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Detection of aminoglycoside-modifying enzyme genes in *Pseudomonas aeruginosa* clinical isolates

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Abstract

Pseudomonas aeruginosa is an opportunistic pathogen that frequently causes infections in immunocompromised patients and is involved in outbreaks of hospital-acquired infections with a high mortality rate. Aminoglycosides are a large category of antibiotics that bind specifically to 16S rRNA in 30S ribosomal subunits and disturb protein translation. This antibiotic class plays a significant bactericidal role against a wide range of Gram-negative bacteria such as *P. aeruginosa*. Among different aminoglycoside resistance mechanisms, inactivation of drugs by plasmid-encoded aminoglycoside-modifying enzymes (AMEs) is a common determinant of aminoglycoside resistance in *P. aeruginosa*. These plasmids are spread worldwide, and they are transferred to a wide range of different species. This study aims to detect resistance mechanisms and identify the most prevalent aminoglycoside resistance genes in *P. aeruginosa* clinical isolates, collected from the University Clinical Centre Tuzla. This study included a total of 230 clinical *P. aeruginosa* isolates. Antimicrobial susceptibility tests were performed using the disk diffusion method and the Vitek2 system. Isolates displaying increased MIC values for aminoglycoside antibiotics were included in the multiplex PCR reaction, for the detection of aminoglycoside-modifying enzyme genes. The most prevalent genotype among isolates was *aac* (6')-I. All *aac* (6')-I genotyped isolates also displayed a high rate of resistance to other classes of antibiotics, and they were characterized as multidrug-resistant (MDR) or extensively drug-resistant (XDR). Results indicate that the aminoglycoside-resistance genes are highly prevalent and could easily spread among *P. aeruginosa* strains.

Keywords

Antibiotic resistance, P. aeruginosa, Aminoglycosides, AME, aac (6')-I

Introduction

Aminoglycosides have been a cornerstone of the treatment of nosocomial infections caused by *Pseudomonas aeruginosa* (*P. aeruginosa)* for over 80 years. They are known for their bactericidal properties and synergistic effects with beta-lactams, and are commonly employed in the treatment of various Gram-negative bacterial infections (Saeli et al., 2024). Aminoglycosides are a large category of antibiotics that bind specifically to 16S rRNA in 30S ribosomal subunits and disturb protein translation. *P. aeruginosa* is the opportunistic pathogen that frequently causes the infections of immune-compromised patients and also is involved in the outbreaks of hospital acquired infections with high mortality rate (Ansari et al., 2016; Huttner et al., 2013). Infections caused by *P. aeruginosa*, can become a difficult to treat due to its natural resistance to different antibiotics, and it also has an extraordinary ability to acquire additional mechanisms of resistance to several classes of antimicrobial drugs, even during treatment. Selection of the appropriate antibiotic to initiate therapy is essential to optimizing the clinical outcome (Lister et al., 2009). *P. aeruginosa* resistance to aminoglycosides is widespread and is present in virtually all areas of the world, but particularly in Europe and Latin America (Poole, 2005). Multiple mechanisms contribute to *P. aeruginosa's* resistance to aminoglycoside antibiotics. The most prevalent involves the enzymatic inactivation by aminoglycoside-modifying enzymes (AMEs) encoded on plasmids (Lee et al., 2007). These enzymes alter aminoglycosides through phosphorylation (aminoglycoside phosphotransferases [APH]), acetylation (aminoglycoside acetyltransferases [AAC]), or adenylation (aminoglycoside nucleotidyltransferases [ANT]). Most prevalent genes that encode AMEs are *aac* (6′)-I, *aac* (3')-I, *aac* (3')-II, *ant* (2″)-I, and *aph* (3′)-VI, and their substrates are the most important antipseudomonal aminoglycosides: amikacin, gentamicin and tobramycin (Vaziri et al., 2011). In *P. aeruginosa*, enzymes that modify the 3′ position (3-N-aminoglycoside acetyltransferases [AAC (3′)]) and the 6′ position (6′-N-aminoglycoside acetyltransferases [AAC (6′)]) were among the first to be identified, and are still the predominant acetyltransferases responsible for resistance in this organism (Torres et al., 2000). Aminoglycoside-modifying enzymes are often encoded by mobile elements that harbour additional resistance determinants (Mugnier et al., 1996). AMEs genes are often associated with plasmids, transposons and/or integrons in *P. aeruginosa*, including plasmids and integrons carrying genes for extended-spectrum beta-lactamases (ESBL) or metallo-beta-lactamases (MBL) (Rubens et al., 1979; Riccio et al., 2003). The growing problem of multidrug resistance, frequently observed in Gram-negative bacteria such as *P. aeruginosa*, requires new studies and further research into the mechanisms of aminoglycoside resistance (El-Far et al., 2023).

