



ORIGINAL ARTICLE

## Prevalence of Antibiotic Resistance Determinants in Carbapenem-Resistant *Pseudomonas aeruginosa*: Focus on Class 1 and 2 Integrons and *blaIMP* Gene

Maryam Behboudipour<sup>1</sup> , Neda Soleimani<sup>1\*</sup> , Negar Azarpira<sup>2</sup> , Neda Soleimani<sup>2,3</sup>

1. Department of Microbiology and Microbial Biotechnology, Faculty of Life Sciences and Biotechnology, Shahid Beheshti University, Tehran, Iran.

2. Transplant Research Center, Shiraz University of Medical Sciences, Shiraz, Iran.

3. Department of Pathology, Shiraz Medical School, Shiraz University of Medical Sciences, Shiraz, Iran.

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### ABSTRACT

As a leading non-fermentative opportunistic bacterium, *Pseudomonas aeruginosa* (*P. aeruginosa*) plays a major role in healthcare-associated infections. The emergence of carbapenem-resistant strains is a serious clinical threat, often associated with integrons and carbapenemases such as *blaIMP*. The present study aimed to assess the distribution of class 1 and 2 integrons and the *blaIMP* gene among clinical isolates of carbapenem-resistant *P. aeruginosa* from hospitals in Shiraz.

Seventy clinical isolates of *P. aeruginosa* were collected from different hospital wards. The identification of the isolates was performed using common microbiology methods. The disk diffusion method was used to evaluate the antimicrobial susceptibility. Minimum inhibitory concentration (MIC) values for imipenem in carbapenem-resistant strains were obtained using E-test strips. Polymerase chain reaction (PCR) was used to identify the resistance determinants including *intI1*, *intI2*, and *blaIMP*.

Of the 70 clinical isolates, 35 (50%) isolates were imipenem-resistant. MIC testing showed that 34 isolates had a resistant MIC (MIC  $\geq 8 \mu\text{g/mL}$ ). PCR results showed that 33 (94.3%) isolates carried the *intI1* gene and 17 (48.6%) isolates carried the *blaIMP* gene. Co-existence of *intI1* and *blaIMP* genes was observed in 17 (48.6%) isolates. The *intI2* gene was not detected in any of the samples. The prevalence of the *intI1* and *blaIMP* genes was higher among the isolates obtained from intensive care units (ICU) and internal medicine wards.

The high prevalence of class 1 integrons and the *blaIMP* gene among carbapenem-resistant isolates suggests the key function of mobile genetic elements in the horizontal spread of resistance factors.

**Keywords:** Integrons, Drug Resistance, Carbapenemase, *Pseudomonas aeruginosa*

\*Corresponding:

Neda Soleimani

Address:

Department of Microbiology and Microbial Biotechnology, Faculty of Life Sciences and Biotechnology, Shahid Beheshti University, Tehran, Iran.

E-mail:

N\_soleimani@sbu.ac.ir

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## Introduction

*Pseudomonas aeruginosa* (*P. aeruginosa*), an important opportunistic organism, poses a serious threat to patient health, especially in hospital settings. This bacterium plays a prominent role in causing healthcare-associated infections, including surgical site infections, urinary tract infections, pneumonia, and bloodstream infections (1, 2). The emergence of innate and acquired resistance in *P. aeruginosa* is mainly due to indiscriminate and improper antibiotic use, leading to the spread of resistant strains.(3).

The consequences of the spread of multidrug-resistant (MDR) isolates include treatment delays, ineffectiveness of antimicrobial regimens, and increased patient mortality (4). The mortality rate from infections associated with this pathogen is relatively high, estimated to be between 18% and 61% (5, 6). Carbapenem antibiotics such as imipenem and meropenem have been identified as the most effective drugs to combat severe infections caused by MDR strains of this bacterium. Unfortunately, the excessive and irrational increase in the use of this class of antibiotics has led to the increasing spread of bacterial resistance to them worldwide (7, 8).

A variety of mechanisms play a role in the resistance of *P. aeruginosa* to carbapenems, including increased expression of efflux pumps (excretion of antibiotics), decreased outer membrane permeability (restriction of drug entry), and production of carbapenemase (Carbapenem hydrolyzing enzymes) (9, 10). Among the hydrolyzing enzymes of classes A, B, and D, class B enzymes -also known as metallo-beta-lactamases (MBLs) are of great importance in medical settings. Verona integron-encoded metallo-β-lactamase (VIM) and imipenemases (IMPs) are the most common class B enzymes in the isolates of this bacterium. These enzymes are transported and spread among bacteria using mobile genetic elements such as plasmids, integrons, transposons, and integrating and conjugative elements.

Most genes encoding metallo-beta-lactamases have been observed as part of the gene cassettes in class 1 integrons(11). Based on the integrase gene (*intI*) sequence, five classes of integrons have been identified in Gram-negative bacteria. However, only the first three classes (*intI1*, *intI2*, and *intI3*) are known to contribute to the development of MDR in human pathogenic species (12). Many sources have

highlighted the clinical importance of class 1 integrons and the high prevalence of these genetic factors among various hospital organisms. It is estimated that humans and animals release approximately  $10^{23}$  copies of these genetic elements into the environment daily (13). This suggests a genetic link between human and animal pathogens and environmental bacteria (14, 15). To effectively and targeted combat drug-resistant pathogens, it is crucial to investigate the mechanisms through which these microorganisms acquire resistance determinants (11).

Carbapenem resistance and integron prevalence in *P. aeruginosa* have been investigated in other studies. Notably, the overall prevalence of MDR *P. aeruginosa* isolates recovered from clinical samples of Iranian patients has been reported to be approximately 70.1%, highlighting the significance of regional surveillance (16). However, molecular data on the co-distribution of class 1 and 2 integrons and the *blaIMP* gene in carbapenem-resistant isolates from southern Iran remain limited. This study aimed to address this gap by evaluating resistance patterns and genetic profiles of clinical isolates collected from hospitals in Shiraz.

