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Characterization of Carbapenem Resistance in *Pseudomonas aeruginosa* and Involvement of the *OprD* Gene in Clinical Isolates Isolated in Abidjan

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Abstract

Carbapenem resistance in *Pseudomonas aeruginosa* (*P. aeruginosa*) represents a major public health concern, as it drastically limits therapeutic options for severe nosocomial infections. This study investigated carbapenem resistance, multidrug resistance, and the presence of the *oprD* gene among 31 clinical isolates of *P. aeruginosa* collected from the National Reference Center for Antibiotics at the Pasteur Institute of Côte d'Ivoire. Isolates were identified using the API 20NE gallery and MALDI-TOF mass spectrometry. Antimicrobial susceptibility testing was performed by the disk diffusion method according to CA-SFM and CLSI recommendations. Results showed that 87.1% and 90.3% of isolates were resistant to imipenem and meropenem, respectively. High levels of cross-resistance were also observed to other β-lactams (cefepime, ceftazidime) and aminoglycosides (gentamicin, tobramycin, amikacin). The *oprD* gene was detected at a frequency of 83.9% among the isolates. Correlation analysis identified several phenotypic profiles combining carbapenem resistance, multidrug resistance, and *oprD* positivity. Sequencing of the *oprD* gene showed substantial polymorphism, including amino acid substitutions and deletions, allowing classification of isolates into five genetic groups. These findings highlight that carbapenem resistance in *P. aeruginosa* is multifactorial, involving both *oprD* gene alterations and associated multidrug resistance mechanisms, significantly reducing available therapeutic options.

Keywords

Carbapenem, Miltidrug Resistance, *OprD*, *P. aeruginosa*, Abidjan

1. Introduction

Pseudomonas aeruginosa is a formidable opportunistic pathogen that poses a growing threat in healthcare settings. It is characterized by its remarkable ability to develop multiple antibiotic resistance mechanisms and to persist in hospital environments, facilitating its spread [1]. Conditions favoring its proliferation-such as moist environments, drains, medical equipment, and contaminated aqueous solutions-contribute to its emergence and transmission in hospital wards, particularly in intensive care units [2]. Responsible for a wide range of severe infections, including urinary tract infections, bacteremia, pneumonia, and device-associated infections, *P. aeruginosa* mainly affects immunocompromised patients or those with prolonged hospital stays. Recent studies estimate that it accounts for approximately 16% of nosocomial pneumonia cases [3]. Historically susceptible to most β-lactams, *P. aeruginosa* has progressively acquired resistance to at least one antibiotic from three different classes (multidrug resistance), and in some cases to all available antibiotic classes, which represents a major therapeutic challenge. Carbapenems, particularly imipenem and meropenem, remain cornerstone agents in the treatment of infections caused by multidrug-resistant strains [4]. However, carbapenem resistance is of particular concern, as it drastically limits available therapeutic options. This resistance relies on multiple mechanisms, including the production of carbapenemases (notably metallo-β-lactamases), altered membrane permeability due to loss or mutation of the *OprD* porin, overexpression of efflux systems, and hyperproduction of *AmpC* β-lactamases [4] [5]. Among these mechanisms, loss or reduced expression of the *OprD* porin-caused by mutations, deletions, or insertions in the *oprD* gene-is the most common cause of intrinsic resistance to imipenem and, to a lesser extent, meropenem [6]. The global emergence of carbapenem-resistant *P. aeruginosa* strains poses a major public health challenge, as it compromises standard antimicrobial therapies and increases mortality associated with severe infections. In this context, the aim of this study is to determine the prevalence of *oprD* gene alterations and assess their contribution to carbapenem resistance in clinical *P. aeruginosa* isolates.

2. Materials and Methods

2.1. Sampling

The biological material used in this study consisted of thirty-one (31) clinical isolates of *P. aeruginosa* obtained from the National Reference Center for Antibiotics at the Pasteur Institute of Côte d'Ivoire. Identification of isolates was performed using the API 20NE gallery (BioMérieux, Marcy l'Etoile, France) and MALDI-TOF mass spectrometry (Microflex, Vitek, BioMérieux, France).

