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SUSCEPTIBILITY TO ANTIMICROBIALS OF *ACINETOBACTER BAUMANNII* AND *PSEUDOMONAS AERUGINOSA* CLINICAL STRAINS AND THEIR *blaVIM* VARIANTS IN ICU OF REGIONAL BURN CENTRE

Citlivosť na antimikróbne látky klinických kmeňov *Acinetobacter baumannii* a *Pseudomonas aeruginosa* i ich *blaVIM* variantov na JIS regionálneho popáleninového centra

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Abstract

Aim. To study in clinical strains of *Acinetobacter baumannii* and *Pseudomonas aeruginosa*, as dominating in ICU of Regional Centre of Thermal Injury, their susceptibility to antibiotics, antiseptics and the incidence of *blaVIM* variants among them.

Materials and methods. Clinical strains of *A. baumannii* and *P. aeruginosa* were collected from 126 patients with burns wounds in the early three days after injury in the period from February 2022 to August 2022. There was determined susceptibility of the 83 *A. baumannii* and 43 *P. aeruginosa* clinical strains to antibiotics using disc-diffusion method, and to antiseptics – by means of double serial dilutions method. Among the isolated clinical strains with phenotypic resistance to carbapenems (double-disk diffusion test with meropenem and imipenem). The molecular identification of VIM genes was performed by polymerase chain reaction (PCR).

Results. The lowest sensitivity of acinetobacteria was found to ceftazidime (4,82%), cefepime (4,82%), piperacillin-tazobactam (34,94%), meropenem (15,66%), imipenem (27,71%), levofloxacin (3,61%), ciprofloxacin and gatifloxacin (4,82%), moxifloxacin (9,38%), ofloxacin (12,5%). Received data demonstrated the highest resistance of *P. aeruginosa* to beta-lactam antibiotics, including carbapenems (imipenem 46,51%, meropenem 53,49%). Among clinical strains of Gram-negative bacteria with phenotypic resistance to carbapenems, the presence of the VIM gene in *A. baumannii* (n = 11; 13,3%), *P. aeruginosa* (n = 14; 33,3%) was found by PCR. The research has shown high sensitivity of *A. baumannii* to decamethoxine, octenidine; and high resistance to antiseptics of *P. aeruginosa*.

Conclusions. Among isolates from patients in burn ICU with perioperative infectious complications *A. baumannii* and *P. aeruginosa* strains, which have low sensitivity to aminoglycosides, fluoroquinolones, inhibitor-protected piperacillin, cephalosporins, as well as phenotypic resistance to carbapenems imipenem and meropenem there is high risk of variants with *blaVIM* genes. However, their high susceptibility to detergents remain great importance of antiseptics in the prevention of infection in burn patients (Tab. 3, Fig. 2, Ref. 21). Text in PDF www.lekarsky.herba.sk.

KEY WORDS: *blaVIM* genes, burn wounds, non-fermenting gram-negative bacteria, resistance to antibiotics.

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Introduction

Infectious and inflammatory complications in patients with surgical pathology occur in 7-10% of cases, according to the WHO. Moreover, despite the fact that their number depends on the level of development of the country, the European Center for Disease Prevention and Control indicates a steady increase in infectious complications as in Developed so in Developing Countries (9, 21). The spectrum of dominant pathogens of infectious and inflammatory complications in surgery has not changed over the past 30 years, but recently, under the war conflict in Ukraine, there has been an increase in the frequency of elimination of gram-negative microorganisms in patients with burns. Of note, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* species are the most common pathogens contributing to primary health-care associated infections in burn patients and are prevalent among critically wounded with burns (13, 16). Thus, it is known that *P.aeruginosa* causes above 17% of infectious complications in patients with burns and especially combat ones, and leads to the 80% of mortality incidence among them (1).

Combat wound infection can be caused by drug-resistant ESKAPE-E (*Enterococcus species*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter cloacae*, and *Escherichia coli*) pathogens, and are a major concern for the Military Healthcare System.

