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ORIGINAL RESEARCH/ОРИГІНАЛЬНІ ДОСЛІДЖЕННЯ

THE CORRESPONDENCE OF THE CARBAPENEMASE GENOTYPE AND PHENOTYPIC ANTIMICROBIAL PROFILES OF *PSEUDOMONAS AERUGINOSA*

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The aim of the study was to determine the correspondence between the carbapenemase genotype and the phenotypic antimicrobial profiles of *P. aeruginosa.*
Materials and methods. The study included 51 clinical isolates of *P. aeruginosa*, isolated from the patients with post-operative complications of the respiratory organs. The final identification of the obtained isolates was performed in the Riesbeck laboratory using MALDI-ToF (Bruker), followed by the determination of their sensitivity to antimicrobial drugs at the EUCAST Development Laboratory (Växjö, Sweden). Determination of the resistance genes was carried out by using polymerase chain reaction in real time (PCR-RF). The antimicrobial resistance index (ARI) was determined according to the method of G.V. de Socio. Statistical analysis was performed using the standard IBM SPSS Statistics software version 22.0 and GraphPad Prism Software 10.1.0. (USA, 2023).
Results. 39 strains of *P. aeruginosa* (76.5%) showed polyresistance, and 26 of them (51.0%) were resistant to all antibiotics. According to research data, *P. aeruginosa isolates* most often carried the *bla*$_{VIM}$ gene. Genetically determined production of oxacillinase group β-lactamase class D among clinical isolates of *P. aeruginosa* occurred somewhat less often. Based on the obtained results, four carbapenemase genetic resistotypes of *P. aeruginosa* as pathogens of respiratory tract complications in critically ill patients were established. We detected the antimicrobial resistance index (ARI) based on the phenotypic characteristics of *P. aeruginosa* at the level of 0.69±0.39. The phenomenon of statistically reliable correlation of the ARI of microorganisms by phenotypic characteristics with their carbapenemase genetic resistotypes was established.
Conclusions. 76.5% of strains of *P. aeruginosa* show polyresistance, and 51.0% of them are resistant to all antibiotics. Four different carbapenemase genetic resistotypes of *P. aeruginosa* as pathogens of respiratory tract complications in critically ill patients were established. There is the phenomenon of statistically reliable correlation of the ARI of microorganisms by phenotypic characteristics with their carbapenemase genetic resistotypes.
Keywords: *P. aeruginosa,* antibiotics, drug resistance, multi-drug resistance, carbapenems, phenotypic profile, genes of resistance.

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Introduction

In its 2017 report, the WHO published a list of microorganisms for which the development of new antimicrobials was critically needed. At the same time, *Pseudomonas aeruginosa* (*P. aeruginosa*) was among the most prioritized among them [1]. After all, representatives of this species of bacteria are the dominant causative agents of healthcare-associated infections and create excessive danger in intensive care units [2]. *P. aeruginosa* has a powerful arsenal of pathogenicity factors and genetically determined mechanisms of resistance to environmental factors, including antibiotics and antiseptics [3, 4]. This pathogen has natural mechanisms of resistance to antibiotics, such as a decrease in the permeability of the outer membrane, the production of enzymes that inactivate antimicrobial agents, and the expression of efflux pumps. However, along with this, they are characterized by horizontal transmission of resistance genes and frequent mutations [5]. It follows that representatives of this species can change the pattern of antibiotic resistance very quickly by changing their genotypic profiles.

Therefore, the aim of the study was to determine the correspondence between the carbapenemase genotype and the phenotypic antimicrobial profiles of *P. aeruginosa.*

Materials and methods

The study included 51 clinical isolates of *P. aeruginosa*, isolated from the patients with post-operative complications of the respiratory organs at Municipal Non-Profit Enterprise Vinnytsia Regional Clinical Hospital named after M. I. Pirogov Vinnytsia Regional Council during 2022-2023. Specimens for further analysis were collected from the site of infection with sterile tampon probes and placed in tubes with Amies transport medium. Cultivation of microorganisms was carried out according to the standard culture method on Columbia agar at a temperature of 37°C. The final identification of the obtained isolates was performed in the Riesbeck laboratory using MALDI-ToF (Bruker), followed by the determination of their sensitivity to antimicrobial drugs at the EUCAST Development Laboratory (Växjö, Sweden) [6].