2.2. Antimicrobial Susceptibility Testing

The susceptibility of isolates to ticarcillin/clavulanic acid (75 + 10 µg), imipenem (10 µg), meropenem (10 µg), ceftazidime (30 µg), cefepime (30 µg), levofloxacin (5 µg), ciprofloxacin (5 µg), gentamicin (10 µg), tobramycin (10 µg), amikacin (10 µg), fosfomycin (5 µg), and colistin sulfate (10 µg) (Bio-Rad®) was determined using the disk diffusion method on Mueller-Hinton agar (Bio-Rad®, France), according to the recommendations of the Antibiogram Committee of the French Society for Microbiology [7]. Using a sterile swab, a bacterial colony from a pure culture of each *P. aeruginosa* isolate was suspended in a glass tube containing 2 mL of 0.85% NaCl saline solution (Api® BioMérieux®). The turbidity of each suspension was adjusted to 0.5 McFarland standard. The inoculum was spread evenly onto Mueller-Hinton agar using a sterile cotton swab. Antibiotic disks were applied manually with a Bio-Rad® disk dispenser, and plates were incubated at 37˚C for 24 h under aerobic conditions. The inhibition zone diameters were read and interpreted automatically using the ADAGIO system (Bio-Rad®, France). Results were recorded on data sheets and entered into Microsoft Excel. Inhibition zone diameters (IZD, in mm) were interpreted as susceptible (S) or resistant (R) by comparing them to critical diameters established for *P. aeruginosa* according to CA-SFM [7] guidelines. For colistin sulfate, interpretation followed CLSI [8] criteria, with inhibition zones ≥ 11 mm considered "susceptible" and ≤10 mm considered "resistant".

2.3. Polymerase Chain Reaction (PCR)

Amplification of the *oprD* gene was carried out by PCR in an Applied Biosystems 9700 thermocycler using a reaction volume of 50 µL. The primers used to amplify the *oprD* gene produced a 160 bp fragment [9].

2.3.1. DNA Extraction

Genomic DNA was extracted from each *P. aeruginosa* isolate using the phenol/chloroform/isoamyl alcohol method described by Sambrook and Russell (2001). A pure bacterial colony from each isolate was suspended in 300 µL of lysis buffer and incubated at 60˚C for 1 h. After incubation, 400 µL of phenol/chloroform/isoamyl alcohol mixture were added, and the tubes were homogenized. Centrifugation at 13,000 × g for 5 min separated the phases. The upper aqueous phase containing DNA was transferred to a new tube. DNA was then precipitated by adding 500 µL of cold absolute ethanol and 50 µL of sodium acetate, followed by incubation at −80˚C for 2 h. After centrifugation at 13,000 × g for 20 min, the supernatant was discarded, and the DNA pellet was washed with 1 mL of 70% ethanol. A second centrifugation at 13,000 × g for 5 min at 4˚C was performed. The supernatant was removed, and the pellet was dried at 65˚C. The purified DNA was finally resuspended in 60 µL of elution buffer and stored at −20˚C until use.

2.3.2. PCR Reaction Mixture (Master Mix) for *oprD* Amplification

The PCR master mix for *oprD* amplification was prepared in an Eppendorf tube

by pipetting the required reagents in the following volumes: 10 µL of PCR buffer, 30.3 µL of nuclease-free water, 0.5 µL of dNTP solution (10 µM), 3 µL of $MgCl_2$ (25 mM), 0.5 µL of forward primer *OprDF* (10 µM), 0.5 µL of reverse primer *OprDR* (10 µM), and 0.2 µL of Taq DNA polymerase. The mixture was vortexed and briefly centrifuged to ensure homogeneity before amplification.

