V.O.1

Comparison of antibiotic resistance and virulence between biofilm-producing and non-producing clinical isolates of *Enterococcus faecium*

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An increase in the antibiotic resistance among *Enterococcus faecium* strains has been observed worldwide. Moreover, these bacteria have the ability to produce several virulence factors and the ability to form biofilm that play an important role in human infections. The aim of this study was to compare the antibiotic resistance and the prevalence of selected virulence genes between biofilm-producing and non-producing *E. faecium* strains.

Ninety *E. faecium* strains were investigated. Identification and susceptibility testing were conducted by VITEK2 system. Biofilm forming ability was determined using two methods: the tube method and Congo red agar method. Then, isolates were divided into two groups: biofilm-positive (BIO+, n=70) and biofilm-negative (BIO–, n=20) strains. Genes encoding surface protein (*esp*), aggregation substance (*as*), surface adhesin (*efaA*), collagen adhesin (*acm*), gelatinase (*gelE*) and hialuronidase (*hyl*) were investigated by PCR amplification. Chi-square test was used in statistical analysis.

BIO+ isolates were resistant to beta-lactams, whereas 10% BIO– strains were susceptible to ampicillin (statistically significant difference, p = 0.007) and 5% – to imipenem. Resistance to gentamicin was detected in 75.7% BIO+ and 60% BIO– strains, to streptomycin – in 91.4% BIO+ and 85% BIO– strains (insignificant differences, p>0.05). Linezolid and tigecycline had the highest activity against tested isolates.

All of *E. faecium* strains carried two or more of the virulence genes. *ace*, *efaA* and *gelE* genes occured more frequently in BIO– strains (*ace* in 50% BIO+ *vs* 75% BIO–, *efaA* – 44.3% *vs* 85%, *gelE* – 2.9% *vs* 15%, respectively), while *hyl* gene appeared more frequently in BIO+ isolates (87.1% BIO+ *vs* 65% BIO–). These differences were statistically significant (p<0.05). No statistically significant differences were found in the case of *esp* and *as* genes.

BIO+ strains are more resistant to antibiotics than BIO– strains, but interestingly, BIO– isolates are characterized by a higher virulence potency.

Key words: *Enterococcus*, biofilm, virulence

Acknowledgements: This work was supported by funds from Leading National Research Center (53-KNOW-2013) in Bialystok.

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V.O.2

Biological activity studies of surfactin – biosurfactant produced by *Bacillus subtilis*

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Introduction: The purpose of the study was to examine the biological activity of cyclic lipopeptide – surfactin, produced by *Bacillus subtilis* strain in certain conditions. This biosurfactant acts as an antibiotic. The anticancer, antibacterial and antifungal properties of surfactin are utilized in medicine and biotechnology. This fact was the starting point to undertake the research in a microbiological laboratory.
Materials and methods: *B. subtilis* liquid culture in mineral medium with 10 g/L glucose as a carbon source and 2.5 g/L $NaNO_3$ as a nitrogen source was incubated for 14 days in 37°C. The culture was centrifuged (4000 rpm, 25°C, 20 min) and acidified to pH 2.0 with 1 M HCl. After that, the culture was left for 24 hours in a fridge and centrifuged again (4000 rpm, 4°C, 10 min). Then, each with 0.1 g of lipopeptide extract was extracted by utilizing $CH_3COOC_2H_5$ (25 mL). The extract was dissolved in MeOH and analyzed by TLC in the development system: $CHCl_3$/MeOH/NH_4OH (80/25/4, v/v/v). The secondary amine groups were visualized with ninhydrin and the value of R_f was calculated. Diffusion – cutters tests were performed with concentrations of surfactin of 2.5–50 mM on selected 5 bacteria stains on plates with a Mueller-Hinton medium. Similarly, tests were also carried out on 5 fungi and molds species on plates with a Sabouraud medium with chloramphenicol.
Results: The results of this study demonstrated, that only *Escherichia coli* and *Fusarium culmorum* are sensitive to surfactin in the examined concentration range. The greatest zone of inhibition of these species was observed with a surfactin concentration of 50 mM. Other species are characterized by resistance to the action of the surfactant in the examined concentration range.

