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ORIGINAL ARTICLE

Correlation of Susceptibility to Antiseptics With Biofilm-forming Properties in Acinetobacter baumannii as a Pathogen of Surgical Infection

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ABSTRACT

Introduction: The purpose of this research was to study the biofilm-forming properties of clinical strains of *A.baumannii*, isolated from burn wounds in patients of ICU, and their sensitivity to antiseptics. **Methods:** 220 clinical strains of *A. baumannii* isolated from intensive care unit infected burn patients were the object of the study. Antiseptic sensitivity of Acinetobacter spp. (decamethoxine, chlorhexidine, miramistin, povidone iodine) was investigated using double serial dilutions according to the standard procedure. The study of biofilm-forming properties of clinical Acinetobacter isolates was performed using the spectrophotometric technique by G.D. Christensen. In order to determine the relationship between the antiseptic sensitivity and biofilm-forming properties of *A. baumannii* strains, we determined the correlation coefficient (r-Pearson), the absolute value of which characterised the binding force. **Results:** Among 435 burn persons, who were involved in the investigated group, representatives of Acinetobacter spp. were found in 220 (50.6%), that has revealed the etiological significance of the opportunistic pathogens of Acinetobacter isolates in the development of infectious complications of burns in intensive care units. Clinical strains of *A. baumannii* have shown variable susceptible to decamethoxine, chlorhexidine, miramistin, povidone iodine and have been found to possess high biofilm-forming properties. The r-Pearson coefficient between sensitivity of *A. baumannii* to investigated antiseptics and biofilm-formation pointed out positive moderate and strong correlations. **Conclusion:** Biofilm-formation of Acinetobacter spp. is correlated with their susceptibility to chlorhexidine and povidone iodine strongly. However, as a more powerful antimicrobial activity of antiseptic against *A. baumannii* was as weaker correlation had been established.

Keywords: *A.baumannii*, Surgical site infection, Antiseptic, Biofilm, Correlation

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INTRODUCTION

According to the data of World Health Organization (WHO) surgical site infection (SSI) is the most frequent type of health care associated infections worldwide and affects up to one third of patients who have undergone a surgical procedure (1). 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 the hospital (2). The spectrum of microorganisms causing SSI varies depending on type of clinic and unit, where patient undergoes medical care and type of surgery. However, *Staphylococcus spp.* (29.4%), *Enterobacteriaceae spp.* (34.1%), gram-negative non-fermentative bacilli (6.7%) are known to be prominent nosocomial pathogens in intensive care unit (ICU) patients including burn and trauma patients and those requiring mechanical ventilation (3, 4).

Nowadays the worldwide emergence of multi-drug resistant bacteria has been contributing to substantial challenges in treatment and caring for patients. Infections caused by them are sometimes life-threatening, especially in ICU patients (5, 6).

Acinetobacter baumannii is among leading clinically significant multi-drug resistant opportunistic nosocomial pathogens (5, 7). It is ubiquitous and can colonise medical devices as well as the skin and the airways of patients and hospital staff (8). Wide range of infections, long-term survival, easy spread in the hospital environment and ability to rapidly acquire resistance determinants against most of antimicrobial agents are closely related

to intrinsic biological properties of *A.baumannii* (5). The ability of *Acinetobacter spp.* to exist in the environment of medical institutions, attach to the surfaces of catheters, probes, respiratory tubes, contact lenses and form biofilms indicates their leading role among known pathogens of infectious complications associated with the medical care. The presence in the biofilms gives pathogens additional resistance to antimicrobial agents and factors of the immune system, as well as new properties, which 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 (2).

As follows, it promotes increasing interest among scientists and practical physicians to overcoming outbreak of multi-drug resistant *A.baumannii* in ICU by monitoring of changes in its biological characteristics and implementation of new antibacterial drugs.

The purpose of this research was to study the biofilm-forming properties of clinical strains of *A.baumannii*, isolated from burn wounds in patients of ICU, and their sensitivity to antiseptics.

