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Original Article

Prevalence of Carbapenem-resistance and Molecular Detection of Carbapenemase Genes of *bla*$_{IMP-1}$ and *bla*$_{VIM-2}$ in *Pseudomonas aeruginosa* isolated from Ventilated Intensive Care Unit Patients

Renas Othman Mohammed [a], **Karzan R. Sidiq** [a]*

[a] Medical Laboratory Science Department, College of Science, Charmo University, Sulaymaniyah, Iraq.

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*** Corresponding Author:**
karzan.sidiq@chu.edu.iq

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Abstract: *Pseudomonas aeruginosa* (*P. aeruginosa*) is a life-threatening pathogen in intensive care unit (ICU). The rise of bacterial resistance to carbapenem is a major global health concern due to dwindling treatment options. This study investigated the prevalence of carbapenem-resistant *P. aeruginosa* (CRPA) and its imipenemase-1 metallo-β-lactamase (bla$_{IMP-1}$) and verona-integron encoded metallo-beta-lactamase 2 (bla$_{VIM-2}$) genes in ventilated ICU patients. From November 2024 to March 2025, 150 respiratory samples (sputum and bronchial wash) were collected from ICU patients in Sulaymaniyah, Iraq. The isolates were identified using standard microbiological examinations, the BD Phoenix™ M50 system and 16S rRNA sequencing for CRPA isolates only. Carbapenemase activity was phenotypically assessed via the modified carbapenem inactivation method (mCIM), while polymerase chain reaction was used to target the carbapenemase-encoding genes. The prevalence rate of *P. aeruginosa* was 33.3% (50/150), including 6.6% (10/150) CRPA among all patients and 20% (10/50) CRPA in *P. aeruginosa* positive cases. Clinically significant antimicrobial resistance patterns of 5 (10%) MDR and 8 (16%) XDR were observed in *P. aeruginosa*, predominately including CRPA in both categories. Genotypic analysis revealed that none of the CRPA isolates carried the bla$_{VIM-2}$ gene, whereas 20% (2/10) isolates harbored the bla$_{IMP}$-1 gene in chromosomal DNA and plasmid-enriched DNA, suggesting potential plasmid association and horizontal gene transfer risk. The mCIM assay also confirmed carbapenemase activity only in bla$_{IMP-1}$ gene positive isolates, suggesting that other resistance mechanisms may play a role in evading carbapenems. This study underscores an urgent need for intensive molecular surveillance in ICU, as the plasmid-mediated bla$_{IMP-1}$ gene could lead to hardly controllable nosocomial infections.

1. Introduction

Pseudomonas aeruginosa (*P. aeruginosa*) is recognized as a Gram-negative opportunistic pathogen in healthcare settings worldwide [1]. It is especially problematic in patients with compromised immunity, chronic respiratory conditions, and those requiring mechanical ventilation, as it significantly contributes to hospital-acquired bronchitis and pneumonia [2]. The clinical management of *P. aeruginosa* infections is complicated by its inherited resistance to different sorts of antibacterials and rapidly acquired additional resistance mechanisms, resulting in frequent therapeutic failures and persistent colonization [3]. Among the available antimicrobial agents, carbapenems have often served as the drugs of last resort against multidrug-resistant (MDR) *P. aeruginosa* [4].

Nevertheless, the global reporting of carbapenem-resistant strains threatens their continued effectiveness and poses severe challenges for both therapy and infection control [5]. The suggested mechanisms of carbapenem resistance in *P. aeruginosa* are often chromosomal, including the reduction or loss of OprD porin channels, the enhancement of efflux pumps, and the depression of genomic AmpC β-lactamase, which can be acquired via the horizontal transfer of carbapenemase genes [6]. The coexistence of these mechanisms often confers MDR or pan-drug-resistant (PDR) phenotype.

Carbapenemases are enzymes capable of hydrolyzing carbapenem antibiotics and are classified primarily as serine β-lactamases and metallo-β-lactamases [4]. The Ambler class B metallo-β-lactamases, such as imipenemase and verona integrin-encoded metallo-β-lactamase, have particular clinical significance in *P. aeruginosa*. The imipenemase-1 metallo-β-lactamase (bla_{IMP-1}) and verona-integron encoded metallo-beta-lactamase 2 (bla_{VIM-2}) genes are commonly embedded within integrons and mobile genetic elements, greatly enhancing their dissemination within hospital populations and between geographic regions [2, 7]. Molecular surveillance has revealed considerable variation in the abundance of these genes among carbapenem-resistant isolates, with some regions seeing sporadic occurrence and others reporting endemic spread [4]. Despite the attention afforded to carbapenemases, multiple studies have demonstrated that many carbapenem-resistant *P. aeruginosa* (CRPA), particularly those in respiratory specimens, lack molecular evidence of these acquired genes and instead harbor chromosomally mediated resistance [1].