Key words: surfactin, *Bacillus subtilis*, biological activity

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V.O.3

Evaluating the immunogenicity and protective efficacy of PLGA (85:15) nanoparticulate *Brucella* vaccine containing rL7/L12 protein of *Brucella abortus*

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There is a compelling need for adjuvants or delivery vehicles suitable for human use to enhance the efficacy of vaccines in the prevention of life threatening infection. In this study, we have tried to explore the immunogenic potential of PLGA (poly lactic-co-glycolic acid) nanoparticles (NPs) entrapping rL7/L12 protein, an immunodominant antigen of *Brucella*. It was observed that the formulation is able to elicit high titers right after the first immunization also there was a good elicitation of the Th1 cytokines: IFN-γ and TNF-α. The formulation therefore primes the Th1 or cellular wing of immune response; isotypes of IgG antibody IgG1/2a ratio showed a mixed Th1/Th2 profile. In the lymphocyte stimulation assay performed *in vitro,* the splenocytes of the immunized mice showed a high proliferation index of 1.5 as compared to the control which reveals that L7/L12 filled PLGA NPs are potent inducer of inflammatory and protective responses necessary to combat *Brucella* infection. CFU estimation done after plating the splenocyte suspension on Tryptic Soya agar (TSA) showed that the mice immunized with the protein filled NPs inhibit infection significantly giving less splenic CFU of *B. abortus* 544 as compared to the control group. So there is much promise in this approach, but there is much work to be done in order to optimize the *in vitro* release kinetics to make it practically a slow releasing vehicle of the best and most potent set of *Brucella* antigens.

Key words: *Brucella*, rL7/L12, PLGA, nanoparticle, Th1 response, IFN-γ, TNF-α

Abbreviations: PLGA, poly lactic-co-glycolic acid; FDA, Food and Drug Administration; APCs, antigen presenting cells; CMI, cell mediated immunity; CFU, colony forming units; TSA, tryptic soya agar; NPs, nano particles; DLS, differential light spectroscopy

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V.O.4

Klebsiella pneumoniae: characteristics of carbapenem resistance and virulence factors

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Klebsiella pneumoniae, known as a major threat on public health, is the most common factor of nosocomial and community acquired infections. In this study, 50 *K. pneumoniae* clinic specimens isolated from bronchobic, urea, blood, catheter, rectal, bile, tracheal and wound cultures were collected. These isolates were identified and carpabenem resistance was determined via automated system, CHROMagar Orientation and CHROMagar KPC. The carbapenemase gene regions (blaIMP, blaVIM, blaOXA, blaNDM and blaKPC) and presence of virulence factors (magA, k2A, rmpA, wabG, uge, allS, entB, ycfM, kpn, wcaG, fimH, mrkD, iutA, iroN, hly ve cnf1) of the isolates were determined by using Multiplex-PCR.

OXA-48 carbapenemase gene region were determined in 33 of 50 *K. pneumoniae* strains. In addition for *K. pneumoniae* isolates only NDM-1 resistance in 1, OXA-48 and NDM-1 resistance in 4 unusual isolates were detected. Virulence gene regions that encountered among *K. pneumoniae* isolates were 88% wabG, 86% uge, 80% ycfM and 72% entB, related with capsula, capsula lipoprotein, external membrane protein and responsible for enterobactin production, respectively. Even, there was no significant difference between resistant and sensitive strains due to the virulence gene regions ($P \geq 0.05$), virulence factors in carbapenem-resistant isolates were found to be more diverse. This study is important that both to prevent spread of carbapenem resistant infections and to plan for developing effective treatments. Moreover, this study is the first detailed study of the carbapenem resistance and virulence factors in *K. pneumoniae* strains.

Keywords: *Klebsiella pneumoniae*, multidrug carbapenem resistance, virulence factors

Acknowledgements: This work was supported by Hacettepe University Scientific Research Project grant 014D01601002 and The Scientific and Technological Research Council of Turkey (TUBITAK) with PhD Scholarship (2211/C).