An important etiological feature of wound infectious complications is the constant change in the biological properties of microorganisms (17). Nowadays the rapid acquisition of resistance to chemotherapeutic agents in bacteria has become the biggest public health crisis. According to the International Nosocomial Infection Control Consortium, the incidence of antibiotic resistance among the total bacterial spectrum of nosocomial infections exceeded 10% (2003-2010) (18). In hospitals and intensive care units, the situation is complicated by the emergence of multidrug-resistant clinical strains of microorganisms, being resistant to two or more pharmacological groups of antibiotics. According to the WHO, *A. baumannii*, *P. aeruginosa* are classified as problematic pathogens of the first level in terms of the prevalence of polyanTIMicrobial resistance to carbapenems. However, carbapenem resistance of acinetobacteria and pseudomonads is a global challenge (8).

Therefore, constant monitoring of antibiotic susceptibility of clinically significant species of microorganisms, causing hospital-acquired infectious and inflammatory complications in intensive care units (ICU) and surgery, becomes important because of detecting main mechanisms of antibiotic resistance development in them to investigate approaches for its overcoming and find new effective antimicrobials.

The aim was to study the susceptibility to antibiotics and antiseptics and determine *blaVIM*-patterns in clinical strains of two dominant species of non-fermenting Gram-negative bacteria, received from ICU burn patients.

Materials and methods

The 126 patients with infectious complications of burn wounds in the period from February 2022 to August 2022, including those who had received combat burns were enrolled in the study. All this patients had a negative result of the Ag test for SARS-CoV-2. Other patients with confirmed SARS-CoV-2 infection were isolated and treated in another department according to current protocols and recommendations for the treatment of SARS-CoV-2 infection (5, 7, 12). Some of them required artificial pulmonary ventilation for the development of bilateral interstitial pneumonia (3, 4, 6, 11). Patients without SARS-CoV-2 infection were treated at the Tertiary Clinical Center for Thermal Injury and Plastic Surgery of the Municipal Non-profit Enterprise "Vinnytsya Regional Clinical Hospital Vinnytsya Regional Council". Biomaterial samples (pus, secretions from wounds, burned mucosa) were collected before the beginning of antibacterial therapy, directly in the ward under aseptic conditions with a sterile swab, which was placed in a sterile test tube and delivered within 2 hours to the bacteriological laboratory. The obtained samples were cultured on meat-peptone agar supplemented with 5% sheep erythrocytes and CHROMagar *Acinetobacter* (Paris, France). The final identification was carried out using NEFERMtest24 (PLIVA - Lachema as Brno, Czech Republic).

This study was approved by the ethics committee of the National Pirogov Memorial Medical University and conformed to the provisions of the Declaration of Helsinki (as revised in Seoul, Republic of Korea, October 2008). Non-fermenting Gram-negative rods, including 83 strains of *A. baumannii* and 43 strains of *P. aeruginosa*, had been isolated from 126 patient samples.

The susceptibility of the studied clinical isolates to antibiotics was determined using the standard disc-diffusion method, and method of double serial dilutions (2). Antimicrobial effectiveness of antiseptics against isolated strains of microorganisms was also studied in the research by means of double serial dilution test. The Muller-Hinton nutrient broth and agar were used for cultivation of bacteria in the study. The analysis of antibiotic susceptibility to antibiotics was guided by the recommendations of the European Committee for the Study of Antimicrobial Susceptibility (EUCAST Expert rules) (19).

Screening of metallo-beta-lactamase (M β L) phenotypes in gram-negative microorganisms was performed by means of well-known double-disk diffusion test with meropenem and imipenem (Double Disk Synergy Test (DDST), by Lee et al. (14).

Among the isolated clinical strains with phenotype of resistance to carbapenems subsequently there was performed the molecular identification of VIM genes, encoding β -lactamases of class B, through which one of the mechanisms of resistance to carbapenems is realized.

Determination of VIM genes was carried out by polymerase chain reaction. Amplification was performed

using the methods of "BioRad iQ 5". The final identification of VIM genes in pure culture of clinical strains of Gram-negative bacteria was performed by real-time PCR according to the manufacturer's instructions (01784-RT-C; LLC NPF "Litech").

