are more stable and diverse in shape and size.

Silver nanoparticles were obtained using an aqueous extract of the moss Sphagum fallax. S. fallax plants were taken from an oligotrophic bog. Spectrophotometric studies showed the presence of a characteristic peak at a wavelength of 420 nm, which confirms the formation of colloidal silver nanoparticles. In addition, the catalytic activity of AgNPs was determined in the reduction reaction of 4-nitrophenol to 4-aminophenol with sodium tetrahydroborate. The absorption of the resulting solution was measured in the range of 200–700 nm (UV-1280, Shimadzu, Japan) with an interval of 1 sec between measurements.

Silver nanoparticles (SNPs) are believed to selectively affect bacterial membranes, are effective as antimicrobial agents at low concentrations, and are not cytotoxic to eukaryotic cells. To analyze the cytotoxic activity of the obtained biogenic silver nanoparticles, their effect on human lung carcinoma cells (A549) after 24 h of incubation at various concentrations was evaluated. It has been shown that the concentration of nanoparticles of 12.5 μg/ml causes the death of 45% of cells. Currently, work is underway to determine the concentrations that are toxic to microorganisms.

This work was supported by the Russian Foundation for Basic Research (project no. 21-74-10034).

MOST COMMON BACTERIAL ISOLATES FROM WOUND SPECIMENS

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Introduction: Bacterial infections of wounds are among the leading causes of morbidity and mortality through out the world. Pathogens that infect wounds can be part of normal flora or aquaried from the hospital environment.

Aim: To determine the most common microorganisms isolated from wound specimens.

Material and methods: A total 337 wounds samples in a period of one year, from 01.01.2022 to 31.12.2022, were processed in the Department of Microbiology at the Center for Public Health Bitola. All samples were inoculated on Columbia blood agar for isolation of aerobic bacteria, Schaedler agar for anaerobic bacteria, Thioglycolate broth for enrichment of the growth and CAN2 (Candida agar) for isolation of yeasts. Standard microbiological methods and Automatized Vitek 2 system was used for identification of all microorganisms.

Results: Of a total 337 samples , positive were 209 (62%) with 288 isolated microorganisms. Of these 142 were gram positive, 128 gram negative, 14 yeasts and 4 strains were anaerobs. 128 (38%) specimens were negative by culture. The most frequently isolated microorganisms were: Staphylococcus aureus 76 (26,4) of which MRSA were 16 (21%), Enterococcus faecalis 48 (16,7%), E.coli 42 (14,5%) of which ESBL positive were 6 (14,2%), Enterobacter cloacae 17 (6%), Pseudomonas aeruginosa 16 (5,5%), Proteus mirabilis 12 (4,2%), Streptococcus **β** haemolyticus 12 (4,2%). From a total positive specimens monobacterial were 70,3% (147/209) and in 29,7% (62/209) were polymicrobial.

Conclusion: High prevalence of bacterial isolates was found, Staphylococcus aureus being the most dominant. Knowing the prevalent type of microorganisms present in infected wounds and their resistance pattern is crucial to choose the adequate treatment.

Key words: wound specimens, bacterial infections, microorganisms, Staphylococcus aureus.

Klebsiella pneumoniae rezistentna na karbapeneme u Sveučilišnoj kliničkoj bolnici Mostar

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Pojava karbapenemaza u enterobakterija predstavlja veliki problem u kliničkoj praksi, zbog velike raznolikosti enzima i činjenice da se rezistencija karbapenemazama može širiti horizontalnim prijenosom gena. Od 2018. godine u Sveučilišnoj kliničkoj bolnici (SKB) Mostar prisutni su izolati Klebsiella pneumoniae rezisteni na karbapeneme (CR). Tokom 2022. i 2023. godine zabilježena je veća učestalost CR K. pneumoniae izolata.

Cilj istraživanja: Kod prikupljenih izolata CR K. pneumoniae odrediti osjetljivost na antibiotike i vrstu karbapenemaze, te usporediti mehanizam rezistencije među prikupljenim izolatima iz 2018., 2022. i 2023. godine.

Materijal i metode: Istraživanje je provedeno u SKB Mostar na 65 prikupljenih izolata CR K. pneumoniae. Izolati su prikupljeni iz različitih kliničkih uzoraka bolničkih pacijenata nezavisno o dobi, spolu i osnovnoj bolesti.

Ispitivanje osjetljivosti na antibiotike napravljeno je metodom disk-difuzije po Kirby-Baueru na pločama Mueller-Hinton agara prema EUCAST (European Committee on Antimicrobial Susceptibility Testing) standardima. Za sve testirane antibiotike, osim kolistina, određen je MIK pomoću Vitek 2. MIK za kolistin je određen metodom mikrodilucije u bujonu.

Prisutstvo i vrsta karbapenemaze određena je in vitro brzim dijagnostičkim testom za detekciju OXA-163, OXA-48, KPC, NDM i VIM karbapenemaza (RESIST – 5 O.O.K.N.V., Coris BioConcept).

Klebsiella pneumoniae rezistentna na karbapeneme u Sveučilišnoj kliničkoj bolnici Mostar

Rezultati: Metodom mikrodilucije na uređaju VITEK 2 kod svih prikupljenih kliničkih izolata K. pneumoniae dokazana je rezistencija na karbapeneme, kinolone, trimetoprim/sulfametoksazol i ostale beta-laktamske antibiotike. Za sve testirane izolate MIK za meropenem i imipenem iznosio je ≥ 8 mg. Većina testiranih izolata osjetljiva je na amikacin i kolistin. Svi testirani izolati posjeduju karbapenemazu OXA-48 kao dominantan mehanizam rezistencije na karbapeneme.

