V.P.1

Genetic and phenotypic analysis of drug-resistance of uropathogenic *Escherichia coli* strains

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Urinary tract infections (UTIs) are one of the most common human infections. Approximately one in two women and one in twenty men will suffer from the UTI in their lifetime. *Escherichia coli* is the primary causative agent of uncomplicated and complicated UTIs. Therapeutic difficulties result in large part from the quick spread of multidrug resistance (MDR) among pathogenic bacteria. This problem concerns the large group of β-lactam antibiotics, but also other compounds, such as fluoroquinolones, aminoglycosides, etc., which are often used for the UTI treatment. Monitoring of drug resistance among microorganisms and rational use of antibiotics may help reducing the prevalence of MDR strains.

We have examined antimicrobial resistance of 127 UTI *E. coli* isolates recovered from patients in a Polish hospital. The isolates were tested against 16 antimicrobials, including β-lactams (penicillins, cephalosporins and carbapenems), aminoglycosides, fluorouinolones, and sulfonamides, by the disk-diffusion method according to EUCAST. The ESBL strains were detected by the DDST test. Resistance patterns were correlated with the presence of 16 genes encoding resistance to the compounds ($bla_{\text{CTX-M-1}}$-, bla_{SHV}-, bla_{TEM}-, bla_{CMY}-, $bla_{\text{OXA-1}}$-like genes, and *gyrA*, *parC*, *qnrA*, *qnrB*, *qnrS*, *aadB*, *aac(3)-II*, *aac(3)-IV*, *sul1*, *sul2*, and *sul3*). The genes were analyzed by PCR and sequencing.

It was shown that the DDST test results revealed 7 strains (9%) with ESBL. Genetic analysis revealed that $bla_{\text{TEM-1}}$, coding for the broad-spectrum β-lactamase TEM-1, was the most common gene. It was detected in 116 (91%) of the isolates, *sul2* was detected with the similar high frequency as 73%. Other genes occurred less frequently among the tested strains. Resistance in several strains could not be correlated with any of the genes tested in this study. In several cases, the presence of a resistance gene (for example $bla_{\text{CTX-M-1}}$, bla_{SHV}, $bla_{\text{TEM-1}}$, *sul1*, *sul2*) did not confer resistance according to the EUCAST breakpoints. This is a preliminary report of a study aimed at identification of resistance phenotypes and mechanisms in a group of uropathogenic *E. coli* clinical isolates. It shows highly differentiated distribution of individual resistance genes and their complex contribution to resistance phenotypes.

Key words: antibiotics, multidrug resistance, uropathogenic *Escherichia coli*, PCR

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V.P.2

Analysis of uropathogenic *Escherichia coli* forming biofilm upon different environment conditions

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The last years studies revealed bacterial ability to form different types of biofilm. This properties lets bacteria for better surviving in an inefficient or toxic environment. The biofilms which forms mushrooms-like structures (which are hydrated and not very strong attached to the surface), flat layers (firmly adhered to the surface), multi-species or intracellular microcolonies are described in the literature. Different structures of biofilms are related with different surfaces and environment for bacterial living.

The aim of this study was the analysis of the biofilm formation of 127 uropathogenic *Escherichia coli* strains under different environmental conditions. The different parameters of growth have included surface (glass or polyurethane), medium (enriched or minimal) and time of incubation (24–72 h). The biofilm formation after 24 h, 48 h and 72 h was determined spectrophotometrically at 550 nm after crystal violet staining and it was correlated with bacterial growth (600 nm). Biofilm formation was also observed on the glass by the live/dead staining (BacLight™, Invitrogen) with epi-fluorescence microscope. The statistical significance was estimated by paired T-test, nonparametric. The observed biofilms were different for the particular strains. They were usually weaker than typical bacterial biofilm of *Pseudomonas* sp. or *Proteus* sp. (data not shown). The biofilm formation was the highest in the rich medium (LB) after 24 h and its level haven't changed in the following time. The relative biofilm formation was higher in minimal medium (artificial urine) in comparison to enriched medium (LB). This results suggest that most bacterial cells prefer to live in biofilm community in the difficult environmental conditions, not as planktonic cells. Moreover, biofilm formation on polyurethane surface did not correlate with biofilm formation on the glass. It suggests that biofilm formation can be correlated with bacterial properties as virulence factors or hydrophobicity. This phenomenon can explain different types of biofilm formation among analyzed collection of *E. coli*.

Key words: biofilm, uropathogenic *Escherichia coli*, growth conditions

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V.P.3

Phosphate starvation enhances the pathogenesis of *Bacillus anthracis*

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Identifying the factors responsible for survival and virulence of *Bacillus anthracis* within the host is prerequisite for the development of therapeutics against anthrax. Host provides several stresses as well as many advantages to the invading pathogen. Inorganic phosphate (P_i) starvation within the host has been considered as one of the major contributing factors in the establishment of infection. Here, we report for the first time that P_i fluctuation encountered by *B. anthracis* at the different stages of its life cycle within the host, contributes significantly in its pathogenesis. In this study, P_i starvation was found to hasten the onset of infection cycle by promoting spore germination. After germination, it was found to restrict growth but favored cell elongation which might be one of the many reasons for the antibiotic tolerance of the pathogen. Interestingly, phosphate starvation enhanced the pathogenicity of *B. anthracis* by augmenting its invasiveness in macrophages *in vitro*. *B. anthracis* grown under phosphate starvation were also found to be more efficient in establishing lethal infections in mouse model as well. Phosphate starvation increased *B. anthracis* virulence by promoting the secretion of primary virulence factors like protective antigen (PA), lethal factor (LF) and edema factor (EF). Thus, this study affirms that besides other host mediated factors, phosphate limitation may also contribute *B. anthracis* for successfully establishing itself within the host. This study is a step forward in delineating its pathophysiology that might help in understanding the pathogenesis of anthrax.

V.P.4

The determination of antibiotic resistance of *Helicobacter pylori* isolated from patients referred to Imam Khomeini Hospital of Sari in 2014

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Introduction: *Helicobacter pylori*, which infects about half the world's population, is assumed as an important risk factor in chronic gastritis, peptic ulcer and gastric cancer. Resistance to antibiotics is the main reason of failure of *H. pylori* treatment. Since the antibiotic resistance patterns of *H. pylori* is varied geographically; therefore, the purpose of this study was to investigate the prevalence and resistance of *H. pylori* to 6 commonly used antibiotic to treat this infection.

Material and methods: The cross-sectional study was conducted on 120 patients with upper GI symptoms which they had referred to endoscopy department of Imam Khomeini Hospital during November 2013 to June 2014. Demographic characteristics of patients were recorded prior to sampling and the situations of resistance to 6 commonly used antibiotics in treating of *H. pylori* infection were studied. The obtained data were recorded in Spss 16 software and descriptive statistics (frequency and mean) were applied to analyze data. Also, the chi-square test was used to determine significance.

Results: A number of 30 isolates were obtained from the culture of gastric biopsy specimens of 120 patients. The resistance level to metronidazole, clarithromycin, tetracycline, amoxicillin and levofloxacin was 63.3%, 16.6%, 6.6%, 10% and 3.3%, respectively. There was not observed any resistance to furazolidone.

Conclusion: According to antibiotic resistance level of this study. It seem that the antibiotic susceptibility tests are always necessary in order to determine the appropriate drug regimen against *Helicobacter pylori*.

Keywords: *Helicobacter pylori*, Antibiotic resistance, disk diffusion

V.P.5

Evaluation of urease activity by measuring the concentration of ammonia generated by two ureaplasmas species

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The aim of this study was to evaluate urease activity of two ureaplasma species: *U. parvum* (Up) and *U. urealyticum* (Uu). Ureaplasmas have been associated with infections of human urogenital tract and with lung disease of neonates. Urease is one of the virulence factors of these bacteria. The urease pathogenic effect is caused by generation of ammonia as a product of hydrolysis of urea.
25 Up and 25 Uu clinical strains studied were originally isolated from tracheal aspirates of preterm infants. The presence of ureaplasmas was confirmed in PPLO broth culture and by PCR with primers specific for Up and Uu.
The pathogenic effect of clinical strains of Up and Uu was studied in two types of cell culture systems (A549-human lung carcinoma epithelial cells and SiHa – human cervical carcinoma epithelial cells) and also in PPLO broth as a control. Urease activity was assessed by evaluating the concentration of ammonia which is based on pH media measurement. Calculations were performed assuming that ammonium hydroxide is completely dissociated, which results from Ostwald's dilution law. The pH measurement was done after 18 hours post inoculation. The average concentrations for Uu and Up was 2651.6 pg/l per 100 CCU and 1692.8 pg/l per 100 CCU, respectively. Uu species generated a higher concentration of ammonia than Up. This effect was statistically significant in comparison with Up ($p=0.02$ U-Mann-Whitney test).
Ammonia concentration in A549 cells inoculated with Uu was significantly higher than in SiHa cells and PPLO medium ($p=0.03$).
The high activity of urease of *U. urealyticum* observed in human lung cells, can explain the damage observed in preterm infants infected vertically by Uu.

Key words: activity of urease, pathogenicity of *U. urealyticum* and *U. parvum*

V.P.6

Neoglycoconjugates of *E. coli* R1 type oligosaccharides with bacterial toxoids

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The lipooligosaccharide (LOS) from *Escherichia coli* rough strain was isolated. The R1 type strain produces two LOS structures, which differ by the number of monosaccharides building core oligosaccharide (OS). The predominant nonasaccharide consists of Kdo and heptoses in inner core and glucoses and galactoses in outer core. Less abundant decasaccharide has an additional inner core glucosamine. Simultaneous presence of both negative and positive charges is a particular feature of this zwitterionic OS. Phosphates, pyrophosphates and ethanolamines substitution renders the R1 glycans even more heterogenic. The nonasaccharides and decasaccharides were isolated from OS mixture with the use of size exclusion chromatography and hydrophilic interaction chromatography. Isolated glycans were further characterized with the use of NMR spectroscopy, oxidized and coupled to carrier proteins. The carriers used were toxoids of well know bacterial toxins: tetanus toxoid and C-terminal part of *Clostridium difficile* toxin B. Clostridial toxoid was recombinant protein produced and isolated from *E. coli*. In order to examine immunogenicity, rabbits were immunized with glycoconjugates. Reactivity of post immunization sera with gram-negative bacteria endotoxins was investigated. Sera react with LOS from *Bordetella pertussis* type 186, *Shigella sonnei* phase II, *E. coli* type R1 and R3 and lipopolysaccharides (LPS) from *Klebsiella pneumoniae* type O1, O4, O5, *Shigella sonnei*, and *E. coli* O6, O18 and O39.

Key words: glycoconjugates, oligosaccharides, LC-MS, HILIC, TcdB, *Clostridium difficile* toxin B, bacterial antigens

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V.P.7

Identification of *Mycobacterium tuberculosis* proteins binding to human IL-8

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Interleukin-8 (IL-8) belongs to the family of C-X-C chemokines and has been implicated in the pathogenesis of several human respiratory diseases, including tuberculosis (TB). We have shown for the first time that tubercle bacilli have the ability to directly bind to human IL-8. The main goal of our studies was to identify the putative proteins of *Mycobacterium tuberculosis* (*M. tb)* that bind to IL-8 and to characterize their role in the modulation of inflammatory response initiated in neutrophils upon infection with *M. tb*. To identify and describe the IL-8 binding-mycobacterial molecules we employed the affinity chromatography technique followed by the liquid chromatography–mass spectrometry (LC-MS) analysis. Based on LC-MS analysis we selected three mycobacterial proteins, that interacted with human IL-8, and identified them as: AslA (probable sulfatase), SahH (S-adenosyl-L-homocysteine hydrolase), GlmU (acetylglucosamine pyrophosphorylase). Further, we cloned mycobacterial genes: *sahH, glmU* and *aslA* into expression vector pHis and purified recombinant proteins: rAslA, rSahH and rGlmU using affinity chromatography. The quantitative analysis of molecular interaction between mycobacterial rSahH, rGlmU and rAslA proteins and IL-8 were estimated by surface plasmon resonance (SPR). Our observation showed that the highest binding affinity was noted for interaction of AslA with IL-8 (ka=1.8×10^3 [1/Ms]). Binding affinity for SahA/IL-8 and GlmU/IL-8 interactions were also significantly strong (ka=769 [1/Ms], and ka=303 [1/Ms], respectively). All experimental data analysis was done using Biacore AB BIA evaluation 3.2 software. Furthermore, we constructed *M. tb* mutants overproducing AslA, SahH and GlmU proteins and *M. tb* mutant strain lacking an intact form of *aslA*. All *M. tb* mutants were employed for studying the role of identified mycobacterial proteins in modulation of inflammatory response initiated in human neutrophils infected with *M. tb*. Performed experiments revealed that overproduction of IL-8 binding proteins in the tubercle bacilli resulted in the attenuation of pathogen mutant strains, in the presence of IL-8, in human neutrophils.

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V.P.8

Molecular characterization of clinical strains of *Staphylococcus aureus* – the host of bacteriophages

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Introduction: Bacteriophages (phages) therapy may offer an innovative means of staphylococcal infections treatment, which can be combined or alternated with antibiotic therapy and may enhance our abilities to treat bacterial infections successfully.
Objectives: The aim of this study was the molecular characterization and drug resistance of clinical isolates of *S. aureus* – the host of bacteriophages.
Method: 26 clinical isolates of *S. aureus* – the host of bacteriophages, from Biomed S.A.'s own collection within the ONKOFAG project, were tested. The distribution of 16 toxin genes were detected by PCR and multiplex PCR method. The β-lactam, macrolide and aminoglycoside resistance was investigated according to EUCAST v.4.0, 2014. The presence of the resistance genes such as: *mec*A, *erm*A, *erm*B, *erm*C, *aac*(6')/*aph*(2"), *aph*(3')-IIIa and *ant*(4')-Ia were performed. Isolates were genotyped by PFGE method.
Results: In this study, 11 (42%) isolates turn to be MRSA. Fifteen isolates (57%) resistant to erythromycin were found. $cMLS_B$ phenotype was predominant (46%; n=12), then $iMLS_B$ (11%; n=3). Only two of all isolates (8%) showed the presence of *aac*(6')/*aph*(2"), *aph*(3')-IIIa and *ant*(4')-Ia genes. The most prevalence virulence factors were *luk*E (85%; n=22) and *sei* (58%; n=15). The gene for PVL was identified in four (15%) strains. The *tst* gene was detected in three (11%), *sea* in one (4%), *seb* in four (15%), *seh* in two (8%), *sej* in four (15%), *sed* in eight (31%), and *seg* in nine (35%) isolates. The *eta*, *edin*A, *edin*B, *sec* and *see* genes were not found. Strains clustering into the largest PFGE clonal group, mainly belonging to MRSA.
Conclusion: The studied strains did not possess high level of virulence genes. Six resistance profiles and fifteen PFGE pattern were identified among 26 *S. aureus* strains. The correlation between MRSA phenotype and PFGE pulsotype was performed.