## Methods

### Bacterial Isolation and Sample Collection

The study was conducted between 2022 and 2023. Clinical isolates were collected over a period from November 1, 2022, to September 21, 2023, during a descriptive cross-sectional study. A total of 70 non-duplicate *P. aeruginosa* isolates were obtained from various departments of the Namazi and Abu Ali Sina hospitals in Shiraz, Iran. The sample size was determined by including all eligible non-duplicate isolates obtained during the defined study period. To avoid strain duplication, only one isolate from each patient was considered. The isolates were recovered from different samples such as: wounds, sputum, blood, urine, double-lumen tube, body fluids, endotracheal tubes (ETT), and other parts. The code of ethics for this study (IR.SUMS.REC.1402.034) was approved by the Ethics Committee of the Shiraz University of Medical Sciences.

### Identification, and Preservation of *P. aeruginosa* Isolates

Initially, *P. aeruginosa* was isolated by culturing all the studied samples on Eosin Methylene Blue

(EMB) agar and blood agar culture media (CONDA, Spain). The initial identification of *P. aeruginosa* was based on the colony morphological characteristics and the production of a characteristic odor. To more accurately identify *P. aeruginosa* isolates, a set of common biochemical tests was used, including Gram staining, catalase, oxidase, citrate, and Triple Sugar Iron (TSI) agar tests (CONDA, Spain).

In addition, the isolates were cultured on a specific Citrimide agar medium (Merck, Germany) for final confirmation. After completing these steps, for molecular testing and further investigation, the identified isolates were prepared in Tryptic Soy Broth (TSB) (CONDA, Spain) supplemented with 30% glycerol and stored frozen at -70°C (10). In addition to phenotypic identification, all isolates were confirmed by molecular methods using PCR amplification of the *toxA* gene (17).

### Determination of Antimicrobial Resistance Profiles

To perform antibacterial susceptibility testing of *P. aeruginosa* isolates, the disk diffusion technique was used in accordance with the guidelines of the Clinical and Laboratory Standards Institute (CLSI M100-Ed31, 2021). For this purpose, Mueller-Hinton agar medium (Merck, Germany) was prepared and standardized bacterial suspensions were inoculated onto the surface of the medium to form lawn cultures.

Antibiotic discs (Padtan Teb Company, Iran) were used based on antibiotic class as follows: Penicillins (Piperacillin, PIP, 100 µg), Penicillin/beta-lactamase inhibitor combinations (Piperacillin/tazobactam, PTZ, 100/10 µg); Cephalosporins (Ceftazidime, CAZ, 30 µg), Monobactams (Aztreonam, AZT, 30 µg), and Carbapenems (Imipenem, IPM, 10 µg); Aminoglycosides (Amikacin, AN, 30 µg and Gentamicin, GM, 10 µg); and Fluoroquinolones (Ciprofloxacin, CP, 5 µg and Levofloxacin, LEV, 5 µg). The standard strains *P. aeruginosa* ATCC 27853 and *E. coli* ATCC 25922 were used to ensure the accuracy and precision of the tests (18).

### Determination of MIC values

The imipenem MIC for the imipenem-resistant *P. aeruginosa* isolates was determined using E-test strips (E-test, Liofilchem, Italy). For this purpose, a suspension with a turbidity of 0.5 McFarland was prepared from freshly grown colonies after overnight culture. Then, the bacterial suspension was spread

evenly on the surface of plates containing Mueller-Hinton agar medium using a sterile swab. The imipenem E-test strip was placed on the surface of the culture medium. After 24 hours in an incubator at 37°C, the presence of an elliptical inhibition zone around the strips was assessed. MIC values were interpreted at the point where the ellipse of inhibition intersected the E-test strip. For the quality control of drug susceptibility testing, the reference strain *P. aeruginosa* ATCC 27853 was utilized (19).

### DNA isolation from bacterial samples

The boiling protocol was used to isolate the bacterial genome. In brief, fresh bacterial cultures were used to prepare a suspension by transferring several colonies into 200 µL of sterile distilled water, followed by vortexing. The mixture was then heated at 100°C for 20 minutes. After the boiling step, the suspension was centrifuged at 4°C at 10,000 rpm to collect cells and solid particles at the bottom of the microtube.

Then, the clear supernatant containing the DNA of the target bacteria was carefully and delicately removed and transferred to sterile microtubes. To prevent DNA damage, these samples were stored at -20°C until further testing. The DNA yield and purity were assessed using a NanoDrop spectrophotometer (Thermo Scientific, USA) (20).

### Detection of Class 1/2 Integrons and blaIMP Gene in Imipenem-Resistant Isolates

The presence of class 1, 2 integrons and the blaIMP metallo-β-lactamase carbapenemase gene in imipenem-resistant isolates was investigated by PCR using the specific primers

*intI1*: F: 5'-GGTCAAGGATCTGGATTTCG -3', R: 5'-GCCAACTTCAGCACATG -3' (newly designed primers),

*intI2*: F: 5'-CACGGATATGCGACAAAAAGGT -3', R: 5'-GTAGCAAACGAGTGACGAAATG -3' and

*blaIMP*: F: 5'-TGAGCAAGTTATCTGTATTTC-3', R: 5'-TAGTTGCTTGGTTTGATG -3' (21, 22). The specificity of each primer was confirmed using NCBI Primer-BLAST.

To perform the reaction, each mixture was prepared in a final volume of 25 µL, including 1 µL of forward primer (10 pmol/µL), 1 µL of reverse primer (10 pmol/µL), 12.5 µL of 2x PCR Master Mix Red - MgCl2 2 mM (Amplicon, Denmark), 5 µL of template DNA, and 5.5 µL of sterile distilled water (23). After

preparing the reaction mixture, the microtubes were placed in a thermal cycler (Eppendorf, Germany) and the reaction was performed under the following conditions: initial denaturation at 95°C for 5 min, then 35 cycles including: denaturation at 95°C for 1 min, annealing of *intI1*, *intI2*, and *blaIMP* at 55, 58, and 51°C for 1 min, extension at 72°C for 1 min, and a final extension at 72°C for 5 min. After the reaction, 5 µl of each PCR product was electrophoresed on a 1.5% agarose gel at a voltage of 85 for 45 minutes. The amplicons were visualized using a Gel Documentation device.