Zaključak: S obzirom da je provedenim ispitivanjem dokazana rezistencija testiranih izolata na više skupina antimikrobnih lijekova isti se mogu okarakterisati kao multiplorezistentni ili multirezistentni. Ovo su prvi višegodišnji podaci o prisutnosti CR K. pneumoniae sa OXA-48 mehanizmom rezistencije unutar SKB Mostar.

Klebsiella pneumoniae rezistentna na karbapeneme u Sveučilišnoj kliničkoj bolnici Mostar

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The appearance of carbapenemases in enterobacteria is a major problem in clinical practice, due to the great diversity of enzymes and the fact that carbapenemases resistance can be spread by horizontal gene transfer. Since 2018, carbapenem-resistant (CR) Klebsiella pneumoniae isolates have been present at the University Clinical Hospital (UCH) Mostar. During 2022 and 2023, there was a higher incidence of CR K. pneumoniae isolates.

Aim of the research: In the collected isolates of CR K. pneumoniae, determine the sensitivity to antibiotics and the type of carbapenemaeses, and compare the mechanism of resistance among the collected isolates from 2018, 2022 and 2023.

Material and methods: The study was conducted at the UCH Mostar on 65 collected isolates of CR K. pneumoniae. Isolates were collected from different clinical samples of hospital patients regardless of age, gender and underlying disease.

The antibiotic sensitivity test was done using Kirby-Bauer disc-diffusion method on Mueller-Hinton agar plates according to EUCAST (European Committee on Antimicrobial Susceptibility Testing) standards. For all tested antibiotics, except colistin, MIK was determined using Vitek 2. MIK for colistin was determined by the method of microdilution in broth.
The presence and type of carbapenemase was determined in vitro by rapid diagnostic test for the detection of OXA-163, OXA-48, KPC, NDM and VIM carbapenemases (RESIST – 5 O.O.K.N.V., Coris BioConcept).

Klebsiella pneumoniae rezistentna na karbapeneme u Sveučilišnoj kliničkoj bolnici Mostar

Results: Resistance to carbapenems, quinolones, trimethoprim/sulfamethoxazole and other beta-lactam antibiotics was demonstrated by the method of microditution on VITEK 2 in all collected clinical isolates of K. pneumoniae. For all tested isolates, MIC for meropenem and imipenem was ≥ 8 mg. Most of the tested isolates are sensitive to amikacin and colistin. All tested isolates possess carbapenemase OXA-48 as the dominant mechanism of resistance to carbapenems.

Conclusion: Given that the conducted study proved resistance of tested isolates on several groups of antimicrobial drugs, they can be characterized as multi-resistant. These are the first multi-year data on the presence of CR K. pneumoniae with OXA-48 resistance mechanism within UCH Mostar.

Mikrobiološki profil infekcija operativnog mesta nakon artroplastika kuka i kolena u tercijarnoj hiruškoj ustanovi

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Uvod: Registrovani mikrobiološki profili infekcija operativnog mest (IOM) veoma variraju među razvijenim i zemljama u razvoju u svetu, kao i u Srbiji. Varijabilitet registrovanih mikroorganizama (MO) postoji i među svih hirurškim procedurana, i nakon artroplastika kuka i kolena.

Cilj: Određivanje prouzrokovača IOM i njihove rezistencije, kod pacijenata kojima su urađene totalne i paricjalne artopastike kuka i kolena

Metod: Sprovedena je prospektivna kohortna studija, na 1018 pacijenata, na Klinici za ortopedsku hirurgiju i traumatologiju (KOHT), Kliničkog centra Srbije u Beogradu, u period maj 2016-april 2018. god. Svim pacijentima je uzet bris rane pod aseptičnim uslovima nakon operacije i određivano postojanje prozrokovača IOM u rani.

Mikrobiološki profil infekcija operativnog mesta nakon artroplastika kuka i kolena u tercijarnoj hiruškoj ustanovi

Rezulatati: Nakon 1018 urađenih artroplastika registrovano je 46 IOM. Iz 36 IOM nakon totalnih artroplastika izolovano je 45 MO. Najfrekventnije je izolovana koagulaza negativni Staphyloccocus (KOnS) (26,7%), od ceča je 58,3% bilo meticilin rezistentno. Zatim, Acinetobacter spp. (24,4%) i Stapylococcus aureus (22,3%), od kojih je 70% bilo meticilin i 10% vankomicin rezistentno. Svi izolati Acinetobacter spp. bili su rezistentni na karbapeneme. Nakon totalnih artroplastika kuka najčešće je izolovan Acinetobacter spp. (30,3%) i KOnS (24,5%); nakon totalnih artroplastika kolena najčešće je izolovan S.aureus (41,7%), od toga 80% meticilin rezistentno i 20% vankomicin rezistentno. Na drugom mestu bio je KOnS (33,4%). Od 10 IOM nakon parcijalnih artroplastika kuka u 46,1% izolovan je Acinetobacter spp. i u po 15,4% Enterococcus spp. i Proteus mirabilis. Izolovano Enterococcus spp. bio je u 50% rezistentan na vankomicin (VRE) i 50% rezistentan na teikoplanine. Svi izolati S. aureus i KOnS nakon parcijalnih artroplastika bili su rezistentni na meticilin.

Zaključak: Mikrobiološki profil izolovanih MO nakon artroplastika kuka i kolena u Srbiji razlikuju se u odnosu na zemlje Evropske unije i druge zemlje sveta. Značajan procenat izolata Staphylococcus-a rezistentnih na meticilin mogu ukazati na neophodnost empirijske primene antibiotika šireg spektra u periodu do dobijanja rezulata iz zasejanih kultura IOM. Takođe, povećana rezistencija može biti posledica nekritičnog korišćenja antimikrobnih lekova, što zahteva adekvatnu i kompletnu primenu preporučenih mera racionalnog korišćenja antibiotika u prevenciji i lečenju IOM.

