5th SYMPOSIUM ON DIAGNOSIS AND THERAPY OF FUNGAL DISEASES

Fungal infections in neonates

5. SIMPOZIJUM DIJAGNOZA I TERAPIJA GLJIVIČNIH OBOLJENJA - Knjiga sažetaka

Urednik: Valentina Arsić Arsenijević, redovni profesor, Medicinski fakultet Univerzitet u Beogradu

Recenzenti: Dragana Janić, redovni profesor, Medicinski fakultet Univerzitet u Beogradu
Nada Suvajdžić Vuković, redovni profesor, Medicinski fakultet Univerzitet u Beogradu
Suzana Otašević, redovni profesor, Medicinski fakultet Univerzitet u Nišu

Izdavač: Valentina Arsić Arsenijević, Društvo medicinskih mikologa Srbije

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Dear Friends and Colleagues,

It is a great pleasure for us to invite you to attend the 5th Symposium on Diagnosis and Therapy of Fungal Diseases (5th DTFD), which will be held in Belgrade, Serbia, 9 and 10 October 2017, in Crowne Plaza Hotel

The next DTFD is the fifth national meeting in the series organized by the Serbian Society of Medical Mycology (SSMM) supported by European Confederation of Medical Mycology (ECMM), Ministry of Education, Science and Technology Republic of Serbia and Faculty of Medicine University of Belgrade.

DTFD has become an important and essential national meeting in the field of fungal diseases, a forum in which mycologists, clinicians and basic researchers from different fields exchange research results and opinions on medical mycology practice. The outstanding scientific program will include plenary sessions on fungal infections and round table sessions.

The meeting is designed for medical microbiologists, infectious disease specialists, hematologists, oncologists, intensive care units doctors, immunologists, dermatologists, pediatricians and all those with interests in medical mycology. We expect the 5th DTFD to be at least as successful as previous symposiums.

The venue for the 5th DTFD is located in Belgrade, Serbia. Belgrade is the capital and largest city of Serbia with easy access from any part of the world thanks to its International Airport, motorway and railway networks. The city lies at the confluence of the Sava and Danube rivers, where the Pannonian Plain meets the Balkans. It has an urban population of more than 2 million people, making it one of the largest cities of Southeastern Europe. Its name translates to white city. Belgrade's wider city area was the birthplace of the largest prehistoric culture of Europe, the Vinča culture, as early as the 6th millennium BC. Now Belgrade has its own autonomous government, has a special administrative status in the territorial organization of Serbia, and is the financial center of Serbia.

We look forward to greeting you in Serbia and discuss new developments in medical mycology!

Prof. Predrag Minic and Prof. Valentina Arsic Arsenijevic
On Behalf of Organizing and Scientific Committee

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DTFD
Diagnosis & Therapy of Fungal Diseases

5th DIAGNOSIS AND THERAPY OF FUNGAL DISEASES

Training Course for Fungal Infections in Neonates

10/10/17 Hotel Crowne Plaza, Belgrade, Serbia; www.dtfd.org www.fems-microbiology.org

Monday, 09 October 2017

16:00-19:00 **REGISTRATION**

POSTER PRESENTED AUTHORS AND TITLES:

P.01 Miljana Misic et al: Monitoring of Microscopic Filamentous Fungi in Indoor Air and in the Neonatal Intensive Care Units (Serbia)
P.02 Myrsini Chatzi et al: Isolation rate of *Candida* species from Clinical Specimens from our hospital in a University Hospital, Thessaloniki (Greece)
P.03 Dragana Ivanovic et al: Education of medical personnel in neonatology units-new technologies for health system/molecular diagnosis (Serbia)
P.04 Dragana Srebro et al: Susceptibility of *Candida* species to fluconazole and butoconasole in pregnant Serbian women: Possible role of maternal screening of vulvovaginal candidiasis and therapy in the prevention of infection of the newborn (Serbia)
P.05 Ionut Pecete et al: Distribution Of *Candida* Species Involved In Systemic Infections Occurred In Hospitalized Patients (Romania)
P.06 Rok Tomazin et al: Screening for azole-resistant *Aspergillus fumigatus* isolates from clinical samples and hospital air (Slovenia)
P.07 Aljosa Obreza et al: Microdilution techniques for susceptibility testing of pathogenic yeasts (Slovenia)
P.08 Biljana Zivaljevic et al: Prevalence and Risk Factors of Vaginal Candidiasis among Pregnant Women: A Single Center Experience in Serbia (Serbia)
P.09 Mirjana Peric et al: Evaluation of a ultarsound assist methods for sampling fungi from the mucosal membrans (Serbia)
P.10 Slavica Dacic et al: Candidaemia and antifungal susceptibility testing of fungi isolated from neonates (Serbia)
P.11 Katarina Rajkovic et al: Modeling and prevention of fungal biofilms formation (Serbia)

Tuesday, 10 October 2017

OPENING

SSMM message: **Predrag Minic** - The excellence in science and role of knowledge in solving today's problems of fungal diseases
FEMS message: **Branka Vasiljevic** - Role of FEMS in the promoting excellence in microbiology
ISHAM message: **Valentina Arsic Arsenijevic** - ISHAM and fungi
ECMM message: **Ester Segal** - ECMM and fungal infections

SESSION 1 08:00-09:30
Major and minor fungal "players" in neonatology
Chairs: Lena Klingspor and Thomas Walsh

1.1 Thomas Walsh: Risk factors and clinical manifestations of fungal infections in neonates (USA)
1.2 Lena Klingspor: The importance of *Candida* and other yeast infections in neonates: epidemiology data and diagnostic options (Sweden)
1.3 Iris Pejicic: Incidence and risk factors for invasive candidiasis in neonatal intensive care unit in the institute of neonatology in Belgrade, Serbia (Serbia)
1.4 Niranjan Nayak: Molecular characterization, virulence determinants and antifungal susceptibility pattern of Trichosporon asahii isolates from new born babies in Nepal (India)

SESSION 2 10:00-11:45
Fighting against fungi outside the host
Chairs: Mihai Mares and Suzana Otasevic

2.1 Mihai Mares: Prevention of *Candida* transmission to neonates - a multifaceted issue (Romania)
2.2 Suzana Otasevic: Maternal genital *Candida* colonization: the major risk for neonates infection. Is it a valid dogma? (Serbia)
2.3 Irene Grant: In Utero & Neonatal Complications with indoor *Aspergillus*, *Penicillium*, *Chaetomium*, *Stachybotrys*, *Trichothecenes* Environmental Exposure (USA)
2.4 Aleksandar Jurisic: Vulvovaginal candidiasis during pregnancy - single centre experience (Serbia)

COURSE POSTERS 12:00-14:00
Training Course - Fungal Infections in Neonates
Posters discussion

Case reports granted by SSMM: Neonates vs. pediatrics cases presentations and laboratory findings
Decision-making and problem-solving of fungal infections in neonates - role of laboratory

LUNCH 14:00-15:00

SESSION 3 15:00-16:30
Which test is the best for diagnosis of fungal diseases?
Chairs: Michaela Lackner and Valentina Arsic Arsenijevic

3.1 Michaela Lackner: Utility of PCR diagnosis of invasive fungal infections in neonates (Austria)
3.2 Miha Skvarc: Early laboratory biomarkers for sepsis prediction: role of BD glucan in neonatology (Slovenia)
3.3 Ljubomir Petricevic: Can *Candida* be fearsome in pregnancy? (Austria)
3.4 Valentina Arsic Arsenijevic: *Candida* and *Fusarium* sepsis in Serbian paediatric and neonatology settings– the role of blood culture (Serbia)
3.5 Jelena Martinovic: Uncommon presentation of *Candida* infection in newborn infants (Serbia)

SESSION 4 16:30-18:00
Antifungals and treatment strategy – What is new?
Chairs: Esther Segal and Dragana Janic

4.1 Esther Segal: Packaging methods of antifungal drugs (Israel)
4.2 Lidija Senerovic: Novel therapeutic strategies for mixed infections: controlling bacterial-fungal interaction, signaling and quorum sensing (Serbia)
4.3 Dragana Janic: Antifungals and current treatment guidelines in pediatrics and neonatology (Serbia)
4.4 Nikola Stojanovic: Virulence factors and susceptibility of *Candida* spp. causative agens of neonatal infections (Serbia)

CLOSING 18:00
Symposium evaluation; Closing ceremony;

1.1. Risk Factors and Clinical Manifestations of Fungal Infections in Neonates

Thomas J. Walsh, MD, PhD (hon), FIDSA, FAAM, FECMM

Founding Director, Transplantation-Oncology Infectious Diseases Program; Chief, Infectious Diseases Translational Research Laboratory; Professor of Medicine, Pediatrics, and Microbiology & Immunology; Weill Cornell Medicine of Cornell University and New York Presbyterian Hospital; Henry Schueler Foundation Scholar; Sharp Family Foundation Scholar in Pediatric Infectious Diseases; Investigator of Emerging Infectious Diseases of the Save Our Sick Kids Foundation, New York, USA

Candidemia and deeply invasive candidiasis are the most common mycoses in neonates. Less commonly encountered pathogens include *Aspergillus* spp., Mucorales, *Fusarium* spp., and *Trichosporon* spp.

Invasive candidiasis is a leading cause of infection-related morbidity and mortality in extremely low-birth-weight (ELBW) (<1000 g) infants. In a study involving a prospective observational cohort of infants <1000 g birth weight at 19 centers of the US National Institutes for Child Health and Development (NICHD) Neonatal Research Network, Benjamin and colleagues quantified the risk factors predicting infection in high-risk premature infants and compared clinical judgment with a prediction model of invasive candidiasis. Nineteen neonatal intensive care units (NICUs) from the Neonatal Research Network enrolled 1515 ELBW infants over three years. Among the infants enrolled, 137/1515 (9.0%) developed invasive candidiasis documented by positive culture from 1 or more of the following sources: blood (n=96), CSF (n=9), urine obtained by catheterization or suprapubic aspiration (n=52), or other sterile body fluid (n=10).

Two models were generated with invasive candidiasis as their outcome. The first model identified potentially modifiable risk factors. In multivariable analysis, potentially modifiable risk factors associated with candidiasis included presence of an endotracheal tube, presence of central catheter, receipt of intravenous lipid emulsion, administration of broad-spectrum antibiotics in the week prior to culture, and intrapartum antibiotics.