This study aims to identify the main resistance mechanisms and the most prevalent aminoglycoside resistance genes in *P. aeruginosa* clinical isolates collected from University Clinical Center Tuzla.

Material and methods

Bacterial isolates

Isolates of *P. aeruginosa* were collected at the University Clinical Center in Tuzla, Department of

Microbiology. Isolates of *P. aeruginosa* were collected at the University Clinical Center Tuzla, Department of Microbiology. The study included a total of 230 clinical *P. aeruginosa* isolates from different clinical samples. The samples originated from patients hospitalized at various Clinics and Departments of the University Clinical Center Tuzla (hospital samples), and from patients examined in outpatient clinics in the area of Tuzla Canton (outpatient samples).

Isolation and identification of bacterial strains

Isolation and identification of clinical isolates of *P. aeruginosa* was performed using standard bacteriological methods (morphological, cultural and biochemical). Identification was done by cultivation on selective and differential media. Clinical samples were inoculated on MacConkey and blood agar (BD, Germany) with a sterile loop or swab, depending on the sample, and plates were incubated aerobically at 35-37°C for 18-24 hours. After incubation period (35-37°C/18-24 h) specific bacterial colonies were observed (round pale pink colonies on McConckey agar, narrow zone of beta haemolysis on blood agar), picked and plated on Cetrimide agar. For *P. aeruginosa* identification on Cetrimide agar, plates were incubated aerobically at 35-37°C for 18-24 hours, and pigment production was observed. Large slimy colonies with pronounced pigment production were selected for further verification using biochemical testing - oxidase test, catalase and citrate decomposition test.

Cultivation on BHI broth (Brain Hart Infusion broth, HiMedia, India) aerobically at 35°C for 24h, was performed to refresh and multiply the strains before additional testing. After isolation, the strains were properly labelled (sample identification number, type of isolate, date of isolation) and stored at -20°C in BHI broth with the addition of 16% glycerol.

Antimicrobial susceptibility of P. aeruginosa clinical isolates

Susceptibility of *P. aeruginosa* clinical isolates to different classes of antibiotics was determined by the standard Kirby-Bauer disk diffusion method on Müeller-Hinton agar in accordance with CSLI standards (Clinical and Laboratory Standards Institute, 2017). Fresh bacterial culture was incubated at 35^0C/24h on a nonselective medium – Tryptone Soy agar (HiMedia, India). Single colonies of the test strain were suspended in 0.9% sterile saline to achieve turbidity equal to 0.5 McFarland standard (1.5 X 10^8 CFU/ml). Then suspension of microorganisms was applied with a sterile swab on surface of Müeller-Hinton agar (Liofilchem, Italy). Seven antibiotics representing different antimicrobial classes were selected for testing, including three aminoglycosides—gentamicin 10 µg (GENT), amikacin 30 µg (AMK), and tobramycin 10 µg (TOB)—and four additional agents from other classes: ceftazidime 30 µg (CAZ), imipenem 10 µg (IMP), piperacillin/tazobactam 100/10 µg (PIP/TZP), and ciprofloxacin 5 µg (CIP) (Mast disc, Mast Group Ltd.). Sensitivity or resistance to selected antibiotic was determined by measuring the zone of inhibition that forms around the antibiotic disc after incubation and comparing it with standard values. Resistance phenotypes were defined as MDR (multidrug resistant) *P. aeruginosa* for an isolate that is non-susceptible to at least one agent in three antimicrobial categories; an XDR (extensively drug-resistant) isolate is non-susceptible to at least one agent in all tested antimicrobial categories.