Key words: bacteriophages, *Staphylococcus aureus*, molecular characterization

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V.P.9

Bacterial tick-borne diseases as a cause of heart damage

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Objectives: Heart disease could be caused by many uncultured (blood-culture negative) bacteria. The aim of presented studies was to establish if any tick-borne pathogens such us: *Borrelia burgdorferi, Coxiella burnetii, Bartonella* spp. and *Rickettsia* spp. can contribute to serious heart disorders which lead to heart damage.
Material and methods: Samples of myocardium, aortic and tricuspid valves of thirty hearts removed from patients undergoing heart transplantation and paired valve/serum samples from 49 patients (taken during cardiac surgery) were tested.
DNA was extracted with the QIAamp Tissue kit (QIAGEN Gmbh, Hilden, Germany) according to the manufacturer's recommendations. Extracted DNA from above mentioned tissues were examined by PCR with primers targeting following genes: OspA and 16S rRNA for *Borrelia burgdorferi* sensu lato, *htpAB* fragment for *Coxiella burnetii* and citrate synthase (*gltA*) for *Bartonella* spp. and *Rickettsia* spp. Specificity of all positive results will be confirmed by sequencing the amplicons with the ABI 377 DNA Analyzer (Applied Biosystem, USA) according to the manufacturer's recommendations. Serum samples were tested with appropriate diagnostic kits.
Results: In 49 patients undergoing cardiac surgery, IgM and/or IgG antibodies to *B. burgdorferi* sl. were detected in 7 (14%) individuals. Specific IgG antibodies to *Bartonella* spp. were detected in 2 patients and IgG antibodies to *Rickettsia* sp. in 5 patients.
Conclusions: Detected pathogens occurring in ticks in natural environment are of clinical importance in cardiology. Obtained results indicate that testing of infections caused by *B. burgdorferi*, Rickettsia sp., *C. burnetii* and *Bartonella* spp. should be standard procedure in patients with cardiac diseases.

Key words: tick-borne diseases, heart

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V.P.10

Development of a real-time PCR method for identification of methanogenic Archaea in cloacal contents of chicken with the use of *mcrA* gene

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Intestinal fermentation is a beneficial process of otherwise indigestible compounds that break down, during which a fair amount of hydrogen is produced. Hydrogen slows down the fermentation; this has an adverse effect on the host. Production of methane is one of the ways to utilize hydrogen. It is done by various types of microbes colonizing in the intestines of chicken, including sulfate-reducing bacteria (mostly *Desulfovibrio*), bacteria of Ruminococcaceae and Lachnospiraceae family, *Wollinella*, *Helicobacter*, *Campylobacter*, *Megamonas*, and methanogenic Archaea.
Our goal was to detect methanogenic Archaea in cloacal contents of chicken, and to quantify this group of microorganisms. In order to do this, we used a *mcrA* gene, which encodes an α-subunit of methyl coenzyme M reductase, an enzyme that is involved in the last step of methanogenesis in methane-producing Archaea, and is specific to just this group of microbes. We developed a real-time PCR method with the use of methane-producing-archaea-specific primer pair mcrA_F (5'-GGT GGT GTM GGD TTC ACM CAR TA-3') and mcrA_R (5'-CGT TCA TBG CGT AGT TVG GRT AGT-3'), with the product of approx. 470 bp in length. The specificity of the primer pair was tested against other gut microbionts, including members of the Firmicutes, Bacteroidetes and Proteobacteria types, and the test came back 'positive'. To the best of our knowledge, this is the first report describing the use of these particular primers in chicken intestinal microbiota studies.

Key words: Archaea, chicken intestinal microbiota, hydrogen sink

V.P.11

Anti-biofilm activity of high-valent ruthenium complexes against *Pseudomonas aeruginosa* strains

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Anti-biofilm activity of high-valent ruthenium complexes are poorly investigated. In this study we analyze benzimidazole complexes of ruthenium in +IV and +VI oxidation states. Obtained results suggest that both complexes can inhibit biofilm of *Pseudomonas aeruginosa* PAO1 and LES B58 strains. A significant reduction of biofilm amount was observed between 31.8% and 58.3%. Moreover, ruthenium complexes act selectively in case of PAO1 biofilm. *Pseudomonas* spp. are producers of extracellular pigments and in this studies interactions with pyocyanin and pyoverdine extracted from LES B58 culture were observed. Spectroscopic studies confirm change in oxidation state of pyocyanin after treatment with 1 mM concentration of ruthenium complexes. To explain mechanism of biofilm inhibition interaction with chromosomal DNA isolated from PAO1 was studied. Electronic absorption spectroscopy obtained spectra proved that ruthenium complexes bind to dsDNA. The investigation show clearly that addition of ruthenium complexes to DNA solution leads to hyperchromism accompanied by the slight bathochromism. In turn, tests with pDsRed2 (3.3 kb) plasmid DNA revealed conformational transitions in 250 μM concentration of ruthenium complexes and DNA cleavage in concentration equal and higher than 500 μM for Ru(IV) and Ru(VI) complexes, respectively. DNA binding tests were performed in water environment at 37°C. Interestingly, micromolar concentrations of ruthenium complexes alter specificity of PCR reactions, when interaction with DNA as well as polymerase occurred. Target of ruthenium complexes action seems to be interaction with peptides thus additional studies for quenching of BSA fluorescence were also demonstrated. As a control for all experiments solutions of $RuCl_3$ (source of Ru ions and ingredient of mother solution), 2-hydroxymethylbenzimidazole and deionised water were used.

Key words: Ruthenium complex, biofilm, *Pseudomonas aeruginosa*, plasmid pDsRed2, DNA binding, UV-Vis spectroscopy

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V.P.12

The balance between proinflammatory and anti-inflammatory cytokines in the immune responses to BCG and DTwP vaccines

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Tuberculosis Bacillus Calmette-Guérin (BCG) and *pertussis* vaccines used on a large scale have been found insufficient and further improvements will be required. In order to develop improved vaccines, a better understanding of the main pathways involved in the protective immunity to the pathogens is crucial. We address the question as to whether the balance between proinflammatory and antiinflammatory cytokine production might affect host responses to BCG and diphtheria-tetanus toxoids – whole cell *pertussis* (DTwP) vaccines. The study population consisted of 157 healthy people, age range 18–27 years, who had been subjected to BCG and DTwP vaccination according to the state policy. Tuberculin skin testing (TST) revealed a delayed type hypersensitivity to PPD (purified protein derivative) in 56% volunteers. PPD-stimulated blood lymphocytes from TST(+) volunteers produced significantly more IFN-γ and less IL-10 than lymphocytes from TST(-) participants. The TST reaction sizes were correlated positively with IFN-γ and negatively with IL-10 production. Inter-individual variations in the phenotype of cells producing IFN-γ in co-cultures of PPD-pulsed dendritic cells and lymphocytes were observed. In some co-cultures CD4(+), CD8(+) and NK cells produced IFN-γ. In 3 out of 12 co-cultures IFN-γ was produced almost exclusively by CD8(+) or NK cells. The levels of anti-*pertussis* toxin IgG were significantly higher in the sera of TST(-) compared to TST(+) volunteers. The data support the concept that a wide-range of inter-individual variation in response to vaccination is one of the reasons for BCG and *pertussis* vaccines insufficiency.

Key words: BCG, *pertussis*, IFN-γ, IL-10, tuberculin skin test, IgG

V.P.13

Proteomic analysis of outer membrane proteins of *Salmonella* Enteritidis strains with different sensitivity to serum by MALDI TOF/TOF spectrometry

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Salmonella Enteritidis are Gram-negative bacteria that invade a broad range of hosts causing both acute and chronic infections. *Salmonella* Enteritidis is the most frequently isolated serovar in Poland, accounting for above 70% of the total cases of human salmonellosis in the last few years. In 2014 year 95 cases of sepsis were caused by *Salmonella* strains in Poland. Children, the elderly and immunocompromised individuals are the groups most likely to present severe forms of the disease.

The sensitivity of Gram-negative bacteria to the bactericidal activity of human serum (HS) depends on the structure and organisation of the bacterial outer membrane. Dynamic changes in the expression of outer membrane proteins (OMPs) play a decisive role in generating the resistance of bacteria to human serum. The objective of this study was to find the correlation between the expression of specific OMPs of *S*. Enteritidis strains resistant and sensitive to HS.

Clinical *S*. Enteritidis strains were isolated from DiaLab Laboratory, Wroclaw. The bactericidal activity of serum was determined using 75% HS taken from healthy volunteers (Regional Center of Blood Donation and Treatment, Wroclaw). OMPs were isolated using Zwittergent Z 3-14® and resolved by two-dimensional gel electrophoresis (2-DE). The 2-DE gels were Coomassie Brillant Blue stained and the protein spots were analyzed by PDQuest software. For protein identification matched spots were extracted from the gel and subjected to in-gel tryptic digest MALDI-TOF tandem mass spectrometry analysis.

Bactericidal activity assay against *S*. Enteritidis strains showed different levels of sensitivity to 75% HS. The analysis of OMPs of resistant and sensitive to HS strains showed differences between the optical density and OMPs presence. One of the identified proteins in the *S*. Enteritidis strain resistant to HS was protein PgtE, previously described in the literature as one of the factors generating the resistance of bacteria to serum.

Key words: *Salmonella* Enteritidis, Serum resistance, Outer membrane proteins

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V.P.14

Immune response gene polymorphisms in tuberculosis

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Tuberculosis (TB), an infectious disease caused by *Mycobacterium tuberculosis* (*M. tb*), remains a leading public health problem in most parts of the world. Despite the discovery of the bacilli over 100 years ago, there are still many unanswered questions about the host resistance to TB. Although one third of the world's population is infected with virulent *M. tb*, about 10% develop active disease within their lifetime. A lot of studies suggest that host genetic factors determine the outcome of *M. tb*-host interactions, however, specific genes and polymorphisms that govern the development of TB are not completely understood. Strong evidence exists for genes encoding pattern recognition receptors (TLR, CD14), cytokines and their receptors (IFN-γ, TNF-α, IL-12, IL-10), major histocompatibility complex (MHC) molecules, vitamin D receptor (VDR), proton-coupled divalent metal ion transporters (SLC11A1), and soluble C-type lectins (MBL). Polymorphisms in these genes have a diverse influence on the susceptibility to or protection against TB among particular families, ethnicities and races. In our paper, we review recent discoveries in human genetic studies and correlate these findings with their effects on macrophage function and influence on TB susceptibility.

Key words: tuberculosis, susceptibility/resistance genes

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V.P.15

Exponential phase lipopolysaccharide of *Salmonella enterica* subsp. *enterica* serovar Enteritidis activates both classical and lectin pathways of the serum complement

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Salmonella infections are still the cause of dangerous food-borne infections especially in developing countries. The most serious outcome of the presence of vital *Salmonella* rods in the human body is sepsis followed by the bacteremia. The molecular mechanisms of sepsis depending on the bacterial pathogens cell structures are not fully understood. Isolates of gram-negative bacteria bear on O-antigen (O-Ag) of lipopolysaccharide (LPS) vary in serum sensitivity. C3 protein activation supports the course of the three pathways of the complement (C) cascade: the alternative pathway (AP), the classical pathway (CP), and the lectin pathway (LP) leading to the destruction of microorganisms and limitation of the infection.

This paper deals with the *Salmonella enterica* subsp. *enterica* serovar Enteritidis (*S*. Enteritidis) strain isolated in 2011 from human fecal sample. We found that: I) the alternative pathway (AP) of C is not effective at killing *S*. Enteritidis strain and therefore the pathogen is resistant to serum killing; II) C3 protein can bind to *S*. Enteritidis when is both in exponential and stationary phase but activation of C3 is influenced by growth phase, with greater C3 molecule breakdown reported against LPS extracted from exponential phase relative to stationary phase; III) LPS isolated from *S*. Enteritidis activates C through induction of the classical/lectin pathway as opposed to the AP. We propose, based on the data, that *S*. Enteritidis susceptibility to serum is the proportion between activation of C proteins outside of the cell and the surface antigen-dependent deposition of C components participating in the cell lysis.

Key words: *Salmonella*, lipopolysaccharide, C3 fragments activation/deposition, serum susceptibility, growth phase

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V.P.16

Helicobacter pylori antigens, acetylsalicylic acid, 7-ketocholesterol – potential role in the destabilization of gastric epithelial cell barrier. Model *in vitro* of Kato III cells

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Helicobacter pylori (Hp) bacteria have a high affinity to gastric epithelium. Chronic Hp colonization may initiate gastric and duodenal ulcers and even gastric cancers. Hp infections promote excessive inflammatory response and damage of gastric epithelial cells. This may be followed by the impairment of their barrier function. The role of individual Hp components in this processes is not specified. Hp cytotoxicity may increase in the milieu of anti-inflammatory drugs such as acetylsalicylic acid (ASA) but also in the presence of diet lipids, including low density lipoprotein (LDL). LDL is a classic risk factor for coronary heart disease and its concentration increases during Hp infection. The aim of this study was to evaluate the cytotoxic effects of Hp antigens as well as ASA and 7-ketocholesterol (7-KCh) towards Kato III cells. The ability on the cells to reduce nitrotetrazolium salt – MTT and morphology of cell nuclei assessed by 4',6-diamidyno-2-fenyloidol (DAPI) staining were selected as cytotoxicity markers. Kato III cells were stimulated for 24h, 37°C, 5% CO_2, with Hp antigens: glycine acid extract (GE), cytotoxin associated gene A (CagA) protein (IRIS, Siena, Italy) and LPS (courtesy of prof. AP Moran) as well as with ASA and 7-kCh (Sigma). The cytotoxic effects against Kato III cells were demonstrated in the cell cultures containing Hp LPS but not GE or CagA. ASA and 7-KCH also showed cytotoxic properties. The cytotoxicity of stimulators used in this study was dose dependent and related to diminished percentage of MTT reducing cells and to increased percentage of cells with DNA damage. This study suggests that *in vivo* damage of gastric epithelial cells during Hp infection may result in impairment of gastrointestinal barrier function and development of chronic inflammatory responses, potentially pathological. These deleterious effects can be enhanced in the milieu of ASA and diet lipids.

Key words: *H. pylori*, acetylsalicylic acid, LDL, gastro-epithelial barrier

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V.P.17

The effect of nickel(II) chloride on biofilm formation by uropathogenic *Escherichia coli* strains

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Escherichia coli is known as causative agent of urinary tract infections. Uropathogenic *E. coli* (UPEC) strains are able to form microcolonies in mucosa lining of urinary bladder known as biofilm. The biofilm formation might be modulate by environmental pollutants like heavy metals. Nickel has many industrial applications and progress in industrialization might led to increase emission of this metal into ecosystems.

