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SENSITIVITY TO CIPROFLOXACIN OF *PSEUDOMONAS AERUGINOSA* STRAINS WITH DIFFERENT LEVELS OF C-DI-GMP BIOSYNTHESIS

Abstract

It was investigated the antibiotics sensitivity of *Pseudomonas aeruginosa* strains with different levels of c-di-GMP biosynthesis: PA01 Δ 100 wspF1 (increased biosynthesis level) and PA01 pJN2133 (low level of biosynthesis) in comparison with the collection *P. aeruginosa* strains: PA01 and ATCC 27853. Not established significant differences between the strains sensitivity to ciprofloxacin. It is shown that the minimum concentration of ciprofloxacin that inhibit biofilm formation is significantly lower than minimum inhibitory concentrations of P. *aeruginosa* growth. It is established that subinhibitory concentrations of ciprofloxacin violates the biofilms morphology.

Key words: biofilm, *Pseudomonas aeruginosa*, ciprofloxacin, antibiotic sensitivity, minimum inhibitory concentration.

Relatively new methods in treatment become fruitless and require correction or development of completely new approaches [2,3]. The study of new possibilities of antibiotics is developing. IV generation of cephalosporins are highly active against *Pseudomonas spp.,* and they should also be included in the study.

Ciprofloxacin is an antibacterial agent of the second fluoroquinolones generation. Suppresses DNA-gyase, disrupts biosynthesis of DNA, growth and division of bacteria; causes pronounced morphological changes [1].

Pseudomonas aeruginosa strains with different levels of c-di-GMP biosynthesis were used in the work as test-microorganisms: PA01 Δ 100 wspF1 (elevated biosynthesis level) and PA01 pJN2133 (reduced biosynthesis level). *Pseudomonas aeruginosa* ATCC 27853 [5] and PA01 were control strains.

Biofilm incubation was performed according to the Swift technique [6] with addition of the 20 µl different ciprofloxacin concentrations: together with bacteria (for biofilm formation effect) and after 24 h of biofilm incubation (in case of mature biofilm). Accounting results were after 24 h in the system biofilm-plankton. For biofilm staining crystal violet was used («µQuant» BioTek, λ=592 nm).

Ciprofloxacin sub-inhibitory concentration (0. 1 µg / ml), or did not affect the formation of biofilm shown for strains *P. aeruginosa* PA01 and PA01 Δ100 wspF1, or reduced its biomass by 2.4 times in case of the strain ATCC 27853 and at 1, 4 times for strain PA01 pJN2133 (Fig. 1).

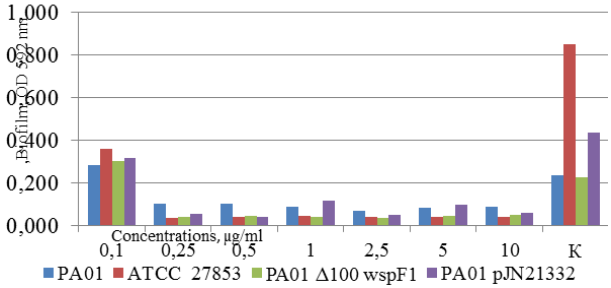

Fig. 1. Ciprofloxacin concentrations influence on *P. aeruginosa* biofilm formation.

The biofilms of the strains uniformly covers the surface and contains a significant amount of microcolonies submerged in the extracellular matrix.

To determine the antibiotics effect on mature biofilm, from plates, in which biofilms were cultivated during 24 h., plankton was removed. The biofilm on the bottom of the wells was washed with a sterile 0.9% NaCl, fresh nutrient medium with antibiotics was added into the wells for 24 hours. The influence of antibiotic was evaluated by the number of cells leaving the biofilm in the liquid medium and the biomass of the biofilm. The results are shown in Fig. 2-3.

According to the data shown on Fig. 2, ciprofloxacin expressly inhibits the release of cells from mature biofilms. At the lowest concentration of antibiotic (0.1 µg/ml) the number of planktonic cells was lower compared with control by 35-55%. With an increase in the content of ciprofloxacin, the inhibitory effect increases and in the range of concentrations of 1-10 µg/ml the amount of planktonic cells was only

7-10% of control in the case of strains *P. aeruginosa* ATCC 27853, PA01 Δ100 wspF1 and PA01 pJN2133. For *P. aeruginosa* PA01, these results were higher (20-30%).

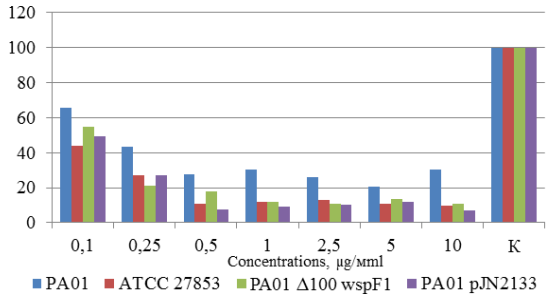

Fig. 2. Ciprofloxacin influence on the number of *P. aeruginosa* planktonic cells.

Ciprofloxacin does not cause a significant reduction in the biomass of the strains mature biofilm, with the exception of *P. aeruginosa* PA01 pJN2133, which has a very low content of c-di-GMP. The biofilm biomass decreasing was dependent on the concentration of antibiotic and was: 18% with its content of 0.1 μg/ml; 37% at 0.25 μg/ml; 40% at 0.5-2.5 μg/ml and 50-55% at 5 and 10 μg/ml, respectively (Fig. 3).

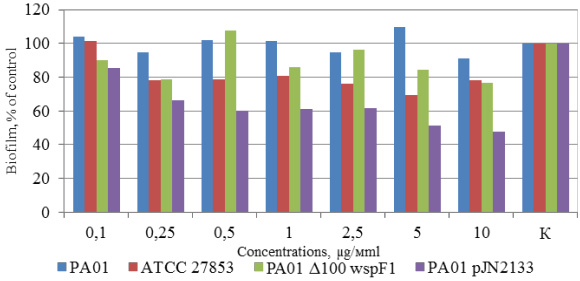

Fig. 3. Effect of various ciprofloxacin concentrations on mature biofilm.

For ciprofloxacin, an increase in this concentration is estimated at about 70%, which corresponds to data on a higher level of bacterial resistance to antibiotics in biofilms [2, 3, 4].

Obtained results showed that all strains mature biofilms are resistant to ciprofloxacin. Under antibiotic influence in no case was the complete destruction of the biofilm. The maximum decrease in the biofilm mass by 55% occurred only in the case of *P. aeruginosa* PA01 pJN2133 in the presence of 10 μg/ml of ciprofloxacin.

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