MATERIALS AND METHODS

Biological samples had been collecting from 435 ICU infected burn patients in N. I. Pirogov Vinnitsa Regional Clinical Hospital during a 7-year period from 2011 to 2018. Bacterial isolates were sampled from patients with second- and third-degree burns (burn area 30.0-85.0 % of body surface) before antimicrobial medication. Patients with immunodeficiency, HIV, diabetes and other chronic diseases in history were excluded from the research.

Samples included swabs from burn wounds, which were plated on 5% sheep blood agar and CHROMagar Acinetobacter (Paris, France). We incubated plates at 37°C for 24 hours. Automatic bacteriological analyser Vitec - 2compact bioMйrieux (France) carried out final identification. This study was approved by the ethics committee of National Pirogov Memorial Medical University and conformed to the provisions of the Declaration of Helsinki (as revised in Seoul, Republic of Korea, October 2008).

Cultivation and final biochemical identification of clinical bacterial strains were performed by the certified scientific bacteriological laboratory of the Department of Microbiology of the National Pirogov Memorial Medical University, Vinnytsya (certificate of the Ministry of Public Health of Ukraine No 049/15 from 02.02.2015). 220 clinical strains of *A. baumannii* isolated from ICU infected burn patients were the object of the study.

Antiseptic sensitivity of *Acinetobacter spp.* (decamethoxine, chlorhexidine, miramistin, povidone iodine) was investigated using double serial dilutions according to the standard procedure (9). The recommendations of European Committee on Antimicrobial Susceptibility Testing (EUCAST Expert rules) were used for the analysis of the susceptibility of *A.baumanii* clinical strains to antiseptics (10).

The study of biofilm-forming properties of clinical *Acinetobacter* isolates was performed using the spectrophotometric technique by G.D. Christensen (MtP microtitre 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 (ODU) using a spectrophotometer (570 nm). The ability of microorganisms to form biofilms was assessed as low (at ODU <0.120), average (at ODU = 0.121-0.239) and high (at ODU > 0.240) (14) (11, 12). The statistical analysis of the results was carried out by standard "STATISTICA+" and "Microsoft Excel 2010" software packages. In order to determine the relationship between the antiseptic sensitivity and biofilm-forming properties of *A. baumannii* strains, we determined the correlation coefficient (r-Pearson), the absolute value of which characterised the binding force.

RESULTS

The findings obtained demonstrated the continuous high level of SSI among burn persons in ICU of N. I. Pirogov Vinnitsa Regional Clinical Hospital during a 7-year period from 2011 to 2018. SSI developed in 172±15 patients of 1000 cases on average for 7 years.

P. aureginosa (57.5±9.24 %), *S. aureus* (17.0±2.18 %), *S. epidermidis* (4.6±1.2 %), *E. feacalis* (3.9±1.9 %), *E. faecium* (2.3±1.42 %) were found as predominant pathogens, in burn patients, who were involved in the investigated group. The study showed representatives of *Acinetobacter spp.* were found in 220 (50.6%) among 435 patients of ICU, that has revealed the etiological significance of the opportunistic pathogens of *Acinetobacter* isolates in the development of infectious complications of burns in ICU.

Clinical strains of *A. baumannii* were the most susceptible to decamethoxine and the MBC (minimum bactericidal concentration) against them was 36.66±13.02 μg/ml (Table I). Miramistin showed lower activity against investigated isolates in comparison to decamethoxine, but results had no statistical significance (p>0.05). Nevertheless, the MBC of chlorhexidine and povidone iodine were comparatively higher than the MBC of decamethoxine 1.9 and 99.7 times respectively.