2.3.3. PCR Conditions

A 45 µL aliquot of the master mix was dispensed into each PCR tube, followed by 5 µL of template DNA. Tubes were placed in an Applied Biosystems 9700 thermocycler and subjected to the following cycling conditions: an initial denaturation at 94˚C for 5 min; 35 cycles of denaturation at 94˚C for 1 min, annealing at 65˚C for 30 s, and extension at 72˚C for 1 min; followed by a final extension at 72˚C for 10 min. Amplification of the *oprD* gene used primers described by Dumas *et al.* (2006), yielding a 160 bp fragment: *OprDF*: 5'-AATTCGAAGGGCTCGACCTC-3' and *OprDR*: 5'-GCGCTGAGGTTATCGGTGA-3'. After amplification, PCR products were stored at 4˚C pending electrophoresis analysis.

2.3.4. Agarose Gel Electrophoresis

Following amplification, PCR products were visualized on 1.5% agarose gel. SYBR® Green nucleic acid stain was added to molten agarose at 5 µL per 100 mL before solidification. Electrophoresis was conducted in 1× buffer at 100 V for 1 h. DNA bands were visualized under UV light using a GEL DOC imaging system. Two microliters of loading dye were added to 10 µL of PCR product before loading onto the gel. A molecular weight marker (100 bp or 1 Kb DNA ladder; Thermo Scientific) was included to estimate fragment sizes. Band sizes were compared to those of control strains: identical sizes were considered positive results, different sizes were interpreted as negative, and close sizes as indeterminate. Technical validation was performed using a negative control (no DNA) and known positive controls.

2.4. Sequencing

Bidirectional sequencing of positive PCR products was performed using the ABI Prism® BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA), following the manufacturer's instructions. Sequence data were analyzed with BioEdit® software version 6.0. The obtained sequences were compared with reference sequences using BLASTN (NCBI, http://www.ncbi.nlm.nih.gov/) and aligned with the *P. aeruginosa* PAO1 reference strain sequences available in GenBank.

2.5. Data Analysis

Data generated in this study were processed using Microsoft Excel 2016 (Microsoft Office™). The percentage of resistance to each antibiotic was calculated as the number of resistant isolates divided by the total number of isolates, multiplied by 100. Similarly, the percentage of *oprD*-positive susceptible isolates was determined as the number of susceptible isolates carrying the *oprD* gene divided by the

total number of isolates, multiplied by 100, while the percentage of *oprD*-positive resistant isolates was obtained as the number of resistant isolates carrying the *oprD* gene divided by the total number of isolates, multiplied by 100.

3. Results

3.1. Antibiotic Susceptibility of *Pseudomonas aeruginosa* Isolates

The antibiotic susceptibility results of *Pseudomonas aeruginosa* isolates are presented in Figure 1. In this study, 87.1% and 90.3% of the isolates were resistant to imipenem and meropenem, respectively. High resistance rates were also observed for cefepime (77.4%), gentamicin (74.2%), and ciprofloxacin (58.1%). No resistance was detected to colistin sulfate.