Globally, screening for CRPA and the key carbapenemase-encoding genes, bla_{IMP-1} and bla_{VIM-2}, is increasingly recognized as a critical area of study [8-11]. CRPA has been designated by the WHO as a priority and pathogen of serious health concern, which necessitates novel therapeutic options [12]. It is observed that ventilated patients suffer from hardly treatable respiratory tract infections in the intensive care unit (ICU) in Sulaymaniyah city. Thus, this study investigated the prevalence of carbapenem resistance and the distribution of carbapenemase genes (bla_{IMP-1} and bla_{VIM-2}) in *P. aeruginosa* isolated from ventilated ICU patients. These microbiological and molecular characterizations of *P. aeruginosa* inform local epidemiological surveillance, guide empirical therapy, and assist in tackling the transmission of MDR strains among high-risk patient populations.

2. Materials and Methods

2.1. Study Population and Sample Collection

A total of 150 clinical respiratory specimens, comprised of sputum and bronchial washes, were collected by authorized medical staff (nurses and physicians) from mechanically ventilated patients who had been admitted to ICU at public and private hospitals in Sulaymaniyah city, Iraq, from November 2024 to March 2025. The samples were collected from mixed ICUs (medical and surgical units) at Shar public hospital, Hiwa public hospital, and Harem private hospital. All patients involved were on mechanical ventilation with clinical evidence of/suspected lower respiratory tract infections at the time of the sample collection, while non-ventilated patients and those with no respiratory infections were excluded. The collected specimens were kept in sterile containers and transported to the clinical microbiology laboratory within two hours of collection under appropriate biosafety conditions. Ethical approval (Reference No. 8B in November, 2024) was provided by the ethics committee at the College of Science, Charmo University.

2.2. Identification of P. aeruginosa

2.2.1. Presumptive Identification

The specimens were aseptically inoculated onto MacConkey and blood agar plates (Liofilchem, Italy) and incubated aerobically at 37 °C for 18–24 hours. Colonies morphologically consistent with *P. aeruginosa*, characterized by their mucoid or non-mucoid appearance, pigmentation, and characteristic grape-like odor, were subjected to Gram staining and preliminary biochemical characterization. In addition, basic biochemical tests, such as the tube-based catalase/oxidase test (Liofilchem, Italy, Catalog no. 88023), were performed to differentiate *P. aeruginosa* from other Gram-negative aerobic bacteria according to standard microbiological protocols. Specific identification was subsequently performed by inoculating the isolates onto Cetrimide agar, a typical selective medium for *P. aeruginosa* [13].

2.2.2. *Automated Identification*

The BD Phoenix™ M50 automated microbiology system (Becton Dickinson, Franklin Lakes, NJ, USA) was used as a reliable method to ensure the identification of the *P. aeruginosa* isolates using the standard protocol and panel type NMIC/ID-431.

2.2.3. *Molecular Identification*

Molecular identification of the *P. aeruginosa* isolates was done using the polymerase chain reaction (PCR) technique for the amplification and sequencing of the 16S rRNA gene as follows:

2.2.4. *DNA Extraction*

Genomic DNA was extracted using a commercial genomic DNA extraction kit (ADD BIO Inc., Daejeon, Republic of Korea, catalog no. 10027), according to the manufacturer's protocol. The concentration and quality of the DNA extracts were evaluated using a Nanodrop Lite spectrophotometer (ThermoFisher, USA). The extracts were preserved at −20 °C for subsequent experiments.

2.2.5. *16S rRNA Gene Amplification*

To confirm species-level identification, the 16S ribosomal RNA (16S rRNA) gene was amplified by PCR (Thermocycler, Bio-Rad Laboratories, Inc., Hercules, CA, USA), using universal bacterial primers. The forward and reverse primer sequences were (7F:5′-AGAGTTTGATYMTGGCTCAG-3′) and (1510R:5′ACGGYTACCTTGTTACGACTT-3′) [14], designed to amplify a 1500 base pair fragment of the 16S rRNA gene. The PCR reaction was implemented in a 20 µl total volume, including 10 µl master mix (BIO-Rada), 0.5 µl of each primer, 1µl of DNA template, and 8µl of double-distilled water (ddH_2O). The thermal cycling parameters consisted of an initial denaturation (94°C/5 minutes), 30 cycles of (denaturation: 94°C/30 seconds; annealing: 58°C/30 seconds; extension: 72°C/1 minute), and the final extension (72°C/5 minutes).

2.2.6. *Gel Electrophoresis and Gel Purification*

Gel electrophoresis was carried out using 1.5% agarose gels in a 1× Tris-acetate-EDTA (TAE) buffer at 100 volts for 40 minutes. The PCR products were visualized with the aid of ethidium bromide under UV transillumination (Gel Doc™ system, Bio-Rad). Amplicon sizes (~1500 bp) were verified with the aid of a 100 bp DNA ladder (Genesand biotech Co., Ltd., China). Gel purification of the PCR amplicons was performed using a commercial gel purification kit (Addbio Inc., Korea), according to the included protocol.

2.2.7. *DNA Sequencing and Bioinformatic Analysis*

The purified 16S rRNA fragments were sequenced by a commercial Sanger sequencing facility (Macrogen, South Korea). The yielded sequences were blasted against the already sequenced 16S rRNA genes in the National Center for Biotechnology Information (NCBI), which helped with species identification and determining the sequence similarity percentages. The accession numbers were allocated to the bacterial isolates by submitting the consensus sequences to the NCBI.