The aim of this study was to evaluate the effect of nickel ions on biofilm formation by 12 UPEC strains. The biofilm formation in TSB growth medium was determined spectrophotometrically at 531 nm after crystal violet staining in correlation with bacteria growth (600 nm). The statistical significance was estimated by Wilcoxon matched-pairs signed rank test of paired T-test., nonparametric.

We observed that nickel ions in growth medium are able to reduce the biofilm formation in 36% of tested UPEC strains. This effect is not correlated with inhibition of bacteria growth.

Taken together, our data demonstrate that nickel ions might inhibit the production of exopolysaccharide matrix by uropathogenic *E. coli*.

Key words: UPEC, nickel, biofilm

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V.P.18

The effects of nickel(II) complexes with imidazole derivatives on pyocyanin and pyoverdine production by *Pseudomonas aeruginosa* strains isolated from cystic fibrosis

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Pseudomonas aeruginosa infection is problematic in patients with cystic fibrosis (CF). *P. aeruginosa* secretes a diversity of pigments, such as pyocyanin and pyoverdine. Pyocyanin generates reactive oxygen species damaging the cilia of respiratory epithelium, stimuli IL-8 secretion by epithelial cells and inhibits T-cell proliferation. Pyoveridine is a siderophore, found to be important for bacterial virulence and biofilm development. The modulatory effect of environmental pollutants like heavy metals (for example nickel complexes) on pyocyanin and pyoverdine production might be important from clinical point of view.

The aim of this study was to evaluate the effects of complexes of nickel(II) on pyocyanin and pyoverdine production by 23 strains of *P. aeruginosa* isolated from CF. The mononuclear nickel(II) coordination compounds, having the formula $[Ni(iaa)_2(H_2O)_2]\cdot H_2O$ (iaa = imidazole-4-acetate anion) and $[Ni(1\text{-allim})_6](NO_3)_2$ (1-allim = 1-allylimidazole), were tested at concentrations in range 15–1000 μM. The absorbance of pyocyanin *was determined spectrophotometrically* at 691 nm in TSB growth medium. The fluorescence of pyoverdine was determined (λ_{ex}=398 nm; λ_{em}=460 nm) in growth medium. Moreover, pyocyanin is accumulating in biofilm matrix during its formation. This effect was used to determined the thickness of formed biofilm by confocal microscopy in the presence of tested metal complexes. The kinetic of bacterial growth of all tested strains was positively correlated with pyocyanin production in contrast to diversified secretion of pyoverdine after 24 h. The nickel(II) complexes had no effect on pyocyanin production by all tested bacterial strains. All tested complexes induced production of pyoverdine by 30% of tested *P. aeruginosa* strains. The ligand 1-allylimidazole alone and in $[Ni(1\text{-allim})_6](NO_3)_2$ complex inhibited production of pyoverdine by 45% of bacterial strains. We concluded that naturally occurs nickel complexes had modulatory effect on bacterial virulence and biofilm development by *P. aeruginosa*. This observation might have clinical implications in *P. aeruginosa* eradication in cystic fibrosis.

Key words: *Pseudomonas* sp., cystic fibrosis, nickel, pyocyanin

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V.P.19

A possible involvement of secreted aspartic proteases of *Candida albicans* in the propagation of inflammation during candidiasis

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Candida albicans is a natural commensal component of human microbiota. However, disturbances of the microbial balance or weakening the immune system of the host can disclose a pathogenic nature of this yeast-like fungus. A propagation of candidal infection depends on a variety of virulence factors. One of the major group of candidal pathogenicity factors is a family of secreted aspartic proteases (SAPs) that can degrade the main host proteins involved in the resistance of the host against pathogens. However, SAPs are also capable of inducing an inflammatory response through mechanisms that are independent of SAP proteolytic activity, e.g., by stimulating the expression of pro-inflammatory cytokines.
Interleukin-8 (IL-8) is a major neutrophil chemotactic factor that also induces the formation of neutrophil extracellular traps (NETs), composed of DNA and microbicidal proteins such as neutrophil elastase (NE). The IL-8 activity is regulated by binding to α-1 proteinase inhibitor (A1PI) which possesses an inhibitory activity against NE. In our current study we found that despite IL-8 resistance to all SAPs tested the availability of IL-8 at the site of infection can change during SAP action on A1PI, the IL-8-binding partner. A1PI was degraded by both the concentrated supernatants from *C. albicans* cultures and the purified SAPs, to form a stable product, shorter than the parent molecule by 51 C-terminal amino acids. This degradation product possessed a lower (by about 50%) ability to bind IL-8, and thus influenced the neutrophil chemotaxis process as well as the NET formation. Furthermore, the cleaved A1PI was less efficient in terms of NE inhibition, a feature that can contribute to uncontrolled degradation of host proteins. Our results indicate that the fungal proteases can contribute to the uncontrolled progression of inflammation.

Key words: *Candida albicans*, secreted aspartic proteases, α-1 proteinase inhibitor, interleukin-8

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V.P.20

Immunogenic form of Enterobacterial Common Antigen (ECA) as a component of universal antienterobacterial vaccine

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Bacterial infections are one of most common reason of medical interventions and are directly linked with serious cases like nosocomial infections and sepsis. Increased drugs resistance of some bacteria like carbapenem-resistance *Enterobacteriaceae* (CRE), stresses the need for new, cross-protective vaccines based on common surface antigens. In case of Gram-negative bacteria we can point a few structural antigens. Capsular polysaccharide (CPS) and lipopolysaccharide (LPS) are major surface, saccharide-based antigens, but unfortunately their structure strongly diverse between species and even strains. Enterobacterial Common Antigen (ECA) occurs on cell surface of all Gram-negative bacteria belonging to *Enterobacteriace* family, such as *E. coli*, *Shigella* spp., and *K. pneumoniae*. ECA is a polysaccharide build of trisaccharide repeating unit [→3)-α-d-Fuc4NAc*p*-(1→4)-β-d-ManNAcA*p*-(1→4)-α-d-GlcNAc*p*-(1→] and occurs in three forms: phosphatydyl-linked ECA_{PG}, free cyclic ECA_{CYC} and hypothetic LPS-linked ECA_{LPS}.
In our work, using ESI-MS and NMR techniques, we have established for the first time the structure of ECA chain linked to the core oligosaccharide of *Shigella sonnei* phase II LPS and directly shown covalent linkage between ECA and LPS [1]. We have also prepared neoglycoconjugate of delipidated ECA_{LPS} with tetanus toxoid and immunized rabbits to obtain protective sera. First data indicates broad cross-reactions with many bacterial LPS, opsonisation of bacterial cell and bactericidal activity in *in vitro* tests as well [2]. The results let us believe that immunogens based on ECA and core oligosaccharide epitopes of ECA_{LPS} are potential components of universal anti-enterobacterial vaccine.

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V.P.21

Ectromelia virus infection affects mitochondrial functions in L929 cells

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Ectromelia virus (ECTV) belongs to the *Poxviridae* family and is a causative agent of mousepox. The aim of our study was to investigate how ECTV infection affect the mitochondrial function in L929 cell line.
In our work we used L929 cells and Moscow strain of ECTV (ECTV-MOS). The JC-1 dye was used for measuring mitochondrial membrane potential (MMP) by flow cytometry. The L929 cells treated with CCCP were used as negative control. At the early stages of ECTV-MOS infection (at 4 hour post infection; 4 h.p.i.) MMP did not differ significantly between non-infected control L929 cells and ECTV-MOS-infected cells. These results may be related to replication cycle of ECTV-MOS. Our previous study have shown, that at 4 h.p.i. ECTV-MOS did not form viral factory (sites of viral replication) in L929 cells and did not affect the mitochondrial morphology. However, at the late stages of ECTV-MOS infection the viral factories as well as changes in the mitochondrial network in L929 cells were observed. Furthermore, our current study indicated statistically significant differences in MMP between non-infected control L929 cells and ECTV-MOS-infected cells at 8 and 24 h.p.i. Presented results show, that ECTV-MOS affects the MMP at late stages of infection. Reduction of MMP was probably related in the progressive mitochondrial damage during ECTV-MOS infection.

Key words: ECTV-MOS, mitochondria, mitochondrial membrane potential

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V.P.22

The prevalence of *Malassezia* species on the skin of HIV-positive patients

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Objectives: Over the last decade there has been a growing number of reports on the prevalence of *Malassezia* species on the skin of healthy individuals and patients with *Malassezia*-associated diseases. However, only scanty information have been provided on the occurrence of *Malassezia* species specifically in patients with a severely impaired immunological status. The purpose of this study was to investigate the species composition of *Malassezia* microflora on the skin of patients positive for human immunodeficiency virus (HIV).
Materials and Methods: The study included 29 HIV-positive subjects (26 males, 3 females, aged 23–54 years; median age: 37 years), in- and out-patients of the Chair of Gastroenterology, Hepatology and Infectious Diseases, Medical College, Jagiellonian University, Kraków. From each patient, samples from four different body sites (head, face, chest, and back) were collected by a swab method. The yeasts were cultured on modified Dixon's agar medium, and the suspected colonies of *Malassezia* were subcultured, and subjected to species identification by using both phenotype-based and molecular methods. Whereas conventional differentiation involved an array of biochemical tests, molecular speciation was done with PCR-sequencing of the internal transcribed spacer (ITS) 1/2 regions within the rDNA operon.
Results: A total of 57 *Malassezia* cultures were obtained from clinical samples, with back being the commonest site of isolation (21 cultures; 36.9% of all *Malassezia* cultures), followed by chest (20; 35.1%), face (8; 14%), and head (8; 14%). The overall positive culture rate of the *Malassezia* yeasts was 49.1% (57 positive samples out of 116 samples tested). *Malassezia sympodialis* was recovered with the highest frequency (i.e. from 27 or 47.3% of the samples), followed by *M. globosa* (17; 29.8%), *M. furfur* (10; 17.5%), *M. dermatis*, *M. restricta*, and *M. slooffiae*, with the latter three being cultured from single samples only. No correlation was found between the *Malassezia* species identified and anatomical site or skin condition. The phenotypic and molecular methods used for species identification gave concordant results for 54 (94.7%) of the strains cultured.
Conclusions: The species composition of *Malassezia* microbiota on the skin of HIV-positive subjects seems not to differ from that observed among individuals of either negative or unknown HIV status. Despite the high concordance of the two identification approaches, PCR-sequencing is a preferred method, as its results are more reliable and more easily and rapidly produced.

Key words: *Malassezia*, human immunodeficiency virus (HIV), species identification

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V.P.23

Emerging bacteria in a tertiary healthcare set up

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Background: Emerging organisms are organisms that have newly appeared in a cohort/population or have existed but are rapidly increasing in incidence, geographic or host range. While, one-tenth of all infectious diseases are attributable to emerging organisms, operationally defining an organism as emerging is a subjective endeavour. As emerging organisms sporadically affect a relatively small percentage of population, they are not studied at large. This study was aimed at studying the characteristics of emerging bacteria at an Indian tertiary care hospital.
Methods: 33836 positive isolates obtained from 132646 processed samples during 2011–2014 were included. Identification percentage >85% along with inbuilt standards for identification comparison were considered for final validation through automated systems. Non repeat positive cultures were interpreted in conjunction with colony characteristics, cellular morphology, disc-diffusion antifungal susceptibility patterns, clinical correlates and environmental surveillance. The frequency of isolation, sources, referring centres, susceptibility profiles and phenotypic characteristics. A literature search was done to identify reports on human pathogenicity and yeasts and algae reported fewer than 100 times on PubMed were defined as emerging.
Results: 26996 (79.78%) Gram negative and 6508 (19.23%) Gram positive bacteria were isolated from 33836 isolates, of which 1190 (3.52%) were emerging bacterial isolates. Emerging enterobacteriaceae comprised of 39 species in 18 genera, non-fermenters 41 species in 22 genera and Gram-positive bacteria 63 species in 15 genera. The emerging bacteria were isolated from multiple sources and centres. Most of the isolates were multi-resistant while only a few were susceptible to commonly used drugs. Environmental surveillance was not corroborative.
Conclusion: Emerging bacteria have the potential to infect compromised hosts, posing difficulty in management due to multidrug resistance. They are likely to evade routine identification or be disregarded as non-contributory. Astute efforts directed at identification of emerging isolates, decisions by clinical microbiologists and treating physicians; and containment of infection are required.

Key words: emerging bacteria, microbial identification, antimicrobial resistance, automated microbiology systems

V.P.24

Modulating effect of IL-18 on mycobacterial antigen stimulated dendritic cell-T cell synapse for BCG vaccinated tuberculin-positive and negative individuals

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Although the current *M. bovis* BCG (Bacille Calmette-Guérin) vaccine has been central to the control of TB, the protection offered by vaccination is insufficient and not life-long. The tuberculin skin test (TST) provides a useful *in vivo* model for studying the effector side of the immune response to mycobacteria. TST allows measuring delayed type hypersensitivity (DTH) to purified tuberculin proteins (PPD). Only slightly more than 50% individuals vaccinated with BCG develop DTH to PPD. The major goal of this study was to explore the role of dendritic cells (DC) in the maintenance of TST positivity through the examination of mycobacterial antigens driven changes in DC phenotypic markers (CD86, CD80, CD40, HLA-DR, DC-SIGN), expression of cytokines (IFN-γ, IL-10, IL-23) and IP-10 chemokine. Specific cellular response of naive and memory CD4(+) T cells was analyzed. DC were stimulated with PPD and two live *M. bovis* strains, a recombinant producing human IL-18 (rBCGhIL-18) and nonrecombinant BCG bacteria. In this way, we could observed that the IL-18 modulated DC functions by allowing them to promote or stop mycobacteria specific immune responses, which may be crucial for the activation of T cells leading to skin induration in TST positive individuals.

Key words: BCG, tuberculin test, dendritic cell, T cell, IL-18

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V.P.25

The importance of AAA+ ClpB chaperone in pathogenicity of the bacterium *Leptospira interrogans*

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Bacterial ClpB is a member of the Hsp100/Clp subfamily of the AAA+ ATPases that mediates the reactivation of aggregated proteins in cooperation with the DnaK chaperone system. The data accumulated over the last few years regarding several bacterial pathogens suggest that ClpB may play an important role in their virulence. However, currently the precise role of the molecular chaperone ClpB in bacterial pathogenesis is not well characterized. This chaperone can function either as a true virulence factor directly involved in causing disease or as a virulence-associated protein that can be essential to enable the pathogen to colonize its host. Recently, it has been demonstrated that ClpB from *L. interrogans* ($ClpB_{Li}$), a spirochete capable of causing in mammals a disease known as leptospirosis, is essential for bacterial survival under stressful conditions and also during infectious process.