### Data analysis

SPSS statistical software version 25.0 (IBM Corp., Armonk, NY, USA) was used to analyze the obtained data. Descriptive statistics were used to express the results; quantitative variables were reported as mean  $\pm$  standard deviation and qualitative variables as frequency and percentage. To assess the association between categorical variables, Fisher's exact test, Chi-square, and Monte Carlo tests were employed where appropriate, particularly in cases with small expected cell counts. A *p*-value  $< 0.05$  was considered statistically significant.

## Results

### Clinical and Demographic Distribution of the Isolates

According to the study findings, among the 70 *P. aeruginosa* isolates confirmed by molecular methods, 39 (55.7%) were from male patients and 31 (44.3%) from female patients. The mean age of the patients was estimated to be 50.5  $\pm$  21.3 years. Regarding the clinical specimen type, 17 isolates (24.3%) were obtained from urine, 12 (17.1%) from sputum, 11 (15.7%) from wounds, 9 (12.9%) from blood, 6 (8.6%) from body fluids, 5 (7.1%) from ETT, 2 (2.9%) from the double lumen tube, and 8 (11.4%) from other samples.

In terms of the distribution of isolates by department, 18 (25.7%) isolates were isolated from the intensive care unit (ICU), 13 (18.6%) isolates from the surgical department, 10 (14.3%) isolates from the internal medicine department, 8 (11.4%) isolates from the emergency department, 5 isolates from the cardiopulmonary cerebral resuscitation (CPCR) room (7.1%), 3 (4.3%) isolates from the oncology

department, 3 (4.3%) isolates from the operating room, and 10 (14.3%) isolates from other departments.

### Antimicrobial susceptibility test

The antibiotic resistance pattern of *P. aeruginosa* isolates obtained from patients is shown against various antibiotics (Figure 1). According to the results, among the studied isolates, the highest resistance rate was observed against imipenem (50%) and the highest sensitivity rate was reported for amikacin (55.7%), gentamicin (52.9%), and ceftazidime (52.9%). Statistical analysis was performed using the Monte Carlo method to assess the association between antibiotic resistance patterns and both hospital departments and clinical specimen types. No statistically significant associations were observed between specimen type and resistance patterns for any of the antibiotics tested (*P* > 0.05) (Table 1)

However, a significant association was found between hospital departments and antibiotic resistance for piperacillin, imipenem, and ciprofloxacin (*P* < 0.05) (Table 2). Resistance to these antibiotics was higher in the ICU, internal medicine, and surgical departments compared with other departments, possibly due to the frequent prescription of these drugs for patients with acute or severe conditions. Among the total isolates, 34 (48.6%) were classified as MDR, while 36 (51.4%) were non-MDR. The differences in antibiotic resistance rates between MDR and non-MDR isolates were statistically significant for all antibiotics tested (*P* < 0.05). MDR isolates exhibited the highest resistance to imipenem, levofloxacin, ceftazidime, and piperacillin, while showing the lowest resistance to aztreonam. There was no statistically significant association between MDR status and either clinical specimen types or hospital departments (*P* > 0.05). However, MDR isolates were relatively more frequent in the ICU and internal medicine departments compared with other wards (Figure 2).

### MIC Values Determined by the E-Test

To confirm carbapenem resistance, the gradient test for imipenem was applied to the resistant strains. Imipenem-resistant *P. aeruginosa* with ( $\text{MIC} \geq 8.0 \mu\text{g/mL}$ ) was defined as Carbapenem-Resistant *P. aeruginosa* (CRPA). It is important to note that among the 35 imipenem-resistant *P. aeruginosa* isolates, 34 (97.1%) isolates had MIC values in the resistant range,

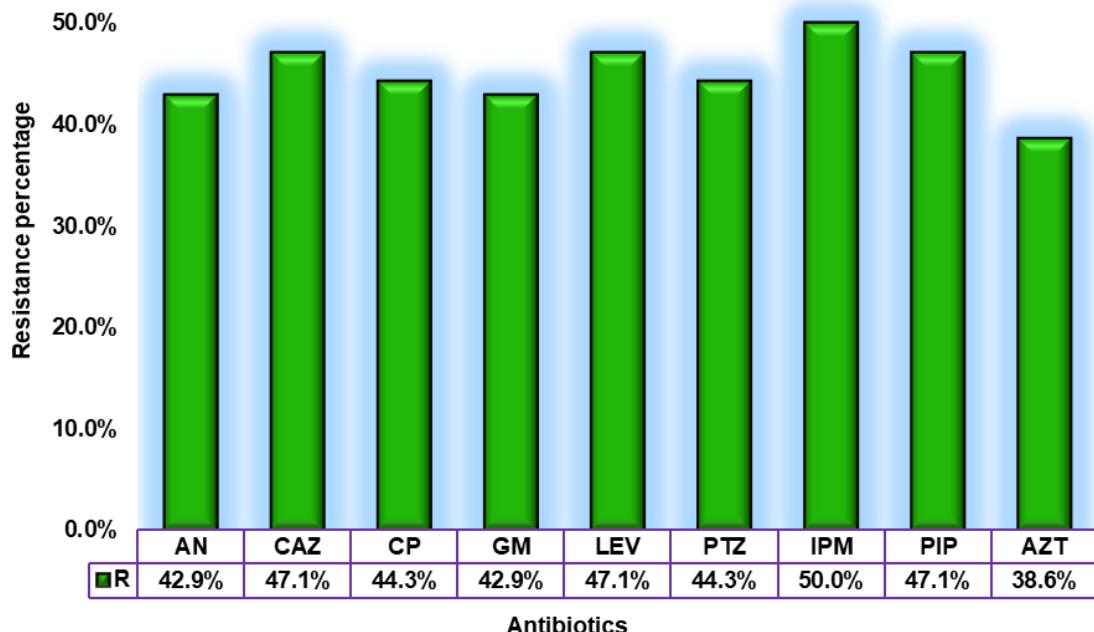
and only 1 (2.8%) isolate was susceptible to imipenem based on MIC.