Ključne reči: infekcije operativnog mesta, artroplastika kuka, artroplastika kolena, mikroorganizmi, rezistencija

Rezistencija Escherichia coli na fluorohinolone u uzorcima urina ambulantnih pacijenata Medigroup sistema

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Infekcije urinarnog trakta (IUT) su česti uzroci ambulantnih poseta pacijenata, a najčešći bakterijski izolat iz urina je Escherichia coli. S obzirom da se IUT često leče empirijski, važno je poznavati lokalne epidemiološke podatke za adekvatnu inicijalnu terapiju.

Cilj: Budući da se podaci za praćenje antimikrobne rezistencije (AMR) obično odnose na bolničke infekcije, cilj ove studije je bio da se ispita fluorohinolonska rezistencija izolata E. coli iz urina ambulantnih pacijena Medigroup sistema, čime bi se doprinelo proceni lokalne antimikrobne osetljivosti izolata E. coli.

Materijal i metode: Sprovedena je retrospektivna studija koja je obuhvatila period od 01. januara 2022. do 31. decembra 2022. Uzorci urina su prikupljeni u laboratorijama Medigroup sistema i poslati u Centralnu laboratoriju gde je urađena identifikacija uropatogena i testiranje na antimikrobnu osetljivost. Antimikrobna osetljivost je urađena u skladu sa smernicama Evropskog komiteta za ispitivanje osetljivosti na antimikrobne lekove (EUCAST). Analizirani su podaci osetljivosti/rezistencije izolata E. coli na fluorohinolone (FH) i dodatno na trimetoprim/sulfametoksazol (TMP/SMX) kao najčešću preporučenu empirijsku terapiju za nekomplikovane infekcije urinarnog trakta.

Rezultati: U ispitivanom periodu je ukupo izolovano 5033 soja E. coli. Od toga je 1145 izolata bilo rezistentno na FH, što predstavlja stopu rezistencije od 22.75 %. Stopa rezistencije na TMP/SMX iznosila je 30.23 %.

Zaključak: Stopa rezistencije urinarnih izolata E. coli među ambulantnim pacijentima pokazala je visok nivo na TMP/SMX, koji za posmatrani period iznosi preko 30%. Neophodno je kontinuirano sprovođenje studija osetljivosti na lokalnom i regionalnom nivou. Uključivanje podataka što većeg broja laboratorija je ključno za uspešno sagledavanje i rešavanje AMR u zajednici.

Ključne reči: Escherichia coli, fluorohinolonska resistencija, urinarne infekcije

Fluoroquinolone resistance rates of Escherichia coli isolates from outpatients urine samples in Medigroup system

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Urinary tract infections (UTIs) are frequent causes of outpatient visits and the most common bacterial isolate from urine is Escherichia coli. Since UTIs are often treated empirically, it is important to know the local epidemiological data for the appropriate initial treatment.

Objective: Given that antimicrobial resistance (AMR) surveillance data are usually related to hospital-acquired infections, the aim of this study was to investigate the fluoroquinolone resistance of E. coli isolates from the outpatient urine samples of the Medigroup system, which would contribute to the assessment of local sensitivity/resistance of E. coli isolates.

Methods: We performed a retrospective study for twelve months period, between January 1st and December 31st, 2022. Urine samples were collected in the laboratories of the Medigroup system and sent to the Central Laboratory where identification and antimicrobial susceptibility testing were performed. The European Committee for Antimicrobial Susceptibility Testing (EUCAST) breakpoints were used for the interpretation of susceptibility testing results. E. coli isolates were evaluated for nonsusceptibility to fluoroquinolones (FH) and additionally to trimetoprim/sulfametoxasole (TMP/SMX) as a frequently recommended empiric therapy for uncomplicated urinary tract infections.

Results: A total of 5 033 E. coli isolates were obtained during the observed period. Out of those, 1145 isolates were resistant to FH, which represents a resistance rate of 22.75%. The rate of resistance to TMP/SMX was 30.23 %.

Conclusion: The resistance rate to TMP/SMX of urinary E. coli isolates among outpatients samples showed a high level, which for the observed period was greater than 30%. It is necessary to conduct susceptibility study on local and regional level. Incorporating data from as many laboratories as possible is critical to successfully understanding and solving AMR in the community.

Keywords: Escherichia coli, fluoroquinolone resistance, urinary tract infections

LISTERIOZA – ZONOTSKI ASPEKT

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Listeria monocytogenes je fakultativni, intracelularni, gram-pozitivni štapić koji je odgovoran za pojavu listerioze. Oboljenje obično nastaje konzumiranjem hrane koja je kontaminirana listerijom, posebno mlečnih proizvoda, lisnatog povrća, ribe i mesa. Listeria se može naći u domaćinstvu, restoranima i drugim mestima kao što su prodavnice ili fabrike za preradu hrane. Patogen je sveprisutan, jer se može naći u zemljištu, vodi i vegetaciji kao i u digestivnom traktu ljudi.