The second model predicted candidiasis at the time of blood cultures. Components of the history, physical exam, and initial laboratory evaluation that predicted candidiasis included vaginal delivery, week of gestational age, Candida - like dermatitis on physical exam, central catheter, lack of enteral feeding, hyperglycemia, days of antibiotic exposure in week prior to culture, and thrombocytopenia. The clinical prediction model was superior to clinical judgment. The clinical prediction model had an area under the receiver operating characteristic curve of 0.79, and was superior to clinician judgment (0.70) in predicting neonatal invasive candidiasis.

Invasive candidiasis increases risk of death; e.g., 47/137 (34%) infants with candidiasis died compared with 197/1378 (14%) without candidiasis ($p<0.0001$). Mortality is highest in the infants from whom *Candida* was isolated from multiple sources (e.g., urine and blood or urine and CSF); e.g., 16/28 (57%) of such infants died ($p=0.32$). Mortality is similar in patients who have *Candida* spp. isolated only from blood and those with Candida isolated only from urine.

Among the moulds causing invasive fungal infections in neonates, mucormycosis is the most lethal. In a systematic review of all published cases of 59 cases were published that fulfilled stringent enrollment criteria. Mucormycosis is a life-threatening infection in neonates with distinct patterns of gastrointestinal and cutaneous involvements and high mortality. Most of the infants (77%) were premature. The most common sites of mucormycosis were gastrointestinal (54%) and cutaneous (36%) tissues. This pattern differs from sinopulmonary and rhinocerebral patterns of older children. Rhizopus spp. are the most common species isolated (72%) in such cases. The cutaneous lesions typically appear as small, black eschars on the back or extremities that then spread rapidly to involve the paraspinal muscles, intercostal muscles, and thorax resulting in extensive loss of soft tissue. Such infections have been linked to sporangiospores contaminating sheets on which the infants are laying or to contaminated bandages or arm boards. As the differential diagnosis is broad, a definitive diagnosis may be delayed, many patients (37%) received no antifungal therapy. Overall mortality of neonatal mucormycosis is 64%. A higher fraction of neonates treated with amphotericin B and surgery survived than those who received no therapy (70% versus 5%). A combination of amphotericin B and surgery is common management strategy in survivors.

1.2.The importance of *Candida* and other yeast infections in neonates: epidemiology data and diagnostic options.

Lena Klingspor, MD, PhD

Dep. of Laboratory Medicin Karolinska Institutet, Karolinska University Hospital, Stockholm, Sweden

Background: Invasive candidiasis is the most frequent neonatal invasive fungal infection in neonates. Candidemia is a leading cause of late-onset sepsis in very-low-birth-weight (VLBW) infants (birth weight < 1,500 g) and is associated with significant morbidity and mortality. It is reported that invasive candidiasis develops in 2%-5% of VLBW infants. Especially in preterm infants, hematogenous Candida meningoencephalitis, is a serious condition associated with an increased mortality rate, as well as neurodevelopmental impair-

ment. This emphasizes the importance of candidiasis in neonates.

Epidemiology: *Candida albicans* and *Candida parapsilosis* are the most common species isolated in neonates. *C. albicans* is most commonly vertically transmitted (from mother to infant), while *C. parapsilosis* is horizontally transmitted (primarily in the presence of a central catheter). *C. parapsilosis* is an increasingly reported pathogen especially in preterm neonates with indwelling catheters. A new emerging pathogen , *Candida auris* , has caused serious invasive infections mostly in adults but also in neonates and infants. Some *C. auris* isolates can be resistant to all three main classes of antifungals.
Malassezia furfur and *Malassezia pachydermatidis* are the two most common Malassezia species that may cause infections in neonates. Nosokomial outbreaks from human to human has been reported and in the cases with *Malassezia pachydermatis* (not strictly lipid dependent) outbreaks has been associated with health care workers' hands after being colonized from pet dogs at home. *Malassezia* species and other rare yeasts may cause infection with similar clinical picture as *Candida* and may be cultured from blood (rarely *Malazzesia furfur*) or septical lesions in different organs. Invasive candidiasis and other yeasts are probably underestimated because IFIs are often difficult to discriminate from other conditions in combination with laboratory diagnostic difficulties.

Diagnostic options: Diagnosis of candidiasis can be made by isolation of the fungus from blood or other normally sterile body fluid. However, in neonates it is difficult to obtain an appropriate volume for analysis. Even with optimal volumes the sensitivity is poor around, 50%-75%. When invasive candidiasis is suspected, urine and CSF culture should always be taken. Rapid identification of yeasts to species level is important and all isolates should undergo antifungal susceptibility testing. However, *Candida auris* , is difficult to identify with standard laboratory methods, and it can be misidentified in labs without specific technology.

The serologic tests (antigen and antibodies) available for the diagnosis of candidiasis, are not sensitive and specific enough. Molecular tests such as PCR may provide faster and more sensitive diagnostics but need to be standardized and evaluated in neonates. In the rare cases with congenital candidiasis diagnoses is made by direct microscopy and culture from the umbilical cord and placenta and from skin lesions and blood cultures from the baby.

Rapid and more sensitive diagnostics are needed in neonates for an early diagnose and early treatment to improve outcome. It is also of outmost importance that we gain more knowledge regarding contemporary epidemiology in neonates : in each country , in different hospitals and that we follow the epidemiology over time.

1.3 INCIDENCE AND RISK FACTORS FOR INVASIVE CANDIDIASIS IN NEONATAL INTENSIVE CARE UNIT IN THE INSTITUTE OF NEONATOLOGY IN BELGRADE, SERBIA

I. Pejčić[1], O. Rakić[1], I. Jovanović[1], M. Ranković Janevski[1], D. Ivanović[2], V. Arsić Arsenijević[3]

[1]Institute of neonatology, Belgrade; [2]Institute of public health of Serbia, Belgrade; [3]Institute of microbiology and immunology, Faculty of medicine, University of Belgrade, Belgrade, Serbia

iris.pejcic@gmail.com

Candida infections in infants are associated with significant mortality and morbidity, including neurodevelopmental impairment among survivors. Clinical signs of invasive candidiasis (IC) are non specific, and are often similar to the other infectious and non-infectious conditions. This is the reason why IC is underdiagnosed and there are no true data about its incidence.

Objectives: To determine the incidence of IC based on positive blood culture (Candida) and to analyse the risk factors for developing IC.

Methods: Retrospective analysis of the incidence of IC in newborns treated in the Institute of neonatology during 7 years period (January 2010 to December 2016). Diagnosis of IC was based on positive blood culture for Candida. As risk factors we analysed: infection and hypertension of mother, mode of delivery, perinatal asphyxia (APN), central venous catheter (CVC), mechanical ventilation (MV), use of antibiotics (AB), total parenteral nutrition (TPN), probiotic and prophylactic use of fluconasol.

Results: In this period we treated 5804 newborns, of whom 51.2% were of gestational age (GA) ≤32 weeks. Sepsis was diagnosed in 1245 (21.5%), while IC was diagnosed (isolation of Candida) in 31 (0.5%) newborns. The main risk factors for IC were: GA ≤32 weeks (80.6%), prolonged use of AB (80.6%) and TPN (74.2%), APN (74.2%), treatment in NICU (64.5%), CVC (61.3%) and MV (61.3%).

Conclusion: Incidence of IC, based on the positive blood culture, in the period 2010-2016 was 0.5%. As blood culture has low sensitivity for diagnosis of IC, we need other diagnostic biomarkers of infection, in order to improve sensitivity and specificity.

1.4 Molecular characterization, virulence determinants and antifungal susceptibility pattern of *Trichosporon asahii* isolates from new born babies in Nepal

[1]Supram Hosuru Subramanya, [1]Niranjan Nayak, [2]Shivaprakash M Rudramurthy, [2]Arunaloke Chakrabarti, [2]Dipika Shaw, Indira Bairy, [1]Shishir Gokhale.

[1]Manipal College of Medical Sciences, Pokhara, Kaski, Nepal; [2]Postgraduate Institute of Medical Education and Research, Chandigarh, India

Background: *Trichosporon asahii* is an emergent opportunistic yeast pathogen, responsible for both superficial and invasive infections. Trichosporonosis has been increasingly recognised in Nepal. Considering the importance of emerging yeasts, and their clinical impact on therapeutic outcome and case management, especially in the newborn this study was conducted to investigate the prevalent genotypes and to determine the *In vitro* virulence determinants, as well as drug susceptibility pattern of clinical *Trichosporon* isolates.

Materials and methods: A total of 31 positive cultures of *Trichosporon* species isolated from various clinical samples like blood, respiratory specimens, peritoneal fluid, plural fluid, pus, urine and indwelling medical devices between 2013 to 2016 at Manipal teaching hospital, the largest tertiary care centre in western Nepal, were studied. The isolates were identified by conventional techniques and genotyped by amplification and sequencing of the intergenic spacer 1 (IGS1) region of r DNA. Virulence determinants were further characterized by phenotypic methods. The *in vitro* antifungal sensitivity testing for planktonic and biofilm cells were carried out by broth microdilution method (CLSI) and XTT reduction assay respectively.

Results: Species identification revealed all the 31 isolates to be *Trichosporon asahii.* Six of the 31 isolates were genotype IV and rest 25 were genotype III. Quantum of *in vitro* virulence activities like cell surface hydrophobicity, superoxide dismutase, proteinase, esterase, deoxyribonuclease, and melanin production were observed in varying degrees. None of the isolates showed either phospholipase or haemolytic activity. All isolates exhibited high biofilm forming abilities in terms of biomass production (OD range: 0.38-1.27) and metabolic activity of biofilm cells (OD range: 0.23- 2.85). MIC50, MIC90, and the geometric mean (µg/ml) MIC of planktonic *T. asahii* cells obtained against various antifungal drugs were as follows, amphotericin B: 2;2;1.74; caspofungin: 0.5;1;0.66; fluconazole: 8;8;6; itraconazole: 0.125;0.42;0.16; voriconazole: 0.125;0.49:0.18; posaconazole: 0.3;0.42;0.25; anidulafungin: 0.5;0.8;0.37; and micafungin: 4;8;5.27. In contrast to this, a remarkably high MICs were observed among biofilm-forming

cells against fluconazole, amphotericin B, and voriconazole.