#### Molecular detection of Class 1/2 Integrons and blaIMP Gene

Of the 35 *P. aeruginosa* isolates that were resistant to imipenem, 33 (94.3%) isolates and 17 (48.6%) isolates contained the *intI1* and *blaIMP* genes,

respectively (Figure 3). The co-occurrence of *intI1* and *blaIMP* was observed in 17 (48.6%) isolates. Class 2 integrons were not detected in any of the studied isolates.

It is also important to note that all the isolates carrying the resistance genes (*intI1* and *blaIMP*) were MDR, while none of the isolates lacking the MDR phenotype harbored these genes.



**Figure 1.** Resistance profiles of *P. aeruginosa* isolates against different antibiotics. AN: amikacin, CAZ: ceftazidime, CP: ciprofloxacin, GM:gentamicin, LEV: levofloxacin, PTZ: piperacillin-tazobactam, IPM: imipenem, PIP: piperacillin, AZT: aztreonam.

**Table 1.** Association of antibiotic resistance with the type of clinical specimen in *P. aeruginosa* isolates.

Antibiotics	Antibiotic resistance No. (%)	Blood No. (%)	Urine No. (%)	Sputum No. (%)	Wounds No. (%)	Fluids No. (%)	ETT No. (%)	Others No. (%)	P-value
<b>Penicillins</b>									
Piperacillin	33 (47.1%)	3 (9.1%)	9 (27.3%)	4 (12.1%)	7 (21.2%)	2 (6.1%)	3 (9.1%)	5 (15.2%)	0.758
<b>Cephalosporins</b>									
Ceftazidime	33 (47.1%)	3 (9.1%)	9 (27.3%)	5 (15.2%)	5 (15.2%)	3 (9.1%)	3 (9.1%)	5 (15.2%)	0.820
<b>Monobactams</b>									
Aztreonam	27 (38.6%)	2 (7.4%)	7 (25.9%)	5 (18.5%)	5 (18.5%)	2 (7.4%)	2 (7.4%)	4 (14.8%)	0.686
<b>Carbapenems</b>									
Imipenem	35 (50%)	3 (8.6%)	9 (25.7%)	5 (14.3%)	7 (20%)	3 (8.6%)	3 (8.6%)	5 (14.3%)	0.600
<b>Penicillins/Beta-lactamase Inhibitors</b>									

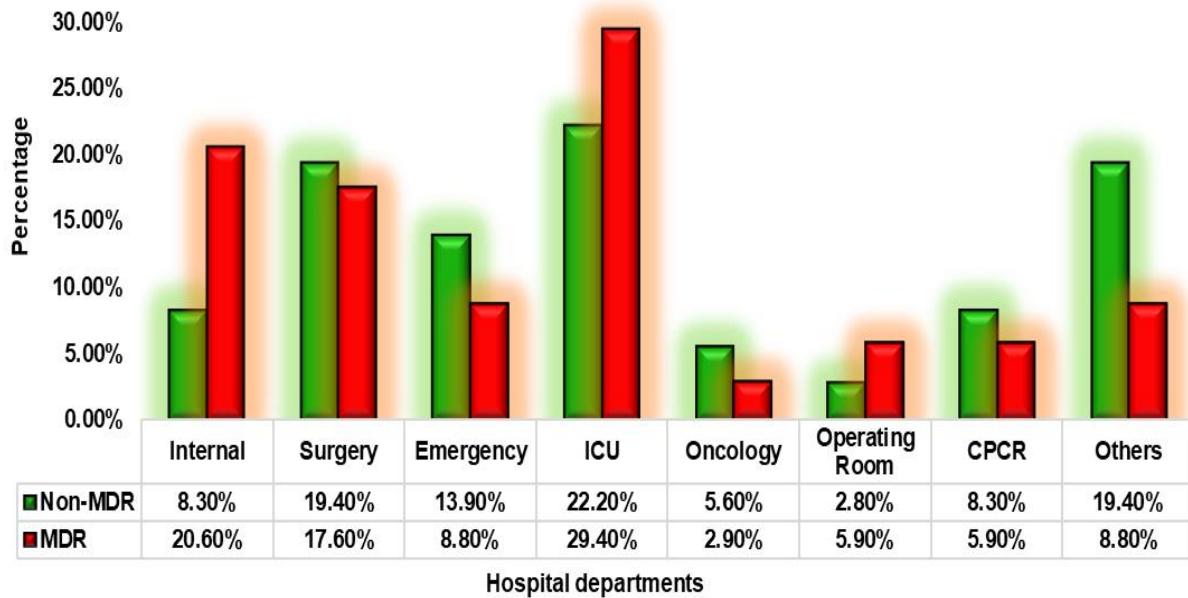
Antibiotics	Antibiotic resistance No. (%)	Blood No. (%)	Urine No. (%)	Sputum No. (%)	Wounds No. (%)	Fluids No. (%)	ETT No. (%)	Others No. (%)	P-value
Piperacillin/tazobactam	31 (44.3%)	3 (9.7%)	8 (25.8%)	4 (12.9%)	7 (22.6%)	3 (9.7%)	1 (3.2%)	5 (16.1%)	0.747
<b>Aminoglycosides</b>									
Amikacin	30 (42.9%)	1 (3.3%)	9 (30%)	4 (13.3%)	7 (23.3%)	2 (6.70%)	2 (6.7%)	5 (16.7%)	0.095
Gentamicin	30 (42.9%)	3 (10%)	9 (30%)	3 (10%)	7 (23.3%)	1 (3.3%)	2 (6.7%)	5 (16.7%)	0.059
<b>Fluoroquinolones</b>									
Ciprofloxacin	31 (44.3%)	3 (9.7%)	9 (29%)	5 (16.1%)	6 (19.4%)	1 (3.2%)	2 (6.5%)	5 (16.1%)	0.665
Levofloxacin	33 (47.1%)	3 (9.1%)	9 (27.3%)	5 (15.2%)	7 (21.2%)	1 (3%)	3 (9.1%)	5 (15.2%)	0.310

Abbreviations: ETT, Endotracheal tube; \* Significant level of *P*-value is <0.05; *P* value was calculated by using Monte Carlo test.