The aim of this study was to provide a better insight into the role of ClpB in bacterial pathogens and answer the question whether $ClpB_{Li}$ may be a target of humoral immune response during leptospiral infections in mammals. For this purpose, we cloned and expressed the coding sequence of the $clpB_{Li}$ gene in the pET system. The purified recombinant $ClpB_{Li}$ was subsequently used in Western blotting and ELISA test to assess its immunoreactivity with sera collected from *Leptospira*-infected animals and uninfected healthy controls.

We demonstrated that $ClpB_{Li}$ is able to provoke an immune response in animals, as evidenced by an increased level of antibodies against $ClpB_{Li}$ in sera of *Leptospira*-infected animals compared to the control group. Additionally, we found $ClpB_{Li}$ in kidney tissues obtained from hamsters experimentally infected with *L. interrogans*. Our results suggest that $ClpB_{Li}$ is both synthesized and immunogenic during infection, further supporting its importance in pathogenicity of *Leptospira*.

Key words: ClpB chaperone, immunoreactivity, *Leptospira*, leptospirosis, pathogenicity

V.P.26

Comparison of virulence factors of *Escherichia coli* strains isolated from reptiles, humans and poultry

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Extraintestinal pathogenic *Escherichia coli* (ExPEC) are a serious threat to human health causing urinary tract infection, meningitis or septicemia. Warm-blooded animals are a reservoir of ExPEC, but there is still little known about epidemiology of ExPEC among cold-blooded animals. Reptiles lives in Zoological Garden as well as pet animals might be carriers of pathogenic *E. coli* and a potential source of infection in humans. The aim of this study was to assess the zoonotic potential of *E. coli* strains [n=13] isolated from intestines of reptiles (RepEC) by comparing virulence factors of uropathogenic *E. coli* (UPEC) [n=13] isolated from urine of patients and avian pathogenic *E. coli,* (APEC) [n=13] isolated from poultry with colibacillosis, in Lower Silesia in Poland. These isolates were subjected to testing virulence of 15 genes related with pathogenicity of ExPEC such as: *iss*-increased survival of bacteria in host serum, *irp2,*-siderophores, *papC*-adhesion to urinary tract or *kps*-capsules, were targeted by three PCR reactions. To assess genetic diversity, phylogenetic groups of *E. coli* were determined by triplex PCR according to Clermont (2001). The antibiotic resistance of tested strains to tetracycline, amoxicillin, fluoroquinolones and cefuroxime, was determined by E-test method. In presented research, bacterial strains which belonged to B2 and D phylo-groups (theoretically including ExPEC) were identified among RepEC as well as APEC isolates, while all UPEC strains were assigned to B2 group. The virotyping of RepEC, APEC, UPEC strains revealed evident differences in virulence genes distribution, such as the prevalence of virulence genes was significantly lower among RepEC than among APEC and UPEC. However in some RepEC strains virulence genes characteristic to ExPEC have been also identified such as: *iss, papC, irp2, iucD* or *fimC*. RepEC strains displayed sensitivity to all tested antibiotic and chemotherapeutics while some of APEC and UPEC strains were resistant to two or more antimicrobials.

Key words: pathogenicity of *E. coli*, ExPEC, virulence genes

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V.P.27

Does Fourier Transform Infrared Spectroscopy (FTIR) may be promising tool in rheumatoid arthritis diagnosis?

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Rheumatoid arthritis (RA) is one of the most common chronic inflammatory disorders affecting joint tissues. It lead to irreversible erosion of bone and destruction of articular cartilage. Fast and correct diagnosis of RA is still problematic.

The aim of this work was to differentiate of RA patients, versus healthy controls, based on infrared spectra of sera.

Sera of the 29 RA patients (Swietokrzyskie Rheumatology Center, Specialist St. Luke Hospital; Konskie) and 28 blood donors (Regional Blood Center, Kielce) were analyzed. Each serum was measure after water evaporate (5 min) using ATR/FT-IR technique in range 3000–700 cm^{-1}. For each sample 50 spectra scans were performed and averaged for calculation of the first derivative and following the statistical analysis (hierarchical cluster analysis – HCA and principal component analysis – PCA).

Based on HCA of the infrared spectrum fragments (3000–2800 and 1800–750 cm^{-1}) analyzed sera were clustered into two distinct groups with 2% error. The first group contains only blood donors sera while the second group includes RA patients sera and one control serum. Using specific window (900–750 cm^{-1}), it was make possible to differentiate analyzed sera (RA patients and blood donors) into two clusters with any misclassification. However, in the first case the distance between clusters was much longer, which make those results more significant.

Distinct differentiation of RA and non-RA sera were noted based on FTIR spectra, which make this technique a convenient option for fast and non-invasive tool in disease diagnosis.

Key words: infrared spectroscopy, rheumatoid arthritis

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V.P.28

The effect of silver nanoparticles on bacterial adhesion, cells aggregation and biofilm structure

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Background: Antimicrobial effect of silver nanoparticles (AgNPs) has been extensively studied in recent years. Due to the broad spectrum of its antimicrobial activity, the nanosilver is utilized as an additive to great number of consumer products. Our study showed that AgNPs is also effective towards biofilms, bacterial communities that are very difficult to eradicate with traditional antibacterials. The results obtained demonstrate the anti-biofilm activity of AgNPs, considering bacterial adhesion to colonized surface.

Materials and methods: *Pseudomonas aeruginosa* ATCC10145 and *Staphylococcus aureus* ATCC29213 were used in this study. AgNPs colloid was obtained from Nano-Tech (Poland). Minimal biofilm inhibitory concentration (MBIC) values were measured by two-fold microdilution method. Subinhibitory concentrations of AgNPs (0.25 and 0.5×MBIC) were applied to study the nanosilver effect on bacterial adhesion to polystyrene plates. Relative bacterial cell surface hydrophobicity in the presence of AgNPs was examined by salt aggregation test. The effect of AgNPs on biofilm structure stained with acridine orange was observed with confocal laser scanning microscopy (NIKON A1R MP) followed by creating 3D visualizations. Morphology of bacterial cells within biofilms treated with AgNPs was examined with transmission electron microscopy (Zeiss Libra 120 EFTEM).

Results: MBICs of AgNPs were 8 micrograms mL-1 for *P. aeruginosa* and 12 micrograms mL-1 for *S. aureus*. 0.5xMBIC of AgNPs diminished bacterial adhesion to polystyrene by 55% for *P. aeruginosa* and by 40% for *S. aureus*. AgNPs also influenced the hydrophobicity of both species by enhancing aggregates formation. Microscope analyses showed significant changes in biofilm structure and morphology of bacterial cells caused by AgNPs.

In conclusion, silver nanoparticles influence the hydrophobicity of cell surface, impede the adhesion to polystyrene and change the biofilm structure. More pronounced anti-biofilm effect was shown in case of *P. aeruginosa*.

Key words: silver nanoparticles, biofilm, antibacterial effect

V.P.29

Synergistic hemolysins of coagulase-negative staphylococci (CNS)

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In earlier studies we showed the production of synergistic hemolysins by two CNS subspecies *S. cohnii. cohnii* and *S. cohnii. urealyticus*. In the present study we examined 104 strains, originating from the collection of the Department of Pharmaceutical Microbiology (ZMF), Medical University of Łódź and belonging to *S. capitis, S. hominis, S. haemolyticus* and *S. warneri*. The *S. c. urealyticus* strain was used as the control. It was shown that 83% of strains produced synergistic hemolysin, however, the percentage of positive strains of *S. haemolyticus, S. warneri, S. capitis* and *S. hominis,* was different – 98%, 78%, 75% and 68%, respectively.

The hemolysins were obtained from culture supernatants by precipitation with ammonium sulphate (0–60% of saturation) of proteins and extraction using a mixture of chloroform and methanol in proportion 2:1. The purity of hemolysins was controlled by TRIS/Tricine PAGE. These preparations were used for the determination of cytotoxicity on the line of human foreskin fibroblasts ATCC Hs27, antimicrobial activity by dilution in solid agar plate method and hemolytic activity with the use of red blood cells from sheep, rabbit, dog, guinea pig and human beings.

All CNS hemolysins possessed cytotoxic activity and lysed red cells from different mammalian species, however the highest activity was observed, when quinea pig, human and dog red blood cells were used. The cytotoxic effect on fibroblasts was observed within 30 minutes.

The antimicrobial activity was examined using four hemolysins of *S. capitis, S. hominis, S. c. cohnii* and *S. c. urealyticus*, since the antimicrobial activity of hemolysins produced by *S. warneri* and *S. haemolyticus* had been tested earlier. Hemolysins of the two *S. cohnii* subspecies did not demonstrate antimicrobial activity. Cytolysins of *S. capitis* and *S. hominis* had a very narrow spectrum of action; out of 37 examined strains only the growth of *Micrococcus luteus, Corynebacterium diphtheriae* and *Pasteurella multocida* was inhibited. None of the hemolysins were able to suppress the *Candida albicans* growth.

Key words: synergistic haemolysins, coagulase-negative staphylococci, biological activity

V.P.30

Evaluation of methods used for isolation of surface proteins of *Clostridium difficile*

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Clostridium difficile pathogen is a cause of the most frequent nosocomial infection which is antibiotic-associated diarrhea. Antibiotic treatment is causing the disruption of microbiome balance which makes the gut a friendly environment for the pathogen. In healthy infants younger than 1 month of age, *C. difficile* has been recovered from an average of 37% of stools, with a range of 25 to 80% of infants harboring *C. difficile* as a harmless commensal. At age of three the rate of colonization reaches 3%, similar to carriage rates previously reported in adults.

Two major toxins (TcdA and TcdB) produced by many *C. difficile* strains are accepted as major virulence factors. The identity and roles of other pathogenicity factors are less well understood. Adhesion is an early, critical step in colonization and the subsequent disease process, in which surface-localized elements play the crucial role. In the literature, there are many methods for surface protein isolation: using 0.2 M glycine pH 2.2, 10 mM EDTA, 1 M LiCl, 8 M urea, lysozyme and others. The method most frequently applied is the one using 10 mM EDTA.

The aim of this study was to compare two methods for surface-protein isolation of *C. difficile* – using 1 M LiCl and 10 mM EDTA, and to determine the applicability of those methods for immunological studies and two-dimensional electrophoresis.

Each method is efficient, fast and easy to perform. Both methods give good results in immunological studies. Proteins obtained by EDTA method are not applicable to 2d electrophoresis because of streaking.

Key words: nosocomial infection, *Clostridium difficile*, 2D electrophoresis

V.P.31

Leonurus cardiaca extract as a new potential therapeutic in staphylococcal infective endocarditis

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Introduction: Pathological changes in infective endocarditis (IE) are usually located on damaged valves or their prosthetics, on which deposits of blood platelets and plasma proteins are formed, preparing excellent surface to the attachment of the pathogens. The difficulties in the treatment of IE arise from both high resistance of microorganisms forming aggregates/biofilm to classic antibiotics and host defense mechanisms, and impairment of physiological repair processes within damaged tissue. Thus, it is necessary to conduct the research on alternative treatment options, including the use of some plant-derived preparations with known beneficial effects on the cardiovascular system as well as antimicrobial and immunomodulatory activity.
Aim: The assessment of antithrombotic activity of *Leonurus cardiaca* polyphenol-rich extract, and its impact on the expression of *Staphylococcus aureus* virulence factors important in the pathogenesis of IE.
Materials and methods: Tested polyphenolic extract from *L. cardiaca* herb (LC). Bacteria: *S. aureus* NCTC 8325-4 and *S. aureus* Wood 46 (ATCC 10832), with known type of MSCRAMMs/SERAMMs expression. Microbial parameters tested *in vitro* under the influence of LC: staphylococcal adhesion to abiotic or fibrinogen/fibrin network coated surfaces (Alamar Blue staining); the release of coagulase (tube method); *S. aureus* aggregation in plasma (spectrophotometric method) and its survival in the blood (CFU counting); the expression of Staphylococcal protein A (SpA; fluorimetric method).
Results: It has been found that *S. aureus* adhesion to abiotic or fibrinogen/fibrin coated surfaces in the environment of the extract (subMIC) was significantly reduced. LC negatively affected also staphylococcal coagulase secretion and SpA expression on microbial cell surface. Moreover, *S. aureus* survival in human blood and aggregation in human plasma have been reduced in the presence of LC.
Conclusions: These few studies on the little known properties of *L. cardiaca* extract provide new data for the future development of alternative therapies of difficult to treat cardiovascular system infections.

Key words: infective endocarditis, natural plant products, *Staphylococcus aureus*

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V.P.32

Regulation of TLR3 mobility and access to dsRNA

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Toll-like receptor 3 (TLR3) is expressed in the cells of the central nervous system (CNS) where it recognizes self and foreign dsRNA in contribution to control infections caused by herpes viruses. Humans or animals with deficiencies in TLR3 have high susceptibility to infections with HSV-1 (herpes simplex virus type 1) or HSV-2 and humans deficient in TLR3 have a rare, but increased susceptibility to viral encephalitis following HSV-1 infection. This suggests that while TLR3 is largely redundant for dsRNA responses in leukocytes, the central nervous system relies on TLR3 to respond to viral dsRNA. TLR3 shows different expression than other nucleic acid recognizing TLRs and engages different adaptor protein for initiation of IRF3 and NF-κB signalling, what suggests that control of neurotropic virus infections in the brain, such as HSV, may require a different regulatory mechanisms.
Preliminary studies in murine astrocytes C8D1A cells indicate that endosomal sorting complex required for transport (ESCRT-0) is ubiquitinated and co-precipitates with TLR3. Transfection of cells with STAM (signal transducing adaptor molecule; ESCRT-0 component) siRNA indicates that silencing of this protein downregulates expression of TLR3 and Hrs (hepatocyte growth factor-regulated tyrosine kinase substrate), another ESCRT-0 component.