Results

Analyzing the results of the susceptibility of the studied strains, the variable sensitivity of clinical strains of *A.baumannii* to antibiotics of different pharmacological groups was revealed. There was found the lowest sensitivity of acinetobacteria to such cephalosporin antibiotics as cefepime (4,82%), ceftazidime (4,82%). In addition, low susceptibility was found to inhibitor-protected beta-lactam antibiotics piperacillin-tazobactam (34,94%). Clinical strains of acinetobacteria demonstrated low sensitivity to carbapenems, in particular to meropenem (15,66%). There were found 65,06% of resistant isolates to imipenem (Tab. 1).

Table 1. Sensitivity to antibiotics of clinical strains of *A. baumannii* (n = 83) isolated from patients (in %).

Antibiotics	Susceptible strains	Moderately resistant strains	Resistant strains
Piperacillin-Tazobactam	34,94	8,43	56,63
Ceftazidim	4,82	2,41	92,77
Cefoperazone-sulbactam	55,42	20,48	24,10
Cefipime	4,82	0,00	95,18
Imipinem	27,71	7,23	65,06
Meropenem	15,66	1,20	83,13
Doxycyclin	50,60	6,02	43,37
Gentamicin	51,81	4,82	43,37
Tobramycin	61,45	6,02	32,53
Amicacin	8,43	9,64	81,93
Aztreonam	16,87	12,05	71,08
Norfloxacin	7,23	2,41	90,36
Ofloxacin	15,66	0,00	84,34
Pefloxacin	1,20	1,20	97,59
Ciprofloxacin	4,82	1,20	93,98
Levofloxacin	3,61	1,20	95,18
Gatifloxacin	4,82	0,00	95,18
Moxifloxacin	8,43	0,00	91,57
Polymyxin B	91,57	2,41	6,02

Low sensitivity of clinical strains to fluoroquinolones was established. Only 4,81% and 6,02% of the studied strains were susceptible and moderately resistant to levofloxacin and ciprofloxacin. Slightly higher sensitivity of *A.baumannii* was found to moxifloxacin (9,38%), ofloxacin (12,5%). Acinetobacteria were resistant to amikacin (81,93 %). However, 51,81% of *A.baumannii* strains demonstrated sensitivity to gentamicin and 61,45% of the studied isolates were susceptible to tobramycin. Similar antimicrobial activity against acinetobacteria was found in doxycycline (50,60%).

Received data demonstrated the highest resistance of *P.aeruginosa* clinical strains to beta-lactam antibiotics, including carbapenems (to imipenem – 46,51% and meropenem – 53,49%) (Tab. 2).

Table 2. Susceptibility to antibiotics of clinical strains of *P. aeruginosa* (n=43) isolated from patients (in %).

Antibiotics	Susceptible strains	Moderately resistant strains	Resistant strains
Piperacillin-tazobactam	32,56	6,98	60,47
Ceftazidim	18,60	2,33	79,07
Cefoperazone-sulbactam	30,23	6,98	62,79
Cefipime	25,58	2,33	72,09
Imipinem	51,16	2,33	46,51
Meropenem	39,53	6,98	53,49
Gentamicin	30,23	2,33	67,44
Tobramycin	39,53	2,33	58,14
Amicacin	36,36	15,15	48,48
Doxycyclin	25,58	4,65	69,77
Aztreonam	20,93	6,98	72,09
Norfloxacin	16,28	0,00	83,72
Oxacillin	18,60	9,30	72,09
Pefloxacin	13,95	4,65	81,40
Ciprofloxacin	23,26	4,65	72,09
Levofloxacin	25,58	4,65	69,77
Gatifloxacin	32,56	2,33	65,12
Moxifloxacin	34,88	4,65	60,47

The sensitivity of *P.aeruginosa* was low to inhibitor-combined penicillins and cefoperazone, and also to the fourth-generation of cephalosporin antibiotic cefepime that did not exceed 30,23%. There was established low susceptibility among the studied clinical strains to piperacillin-tazobactam (32,56%) and ceftazidime (18,60%).