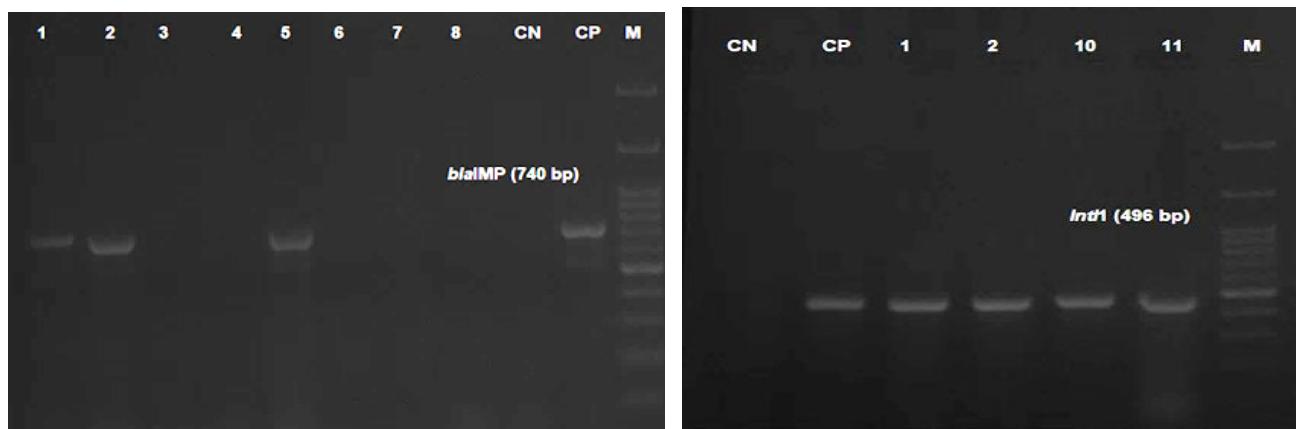
**Table 2. Association of antibiotic resistance with different hospital wards in *P. aeruginosa* isolates.**

Antibiotics	Antibiotic resistance No. (%)	Intensive care unit (ICU) No. (%)	Internal No. (%)	Surgery No. (%)	Emergency No. (%)	CPCR No. (%)	Oncology No. (%)	Operating room No. (%)	Others No. (%)	P-value
<b>Penicillins</b>										
Piperacillin	33 (47.1%)	10 (30.3%)	7 (21.2%)	6 (18.2%)	3 (9.1%)	2 (6.1%)	1 (3%)	1 (3%)	3 (9.1%)	0.012*
<b>Cephalosporins</b>										
Ceftazidime	33 (47.1%)	11 (33.3%)	7 (21.2%)	5 (15.2%)	3 (9.1%)	2 (6.1%)	1 (3%)	1 (3%)	3 (9.1%)	0.578
<b>Monobactams</b>										
Aztreonam	27 (38.6%)	11 (40.7%)	5 (18.5%)	4 (14.8%)	3 (11.1%)	2 (7.4%)	1 (3.7%)	0 (0%)	1 (3.7%)	0.088
<b>Carbapenems</b>										
Imipenem	35 (50%)	11 (31.4%)	7 (20%)	6 (17.1%)	3 (8.6%)	2 (5.7%)	1 (2.9%)	2 (5.7%)	3 (8.6%)	0.001*
<b>Penicillins/Beta-lactamase Inhibitors</b>										
Piperacillin/tazobactam	31 (44.3%)	10 (32.3%)	6 (19.4%)	6 (19.4%)	2 (6.5%)	2 (6.5%)	1 (3.2%)	2 (6.5%)	2 (6.5%)	0.869
<b>Aminoglycosides</b>										
Amikacin	30 (42.9%)	9 (30%)	7 (23.3%)	5 (16.7%)	2 (6.7%)	2 (6.7%)	0 (0%)	2 (6.7%)	3 (10%)	0.599
Gentamicin	30 (42.9%)	8 (26.7%)	6 (20%)	6 (20%)	2 (6.7%)	2 (6.7%)	1 (3.3%)	2 (6.7%)	3 (10%)	0.682
<b>Fluoroquinolones</b>										
Ciprofloxacin	31 (44.3%)	9 (29%)	7 (22.6%)	6 (19.4%)	2 (6.5%)	2 (6.5%)	1 (3.2%)	1 (3.2%)	3 (9.7%)	0.007*
Levofloxacin	33 (47.1%)	9 (27.3%)	7 (21.2%)	6 (18.2%)	3 (9.1%)	2 (6.7%)	1 (3%)	2 (6.1%)	3 (9.1%)	0.693

Abbreviations: CPCR, Cardiopulmonary cerebral resuscitation; \* Significant level of *P*-value is <0.05; *P* value was calculated by using Monte Carlo test.



