EV0351

ePoster Viewing

Biofilm-related infections

The effect of ampicillin on biofilm created by *Corynebacterium striatum in vitro* studies

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Objectives: Corynebacterium striatum is a Gram-positive bacillus, which may be a component of human microbiome causing opportunistic infections at various sites. An important feature conducive to infections is the ability to produce biofilm on plastic surfaces, causing e.g. catheter-related bloodstream infections, urinary tract infections and infections associated with its presence on surfaces of implanted prostheses. The effective antibiotic treatment is a basic action reducing creation of biofilm resulting in development of infections. The objective of this study was to evaluate the effect of ampicillin on C. striatum biofilm in vitro based on the image analysis with a confocal microscope.

Methods: The study used C. striatum ATCC 6940, sensitive to all antibiotics. Biofilm culture (37°C) was performed on the surface of the LAB-TEK Chaber Sidle System in TSB with 5% FCS, using the input inoculum of 0.5 McFarland diluted 1:100. After 48 h of culture the ampicillin solution was added AMPI1= 2xMIC (1,0 µg/ml) and AMPI2 = 4xMIC (2,0 µg/ml). Biofilm was stained with two dyes (SYTO13 propidium iodide).The surface occupied by biofilm, the intensity of illumination and the value of illumination maxima of the biofilm surface against the control culture were determined. The resulting images (8-bit grayscale).

Results: The biofilm surface was analyzed based on 245 scanned parts of individual fragments covering the whole image visible in the microscope, which allowed for treatment of the measurements results of maxima values as a statistical whole. An increase of the surface of live bacteria was found under the influence of ampicillin at a concentration AMPI2, which made an impression of a paradoxical increase of the area of biofilm surface (“flattening” or “collapsing” of the created structures, coverage of a larger area). The effect of ampicillin leads to exposing of a greater number of living cells than in more compact biofilm of the control culture. Possibly such “exposing” of the living cells may be affected by detachment of the disintegrating bacteria from the surface. The analysis of the average maxima values ??of fluorescent illumination of dead cells showed a significant difference between the two concentrations of ampicillin and the control culture, which may result from changes in the structure of cell walls and a higher dye binding. A good indicator describing the effect of an antibiotic on biofilm is the ratio of the surface of live cells to the surface of dead cells of biofilm.

Conclusion: The effect of multiple MIC doses of ampicillin causes destruction of biofilm, i.e. changes in the area of its surface, detachment and exposing of subsequent live bacteria and changes of cell wall properties. The presented model allows for statistical assessment of the image in the confocal microscope, which may be applied to studies of other experimental models.