The minimum inhibitory concentration (MIC) for tested antibiotics was determined using an automated system - the Vitek2 Compact system (bioMérieux, Marcy l'Etoile, France, Version 07.01), using AST-N240 cards for testing the susceptibility of *P. aeruginosa*. This system automatically reads and interprets results representing the minimum inhibitory values for antibiotics, and the results were interpreted in accordance with EUCAST standards (MIC Interpretation Guideline EUCAST_NOV_2019).

Genotypic detection of antibiotic resistance

P. aeruginosa strains that showed resistance to aminoglycosides by the disc diffusion method, and with elevated MIC values (for amikacin >16 µg/mL, and for gentamicin and tobramycin >8 µg/mL), were included in multiplex PCR reactions for the molecular detection of genes responsible for aminoglycoside resistance. The primers needed for multiplex PCR were selected from gene databases (Zainab and Syoof, 2015). Primer sequences and corresponding amplicon sizes are listed in Table 1. Extraction of genomic DNA for use in the PCR method was performed using a commercial bacterial DNA isolation kit (QIAamp DNA Mini Kit, 50 QIAamp Mini Spin Columns, Qiagen, Germany). The procedure was carried out according to the manufacturer's instructions. DNA isolation was verified by electrophoresis on a 2% agarose gel under the following conditions: 100 V for 20 minutes.

The reaction was performed as single multiplex PCR reaction, including all primers for listed genes (*aac* (3')-I, *aac* (3')-II, *aac* (6')-I, *aph* (3′)-VI). The reaction mixture (50 µL) contained: 1× PCR buffer, 10 mmol/L Tris–HCl [pH 8.3] (Sigma-Aldrich, Germany), 1.5 mmol/L $MgCl_2$ (Sigma-Aldrich, Germany), 0.2 mmol/L deoxynucleotide triphosphate mix (Sigma-Aldrich, Germany), 10 pmol of each primer, and 2 U of AmpliTaq Polymerase (Sigma-Aldrich, Germany). 2 µL of the total isolated DNA per sample was added to each multiplex PCR mixture. Amplification was performed in a Thermal cycler (Applied biosystems, 2720 Thermal Cycler), under the following conditions: initial denaturation at 95^0C for 5 minutes, followed by 36 cycles that included denaturation at 95^0C for 30 seconds, then primer binding at 52^0C for 40 seconds and elongation at 72^0C for 50 seconds. The last stage included the final elongation at 72^0C for 5 min. Amplification products were detected electrophoresis on a 2% agarose gel with the addition of ethidium bromide (0.05 µL/ml). Electrophoresis took place in a 100V field for 45 minutes. A 100bp marker (DNA Ladder-Sigma Aldrich) was used to determine the length of the obtained fragments during amplification, and visualization of the fragments carried out using a UV transilluminator (VWR GenoMini).

Table 1. Primers used in the multiplex PCR reaction for detection of aminoglycoside resistance gene

Genes		Primer nucleotide sequences	Product size	Gene database no.
aac (3')-I	F	AGTTTGAGCAAGCGCGTAGT	164 bp	AF263520.1
	R	GGGATCGTCACCGTAATCTG		
aac (3')-II	F	CAAACGATGGGTGACGTATG	212 bp	AF466526.1
	R	CGTCGAACAGGTAGCACTGA		
aac (6')-I	F	TCCGTCACTCCATACATTGC	304 bp	DQ174113.1
	R	CGGTACCTTGCCTCTCAAAC		
aph (3')-VI	F	CCGAAGACGACATCGGTATG	410 bp	DQ315788.1
	R	TGCCTTCTCATAGCAGCGTA		

Results and Discussion

P. aeruginosa clinical isolates were initially identified phenotypically based on their characteristic growth on differential and selective media, and confirmed by biochemical tests: production of catalase and oxidase enzymes, utilization of cetrimide and breakdown of citrate in the nutrient medium.

The highest number of *P. aeruginosa* isolates originated from respiratory tract samples overall (112/230), including bronchial and tracheal aspirates (64/230), sputum (46/230), and bronchoalveolar lavage (2/230). The second most frequent were isolates from urine samples (56/230), followed by throat swabs (16/230). Samples with lower prevalence included wound swabs (6/230), cannula swabs (6/230), and other samples.

Results of antibiotic susceptibility by the Kirby-Bauer disk diffusion method are presented in Table 2.