Keywords: TLR3, ESCRT-0, HSV

V.P.33

Modulation of monocyte/macrophage function by *H. pylori* LPS *versus* downregulation of lymphocyte expansion

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We evaluated the effectiveness of monocytes/macrophages as antigen presenting cells (APC) in promoting the blastogenic response of splenocytes from guinea pigs uninfected or infected with *H. pylori*, in response to its glycine extract (GE), CagA protein (IRIS), urease A subunit and lipopolysaccharide (LPS) (courtasy of A. P. Moran). Lymphocyte expansion was assessed in cell cultures, containing: monocytes or mature macrophages derived from bone marrow monocytes, unstimulated or stimulated with *H. pylori* antigens. The lymphocyte proliferative potential was estimated in the presence of phytohemagglutinin (PHA). The proliferation indication was the ratio of [^{3}H]-thymidine incorporation into DNA of antigen-stimulated to unstimulated cells, whereas the DNA damage by DAPI staining. TGF-β and IFN-γ concentrations were assessed in cell culture supernatants. Lymphocytes of control and *H. pylori*-infected animals proliferated in response to PHA, and in co-cultures with GE, CagA or UreA treated monocytes/macrophages. In the cultures containing APC preincubated with *H. pylori* LPS or *E. coli* LPS, the lymphocyte division was inhibited with relation to monocytes DNA damage. In response to *H. pylori* LPS or *E. coli* LPS 80% and 95% cells showed DNA damage, respectively. Mature macrophages of control animals were less susceptible to LPSs cytotoxicity. The amount of macrophages with damaged DNA reached 70% in response to *H. pylori* LPS and 60% to *E. coli* LPS. The most resistant were mature macrophages derived from *H pylori*-infected animals. The number of such macrophages with affected DNA upon treatment with *H.pylori* LPS was 50%, while with *E. coli* LPS – 30%, which may be related to effectiveness of DNA repair. In the cultures containing monocytes/macrophages treated with LPSs and in their co-cultures with lymphocytes, the concentration of regulatory TGF-β, but not proinflammatory IFN-γ, was increased. Impaired homeostasis of monocytes/macrophages, following DNA damage and TGF-β release, in response to *H. pylori* LPS may lead to the suppression of adaptive immunity against *H. pylori* and persistent infection.

Key words: *H. pylori*, antigen presenting cells, lymphocyte expansion

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V.P.34

Role of *Helicobacter pylori* infection in cancer and metastasis of bone marrow derived-mesenchymal stem cells – an *in vitro* study

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Introduction: *Helicobacter pylori* (*H. pylori*) has effective role on mesenchymal stem cells (MSCs) migration to the gastric wounded tissue. We try to find the effects of *H. pylori* as bacterial microenvironments on MSCs that could be the case of cancer stem cells and gastric adenocarcinoma and also the effect of the *H. pylori* on the metastasis of the tumor cells.

Material and Methods: MSCs were followed for evaluation by flow cytometeric analysis with the hAbs and treated under the osteogenic and adipogenic differentiation medium.

BMD-MSCs were co-cultured with *H. pylori* (ATCC 26695) and Gastric epithelial cell line (AGS) for 72 h in (DMEM; Gibco) with 10% FBS (Sigma). The expression of MMP2, MMP9, P53 and bcl2 was examined by RT-PCR.

Results and Conclusion: *H. pylori* chronic and acute infection triggers inflammation factors which case migration of the MSCs. When stem cells attract to the site for their tissue healing function on the wound tissue, they will trapped under special microenvironment by *H. pylori* bacteria. This study demonstrated that *H. pylori* increased the anti-apoptosis factor bcl-2 to survive stem cells and lead them to cancer stem cells by change the regulation of p53. *H. pylori* increased the metastatic proteins MMP2 that show the role of *H. pylori* in metastasis. Focus on *H. pylori*-induced molecular pathogenesis and the impact of microenvironment in gastric progenitor cells or MSCs will be crucial to identify the molecular targets in tumor initiation and the origin of gastric cancer.

V.P.35

Bacillus subtilis metabolic products antimicrobial activity on planktonic and sessile forms of uropathogenic bacteria

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Biosurfactants produced by *Bacillus subtilis* strains are lipopeptides belonging to surfactin, iturin and fengycin families. These compounds exhibit antibacterial, antiadhesive activities and have also an influence on the biofilm dispersion process.
The aim of the study was to investigate the antimicrobial effect of compounds secreted by *Bacillus subtilis* strain on uropathogenic bacteria (planktonic cells, prevention of biofilm formation and eradication of mature biofilm).
B. subtilis IETU-I'1a was isolated from soil heavily contaminated with hydrocarbon. Its lipopeptide biosurfactants secretion was confirmed by LC-MS/MS. Cell free supernatants were obtained after *B. subtilis* culturing in LB for 48 h at 28ºC, 150 rpm. 32 strains belonging to 12 different species of Gram-negative and Gram-positive bacteria isolated from biofilm on urinary catheters were selected for antimicrobial assays. The microdilution method and agar well diffusion assays were used to study the effect of *B. subtilis* extracellular products mixture on uropathogens planktonic forms. The examination of *B. subtilis* antimicrobial activity on biofilms was performed using microplates and MTT assays. Confocal laser scanning microscopy (CLSM) was used to visualize the uropathogens biofilm after the treatment with *B. subtilis* cell free supernatant.
In the case of 62% studied uropathogens the metabolic activity of planktonic cells was inhibited by 89% after the treatment with *B. subtilis* cell free supernatant. *B. subtilis* products also affected the process of biofilm formation, an average reduction of 95% was observed in the case of 19 uropathogenic strains. It was also shown that the cell free supernatant was active against mature biofilms, in 15 tested strains the reduction of about 79% in biofilm biomass was noted. The inhibitory activity of *B. subtilis* extracellular products was not observed in the case of several species: *Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus epidermidis* and *Enterococcus faecalis*. The above-described results were confirmed by CLSM (for selected strains).

Key words: uropathogens, biofilm, biosurfactant

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V.P.36

The *in silico* prediction of foot-and-mouth disease virus (FMDV) epitopes on the South African Territories (SAT)1, SAT2 and SAT3 serotypes

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Foot-and-mouth disease (FMD) is an economically important disease that affects even-toed hoofed mammals. The FMD virus (FMDV) is the causative agent of FMD, of which there are seven clinically indistinguishable serotypes. Three serotypes, namely, South African Territories (SAT)1, SAT2 and SAT3 are endemic to southern Africa and are the most antigenically diverse among the FMDV serotypes. Consequently, infection or vaccination with one virus may not provide immune protection from other strains. The identification of B-cell epitopes is therefore key to the rational design of high-crossover vaccines that recognize the immunologically distinct strains present within the population. Computational epitope prediction methods have been proposed as a cost and time-effective alternative to the classical experimental methods. The aim of this study is to employ *in silico* epitope prediction programmes to predict B-cell epitopes on the capsids of the SAT serotypes. Sequence data for 18 immunologically distinct strains from across southern Africa whose X-ray crystal structures are unavailable was collated. Homology models of the 18 virus capsids were built and subsequently used as input to two different epitope prediction servers, namely *Discotope1.0* and *Ellipro*. Only those epitopes predicted by both programmes were defined as epitopes. Both previously characterised and novel epitopes were predicted on the SAT strains. Some of the novel epitopes are located on the same loops as experimentally derived epitopes, while others are located on a putative novel antigenic site, which is located close to the five-fold axis of symmetry. A consensus set of 11 epitopes that are common on at least 15 out of 18 SAT strains was collated. Those predicted epitopes found to be true epitopes may consequently be used in the rational design of broadly reactive SAT vaccines.

Key words: foot-and-mouth-disease, foot-and-mouth disease virus, South African Territories (SAT), viral protein (VP), epitope, epitope prediction, immunoinformatics, homology model, *Discotope*, *Ellipro*

V.P.37

Quality of HIV testing in Zambia (2009–2012): findings from the national quality assurance system

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Background: Task-shifting and scale-up of HIV testing services in high HIV prevalence countries challenge testing quality. The Zambia National Quality Assurance program was launched in 2009 to monitor HIV testing performance countrywide. This study aimed to evaluate quality of laboratory and non-laboratory HIV rapid testing in Zambia and to what extent the national quality assurance (QA) system has affected performance over time.
Methods: HIV testing performance was investigated among rural and urban HIV testing sites participating in four annual national HIV proficiency testing (PT) exercises conducted in 2009 (n=282), 2010 (n=488), 2011 (n=500) and 2012 (n=404). Testers included lay **counselors**, nurses, laboratory personnel and others. PT panels of five dry tube specimens were issued to sites by the national reference laboratory. Site accuracy level was assessed by comparison of reported results to expected results. Non-parametric rank tests and multiple linear regression models were used to assess variation in accuracy between tester groups and examine factors associated with accuracy respectively.
Results: The QA system reached 30% of registered sites countrywide. Response rates varied by year: 51.3%, 71.8%, 84.5% and 63.0% between 2009 and 2012. The overall accuracy level was 93.1%, 96.9%, 96.0%, 96.2% between 2009 and 2012. Frequency of participation, experience and adherence to the national HIV testing algorithm were associated with higher accuracy over time. Accuracy varied by tester groups in 2009 with laboratory personnel being more accurate than non-laboratory personnel, while from 2010 onwards no variations were seen. Through the years, lay **counselors** maintained an upward performance, while nurses, laboratory personnel and others maintained a closely stable performance.
Conclusions: The study showed improvements in overall accuracy level and particularly among lay counselors over time. Further improvement is needed and the QA system seems to be important in this regard which needs urgent strengthening to effectively monitor HIV testing countrywide.

Key words: Task-shifting, HIV testing, quality assurance, Zambia

V.P.38

The first Polish report of azole resistant clinical *Aspergillus fumigatus* isolate

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Aspergillus fumigatus is one of the most prevalent airborne fungal pathogens causing infection worldwide. Most *Aspergillus fumigatus* strains are susceptible to azoles, which are applied as the first line therapeutics. However, during last decade the acquired resistance for triazoles species has been described. There is a number of publications concerning the examination of clinical *A. fumigatus* strains from different countries, however there is no Polish report. Here, we describe first examination of 17 clinical and 11 environmental *A. fumigatus* strains isolated during 2007-2014 period from the collection of Medical University in Wrocław. Their susceptibility for itraconazole, voriconazole and posaconazole has been examined. The MIC values of triazoles for one of the examined isolates were respectively: >8 mg/L for itraconazole, 2 for voriconazole and 0,5 for posaconazole. The CYP51A gene with promoter region of all isolates was sequenced. The mutation TR34/L98H was confirmed in the resistant isolate. Although the presence of CYP51A mutation correlation with azole resistance has been previously proved, the other resistance mechanism cannot be excluded.

Key words: *Aspergillus fumigatus*, azole resistance, CYP51A

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V.P.39

Probiotics protect against 2-amino-1-methyl-6-phenyl-1H-imidazo[4,5-b] pyridine (PhIP) – an *in vitro* study

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Several studies have suggested that diet has an impact on the incidence of human colorectal cancer, with DNA damage playing a key role in cancer development. Heterocyclic aromatic amines (HAAs) are carcinogenic substances, which are present in a typical Western diet rich in thermally processed meat. These factors can modulate the cytotoxicity of faecal water (FW) and induce tumours in the human gastrointestinal tract. According to the International Agency for Research on Cancer (IARC), PhIP is classified as a possible human carcinogen (class 2B). Usually from one to several micrograms of each HAA is consumed per day by individuals eating well-done cooked meat, and it depends on the country and eating habits. Supplementation with probiotics is promising in terms of reducing the harmful effects of HAAs in the human body. The aim of the research was *in vitro* assessment of the protective activity of the probiotic strains *Lb. rhamnosus* 0900, *Lb. rhamnosus* 0908 and *Lb. casei* 0919 against PhIP (2-amino-1-methyl-6-phenyl-1H-imidazo[4,5-b]pyridine) after incubation with faeces from 15 persons aged 4 months to 82 years (children, adults, and the elderly). The highest mean cytotoxicity of FW was observed for the elderly (63.2%±3.7) and the lowest for children (28.0%±9.5), as estimated by a neutral red uptake assay. The probiotics lowered the average cytotoxicity of FW exposed to PhIP. The concentration of PhIP in FW was most effectively reduced by *Lb. casei* 0919 (45.8%), as determined by high performance liquid chromatography. In an alkaline comet assay, *Lb. casei* 0919 and *Lb. rhamnosus* 0908 displayed the strongest protective effect against PhIP (up to 80% reduction of DNA damage). The protective activity of the probiotic strains was specific to a given person's FW, which implies the involvement of intestinal microbiota in the process.

Key words: probiotic, cytotoxicity, heterocyclic aromatic amines, DNA damage, HPLC

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V.P.40

Probiotic bacteria inhibit adherence of enteric pathogens to human colon adenocarcinoma cell line Caco-2

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The important criteria for a prospective probiotic strain are the ability to adhere to mucosal surfaces of the gastrointestinal tract. The adherence to cells of the gastrointestinal tract is a main process for bacteria to survive and colonise the niche. Adherence prevents probiotic cells from being washed out, and enables temporary colonisation, immune modulation and competitive exclusion of pathogens. Some probiotics can inhibit gastrointestinal infections by blocking the adherence of pathogens to the intestinal epithelium. The aim of this study was to determine the anti-adherence properties (*in vitro*) of three probiotic *Lactobacillus* strains (*Lb. rhamnosus* 0900, *Lb. rhamnosus* 0908, and *Lb. casei* 0919), and their mixture against pathogens: *Escherichia coli* ATCC 10536, *Salmonella entrica* serovar Typhimurium ATCC 14028, and *Candida albicans* ATCC 10231 using Caco-2 human colon adenocarcinoma cells. All strains of lactobacilli and the probiotic mixture to the greatest extent inhibited adherence of *S*. Typhimurium, up to 91%. *Lb. rhamnosus* 0900 inhibited *E. coli* by 75.9%, and *Lb. casei* 0919 decreased adherence of *C. albicans* by 49%. Our findings indicate that tested lactobacilli have good anti-adherence activity against *S*. Typhimurium ATCC 14028, *E. coli* ATCC 10536 and *C. albicans* ATCC 10231. *Lb. rhamnosus* 0900 possess the strongest, while *Lb. rhamnosus* 0908 the weakest anti-pathogenicity, thus its presence in the composition of the probiotic mixture should be reconsidered. The ability of adherence inhibition depended on the specific probiotic strain and the pathogen.

Key words: probiotics, adherence, intestinal microbiota, diarrhea, pathogens, Caco-2 cells

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V.P.41

Antigenotoxicity of prebiotics and end-products of their fermentation – an *in vitro* study

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Components of diet – lactic acid bacteria and prebiotics are able to modulate the intestinal microbiota and are thought to be involved in reduction of colorectal cancer risk. Human gastrointestinal tract (GI) is the natural endosymbiotic habitat for lactic acid bacteria. Many of these bacteria, primarily from the *Lactobacillus* and *Bifidobacterium* genera belong to probiotics and are generally regarded as being helpful to host health. The presence of short chain fatty acids (SCFA) in GI tract was found to be very beneficial for host organism. SCFA production is one of the proposed mechanisms of anticancer action of probiotics. In the study, antigenotoxic activity of some prebiotics and end-products of their fermentation by probiotics towards faecal water (FW) were estimated in the comet assay, together with SCFA production by these bacteria, what was measured with HPLC. End-products of seven prebiotics fermentation conducted by *Lactobacillus casei* DN 114-001 showed the highest antigenotoxic potency towards FW, among which fermented inulin lowered the genotoxicity by 75%. The strain produced the highest amounts of butyrate after fermentation of prebiotics, especially from resistant dextrin (4.09 μM/ml). Fermented resistant dextrin improved DNA repair by 78% after pre-treatment with 6.8 μM methylnitronitrosoguanidine (MNNG). Fermented inulin could better induce DNA repair after pre-treatment with mutagens (FW, 25 μM hydrogen peroxide and MNNG) than non-fermented, and the efficiency of DNA repair after 120 min of incubation decreased by 71, 50 and 70%, respectively. The different degree of inhibition of genotoxicity observed with the various combinations of bacteria and prebiotics suggest that this can be a consequence of carbohydrate type, yields and ratio between the end-products of prebiotics fermentation. The use of probiotic and prebiotic combinations (synbiotics) should be promoted, as a strategy to improve health and prevent colorectal cancer risk at early stages.

