Annotation. High antimicrobial properties of antiseptics determine the scientific interest in the study of their effect on the biofilm formation of clinically significant pathogens and the detection of a probable dependence between the sensitivity of bacteria to them and the ability to form biofilms. The aim was to study the biofilm-forming properties of clinical strains of *P. aeruginosa*, depending on their sensitivity to various antiseptic medicines. 30 clinical strains of *P. aeruginosa* were isolated and identified from the patients with infectious complications of different areas who had been treated at the burn, intensive care and surgical units. The study of biofilm-forming properties of clinical isolates of *P. aeruginosa* was performed by using the spectrophotometric technique by G.D. Christensen (MTP microtiter plate test). The sensitivity assessment of the derived strains to antiseptics (decamethoxine, decasan, miramistin, chlorhexidine) was done by double serial dilutions according to the standard procedure approved by the Order №167 of the Ministry of Public Health of Ukraine. The statistical processing of the results obtained was carried out by "Microsoft Excel 2010" software package. In order to determine the relationship between the biofilm formation and sensitivity to antiseptic medicines of *P. aeruginosa* strains, we determined the correlation coefficient (r-Pearson coefficient). The clinical strains of *P. aeruginosa* were found to be the most sensitive to decasan and miramistin, and their MICs did not differ 70,3±25,98 mg/ml and 68,5±33,20 mg/ml respectively. Chlorhexidine has been shown a reducing of antimicrobial efficiency against *P. aeruginosa* (MIC 115,62±59,75 mg/ml). High biofilm-forming properties of clinical isolates *P. aeruginosa* have been proved. The optical density of the biofilms formed by these microorganisms was 0,674±0,17 density units for 24 hours of cultivation. A direct correlation between the sensitivity of strains *P. aeruginosa* to decamethoxine, decasan, miramistin and their biofilm-forming properties was established. The sensitivity of the clinical strains to chlorhexidine did not depend on their biofilm potential. Clinical strains of *P. aeruginosa* have high biofilm-forming properties and retain sensitivity to antiseptic medicines based on cationic surfactant antiseptics. Decamethoxine and miramistin provide the highest antimicrobial action on *P. aeruginosa* isolates. The sensitivity of *P. aeruginosa* to decamethoxine, decasan, miramistin has shown to be in a direct correlation with their biofilm-forming properties.

Keywords: antiseptics, biofilms, decamethoxine, decasan, miramistin, chlorhexidine.

Introduction

The last decade marked the emergence of new data on the role of opportunistic microorganisms, that can form biofilms, in the development of various nosological forms of purulent-inflammatory diseases with severe course during a treatment in hospital. Gram-negative non-fermenting microorganisms occupy a significant place among them. The ability of *Pseudomonas* spp. to exist in the environment of medical institutions, attach to the surfaces of catheters, probes, respiratory tubes, contact lenses and to form biofilms indicates their leading role among known pathogens of infectious complications associated with the medical care. The presence in the biofilms gives the pathogens additional resistance to antimicrobial agents and factors of the immune system, as well as new properties that are not characteristic for planktonic forms of bacteria. Therefore, it is naturally that the acquisition of antibiotic resistance is increasing among high-biofilm forming strains of bacteria [1, 3, 9, 10].

Taking into account these features, the development and implementation of new effective antimicrobial agents against microorganisms in planktonic and biofilm forms are the important measures on the way of overcoming the resistance of microorganisms to chemotherapeutic drugs. Nowadays, there is the positive experience of clinical use in various fields of medicine a new group of medicines based on cationic surfactant antiseptics, which belong to the most effective ones, according to scientific literature. These drugs possess a multi-vector mechanism of antimicrobial action, because of their high surface activity, washing and emulsifying properties, ability to change the surface tension of the bacterial cell and the violation of its osmotic balance, resulting in a "osmotic shock" due to their diphilic structure of the molecule. It provides the death of the microorganism [2, 4, 6, 7, 8].

High antimicrobial properties determine the scientific interest in the study of the activity of antiseptics on the biofilm formation of clinically significant pathogens and the detection of a probable dependence between the level of bacterial sensitivity to them and the ability to form biofilms. This work was aimed to study the biofilm-forming properties of clinical strains of *P. aeruginosa*, depending on the sensitivity to various antiseptic medicines.
**Material and methods**

There were isolated from the patients with severe burns and patients of intensive care and surgical units and identified 30 clinical strains of *P. aeruginosa*. Clinical strains of *P. aeruginosa* were previously identified by morphological, tinctorial, cultural and biochemical properties and had been possessed resistance to antibiotics. Isolation and identification of microorganisms were carried out according to the standard method using NEFERM test-24 (PLIVA - Lachema a.s. Brno, Czech Republic).