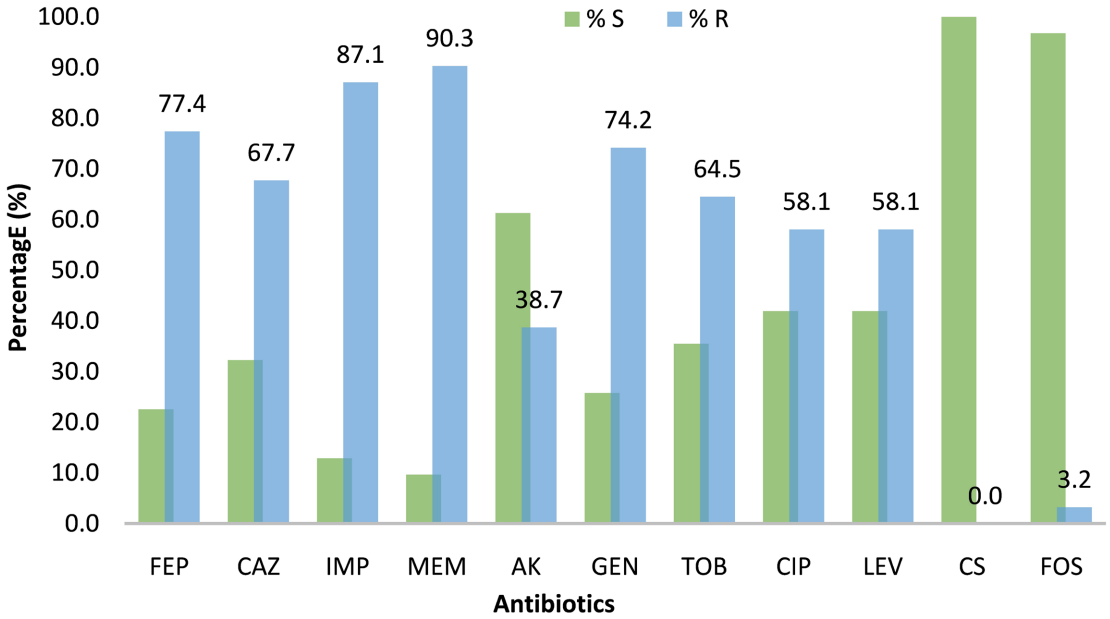

Figure 1. Percentage of resistance of *P. aeruginosa* isolates to the tested antibiotics.

3.2. Cross-Resistance between Carbapenems and Other Antibiotic Classes

The results of cross-resistance between carbapenems (imipenem and meropenem) and other antibiotic classes are presented in Table 1. *P. aeruginosa* isolates resistant to meropenem showed high resistance rates to cefepime (82.1%) and gentamicin (78.6%), while resistance to other β-lactams (ceftazidime) and aminoglycosides (amikacin and tobramycin) ranged from 39.3% to 67.9%. Similarly, isolates resistant to imipenem exhibited resistance rates of 77.8% and 74.1% to cefepime and gentamicin, respectively, while resistance to the other tested antibiotics ranged from 40.7% to 70.4%. These findings indicate that carbapenem-resistant isolates frequently display cross-resistance to other antibiotic classes, particularly β-lactams and aminoglycosides, thereby considerably limiting therapeutic options for the management of *P. aeruginosa* infections.

Table 1. Cross-resistance of carbapenems with other antibiotics.

Carbapenem resistance phenotypes (n)	Other antibiotics								
	FEP	CAZ	AK	GEN	TOB	CIP	LEV	CS	FOS
IMP R (27)	21	19	11	20	18	17	16	0	1
%	77.8	70.4	40.7	74.1	66.7	63.0	59.3	00	3.7
MEM R (28)	23	20	11	22	19	16	17	0	1
%	82.1	71.4	39.3	78.6	67.9	57.1	60.7	00	3.6

Abbreviations: FEP: cefepime, CAZ: ceftazidime, IMP: imipenem, MEM: meropenem, AK: amikacin, GEN: gentamicin, TOB: tobramycin, CIP: ciprofloxacin, LEV: levofloxacin, CS: colistin, %: percentage, (n): number of isolates

3.3. Detection of *oprD* genes in *P. aeruginosa* Isolates

The results of agarose gel electrophoresis of the PCR products from *Pseudomonas aeruginosa* isolates are presented in Figure 2. Twenty-six (26) isolates, corresponding to 83.9%, tested positive for the *oprD* gene, displaying amplicons of 160 base pairs (bp). These isolates were interpreted as *oprD*-positive, corresponding to wells 4, 5, 7, 9 - 26. Five isolates (16.1%) showed no band at 160 bp and were therefore considered *oprD*-negative; wells 3, 6, and 8 correspond to these negative isolates. Wells 1 and 2 represent the negative and positive controls, respectively, while lane M indicates a 100 bp DNA molecular weight marker.