Key words: probiotics, prebiotics, genotoxicity, colorectal cancer, SCFA

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V.P.42

Adhesive properties and biofilm formation by clinical strains of Streptococcus Anginosus Group

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Three species of streptococci, *S. anginosus*, *S. constellatus*, *S. intermedius*, form separate division known as Streptococcus Anginosus Group – SAG (formerly milleri). SAG strains are isolated from various human body sites, such as oral cavity, gastrointestinal and genitourinary tracts. And although the association between species and abscesses forming was observed, the bacteria are still regarded as commensal streptococci. This opinion is particularly risky while consider increasing number and severity of SAG infections.

Medical reports not only showed SAG as the major factor of particular infections, but also presented SAG as co-existing with *Pseudomonas aeruginosa* in cystic fibrosis patients. Moreover, such a co-existing population of SAG exhibit less susceptibility for antibiotic treatment. Another research showed that not accurate dose of antibiotic could stimulate bacteria to biofilm formation. The pool of molecular factors which stimulate bacteria to adhesion and biofilm formation, is quite diverse. Extracellular receptors, quorum sensing molecules and transcription regulators were considered as the major agents.

In this study we investigated adhesion properties and biofilm formation of clinical SAG strains obtained from various infections: isolated from abscesses, respiratory tract, blood or central nervous system. Adhesive properties to ECM compounds and to lung fibroblast were examined. We tested influence of catabolite control protein A in regulation of biofilm formation. Moreover, we searched genomic sequences of these strains for other putative adhesion factors.

Key words: *Streptococcus anginosus*, adhesion, biofilm, *cpA*

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V.P.43

The influence of main virulence factors of *Candida albicans* on human gingival fibroblasts

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Candida albicans belongs to normal constituents of human microbiota and can be isolated from the oral cavities of 60% of healthy adults. Besides oral mucosa, *C. albicans* is also present in gingival tissues and periodontal pockets. This dimorphic fungus can become a dangerous pathogen for the human host, especially for individuals with severe defects in the immune system. *C. albicans* uses numerous virulence factors to impair host defense responses, and to colonize and invade host tissues. The main candidal virulence factors include secreted hydrolases such as aspartic proteases (Saps) as well as a variety of cell wall components such as glucans, mannans and adhesins.
Within the gingival tissue, fibroblasts are the most common cell type that regulate the homeostasis of the connective tissue, and also have an ability to respond to pathogen associated molecular patterns (PAMPs) and produce inflammatory mediators. These activities, as well as the obligatory contact of fibroblasts with periodontal pathogens, make these host cells important players in the periodontal tissue in both health and disease.
The aim of our current study was to determine if *C. albicans* virulence factors can modulate the inflammatory response of fibroblasts, including the production of chemokines and cytokines. We compared the responses of human gingival fibroblasts isolated from healthy individuals and from chronic periodontitis patients. The cells were stimulated with a mixture of Saps, released to culture medium or purified Saps, overproduced in *Pichia pastoris*, as well as with major *C. albicans* cell wall components, including mannans, glucans, and proteins. Among the compounds tested, Saps caused a strong induction of interleukin-1β, -6 and -8 (Il-1β, Il-6, and Il-8), and tumor necrosis factor alpha (TNF-α) in both fibroblast batches, suggesting that specific virulence mechanisms of *C. albicans* and immune responses of gingival fibroblasts can contribute to the chronic inflammation associated with periodontitis.

Keywords: *Candida albicans,* fibroblasts, inflammatory mediators, inflammati on, periodontitis

V.P.44

Proteus mirabilis RMS 203 as a new representative of O13 Proteus serogroup. The importance of N$^{\varepsilon}$-[(S/R)-1-Carboxyethyl]-L-lysine residue in the serospecificity of *Proteus* lipopolysaccharides

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Lipopolysacharides (LPSs) of opportunistic *Proteus* species bacilli often contain glucuronic or galacturonic acid, which can be additionally amidated with amino acids, like L-lysine or L-alanine. The unique feature of some *Proteus* species O-polysaccharides is occurrence of N$^{\varepsilon}$-[(S/R)-1-Carboxyethyl]-L-lysine. An amide of GalA with L-lysine was previously indicated as a crucial cross-reactive epitope located in the O-antigens or core regions of *Proteus* LPSs. The results of presented serological studies with reference to published O-antigens structures, suggest that N$^{\varepsilon}$-[(S/R)-1-Carboxyethyl]-L-lysine can be sufficient for cross-reactions of O13 *Proteus* antisera, and LPSs of different *Proteeae* tribe representatives. The studies also revealed that *Proteus mirabilis* RMS 203 strain can be classified into the O13 serogroup, represented by two strains: *Proteus mirabilis* (26/57) as well as *Proteus vulgaris* (8344). However, the obtained data also indicated differences in the reactivity of O13 antiserum with low molecular weight fractions of LPSs, corresponding to the core region of lipopolysaccharides. All serological studies were conducted using ELISA, PAGE and Western blot technique with the purified LPSs preparations and rabbit polyclonal sera specific to selected *Proteus* strains.
Proteus O13 serogroup had previously been reported as one of the most frequent among *Proteus* clinical isolates. Finding the LPSs of different species from the tribe *Proteeae* cross-reacting with *Proteus* O13 antisera is of high importance in the future search for vaccine antigens common for different bacteria species.

Key words: *Proteus*, O-polysaccharide, cross-reaction

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V.P.45

Molecular characterization of clinical strains of *Pseudomonas aeruginosa* – the host of bacteriophages

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Introduction: Bacteriophages (phages) therapy may offer an innovative means of *Pseudomonas aeruginosa* infections treatment, which can be combined or alternated with antibiotic therapy and may enhance our abilities to treat bacterial infections successfully.
Objectives: The aim of this study was the molecular characterization of clinical isolates of *Pseudomonas aeruginosa* – the host of bacteriophages.
Method: 19 clinical isolates of *P. aeruginosa* – the host of bacteriophages, from Biomed S.A.'s own collection within the ONKOFAG project, were tested. The resistance of isolates were phenotypically examined according to EUCAST v. 4.0 (2014). The genes coding exotoxins (*exo*A, *exo*S, *exo*T, *exo*U), elastase B (*las*B), alkaline phosphataseA (*apr*A), phenazine biosynthetic operon (*phz*ABCDEFG) and two phenazine modifying genes (*phz*M and *phz*S) were detected by PCR and multiplex PCR. The isolates were genotyped by PFGE.
Results: Among 19 isolates of *P. aeruginosa* none showed the resistance phenotypes. The most common genes of exotoxins were *exo*A and *exo*T (100%), followed by *exo*Y (95%), *exo*S (74%), *exo*U (26%). In this study, the operon *phz*ABCDEFG was detected at one strain (5%) while *phz*M and *phz*S appeared at 100% of isolates. Six out of nineteen strains (32%) were positive for the presence of *las*B gene, while *apr*A gene was found in all of the tested isolates (100%). High genetic diversity within *P. aeruginosa* strains was observed using the PFGE method.
Conclusion: According to the results of the present study, the high genetic diversity and high frequency of virulence factors genes among clinical isolates of *P. aeruginosa* – the bacteriophage hosts, were performed.

Key words: *Pseudomonas aeruginosa*, bacteriophage hosts, molecular characterization

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V.P.46

The involvement of two orphan response regulatory proteins Rv0818 and Rv2884 in physiology of mycobacteria

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Mycobacterium tuberculosis, the causative agent of tuberculosis, the most successful human pathogen, had spread intensively worldwide causing millions of deaths each year. *Mycobacterium tuberculosis* possesses a great ability to adapt to a wide range of living conditions inside as well outside the human host. Bacteria are able to sense and respond to environmental changes such as hypoxia, reactive oxygen and nitrogen intermediates and pH alterations *via* two component signal transduction systems (TCSSs). The genome of *Mycobacterium tuberculosis* possesses information for 6 orphan regulatory proteins only vaguely characterized till now. Hence, we created *Mycobacterium smegmatis* unmarked mutant strain lacking one of orphan regulatory components, MSMEG_5784 protein, a homologue of Rv0818 of *M. tuberculosis* as well as merodiploid strains overproducing Rv0818 and Rv2884 proteins in *M. tuberculosis*. The kinetic of growth, viability and morphological changes were analyzed comparing to "wild type" strain. We also optimized the conditions for purifications of Rv0818 and Rv2884 recombinant proteins in *E. coli* system.

V.P.47

Aminoadipate aminotransferases in the pathogenic fungus *Candida albicans*

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Aminoadipate aminotransferase (AmAA) is an enzyme of alpha-aminoadipate pathway (AAP) for L-lysine biosynthesis that catalyzes reaction of alpha-ketoadipate conversion to alpha-aminoadipate. AmAA may also participate in biosynthesis or degradation of aromatic amino acids and in D-tryptophan based pigment production (possible implications for *C. albicans* pathogenesis). The AAP is unique for fungal microorganisms. Enzymes involved in this pathway can be used as a potential molecular markers or may also become novel targets for antifungal chemotherapy. Understanding the pathway regulation mechanism may also broad the knowledge of β-lactam antibiotics biosynthesis, since aminoadipate is the essential intermediate of their production.
Search of the NCBI *Candida albicans* database resulted in identification of the two ORFs containing putative aminoadipate aminotransferase genes: *ARO8* (ORFs 19.2098 and 19.9645), and *YER152C* (ORFs 19.1180 and 19.8771). *ARO8* from *C. albicans* exhibits 65% identity to *ARO8* from *S. cerevisiae* while *YER152C* exhibits 37% identity to *ARO8* and 52% to *YER152C* from *S. cerevisiae*. The *ARO8* and *YER152C* genes were amplified from the *C. albicans* genome. Both were cloned and expressed as His-tagged fusion proteins in *E. coli*. Basic molecular properties of the purified proteins were determined, including their molecular weight (Aro8CHp 55 kDa, YerCHp 48 kDa*)* and oligomeric structure (Aro8CHp is a dimer). The purified *ARO8* gene product, Aro8CHp revealed aromatic and α-aminoadipate aminotransferase activity and exhibited a broad substrate spectrum. Catalytic parameters of this enzyme were determined: $K_{m(L\text{-}Phe)}$ 0.052±0.001, $K_{m(L\text{-}Tyr)}$ 0.106±0.01, $K_{m(L\text{-}AA)}$ 0.032±0.006) and it was shown that it may also use oxoadipate and alpha-ketoglutarate as amino acceptors. Aro8CHp exhibited pH optimum 8 which is similar to that of aminoadipate aminotransferase from *S. cerevisiae*. Presence of L-lysine and L-canavanine in the assay mixture up to 10 mM had no effect on the enzyme activity whereas L-norleucine and oxalic acid appeared weak inhibitors (IC_{50} = 2.8 mM, 6.3 mM, respectively).

Key words: L-lysine biosynthesis, aminoadipate aminotransferase, *Candida albicans*

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V.P.48

Immunochemical studies of exopolysaccharide produced by *Tsukamurella pulmonis*

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Tsukamurella are strictly aerobic, Gram-positive rods that are weakly or variably acid-fast and are included in the order *Actinomycetales*. Species in the *Tsukamurella* genus share many phenotype features with other species from genera: *Corynebacterium, Nocardia*, *Rhodococcus*, and *Mycobacterium*. *Tsukamurella* are known to be opportunistic pathogen causing severe infection in the humans with immunosuppression such as chronic pulmonary disease, HIV infection, patients which use corticosteroid and carrying foreign bodies like long term indwelling central venous catheters. Until now the genus *Tsukamurella* include 12 species.
The strain of *T. pulmonis* was obtained from Polish Collection of Microorganisms (PCM 2578). The bacteria were identified morphologically by Gram staining, scanning electron microscopy and by MALDI-TOF mass spectrometry. The polysaccharides were extracted by trichloroacetic acid from dry bacterial cell mass and purified by anion exchange and gel permeation chromatography. Sugar composition was determined by gas liquid chromatography-mass spectrometry (GLC-MS). The monoclonal antibodies against polysaccharide of *T. pulmonis* were obtained by the hybridoma technique.
The scanning electron microscopy showed a variation of the rod shape of the bacteria. The bacteria possess hydrophobicity and by exposing the bacteria on xylene the aggregation of the bacteria resulting in the dispersion diminished by 32.7 %, as shown by the reduction in light absorbance of the aqueous phase of bacterial suspension. Purified polysaccharide of *T. pulmonis* consist of arabinose, mannose, and glucose. Monoclonal antibody obtained against polysaccharide was IgM type. Polysaccharide antigen of *T. pulmonis* precipitated in 1.5 μg/ml of monoclonal antibody by quantitative immunoprecipitation test. Results indicate that monoclonal antibodies and polysaccharide could serve as tools for diagnostic purposes.

V.P.49

Interactions between adhesive proteins exposed at the surface of *Candida albicans* cells with human plasma proteins that comprise the contact-activated kinin-generating system

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Bradykinin-related peptides (kinins) are universally involved in inflammatory reactions in the human organism, including those triggered by microbial infections. Kinin formation depends on the simultaneous adsorption of three plasma proteins – high molecular mass kininogen (HK), prekallikrein (PK) and factor XII (FXII) – on the surfaces of blood cells. During infections, the activation of this kinin-generating system (also known as the "contact system") also occurs on the pathogen surfaces. We have recently reported the tight binding of HK by *Candida albicans* – a major fungal pathogen in humans – and identified the fungal cell wall proteins (CWPs) involved in this adsorption phenomenon.

The first aim of our current study was to perform a similar qualification of CWPs in terms of the affinity to two other contact factors, PK and FXII. CWPs were extracted from *C. albicans* hyphae with β-1,3-glucanase. By adsorption of CWPs on PK- or FXII-coupled agarose gels and mass spectrometric analysis of the eluted material, major putative contact factor-binding proteins were identified. These included: (i) typical adhesins from agglutinin-like sequence protein family (ALS3, ALS1), and (ii) atypical (cytoplasm-derived) surface-exposed proteins such as enolase (ENO), elongation factor 2 (EF2), triosephosphate isomerase (TPI), glucose-6-phosphate isomerase (GPI) and phosphoglycerate mutase (GPM).

