ANTIBACTERIAL DRUGS EFFECTS ON *K. PNEUMONIAE* PLANKTONIC CELLS ABILITY TO FORM SECONDARY BIOFILMS

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Biofilms are complex communities of surface-associated cells enclosed in a polymer matrix containing open water channels. Bacteria growing in biofilms exhibit increased resistance to antimicrobials and host immune response compared to their freeliving, planktonic counterparts due to several reasons like restricted penetration of antimicrobials into a biofilm, decreased growth rate, and expression of possible resistance genes. Effective strategies to prevent or control biofilms on medical devices must take into consideration the unique and tenacious nature of biofilms. *Klebsiella pneumoniae* is an important biofilm forming organism responsible for a wide range of infections placing it among the eight most important nosocomial pathogens. Current intervention strategies are designed to prevent initial device colonization, minimize microbial cell attachment to the device, penetrate the biofilm matrix and kill the associated cells, or remove the device from the patient. The threat of antibiotic resistance and their inability in breaking the biofilms has increased the likelihood that novel strategies for preventing or delaying the biofilm growth mode are urgently needed.

The aim of the study was to determine effects of gatifloxacinum, amikacin and cefoperazon+sulbactam on ability of *K.pneumoniae* planktonic cells to form secondary biofilms.

Materials and methods. *K. pneumoniae* biofilms were grown in 96-well microtiter plate, the optical density of initial bacterial suspension was performed using "Densi-La-Meter" according to McFarland turbidity levels after 24-hour incubation at 37 ^{0}C. Optical density of the secondary biofilms formed by planktonic cells without and in presence of antibacterial drug was measured after 24-hour incubation at 37 ^{0}C and staining on photometer "Multiskan EX 355" at a wavelength of 540 nm and evaluated in conventional units of optical density. All experiments were performed in duplicate and repeated at least three times on different days. The effect of different treatments on biofilm eradication was evaluated by the Student's t-test and $P < 0.05$ was considered significant. Data were analyzed using Excel software.

Results. *K.pneumoniae* suspension cultures were incubated at 37 ^{0}C during 24 hours in the presence of gatifloxacinum, amikacin and cefoperazon+sulbactam. Ability to form secondary biofilms was studied by inoculation of 200 µl of selected planktonic cells into polystyrene microtiter plates with addition of nutrient broth in 4 replicates and further incubation overnight at 37 ^{0}C in wet chamber. After biomass removing *K.pneumoniae* secondary biofilms were identified

by a quantitative expression of the degree of biofilm in absorbance values (units of optical density). These data indicated that antimicrobial effect of gatifloxacinum decreases an ability to form secondary biofilms in 17.4 times (0,074 ± 0,008 units of optical density), amikacin – in 12.3 times (0,105 ± 0,002 units of optical density) and cefoperazon+sulbactam – in 11.5 times (0,112 ± 0,003 units of optical density) compared with positive control (secondary biofilm formation without adding antimicrobial drugs) that was 1,29 ± 0,02 units of optical density. Also considering the results of effects of these drugs on ability to form primary biofilms of *K.pneumoniae* isolates such as with gatifloxacinum biofilm formation was inhibited in 6.8 times (0,191 ± 0,064 units of optical density), amikacin – in 14.7 times (0,089 ± 0,006 units of optical density) and cefoperazon+sulbactam – in 3.4 times (0,385 ± 0,054 units of optical density) compared with the control , it can be concluded that the most effective drug that blocks the ability to form primary biofilms by *K.pneumoniae* isolates is amikacin, gatifloxacinum inhibits an ability to form secondary biofilms by *K.pneumoniae* planktonic cells, that seems to be associated with the mechanism of action of these drugs.

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