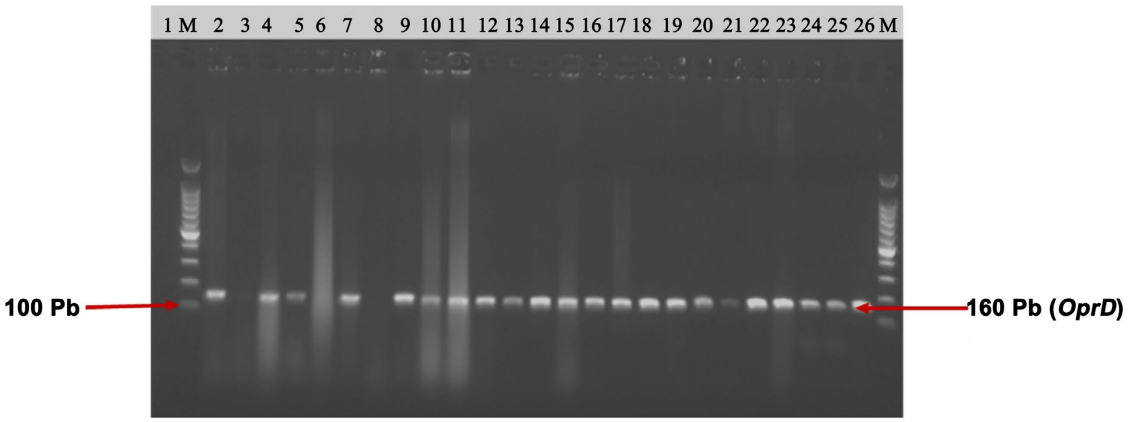

Figure 2. Electrophoretic profile showing a simplex PCR for the detection of the *OprD* gene.

3.4. Correlation between Carbapenem Resistance, Multidrug Resistance (MDR), and *oprD* Gene Detection

Multidrug resistance (MDR) was defined as resistance to at least three classes of antibiotics. Table 2 presents the correlation between carbapenem resistance (CarbaR), MDR, and the presence of the *oprD* gene. Seven (7) correlation patterns were observed in this study, labeled A, B, C, D, E, F, G, and H, as shown in Table 2. The majority of isolates belonged to profiles A, B, and C, characterized by simultaneous resistance to both imipenem and meropenem, often associated with MDR and the loss of the *oprD* gene. Some isolates, corresponding to profiles E, F,

and G, exhibited resistance to only one carbapenem while retaining the *oprD* gene, suggesting the involvement of alternative resistance mechanisms. Finally, profile H included isolates sensitive to one carbapenem but resistant to the other, without *oprD* expression. In total, 2 isolates (6.67%) belonged to type F and 1 isolate (33.33%) to type G.

Table 2. Correlation between carbapenem resistance, multidrug resistance (MDR), and *OprD* gene detection.

Analysis Codes	IMP	MEM	MDR	*OprD*	Correlation Codes
11235c/12, 1810c/12, 1076c/12, 1014c/12, 1060c/12, 1354c/12, 1175/c12, 1953c/13, 2441c/15, 885c/15, 2568c/15, 2548c/15, 2563c/15, 2562c/15, 2583c/15, 1078c/15	R	R	+	+	**A, B, C**
957c/12, 2589c/15	R	S	−	+	**G**
1217c/12, 2425c/15, 792c/15, 795c/15	R	R	−	+	**A, D**
255c/12	R	R	−	−	**A**
1780c/12, 1635c/12	R	R	+	−	**A, E**
1245c/13, 2038c/15	S	R	−	+	**E, F**
2359c/15	R	R	−	+	**A, F**
1570c/15, 2440c/15, 2415c/15	S	R	+	−	**E, H**