Conclusion: Our results suggested that genotype III is the most predominant genotype among the clinical isolates of *T. asahii* in western Nepal. Triazoles like voriconazole, itraconazole, and posaconazole exhibited optimally good *in vitro* activity against planktonic *T. asahii* cells. High rate of biofilm formation among *T asahii* strains and the resistance of biofilm cells to antifungal drugs might play a crucial role in the clinical outcome in neonates admitted to ICUs with infections associated with indwelling medical devices.

2.1 Prevention of *Candida* transmission to neonates – a multifaceted issue

Mihai Mares, Laboratory of Antimicrobial Chemotherapy

Ion Ionescu de la Brad University – Iasi, Romania

Invasive fungal infections are redoubtable diseases in preterm new-borns and neonates. The main players are *Candida albicans*, *Candida parapsilosis*, and other *Candida* species (i.e. *Candida glabrata*, *Candida tropicalis*, the new emerging multi-resistant species *Candida auris*).

In the very first period after birth, both innate and adaptive immune systems are deficient making the baby vulnerable to infections. Neonatal neutrophils, antigen presenting cells, and other immune effectors are characterized by quantitative and qualitative deficiencies. In preterm infants, these conditions are even worse and they show a high risk for invasive candidosis. Fungal colonization of skin, gastro-intestinal tract and respiratory mucosa is representing the first step which is then followed by an iatrogenic promoted dissemination (broad-spectrum antibiotics, postnatal steroids, histamine type-2 antagonists, central vascular catheters for parenteral nutrition).

Transmission pattern includes vaginal delivery, patient to patient passage, health care workers colonization, indoor environment contamination, contaminated infusates etc. The preventive measures include general and specific rules (hygiene, disinfection, catheter management, antifungal prophylaxis, pre-birth vaginal decolonization in mothers). Appropriate interventions are needed for prevention of *Candida auris* outbreaks in neonates intensive care units.

2.2 Maternal genital *Candida* colonization: The major risk for neonates' infection. Is it a valid dogma?

Suzana Otašević

Public Health Institute Niš, Niš, Serbia

Although neonatal care has been improved significantly in recent years, fungal infections still remain leading cause of sepsis in very low birth weight (VLBW≤ 1.500 g) preterm infants and extremely low birth weight (ELBW≤ 1.000 g) ones. Moreover, *Candida*-attributable mortality in preterm infants can reach up to even 43%.

The most fungal infections in preterm neonates are caused by *Candida* species from which *Candida (C.) albicans* and *C. parapsilosis are responsible for* approximately 80-90% of infant's infections. Additionally, the incidence of infection caused by other *Candida* species, particularly *Candida glabrata*, has also increased dramatically during the past two decades. Recovered non-albicans representatives of *Candida* spp., such as *C. glabrata* and *C. krusei*, are of great importance since they may harbor innate resistance to the azole class of antimycotic drugs.

The first link in the pathogenetic chain of fungal infections in neonates is adherence followed by colonization and dissemination. Adherence factors of fungi, the number of microorganisms and multiple site colonization are the most important virulence properties and risk factors which could influence infection. Colonization and many risk factors as moist skin, mucosal or skin damage, immature immune defense associated with possible coinfection, necrotising enterocolitis, intestinal perforation, required interventional care as abdominal surgery, administration of antibacterial drugs, steroids, H2 antagonists, parenteral nutrition**,** necessity of catheters use, and tubes application are mostly *predisposing* conditions which could induce the occurrence of yeast dissemination and onset of infection.

Generally accepted attitude is that maternal genital *Candida* colonization is the major risk for neonate's infection. Based on molecular evidence, *Candida* vaginal infection is strongly associated with congenital candidosis, has the great impact on outcomes of pregnancy with a history of cerclage and can be the large problem in attempts of *in vitro* fertilization. However, horizontal acquisition of *Candida* infection in neonates has also been proven and currently is consider as probably the most common mode of transmission for *C. parapsilosis strain*. Medical equipments and procedures, contaminated instruments, needles, dressings, or contaminated gloves that are not changed between patients are often responsible for patient to patient or health workers to patient horizontal transmission.

Currently, therapies for systemic fungal diseases, neonatal invasive candidosis (NIC) included, are not universally successful, morbidity and mortality of NIC remains high and management is still faced with numerous challenges. Regarding this scenario, preventive measures are of great significance and so far, the efforts are focused on preemptive diagnostic approach and chemoprophylaxis or probiotic prophylaxis with aim to decrease index of colonization.

Preventive measures have significant influence on incidence and prevalence of invasive mycoses. In neonatal *Candida*-invasive infection, preventive procedures with the goal to interrupt the process of agent transmission represent the new challenge. Strategy plans have to start with identification of risk and protective factors for vertical and horizontal transmission of yeast, followed by development of preventive test strategy and its widespread adoption.

Key words: Neonatal candidosis, preventive measures, transmission

2.3. In Utero & Neonatal Complications with indoor Aspergillus, Penicillium, Chaetomium, Stachybotrys, Trichothecenes Environmental Exposure

Irene H. Grant MD

Kaplan University, Davenport,USA

Introduction: Indoor toxin-producing, infectious fungi are recognized health hazards. Once established, Aspergillus/Penicillium (A/P), Chaetomium (CH), Stachybotrys (ST) produce myriad harmful products including carcinogenic, neurotoxic, immunosuppressant mycotoxins (MCTs), toxic debris, volatiles, particulates on materials for years without further intrusion. Despite disinfection, remaining environmental ST spores, Trichothecenes (Ts) damage any contacted cell (skin/mucosa/neurons/phagocytes). Little data on human illness exists since studies are unethical.
Innate immunity preventing fungal infection is weakened during pregnancy and neonatal periods. Gestational exposure may result in milk mycotoxin contamination. Endogenous mycotoxin production is plausible.

Aims: Analyze gestational/neonatal outcomes associated with documented indoor mycotoxin and hazardous mold exposures to determine clinical markers for such exposures.

Methods: Spore trap/microscopy/Mold-PCR/ERMI analysis/MCT measurement. Medical examination/record analysis (exposure-timing/severity/mold species/maternal-pediatric chronology/outcomes), fungal IGGs, MCT excretion.

Results: Analysis of indoor gestational mold exposures in 12 pregnancies (6M/4F/2 embryo) for 5 mothers in 4 mold contaminated homes (A/P, CH, Trichoderma, Mucor [see Table 1]) found exposure timing/severity correlated with adverse pregnancy, placental, congenital, postnatal and lactation outcomes (see Tables 2 & 3).

Conclusions: Gestational exposure to toxin-producing, infectious molds and/or hazardous products appears to cause in-utero complications, congenital defects and placental abnormalities.

Exposure timing/severity correlated with maternal debility/congenital/placental defects/postnatal/lactation outcomes. Neurological/cognitive and dermatological complications appear frequent.

Adverse lactation consequences occurred in all breastfed: grey-black oronasal drainage, projectile vomiting, choking, oropharyngeal neurologic damage, apnea/respiratory arrest, Intussusception, refractory perirectal rashes. Breastfeeding associated with hazardous indoor mold contamination appears hazardous

Mycotoxin breast milk screening appears an important advance. Neonatal Fungal IgG's unreliable.

Neonatal Trichothecenes excretion matched environmental Stachybotrys/ Trichoderma exposure and Environmental Trichothecenes. Environmental/human MCT testing appears useful identifying contamination +/or exposure. Ochratoxin excretion appears endogenous and a marker for persistent Aspergillus/Penicillium colonization/ infection. Epidemiological studies are urgently needed.

TABLE 1

INDOOR ENVIRONMENTAL EXPOSURES	# Children	
Severe contamination with hyphae	9	
Aspergillus/Penicillium	12	11 A. niger, 11 P. brevicompactum
Chaetomium	12	
Stachybotrys	11	
Mucor	5	
Environmental Trichothecenes Exposure	10	
Environmental Ochratoxin Exposure	0	
Environmental Aflatoxin Exposure	0	

TABLE 2		
CLINICAL OUTCOMES		
Mothers	5	
Total Pregnancies	12	
Miscarriages	2 (17%)	
Births (6 male, 4 female)	10	
Exposure timing unknown	6	
Exposure 1st trimester	3	
2nd trimester	2	
3d trimester	1	
Gestational Complications	4 (40%)	2 miscarriages, 1 preterm labor, 1 pre-eclampsia
Birth complications		
Placenta Abnormalities	2	calcification, chronic villitis with placental infarcts, double placenta, gritty membranitis.
Birth Defects	5 (50%)	2 Cardiac (murmur, VSD), 1 renal hypertrophy, 1 ptosis, diffuse permanent black grey mottling "goose-bump" texture
Dermatologic complications	5 (50%)	severe refractory eczema, Bleeding refractory "diaper rash"
Neurological complications	4 (40%)	Developmental delay, Cognitive Impairment, ptosis, oropharyngeal hypotonia
Specific Mold IGG response	0/3 tested	
Urine Trichothecenes	4/5 tested	
Urine Ochratoxin	3/5	
Urine Aflatoxin	0/5	

TABLE 3	
LACTATION COMPLICATIONS	
Lactation Exposure	6 (60%)
Milk Intolerance	5 (83%)
Projectile Vomiting	3
Choking	2
Grey/Black tongue	2* (Trichothecenes documented in milk in 1)
Perinatal Loss of oropharyngeal motor innervation	1
Apnea	1
Intussusception	1
Refractory bleeding perirectal "diaper rash"	1

2.4 Vulvovaginal Candidiasis complications of pregnant women and risk for newborns

Aleksandar Jurišić[1], Žaklina Jurišić[2], Dejan Dinić[3] Valentina Arsić Arsenijević[4]

[1]University of Belgrade Medical School, Narodni Front Obstretics and Gynecology Hospital, Belgrade, Serbia; [2]Obstretics and Gynecology Polyclinic Jurisic, Belgrade, Serbia; [3]CC Zemun, Belgrade, Serbia; [4]Medical Mycology Reference Laboratory, University of Belgrade Medical School, Insititute of Microbiology and Immunology, Belgrade, Serbia

Problem Statement: The aim of this study was to determine frequency of different *Candida* species and their susceptibility profile among pregnant women.