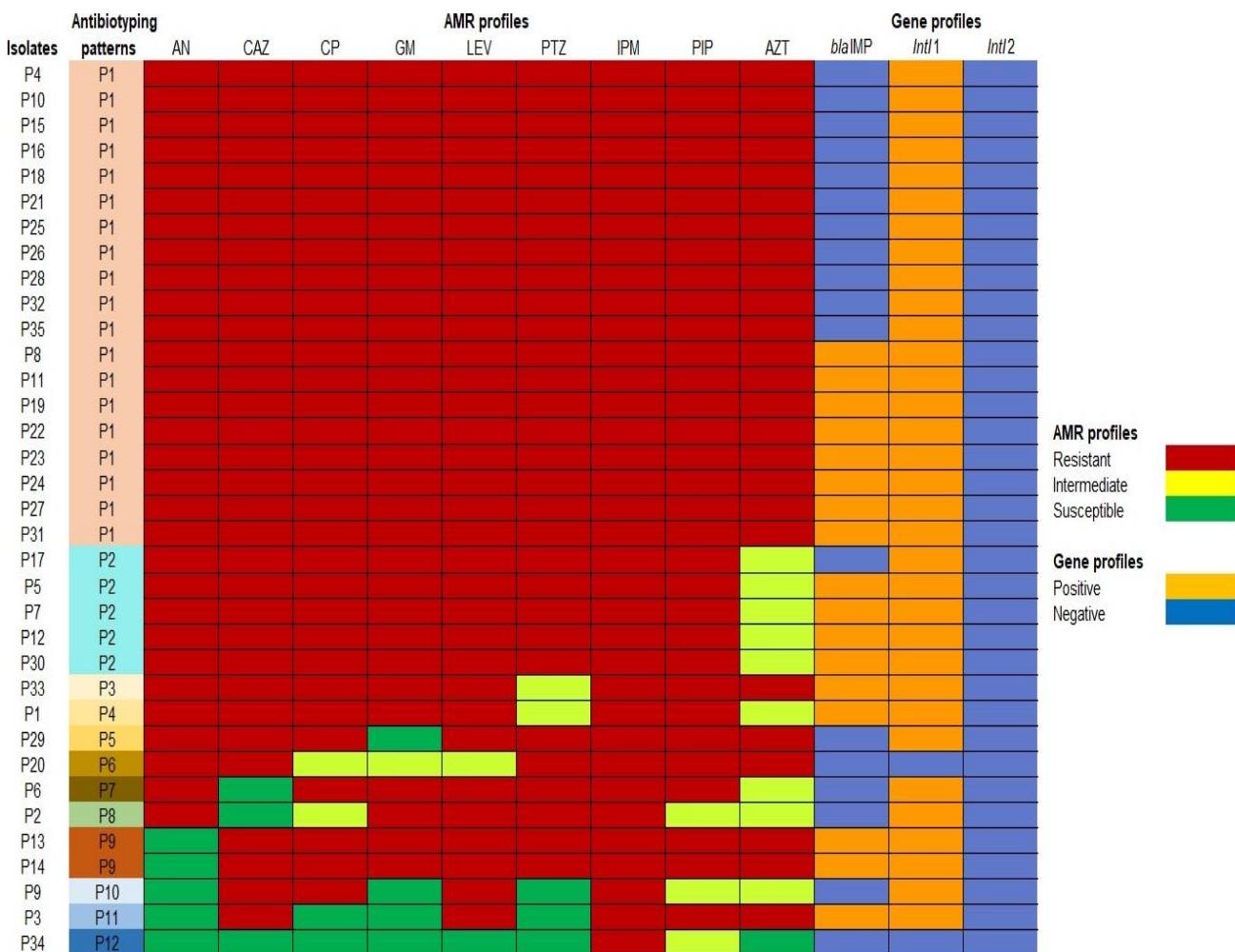
**Figure 2.** Distribution of MDR and non-MDR isolates across different hospital departments. MDR: Multidrug-resistant, CPCR: Cardiopulmonary cerebral resuscitation, ICU: Intensive care unit.



**Figure 3.** Gel electrophoresis image of PCR products of the *intI1* (496 bp) and *blaIMP* (740 bp) genes. M (ladder DNA 100 bp), CN (negative control), CP (positive control).

The heat map drawn in this study shows the phenotypic distribution pattern of antimicrobial resistance and the presence of genes involved in resistance in *P. aeruginosa* isolates (Figure 4). According to the heat map, most carbapenem-resistant isolates also carried class 1 integrons and exhibited resistance to several other antibiotics, including aminoglycosides and fluoroquinolones, suggesting a notable co-occurrence between the presence of integrons and the MDR phenotype. However, some isolates, despite harboring resistance genes, demonstrated relative susceptibility to certain antibiotics, indicating diversity in phenotypic

expression. Overall, 12 distinct antibiotic patterns were identified, with patterns 1 (P1) and 2 (P2) being the most common and including the largest number of isolates. Note that the heat map illustrates correlation patterns and does not establish causality between the variables. The distribution of the *blaIMP* gene among isolates with and without class 1 integrons is shown (Table 3). Notably, all isolates harboring the *blaIMP* gene also carried class 1 integrons, whereas the reverse was not observed. However, Fisher's exact test showed no statistically significant association between the presence of class 1 integrons and the *blaIMP* gene ( $p = 0.486$ ), which is likely due to the limited sample size.



**Figure 4. Heatmap of the antibiotic resistance profiles among the imipenem-resistant *P. aeruginosa* isolates. Each row represents an individual isolate, while each column corresponds to a specific antibiotic or resistance gene. 12 distinct antibiotypes (P1–P12) were identified based on the resistance patterns.**

**Table 3. Correlation between the presence of the blaIMP gene and class 1 integrons in the imipenem-resistant *P. aeruginosa* isolates.**

Gene	blaIMP (Positive)	blaIMP (Negative)	Total
<b>intI1 (Positive)</b>	17(51.5%)	16(48.5%)	33(94.3%)
<b>intI1 (Negative)</b>	0(0.0%)	2(100%)	2 (5.7%)
<b>Total</b>	17(48.6%)	18(51.4%)	35(100%)
<b>p-value (Fisher's exact test)</b>			0.486

\*Significant level of *P*-value is < 0.05.

The distribution of *P. aeruginosa* isolates harboring class 1 integrons and the blaIMP carbapenemase gene is presented according to the clinical specimen types and hospital departments (Table 4). A statistically significant association was observed between the presence of *intI1* and the type of clinical specimen ( $P = 0.016$ ), with higher detection

rates in urine (27.3%), wound (21.2%), and sputum (15.2%) samples. In contrast, no significant association was found between the presence of the blaIMP gene and specimen type ( $P = 0.14$ ).

However, blaIMP-positive isolates were most frequently identified in urine samples (23.5%), followed by blood and ETT (17.6%). Furthermore, the

distribution of *intI1* and *blaIMP* genes across various hospital departments did not demonstrate statistically significant differences ( $P > 0.05$ ). However, *intI1*-positive isolates were most frequently identified in the ICU (27.3%) and internal medicine wards (21.2%), whereas *blaIMP*-positive isolates were more

commonly detected in the ICU (23.5%) and CPCR (23.5%) departments. Considering that all *blaIMP*-positive isolates also carried the *intI1* gene, isolates harboring both genes were not analyzed separately, as their distribution pattern was identical to that of the *blaIMP*-positive isolates.