R: Resistant; **S:** Susceptible; **+**: Presence; **−**: Absence; **A:** carbapenem-resistant with multidrug resistance (CarbaR-MDR); **B:** carbapenem-resistant, *oprD*-positive (CarbaR-*OprD*$^+$); **C:** carbapenem-resistant with multidrug resistance, *oprD*-positive (CarbaR-MDR-*OprD*$^+$); **D:** carbapenem-resistant, *oprD*-positive (CarbaR-*OprD*$^+$); **E:** carbapenem-resistant, *oprD*-negative (CarbaR-*OprD*$^-$); **F:** imipenem-susceptible, meropenem-resistant, *oprD*-positive (ImpS-MEMR-*OprD*$^+$); **G:** imipenem-resistant, meropenem-susceptible, *oprD*-positive (ImpR-MEMS-*OprD*$^+$); and **H:** imipenem-susceptible, meropenem-resistant, *oprD*-negative (ImpS-MEMR-*OprD*$^-$).

3.5. *OprD* Gene Sequencing

The PCR products of the *OprD* gene were fully sequenced as described above, and the resulting sequences were compared to the *OprD* sequence of the reference strain PAO1 (GenBank). Two types of mutations were observed in the *oprD* sequences of all imipenem-resistant isolates: substitutions and deletions. Comparison of the *OprD* sequences of the studied isolates with that of the wild-type PAO1 strain revealed significant polymorphism in this gene. However, no mutations were detected in the *oprD* sequences of two isolates. Depending on the number and the presence or absence of substitutions and deletions within the gene sequence, the isolates studied were classified into five genetic groups: Group 1 consisted of seven isolates with 22 amino acid substitutions and 2 deletions; Group 2 included five isolates with 27 amino acid substitutions and 2 deletions; Group 3 comprised three isolates with 23 substitutions and 2 deletions; Group 4 included two isolates with only 14 substitutions; and Group 5 contained two isolates with

no detectable mutations.

4. Discussion

In this study, *P. aeruginosa* isolates exhibited very high resistance rates to carbapenems, with 87.1% for imipenem and 90.3% for meropenem, as well as marked resistance to cefepime (77.4%), gentamicin (74.2%), and ciprofloxacin (58.1%). In contrast, no resistance to colistin was observed (0%). These rates exceed those reported by Ramatla *et al.* [4] and Saha *et al.* [5], who described global carbapenem resistance levels ranging from 30% to 50%, but are consistent with findings from Eid *et al.* [10] and Yin *et al.* [11], where resistance exceeded 85% in certain intensive care units. The absence of colistin resistance aligns with data from Zhu *et al.* [12] (2025) and other recent studies, confirming that this drug remains one of the few effective therapeutic options against multidrug-resistant strains. These differences reflect the influence of local context, including antibiotic pressure, clonal dissemination of resistant strains, and variability in molecular mechanisms such as loss or mutation of the *oprD* gene, production of carbapenemases, and overexpression of efflux pumps. These results highlight the need for continuous surveillance and antimicrobial stewardship policies to limit the spread of multidrug-resistant strains in hospital settings. Analysis of **Table 2** revealed marked phenotypic heterogeneity among the 31 isolates studied. Dominant profiles included type A (*CarbaR-MDR*), observed in 20 isolates (66.7%), and type B (*CarbaR-oprD*$^+$), present in 21 isolates (70%). These profiles indicate a high prevalence of strains combining carbapenem resistance and multidrug resistance while retaining detectable *oprD*. Profile C (*CarbaR-MDR-oprD*$^+$), detected in 2 isolates (6.67%), illustrates the coexistence of multiple resistance mechanisms, suggesting synergy between multidrug resistance and functional *OprD* porin preservation. Profiles D (*CarbaR-oprD*$^+$, 60%) and E (*CarbaR-oprD*$^-$, 10%) indicate that carbapenem resistance is not exclusively dependent on *oprD* loss: most resistant strains remain *oprD*-positive, suggesting structural alterations or reduced porin expression rather than complete loss. Atypical phenotypes F (*ImpS-MemR-oprD*$^+$, 6.67%) and G (*ImpR-MemS-oprD*$^+$, 3.33%) reflect differential resistance between imipenem and meropenem, likely due to structural variations in the *OprD* channel or differential expression of carbapenemases. Finally, profile H (ImpS-MemR-*oprD*$^-$), observed in one isolate (3.33%), illustrates a rare combination of *oprD* loss and selective meropenem resistance. Overall, 83.9% of *P. aeruginosa* isolates were positive for the *oprD* gene, consistent with Saleh *et al.* [13] (2023), who reported 83.3% positivity in their clinical cohort. This gene encodes an outer membrane porin essential for carbapenem entry, particularly imipenem. Loss, mutation, or down regulation of *oprD* reduces membrane permeability and contributes to resistance, as demonstrated by Wang *et al.* [14]. The absence of the 160-bp PCR band in *oprD*-negative isolates confirms the specificity of the PCR method, widely used for detecting this gene and characterizing resistance profiles [13]. The findings confirm the diversity of mechanisms involved in carbapenem resistance in *P. ae-*