Methods: During four-month period (February–May 2015), swabs samples of vaginal discharge were taken from 34 pregnant women. Mycological investigation was done in National Reference Medical Mycology Laboratory. Vaginal swabs cultured on triple agar media containing: Sabouraud's dextrose agar (HiMedia, India), HiChrom *Candida* Differential agar (HiMedia, India) and Blood agar (Institute of Virology, Vaccines and Sera Torlak, Serbia). Isolates were identified by color of the colonies according to manufacturer's instructions. Antifungal susceptibility of the *Candida* isolates was determined by disk diffusion test against butoconazole (Richter Gedeon Nyrt., Hungary) and additionally against fluconazole, miconazole, clotrimazole and nystatin (all from Bio-Rad, France). Demographic and epidemiological data were collected. Descriptive statistics and Chi- square test were used to analyze the data.

Results: Vaginal swab cultures were taken from 34 pregnant women (mean age 31.94 ± 3.46, range 24-38 years) that participated in the study, of which 11 (32.35%) swab cultures yielded *Candida* spp. and 1 (2.94%) yielded *Saccharomyces cerevisiae*. Among 12 (35.29%) isolates the species distribution was as follows: C. albicans (n=10/12,83.33%), *C. tropicalis* (n=1/12, 8.33%), S. cerevisiae (n=1/12, 8.33%). Majority of strains have been shown to be susceptible to butoconazole (10/12, 83.33%), with only 2/12 (16.67%) being susceptible dose-dependent. However, among *C. albicans* strains, high rate of susceptible dose-dependent (7/12, 58.33%) to other azoles was observed. All strains were susceptible to nystatin (12/12, 100%). Resistant *C. albicans* strains were not detected. Based on epidemiological data, symptoms of vaginal discharge and risk factors associated with VVC were not statistically significant between *Candida*-positive and *Candida*-negative pregnant women due to small size of investigated group.

Conclusion: *C. albicans* was the most common cause of VVC in pregnant women and showed high susceptibility rate to azoles, especially to buto-

conazole. Larger controlled study is required to determine the efficiency of butoconazole in nonalbicans species and its usefulness in VVC therapy of pregnant women.

3.1. Utility of PCR diagnosis of invasive fungal infections in neonates

Michaela Lackner

Medical University, Innsbruck, Austria

Neonatal infections continue to cause morbidity and mortality in infants. Among approximately 400.000 infants followed nationally, the incidence rates of early-onset sepsis infection within 3 days of life are 0.98 cases per 1000 live births. Newborn infants are at increased risk for infections because they have relative immunodeficiency.

Invasive candidiasis is a leading infectious cause of morbidity and mortality in premature infants. Invasive candidiasis typically occurs in the first 6 weeks of life and presents with nonspecific signs of sepsis. Definitive diagnosis relies on the growth of Candida in blood culture, but this may identify fewer than half of cases. Several test assays are on the market, the sensitivity and specificity varies between centre to centre, patient population investigated and PCR format applied.

	Study population	Patients, *n*	Sample volume	Sensitivity	Specificity
Blood culture	Adults	37		50%	100%
Fungal antigens					
Mannan	Neonates	70	300 µL	92%	84%
Mannan	Chemotherapy	51		71%	77%
Mannan	Children and adults	92	300 µL	41%	100%
1->3 β-D glucan	Chemotherapy	51		76%	60%
1->3 β-D glucan	Children and adults	92	500 µL	47%	100%
Fungal antibodies					
Anti-mannan	Chemotherapy	51		43%	90%
Anti-mannan	Children and adults	92		47%	100%
PCR					
Semi-nested	Children and adults	92		88%	100%
Nested	Hospitalized patients	110 (24 neonates)	200 µL	86%	54%
Real-time	Hospitalized patients	110 (24 neonates)	200 µL	81%	96%
Real-time	Inpatients	23		93%	66%
Real-time	Inpatients	23		77%	100%
Real-time	Immunocompromised	384	2500 µL	88%	94%

The efficiency of these in-house assays has not been widely studied, lacks thoroughly clinical evaluation and therefore can't be recommended as stand-alone, single approach in clinical routine diagnostics. Molecular-based diagnostic assays can be recommended as valuable add on tools that complement conventional diagnostic procedures.

3.2 Early laboratory biomarkers for sepsis prediction: role of 1,3-β-D-glucan in neonatology

Asist. prof. Miha Skvarč, MD, PhD, specialist in clinical microbiology

Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana, Slovenia

Considerable progress has been made in the prevention, diagnosis, and management of pediatric patients with invasive fungal disease (IFD). The reported decreasing trend in the incidence of invasive candidiasis (IC) over the past 15 years. Nevertheless, due to the growing number of immunocompromised children at risk for IFD, this disease continues to be associated with significant morbidity and death and with increased financial burden to the health care system. Two most common pediatric IFDs are IC and invasive aspergillosis (IA).

Recognizing them can be difficult, because nonspecific clinical signs and symptoms or isolated fever is frequently the only presenting features. Risk factors for neonatal invasive candidiasis include many of the standard risks that are known in adult patients, such as central venous catheter use, acid blockers, and antibacterial use. However, in the neonates, both incidence and outcomes are also linked to the gestational age and the birthweight of the baby.
One of the most important thing in preventing grave outcomes of invasive candidiasis is prompt diagnostics. If we ask ourselves what clinical features, laboratory tests, and imaging studies are useful to identify a fungal cause for deteriorated health of a child despite broad-spectrum antibiotics, we could use the same analogy as in adults. Be quick and accurate. With biomarkers, we can predict invasive fungal infection. Measurement of biomarkers can be performed by the patient. We can use serum galactomannan to predict IA or 1,3-β-D-glucan (BG) to predict IC. Before we order pan fungal real time polymerase chain reaction (PCR) testing in blood, we should perform computed tomography (CT) of the lungs or consider imaging of abdomen in patients without localizing signs or symptoms. CT of sinuses is useful in patients with localizing signs or symptoms. With such procedures, the diagnostic accuracy of our diagnostic methods becomes higher.

The most important advice is. Take the samples for microbiological analysis before antifungal therapy. Even one or

two doses of them can influence on the results and then we do not know what is real disease, contamination or colonization.

How we could prevent IFD? Prophylactic systemic antifungal therapy reduces the incidence of invasive fungal infection in very preterm or very low birth weight infants. Too much therapy in high doses for long time only leads to emergence of organisms with antifungal resistance. We should never treat high concentrations of the biomarkers but patient as a hole. Reconsideration and possible de-escalation of the therapy is necessary especially in vulnerable preterm children.

3.3 Can *Candida* be fearsome in pregnancy?

Prof Dr Ljubomir Petricevic

Medical University of Vienna, Austria

The colonization with *Candida* spp. occurs more frequently in pregnant compared to non-pregnant women (31.4 vs. 19.9%). In particular, pregnant women are more often suffering from asymptomatic *Candida* spp. colonization than their non-pregnant counterparts (46.5 vs. 16.0%). Vaginal colonization with *Candida* spp. during pregnancy has been associated with impaired pregnancy outcomes. There is a reduction in spontaneous preterm birth among women with current asymptomatic colonization of *Candida* who were treated with clotrimazole.

3.4. *Candida* and *Fusarium* sepsis in Serbian paediatric and neonatology settings– the role of blood culture

Valentina Arsic Arsenijevic

National Medical Mycology Reference Laboratory, Institute of Microbiology, Faculty of Medicine University of Belgrade, Belgrade, Serbia

A fungal bloodstream infection (BSI) is an subgroup of infectious process characterized as a systemic inflammatory response syndrome (SIRS), severe sepsis or septic shock. Misdiagnosing, mistreating or late initiation of appropriate therapy commonly results in a high mortality rate in patients with fungal BSI in Serbia.

In a fungal BSI viable fungi are present in blood and the density in peripheral blood can be below 10/per milliliter in adults and about 100/per milliliter in children. Blood cultures (BC) are sensitive to detect these low amounts of fungi. However, density varies during the course of disease, and therefore, BC diagnostics yield positive results in about 50% of cases. If positive it means

the proven fungal BSI, therefore, BC with sufficient quantities of blood is recommended in patients with suspected sepsis because BC have several advantages: (i) they have been in use for more than 100 years and are well integrated in the clinical workflow and clinical guidelines; (ii) semi-automated BC systems have greatly simplified handling in the microbiological laboratory which results in a short hands-on time; (iii) a wide range of bacterial and fungal pathogens can be isolated and identified; (iv) isolation of the pathogen is a prerequisite to phenotypic susceptibility testing which enables clinicians to initiate targeted antimicrobial therapy.

However, BC diagnostics has limitations: (i) detection is limited to pathogens that are able to grow in BC and some fungi, such as *Aspergillus* spp., grow poorly; (ii) antifungals may inhibit growth and relevant pathogens may thus go undetected, after the initiation of antifungalsal therapy; (iii) BC diagnostics requires one week until results are available and many clinicians feel that results are available too late to guide therapy.

So molecular techniques have been developed to replaced BC but almost a decade later, molecular techniques have not replaced BC and it is still not clear how to best integrate them in diagnostic pathways. Traditionally, in Serbian pediatrics and neonatology settings the BC is focusing on bacteria pathogens. Recently fungal BSI become more and more important especially because the BC makes it detection possible. Based on this we reported two nosocomial oubrake in Serbian: ***Candida*** sepsis and neonatology settings complicated with osteomyelitis and ***Fusarium*** sepsis in pediatric setting. Based on timely cooperation between clinicians and laboratory both outbrakes had sucessful oucomes.