Aminoglycosides are broad-spectrum antibiotics of high potency that have traditionally been used to treat infections caused by Gram-negative bacteria, such as *P. aeruginosa* (Asghar and Ahmed, 2018). Despite their continued usefulness as a group of antipseudomonal agents, the issue of resistance remains a growing concern, with notable geographical variations (Greco et al., 2022). Among the 240 tested isolates, the resistance profile revealed that 27.8% (64/230) were resistant to one or more aminoglycoside antibiotics. Resistance to amikacin was observed in 9.5% of isolates, while 25.5% and 22.1% showed resistance to gentamicin and tobramycin, respectively. These findings are in accordance with other reports. Asghar and Ahmed (2018) reported a high resistance rate to gentamicin and tobramycin, which was also observed by Abdelaziz et al. (2024) in their study.

One of the most important factors contributing to the emergence of resistant bacteria is overuse of antibiotics. According to the study by Mladenovic et al. (2016) based on 10-year data of local monitoring of antibiotic resistance of *P. aeruginosa* and consumption of antibiotics in the Niš Clinical Center - the third largest intensive care unit in Serbia—a significant correlation was found between aminoglycoside consumption and resistance to amikacin and gentamicin. *P. aeruginosa* isolates demonstrated resistance to other classes of antibiotics, including cephalosporines (ceftazidime 34%), carbapenems

Table 2. Antibiotic susceptibility profiles determined by the disk diffusion method for *P. aeruginosa* isolates according to EUCAST guidelines (EUCAST, 2019).

Antibiotics	Antibiotic susceptibility		
	S N (%)	I N (%)	R N (%)
Aminoglycoside resistance in total	157 (68.2)	9 (3.9)	64 (27.8)
Amikacin	203 (88.2)	9 (3.9)	22 (9.5)
Gentamicin	171 (74.3)	0	59 (25.5)
Tobramycin	179 (77.8)	0	51 (22.1)
Ceftazidime	196 (85.2)	0	34 (14.7)
Imipenem	164 (71.3)	8 (3.4)	58 (25.2)
Ciprofloxacin	182 (79.1)	0	48 (20.8)
Piperacillin/tazobactam	171 (74.3)	0	59 (25.6)

*S – sensitive, I – intermediate, R-resistant

(imipenem 58%), fluoroquinolones (ciprofloxacin 48%), and penicillin with beta-lactamase inhibitors (piperacillin/tazobactam 48%). The origin and distribution of *P. aeruginosa* isolates resistant to aminoglycosides are presented in Table 3.

Table 3. Origin and distribution of *P. aeruginosa* isolates resistant to aminoglycosides

Antibiotic	Isolates resistant to aminoglycosides (%)					
	Urine samples	Aspirates	Sputum	Pleural punctate	Gastric lavage	Wound swabs
Amikacin	6.20%	10.90%	4.60%	1.50%	0.00%	1.50%
Gentamicin	31.2%	23.40%	6.20%	3.10%	4.60%	6.20%
Tobramycin	31.2%	17.10%	4.40%	3.10%	4.60%	4.60%

Numerous studies investigating the genetic basis of *P. aeruginosa* resistance have reported a high rate of resistance to multiple classes of antibiotics: cephalosporins, carbapenems, fluoroquinolones, and penicillin with beta-lactamase inhibitors (Mendes et al., 2024; Abdelaziz et al., 2024; Vaziri et al., 2011) including aminoglycosides.

Analysis of the resistance profile within the aminoglycoside class revealed the highest percentage of cross-resistance between gentamicin and tobramycin; 94% of strains resistant to gentamicin were also resistant to tobramycin.

Vitek2 system, are presented in Table 5. For amikacin MIC values above 32 mg/L were observed in 24.6% of isolates, while 61.5% and 66.1% of isolates showed MIC values greater than 8 mg/L for gentamicin and tobramycin, respectively.

