Effects of antimicrobials on *Pseudomonas aeruginosa* biofilm formation

U.M. Nemchenko¹, K.O. Sitnikova¹, N.L. Belkova¹, E.V. Grigorova¹, N.M. Voropaeva¹, M.V. Sukhoreva², E.S. Sukhareva², E.D. Savilov¹, ³

¹ Scientific Centre for Family Health and Human Reproduction Problems, Irkutsk, Russia
² City Ivano-Matreninskaya Children’s Clinical Hospital, Irkutsk, Russia
³ Irkutsk State Medical Academy of Postgraduate Education – Branch Campus of the Russian Medical Academy of Continuing Professional Education of the Ministry of Healthcare of the Russian Federation, Irkutsk, Russia

Abstract. *Pseudomonas aeruginosa* is one of the most problematic pathogens in medical institutions, which may be due to the ability of this microorganism to exist in a biofilm, which increases its resistance to antimicrobials, as well as its prevalence and survival ability in the external environment. This work aimed to evaluate the antimicrobial susceptibility of *P. aeruginosa* strains in planktonic and biofilm forms. We studied 20 strains of *P. aeruginosa* collected during 2018–2021 by specialists from the Laboratory of Microbiome and Microecology of the Scientific Centre for Family Health and Human Reproduction Problems. The identification of strains was carried out using test systems for differentiating gram-negative non-fermenting bacteria (NEFERMtest 24 Erba Lachema s.r.o., Czech Republic), and confirmed by mass spectrometric analysis and 16S rRNA gene sequencing. Antimicrobial activity was assessed by the degree of inhibition of cell growth in planktonic and biofilm forms (on a flat-bottomed 96-well plastic immunological plate). All clinical isolates of *P. aeruginosa* were biofilm formers, 47.6 % of the isolates were weak biofilm formers, and 52.4 % of the isolates were moderate biofilm formers. Planktonic cells and the forming biofilm of the tested *P. aeruginosa* strains were carbapenems-resistant. Biofilm formation was suppressed in more than 90 % of cases by the agents of the cephalosporin and aminoglycoside groups. Antimicrobial susceptibility of *P. aeruginosa* strains in the formed biofilm was significantly lower (*p < 0.05*). Carbapenems and cephalosporins did not affect the mature biofilms of the tested *P. aeruginosa* strains in more than 60 % of cases. Only non-beta-lactam antibiotics (ciprofloxacin and amikacin) suppressed the growth of planktonic cells and destroyed the mature biofilm. The revealed differences in the effect of the tested antimicrobials on the *P. aeruginosa* strains biofilms correlate with resistance to a number of antibiotics. To prevent biofilm formation in the hospital strains of *P. aeruginosa*, the use of cefazidime may be recommended, and antimicrobials such as ciprofloxacin and amikacin may be used to affect mature biofilms of *P. aeruginosa*.

Key words: *Pseudomonas aeruginosa*; biofilm formation; antimicrobial drugs; antibiotic resistance.

For citation: Nemchenko U.M., Sitnikova K.O., Belkova N.L., Grigorova E.V., Voropaeva N.M., Sukhareva E.S., Savilov E.D. Effects of antimicrobials on *Pseudomonas aeruginosa* biofilm formation. *Vavilovskii Zhurnal Genetiki i Selektii* = *Vavilov Journal of Genetics and Breeding.* 2022;26(5):495-501. DOI 10.18699/VJGB-22-60

Будьмо антимікробних препаратів на біопленкообразування *Pseudomonas aeruginosa*

У.М. Немченко¹, К.О. Ситникова¹, Н.Л. Белькова¹, Е.В. Григорова¹, Н.М. Воропаєва¹, М.В. Сухорева², Е.С. Сухарева², Е.Д. Савілов¹, ³

¹ Науковий центр проблем здоров’я сім’ї та репродукції людини, Іркутськ, Росія
² Городська Івано-Матренинська дитяча клінічна більниця, Іркутськ, Росія
³ Іркутська державна медична академія послепідготовного освіти – філія Російської медичної академії неперервного професійного освіти Міністерства здравоохорони Російської Федерації, Іркутськ, Росія