Acinetobacter strains, infecting burn wounds of ICU patients, have been found to possess high biofilm-forming properties. The optical density of the biofilms

Table I: Susceptibility of A. baumannii (n=220) to antiseptics, M+m

Antiseptic	MIC (μg/ml)	MBC (μg/ml)
Decamethoxine 0,1%	20,87±7,73	36,66±13,02
Chlorhexidine 0,05%	45,07±23,28	69,41±36,42*
Miramistin 0,01%	41,35±11,95	57,21±17,65
Povidone iodine 10%	2271,64±1465,26	3653,85±2768,87*

Note: MIC - minimal inhibiting concentration; MBC - minimum bactericidal concentration; * - reliability of results differences of the MBC of chlorhexidine, povidone iodine comparatively to the MBC of decamethoxine, p<0.05.

formed by these microorganisms were 0.45 ± 0.13 ODU.

The r-Pearson coefficient (+0.67) between sensitivity of *A. baumannii* to decamethoxine and biofilm-formation pointed out a positive moderate correlation (Fig. 1). That is, with the increase sensitivity of the microorganisms, their ability to form biofilms increases.

Nevertheless, relationship between sensitivity of clinical strains to miramistin and their biofilm-forming properties tends to the previous results with decamethoxine. The correlation value was r=+0.63 in this case, that proved a positive moderate correlation between investigated properties (Fig.2).

The correlation coefficient between sensitivity of *Acinetobacter* strains to Povidone iodine and their biofilm formation was a little bit higher (+0.82), than r-Pirson coefficients with decamethoxine and miramistin. Its value pointed out a positive strong correlation (Fig.3). Ultimately, the r-Pearson coefficient (+0.9) between sensitivity of *Acinetobacter* strains to chlorhexidine and their biofilm formation has proved perfect strong positive correlation (Fig.4).

DISCUSSION

Our study was focused on some of the most commonly used antiseptics in ICU patients: chlorhexidine, povidone iodine, miramistin and decamethoxine. The 0.5% chlorhexidine digluconate is a "gold standard" in surgery. It is recommended in infected wounds, common skin infections as antiseptic agent. Chlorhexidine is used in healthy skin before and during minor surgeries (13). According to our results, chlorhexidine has shown lower effectiveness against *Acinetobacter* strains compared to other investigated antiseptics. It agrees with literature data about a steady increase in resistance to chlorhexidine among gram-negative pathogens (14, 15). It is due to a high frequency of qac genes in *Acinetobacter* baumannii, which promotes markedly reducing of its susceptibility to this antiseptic (15). In turn, biofilm-forming properties of investigated clinical strains demonstrate the highest (perfect) positive correlation with their susceptibility rate to chlorhexidine. It provides ease of use and prognosis in combating SSI.

The povidone iodine consists of iodine and polyvinylpyrrolidone. It is commonly applied on healthy

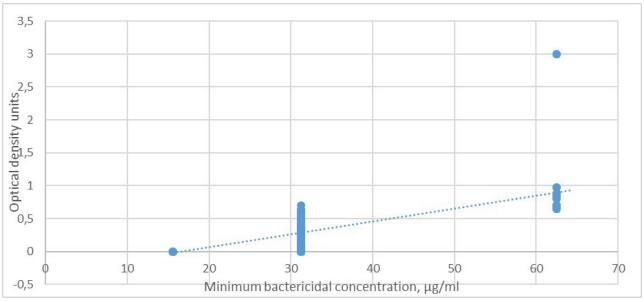

Figure 1: Correlation between sensitivity to decamethoxine and biofilm formation of A. baumannii (n = 220)

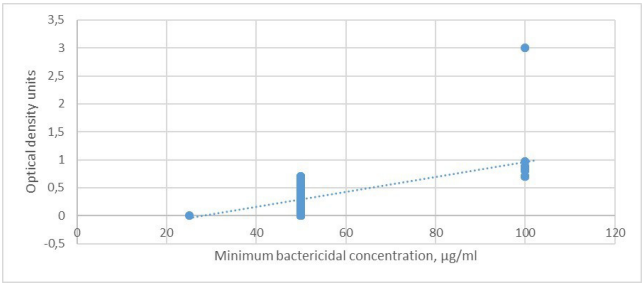

Figure 2 Correlation between sensitivity to miramistin and biofilm formation of A. baumannii (n = 220)