Namirnice koje imaju najveću stopu infekcija povezanih sa L. monocytogenes uključuju: sirove klice, nepasterizovano mleko, meki sirevi, hladno meso, hladne viršle i dimljeni morski plodovi. L. monocytogenes raste na temperaturama frižidera, tačnije, niske temperature indukuju enzime kao što je RNK helikaza koja poboljšava aktivnost i replikaciju L. monocytogenes na niskim temperaturama dok sposobnost proizvodnje biofilma povećava sposobnost L. monocytogenes da preživi u teškim uslovima. Ona takođe koristi flagele na nižim temperaturama koje joj omogućavaju da se pokreće i uhvati za enterocite u ranoj fazi infekcije koje se gube kada je bakterija duže izložena višim temperaturama. Ljudi koji su u najvećem riziku da budu pogođeni listeriozom su trudnice, mala deca, odrasli stariji od 60 godina i osobe sa oslabljenim imunološkim sistemom. Praćenje smernica za bezbednost hrane može smanjiti šansu za dobijanje i širenje listerioze.

Nadzor listerioze kod ljudi u EU se fokusira na invazivne oblike L. monocytoges infekcija, koja se uglavnom manifestuje kao septikemija, simptomi slični gripu, meningitis ili spontani pobačaj. Dijagnoza listerijskih infekcija kod ljudi se generalno sprovodi kultivacijom uzoraka krvi, cerebrospinalne tečnosti i vaginalnih briseva ili detekcijom nukleinske kiseline. Prema Centru za kontrolu bolesti (CDC), otprilike 1.600 ljudi oboli od listerioze svake godine, a oko 260 ljudi umre od te bolesti. Bolest je najčešća kod trudnica, odojčadi, imunokompromitovanih i starijih (65 i više godina).

LISTERIOZA – ZONOTSKI ASPEKT

Trudnice su takođe izložene riziku jer mogu dobiti L. monocytogenes i preneti je na nerođeni fetus. Prema podacima EFSA Journal u 27 država tokom 2021.godine prijaviljeno je 2.183 potvrđenih slučaja invazivne listerioze kod ljudi u Evropi. Tokom našeg istraživanja u poslednjih pet godina ispitano je preko 5000 uzoraka. L. monoctogenes je izolovana i identifikovana u manje od 1% uzoraka i to u mesnih prerađevina (kao npr. dimljena hamburška slanina, viršla, mortadela, dimljena roštilj kobasica), u mlečnih prerađevina (sir), zatim uzorcima dimljenog lososa i briseva radnih površina u proizvodnim pogonima.

Naime subjekat u poslovanju hranom je u obavezi da shodno Pravilniku o opštim i posebnim uslovima higijene hrane u bilo kojoj fazi proizvodnje, prerade i prometa (Sl.gl.RS br.72/10) i Pravilnik o izmeni i dopuni Pravilnika o opštim i posebnim uslovima higijene hrane u bilo kojoj fazi proizvodnje, prerade i prometa (Sl.gl.RS br.62/18) i HACCP planu vrši ispitivanje na parametre bezbednosti hrane kojim se definiše prihvatljivost nekog proizvoda ili proizvodne partije u šta spada i L. monocytogense.

LISTERIOSIS – ZONOTIC ASPECT

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Listeria monocytogenes is a facultative, intracellular, gram-positive rod which is responsible for the occurrence of listeriosis. Illness is usually caused by eating food contaminated with listeria, especially dairy products, leafy greens, fish and meat. Listeria can be found in households, restaurants, and other places such as grocery stores or food processing plants. The pathogen is ubiquitous, as it can be found in soil, water and vegetation as well as in the digestive tract of humans. Foods that have the highest rates of L. monocytogenes related infections include: raw sprouts, unpasteurized milk, soft cheeses, cold meats, cold hot dogs, and smoked seafood. L. monocytogenes grows at refrigerator temperatures, specifically, low temperatures induce enzymes such as RNA helicase that enhances the activity and replication of L. monocytogenes at low temperatures while the ability to produce biofilms increases the ability of L. monocytogenes to survive in harsh conditions. It also uses flagella at lower temperatures that allow it to move and latch on to enterocytes early in the infection, which are lost when the bacterium is exposed to higher temperatures for longer.

The people most at risk of contracting listeriosis are pregnant women, young children, adults over 60, and people with weakened immune systems. Following food safety guidelines can reduce the chance of getting and spreading listeriosis. Surveillance of human listeriosis in the EU focuses on invasive forms of L. monocytoges infections, which mainly manifest as septicaemia, flu-like symptoms, meningitis or abortion. Diagnosis of listeria infections in humans is generally performed by culture of blood samples, cerebrospinal fluid, and vaginal swabs or by nucleic acid detection. According to the Centers for Disease Control (CDC), approximately 1,600 people contract listeriosis each year, and about 260 people die from the disease. The disease is most common in pregnant women, infants, the immunocompromised and the elderly (65 and older). Pregnant women are also at risk because they can contract L. monocytogenes and pass it on to the unborn fetus.

LISTERIOSIS – ZONOTIC ASPECT

According to EFSA Journal data, 2,183 confirmed cases of invasive listeriosis in humans were reported in 27 countries in 2021 in Europe. During our research in the last five years, over 5000 samples were examined. L. monoctogenes was isolated and identified in less than 1% of samples, such as meat products (such as smoked Hamburg bacon, hot dogs, mortadella, smoked barbecue sausage), in dairy products (cheese), then in samples of smoked salmon and swabs of working surfaces in production facilities.

The subject in the food business is obliged to comply with the Rulebook on general and special conditions of food hygiene in every phase of production, processing and circulation (Official Gazette of RS No. 72/10) and the Rulebook on amendments and additions according to the Rulebook on general and special food hygiene conditions at any stage of production, processing and circulation (Official Gazette of RS No. 62/18) and the HACCP plan, testing of food safety parameters is carried out, which defines the acceptability of the product or the production batch, which includes L. monocytogenes.