Анотація. Синегнойна палочка (*Pseudomonas aeruginosa*) відносяться до найбільш проблемним патогеном в лікарських установах, що може бути пов’язано зі способністю цього мікроорганізму суттєво змінювати біопленку, яка підвищує його устойливість до антимікробних препаратів, а також розповсюдженню і виживанню за межами середовища. Цілі цієї праці – оцінка чутливості штамів *P. aeruginosa*, що розміщуються у планктонній формі та формі біопленки, до антимікробних препаратів. Існує 20 штамів *P. aeruginosa* для використання збірки лабораторії мікроорганізму і мікроекології Наукового
Introduction

Pseudomonas aeruginosa invariably occupies the leading place among pathogens of nosocomial infections in the Russian Federation and is included in the group of opportunistic bacteria, united by the term ESKAPE (Skleenova et al., 2018). The presence of a wide range of pathogenic factors, genetic flexibility, and the ability to rapidly acquire resistance to different antibiotic groups makes P. aeruginosa one of the most problematic pathogens in healthcare settings (Edelstein et al., 2019). Patients with compromised immune systems, eye burns and trauma, and those with internal medical devices are primarily at risk of developing a pseudomonal infection (Diggle, Whiteley, 2020). Pseudomonal infections are particularly dangerous in patients with cystic fibrosis (Kosztolowicz et al., 2020; Scherz et al., 2021).

Treatment of infections caused by P. aeruginosa is complicated by the ability of these bacteria to exist in a biofilm, which increases their resistance to antibiotics, their prevalence, and survival ability (de Abreu et al., 2014; Olives et al., 2020). Destruction of bacterial biofilms formed in the secretions of cystic fibrosis patients was shown to be a serious problem, since diffusion of antibiotics into biofilm structures is poor, and their antibacterial activity can stimulate drug resistance (Kosztolowicz et al., 2020). Classical methods for determining antibiotic sensitivity (broth or agar dilution methods and disc diffusion method) are performed on non-adhering bacteria. The results obtained with these methods cannot determine the sensitivity of biofilm bacteria to antibiotics (Olives et al., 2020).

According to the experts of the European Committee on Antimicrobial Susceptibility Testing (EUCAST), the variety of antipseudomonal antibiotics, sensitivity to which is evaluated under in vitro conditions, includes penicillins, cephalosporins, carbapenems, monobactams, fluoroquinolones, aminoglycosides and polymyxins. In this regard, we studied the effect of the above groups of antimicrobial agents (AMAs) (ceftazidime, cefepime, imipenem, meropenem, ciprofloxacin and amikacin) on plankton cell growth, forming and mature P. aeruginosa biofilm.

The aim of the study was to evaluate the sensitivity of P. aeruginosa strains in the planktonic form and in the biofilm form to antimicrobial agents.

Materials and methods

The objects of the study were 20 strains of P. aeruginosa with confirmed drug resistance to antimicrobials from the collection of the Laboratory of Microbiome and Microecology of the Scientific Centre for Family Health and Human Reproduction Problems, accumulated during 2018–2021. Type strain P. aeruginosa ATCC 27853 (Scientific Centre “Kurchatov Institute” – Research Institute for Genetics and Selection of Industrial Microorganisms) was used as a control.

Hospital strains were isolated from patients from two medical institutions in Irkutsk according to the principle “one patient–one isolate”. Eight cultures were obtained from the Irkutsk State Regional Children’s Clinical Hospital (Noskova et al., 2020) and 12 cultures were obtained from the City Ivano-Matreninsky Children’s Clinical Hospital. Cultures were gathered from patients with different types of diseases (sepsis, acute hematogenous osteomyelitis, peritonitis, pneumonia, etc.) and isolated from oropharynx, liquor, wound, endotracheal tubes, tracheostomy, central venous catheter (14 cultures). A separate group consisted of 6 cultures isolated from the sputum of patients with such a genetic disease as cystic fibrosis (CF).