Screening phenotypic manifestations metallo- β -lactamase activity of carbapenem-resistant Gram-negative microorganisms confirmed the existence of such properties in clinical strains *A. baumannii* (n = 83), *P. aeruginosa* (n = 43).

Thus, among clinical strains of Gram-negative bacteria with phenotypic resistance to carbapenems, the presence of the *blaVIM* gene, which encodes the production of metallo- β -lactamases of molecular class B in *A. baumannii* (n = 11; 13,3%), *P. aeruginosa* (n = 14; 33,3%) was found by PCR (Fig. 1, 2).

On the schedule *blaVIM*-dependent resistance to carbapenem antibiotics in the observed pathogens showed gene amplification curves, which clearly indicated the identification of these strains of *A.baumannii* and *P.aeruginosa* *blaVIM* gene, compared with the curves of internal control.

In the study there was established the susceptibility of clinical isolates to all investigated antiseptics. The research has shown high sensitivity of clinical strains of *A.baumannii* to decamethoxine (DCM), octenidine, as evidenced by the value of the minimum bactericidal

Figure 1. Amplification curve of *blaVIM*-positive strains of non-fermenting gram-negative bacteria *A. baumannii*.

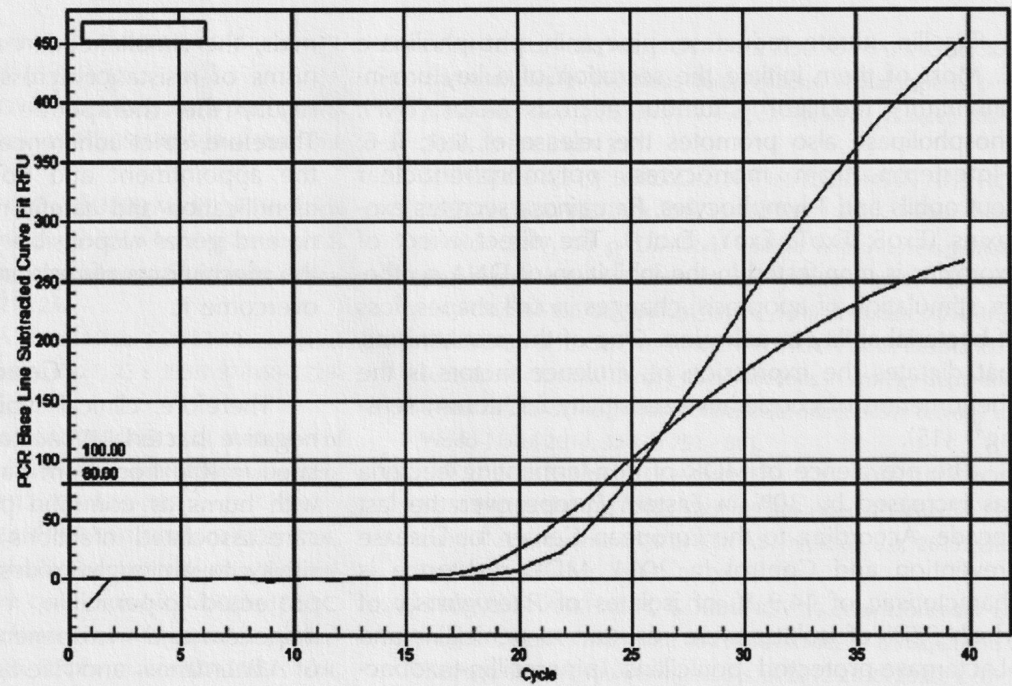
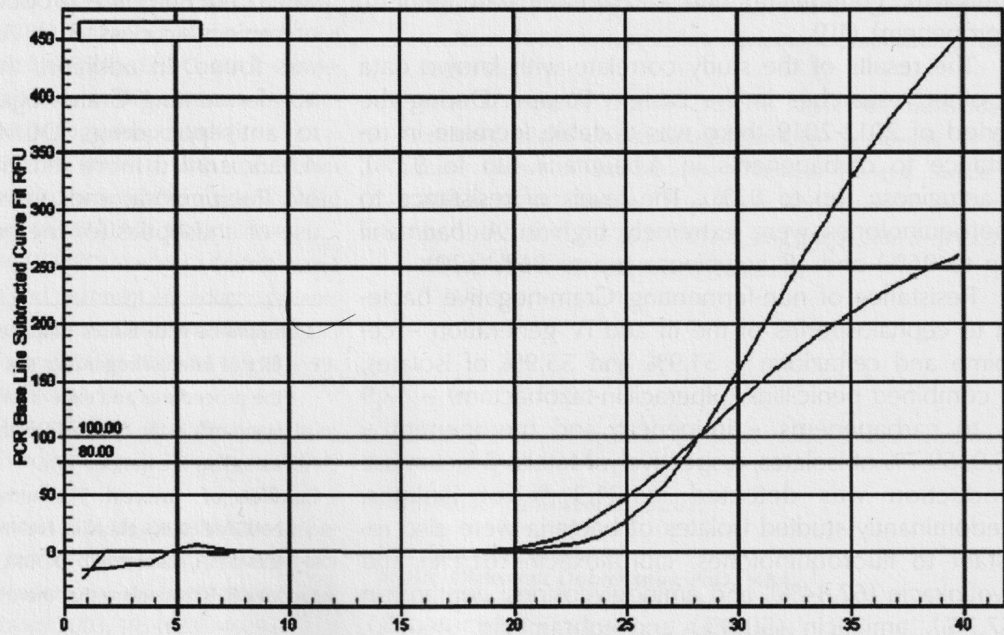