Written informed consent was obtained from each patient after providing a detailed explanation of the aims and protocol of the study. The study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki on Ethical Principles for Medical Research Involving Humans. It was approved by the Biomedical Ethics Committee of Poltava State Medical University (minutes No. 210 as of November 23, 2022) and the Bioethics Committee of National Pirogov Memorial Medical University, Vinnytsya (minutes No. 11 as of November 10, 2022).

The sensitivity of clinical isolates of Gram-negative bacteria to antibacterial drugs was determined using the standard Kirby-Bauer disc diffusion method according to the EUCAST methodology. The isolates were categorized as sensitive (S), resistant (R) and sensitive under increased exposure (I), according to the tables of the limit indicators of the diameters of the growth retardation zones of microorganisms in the presence of the antibiotic (EUCAST Version 13.0, valid from 2023-01-01) [7].

Determination of the resistance genes was carried out by using polymerase chain reaction in real time (PCR-RF) in accordance with methodical instructions for the kit for determining genes of resistance to of carbapenems VIM in plasmid DNA of bacteria (Fluoropol-RV format; 01784-RV-S; NPF "Litekh" LLC). Amplification of the corresponding section of the studied genes was performed with amplifier "BioRad iQ 5".

The antimicrobial resistance index (ARI) was determined according to the method of G.V. de Socio (2019) [8]. For this purpose, on the basis of the previously conducted disk diffusion method, the value "0" was assigned to each individual isolate when determining sensitivity to the antibiotic, "0.5" when determining sensitivity under increased exposure to the antibiotic, and "1" when determining resistance. The obtained indicators regarding the sensitivity of each individual isolate to all antibiotics used in the study were added, followed by division by the number of antibiotics (arithmetic mean).

Thus, ARI with a value of "0" corresponded to microorganisms completely sensitive to all antibiotics, ARI equal to "1" - absolutely resistant strains.

The normality of the data distribution was assessed using the Shapiro-Wilk test. Hypothesis testing was conducted using a two-sided approach. The data are expressed as mean (SD) and median (minimum-maximum), numbers and percentages (n, %). A significance level of $P < 0.05$ was considered statistically significant.

One-way analysis of variance (ANOVA: one factor) was used to compare the results of three or more groups of data. The Bonferroni correction adjusted the level of significance to control the overall error probability (false positive) for testing multiple hypotheses. The result was considered reliable if the p-value was less than 0.05. Statistical analysis was performed using the standard IBM SPSS Statistics software version 22.0 and GraphPad Prism Software 10.1.0. (USA, 2023).

Results

The study identified variable sensitivity to antibiotics among clinical isolates of the genus *Pseudomonas*, isolated from seriously ill patients experiencing in-

fectious complications of the respiratory system (Fig. 1). It is worth noting that 39 strains of *P. aeruginosa* (76.5%) showed polyresistance, and 26 of them (51.0%) were resistant to all antibiotics.

We found (abs. 19) 37.3% *Pseudomonas* spp., which retained sensitivity to piperacillin/tazobactam. The sensitivity of representatives of this genus to cephalosporins varied from 0.0% to 41.2%. The worst result was the test with cefazolin, because all tested strains of *P. aeruginosa* showed resistance to it. Cephalosporins of subsequent generations had better activity: the shares of resistant *P. aeruginosa* isolates to cefepime and ceftolazan/tazobactam were 66.7% and 60.7%, respectively. It turned out to be interesting that the sensitivity of representatives of this genus to ceftazidime (33.3%) increased to 41.2% when testing the protected form of the antibiotic with avibactam.

We obtained similar results on the sensitivity of *Pseudomonas* spp. to fluoroquinolones. Despite the fact that the share of isolates sensitive to ciprofloxacin (23.5%) exceeded the share of those sensitive to levofloxacin (19.6%), resistance among *P. aeruginosa* to both antibiotics was the same and amounted to 76.5%.

The studied representatives of the genus *Pseudomonas* were resistant to imipenem in 74.5% of cases. The proportion of pseudomonads resistant to meropenem was 58.8%, which was generally one of the lowest results of the development of resistance among *P. aeruginosa*.