Identification of P. aeruginosa strains. Primary differentiation of P. aeruginosa strains was performed by colony morphology, pigment on blood agar, and Gram staining. Biochemical identification of selected cultures was performed using tests systems for differentiation of Gram-negative non-

1European Committee on Antimicrobial Susceptibility Testing [electronic source]. Clinical breakpoints – breakpoints and guidance. URL: http://www.eucast.org/clinical_breakpoints/ (accessed on: 15 October 2021).
fermenting bacteria NEFERMtest 24 (Erba Lachema s.r.o., Czech Republic), and confirmed by MALDI-TOF using direct protein profiling of nonfermenting microorganisms. Mass spectrometric analysis was performed on the Bruker Ultraflex Xtreme mass spectrometer (Bruker Daltonics, Germany). Additionally, cultures were identified by a fragment of the ribosomal operon containing the V1–V4 variable regions of the 16S rRNA gene. Full-length 16S rRNA gene fragments of *P. aeruginosa* strains were registered in the international GenBank database under numbers OL616031–OL616034.

To assess the effect of AMA on biofilm formation and destruction of the formed biofilms, antibiotics of the following groups were used: cephalosporins, carbapenems, aminoglycosides, fluoroquinolones, in the form of standard cardboard disks with antimicrobial drugs DI-PLS-50-01, (NICP, Research Centre for Pharmacotherapy, Russia), Hi-Media Laboratories Pvt. Limited (India).

**Determination of biofilm formation capacity and biofilm resistance to AMAs using 96-well plastic plates.** A 24-hour culture was used for the assay. The inoculum was densified in meat-peptone broth (MPB) to 10⁶ CFU/mL. Strains were prepared, culture optical density (OD) was measured, biofilms were stained, and the biofilm formation intensity was determined by measuring the optical density with gentian violet/ ethanol extracts, and the biofilm formation coefficient (BFC) was calculated according to the previously described methods (Nemchenko et al., 2020; Grigorova et al., 2021).

**Evaluation of the ability of AMA to affect plankton cell growth and biofilm formation.** To determine the ability of AMAs to affect plankton cells and the forming biofilm, one AMA disk with the required antibiotic concentration was added to the plate simultaneously with a 24-hour culture: ceftazidime – 10 μg, cefepime – 30 μg, imipenem – 10 μg, meropenem – 10 μg, ciprofloxacin – 5 μg, amikacin – 30 μg. Sterile MPB served as a control. After 30 min, the disks were removed (Tapalskiy, Bilskiy, 2018), the plates were cultured in the thermostat for 24 h, then the experiments were conducted as previously described (Nemchenko et al., 2020; Grigorova et al., 2021).

**Evaluation of the ability of AMA to destroy mature biofilms.** To determine the ability of AMA to destroy a mature biofilm, plankton cells were removed from the culture plate after 24 h of incubation, washed three times with sterile distilled water, and 150 μL of sterile MPB and one AMA disk were added to each well, including control wells. The disks were removed after 30 min. The plates were incubated for another 24 h. Furthermore, the procedure was similar to that previously described (Nemchenko et al., 2020; Grigorova et al., 2021).

**Registration of experimental results.** The biofilm formation coefficient (BFC) was calculated after measuring the optical density of the ethanol extract of the stained wells in all plates as the ratio of the optical density of the experiment extract and optical density of the control extract. When the obtained BFC values were less than 2.0, strains were classified as weak biofilm formers, with values of 2.0–3.9, as moderate biofilm formers, and above 3.9, as strong biofilm formers (Nemchenko et al., 2020; Grigorova et al., 2021). The effect coefficient of AMA on forming and mature biofilms was calculated using the formula

\[
\text{OD BF}_{\text{form}}/\text{OD BF}_{\text{without AMA}} \text{ or } \text{OD BF}_{\text{mature}}/\text{OD BF}_{\text{without AMA}}
\]

where \( \text{OD BF}_{\text{form}} \) or \( \text{OD BF}_{\text{mature}} \) is the optical density of the ethanol extract of the biofilm influenced by AMA, \( \text{OD BF}_{\text{without AMA}} \) is the optical density of the ethanol extract of biofilm cultures without the AMA effect. With a ratio < 0.9, AMA was considered to affect the biofilm; from 0.9 to 1.0, AMA had little effect on the biofilm; from 1.0 and above, AMA had no effect on the biofilm.