3.5 UNCOMMON PRESENTATION OF CANDIDA INFECTION IN THE NEWBORN INFANTS

Jelena Martić [1, 2], Iris Pejčić [3], Valentina Arsić [2, 4], Borisav Janković [1, 2], Zorica Rakonjac [1], Lidija Avramović[5], Svjetlana Đukić Maglajlić [2, 6]

[1]Mother and Child Health Care Institute of Serbia „dr Vukan Čupić", Belgrade; [2]Medical Faculty, University of Belgrade; [3]Institute of Neonatology, Belgrade; [4]Institute of Microbiology and Immunology; [5]Clinic of Obstetrics and Gynecology "Narodni front"; [6]University Children's Hospital Tiršova, Belgrade, Serbia

Introduction: *Candida* is an important cause of neonatal infections with significant morbidity and mortality, especially in premature infants. The clinical manifestations of *Candida* infection in the neonate vary, ranging from localized skin and mucous infections to life-threatening systemic infection. *Candida* arthritis is rare presenting feature of systemic candidiasis. The aim of study was to report a case series of newborn infants with *Candida* arthritis.

Methods: four cases of septic arthritis caused by *Candida* were threated in our institutions in 2009. Diagnosis were made on the basis of clinical and radiological signs of arthritis and isolate of *Candida* from joint aspirate, debridement tissue culture or blood culture.

Results: Two patients were male and two female. Three infants were born in late preterm gestation (36.4 ± 0.23 weeks) and one was born at term. Three of four infants were born by cesarean section and one vaginally. Early presentation (2nd and 3rd day of life) with nonspecific signs of infection was present in three patients and all infants were treated with antibiotics. Signs of arthritis were presented in second and third week of life. All infants presented with swelling of the knee and one patient had swelling of the elbow too. Culture reports revealed *Candida* in blood in two, and in joints in three patients. Therapy with fluconazole and amphothericin B was performed as well as surgical treatment.

Conclusion: Arthritis is an uncommon presentation of candida infection in newborns. All infants with invasive *Candida* infection must be evaluated for signs of joint infection. In infants with arthritis amphotericin B is the recommended as the preferred initial therapy in neonates with *Candida* arthritis. Alternate therapy includes fluconazole or combination of these two agents.

4.1. Packaging of Antifungal Drugs

Esther Segal

Dep. Clinical Microbiology and Immunology, Sackler School of Medicine, Tel-Aviv University, Tel-Aviv, Israel

The arsenal of antifungal drugs has been expanded over the years and consists of drugs assigned to four major classes: the polyenes which bind to ergosterol and disrupt the fungal cell membrane, the azoles which inhibit ergosterol-biosynthesis, the allylamines which inhibit ergosterol- biosynthesis at the early steps, and the echinocandins which act on the fungal cell wall by blocking the enzyme glucan synthase. However, due to various shortcomings of the drugs, such as toxicity leading to significant adverse side effects, problems with delivering the active drugs to the site of infection, mode of administration or development of resistance, still necessitate a search for new drugs.

An alternative approach to be explored could be improvement of delivery systems or development of new formulations via different packaging of the available antifungals. Thus, possibly bypass problems of solubility of drugs, reduce toxicity and improve administration mode. The latter alternative approach is the focus of this presentation.

The classical packaging system is association of polyenes with lipids, such as incorporation of Amphotericin B (AMB) into liposomes - a formulation used clinically for over a decade.

Another different, experimental lipid-based formulation of AMB is the Cochleate AMB- a solid lipid bi-layer sheet rolled into a spiral, which has the potential for oral administration. A different experimental approach was combining AMB with arabinogalactan, which yielded a soluble conjugate. B-PEG AMB is an additional experimental conjugate with increased water solubility and lower toxicity. However, none of these experimental formulations is thus far in clinical use.

Association with lipids was also attempted in other classes of antifungals. Combining Fluconazole with lipid nanoparticles resulted in a preparation that was non-recognizable by efflux pump proteins and thereby prevented the efflux of the drug in Fluconazole-resistant *Candida*. Changing formulation of azoles, such as use of an aqueous nano-suspension of Itraconazole led to improvement in treatment of bronchopulmonary aspergillosis.

Echinocandins are available for IV use only. Recent studies reported on development of a preparation -SCY-078, which has a similar mode of activity as echinocandins but with a different chemical structure and which exhibits antifungal activity and good oral bioavailability.

The research of the author's group focused since a number of years on exploration of polyene–lipid investigative formulations involving association of Amphotericin and Nystatin (NYT) with the clinically used food supplement – Intralipid (IL). We developed two formulations by associating AMB and NYT with Intralipid: AMB-IL and NYT-IL. AMB-IL and NYT-IL are affordable, stable, standardized preparations, active *in vitro* against *Candida, Aspergillus* and *Mucorales species*. AMB-IL and NYT-IL were active also *in vivo* in experimental invasive murine *C. albicans* and *A. fumigatus* infections. Intravenous administration of NYT-IL was well tolerated *in vivo*, thereby enlarging the arsenal of drugs against invasive mycoses. Recently, the *in vivo* activity of NYT-IL was explored against *Mucorales species* in a *Galleria mellonella* model, indicating efficacy. Combinations of NYT-IL with other antifungals revealed a synergistic effect *in vitro* against *A. terreus*. Currently, activity of NYT-IL is being assessed against additional fungi. Thus, Amphotericin B – Intralipid and Nystatin–Intralipid bear the promise to be considered as candidates for therapy of invasive mycoses.

4.2 Novel therapeutic strategies for mixed infections: controlling bacterial-fungal interaction, signaling and *quorum sensing*

Lidija Senerovic

Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Belgrade, Serbia

Fungi and bacteria often inhabit the same niche, whether in the environment, or in plant or animal hosts. Instead of existing as single-species planktonic forms, the majority of microorganisms form complex communities attached to the surfaces called biofilms. Biofilms, the cell aggregates embedded in a self-produced extracellular matrix, are often implicated in low sensitivity or resistance to antimicrobials. Biofilm-related infections have been encountered in chronic diseases such as cystic fibrosis, otitis media, ventilator-associated pneumonia, tuberculosis, or in chronic wounds that have an impaired blood supply. Most of these diseases have polymicrobial nature involving bacteria and pathogenic fungi. Polymicrobial infections, accompanied with mixed species biofilms are harder to treat and usually have increased mortality outcomes compared with their monomicrobial counterparts and thus, constitute a significant health care burden.

Virulence and biofilm formation of many pathogenic bacteria are under control of quorum sensing system (QS). In fungi, growth, stress resistance, morphogenesis and biofilm formation are also regulated by QS. QS allows bacteria and fungi to monitor their cell density through the release of specific signaling molecules called autoinducers. At a high cell density, autoinducers' concentration reaches threshold initiating the signaling cascade that regulates expression of different genes, many of them being required for microbial pathogenicity. QS signaling molecules mediate also interspecies communication during polymicrobial infections and, depending on the type of interaction between bacteria and fungi, influence the disease progression. Therefore, the application of QS modulators can be considered a promising strategy for efficient control of polymicrobial infections.

The rich sources of novel bioactive molecules are plants extracts or essential oils. Essential oils (EOs) are complex mixtures of volatile compounds extracted from plants. Numerous EOs have different biological activities including antibacterial, antifungal and anti-QS. Specific combinations of the major components of EOs have been reported to potentiate activity of conventional antimicrobials improving their effectiveness.

The antimicrobial potential of selected citrus essential oils (EOs), namely pompia and grapefruit, was evaluated against different human pathogens and their ability to prevent and treat mixed

species biofilms was analyzed. Pompia (*Citrus x monstrousa*) and grapefruit (*Citrus x paradisi*) EOs efficiently inhibited fungal growth, whereas no effect on *Pseudomonas aeruginosa* growth was observed. Both citrus EOs inhibited formation of bacterial and fungal monomicrobial biofilms and were efficient in potentiating the activity of clinically used antimicrobials. Citrus EOs inhibited formation of mixed biofilms composed of medically important pathogens *P. aeruginosa* and *Aspergillus fumigatus* or *Scedosporium apiospermum*. Citrus EOs affected quorum sensing in *P. aeruginosa* and caused fast permeabilisation of *Candida albicans* membrane. These results suggest that pompia and grapefruit EOs could be used for prevention and treatment of biofilm- associated chronic polymicrobial infections.

4.3. Antifungals and current treatment guidelines in pediatrics and neonatology

Dragana Janic

University Children`s Hospital, Belgrade, Serbia

Making decision on antifungal treatment in children proves to be significantly more difficult than in adults. Available diagnostic tools for fungal infections are of lower reliability in children and, additionally, appropriately designed large clinical studies to support high level recommendations are lacking. Pharmacokinetic is substantially less investigated in children and many of the drugs have limitation for use depending on the age of the child.

In the ever enlarging populations of children with hematological malignancies being treated with intensive anticancer therapy as well as stem cell transplant recipients most concerning are infections with aspergillus species, while in sick neonates, in patients with central venous catheter and patients hospitalized in intensive care units candida makes large majority of all fungal infections. Invasive candidiasis and aspergillosis are leading causes of fungal infectious morbidity and mortality among previously mentioned pediatric patients populations but rare fungal infections are also increasingly being diagnosed.

Existing guidelines from three largest societies/organizations dealing with the problem of antifungal treatment, although differing in many respects, are still the beacon for the clinician in the murky and dangerous waters of invasive fungal disease in children. Infectious Diseases Society of America (IDSA), the European Conference on Infection in Leukemia (ECIL) and the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) guidelines understanding is crucial in clinical decision on when to start anti-

fungal treatment and which drugs to use. Explaining reasons for similarities and differences in mentioned recommendations possibly directs clinician which guidelines to choose in a particular patient. Role of preemptive therapy, need for therapeutic drug monitoring, how to treat relapsed fungal disease are examples of open questions in this field discussed in this presentation.

4.4. VIRULENCE FACTORS AND SUSCEPTIBILITY OF *CANDIDA* SPP. CAUSATIVE AGENTS OF NEONATAL INFECTIONS

N. Stojanović[1], P. Stojanović[1,2], S. Otašević[1,2], V. Arsić-Arsenijević[3]

[1]Faculty of Medicine, University of Niš, Niš, Serbia; [2]Institute for Public Health Niš, Center of Microbiology, Niš, Serbia; [3]Faculty of Medicine, University of Belgrade, Belgrade, Serbia

Introduction: *Candida* spp. frequently cause hospital-acquired bloodstream infections (BSI) with a high mortality rate, up to 70%, whereas in neonates this mortality is around 50%. The switch from *C. albicans* to *C.* non-*albicans* (NAC), especially during last decades, as a causative agent of neonatal infections redirects the researchers focus to the NAC antifungal susceptibility and virulence characteristics.