The second aim of this study was to isolate several fungal surface proteins and characterize their interactions with the three contact system proteins using the surface plasmon resonance (SPR) measurements. The purified proteins were immobilized on CM5 sensor chips of the BIACORE 3000 system and analyzed for HK-, FXII- or PK-binding affinity and rate constants. With this approach, we directly confirmed the binding affinity of HK and FXII to ALS3, ENO and TPI, and of PK to ENO, TPI and GPI.

The current characteristics of the interactions of the host contact system with the fungal pathogen cells indicates potential targets for novel therapeutic approaches.

Key words: contact system, *Candida albicans* adhesins, surface plasmon resonance

V.P.50

Structural basis for the neutralization mechanism of hepatitis E virus by monoclonal antibodies

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Infectious hepatitis viral diseases are major health problems in both developing and developed countries, and are transmitted *via* parenteral and faeco-oral routes. Hepatitis E virus (HEV) is an important cause of severe hepatitis in humans and is responsible for unusually high rates of mortality in pregnant women because of the development of fulminant liver disease. In our work, we have demonstrated that the HEV capsid comprises capsomeres of a homodimeric structural capsid protein (E2) that forms a partially enclosed shell. Using information garnered from the crystal structure, we verified the mechanism by which the E2s domain protrudes from the viral surface to engage with host cells to initiate infection. Following this, we determined the crystal structure of the HEV E2s (I) domain in complex with the 8C11 Fab, a genotype specific neutralizing antibody, at 1.9 Å resolution to identify the 8C11 epitope(s) involved in the binding. Through mutational analysis and cell model assays, we identified the most crucial residue for HEV interaction and the genotype I preferred neutralization by 8C11. Very recently we have studied the broad neutralization of all genotypes of HEV by a cross genotype mAb 8G12. The antibody: antigen complex structure, mutational studies and cell model assays showed that 8G12 equally recognizes all 4 genotypes of HEV and neutralizes the infection. Overall, these findings provided data that was instrumental in the creation of a vaccine for HEV, and will lead to the development of antibody-based specific drugs for the treatment against HEV.

V.P.51

Introducing lipid rafts in pathogenic bacteria with its characterization in *Bacillus anthracis*

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Lipid rafts are dynamic assemblies of specific proteins and lipids, distributed heterogeneously on membrane. Flotillin-1, a conserved raft marker protein in eukaryotes plays a significant role in cellular processes. It comprises of characteristic N-terminal SPFH (Stomatin, Prohibitin, F, and HflK/C) domain and C-terminal oligomerization domain with coiled-coil region. It initiates with transmembrane region and is preceded by NFeD domain of adjacent gene. The domain showing highest identity to above SPFH has been designated as SPFH2a in prokaryotes. In this study, presence of above sequence encoded features of raft marker protein was examined in all pathogenic bacteria (PB). Analysis of 300 pathogenic strains revealed 66 isolates of 20 species with SPFH2a domain, representing its wide but non-ubiquitous distribution in PB. One representative of each species was selected for successive study of Flotillin/oligomerization domain and NFed domain of adjacent gene. Based on identity to above attributes, *Bacillus thuringiensis* and *Bacillus anthracis* (*BA*) appeared to be better candidates for microdomain investigation in PB.

Overwhelming threat of bioterror across the globe led us to further investigation of *BA BAS0525* encoding FlotP. *In silico* and *in vitro* analyses shows its identity to eukaryotic Flotillin-1. *In vivo* studies revealed FlotP as a membrane protein restricted to Detergent Resistant Membrane (DRM) fractions, favoring its presence in lipids and signaling proteins rich regions. Heterogeneous distribution of FlotP was observed on membrane in punctuate manner. Constitutive expression of FlotP at RNA and protein levels suggested its critical role in vital cellular processes. Simultaneously, we also observed the effect of various sterol inhibitors on membrane rigidity as well as signalling responses. All of these features cumulatively appear to favor eukaryotic microdomain kind of entity. This is the first report of any raft marker protein in PB of global concern. Its cellular needs, membrane exposed attributes and microdomain association is likely to provide an attractive approach to control bacterial infections and better therapeutic management by targeting lipid microdomains and FlotP in such Flotillin harboring pathogens.

Key words: pathogen, *Bacillus anthracis,* Flotillin, SPFH, DRM, FlotP, Microdomain, Lipid Raft

V.P.52

Prevalence of intestinal parasitic infections in rehabilitation centers in Golestan Province North of IRAN

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The overall infection rate of intestinal parasite was 12.3% (24 out of 196 subjects; 7.1% in males and 5.2% in females). *Blastocystis hominis* (4.2%) and *Giardia lamblia* (3.1%) were found the most commonly intestinal protozoan infection and the lowest prevalence of *Chilomastix mesnili* (0.5%). In this study prevalence of the pathogenic protozoan and worm were 9/3%. Prevalence of parasitic infections were higher particularly intestinal protozoa under 9 years of age and 29–20 years (4.1%) than in 40 years (0.5 %) ($P<0.001$). Parasitic infection in mentally rehabilitant was 9.2% and the physically exercise – mentally 2.5%. Intestinal parasites infection among the disabled Elderly and people with Down syndrome were not observed. Specific infection rate in mentally handicapped was 75% and physically exercise - mentally 21%.

Although the prevalence of intestinal parasites among disabled rehabilitation centers is not high compared to other social groups in Golestan province. However, due to complications from diseases such as Amoebiasis, Giardiasis and other parasitic disease, personal and social hygiene is essential.

Key words: intestinal parasitic infections, rehabilitation centers; Golestan Province

V.P.53

Genotype Identification of *Echinococcus granulosus* from paraffin-embedded tissues of hydatid cysts isolated from human by PCR-RFLP

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Background: Identifying the various genotypes of *Echinococcus granulosus* as the agent of hydatid cysts in endemic areas can influence the disease control programs, particularly in humans. Therefore, this study was conducted to identify the different genotypes of *Echinococcus granulosus* from paraffin-embedded tissues of hydatid cysts isolated from human by PCR-RFLP.
Materials and Methods: To identify the molecular characteristics of *Echinococcus granulosus*, tissue samples from 30 human patients infected with hydatid cysts were collected from hospitals across the province of Golestan. DNA was extracted and characterized by PCR-RFLP method. In this study, 3 restriction endonuclease enzymes were used.
Results: PCR product obtained from amplification of *Echinococcus granulosus* rDNA-ITS1 from human hydatid cysts showed two different patterns of DNA bands in human isolates. In spite of the difference between human isolates in the size of DNA bands (1000 base pairs), the use of BD1/4S and EGF1/EGR2 primers showed that these isolates are to some extent similar in the size of band (391 base pairs). PCR products by RFLP method showed a different pattern of genotype or strain with Taq1 restriction enzyme in human isolates. No change in the size of DNA bands were observed with Msp1 and Alu1 restriction enzyme in human isolates.
Conclusions: Therefore, genotypic differences and similarities between the size of DNA bands of *Echinococcus granulosus* from human isolates with PCR-RFLP method indicated the occurrence of different genotypes of *Echinococcus granulosus* in different parts of Golestan Province.

V.P.54

Epitopes common for pathogens and some allergens from food

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The epitopes from 4 sources were searched in the UniProt database: wheat, egg, Baltic cod and shrimp proteins. The shortest sequences (5–7 amino acid residues) were found in a lot of proteins being not homologues of primary precursors of these epitopes (cod parvalbumins, wheat gliadins, shrimp tropomyosin or ovomucoid and ovoalbumins from egg white). Short sequences are present inter alia in proteins of pathogens and parasites according to hygiene hypothesis. There is also a theory that short sequences (pentapeptides) can be a basic fragments responsible for interaction between proteins and antibodies. The longer sequences (eight and more amino acid residues) are present only in proteins homological to primary precursors.

Key words: allergens, epitopes, proteins

Acknowledgements: This work was supported from the own founds of the University of Warmia and Mazury in Olsztyn.

V.P.55

The influence of ectromelia virus infection on the distribution and activation of NF-κB transcription factor in BALB/3T3 murine fibroblasts

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Ectromelia virus (ECTV), a causative agent of mousepox, is a member of *Poxviridae* family.
Poxviruses are human and animal pathogens, which can be used as vaccine vectors. Poxviruses are also extensively studied due to their immunomodulatory properties. NF-ϰB, a pleiotropic transcription factor, is a regulator of inflammatory response and cell survival. It is also a target for pathogens, including poxviruses.
The aim of our study was to investigate the effect of ECTV on NF-ϰB transcription factor intracellular distribution and activation in BALB/3T3 murine fibroblasts.
In our work, a highly virulent Moscow strain of ECTV (ECTV-MOS) was used. BALB/3T3 cells were infected with ECTV-MOS at m.o.i.=2. At 18 h.p.i. cells were either left unstimulated or were treated with TNF-α (2 ng/ml, 10 or 30 min). Cells were fixed with 4% PFA, stained, and analyzed by immunofluorescence. For Western Blot analysis of inhibitor ϰBα (IϰBα) and phopsho-IϰBα content, total cell extracts were prepared.
We observed that p65 NF-ϰB is not recruited to viral factories and ECTV does not affect NF-ϰB p65 colocalization with F-actin. ECTV also prevented TNF-α-induced IϰBα phosphorylation. We could also observe that syncytia formation during ECTV infection was correlated with NF-ϰB inhibition. Studying viral interference with cellular pathways may serve drug target identification and may be helpful in vaccine development.

Key words: ectromelia virus, BALB/3T3 cell line, NF-ϰB

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V.P.56

Physical state of human papillomavirus (HPV-16) in cervical intraepithelial lesions and cancers determined by two different quantitative real-time PCR methods

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Determination of physical state of HPV-16 genome may be used to assess the risk for progression to cervical neoplasia and cancer. Therefore the aim of the study was to analyse the correlation between real-time PCR (qPCR) and multiplex real-time PCR (multiplex qPCR) for detection of HPV-16 status.
The study was performed on 100 HPV-16 positive samples with cervical intraepithelial lesions and carcinomas. TaqMan-based qPCR and multiplex qPCR were used to assess viral E2 and E6 sequences. To generate standard curves for these sequences the serially diluted DNA plasmid (pBR322-HPV-16, ATCC 45113) was used in three replicates for each dilution point.
The load of E2 and E6 were determined by the number of copies normalized to the number of cells, assessed by qPCR targeting the RNase P open reading frame. The physical state of viral genome was estimated as a ratio of E2 copies number per cell to E6 copies number per cell.
Among 100 analysed samples, there were no statistically significant differences in the E2 and E6 viral load evaluated by qPCR and multiplex qPCR ($p=0.41$ and $p=0.34$), the correlation coefficients were at the level of 0.94 and 0.97, respectively. There were 96% of samples with mixed and 4% with episomal state of viral genome detected by qRT-PCR, e and 90% and 10% found by multiplex qPCR. These differences were not statistically significant ($p=0.1$). The sensitivity and specificity of multiplex qPCR were 93.7% and 100% as compared to qRT-PCR, positive predictive value was 100%.
In summary, the multiplex qPCR seems to be a better method compared to qPCR, which could be used for cervical cancer screening because it allows for simultaneous evaluation of the two HPV genes in one reaction tube thereby reducing the cost of the test.

Key words: HPV-16 physical status, cervical carcinogenesis, multiplex real-time PCR

V.P.57

Determination of the influence of the resistant dextrins on the rat's intestinal microflora

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The objective of the present study was to observe whether the diet based on the consumption of resistant dextrin can show differences in the gut microbiota of rats in comparison with microbiota of rats based on a basic diet. Rats were reared in the Institute of Animal Reproduction and Food Research in Olsztyn.
Resistant dextrin, that was added to the forage fed to dextrin group of rats, was obtained during the process of pyroconversion and chemical modification of potato starch at Jan Dlugosz Academy in Czestochowa. Pyroconversion was carried out for 120 minutes at the temperature equal 130°C. Chemical modification was done with the use of 40% tartaric acid and 0.1% HCl as a catalyst for the reaction.
The project was performed by observations and calculations of colony forming units: total number of bacteria, *Lactobacillus*, *Bifidobacterium*, *Enterobacteriaceae*, *Enterococcus*, *Clostridium*, *Escherichia coli*.
After obtaining the results, the influence of the uptake of resistant dextrin was observed. The slight increase in beneficial *Bifidobacterium* amount together with significant boost of another favourable bacteria *Lactobacillus* of magnitude for dextrin group in comparison to control one revealed, that resistant dextrin can be utilised by those bacteria in order to stimulate their growth. On the other hand, the decrease in the rest of bacteria was observed in rats' feaces after the consumption of the diet rich in resistant dextrin. The most significant difference was observed in the amount of *Clostridium*, which turned out to be lower for dextrin-fed rats than control groups by more than one order of magnitude.
From obtained results it can be concluded, that analysed resistant dextrin display prebiotic properties. It increased amount of beneficial bacteria (*Lactobacillus* and *Bifidobacterium*), decreasing simultaneously unfavourable ones (*Clostridium*).

Key words: resistant dextrins, microflora, rats

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V.P.58

Probiotic preparation reduces faecal water genotoxicity and cytotoxicity in chickens fed ochratoxin A contamined feed (*in vivo* study)

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The aim of the present study was to assess the genotoxicity and cytotoxicity of the faecal water of chickens fed ochratoxin A (OTA) contaminated feed with and without probiotic preparation. The probiotic preparation consisted of (per 1 kg): 10^{10} *Lactobacillus* cells and 10^6 yeast *Saccharomyces cerevisiae*.
The study was performed on 20 healthy female Ross broiler chickens divided into 6 groups, 5 animals each, kept separately at the Institute of Animal Physiology and Nutrition. The groups were: control – chickens fed with non-supplemented feed or with the probiotic preparation; OTA – chickens fed feed contaminated with 1 or 5 mg OTA/kg; OTA + probiotic – chickens fed feed contaminated with 1 or 5 mg OTA/kg and supplemented with the probiotic preparation.
Genotoxicity was measured using the comet assay. Feces of chickens fed with feed without additives, and supplemented with probiotic preparation caused DNA damage level 5–6%. Feces of chickens fed with feed with ochratoxin A, bothat 1 and 5 mg/kg level of DNA damage generated to 8–9%. The addition of probiotic preparation for OTA contaminated feed contributed to a statistically significant reduction of genotoxicity, even to the level found in control samples.
Cytotoxicity was measured using the MTT tests. Feces of chickens fed with feed without additives, and supplemented with probiotic preparation exhibited cytotoxicity in relation to the Caco-2 cells, respectively 28% and 22%. The cytotoxicity of fecal contaminated feed chickens fed with 1 mg OTA/kg was 42%, and in the case of a higher dose of toxin 54%. The addition of the probiotic preparation to feed contaminated with 1 or 5 mg OTA/kg reduced the cytotoxicity of fecal to 28% for the lower dose of the toxin, and to 39% at 5 OTA mg/kg of feed. Feed supplementation with probiotic preparation is therefore significantly reduced the cytotoxicity of feces.