The growth of plankton cells in the plate wells was determined as the ratio of the optical density of the bacterial plankton cell suspension after 24 h of cultivation to the initial density; the result was interpreted as previously described (Nemchenko et al., 2020; Grigorova et al., 2021).

Statistical processing of the data was performed using licensed MS Excel 2007 for Windows 7 applications. Non-parametric criteria were used to assess the significance of differences between the two groups according to the level of any criterion: \( \chi^2 \), Mann–Whitney \( U \)-criterion. Absolute and relative (percentage) values were calculated for the qualitative variables. The significance level for statistical hypothesis testing \( (p) \) was assumed to be 0.05.

**Results**

It was found that under laboratory conditions without AMA exposure, the planktonic cells of *P. aeruginosa* had a significant growth rate (Table 1). The density of microbial cells increased in 24 h of cultivation more than ten-fold compared to the initial density \( (U_{\text{emp}} = 0, \text{differences significant between the initial density and the density after 24 h, Mann–Whitney test}) \).

The OD of *P. aeruginosa* biofilm cultures isolated from sputum in such a severe, genetically determined disease as cystic fibrosis was significantly greater than that of the type strain \( (p < 0.01) \) and cultures isolated in other diseases (see the Figure). A similar pattern was observed when comparing BFCs. The mean BFC of cystic fibrosis *P. aeruginosa* was 2.79 ± 0.78; *P. aeruginosa* in other diseases was 2.01 ± 0.69; *P. aeruginosa* ATCC 27853 was 1.56.

Evaluation of biofilm formation ability by the amount of dye bound to the biofilm showed that the strains studied, including the *P. aeruginosa* ATCC 27853 type strain, were weak biofilm formers in 47.6 %, in 52.4 % of cases were moderate biofilm formers (see Table 1).

A comparison of the optical densities of cultures growing without and under the AMA effect showed that planktonic cells were resistant to AMA imipenem (5 % of sensitive cultures, \( U_{\text{emp}} = 46 \)) and meropenem (5 % of sensitive cultures, \( U_{\text{emp}} = 64.5 \); there is a difference between the initial density and the density after 24 h, Mann–Whitney test, \( p < 0.05 \)). The other drugs inhibited the growth of planktonic cells, the most effective were amikacin (60 % of sensitive cultures, \( U_{\text{emp}} = 180.5 \)) and ciprofloxacin (50 % of sensitive cultures, \( U_{\text{emp}} = 191.5 \)), cefepime affected 40.0 % of cultures \( (U_{\text{emp}} = 191.5) \), and ceftazidime suppressed the growth of *P. aeruginosa* cultures in 35 % of cases \( (U_{\text{emp}} = 179.0) \) (no difference between the initial density and the density after 24 h, Mann–Whitney test, \( p > 0.05 \)).
Effects of antimicrobials on Pseudomonas aeruginosa biofilm formation

Table 1. Characterization of the tested P. aeruginosa strains by growth rate and biofilm formation intensity

| Indicator                        | Indicator gradation | Strains, % |
|----------------------------------|---------------------|------------|
| Growth rate                      | No                  | 0          |
|                                  | Small               | 0          |
|                                  | Significant         | 100        |

Biofilm formation intensity

| Factor                          | P. aeruginosa in cystic fibrosis | P. aeruginosa in other diseases | P. aeruginosa ATCC 27853 |
|---------------------------------|---------------------------------|---------------------------------|--------------------------|
| Mean optical density of the biofilm\(^1\) | 0.137                           | 0.098                           | 0.073                    |
| Mean optical density of MPB (control) | 0.047                           | 0.047                           | 0.047                    |
| Mean value of BFC               | 2.79                            | 2.01                            | 1.56                     |

Note. \(^1\)The difference is significant between the optical density of gentian violet/ethanol extracts of the cultures in cystic fibrosis and the optical density of gentian violet/ethanol extracts of P. aeruginosa ATCC 27853, \(U_{\text{emp}} = 1\) Mann–Whitney test, \(p < 0.01\). MPB – meat-peptone broth; BFC – biofilm formation coefficient.