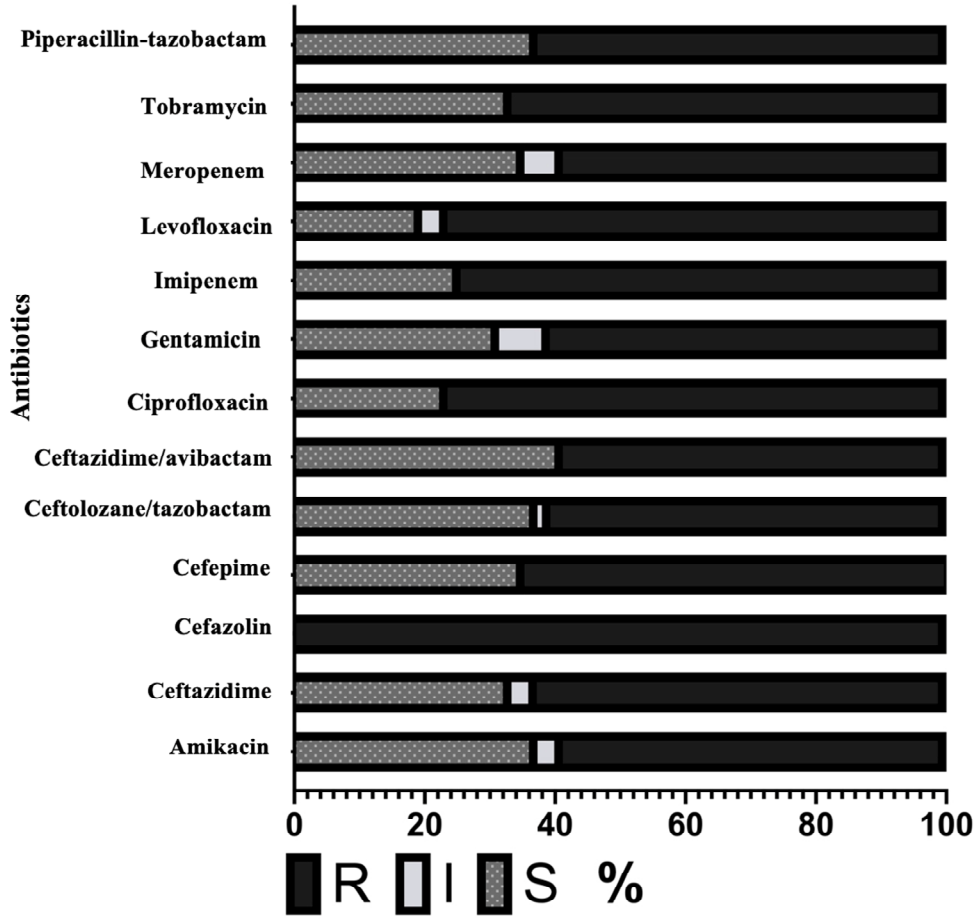

Fig. 1. Sensitivity of clinical isolates of P. aeruginosa (n=51) to antibiotics, % (R – resistant, I – sensitive at increased exposure, S – sensitive).

The sensitivity of *P. aeruginosa* isolated from patients with respiratory complications to aminoglycosides was evaluated by their sensitivity to amikacin, gentamicin and tobramycin, according to EUCAST recommendations. Among them, amikacin showed the best results, since the development of resistance among *Pseudomonas* spp. to it was determined at the level of 58.8%, while the share of sensitive strains was 37.3%. The sensitivity of *P. aeruginosa* strains to gentamicin and tobramycin was 31.4% and 33.3%, respectively, while the percentage of resistant isolates of this genus exceeded 60.0%.

According to research data, *P. aeruginosa isolates* most often carried the bla_{VIM} gene, which determined the production of integron-encoded metallo-β-lactamase class B (Table 1). We found almost 50.0% of carriers of this gene among investigated

pathogens. Genetically determined production of oxacillinase group β-lactamase class D among clinical isolates of *P. aeruginosa* occurred somewhat less often.

Table 1
Characteristics of the carbapenemase genetic profile of P. aeruginosa strains

Microorganisms	Resistance genes	Quantity, abs./%
P. aeruginosa (n=51)	VIM	25/49.0
	OXA-23	6/11.8
	OXA-40	9/17.6

Based on the obtained results, the following carbapenemase genetic resistotypes of *P. aeruginosa* as pathogens of respiratory tract complications in critically ill patients were established:

a) carriers of all three carbapenem resistance genes at the same time (abs. 4; 7.8%);

b) carriers of bla_{VIM} snd bla_{OXA-23} genes (abs. 2; 3.9%);

c) carriers of bla_{VIM} та bla_{OXA-40} genes (abs. 5; 9.8%);

d) carriers of bla_{VIM} genes (abs. 15; 29.4%).