Key words: probiotic, ochratoxin A, genotoxicity and cytotoxicity, chickens

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V.P.59

Increased concentration of Taq DNA polymerase as a solution for GC-rich templates from clinical and environmental samples

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DNA polymerase is an enzyme which plays crucial role in replication and DNA repair. It found application in PCR (polymerase chain reaction) where catalyses process of *in vitro* DNA synthesis. To meet the demands posed by modern diagnostic, molecular biology or genetic engineering it is necessary to improve DNA polymerases to obtain new or better features useful in these fields. So far implemented modifications in majority are based on improved reaction buffers, PCR enhancers and mutagenesis of these proteins. These modification lead to obtaining enzymes with higher thermostability, used for long or difficult amplicons (like GC-rich templates) or resistant to inhibitors from clinical and environmental samples.

The aim of our study was determined the influence of native *Taq* DNA polymerase concentration on its properties to amplification difficult amplicons, especially GC-rich temples. *Taq* DNA polymerase was produced in *E. coli* cell, purified with using metal affinity chromatography and concentrated to a dozen units per microliter. Amplification difficult amplicons was determined by PCR with GC-rich templates in the gradient concentration of *Taq* DNA polymerase. Obtained results will be compared to commercial modified polymerases with particular reaction buffer for difficult amplicons.

The present studies suggest that it is not necessary to used modified polymerase or improved reaction buffer to amplification difficult amplicons (GC-rich templates). A solution is used to exceeded amount of polymerase.

Key words: *Taq* DNA polymerase, GC-rich templates, PCR

V.P.60

Prevalence of bacterial agents isolated from different parts of the hospital staff Hand and jewelry in Hakim Jorjani Hospital of Gorgan North of IRAN

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Introduction: Hospital acquired infections (HAI) are a significant cause of increased morbidity and mortality in hospitalized patients. Although eradication of HAI is impossible, a well-conducted surveillance and prevention program may significantly reduce HAI and as sociated costs.

Methods: During a three-month period, using the swab method, 100 persons were taken from different parts of the hospital. All samples during the three-stage sampling (In the beginning, at working, in the end work) were examined for the presence of bactria spp. After isolation, bacteria spp. were identified using several phenotypic and biochemical tests. Antibiotic susceptibility test was done by disk diffusion method according to CLSI-2011 guidelines.

Result: In this study from 100 swab samples, 35 men (35%) and 65 women (65%) respectively. In total, the result of 65 (65%) persons were positive. in three stages 159 isolates were obtained that 40 people (61.53%) were belonging to gram positive coagulase-negative *Staphylococcus* and 12 people (18.46%) were *Staphylococcus aureus* and 13 people (20%) were Gram negative. Most of the isolates were obtained from Hand and jewelry of staff, respectively.

Discussion: As for increasing rate of Health care Associated Infections (HAI) caused by bacterial spp. and spread of distribution of the high resistance strains in the hospital environments, it seem necessary employment of infection control procedures to eliminate potential sources of infection for preventing of HAI.

V.P.61

Coordination compounds of Co (III) with N, N'-donor organic ligands exhibit significant antimicrobial activity

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The cobalt coordination compounds with organic ligands are interesting and attractive as potential antimicrobial and antibiofilm agents. Two types of coordination complexes trans-$[Co(N,N)_2Cl_2]Cl$, where N,N is ethylenediamine, and 1,3-diaminopropane as the chelating moiety are new variants of this class of chemical compounds. Recent studies involving biological tests with coordination compounds of Co(III) with N,N'-donor organic ligands (using microbroth dilution method and MicroPlates, Phenotype Microarrays for Microbial Cells) have revealed antibacterial and antifungal activity. The results also demonstrated susceptibility of bacteria forming biofilms on this type of coordination compound. The search for resistant clinical strains of bacteria on complexes of Co (III) with organic ligands was performed on a broad spectrum of clinical strains of both Gram positive (*Staphylococcus aureus* MRSA, *Enterococcus faecium* vancomycin/linezolid-resistant, *Enterococcus faecalis* vancomycin-resistant) and Gram negative (*Escherichia coli, Proteus mirabilis, Proteus vulgaris, Pseudomonas aeruginosa, Serratia marcescens, Klebsiella pneumoniae*) bacteria. All of the tested clinical strains were sensitive, with the exception of clinical isolates of *Enterococcus faecium* and *Enterococcus faecalis*.
For comparison purposes, the antimicrobial activity of the Co (III) complexes was determined by fluorescence optical respirometry (FOR). FOR is based on the analysis of a fluorescence oxygen sensitive biosensor, the fluorescence of which is dependent on the amount of oxygen in the sample under test. Molecular oxygen is a fluorescence quencher. Growing microorganisms consume the oxygen in the culture, thus affecting the intensity of fluorescence of the sample. By analysing the variation in the intensity of fluorescence in the cultures one can track the metabolic activity of microorganisms, and thereby examine the effect of different chemicals. The method allows for more efficient samples analysis and significantly reduces time compared to the traditional method.
Studies using Transmission Electron Microscopy (TEM) exhibited well visible contraction of the bacterial cells after the application of the tested complexes.

Key words: Co(III) complexes, antimicrobial activity, fluorescence optical respirometry

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V.P.62

Monitoring of *H. pylori* infection in guinea pig model by culture and molecular ureC/cagA determinants

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Helicobacter pylori (Hp) causes gastric diseases in humans. For studying Hp pathogenesis an appropriate animal model is required. In this study we adjusted conditions for monitoring Hp infection in guinea pigs inoculated with these bacteria. We used culture method and polymerase chain reaction (PCR) for amplifying of Hp *cagA* and *ureC* gene sequences. Guinea pigs were inoculated *per os* with Hp CCUG17874-Hp74 (6) or ATCC700312-HP12 (6) in 0.85% NaCl (3) or *Brucella* broth (3). Control animals received only salt (2) or broth (2). After 7 or 28 days post infection, the animals were euthanized. Stomachs were isolated and gastric mucus as well as pylorus, body and bottom specimens were collected. Tissues were homogenized and plated on agar medium for Hp cultivation. Colony morphology was evaluated after 7 day culture (37°C, microaerophilic conditions). Bacteria were stained by Gram-staining procedure and the urease, catalase and oxidase activity was determined. DNA was isolated from tissues. Hp *ureC* and *cagA* sequences were amplified, electrophoresed through agarose gel with propidium iodide and identified in UV light. Hp was not detected by culture and PCR in control animals. By comparison guinea pigs inoculated with Hp74 or Hp12 (as broth or salt suspensions) were colonized with these bacteria predominantly in the pylorus area. Bacteria isolated from gastric specimens, which were collected 7 and 28 days post infection, produced catalase and oxidase. Tree isolates derived from animals 7 days post infection were urease-negative similarly as 2/12 samples of gastric mucus. DNA from animals inoculated with Hp contained *ureC* and *cagA* sequences. Culture and PCR methods indicated that guinea pigs were successfully colonized with Hp 7 and 28 days post infection. These methods enable monitoring of Hp infection in guinea pigs, which consist a suitable model for investigation of Hp-related immune responses.

Key words: *H. pylori,* guinea pig model, culture, PCR

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V.P.63

The Bcl-2 family protein expression in L929 fibroblasts after ECTV-MOS infection: research *in vitro*

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Apoptosis is strictly controlled by molecular pathways that involve pro- and anti-apoptotic proteins. Bcl-2 family includes proteins that play regulatory role in the process of apoptosis induction *via* mitochondrial pathway. Bax and Bak are pro-apoptotic members of Bcl-2 family while Bcl-2 and Bcl-xL are anti-apoptotic ones. Cells susceptibility to apoptotic stimuli depends on the ratio between pro- and anti-apoptotic proteins, such as Bax and Bcl-2, respectively. Ectromelia virus (ECTV) belongs to the *Poxviridae* family and *Orthopoxvirus* genus and it is closely related to Variola virus (VARV). The aim of this study was to determine the expression of proteins from Bcl-2 family after ECTV-MOS infection. We used highly virulent Moscow strain of ECTV (ECTV-MOS) to infect L929 cells (ATTC CCL-1). In cells at 4, 8, 12, 18 and 24 hour post infection (h.p.i) as well as in control cells, the level of Bcl-2 family protein were measured using flow cytometry. In the first 18h.p.i we did not observe any significant changes in the cellular level of Bcl-2 and Bcl-xL in comparison to control. At 24 h.p.i. the level of both Bcl-2 and Bcl-xL markedly increased. Our results indicate the participation of anti-apoptotic Bcl-2 family members in the viral strategy of apoptosis inhibition and correspond with our previous immunofluorescence observation that the percentage of apoptotic cell in L929 fibroblasts population did not significantly change.

Key words: apoptosis, Bcl-2; Ectromelia

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V.P.64

Chaperone properties of HtrA homologs from *Helicobacter pylori, Escherichia coli* and *Stenotrophomonas maltophilia*

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Bacterial populations are frequently exposed to fast and drastic changes of their environment. The unfavorable conditions e.g. heat or oxidative stresses, usually lead to formation of improperly folded proteins, which can exhibit cytotoxic effects. To survive, bacteria produce numerous stress proteins, including chaperones and proteases. To this group belongs HtrA, a periplasmic protein displaying serine protease and chaperone-like activities. The presence of HtrA was shown to be necessary for many pathogenic bacteria to endure stressful conditions and colonize a host organism.

Since the HtrA protein in a major periplasmic protein quality control protease whose synthesis and activity is regulated in response to extracytoplasmic stresses, we assumed that biochemical properties of HtrA should reflect adaptation to variable environmental conditions.

I have tested the chaperone activity of HtrA from three bacterial species: *Helicobacter pylori*, *Escherichia coli* and *Stenotrophomonas maltophilia.* Although all these species can inhabit human body, their optimal niches differ significantly. Thus, the biochemical properties of HtrA may differ as well.

For assays the proteolytically inactive HtrA variants and reduced lysozyme as a substrate were used. The HtrA protein from *E. coli,* a well described homolog served as a control. The relationship between the activity, temperature and pH was studied at 30, 37 (temperature of the human body) and 42ºC at pH 5.0; 6.2; 7.0 and 8.0 (pH 6.2 is a value characteristic for the periplasm of *H. pylori*). The dependence on pH was tested at the range of 5 to 9.5 and at a temperature of 37ºC.

My studies showed that all HtrA homologs exhibited chaperone activity and prevented aggregation of lysozyme at all tested pH values. In contrast to HtrA from *E. coli* and *H. pylori*, HtrA from *S. maltophilia* was not able to suppress the substrate aggregation at elevated temperature (42ºC).

Key words: chaperone, protein aggregation, stress factor

V.P.65

Multiplex real-time PCR for distinguishing CMV reactivation from reinfection with different strains after allogeneic haemopoietic stem cell transplantation (allo-HSCT)

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Human cytomegalovirus (CMV) infection remains the leading cause of serious infectious complications after allo-HSCT. These infections in CMV-seropositive recipients can be due to reactivation or reinfection.

Different CMV strains were identified by determining the genotypes isolated from repeatedly tested patients. The UL55 sequences encoding the glycoprotein B (gB) have been chosen as the target gene. The region in which the gB precursor protein is cleaved into two fragments by cellular endoprotease, is characterized by genetic variability, and based on that CMV is classified into four major genotypes: gB1, gB2, gB3 and gB4. For CMV gB, genotyping multiplex real-time PCR assay was used, in which the mixture of primers and probes allowed the simultaneous detection of not only the genotypes but also their quantitative assessment, which in case of co-infection gave the opportunity to determine the mutual quantitative proportions between genotypes.

The study was carried out in 30 allo-HSCT recipients from whom 105 isolates of CMV DNA were genotyped. In 40% of recipients superinfections were detected. Genotype gB1 dominated, and for mixed infections it was characteristic that the genotype gB3 or gB4 was always present. Although there were no significant differences in the values of load for each genotype, in case of co-infection the number of copies of gB1 genotype was significantly higher as compared to singular gB1 infection ($p=0.02$). In patients with mixed genotypes, chronic CMV infections were observed more often, as well as acute/chronic form of GvHD (graft versus host disease), and also antiviral treatment results were less effective. This adverse effect observed in cases with mixed infections could be related to the presence of gB3 and gB4 genotypes, which gives, as already suggested by other authors, greater immuno- and myelosuppression in comparison to genotypes gB1 and gB2.

Key words: CMV superinfection, gB genotypes, viral load

V.P.66

Fibrinolytic activity of novel extracellular protease of *Microbacterium* sp.

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The fibrinolysis plays a crucial role in maintaining of cardiovascular system homeostasis by equilibration of coagulation cascade. The process is driven by endogenous factors present in blood and tissues that dissolve fibrin clots. However, increased fibrinolysis can also occur during bacterial infection as a result of pathogen proteases action. Such an activity against fibrin clots exhibits culture media after *Microbacterium* sp. strain JK6B cultivation.

The total pool of the extracellular proteins secreted to the culture medium was precipitated with ammonium sulphate and purified using ion exchange chromatography and gel filtration. The proteolytic activity was tested with respect to proteins and synthetic chromogenic substrates. Fibrinolytic activity was measured by spectrophotometric monitoring of degradation of fibrin clots formed in 96-well plates from fibrinogen treated with thrombin and zymography with fibrinogen. The effect of protease inhibitors and metal ions on the activity of the enzyme was examined. Protease gene was sequenced and cloned into pLATE31 vector and expressed in *Escherichia coli* BL21(DE3).

Culture media and purified protease were active towards to fibrinogen, azocasein, synthetic substrates S-2251 and S-2765, as well as have an ability to dissolve fibrin clots. Sequencing of N-terminal part of purified protein allowed to find its homologues in other *Microbacterium* strains annotated as metallopeptidases. Metallopeptidase inhibitors (EDTA, phenanthroline) substantially reduced the activity towards azocasein and S-2251, whereas metal ions (Ca^{2+}, Mg^{2+}) caused a minor increase in the activity. The recombinant protein was produced mainly in insoluble form as inclusion bodies. However the fibrinolytic activity was present also in the soluble fraction of cell lysate as confirmed by zymography with fibrinogen.

Fibrinolytic activity of the metalloprotease from *Microbacterium sp.* strain JK6B was confirmed. Both, purified from *Microbacterium* and recombinant proteases, are enzymatically active and able to fibrin degradation.

Key words: bacterial protease, fibrinolytic activity, metalloprotease

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