**Table 4. Frequency of *P. aeruginosa* isolates harboring class I integrons and *blaIMP* carbapenemase gene based on clinical samples and different departments.**

Gene	Specimen type								P-value
	Blood no. (%)	Sputum no. (%)	Wounds no. (%)	Urine no. (%)	Fluids no. (%)	ETT no. (%)	Others no. (%)		
<i>intI1</i> (P) 33 (94.3%)	3 (9.1%)	5 (15.2%)	7 (21.2%)	9 (27.3%)	1 (3%)	3 (9.1%)	5 (15/2%)	0.016*	0.14
<i>intI1</i> (N) 2 (5.7%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (100%)	0 (0%)	0 (0%)		
<i>blaIMP</i> (P) 17 (48.5%)	3 (17.6%)	1 (5.9%)	2 (11.8%)	4 (23.5%)	1 (5.9%)	3 (17.6%)	3 (17/6%)	0.016*	0.14
<i>blaIMP</i> (N) 18 (51.4%)	0 (0%)	4 (22.2%)	5 (27.8%)	5 (27.8%)	2 (11.1%)	0 (0%)	2 (11.1%)		
Gene	Wards								P-value
	ICU	Surgery	Emergency	Internal	CPCR	Oncology	Operating room	Others	
<i>intI1</i> (P) 33 (94.3%)	9 (27.3%)	6 (18.2%)	3 (9.1%)	7 (21.2%)	2 (6.1%)	1 (3%)	2 (6.1%)	3 (9.1%)	0.69
<i>intI1</i> (N) 2 (5.7%)	2 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	
<i>blaIMP</i> (P) 17 (48.5%)	4 (23.5%)	2 (11.8%)	2 (11.8%)	4 (23.5%)	1 (5.9%)	1 (5.9%)	1 (5.9%)	2 (11.8 %)	0.93
<i>blaIMP</i> (N) 18 (51.4%)	9 (38.9%)	4 (22.2%)	1 (5.6%)	3 (16.7%)	1 (5.6%)	0 (0%)	1 (5.6%)	1 (5.6%)	

Abbreviations: P, Positive; N, Negative; CPCR, Cardiopulmonary cerebral resuscitation; ETT, Endotracheal tube; \* Significant level of  $P$ -value is  $< 0.05$ ;  $P$  value was calculated by using Monte Carlo test.

## Discussion

Antibiotic Resistance remains a major challenge and serious threat to healthcare systems worldwide, with alarming increases in morbidity, mortality, and economic burden (24). The widespread and prolonged use of antibiotics is one of the main reasons for the emergence of antibiotic-resistant strains (25). *P. aeruginosa*, as one of the most important hospital pathogens, is no exception, and today we are

witnessing an increase in its MDR and extensively drug-resistant (XDR) strains in clinical settings. The acquisition of genes producing extended-spectrum beta-lactamases (ESBLs) and MBLs, which are associated with the hydrolysis and inactivation of a wide range of beta-lactam and carbapenem antibiotics, has led to the spread of these strains (26). The location of both ESBL and MBL genes on mobile genetic elements such as integrons, plasmids, and transposons has led to their high genetic mobility. These features

allow for their horizontal spread between different bacterial species and strains (27, 28). Carbapenem resistance genes have been increasingly reported across the globe, particularly in regions of Europe, Asia, and South America (29). While numerous investigations have explored the role of carbapenemase genes in *P. aeruginosa*, the findings remain inconsistent, largely influenced by geographical, epidemiological, and population-specific factors (30). The prevalence of infections caused by *P. aeruginosa* has been reported to be relatively high in different regions of Iran, and this bacterium is recognized as the main cause of infection in many hospitals across the country (31, 32). Therefore, considering the key role of integrons in the transfer of antibiotic resistance genes, including carbapenemases, this study investigated the frequency of class 1 and 2 integron genes, as well as the *blaIMP* gene, as one of the most common carbapenemase genes, among the carbapenem-resistant clinical isolates of *P. aeruginosa*.

The highest isolation of *P. aeruginosa* were reported from urine (24.3%), sputum (17.1%), and wound (15.7%) samples. These findings highlight the bacterium's ability to cause infections in the urinary tract, respiratory system, and open wounds. The extensive genetic capabilities and remarkable biological flexibility of *P. aeruginosa* enable it to survive in diverse physical conditions and cause a wide range of infections (33).

Most isolates were recovered from the ICU (25.7%), surgical (18.6%), and internal medicine wards of the hospital (14.3%). This highlights the significant role of *P. aeruginosa* in hospital-acquired infections, especially among critically ill patients, where conditions favor the spread of resistant strains. Furthermore, a statistically significant association was observed between hospital wards and resistance to antibiotics such as imipenem, ciprofloxacin, and piperacillin ( $P < 0.05$ ). Resistance to these antibiotics was higher in isolates recovered from the ICU, internal medicine, and surgical wards. This may be attributed to the higher antibiotic pressure and more frequent use of broad-spectrum antibiotics in these wards.

Due to genetic changes caused by inappropriate antibiotic prescribing, the resistance profile may vary across regions. Therefore, susceptibility testing is essential to prevent the emergence of new resistant strains (34-36). Based on the findings, *P. aeruginosa* isolates exhibited the highest susceptibility to amikacin

(55.7%) and gentamicin (52.9%), while the highest resistance was observed against imipenem (50%). In addition, 34 (48.6%) of the isolates were identified as MDR. The resistance patterns differed significantly between the MDR and non-MDR groups for all tested antibiotics ( $P < 0.05$ ), suggesting that MDR strains represent a greater therapeutic challenge due to their broader resistance spectrum.