In the course of the study, we established the antimicrobial resistance index (ARI) based on the phenotypic characteristics of *P. aeruginosa* at the level of 0.69±0.39.

As a result of the statistical analysis of the obtained results, the phenomenon of statistically reliable correlation of the ARI of microorganisms by phenotypic characteristics with their carbapenemase genetic resistotypes was established.

Thus, ARI according to phenotypic characteristics among *P. aeruginosa* isolates, which were included in the dominant genetic resistotypes of patients with complications of the respiratory system, significantly exceeded the ARI of isolates without resistance genes (Fig. 2). ARI of *P. aeruginosa* strains included in resistotypes with all resistance genes (0.99±0.02), bla_{VIM} gene carriers (0.97±0.06) and bla_{VIM} and bla_{OXA-40} gene carriers (0.91±0.13) significantly exceeded the total ARI of isolates of this genus by 7.5-8.1 times without the presence of carbapenem resistance genes ($p<0.001$).

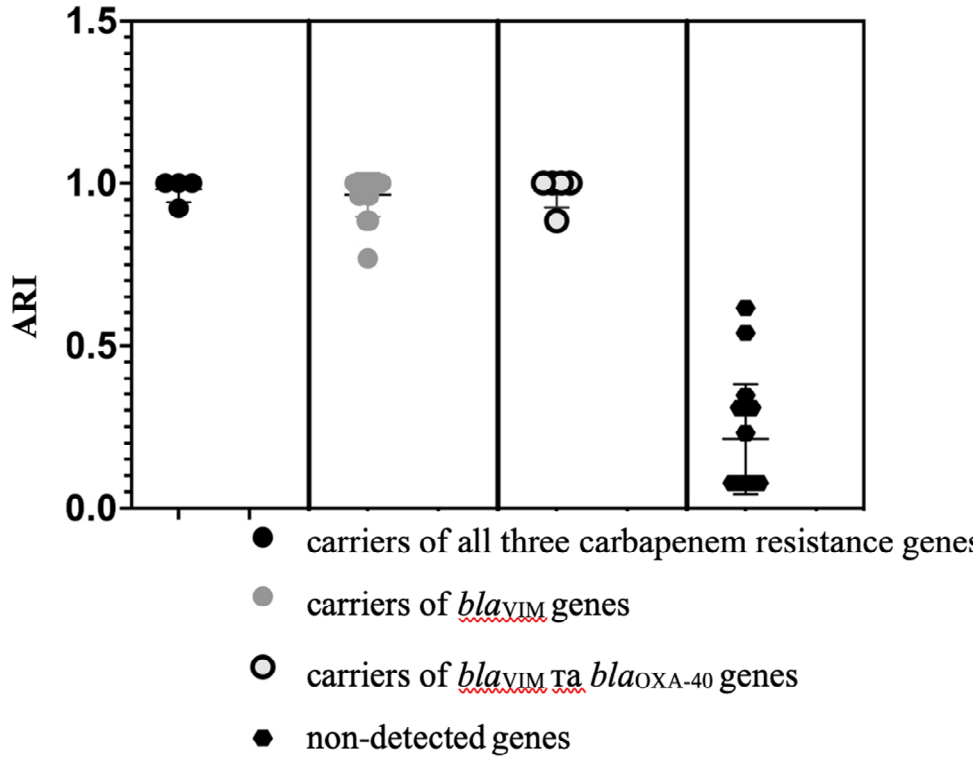

Fig. 2. Antimicrobial resistance index (ARI) of P. aeruginosa (n=51), depending on their carbapenemase genetic resistotypes

Discussion

Despite the fact that *P. aeruginosa* is an opportunistic microorganism, recently, in connection with its wide arsenal of pathogenicity factors and mechanisms of resistance to chemotherapeutic drugs, critically severe infections have been associated with healthcare [4]. A number of studies indicate the dominant role of *P. aeruginosa* in the development

of postoperative complications, including during wartime [9, 10].