Aims: This work aims to evaluate and compare the susceptibility patterns and virulence characteristics of two *Candida* spp. (*C. albicans* and *C. lusitaniae*) isolated from bloodstream of neonates originating from the intensive care unit at the clinical center Nis.

Methods: Complete data for both patients were collected from patients' history. Strain identification was confirmed using both conventional (microscopic, Chrome-agar cultivation and biochemical characteristics determination) and contemporary [MALDI-TOF MS (Bruker, Dultonics, Bremen Germany)] methods. Antifungal susceptibility of two mentioned isolates was performed following CLSI methodology, using a commercial microdilution test Micronaut-AM MHK-2. The *Candida* isolates were screened and quantified for their ability to form biofilms under static conditions in 96-well microtiter plates by a crystal violet method. The ability of isolated rat macrophages to kill and/or engulf yeast particles, as well as the influence of *Candida* on macrophages viability and ability to produce myeloperoxidase were studied using a standard laboratory protocols.

Results: Neonates with *C. albicans* and *C. lusitaniae* blood stream infection were hospitalized in intensive care units and were both prematurely born, with low birth weight. Antifungal susceptibility assay revealed that both *C. albicans* and *C. lusitaniae* were not resistant to any of 9 tested antifungal drugs and the patients were cured after administration of adequate antifungal therapy. Biofilm producing ability of *C. albicans* was more intense than the

ability of *C. lusitaniae*. Both strains were able to reduce macrophage viability up to ca. 75% (76 and 78%) and increase myeloperoxidase activity. Additionally, the ability of macrophages to kill *Candida* isolates was almost the same for the two evaluated strains, while their capability to phagocyte the yeast particles was much more pronounces in the case of NAC.

Conclusions: The results of the preliminary experimental analysis of virulence factors found for *C. albicans* and *C. lusitaniae* points to modest, but important, differences between the two evaluated strains. Although we are aware of the low number of strains that were evaluated and compared one cannot diminish the importance of the results obtained here, since they point to the potential difference in disease causing mechanisms of these two strains.

POSTERS

P.01 Monitoring of Microscopic Filamentous Fungi in Indoor Air and in the Neonatal Intensive Care Units

Miljana Misic[1], Katarina Milincic[2], Iris Pejcic[3], Milica Rankovic[3], Miroljub Milincic[1]

[1]University of Belgrade School of Geography, Department of Environmental Ecology, Belgrade, Serbia; [2]University of Belgrade School of Biology, Belgrade, Serbia; [3]Institute of Neonatology, Belgrade, Serbia

OBJECTIVES Nosocomial invasive mold infections, particularly aspergillosis and zygomycosis, are an increasing problem in immunocompromised patients, including low birth weight (LBW) neonates. The presented study evaluates filamentous fungal diversity and the presence of *Aspergillus* spp and order Mucorales in indoor air samples at the Institute of Neonatology, Belgrade, Serbia.

MATERIALS AND METHODS The air samples were obtained from the (i) reception room; (ii) department of the neonatal intensive care units; (iii) the incubators. The MAS-100 air sampler (Merck, GER) a sterile-90 mm triple plates with Sabouraud-Pateto-Rice agar were used. The fungi were identified by macro- and micromorphology and the number of colony forming units (CFU) were determined according to mycology criteria.

RESULTS In total, 48 samples of the indoor air were taken from the reception room (n=8); Department of the neonatal intensive care units (n=8) and the incubators (n=32). In 4 cases (4/48; 8.33%) the cultivation verified the presence of microscopic filamentous fungi: in the reception room (3/4; 75%) and in neonatal intensive care units (1/4; 25%) and no presence observed in the incubators (0/4; 0%). The established genus was *Aspergillus* spp. (4x), followed by *Trichoderma* spp. (2x) and *Penicillium* spp. (2x), *Paecilomyces* spp., and *Chrysonilia* spp. (1x each). In 1 case the cultivation established sterile aerial mycelium. No molds from order Mucorales found in this study. The observed fungal number was from 3 to 5 CFU per plate.

CONCLUSIONS Relative low number of microscopic molds revealed during this study suggesting good air quality control measure at the Institute of Neonatology but this data is difficult to compare because the criteria for the number of fungi allowed in the hospital air has not been determined yet. With the continuing increase in the number of severely immunocompromised patients including LBW neonates, hospitals are faced with the growing problem of fungal infections, including molds. Therefore, the preventive monitoring of hospital environmental and mold air contaminants is of major importance, as well as indoor air quality control.

P.02 Isolation rate and antifungal susceptibilities of Candida species from clinical specimens in a University Hospital, Thessaloniki, Greece

Myrsini Chatzi, Efthymia Protonotariou, Olia Vasilaki, Ioanna Gkeka, Vasilis Draganoudis, Antonia Landa, Georgia Kagkalou, Lemonia Skoura

Department of Microbiology, AXEPA University Hospital, Thessaloniki, Greece

Aim *Candida* sp are the most common cause of fungal invasive infections in humans worldwide. *Candida* infections especially candidemias are related with high morbidity and mortality rates. The objective of our study was to assess the species distribution and to evaluate the in vitro antifungal activity among *Candida* isolates recovered from hospitalized patients in AHEPA University Hospital.

Methods From January 2015 up to September 2017,294 non-duplicate isolates of *Candida* spp were isolated from following materials: 93 from urine (31.63%), 74 from blood cultures (25.17%), 46 from central vein catheter (15.65%), 38 from pus(12.92%), 14 from peritoneal fluid (4.76%) and 29 from other materials(9.86%). These strains were isolated of patients hospitalized in different departments of AHEPA hospital, including internal, surgical and ICU department. Bacterial identification and antifungal susceptibility testing to fluconazole, amphotericin B, flucytocine and voriconazole were performed by the VITEK 2 automated system.

Results During the study period 182 *C. albicans (62.32%)* and 112 *C. non albicans* (37.67%) were recovered. Of 112 Candida non albicans species distribution was 64 (57.14%) for *C. parapsilosis*, 30 (26.78%) for *C. glabrata*, 7(6.25%) *C. tropicalis*, 6(5.36%) *C. lusitaniae* and 5(4.46%) *C. krusei.* In vitro activity showed that 15 (8.24%) *C. albicans* strains were resistant to antifungal drugs;5 (2.74%) of them were resistant to fluconazole, 7 (3.84%) to amphotericin B, 2 (1.09%) to flucytocine and 1 (0.55%) to voriconazole. Concerning *C. glabrata*, *C. tropicalis* and *C. lusitaniae* strains they were found to be susceptible to every antifungal drug tested. As for *C. parapsilosis*, 7 strains (10.93%) were found resistant to fluconazole and 4 strains (6.25%) to amphotericin B and susceptible to the other antifungal drugs. As regards *C. krusei,* 4 strains (80%) were resistant to fluconazole and susceptible to the other antifungal drugs.

Conclusion *C. albicans* was the most common cause of fungal infection, while *C. parapsilosis* was the most common cause of fungal infection among the non albicans species in our institution. All *Candida* isolates-except few cases of *C. albicans,C. parapsilosis* - showed very good *in vitro* susceptibility activity to all tested antifungals. *C. krusei* isolates are inherently or secondarily resistant to fluconazole.

P.03 Education of medical personnel in neonatology units-new technologies for health system/molecular diagnosis

Dragana Ivanovic[1], Slavica Dacić[1],Suzana Otašević[2], Valentina Arsić Arsenijević[3]

[1]Institute of Public Health, "Dr Milan Jovanović Batut"Belgrade , Serbia; [2]Institute of Public Health Niš,Center of Microbiology, Niš, Serbia; [3]National Medical Mycology Reference Laboratory, Belgrade, Serbia

Aim Fungal diseases (FD) are common nowadays but the diagnosis is the significant problem because is unavailable or delayed. The deployment of molecular biology techniques for diagnosis faced with a number of challenges, including the cost of equipment and reagents, as well as the interpretation of molecular-based diagnosis, especially in pediatric and neonatology settings. Therefore, education of medical personnel in neonatology units-new technologies for health system/molecular diagnosis is important but still limited.

Materials and Methods We searched PubMed database for the terms relevant for fungal diseases diagnosis: Education of medical personnel in fungal diagnosis; Education of medical personnel in fungal biomarker-based diagnosis; Education of medical personnel in beta d glucan based diagnosis; Education of medical personnel in diagnosis of *Candida* infection; Education of medical personnel in diagnosis of *Aspergillus* infection; In order to compare obtained data we searched PubMed database for terms relevant for viral diseases diagnosis Education of medical personnel in diagnosis of HIV infection and Education of medical personnel in diagnosis of viral infection.

Results The results obtained showed a big difference between the number of publications regarding Education of medical personnel in fungal diseases and Education of medical personnel in viral diseases. Results are presented at the Table.

Terms for Pubmed search	Results
Education of medical personnel in fungal diagnosis	**0**
Education of medical personnel in fungal biomarker-based diagnosis	**0**
Education of medical personnel in beta d glucan based diagnosis	**0**
Education of medical personnel in diagnosis of Aspergillus infection	**2**
Education of medical personnel in diagnosis of Candida infection	**20**
Education of medical personnel in diagnosis of HIV infection	**664**
Education of medical personnel in diagnosis of viral infection	**1058**

Conclusion The types of fungal infection that occur in the patients described vary accordingly with the type of underlying disease of the host, as well as with their epidemiological exposure. Education of medical personnel in neonatology units-new technologies for health system/molecular diagnosis is limited but it seems very much needed, especially in neonatology settings.