P. aeruginosa strains displaying increased MIC values above the ECOFF limit (>16 µg/mL for amikacin, and >8 µg/mL for gentamicin and tobramycin) were included in further molecular analyses to detect genes responsible for aminoglycoside resistance. Isolates with MIC values for amikacin below this threshold (amikacin-sensitive) were also included in genotyping. The resulting PCR products were 164bp for *aac* (3')-I, 212bp for *aac* (3')-II, 304bp for *aac* (6')-I. The 410 bp fragment corresponding to *aph* (3')-VI was not detected in any of the tested

Table 4. Cross-resistance of clinical isolates of *P. aeruginosa*

Antibiotic	Total	AMK	GENT	TOB	CAZ	IMP	CIP	PIP/TZP
AMK	22		15 (68%)	13 (59%)	11 (50%)	11 (50%)	12 (54%)	11 (50%)
GEN	59	15 (25%)		48 (81%)	19 (32%)	19 (32%)	33 (55%)	28 (47%)
TOB	51	13 (22%)	48 (94%)		21 (41%)	25 (49%)	28 (54%)	25 (49%)
CAZ	34	11 (32%)	19 (55%)	21 (61%)		18 (52%)	15 (44%)	22 (64%)
IMP	58	11 (19%)	19 (32%)	25 (43%)	18 (31%)		19 (32%)	22 (37%)
CIP	48	12 (25%)	33 (68%)	38 (79%)	15 (31%)	19 (39%)		25 (52%)
PIP/TAZ	59	11 (18%)	28 (47%)	25 (42%)	22 (37%)	22 (37%)	25 (42%)	

Cross-resistance to other classes of antibiotics is also presented in Table 4. Minimum inhibitory concentrations (MICs) of aminoglycoside-resistant *P. aeruginosa* isolates, determined using the

strains. PCR products were separated on a 2% agarose gel using a 100-base pair DNA ladder (Figure 1 and Figure 2). A total of twenty *P. aeruginosa* clinical isolates were genotyped using the

Table 5. EUCAST Breakpoint values for *P. aeruginosa* determined by the Vitek2 system

Antibiotics	*MIC 50*	*MIC 90*	*EUCAST BREAKPOINT VALUES*			*No of isolates*	
			S ≤	***R >***	***ECOFF****	**S**	**R**
AMK	4	>32	S≤8	R>16 mg/L	16 mg/L	43 (66.1)	22 (33.8)
GEN	>8	>8	S≤4	R>4 mg/L	8 mg/L	16 (24.6)	49 (75.3)
TOB	>8	>8	S≤4	R>4 mg/L	2 mg/L	20 (30.7)	40 (61.5)
CAZ	8	>32	S≤8	R>8 mg/L	8 mg/L	43 (66.1)	22 (33.8)
IMP	2	>8	S≤4	R>8 mg/L	4 mg/L	32 (49.2)	33 (50.7)
CIP	>2	>2	S≤0.5	R>0.5 mg/L	0.5 mg/L	24 (36.9)	41 (63.0)
PIP/TZP	16	>64	S≤16 mg/L	R>16 mg/L	16 mg/L	25 (38.4)	40 (61.5)

*AMK-amikacin, GEN-gentamicin, TOB-tobramycin, CAZ-ceftazidime, IMP-imipenem, CIP-ciprofloxacin, PIP/TZP-piperacillin/tazobactam, ECOFF – epidemiological cut-off value, MIC above which bacterial isolates have phenotypically detectable acquired resistance mechanisms

molecular PCR method. Among these, the *aac* (6')-I gene was detected in 17 isolates, two isolates were positive for the *aac* (3')-I gene, and only one isolate harboured the *aac* (3')-II gene. All tested isolates were negative for the *aph* (3')-VI gene.

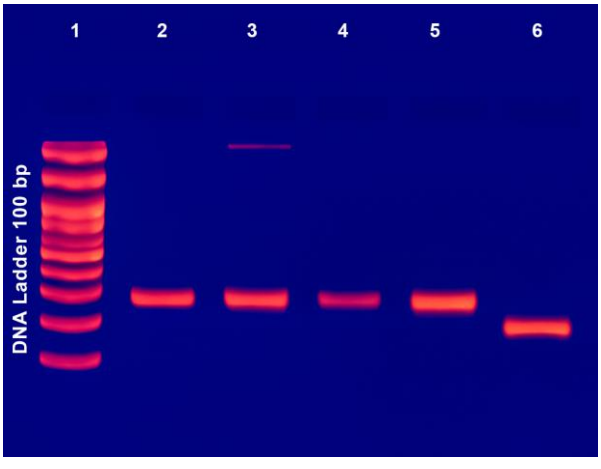

Figure 1. 1 - DNA Ladder 100 bp; 2 - PA 25031 *aac* (6')-I positive; 3 - PA 20043 -*aac* (6')-I positive; 4 - PA 9379 *aac* (6')-I positive; 5 - PA 54160 *aac* (6')-I positive; 6 - PA 29627 *aac* (3')-II positive