The sensitivity of P. aeruginosa cells in a mature biofilm to AMA exposure was lower than that of biofilm formation (Mann–Whitney test, difference significant between the optical density of a forming biofilm and a mature biofilm, \(p < 0.05\)). AMAs ceftazidime, cefepime, imipenem, and meropenem had little or no effect on P. aeruginosa biofilms; the BF\(_{\text{mature}}\)/BF\(_{\text{without AMA}}\) ratio was 0.9 or higher in more than 60 % of cases. Only non-beta-lactam antibiotics, such as amikacin and ciprofloxacin, affected the formed biofilm (Table 3).

Comparison of the AMAs effects among themselves showed that amikacin was more effective than ceftazidime \((\chi^2 = 5.01)\) and meropenem \((\chi^2 = 10.98)\), ciprofloxacin was more effective than meropenem \((\chi^2 = 7.62)\).

The BFC of P. aeruginosa strains in the formed biofilm was significantly higher than BFC of cultures exposed to AMAs at the stage of biofilm formation, which also confirms the resistance of the mature biofilm. BFC for ceftazidime \(U_{\text{emp}} = 48.5\); cefepime \(U_{\text{emp}} = 58\); imipenem \(U_{\text{emp}} = 97\); amikacin \(U_{\text{emp}} = 50\). There is a difference between the BFC value of the forming and BFC value of the mature biofilm, Mann–Whitney test, \(p < 0.01\).

Discussion

The experiment showed that not all AMAs inhibited the growth of planktonic cells of clinical P. aeruginosa isolates. Resistance to cephalosporins (ceftazidime and cefepime) was demonstrated by 65 and 60 % of the tested strains, respectively. Resistance to carbapenems (imipenem and meropenem) was observed in almost all isolates. Resistance to non-beta-lactam antibiotics (amikacin and ciprofloxacin) was shown by 40 and 50 % of the strains, respectively. The findings are consistent both with our previous studies (Noskova et al., 2020) and with a multicentre epidemiological study of antibiotic resistance of...
nosocomial pathogens (“MARATHON” 2015–2016), which observed an increase in resistance of nosocomial *P. aeruginosa* strains to most AMAs, including carbapenems (Edelstein et al., 2019).

The strains studied, especially those isolated from patients with cystic fibrosis, were biofilm-forming (see Table 1). This served as the basis for us to evaluate the effectiveness of AMAs against the forming biofilm of nosocomial pathogens. The experiment showed that compared to other antibiotics, ceftazidime was the most effective drug inhibiting biofilm formation (see Table 2).

As recent studies show, in addition to classical resistance mechanisms, bacteria are able to withstand exposure to high antibiotic concentrations by exhibiting so-called tolerance (Brauner et al., 2016; Yan, Bassler, 2019). Tolerant bacteria grow more slowly than their non-tolerant counterparts and may avoid death by antibiotic treatment (Brauner et al., 2016). Another form of tolerance, which does not result from inherited mutations but rather from phenotypic differentiation, is commonly referred to as persistence. Time-dependent destruction of the bacterial population by antibiotics shows that actively growing cells die first, while persistent cells die in the second phase at a much lower rate. It is this subset of microorganisms that survives antibiotic exposure and recovers after antibiotic withdrawal (Balaban et al., 2004).

It has been suggested that the ability of biofilms to contain tolerant and persistent cells underlies the difficulties encountered in eliminating biofilms (Lewis, 2012). It is likely that the increased antibiotic tolerance arises from altered biofilm cell physiology. It has been suggested that cells within biofilms are in a stationary phase where the penetration of nutrients and oxygen is limited due to consumption by the cells located peripherally (Yan, Bassler, 2019). The presence of persistent cells can be dangerous in certain groups of patients, such as those with cystic fibrosis, when highly persistent mutants are released after long-term antibiotic treatment (Lewis, 2012).

The studies presented showed that the sensitivity of cells in mature biofilms to AMAs was significantly lower; the antibiotics generally failed to destroy biofilm cultures of *P. aeruginosa*. The BFC of cultures in mature biofilms was higher than that of cultures that were affected by AMA during biofilm formation (*p < 0.01*).

Of all AMAs tested, only non-beta-lactam antibiotics (ciprofloxacin and amikacin) inhibited the growth of plankton cells and destroyed the mature biofilm, which may be related to the mechanism of the effect of different classes of antibiotics. The cells in the biofilm decrease the rate of cell division, making them less sensitive to beta-lactam antibiotics affecting the cell wall, while the effect of ciprofloxacin and amikacin does not require actively dividing cells since it targets transcription and translational processes (Sidorenko et al., 2013; Thieme et al., 2021).