In the present study, 50% of the isolates were resistant to carbapenem (imipenem), which aligns with the findings of Park and Koo (47.6%) (19). Similarly, a report from India showed resistance rates ranging from 40% to 47% (37). In contrast, Maleki et al. reported a much higher resistance rate of 100% (38). Other studies have reported resistance levels of 43.4% in China and up to 66% in Latin American countries (39, 40). These variations may be attributed to differences in the geographic location, hygiene standards, sample types, and infection control practices (41). The key function of integrons in the development and spread of antibiotic resistance in *P. aeruginosa* isolates is undeniable (42, 43). Several studies have shown a high prevalence of class 1 integrons in hospital-acquired *P. aeruginosa*. For instance, Shojapour et al. reported that 95% of the isolates from patients in Markazi, Iran, carried the *intI1* gene, while none carried the *intI2* gene (44). Similarly, in the present study, class 1 integrons were detected in 94.3% of the carbapenem-resistant *P. aeruginosa* isolates, whereas class 2 integrons were not found in any of the samples. The absence of class 2 integrons in our isolates is unlikely to be due to technical issues, as previously validated primers were used and positive controls were included. Moreover, the low or undetectable prevalence of class 2 integrons in *P. aeruginosa* has been reported in several other studies.

A study conducted in Nigeria on MDR *P. aeruginosa* isolates reported a lower prevalence of class 1 integrons (57%, 31 isolates), but consistent with our findings, none of the resistant isolates carried class 2 integrons (45). Another study by Khademi et al. reported the frequency of class 1 integron genes among *P. aeruginosa* strains resistant to doripenem, meropenem, and imipenem as 44.7%, 62.7%, and 85.5%, respectively (46). A high prevalence of class 1 integrons has also been reported in other non-fermentative Gram-negative bacteria. For example, Hallaji et al. (2018) found that 63.9% of clinical *Acinetobacter baumannii* isolates from hospitalized

patients in Isfahan, carried class 1 integrons, highlighting their role in the dissemination of multidrug resistance among nosocomial pathogens in the region(47).

The pivotal and key function of integrons in the transmission of antimicrobial resistance genes is now well-established (48). For example, *blaIMP* genes are commonly located in class 1 integrons embedded in accessory genetic elements (AGEs) (49). In this study, the presence of class 1 integrons was confirmed in all isolates carrying the *blaIMP* carbapenemase gene. This finding may indicate the important role of integrons in the spread of these genes among bacterial species. However, no significant association was observed between the presence of class 1 integrons and the *blaIMP* gene ( $p = 0.486$ ). This lack of statistical significance may be attributed to the relatively small sample size, which reduces the power of the analysis to detect true associations.

As such, the possibility of a genuine relationship between these two variables cannot be excluded. Further studies with larger sample sizes are recommended to clarify this potential association. *blaIMP* genes are commonly found within integrons alongside other resistance genes, such as aminoglycoside resistance genes and *blaOXA*. This coexistence of genes in a single genetic unit leads to multidrug resistance and difficulty in treatment (49). Therefore, it is not surprising that strains carrying class 1 integrons are associated with resistance to various antibiotics, including aminoglycosides, quinolones, and beta-lactam compounds (carbapenems) (50). In our study, this relationship was also evident, as most carbapenem-resistant isolates showed resistance to almost all tested antibiotics, which is consistent with the findings of Moazami-Goudarzi and Eftekhar (51).

In this study, the *blaIMP* gene was identified in 48.6% of the carbapenem-resistant *P. aeruginosa* isolates. This prevalence was higher than that in a study in Iraq where only 25% (3 out of 12 MBL-producing phenotypic strains) had the *blaIMP* gene (52). Furthermore, a more detailed analysis revealed that the prevalence of the *intI1* and *blaIMP* genes was higher among the isolates obtained from the ICU and internal medicine wards. The findings of Asghari Gharakhyli et al. also indicated a prevalence of 58.98% of isolates carrying class I integrons in the ICU (53). Notably, the *blaIMP* gene was also detected in the isolates from ETT samples. Such findings raise concerns about

colonization or infection with resistant strains in patients undergoing mechanical ventilation.

In our study, almost half of the carbapenem-resistant isolates that harbored class I integrons did not carry the *blaIMP* gene. This observation suggests that other resistance determinants may be involved in the carbapenem resistance phenotype in these isolates. Although this study focused on the *blaIMP* gene because of its previously reported high prevalence in regional isolates and its clinical relevance, other carbapenemase genes such as *blaNDM*, *blaVIM*, *blaOXA*, or *blaSPM* may also play significant roles. Future studies should include these genes to provide a more comprehensive resistance profile. In addition to the unidentified carbapenemase genes, non-enzymatic mechanisms such as the overexpression of efflux pumps (MexAB-OprM) or the loss of porin proteins like OprD could also contribute to carbapenem resistance.

These findings underscore the multifactorial and intricate nature of the carbapenem resistance mechanisms in *P. aeruginosa*. Although these mechanisms were not investigated in the current study, this represents a limitation that may prevent a comprehensive depiction of the resistance landscape. Further molecular analyses targeting these alternative pathways are recommended. Moreover, the relatively small sample size in this study may limit the generalizability of the findings. Larger multi-center studies are needed to validate these results.

However, these findings have important clinical implications. The high prevalence of carbapenem-resistant *P. aeruginosa* isolates in the ICU and internal medicine wards highlights the need for enhanced infection control protocols in these high-risk departments. Molecular screening of integrons and carbapenemase genes can facilitate the early detection and containment of outbreaks. Moreover, due to the high levels of multidrug resistance, empirical antibiotic therapy in the ICU should be guided by antibiograms and tailored to the resistance patterns. This approach can reduce the risk of treatment failure and limit the selection of further resistant strains.

The study's evaluations showed that class 1 integrons are highly prevalent among carbapenem-resistant *P. aeruginosa* isolates and could be considered as one of the key factors in the spread of gene resistances, including the *blaIMP* gene, which was identified in about half of the isolates with

integrons. These findings highlight the functional role of mobile genetic elements in the horizontal transfer of resistance genes and emphasize the need for continuous monitoring of resistance genes and their transmission mechanisms in healthcare facilities. Since the production of carbapenemases is correlated with resistance to other antibiotic groups and clinical problems such as treatment failure, adopting careful infection control strategies, rational and responsible selection of antibiotics, and the use of molecular methods for early identification of resistant strains can help in curbing the spread of multidrug resistance. Specifically, routine molecular screening for *intI1* and *blaIMP* genes, particularly in the ICU and other high-risk hospital wards, is recommended to facilitate timely intervention and reduce the dissemination of resistant strains period.

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