HELICOBACTER PYLORI INFEKCIJA

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Helicobacter pylori predstavlja proteobakteriju koja je prvi put opisana 1983. godine kao pripadajuća vrsta roda Campylobacter, šest godina poslije, 1989. godine, izdvojena je iz tog roda i svrstana u rod Helicobacter. H. pylori najčešći je uzročnik infekcija kod pacijenata koji od simptoma navode bol u gornjem abdomenu.Dokazano je da godinama može živjeti u ljudskom organizmu, a hoće li se njena prisutnost manifestirati kao bolest sa simptomima ili ne, često ovisi i o samom imunološkom sistemu i općenitom fizičkom stanju pacijenta. U imunokompromitiranih osoba može stvarati velike probleme i neugodnosti, dok je eradikacija uglavnom dugotrajna i iscrpljujuća za samog pacijenta.

Brojnim naučnim istraživanjima utvrđeno je da H. pylori ima ulogu u razvoju gastritisa, želučanog i duodenalnog ulkusa, odnosno karcinoma samog želuca, kao i ekstranodalnog β–ćelijskog limfoma marginalne zone (MALT limfoma). Helicobacter pylori, gram-negativna bakterija, vrlo je značajna jer je tom bakterijom zaraženo više od 50 % populacije u svijetu. Oko 90 % slučajeva akutnog i hroničnog gastritisa izazvala je bakterija H. pylori. Prisutna je u 95 % kod duodenalnih ulkusa i u 70 % kod želučanih ulkusa. Veza između Helicobacter pylori i GERB-a vrlo je kompleksna i nepotpuno istražena. Kod osoba koje su genski predisponirane, H. pylori infekcija može dovesti do autoimunih procesa van probavnog sistema i to najčešće kod perifernih krvnih žila, kože ili tkiva štitne žlijezde. Infekcija H. pylori, ako se ne liječi, može trajati doživotno. Iz tog razloga je važan ozbiljan pristup dijagnostici i primjena odgovarajuće terapije u liječenju. Primarni cilj liječenja je da se umanje postojeće tegobe bolesnicima kod kojih je pronađen izolat H. pylori te spriječe daljnje komplikacije koje mogu nastati nepridržavanjem utvrđenih smjernica liječenja. Ishod bolesti rezultat je složene interakcije između domaćina i bakterije. Polimorfizmi imunog gena domaćina i izlučivanje želučane kiseline uveliko određuju sposobnost bakterije da kolonizira specifičnu želučanu nišu.

Majmunske boginje sa kliničkom slikom fimoze: prikaz slučaja

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Virus majmunskih boginja (MPX) je DNK virus koji pripada porodici Ortopoxvirusa i srodan je virusima Variole, Vaknicije i virusu kravljih boginja. Prvi slučaj majmunskih boginja kod čoveka je zabeležen 70-ih godina prošlog veka u Africi nakon čega je zabeležno više sporadičnih epidemija na tlu kontinenta i dugo je smatran endemskim virusom zapadne Afrike. Broj slučajeva i rasprotanjenost majmunskih boginja godinama unazad raste sa kulminacijom tokom epidemije 2022. godine sa više desetina hiljada novih slučajeva globalno.

Veliki broj novih pacijenata je doveo do novih saznanja o kliničkoj prezentaciji i načinu prenošenja bolesti što je dovelo do relativno brzog suzbijanja epidemije. U ovom prikazu slučaja govorimo o ranije zdravom sredovečnom čoveku koji se javio u lokalni zdravstveni centar zbog promena na prepucijumu. Postavljena je dijagnoza fimoze i pacijent je brzo operisan. U postoperativnom toku dolazi do nastanka promena po tipu pustula sa centralnom umbilikacijom po celom telu kao i na jeziku i sluzokoži usne duplje. Uz navedene kožne promene dolazi i do formiranja konglomerata limfnih čvorova ingvinalno desno. Pacijent nije imao opšte tegobe u vidu visokih temperatura, astenije, glavobolje ili drugih neuroloških i respiratornih manifestacija. U epidemiološkoj anamnezi navodi rizične seksualne odnose. Laboratorijske analize su bile neupadljive sa normalnom krvnom slikom i blago povišenim vrednostima markera zapaljenja. Iz više kožnih promena uzet je uzorak radi ispitivanja na MPX uz dodatna ispitivanja za druge polno prenosive bolesti. Ubrzo je postavljena dijagnoza MPX udruženog sa novootkrivenim AIDS-om i Sifilisom. Započeto je lečenje kristalnim Penicilinom, Doksiciklinom i Klindamicinom uz početak ARV terapije. I pored primenjene terapije konglomerat limfnih čvorova i uznapredovale kožne promene perzistiraju više od 40 dana nakon čega dolazi do postepenog jenjavanja tegoba.
U navedenom slučaju pretpostavljamo da je hirurška manipulacija kožnih promena udružena sa izrazitom imunideficijencijom usled AIDS-a dovela do diseminovane i uporne forme bolesti koja je i pored primenjene terapije perzistirala neuobičajeno dugo.

Monkeypox presenting as phimosis: a case report

The Monkeypox virus (MPX) is a DNA virus that belongs to the Orthopoxvirus family and its related to Variola, Vaccinia, and Cowpox virus. The first case of monkeypox in humans was recorded in the 1970s in Africa, after which several sporadic epidemics were recorded on the continent, and it was considered an endemic virus in West Africa.

The number of cases and the spread of monkeypox has been increasing for years, with a peak during the 2022 epidemic, with tens of thousands of new cases globally. The large number of new patients has led to new insights into the clinical presentation and transmission of the disease, resulting in relatively rapid suppression of the epidemic. In this case report, we discuss a previously healthy middle-aged man who presented to a local health center due to changes on his foreskin.