Figure 2. Amplification curve of *blaVIM*-positive strains of non-fermenting gram-negative bacteria *P. aeruginosa*



concentration (MBcC) of drugs. At the same time, the MBcC against acinetobacteria in DCM was found to be 1.5 times less than miramistin and 1.8 times less than chlorhexidine (Tab. 3).

Table 3. Sensitivity of gram-negative non-fermenting bacteria isolated from patients to antiseptics. MBcC*, µg/ml (M ± m).

Micro-organisms (n)	Antiseptics			
	deca-methoxine 0.1%	octeni-dine 0.1%	miramistin 0.01%	chlorhexidine bigluconate 0.05%
<i>A. baumannii</i> (n = 83)	24.47 ± 3.49	26.03 ± 3.55	37.5 ± 5.98***	47.91 ± 8.95***
<i>P. aeruginosa</i> (n = 43)	128.47 ± 25.75	114.58 ± 27.56	87.5 ± 12.5	236.11 ± 55.88

Legend: * MBcC - minimum bactericidal concentration; *** - the reliability of the difference to DCM (p <0,001).

There was found that clinical strains of *P.aeruginosa*, which caused infectious and inflammatory complications in surgical patients are highly resistant to antiseptics, as evidenced by their high MBcC. Thus, the lowest MBcC for *P.aeruginosa* were found in miramistin, however, despite the higher bactericidal concentrations of DCM and chlorhexidine, but the difference was not significant (p>0.05).

Discussion

The pathogenicity of non-fermenting Gram-negative bacteria is determined by the ability to invade and persist in tissues, as well as to the cytotoxic effect and stimulation of the generalized inflammatory response. Factors, that directly affect the formation of local and systemic inflammation are lipopolysaccharide, exotoxin

S, flagellin, nitrate reductase, piocyanin, phospholipase C. Most of them initiate the secretion of a key pro-inflammatory mediator – tumour necrosis factor (TNF); phospholipase also promotes the release of IL-1; IL-6; γ -interferon from monocytes, polymorphonuclear neutrophils and T-lymphocytes. *Paruginosa* secretes exotoxins (ExoS; ExoT; ExoY; ExoU). The direct effect of exotoxins is manifested in the inhibition of DNA synthesis, stimulation of apoptosis, changes in cell shapes, loss of bacterial ability to adhesion. One of the mechanisms, that dictates the expression of virulence factors is the phenomenon of cooperative sensitivity („Quorum sensing“) (15).