The most effective approach to prevent biofilm formation would be to inhibit the adhesive capacity of cells (Olivares et al., 2020). For example, a study by S. Otani et al. (2018) showed that subinhibitory minimal suppressive concentrations of ceftazidime reduced biofilm mass, suppressed motility and expression of genes involved in bacterial adhesion and *P. aeruginosa* PA01 matrix production (Otani et al., 2018). Previously, S. Roudashti et al. (2017) observed the effects of cephalosporins in *P. aeruginosa* QS systems providing motility and biofilm formation in these microorganisms (Roudashti et al., 2017). In our study, ceftazidime also showed the highest antibiofilm effect compared with other AMAs. However, the mechanism of biofilm resistance to AMAs is complex, multifactorial, and contradictory. This point is supported by numerous studies that demonstrate that low doses of antimicrobials in the centre of infection can increase the risk of mutagenesis and initiate biofilm formation (Kaplan, 2011; Ciolfu et al., 2015; Olivares et al., 2020).

---

**Table 2. Ability of AMAs to affect biofilm formation of the tested *P. aeruginosa* strains (absolute value/%)**

| Antimicrobial agent | Ratio OD BF form/OD BF without AMA |
|---------------------|-----------------------------------|
| Cefazidime          | 19/95 1/5                         |
| Ceftazidime         | 18/90 – 2/10                      |
| Amikacin            | 18/90 – 2/10                      |
| Ciprofloxacin       | 15/75 – 5/25                      |
| Imipenem            | 13/65 2/10 6/30                   |
| Meropenem           | 12/60 2/10 6/30                   |

1 Cefazidime affects biofilm formation compared to imipenem and meropenem, *p < 0.05*; 2 no difference when comparing the effect between other AMAs, *p > 0.05*; OD BF form – optical density of the forming biofilm under the effect of AMA; OD BF without AMA – optical density without AMA exposure; AMAs – antimicrobial agents.

**Table 3. Ability of different AMAs to affect the mature biofilm of *P. aeruginosa* strains (absolute value/%)**

| Antimicrobial agent | Ratio OD BF mature/OD BF without AMA |
|---------------------|-------------------------------------|
| Amikacin            | 12/60 3/15 5/25                   |
| Ciprofloxacin       | 10/50 – 10/50                     |
| Cefazidime          | 5/25 3/15 12/60                   |
| Ceftazidime         | 8/40 – 12/60                      |
| Imipenem            | 6/30 – 14/70                      |
| Meropenem           | 2/10 4/20 14/70                   |

1 Amikacin destroys the mature biofilm compared with ceftazidime (*p = 0.02*) and meropenem (*p < 0.001*); 2 ciprofloxacin destroys the mature biofilm compared with meropenem (*p < 0.03*); OD BF mature – optical density of the mature biofilm under the AMA effect; OD BF without AMA – optical density of the biofilm with no AMA effect; AMAs – antimicrobial agents.
Conclusion
Thus, the study of the effect of AMAs of the groups of cephalosporins, carbapenems, fluoroquinolones and aminoglycosides on the biofilms of the tested hospital *P. aeruginosa* strains showed that the antipseudomonal drugs mainly prevented the formation but did not destroy the already formed biofilm. The significant differences detected in the effect of the tested AMAs both on the mature biofilm of *P. aeruginosa* strains and on the process of its formation to a certain extent correlate with the resistance of this microorganism to a number of antibiotics (Edelstein et al., 2019; Adzhieva et al., 2021). Additional research aimed at detecting tolerant and persistent cells is needed to elucidate the mechanisms involved, which will optimise the overall use of antimicrobials for treating biofilm-related infections (Yan, Bassler, 2019). The use of ceftazidime may be recommended to prevent biofilm formation in the hospital strains of *P. aeruginosa*, and amikacin and ciprofloxacin may be recommended for affecting mature *P. aeruginosa* biofilms.