These results are consistent with findings from other studies. Zainab and Syoof (2015) reported that 64% of clinical *P. aeruginosa* strains were resistant to at least one aminoglycoside antibiotic. Molecular identification revealed genes encoding aminoglycoside-modifying enzymes, with *aac* (6)-I (21.88%), *aac* (3')-I (15.63%), and *aac* (6')-IIb (8.33%).

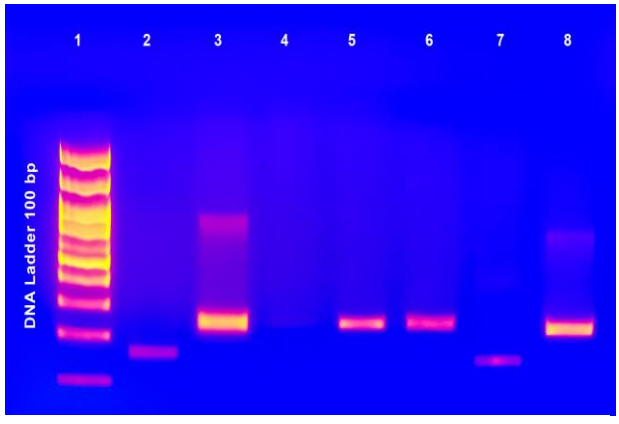

Figure 2. 1 - DNA Ladder 100 bp; 2 - PA 28440 *aac* (3')-I positive; 3 - PA 34423 *aac* (6')-I positive; 4 - PA 54291 negative; 5 - PA 36637 *aac* (6')-I positive; 6 - PA 26659 *aac* (6')-I positive; 7 - PA 40539 *aac* (3')-I positive; 8 - PA 35605 *aac* (6')-I positive

Similarly, Asghar and Ahmed (2018) found that 46.1% of *P. aeruginosa* isolates were resistant to one or more aminoglycosides, and 43.3% of these resistant isolates carried aminoglycoside resistance genes, with *aac* (6')-I being the most prevalent. In European studies, the most common aminoglycoside resistance determinants identified were the *aac* (6')-II and *ant* (2")-I genes (Dubois et al., 2008).

All genotyped isolates exhibited resistance to other classes of tested antibiotics in addition to aminoglycosides (Table 6). Increased MIC values for all tested antibiotics and broad-spectrum resistance to other antibiotic classes was observed

Table 6. Aminoglycoside resistance genes and resistance profiles of *P. aeruginosa* clinical isolates

Isolate	Aminoglycoside resistance			Resistance to other tested antibiotics	Genes detected
	AMK(MIC)	GEN(MIC)	TOB(MIC)		
PA 25031	R (32)	R (8)	R (>8)	Imp, Pip/Tzp	*aac* (6')-I
PA 40539	S (4)	R (>8)	R (>8)	Caz, Imp, Pip/Tzp	*aac* (3')-I
PA 20043	R (16)	R (>8)	R (>8)	Caz, Pip/Tzp	*aac* (6')-I
PA 9379	R (16)	R (8)	S (<=1)	Caz, Imp, Cip, Pip/Tzp	*aac* (6')-I
PA 54160	R (>32)	R (8)	R (>8)	Imp, Cip	*aac* (6')-I
PA 29627	S (4)	R (>8)	R (>8)	Imp, Cip	*aac* (3')-II
PA 34838	R (>32)	R (8)	R (>8)	Caz, Imp, Cip, Pip/Tzp	*aac* (6')-I
PA 46747	R (>32)	R (8)	R (>8)	Caz, Imp, Cip, Pip/Tzp	*aac* (6')-I
PA 37887	R (>32)	R (8)	R (>8)	Caz, Imp, Cip, Pip/Tzp	*aac* (6')-I
PA 36637	R (>32)	R (8)	R (>8)	Caz, Imp, Cip, Pip/Tzp	*aac* (6')-I
PA 35605	R (>32)	R (8)	R (>8)	Caz, Imp, Cip, Pip/Tzp	*aac* (6')-I
PA 34423	R (>32)	R (8)	R (>8)	Caz, Imp, Cip, Pip/Tzp	*aac* (6')-I
PA 7094	R (>32)	R (8)	R (>8)	Caz, Cip, Pip/Tzp	*aac* (6')-I
PA 28440	S (4)	R (>8)	R (>8)	Caz, Pip/Tzp	*aac* (3')-I
PA 17110	R (16)	R (>8)	R (>8)	Caz, Imp, Cip, Pip/Tzp	*aac* (6')-I
PA 41143	R (>32)	R (8)	R (>8)	Caz, Imp, Cip,Pip/Tzp	*aac* (6')-I
PA 47278	R (>32)	R (8)	R (>8)	Caz, Imp, Cip, Pip/Tzp	*aac* (6')-I
PA 52567	R (>32)	R (8)	R (>8)	Caz, Imp, Cip, Pip/Tzp	*aac* (6')-I
PA 26659	R (>32)	R (8)	R (>8)	Caz, Imp, Cip, Pip/Tzp	*aac* (6')-I
PA 55301	R (>32)	R (8)	R (>8)	Caz, Cip	*aac* (6')-I