The diagnosis of phimosis was made, and the patient was quickly operated on. In the postoperative period, changes appeared in the form of pustules with a central umbilication all over the body, as well as on the tongue and oral mucosa. Along with these skin changes, conglomerates of lymph nodes formed on the right inguinal area. The patient did not have any general symptoms such as high fever, asthenia, headache, or other neurological and respiratory manifestations. The patient stated that he had unprotected intercourse. Laboratory analyses were unremarkable with a normal blood count and slightly elevated inflammatory markers. A sample was taken from several skin lesions for MPX testing and other sexually transmitted diseases. The diagnosis of MPX associated with newly discovered AIDS and syphilis was made shortly thereafter. Treatment was initiated with crystalline Penicillin, Doxycycline, and Clindamycin, along with the start of ARV therapy.

Despite the applied therapy, the conglomerate of lymph nodes and advanced skin changes persisted for more than 40 days, after which the symptoms gradually decreased. In this case, we assume that surgical manipulation of skin lesions, combined with severe immunodeficiency due to AIDS, led to a disseminated and persistent form of the disease that persisted unusually long despite the applied therapy.

Uticaj pandemije COVID -19 na vanbolničku i boničku potrošnju antibiotika i stopu antimikrobne rezistencije

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Uvod - Antimikrobna rezistencija danas predstavlja jednu od najvećih prijetnji za globalno zdravlje. Glavni pokretač razvoja rezistencije na antibiotike je neracionalna upotreba antibiotika. Postoje indicije da se ovaj problem značajno pogoršava usljed povećanog propisivanja antibiotika pacijentima sa COVID-19. Cilj ovog rada je da ispita kako je pandemija COVID-19 uticala na povećanu potrošnju antibiotika u vanbolničkim i bolničkim uslovima u sarajevsko-romanijskoj regiji Republike Srpske u 2020. i 2021. godini.

Materijal i metode - Podaci o izolovanim bakterijama i njihovoj senzitivnosti na antibiotike su prikupljeni retrospektivno iz protokola odeljenja za mikorobiologiju Bolnice „Srbija" Istočno Sarajevo. Podaci o potrošnji antibiotika u bolnici su dobijeni iz baze podataka bolničke apoteke za i izraženi su u definisanim dnevnim dozana na 100 bolničkih dana (DDD/100BD). Podaci o vanbolničkoj potrošnji antibiotika dobijeni su od Instituta za javno zdravlje Republike Srpske, izraženi su u DDD/1000 stanovnika/dan.

Rezultati - Ustanovili smo značajan porast potrošnje većine rezervnih antibiotika u toku COVID-19 pandemije (2020. i 2021.) u odnosu na raniji period. U bolničkim uslovima, registrovan je porast potrošnje karbapenema (meropenem: 1,42; 5,18 DDD/100 BD za 2020. i 2021, imipenem 1,24;1,57 DDD/100 BD za 2020 i 2021.), glikopeptida (vankomicin:1,53; 3,26 DDD/100 BD 2020 i 2021.), linezolida 0,37; 0,44, DDD/100 BD za, 2020 i 2021. Karakteristična je promjena potrošnje azitromicina koja je drastično porasla u prvoj pandemijskoj godini, a značajno pala tokom 2021. god(7,92; 0,78 2020 i 2021.). U vanbolničkim uslovima zabilježen je porast potrošnje ko- amoksiklava, azitromicina, levofloksacina,moksifloksacina i cefiksima.

Uticaj pandemije COVID -19 na vanbolničku i boničku potrošnju antibiotika i stopu antimikrobne rezistencije

Zaključak - U periodu pandemije COVID-19, posebno na početku antibiotska terapija je primjenjivana kod svih pacijenata bez obzira na težinu kliničke slike, što je razlog rasta potrošnje posebno rezervnih grupa antibiotika i stope rezistencije. 2020. godinu obilježio je enorman porast potrošnje azitromicina i u vanbolničkim i u bolničkim uslovima, kao i značajan porast potrošnje hinolona i cefalosporina u primarnoj zdravstvenoj zaštiti. U 2021. godini u bolničkim uslovima nastavljen je trend rasta potrošnje rezervnih antibiotika. Neophodan je stalni sistem praćenja i rukovođenja antimikrobnim lijekovima.

Effects of the COVID-19 pandemic on outpatient and in-hospital use of antibiotics and antimicrobial resistance rate

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Introduction - Antimicrobial resistance currently constitutes one of the major global health threats. The chief trigger for developing resistance to antibiotics is an irrational (excessive) use of antibiotics and insufficient patient information about antibiotics and antibiotic resistance. There are indicia that the issue has been worsening significantly since the outbreak and spread of COVID-19 pandemics, due to the intensified prescription of antibiotics to COVID-19 patients [1].

The hitherto conducted studies show that the use of antibiotics by patients with COVID-19 largely exceeds the number of proven bacterial superinfections [2]. Just as the clinical features and history of disease in COVID-19 patients differ from the diseases caused by other respiratory viruses, so do co-infectious pathogens. The objective of this article is to examine whether the COVID-19 pandemic has brought about an increased use of antibiotics in outpatient and hospital settings in the Sarajevo-Romanija region of Republika Srpska in the years 2020 and 2021. Another objective was to determine the state of antimicrobial resistance and the presence of multiresistant bacteria strains in the samples taken from the hospitalized patients in the region.

Materials and methods - Data on the isolated bacteria and their sensitivity to antibiotics have been obtained retrospectively from the book of protocol kept at the Department of Microbiology at the Srbija Hospital in East Sarajevo for the year 2021. Bacterial sensitivity or resistance to antibiotics is presented in percentages in relation to the overall number of individual bacteria isolates. Data on the use of antibiotics in the hospital have been obtained from the database kept at the hospital pharmacy for the years 2020 and 2021, presented in defined daily doses per 100 hospital days (DDD/100BD). Data on outpatient use of antibiotics have been obtained from the Republika Srpska Public Health Institute, shown in DDD/1000 residents/day.