The prevalence of MDR of non-fermenting bacteria has increased by 30% in Eastern Europe over the last decade. According to the European Center for Disease Prevention and Control in 2014, MDR resistance is characteristic of 14,9 % of isolates of *P.aeruginosa*, of which 5,5% of isolates were resistant to penicillins and β -Lactamase-protected penicillins (piperacillin-tazobactam), fluoroquinolones (levofloxacin), aminoglycosides (amikacin, gentamicin) and carbapenems (imipenem, meropenem) (10).

The results of the study correlate with known data of other researches in the Eastern Europe. During the period of 2012-2019 there was a stable increase in resistance to carbapenems in *A.baumannii* (up to 93%), *P. aeruginosa* (up to 83%). The levels of resistance to fluoroquinolones were extremely high in *A. baumannii* (up to 96%) and *P. aeruginosa* (up to 86%) (20).

Resistance of non-fermenting Gram-negative bacteria to cephalosporins of the III and IV generation – ceftipime and ceftazidim – 51,9% and 55,9% of isolates, to combined penicillins (piperacillin-tazobactam) – 57,9 %, to carbapenems – imipenem and meropenem – 65,0, 59,7% of isolates, respectively. Metallo- β -lactamase production was detected in 21,3 % of isolates. Predominantly studied isolates of bacteria were also resistant to fluoroquinolones: ciprofloxacin (61,1%) and levofloxacin (62,84%), and aminoglycosides: gentamicin (57,7%), amikacin (50,5%) and tobramycin (46,4%). Phenotypic multiple resistance (MDR resistance) was possessed by 83,2% of isolates, phenotypic extreme resistance (XDR resistance) – 51,4% of isolates. The presence of phenotypic manifestations of resistance to carbapenems associated with the M β L mechanism was determined in clinical strains of *P. aeruginosa* (46,5%), *A. baumannii* (21,6%). It is proved that among the leading gram-negative pathogens of infectious complications with the M β L mechanism of antibiotic resistance, carriers of VIM genes have been circulating (*P. aeruginosa* 11,8%, *A. baumannii* 2,7%). In the general structure of Gram-negative pathogens of infectious complications, the presence of VIM-dependent resistance to carbapenem antibiotics in *P. aeruginosa* (3,1%), *A. baumannii* (1,2%) was determined.

Increasing resistance to antibacterial drugs due to the influence of plasmid-induced mechanisms is a serious problem in treatment. Due to the activity of plas-

mids, the simultaneous coexistence of several mechanisms of resistance (cross-resistance) is possible, as a result, the therapeutic choice is initially limited. Therefore, strict adherence to the recommendations for the appointment and dosage of antibacterial drugs, identification and careful monitoring of resistant bacteria and genes responsible for it, are necessary to study the mechanisms of resistance and find possible ways to overcome it.

Conclusion

Therefore, clinical strains of non-fermenting Gram-negative bacteria *A.baumannii* and *P.aeruginosa*, isolated in ICU from burn patients and critically wounded with burns as common pathogens of primary health-care associated infections complications have low sensitivity to aminoglycosides, fluoroquinolones, inhibitor-protected piperacillin, cephalosporins, as well as imipenem and meropenem. Among the clinical strains of *A.baumannii* and *P.aeruginosa* with phenotypic resistance to carbapenems, the presence of the VIM gene, which encodes the production of metallo- β -lactamases of molecular class B in *A.baumannii* and *P.aeruginosa*, was found. In addition, the studied clinical isolates of non-fermenting Gram-negative bacteria remain sensitive to antiseptic drugs DCM, chlorhexidine, miramistin. *A.baumannii* is more sensitive to these drugs than strains of *P.aeruginosa*, and proves great importance of early use of antiseptics for the prevention of infection in burn patients.*

***Compliance with Ethics Requirements:** The authors declare no conflict of interest regarding this article. The authors declare, that all the procedures and experiments of this research respect the ethical standards in the Helsinki Declaration of 1975, as revised in 2008 (5), as well as the national law.

Conflicts of Interest. The authors Oleksandr NAZARCHUK, Vasyl NAGAICHUK, Nataliia BAHNIUK, Halyna NAZARCHUK, Olena RYMSHA, Oleksandr DOBROVANOV, Hennadii TULCHYNSKYI, Vira BEBYK declare that there is no conflict of interest.

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