P.04 Susceptibility of *Candida* species to fluconazole and butoconasole in pregnant Serbian women: Possible role of maternal screening of vulvovaginal candidiasis and therapy in the prevention of infection of the newborn

Dragana Srebro[1], Aleksandra Trajković[2], Biljana Živaljević[3], Aleksandar Jurišić[4]

[1]Department of Pharmacology, Clinical Pharmacology and Toxicology, Faculty of Medicine, University of Belgrade, Belgrade, Serbia, Dr Subotica 1, 11129 Belgrade, Serbia; [2]Faculty of Medicine, University of Belgrade, Belgrade, Serbia; [3]Gynecology Practice „Dr. Biljana Živaljević"; [4]Obstetrics and Gynecology Clinic "Narodni Front", University of Belgrade, Belgrade, Serbia

Aim: Vulvovaginal candidiasis (VVC) is an infection of the genital mucosa caused by genus *Candida* (*C.*). The most common causative agent is *C. albicans* but the prevalence of non-*albicans Candida* (NAC) species is increasing and can causes serious infection in the newborns. This study aimed to (i) assess the prevalence of *Candida* spp. in the cervical-vaginal mucosa of pregnant women with VVC before delivery; (ii) to obtaine the data regarding susceptibility of the isolates against fluconazole and butoconazole nitrate, the most common use antifungals in Serbia.

Materials and Methods: This prospective cohort study was carried out during a six months in 2016. Swab samples of vaginal secretion were collected from pregnant women with VVC (n = 61): the control group with non-recurrent VVC (VVC/CG; n = 45; mean age 34.6± 4.5 years) and the study group with recurrent VVC (VVC/SG; n = 16; mean age 34.6± 4.5 years). The relation between other risk factors, such as diabetes mellitus, antibiotic and corticosteroid use, history of sexually transmitted diseases and contraceptive methods, was recorded. *Candida* spp. was identified by conventional methods. The samples were cultured on Sabouraud dextrose agar and on CHROMagar *Candida* and species were identified by culture. Antifungal susceptibility testing (AFST) to fluconazole (Pfizer, USA) and butoconazole nitrate (Richter Gedeon, HU) was determined by CLSI M27-A3 broth dilution method. *C. parapsilosis* ATCC 22019 and *C. krusei* ATCC 6258 were used as a quality control strains.

Results: Overall, the prevalence laboratory-proven positive *Candida* findings was 29.5% (18/61) in our pregnant women and *C. albicans* was the most common in CG (77.8%; 7/9 cases), followed by NAC species (22.2%; 2/9 cases) represented with *C. kruzei*. NAC species dominated in SG (55.6%; 5/9 cases) represented with *C. krusei* (3/5 cases), *C. tropicalis* (1/5 cases) and *C. glabrata* (1/5 cases) and *C. albicans* was less common (44.4%; 4/9 cases). AFST of *C. albicans* showed sensitivity to fluconazole and to butconazole equally in both groups (from 70 to 75% strains). The difference was obtained for NAC species, 50-60% NAC species were sensitivity to but-

conazole in CG and in SG, while sensitivity to fluconasole was lower (about 40%) in SG.

Conclusion: Non-recurrent and recurrent VVC in pregnant women in Serbia are common problem but the resistance of *Candida* strains for both azoles is still uncommon. In vitro sensitivity of NAC strains to butoconazole was higher in comparison to *C. albicans* strain. Therefore, the maternal screening of vulvovaginal candidiasis and detection of NAC strains is important especially for proper therapy. The prevention of this infection of the newborn seems important and required.

P.05 DISTRIBUTION OF *CANDIDA* SPECIES INVOLVED IN SYSTEMIC INFECTIONS OCCURRED IN HOSPITALIZED PATIENTS

Ionut Pecete[1,3], Violeta Corina Cristea[1,2], Irina Gheorghe[3], Carmen Murariu[4], Mariana Carmen Chifiriuc[3], Tatiana Vassu[3]

[1]Synevo Romania, Medicover; [2]University of Medicine and Pharmacy "Carol Davila", Bucharest, Romania; [3]Faculty of Biology, University of Bucharest; [4]Emergency Hospital for children "Marie S. Curie", Bucharest, Romania

Aim Fungal infections account for an important contribution to the morbidity and mortality of hospitalized patients. Their incidence is dependent on the associated pathology and age, the children and elderly being more exposed. Our study aimed to determine the etiology and incidence of fungal systemic infections produced by *Candida* species.

Materials and methods A total number of 44 blood isolates from patients hospitalized for clinical suspicion of candidemia have been studied. The samples were collected in blood culture bottles in Bactec system (Bactec Plus Aerobic F Medium/ Bacter Mycosis IC/F Medium – Becton Dickinson) and incubated for 7 and 14 days, respectively. The positive samples were seeded on Sabouraud culture medium supplemented with chloramphenicol and incubated at 35^0 C. The obtained culture were identify by mass spectrometry using MALDI Biotyper (Bruker).

Results The study revealed a 20% positivity of blood cultures, with a gender distribution of 61% males and 39 % females. Distribution by age group demonstrated the predominance of fungal infections in pediatric patients (38 cases) compared to adults (6 cases). In the pediatric group, the distribution of *Candida* species was the following: *C. parapsilosis* (50%), followed by *C. albicans* (34%), *C. lusitanie* (10%), *C. tropicalis* (3%) and *C. pelliculosa* (3%). In adults (average age 51 years), *C. albicans* (50%) prevailed, followed by one strain of *C. parapsilosis*, *C. kefyr* and *C. inconspicua*.

Conclusion It is mandatory to monitor systemic fungal infections produced by

species of the genus *Candida*, especially due to the emergence of non-*albicans* species in this type of pathology. In our study *C. parapsilois* was prevalent in the pediatric pathology, while in the adult age group, *C. albicans* is still responsible for half of the cases. Laboratory diagnosis, respectively, genus and species identification, along with antifungal susceptibility testing are essential for establishing an appropriate treatment.

P.06 SCREENING FOR AZOLE-RESISTANT *ASPERGILLUS FUMIGATUS* ISOLATES FROM CLINICAL SAMPLES AND HOSPITAL AIR

R. Tomazin*, M. Ločniškar, J. I. Pilih, T. Matos

Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

*Correspondence: rok.tomazin@mf.uni-lj.si

Aim. Azole-resistance in *Aspergillus fumigatus* is gaining on importance in the last few years, mostly because of emergence of resistant isolates in the azole-naïve patients. These isolates appear to be associated with the use of azole-fungicides in agriculture and TR_{34}/L98H mutations in the *CYP51A* gene. The aim of this study was to screen respiratory tract samples and samples of hospital air for azole-resistant *A. fumigatus* in Slovenia and assess the prevalence of azole-resistance in clinical environment.

Material and Methods. 226 samples were included in the study. These included *A. fumigatus* strains isolated from respiratory tract samples from patients hospitalized in the University Medical Center Ljubljana (Ljubljana, Slovenia) and air samples from different departments of the same medical center. These samples were collected from 10.5.2016 to 15.4.2017. The study also included *A. fumigatus* strains from the culture collection of the Laboratory for Diagnostics of Fungal Infections, (Faculty of Medicine in Ljubljana, Slovenia). Inclusion criteria for culture collection strains were voriconazole and/or itraconazole Minimal Inhibitory Concentrations of ≥ 0.25 µg/mL. In total there were 167 clinical isolates. Air samples were collected with DUO SAS Super 360 sampler (VWR, USA). Each sample consisted of 0.5 m^3 of air collected on Sabouraud and Czapek RODAC-agar plates, which were incubated at 37°C and expected for *A. fumigatus* after 48 h. In total there were 59 air samples. All *A. fumigatus* strains were further analyzed: a suspension of 0.5 McF was prepared in saline and 25 µL were transferred to each well of VIPcheck azole-resistance screening plates (VIPcheck, The Netherlands). Inoculated VIPcheck plates were incubated at 37°C and expected daily for growth. If there was no growth after 2 days of incubation, the sample was considered negative. Saline was used as negative

control. Positive controls included 2 azole-resistant *A. fumigatus* strains: *A. fumigatus* TR_{34}/L98H mutation positive and *A. fumigatus* TR_{46}/Y121F/T289A mutation positive. Samples considered positive were further analyzed: molecular detection of 2 point mutations of *CYP51A* gene, L98H and M220T, and tandem repeat, TR_{34}, in the promoter region of the same gene was carried out as previously described (Spiess et al., 2012, AAC).

Results. The prevalence of azole-resistant *A. fumigatus* strains in Slovenia is < 10 % ($p < 0.001$). The prevalence of voriconazole and itraconazole resistant strains of *A. fumigatus* is 3.1 % ± 2.3 % and 0.9 % ± 1.2 %, respectively. We have found a resistant strain carrying the common mutation TR_{34}/L98H.

Conclusion. The prevalence of azole-resistant *Aspergillus fumigatus* remains low in Slovenia, being less than 10 %. Consequently, azole antifungal drugs can remain the first-choice therapy in cases of aspergillosis. We have shown that the widely described resistance-associated *CYP51A* mutation, TR_{34}/L98H, is present in the *A. fumigatus* population in Slovenia.

P.07 MICRODILUTION TECHNIQUES FOR SUSCEPTIBILITY TESTING OF PATHOGENIC YEASTS

A. Obreza*, R. Tomazin, T. Matos

Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

*Correspondence: aljosa.obreza@mf.uni-lj.si

Aim In case of candidosis early antifungal treatment significantly reduces mortality and morbidity. Choice of appropriate therapy depends also on antifungal susceptibility testing, which is often performed with commercially available tests. The most accurate results are obtained by reference methods, like broth microdilution method according to EUCAST-AFST (European Committee on Antimicrobial Susceptibility Testing – Antifungal Susceptibility Testing), which are technically demanding and time consuming. The aim od this study was to find the modified/simplified microdilution technique that provides accurate results that are as close as possible to those obtained by the reference EUCAST-AFST method.

MM 38 isolates were included in the study. These isolates came from the UK NEQAS (United Kingdom National External Quality Assessment Service) scheme for Antifungal Susceptibility that the Institute of Microbiology and Immunology (Faculty of Medicine, University of Ljubljana, Slovenia) received for the external quality control from 2008 to 2017. These included 33 isolates of *Candida* spp, two isolates of *Saccharomyces cerevisiae*, two isolates of *Trichosporon asahii* and one isolate of *Rhodotorula mucilaginosa*. Inocu-

lums were made from 24h-old cultures grown on Sabouraud dextrose agar (BioMérieux, France) in sterile saline, adjusted to 1-5 x 106 CFU/mL and diluted in RPMI 1640 broth with 2.0 % glucose to final yeast concentration of 0.5-2.5 x 105 CFU/mL. We dispensed 200 µL of so prepared inoculum to each well of the commercially available microdilution plate (Merlin Diagnostika, Germany) with lyophilized fluconazole, amphotericin B, flucytosine, voriconazole, itraconazole, posaconazole and caspofungin in ranges of 0.002-128 µg/mL, 0.031-16 µg/mL, 0.0625-32 µg/mL, 0.008-8 µg/mL, 0.031-4 µg/mL, 0.008-8 µg/mL and 0.002-8 µg/mL, respectively. Inoculated plates were incubated at 37 °C for 24 h without agitation. After incubation we've determined MIC with microdilution plate reader by measuring absorbance at a wavelength of 495 nm. We've compared our MIC values with reference, UK NEQAS, values and it was considered appropriate if they did not differ by more than 2 two-fold dilutions according to the reference value.