ruginosa. The predominance of types A (*CarbaR-MDR*) and B (*CarbaR-OprD*$^{+}$) suggests that multidrug resistance is closely associated with carbapenem resistance, without relying solely on porin loss. These observations corroborate Zhu *et al.* [15] and Wang *et al.* [16], showing that many clinical strains retain detectable but altered *OprD*, while complete gene loss remains rare. Carbapenem resistance thus results from multifactorial interactions, combining chromosomal mechanisms (mutations, IS insertions in *OprD*) and acquired mechanisms (carbapenemases, integrons, efflux). Profiles C and D support this hypothesis, indicating resistance can occur even with functional *OprD* due to overexpression of efflux systems such as *MexAB-OprM* or *MexXY* or reduced permeability. Profiles F and G highlight selective carbapenem resistance, likely caused by conformational changes in *OprD* affecting imipenem and meropenem differently [17] [18]. These observations confirm that carbapenem resistance in *P. aeruginosa* is multifactorial and adaptive, combining intrinsic mechanisms (mutations, transcriptional regulation of *OprD*) and acquired mechanisms (carbapenemases, efflux pumps, integrons) [14] [17] [19]. The persistence of CarbaR–MDR strains represents a major public health threat, especially in resource-limited settings where antibiotic misuse and inadequate infection control promote their spread [20].

Furthermore, our results reveal notable polymorphism of the *OprD* gene in imipenem-resistant strains, characterized by various amino acid substitutions and deletions. These findings align with Wang *et al.* [16], who reported point mutations and IS256 insertions in *OprD*, leading to porin loss and increased resistance. Classification of isolates into genetic groups based on mutation type and number reflects the diversity of resistance mechanisms. Groups with amino acid substitutions and deletions resemble profiles reported in other studies. In contrast, isolates without detectable mutations suggest the involvement of additional mechanisms, such as efflux pump overexpression or carbapenemase production [21]. Some mutated isolates do not exhibit proportional imipenem resistance, possibly due to compensatory mechanisms like MexAB-OprM overexpression regulated by *mexR*, which increases resistance independently of *OprD* [22]. Overall, this study highlights the complexity and plasticity of carbapenem resistance mechanisms in *P. aeruginosa*, emphasizing the importance of combining phenotypic and molecular analyses to better understand, monitor, and control the dissemination of highly resistant strains in hospital settings.

5. Conclusion

This study demonstrates a high prevalence of carbapenem resistance among clinical *P. aeruginosa* isolates, often associated with multidrug resistance to other antibiotic classes, particularly β-lactams and aminoglycosides. Detection and sequencing of the *OprD* gene revealed significant polymorphism, with amino acid substitutions and deletions contributing to imipenem resistance, while some isolates without mutations suggest involvement of additional mechanisms such as efflux pump overexpression or carbapenemase production. These findings under-

score the complexity of resistance mechanisms and the need for rigorous microbiological and molecular surveillance. They also highlight the importance of infection prevention and control strategies, as well as rational antibiotic use, to limit the spread of multidrug-resistant strains and preserve the efficacy of available treatments against *P. aeruginosa* infections.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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