References
Adzhieva A.A., Danilova T.A., Danilina G.A., Shevyagina N.V., Minko A.G., Zhukhovitsky V.G. Influence of antibiotics on biofilm formation by *Streptococcus pyogenes* in vitro. Zhurnal Mikrobiologii, Epidemiologii i Imunnologii = Journal of Microbiology, Epidemiology and Immunobiology. 2021;98(1):59-64. DOI 10.36233/0372-9311-64. (in Russian)
Balaban N.Q., Merrin J., Chait R., Kowalik L., Leibler S. Bacterial persistence as a phenotypic switch. Science. 2004;305(5690):1622-1625. DOI 10.1126/science.1099390.
Brauner A., Fridman O., Gefen O., Balaban N.Q. Distinguishing between resistance, tolerance and persistence to antibiotic treatment. Nat. Rev. Microbiol. 2016;14(5):320-330. DOI 10.1038/nrmicro. 2016.34.
Ciofu O., Tolkær-Nielsen T., Jensen P.O., Wang H., Hoiby N. Antimicrobial resistance, respiratory tract infections and role of biofilms in lung infections in cystic fibrosis patients. Adv. Drug Deliv. Rev. 2015;85:7-23. DOI 10.1016/j.addr.2014.11.017.
de Abreu P.M., Farias P.G., Paiva G.S., Almeida A.M., Morris P.V. Persistence of microbial communities including *Pseudomonas aeruginosa* in a hospital environment: a potential health hazard. BMC Microbiol. 2014;14:118. DOI 10.1186/1471-2180-14-118.
Diggie S.P., Whiteley M. Microbe Profile: *Pseudomonas aeruginosa*: opportunistic pathogen and lab rat. Microbiology. 2020;166(1):30-33. DOI 10.1099/mic.0.008860.
Edelstein M.V., Shek E.A., Sukhorukova M.V., Skleenova E.Yu., Ivanchik N.V., … Zvonaryova O.V., Kornilova P.A., Krygla V.G., Portnyagina U.S., Shamaeva S.Kh. Antimicrobial resistance, carbapenemase production and genotypes of nosocomial *Pseudomonas aeruginosa* isolates in Russia: results of multicenter epidemiological study “MARATHON 2015–2016”. Klinicheskaya Mikrobiologiya i Antimikrobnyaya Khimioterapiya = Clinical Microbiology and Antimicrobial Chemotherapy. 2019;21(2):160-170. DOI 10.36488/ cmac.2019.2.160-170. (in Russian)