Effects of the COVID-19 pandemic on outpatient and in-hospital use of antibiotics and antimicrobial resistance rate

Results - We have established a significant increase in the use of most of reserve antibiotics during the COVID-19 pandemic (2020 and 2021) in relation to the previous period. A steady increase of the use of carbapenems was registered in hospital settings (meropenem: 1.42; 5.18 DDD/100 BD in the years 2020 and 2021 respectively, imipenem 1.24;1.57 DDD/100 BD in 2020 and 2021 respectively), glycopeptides (vancomycin: 1.53; 3.26 DDD/100 BD in 2020 and 2021 respectively), linezolids 0.37; 0.44, DDD/100 BD in 2020 and 2021 respectively. Also characteristic is a change in the use of azithromycin, which has increased dramatically during the first pandemic year, but fell considerably during 2021 (7.92; 0.78 in 2020 and 2021 respectively). Also reported in outpatient settings was an increase in the use of co-amoxiclav, azithromycin, levofloxacin, moxifloxacin and cefixime.

Conclusion - During the COVID-19 pandemic, especially at its outset, antibiotic therapy was administered to all patients regardless of their clinical features, which is the reason for the increase in the use of reserve groups of antibiotics and rate of resistance. The year 2020 was marked with an enormous growth of the use of azithromycin in both outpatient and hospital settings, as well as a significant increase in the use of quinolones and cephalosporins in the primary healthcare system. In 2021, the reserve antibiotic use growth trend continued in hospital settings. It is necessary to maintain a continuous antimicrobial monitoring and management system. It is necessary to develop and introduce recommendations and guidelines for the administration of antimicrobial therapy based on national guidelines and on the sensitivity of bacterial pathogens to the choice of antibiotics.

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OPPORTUNISTIC PATHOGEN PSEUDOMONAS AERUGINOSA – DETERMINATION OF LIMIT OF DETECTION OF PH. EUR. 10TH METHOD

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Nasal spray solutions are typically isotonic or hypertonic solutions and can also contain additional excipients. There are studies demonstrating the contaminations of the nasal spray solutions after some days of use with microorganisms that are part of normal skin and nasal flora and also contaminated with Proteus spp., Escherichia coli and Pseudomonas aeruginosa. Pseudomonas aeruginosa is Gram-negative bacteria, an opportunistic pathogen that contributes to frequent infection in humans. Severe infections are usually difficult to treat because Pseudomonas aeruginosa can exhibit resistance to many antibiotics, and therefore timely and reliable analysis of the source of infection is essential to implement preventive measures.

Two levels of artificial contamination and also non-contaminated controls were prepared to determine the limit of detection (LOD) of Pseudomonas aeruginosa. The suspension of Pseudomonas aeruginosa ATCC 9027 of optical density 0.4 McF and successive decimal dilutions were prepared.
The samples of nasal spray products with an active substance (benzalkonium chloride) were obtained from a local pharmacy and used for artificial contamination. Each 10 ml of sample was homogenized with 90 Trypton soy broth with neutralizer. After neutralization, Pseudomonas aeruginosa was added to perform contaminations of every sample, except six control samples. For a low level of contamination was used 1 ml of 10-7 dilution of the bacterial suspension. For a high level of contamination was used 1 ml of 10-6 dilution of the bacterial suspension. Detections of Pseudomonas aeruginosa were performed as described in European Pharmacopeia 10th (Ph. Eur. 10th). LOD was calculated by the PODLOD_ver11.xls EXCEL program by Wilrich and Wilrich (Germany). The calculation showed an acceptable matrix effect statistic, LOD50% was 0.421□□0.235; 0.755 cfu/ml, LOD95% was 1.821 □ 1.017; 3.262□ cfu/ml.
The obtained results contribute to a better understanding of the performance of the method for the detection of Pseudomonas aeruginosa.

Key words:, limit of detection (LOD), Ph. Eur. 10th, Pseudomonas aeruginosa

OPORTUNISTIČKI PATOGEN PSEUDOMONAS AERUGINOSA – ODREĐIVANJE LIMITA DETEKCIJE PH. EUR. 10TH METODE

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Sprej rastvori za nos su tipični izotonični ili hipertonični rastvori i mogu da sadrže dodatne ekscipijente. Postoje studije koje ukazuju da sprej rastvori za nos mogu biti kontaminirani nakon nekoliko dana korišćenja mikrooranizmima koji su prirodno prisutni na koži i nosu, ali takođe kontaminirani i sa Proteus spp., Escherichia coli i Pseudomonas aeruginosa. Pseudomonas aeruginosa je gram-negativna bakterija, oportunistički patogen, koji često uzrokuje infekcije kod ljudi. Teške infekcije je obično teško lečiti jer Pseudomonas aeruginosa može pokazati otpornost na mnoge antibiotike i stoga je pravovremena i pouzdana analiza izvora infekcije neophodna za provođenje preventivnih mera.

Za određivanje granice detekcije (LOD) met Pseudomonasa aeruginosa, pripremljena su dva nivoa veštačke kontaminacije i nekontaminirane kontole. Pripremljena je suspenzija Pseudomonas aeruginosa ATCC 9027 optičke gustine 0,4 McF i sukcesivna decimalna razblaženja.