for all *aac* (6')-I genotyped isolates. The isolates were categorized as multidrug-resistant (MDR; 8/20) and extensively drug-resistant (XDR; 12/20). Numerous studies (Vaziri et al., 2011; Saeli et al., 2024; Thacharodi and Lamont, 2022) have confirmed that aminoglycoside resistance often arises through the acquisition of genes encoding inactivating enzymes. This mechanism represents the most common mode of aminoglycoside resistance in Gram-negative organisms (Mingeot-Leclercq et al., 1999). Aminoglycoside-modifying enzymes (AMEs) are frequently encoded by mobile genetic elements that also carry additional resistance determinants. The *aac* (3') genes are commonly associated with transposons and/or integrons in *P. aeruginosa*, including integrons harboring genes for extended-spectrum beta-lactamases (ESBLs), metallo-beta-lactamases, and other aminoglycoside-modifying enzymes. The results of this study indicate that the detected AME genes are located on mobile genetic elements.

Isolates with confirmed presence of AME genes were characterized as multidrug-resistant (MDR) or extensively drug-resistant (XDR), as they exhibited resistance to three or more different classes of antibiotics, including carbapenems and beta-lactams. The presence of mobile genetic elements carrying resistance genes represents a serious concern due to their potential for horizontal transfer between strains, facilitating rapid dissemination in the environment, particularly in hospital settings.

In recent years, there has been an increasing prevalence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) *P. aeruginosa* strains, with rates ranging from 15% to 30% in certain geographical areas (Peña et al., 2015). Most European countries report resistance rates exceeding 10% across all antimicrobial groups (ECDC, 2020), with a particularly notable rise in Eastern European countries (Ruiz-Garbajosa & Cantón, 2017). This trend is also confirmed by the

present study, which detected a high percentage of MDR and XDR isolates.

Conclusion

Recently, the treatment of infectious diseases has become an increasing challenge, particularly in hospital-acquired infections caused by *P. aeruginosa*. Thanks to its remarkable adaptability, this pathogen can rapidly develop simultaneous resistance to multiple classes of antibiotics. This study confirms the presence of aminoglycoside-modifying enzyme (AME) genes in MDR and XDR clinical *P. aeruginosa* isolates, indicating the involvement of mobile genetic elements in the transfer of resistance genes among clinical strains. These findings raise serious concerns for healthcare-associated infections. For the effective application of antimicrobial therapy and to assess and control the spread of resistance among *P. aeruginosa* strains, molecular detection and continuous monitoring of resistance genes are becoming critically important.

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Authors' contributions

D. Husejnagić: **Conceptualization;** Formal analysis; Investigation; Methodology; Writing – original draft.

A. Avdić: **Formal analysis;** Methodology; Writing – review & editing

S. Širanović: Formal analysis; Methodology; Writing – review & editing.

A. Hercegovac: Writing – review & editing.

N. Tihić: Supervision; Writing – review & editing.

Conflict of interest

No conflict of interest was declared by the authors.

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