Grigorova E.V., Nemchenko U.M., Voropaeva N.M., Bel’kova N.L., Noskova O.A., Savilov E.D. Effect of disinfectants with different active ingredients on biofilm formation in *Pseudomonas aeruginosa*. Bull. Exp. Biol. Med. 2021;171(6):745-749. DOI 10.1007/s10517-021-05308-y.
Kaplan J.B. Antibiotic-induced biofilm formation. Int. J. Artif. Organs. 2011;34(9):737-751. DOI 10.5301/ijao.5000027.
Kosztolowicz T., Metzler R., Wąsik S., Arabski M. Modelling experimentally measured of ciprofloxacin antibiotic diffusion in *Pseudomonas aeruginosa* biofilm formed in artificial sputum medium. PLoS One. 2020;15(12):e0243003. DOI 10.1371/journal.pone.0243003.
Lewis K. Persistor cells: molecular mechanisms related to antibiotic tolerance. In: Coates A. (Ed.). Antibiotic Resistance. Handbook of Experimental Pharmacology. Berlin; Heidelberg: Springer, 2012; 211:121-133. DOI 10.1007/978-3-642-28951-4.
Nemchenko U.M., Kungurtseva E.A., Grigorova E.V., Bel’kova N.L., Markova Y.A., Noskova O.A., Chemezova N.N., Savilov E.D. Simulation of bacterial biofilms and estimation of the sensitivity of healthcare-associated infection pathogens to bactericide Sekuset active. Klinicheskaya Laboratornaya Diagnostika = Clinical Laboratory Diagnostics. 2020;65(10):652-658. DOI 10.18821/0869-2084-2020-65-10-652-658. (in Russian)
Noskova O.A., Savilov E.D., Chemezova N.N., Bel’kova N.L. Antibiotic resistance of pathogens of generalized purulent septic infections in children. Epidemiologiya i Vaksinoprofilaktika = Epidemiology and Vaccinal Prevention. 2020;19(6):56-61. DOI 10.31631/2073-3046-2020-19-6-56-61. (in Russian)
Olivares E., Badel-Berchoux S., Provot C., Prévost G., Bernardi T., Jehl F. Clinical impact of antibiotics for the treatment of *Pseudomonas aeruginosa* biofilm infections. Front Microbiol. 2020;10:2894. DOI 10.3389/fmicb.2019.02894.
Otani S., Hiramatsu K., Hashinaga K., Komiyi K., Umeki K., Kishi K., Kadota J-I. Sub-minimum inhibitory concentrations of cefazidime inhibit *Pseudomonas aeruginosa* biofilm formation. J. Infect. Chemother. 2018;24(6):428-433. DOI 10.1016/j.jiac.2018.01.007.
Roudashski S., Zeighami H., Mirshahabi H., Bahari S., Soltani A., Haghi F. Synergistic activity of sub-inhibitory concentrations of curcumin with cefazidime and ciprofloxacin against *Pseudomonas aeruginosa* quorum sensing related genes and virulence traits. World J. Microbiol. Biotechnol. 2017;33(3):50. DOI 10.1007/s11274-016-2195-0.
Scherz V., Caruana G., Tâfè P., Brouillet R., Bertelli C., Jatton K., Asner S.A. Unexpected associations between respiratory viruses and bacteria with Pulmonary Function Testing in children suffering from Cystic Fibrosis (MUCOVIB study). J. Cyst. Fibros. 2022;21(2): e158-e164. DOI 10.1016/j.jcf.2021.10.001.
Sidorenko S.V., Partina I.V., Ageeets V.A. Impinen: 30-year experience in therapy. Antibiotiki i Khimioterapiya = Antibiotics and Chemotherapy. 2013;58(5-6):55-61. (in Russian)
Skleenova E.Yu., Azizov I.S., Shek E.A., Edelstein M.V., Kozlov R.S., Dekhnic A.V. *Pseudomonas aeruginosa*: the history of one of the most successful nosocomial pathogens in
Влияние антимикробных препаратов на биопленкообразование Pseudomonas aeruginosa

У.М. Немченко, К.О. Ситникова, Н.Л. Белькова ...
М.В. Сухорева, Е.С. Сухарева, Е.Д. Савилов

Russian hospitals. Klinicheskaya Mikrobiologiya i Antimikrobnaya Khimioterapiya = Clinical Microbiology and Antimicrobial Chemotherapy. 2018;3: 164-171. DOI 10.36488/cmac.2018.3.164-171. (in Russian)

Tapalskiy D.V., Bilsky I.A. Antimicrobial susceptibility testing by broth microdilution method: widely available modification. Klinicheskaya Mikrobiologiya i Antimikrobnaya Khimioterapiya = Clinical Microbiology and Antimicrobial Chemotherapy. 2018;1:62-67. DOI 10.36488/cmac.2018.1.62-67. (in Russian)

Thieme L., Hartung A., Tramm K., Graf J., Spott R., Makarewicz O., Pletz M.W. Adaptation of the Start-Growth-Time method for high-throughput biofilm quantification. Front. Microbiol. 2021;12: 631248. DOI 10.3389/fmicb.2021.631248.

Yan J., Bassler B.L. Surviving as a community: antibiotic tolerance and persistence in bacterial biofilms. Cell Host Microbe. 2019;26(1):15-21. DOI 10.1016/j.chom.2019.06.002.

ORCID ID
U.M. Nemchenko orcid.org/0000-0002-7656-342X
K.O. Sitnikova orcid.org/0000-0001-7717-906X
N.L. Belkova orcid.org/0000-0001-9720-068X
E.V. Grigorova orcid.org/0000-0001-6388-2591
N.M. Voropaeva orcid.org/0000-0001-7026-2522
E.D. Savilov orcid.org/0000-0002-9217-6876

Conflict of interest. The authors declare no conflict of interest.
Received March 31, 2022. Revised June 30, 2022. Accepted June 30, 2022.