Results Categorical agreement of minimal inhibitory concentration (MIC) values between modified broth microdilution method and reference values was 96 % for amphotericin B, 76 % for flucytosine, 83 % for caspofungin, 86 % for fluconazole, 68 % for itraconazole and 57 % for voriconazole.

Conclusion Modified broth microdilution method showed high categorical agreement of more than 95 % for amphotericin B, and relatively low agreements, varying from 57-86 %, for other antifungal agents. Modified EUCAST method is therefore not suitable for routine susceptibility testing of yeasts, except for amphotericin B.

P.08 PREVALENCE AND RISK FACTORS OF VAGINAL CANDIDIASIS AMONG PREGNANT WOMEN – A SINGLE CENTER EXPERIENCE IN SERBIA

Biljana Živaljević[1], Aleksandra Trajković[2], Milica Petrović[2], Valentina Arsić Arsenijević[4]

[1]Obstretics and Gynecology Polyclinic Živaljević, Belgrade, Serbia; [3]CC Zemun, Belgrade, Serbia; [4]Medical Mycology Reference Laboratory, University of Belgrade Medical School, Insititute of Microbiology and Immunology, Belgrade, Serbia

Introduction

Vulvo-vaginal candidiasis (VVC) is common and complex disease which can cause physical and psychological distress. Pregnant women are especially vulnerable group, in which VVC occurs more often and may cause more significant consequences for newborns if not treated. The aim of this study was to determine prevalence and risk of VVC in pregnant women with laboratory proved VVC.

Material and methods

Descriptive case series study was done in period January-February 2016., on 52 pregnant women, aging from 24-47 years. Specimens collection and data analyzing were done at the Institute for Epidemiology and Institute for Microbiology, National Reference Laboratory for Medical Mycology.

Results

Average age of patients was 34,5 ± 6,9 years. Confirmed VVC was done based on positive *Candida* culture in 23% of tested patients. *Candida albicans* (75%), *C. glabrata* (16.6%) and *C. krusei* (8.4%) were isolated. In pregnant women with laboratory confirmed VVC dominating symptoms and signs were: itching (50%), enhanced discharge (100%), erythema (40%), oedema (60%) and "worry/concern" (50%) which stood out in comparison to patients without positive *Candida* culture ($p<0,01$; *Fisher* test).

Conclusion

Obtained results showed presence of *Candida* in approximately a quarter of patients. They had considerably higher presence of certain subjective and objective symptoms and signs of infection which can affect the quality of life. The most virulent species, *C. albicans, C. krusei* and *C. glabrata* were isolated, suggesting need for antifungal treatment in order to enhance the quality of life and to prevent colonisation and infection of newborns, as well.

P.09 Evaluation of a ultrasound assist methods for sampling fungi from the mucosal membranes

Perić M[1], Živković R[1], Milić Lemić A[1], Radunović M[2], Arsić Arsenijević V[3]

[1]Department of Prosthodontics, School of Dentistry University of Belgrade, Belgrade, Serbia; [2]Department of Microbiology, School of Dental Medicine, University of Belgrade, Belgrade, Serbia; [3]Institute of Microbiology and Immunology, Faculty of Medicine, University of Belgrade, Belgrade, Serbia

Background: *Candida* infection of mucosal membranes are common in newborns and the disease is characterized with inflammation and erythema of the mucosa. The role of different *Candida* species is not clear, manly because the sampling and testing procedure are limited. The aims of the study were to evaluate the ultrasound method (USM) that improves detection of the non-*albicans Candida* (NAC) and mixed *Candida* spp. and to exam the severity of inflammation caused with NAC and mixed *Candida* spp. in patient with denture.

Material/methods: The cross-sectional study was conducted at the Clinic for Dental Prosthetics, Belgrade, Serbia, and enrolled edentulous patients with denture (n=250). The patient's data were collected and patients with DS were selected and divided into study groups (SGs): SG *Candida*+ and SG *Candida*-. The SGs were classified in three

groups as high, medium and low grade inflammation. Two sampling methods were applied: mucosa swab and USM. For primoisolation and identification was used *Candida* chromogenic media. The sensitivity and specificity of USM were shown by using the ROC curve and the area under the curve (AUC).

Results: By using USM sampling method: (i) the highest number of *Candida* positive patients compared to other sampling methods (p=0.000); (ii) the highest number of *Candida* CFU/ml (10^5) compared to oral rinse method (p=0.000); and (iii) the increase ability to detect NAC and mixed *Candida* samples by applying USM and chromatogenic *Candida* media.

Conclusions: The new diagnostic plathformes performed with USM and chromatogenic *Candida* media are simple, accurate and sensitive and can be easily applied. This diagnostic platform allows the increasing detection of NAC and mixed *Candida* spp after UMS and sample cultivation on *Candida* chromogenic media. Futhermore, the obtained data present the association between sever form of infection and NAC or mixed *Candida* spp.

P. 10 CANDIDAEMIA AND ANTIFUNGAL SUSCEPTIBILITY TESTING IN NEONATES

S. Dacić, Lj. Pavlović, D. Ivanović,

Institute of Public Health of Serbia „Dr Milan Jovanović Batut", Belgrade, Serbia

e-mail: dacicslavica@gmail.com

Introduction: Invasive infection due to *Candida* species is mainly a condition associated with medical progress, and is widely recognized as a major cause of morbidity and mortality in the healthcare environment. Candidaemia has an attributable mortality of 10-15% for neonates. There are at least 15 distinct *Candida* species that cause human disease, but more than 90% of invasive disease is caused by the 5 most common pathogens, *C. albicans*, *C. glabrata*, *C. tropicalis*, *C. parapsilosis*, and *C. krusei*. *C. albicans* is the most frequent species, but considerable differences are found between the number of cases caused by non-*Candida albicans* species.

Objectives: The aim of this study was to establish distribution pattern and antifungal susceptibility profile of *Candida* species isolated from blood cultures of neonates, sent for mycological examination.

Methods: A retrospective review of neonatal patients reports of mycological blood culture results from the Institute of Public Health of Serbia laboratory information management system for the period from 2011-2015 was conducted. All yeasts isolated from blood cultures were identified using a commercial yeast identification kit. Antifungal susceptibility testing of the yeast iso-

lates to five different antifungal agents (amphotericin B, flucytosine, fluconazole, itraconazole, and voriconazole) was also performed using a commercial antifungal susceptibility test.

Results: Twenty-four *Candida* isolates (16,1%) were recovered from one hundred and forty-nine neonatal blood specimens with twenty-one isolates belonging to the *C. albicans* species, one to *C. glabrata* and two to *C. dubliniensis*. Out of twenty-four *C. albicans* neonatal isolates one was resistant to fluconazole and itraconazole, both *C. dubliniensis* isolates were susceptible to all antifungal agents tested, while *C. glabrata* neonatal isolate showed intermediate susceptibility to itraconazole and was susceptible to other four antifungal agents.

Conclusion: *C. albicans* was the dominant species in neonatal blood samples sent for mycological examination and generally remains susceptible to antifungal agents, but on the other hand, there is a concern about the appearance of one isolate resistant to fluconazole and itraconazole. Local data on the susceptibility of invasive *Candida* species to antifungal drugs can assist the clinician in deciding which antifungal drug to apply before receiving the antimycogram results.

P.11 Modeling and prevention of fungal biofilms formation

Katarina Rajković[1,2], Valentina Arsić Arsenijević[1], Nebojša Milošević[3], Suzana Otašević[4]

[1]Faculty of Medicine University of Belgrade, Institute of Microbiology and Immunology Serbia; [2]High Technical and Technological School for Professional Studies, Kruševac, Serbia; [3]Department of Biophysics, Faculty of Medicine, University of Belgrade, Serbia; [4]Institute of Microbiology and Immunology, Faculty of Medicine University of Niš, Serbia; Public Health Institute-Niš, Niš, Serbia

Objectives: *Candida* species are the most common etiologic agent of fungal-related biofilm infection. The aim of this study was to the image analysis complemented with kinetic model of growth have been used to 'describe' fungal growth for further studies of mold biofilm dynamics.

Methods: The clinically important *C. albicans* was obtained from the collection of the National Reference Medical Mycology Laboratory, Institute of Microbiology and Immunology, Faculty of Medicine, University of Belgrade (Belgrade, Serbia). The spore suspensions were placed on tree agar plates, covered with microscopic slide and incubated at 37°C. During these time periods germination of the fungi spores occurred forming hyphae on the bottom side of the microscopic slide. Each microscopic slide removed at different times of incubation (19, 20, 21, 22, 23 and 24h)

and stained with methylene blue for 30 minutes, then set to be analyzed. A gray scale digital images were obtained with a microscope (Leica) magnification of 40X and photographed with a 5mp Camera for analysis.

Results: The growth *C. albicans* was estimated by measuring the area of hephae on image (*A*). Logistic kinetic model was used to describe the increase of *A* during the growth of fungus. Results suggested that the logistic kinetic model was valid for the prediction of the *A* of the image fungi during the time of growth. Parameters *A* used describe the size of the fungi, while logistic function has been used for calculation of fungal growth rate.

Conclusion: A novel, cost-effective and simple method has been used in further studies for quantification of the size and prediction of the biofilm dynamics. Prediction of the biofilm dynamics can provide useful insight for local eradication of formed biofilms on different structures, such as human skin/mucosa and medical device surfaces. This can reduce the risk for entering of fungi in the patient bloodstream and prevent of severe fungal infections.