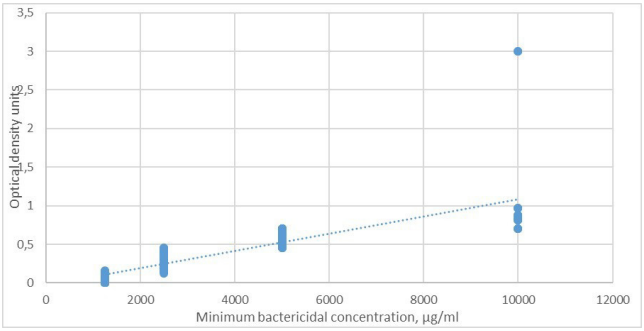

Figure 3: Correlation between sensitivity to povidone iodine and biofilm formation of A. baumannii (n = 220)

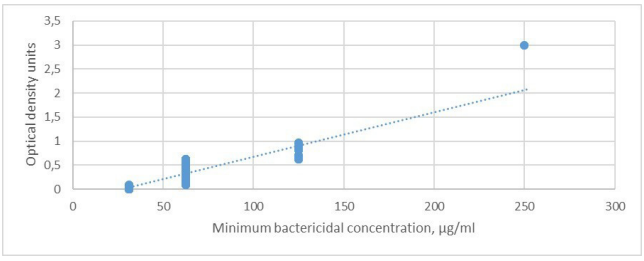

Figure 4: Correlation between sensitivity to chlorhexidine and biofilm formation of A. baumannii (n = 220)

and contaminated skin before surgery, preoperative shower in its purest form. For the scrubbing of wounds, it is used in diluted form (13). The povidone iodine has demonstrated week effectiveness against *Acinetobacter* spp., however susceptibility to it shows very strong correlation with ability to form biofilm. In spite of frequent use of the povidone iodine in ICU worldwide, high MIC against SSI pathogens and individual iodine hypersensitivity would diminish its importance.

miramistin is a member of the cationic surface-active detergents. Although it has been known as an effective

antiseptic for the local treatment of infected wounds with low side effects since 1993, it is only used in Eastern Europe (16, 17). In our study, miramistin has shown high antimicrobial activity against *Acinetobacter* strains. Moreover, our results prove positive strong correlation between biofilm formation of *A. baumannii* and its susceptibility to miramistin. Nevertheless, it is a known fact, that miramistin shows significant cytotoxic effects *in vitro* (16).

Decamethoxine is another member of the cationic surface-active detergents. Its antibacterial, antifungal, antiviral activities were proved by many-year studies and clinical trials (18, 19). Decamethoxine poins out the most powerful bactericidal effect on *Acinetobacter spp.* Additionally, susceptibility of investigated isolates to it are strongly correlated with biofilm-forming potential of A. baumannii. It obviously makes decamethoxine more predicted for use in treatment of SSI.

Results obtained during investigation have demonstrated strong correlation between biofilm-forming properties of *Acinetobacter spp*. with susceptibility to antiseptics, which antibacterial activity was much lower. It means that all isolates with high ability to form biofilms were less sensitive to povidone iodine and chlorhexidine. In turn, moderate correlation between susceptibility of *A. baumannii* to decamethoxine and miramistin and biofilm-formation of these microorganisms has proved that some clinical strains with high ability to form biofilms maintain sensitivity to mentioned antiseptics.

CONCLUSION

Decamethoxine, miramistin and chlorhexidine showed high antimicrobial activity against *A. baumannii* at low concentrations. Biofilm-formation of *Acinetobacter spp*. is correlated with their susceptibility to chlorhexidine, povidone iodine, miramistin and decamethoxine perfectly and strongly. However, as more powerful antimicrobial activity of antiseptic against *A. baumannii* was as weaker correlation had been established.

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