Uzorci sprej rastvora za nos sa aktivnom supstancom (benzalkonijum hlorid) nabavljeni su u apoteci i korišćeni za veštačku kontaminaciju. Po 10 ml uzorka je homogenizovano sa 90 ml tripton soja bujona sa neutralizatorom. Nakon neutralizacije, Pseudomonas aeruginosa je dodat u homogenate, izuzev šest kontrolnih uzoraka. Za nizak nivo kontaminacije korišćeno je 1 ml razređenja 10-7 bakterijske suspenzije. Za visok nivo kontaminacje korišćen je 1 ml razređenja 10-6 bakterijske suspenzije, Određivanje Pseudomonas aeruginosa provedeno je prema European Pharmacopeia 10th (Ph. Eur. 10th). LOD je izračunat u PODLOD_ver11.xls EXCEL programu Wilrich-a i Wilrich-a (Nemačka). Proračun je pokazao prihvatljivu statistiku efekta matriksa, LOD50% je bio 0,421□□0,235; 0,755□ cfu/ml, LOD95% je bio 1,821 □ 1,017; 3,262□ cfu/ml.
Dobijeni rezultati su doprinos boljem razumevanju performansi metoda za određivanje Pseudomonas aeruginosa.

Ključne reči:, limit detekcije (LOD), Ph. Eur. 10th, Pseudomonas aeruginosa

/ Prikaz pedijatrijskog bolesnika sa neuobičajenom kliničkom prezentacijom varicele

/ A Case Report of a Pediatric Patient with Atypical Form of Chickenpox

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/ Dijagnostikovanje i lečenje opsipnih groznica u pedijatrijskoj populaciji je uvek značajan izazov u svakodnevnoj praksi pedijatra. Virus majmunskih boginja, koji je incijalno smatran endemskim u određenim područjima Afrike, se tokom 2022. godine proširio i na druge kontinente. Naš prikaz predstavlja prethodno zdravog petogodišnjeg dečaka koji je razvio atipičnu formu varicele koja može biti diferencijalno dijagnostički problem, posebno u doba epidemije majmunskih boginja.

/ Successful diagnosis and treatment of rash fevers in the pediatric population have always been a significant challenge in everyday pediatric practice. The human monkeypox virus, which was previously considered an endemic infection in African regions, in 2022. had a worldwide spread. Our case represents a previously healthy five-year-old boy developing a non-typical form of a chickenpox infection which can be a clinical diagnostic problem, especially in the monkeypox epidemic.

/ Sistemski oblik bolesti mačije ogrebotine - diferencijalna dijagnoza produžene febrilnosti kod imunokompetentne dece

/ Systemic form of cat scratch disease - differential diagnosis of prolonged fever in immunocompetent children

Stefan Kotlajic[1], Tijana Đerić[1], Milica Zečević[1], Gordana Petrović[1], Srđan Pašić[1,2]

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/ Bolest mačije ogrebotine je relativno česta infekcija u pedijatrijskoj populaciji. Izazivač je gram-negativna aerobna bakterija Bartonella henselae. Kao rezervoar infekcije najčešće se opisuju mačke i psi. Subakutni samoograničavajući lifadenitis proksimalno od mesta prethodne ogrebotine ili ujeda mlade mačke je tipična klinička prezentacija ove bolesti kod imunokompetentnih bolesnika. U znatno ređem, sistemskom obliku, bolest se kod imunokompetentnih pacijenata ispoljava kao produženo febrilno stanje sa mikroapscesima jetre i slezine. Kao netipične forme bolesti opisuju se i osteomijelitis, Perinaudov okuloglandularni sindrom, epileptički napadi i encefalopatija kod zahvatanja centralnog nervnog sistema, artralgije, pleomorfni osipi i druge. Kod imunokompromitovanih bolesnika može doći do razvoja bacilarne angiomatoze i pelioze jetre i slezine. Cilj autora je da ovim posterom ilustruju potrebu i važnost da pedijatri i ostali kliničari koji učestvuju u lečenju deteta sa nejasnom febrilnom boleščću i ubrzanom sedimentacijom, pored ostalih bolesti sa ovom kliničkom slikom, sumnju usmere i na sistemsku bartonelozu.

/ Cat scratch disease (CSD) is a relatively common infection in the pediatric population caused by the gram-negative aerobic bacterium Bartonella henselae. In the most of cases the reservoir of infection for humans are cats and dogs. Subacute self-limiting lymphadenitis proximal to site of earlier kitten scratch or bite is a typical clinical presentation of SCD in immunocompetent children.

/ Sistemski oblik bolesti mačije ogrebotine - diferencijalna dijagnoza produžene febrilnosti kod imunokompetentne dece

/ Systemic form of cat scratch disease - differential diagnosis of prolonged fever in immunocompetent children

Systemic form of cat scratch disease is the less common. Fever of unknown origin or prolonged fever with microabscesses of liver and spleen are most often clinical presentation of systemic CSD. There are other atypical presentations of this infective disease such as: osteomyelitis, Parinaud oculogladular syndrome, central nervous system disease with seizures and encephalopathy, artralgias, pleomorphic rash and others. Immunocompromised patients may have bacillary angiomatosis and/or peliosis of the liver and spleen as a forms of the same disease. The goal of this poster is to highlight the significance and need for clinical suspicion of systemic bartonellosis in children with prolonged febrile illness and a high erythrocyte sedimentation rate after other causes of febrile illness have been ruled out.

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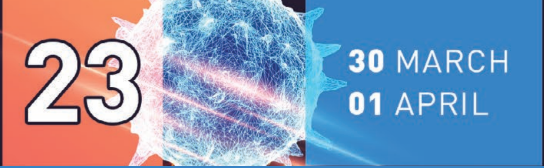
International Standard Book Number (ISBN)

Assigned number

ISBN-978-86-914897-9-3

UMS SERIES 23



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