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V.O.5

The surface expression of CD25 (IL-2R) as a molecular marker of NK cell's cytotoxic activity

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Cytotoxic activity is one of the major functions of Natural Killer (NK) cells and critical effector mechanism of innate immune responses against bacteria and virus infected or cancer cells. We can measure NK activity on the basis of their direct lytic effect on target cells, quantification of soluble cytotoxicity-related markers and cytokines, or indirectly be the estimation of viable target cells prior cytotoxic assay. Still the utility of CD25 marker as an indicator of cytotoxic activity has not been yet established.
Since, NK cells express IL-2R we aimed to evaluate, whether the variations in CD25 (IL-2R) expression on human NK cells is correlated with their functional cytotoxic activity measured by classic methods and if this surface molecule might serve as a new tool to quantify NK cell activity.
Peripheral blood mononuclear cells from 40 donors were isolated and cultured overnight with or without the standard *E. coli* O:55 lipopolysaccharide (LPS) (25 ng/ml). Nonadherent lymphocyte fractions were used as effector cells towards HeLa targets in 4-hour cytotoxic assay measured by MTT reduction assay. Simultaneously lymphocytes were stained (30 min, 4°C) with murine anti-CD3-Cy-5, anti-CD25-PE and anti-CD56-FITC monoclonal antibodies (eBioscience) and analyzed by flow cytometry (LSR2, BD) to quantify CD25-positive cells within total NK cell population ($CD3^{-}CD56^{+}$). Granzyme B, FasL (GenProbe), IFN-γ and IL-2 (R&D Systems) were measured in supernatants by ELISA.
Our results revealed positive correlation between LPS-driven enhancement of NKs cytotoxic activity measured by MTT assay and the expansion of CD25-positive NK cells ($p<0.05$, $r<0.5$). Positive correlation was also observed between CD25+ NKs and the upregulation of granzyme B ($p=0.0003$), IFN-γ ($p=000.4$) and IL-2 ($p=0.0009$) ($p<0.05$, $r<0.5$) but not in regard to the FasL production ($p>0.05$, $r>0.5$).
This phenomenon is a result of LPS-induced NK cell activation, and according to our study, may serve as a surface marker of bacteria-activated NK cells.

Key words: Natural Killer cells, cytotoxic activity, CD25, MTT, cytokines

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V.O.6

Multiresistant *Elizabethkingia meningoseptica* infections in tertiary care

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Background: *Elizabethkingia meningoseptica* is a ubiquitous Gram negative bacteria which is paradoxically susceptible to antibacterials for Gram positive bacteria. Though found in diverse environments, it does not constitute human microflora and is an emerging multiresistant pathogen known to cause a multitude of infections especially in immunodeficient hosts.

Results: *Elizabethkingia meningoseptica* was isolated from pleural fluid of a renal allograft recipient, continuous ambulatory peritoneal dialysis fluid in a patient of chronic kidney disease and post-surgery blood cultures in salpingectomy and mitral valve replacement cases. Coexistent ESBL, AmpC and MBL alongwith resistance to polymyxins and tigecycline were observed. Paradoxical susceptibility to sulfamethoxazole-trimethoprim (SXT) and cefoperazone-sulbactam facilitated treatment.

Discussion: Multiresistant *Elizabethkingia* infections are known to occur under aggressive Gram negative antimicrobial cover and can be potentially untreatable. Alternative prolonged combination therapy with SXT, rifampicin, quinolones, piperacillin-tazobactam, minocycline, macrolides, clindamycin and novobiocin is dependent on paradoxical susceptibility. Antimicrobial susceptibility testing is difficult as these drugs are neither routinely considered for Gram negative organisms nor they are available in automated system panels. Further, no CLSI guidelines exist for testing and interpretation. Dedicated efforts targeted at early diagnosis and surveillance are required to optimize management and control of *Elizabethkingia* infections.

Key words: *Elizabethkingia meningoseptica*, paradoxical susceptibility, metallo-β-lactamase (MBL), extended spectrum β-lactamase (ESBL)

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