This species is often resistant to multiple antibiotics at the same time and is in the "critical" category of the WHO list of priority pathogens for research and development of new antibiotics. *P. aeruginosa* can acquire resistance through chromosomal mutations and gene acquisition. This microorganism has one of the largest bacterial genomes and has a significant assortment of genes obtained by horizontal gene transfer, which are often located in integrons, transposons, insertion sequences, genomic islands, plasmids and conjugative elements. This genomic diversity results in a non-clonal population structure interspersed with specific clones that are associated with significant morbidity and mortality worldwide, the so-called high-risk clones [11].

The results obtained by us fully reflect the criticality of the emerging situation. After all, a significant part of the isolates showed signs of multi-resistance to antibiotics or even complete resistance.

According to the literature data, 10–30% of *P. aeruginosa* isolates are carbapenem-resistant in the United States, whereas worldwide this percentage varies considerably [12]. The fact that the general phenotypic profile of resistance of *P. aeruginosa* depends on the available genetic determinants of carbapenem resistance turned out to be interesting. That is, it follows that with the increase of genes for resistance to carbapenems, the sensitivity of *P. aeruginosa* not only to antibiotics of this group decreases. Presumably, this effect is related to the adjacent transfer of resistance genes to other classes of antibiotics between microorganisms. And it is this question that needs further study.

Conclusions

76.5% of strains of *P. aeruginosa* show polyresistance, and 51.0% of them are resistant to all antibiotics.

Based on the obtained results, four different carbapenemase genetic resistotypes of *P. aeruginosa* as pathogens of respiratory tract complications in critically ill patients were established.

There is the phenomenon of statistically reliable correlation of the ARI of microorganisms by phenotypic characteristics with their carbapenemase genetic resistotypes.

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Authors declare no conflict of interests.

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ВІДПОВІДНІСТЬ КАРБАПЕНЕМ-ГЕНОТИПУ ТА ФЕНОТИПОВИХ АНТИМІКРОБНИХ ПРОФІЛІВ *PSEUDOMONAS AERUGINOSA*

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Мета дослідження — визначити відповідність карбапенем генотипу фенотиповим антимікробним профілям *P. aeruginosa*.
Матеріали та методи. Досліджено 51 клінічний ізолят *P. aeruginosa*, виділених від хворих з післяопераційними ускладненнями органів дихання. Остаточну ідентифікацію отриманих ізолятів проводили в лабораторії Riesbeck за допомогою MALDI-ToF (Bruker) з наступним визначенням їх чутливості до антимікробних препаратів відповідно до стандартів EUCAST у EUCAST Development Laboratory (Швеція). Визначення генів резистентності проводили за допомогою полімеразної ланцюгової реакції в реальному часі (ПЛР-РЧ). Індекс протимікробної резистентності (API) визначали за методикою Г.В. де Copio. Статистичний аналіз проводили за допомогою стандартного програмного забезпечення IBM SPSS Statistics версії 22.0 та GraphPad Prism Software 10.1.0. (США, 2023).
Результати. Полірезистентність виявляли 39 штамів *P. aeruginosa* (76,5%), з них 26 (51,0%) – були стійкі до всіх антибіотиків. За даними досліджень, ізоляти *P. aeruginosa* найчастіше несли ген bla_{VIM}. Дещо рідше зустрічалася генетично детермінована продукція групи оксациліназ β-лактамаз класу D серед клінічних ізолятів *P. aeruginosa*. На основі отриманих результатів встановлено чотири карбапенемазні генетичні резистотипи *P. aeruginosa* як збудників ускладнень дихальних шляхів у важкохворих. Індекс протимікробної резистентності (API) за фенотиповими ознаками *P. aeruginosa* нами виявлено на рівні 0,69±0,39. Встановлено феномен статистично достовірної кореляції API мікроорганізмів за фенотиповими ознаками з їх карбапенемазними генетичними резистотипами.
Висновки. 76,5% штамів *P. aeruginosa* виявляють полірезистентність, а 51,0% з них стійкі до всіх антибіотиків. Встановлено чотири різні карбапенемазні генетичні резистотипи *P. aeruginosa*. Існує феномен статистично достовірної кореляції API мікроорганізмів за фенотиповими ознаками з їх карбапенемазними генетичними резистотипами.
Ключові слова: P. aeruginosa, антибіотики, лікарська стійкість, мультирезистентність, карбапенеми, фенотиповий профіль, гени резистентності.