The sensitivity assessment of the derived strains to antiseptic medicines (decamethoxin, dekasan, miramistin, chlorhexidine) was done by double serial dilutions according to the standard procedure approved by the Order №167 of the Ministry of Public Health of Ukraine on "On Approval of Training Guidance "Assessment of the sensitivity of microorganisms to antibiotics", dated by April, 5, 2007. Minimum inhibitory concentration (MIC) was determined at the lowest concentration of antiseptic solution that was able to suppress the apparent growth of the microorganism. The minimum bactericidal concentration (MBC) of antiseptics was determined at the lowest concentration of antiseptic, which completely destroyed the bacterial culture, by inoculating microorganisms from a broth medium, where the growth of the microorganism was delayed, on an agar medium [5].

The study of biofilm-forming properties of clinical strains was performed by using the spectrophotometric technique by G.D. Christensen (MIP microtiter plate test). Biofilms were reproduced in wells of a sterile, flat-bottom 96-well polystyrene tray (Corning, USA) and stained with 1% solution of crystalline violet. Properties of the microorganisms to form a biofilm were evaluated by the degree of dye absorption in optical density units using a spectrophotometer (570 nm). Interpretation of the results was carried out according to the conventional methodology. Thus, the ability of microorganisms to form biofilms was assessed as low (at optical density <0,120), average (at optical density = 0,121-0,239) and high (at optical density > 0,240) [11].

The statistical processing of the results obtained was carried out by standard "STATISTICA+" and "Microsoft Excel 2010" software packages. In order to determine the relationship between the sensitivity to antiseptics and biofilm formation of investigated microorganisms, we determined the correlation coefficient (r-Pearson coefficient), the absolute value of which characterized the binding force.

**Results. Discussion**

According to the results of the research, it was found that the highest activity on the clinical strains of *P. aeruginosa*, isolated from severely ill patients, was decasan and miramistin, their MICs were almost identical (70,31±25,98 mcg/ml, 68,5±33,20 mcg/ml respectively (table 1)). In turn, the MIC (115,6±59,75 mcg/ml) and MBC (222,5±110,80 mcg/ml) of chlorhexidine for investigated strains were the highest, that indicated a decreasing of the effectiveness of this antiseptic in the treatment of infections, caused by *P. aeruginosa*.

As a result of the research, high biofilm-forming properties of clinical isolates of *P. aeruginosa* have been established. The degree of dye absorption of biofilms formed by investigated pathogens was 0,674±0,17 optical density units (ODU) for 24 hours of cultivation. However, it should be noted that this indicator was significantly different from the MIC for antiseptics, and this is the reason why it is necessary to take into account the study of the biofilm properties of clinical isolates of *P. aeruginosa*.
for different clinical strains. So, some of them have shown the average ability to form biofilms.

A direct correlation between the sensitivity of isolates of *P. aeruginosa* to decamethoxine and their ability to form biofilms was established. The r-Pearson coefficient for these indicators amounted 0.74 (Fig. 1). It was quite naturally that the binding force between the formation of *Pseudomonas* strains and their sensitivity to decasan (r-Pearson 0.78) was similar to those indicators of sensitivity referring to decamethoxine (Fig. 2).

Moreover, direct correlation between sensitivity of *P. aeruginosa* strains to miramistin which was active against them and their biofilm-forming properties was established. The r-Pearson coefficient was found to be 0.77 (Fig. 3).

In turn, the properties of biofilm formation of clinical strains of *P. aeruginosa* were poorly correlated with their sensitivity to chlorhexidine. This was pointed by the low r-Pearson coefficient (0.55). So, the sensitivity of the clinical strains of *P. aeruginosa* to this antiseptic did not depend on their biofilm-forming potential (Fig. 4).

**Conclusions and prospects for further research**

1. Clinical strains of *P. aeruginosa* as pathogens of infectious complications, have high biofilm-forming properties and preserve sensitivity to antiseptic medicines based on cationic surfactants. The highest antimicrobial activity against isolates of *P. aeruginosa*, isolated from severely ill patients, was shown by decamethoxine and miramistin. It has been established that the sensitivity of *P. aeruginosa* to decamethoxine, decasan and miramistin has a direct correlation with their biofilm-forming properties. It means, the higher ability to form a biofilm has a pathogen, the higher bacteriostatic concentration of antiseptic should be applied to it. The sensitivity of clinical strains of *P. aeruginosa* to chlorhexidine does not depend on the biofilm-forming properties of these isolates.

Prospects for further research are based on the in-depth study of the effect of antiseptic medicines on *P. aeruginosa*, to overcome the high adaptive properties of these pathogens, which are aimed to make a treatment of the infectious complications caused by it more effective.

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