2.2.8. *Construction of Phylogenetic Tree*

The bacterial isolates were taxonomically characterized based on the accession numbers retrieved from GenBank. ClustalX 2.1 was used for sequence matching, and MEGA 12 helped to generate the neighbor-joining phylogenetic tree by applying the Kimura 2-parameter model, with 1000 bootstrap repetitions [15].

2.3. *Antimicrobial Susceptibility Testing*

Antimicrobial susceptibility testing and the determination of minimum inhibitory concentration (MIC) were both executed using the BD Phoenix™ M50 automated system that depends on the broth microdilution method. The panel type was NMIC/ID-431. The MIC breakpoints were interpreted as susceptible, intermediate, or resistant depending on the Clinical and Laboratory Standards Institute (CLSI M100, 34th edition, 2024). The *P. aeruginosa* isolates were classified as carbapenem-resistant or

carbapenem-susceptible based on the MICs of meropenem and imipenem. In addition, using standardized criteria [16]. *P. aeruginosa* isolates were also categorized as MDR (MDR: non-susceptible to ≥1 agent in ≥3 antibacterial classes) and extensively drug-resistant (XDR: susceptible to ≤2 antibacterial classes). In this study, an automated system was used for the antimicrobial susceptibility testing. Because of the unreliability of the automated system (BD-Phoenix) for determining colistin susceptibility, colistin has been excluded.

2.4. Detection of Carbapenem-resistant P. aeruginosa

2.4.1. Phenotypic Detection of Carbapenemase Enzyme

The modified carbapenem inactivation method (mCIM) was employed to explore carbapenemase activity in the bacteria [17, 18]. As described in CLSI M100-S31, a 10 µL inoculum of *P. aeruginosa* culture (0.5 McFarland standard) was aspirated onto a meropenem disk (10 µg; Bioanalyse, Turkey) in a petri dish and incubated at 37 °C for 4 hours. The disk was then placed on a Mueller-Hinton agar plate (Liofilchem, Italy), already lawned with *Escherichia coli* ATCC 8739 (a carbapenem-susceptible control strain adjusted to 0.5 McFarland), and incubated at 37°C for 16–18 hours. Bacterial isolates showing inhibition zones of 6–15 mm or the appearance of colonies within a 16–18 mm zone was deemed to be carbapenemase producers (mCIM positive) [19], while isolates with clear inhibition zones of ≥19 mm was considered carbapenemase-negative. Those with 16–18 mm zones were considered indeterminate. The diameter of the inhibition zone was measured and interpreted according to the Clinical and Laboratory Standards Institute (CLSI M100, 31st edition, 2021).

2.4.2. Genotypic Detection of Carbapenemase-encoding Genes

The detection of carbapenemase-encoding genes in *P. aeruginosa* was accomplished using uniplex PCR assays to detect the Verona integron-encoded metallo-β-lactamase (bla_{VIM-2}) gene [20, 21] and the Imipenemase-1 metallo-β-lactamase (bla_{IMP-1}) gene [22], in both chromosomal and plasmid DNA. The primers for bla_{VIM-2} and bla_{IMP-1} detection are listed in table 1.

2.4.2.1. Detection of bla_{IMP-1} and bla_{VIM-2} from Genomic DNA

A uniplex PCR was carried out in a 25 µL reaction, comprised of 12.5 µL of PCR master mix (Genesand Biotech, China), 0.7 µL of each specific primer, 0.7 µL of the genomic DNA template, and nuclease-free water to adjust the final volume. Thermal cycling was performed with an initial denaturation (94 °C/3 minutes), followed by 35 cycles of (denaturation: 94 °C/30 seconds; annealing: 60.2 °C/30 seconds; extension: 72 °C/15 seconds), and a final extension (72 °C/5 minutes). The amplified products were analyzed by agarose gel electrophoresis, and the expected amplicon sizes were confirmed.

2.4.2.2. Detection of bla_{IMP-1} and bla_{VIM-2} from Plasmid DNA

To determine whether the carbapenemase genes (bla_{IMP-1} and bla_{VIM-2}) are plasmid-encoded, plasmid DNA was extracted from the bacterial isolates using a commercial kit (Roti®-Prep Plasmid MINI-XL, Carl Roth, Germany, SKU: 8546.2), according to the manufacturer's protocol. The plasmid DNA was utilized as a template in the PCR assays using the same master mix, primers, and amplification conditions described above.

Table 1: The list of primers for the detection of carbapenem-resistant genes in *P. aeruginosa*.

Primers	Sequence (5′-3′)	Amplicon size (bp)	References
bla_{VIM-2}	**F:** 5′ATGTTCAAACTTTTGAGTAAG3′ **R:** 5′-CTACTCAACGACTGAGCG-3′	801	[20, 21]
bla_{IMP-1}	**F:** 5′-CATGGTTTGGTGGTTCTTGT-3′ **R:**5′-ATAATTTGGCGGACTTTGGC-3′	488	[22]

2.5. *Statistical Analysis*

Descriptive statistical analysis was conducted using Microsoft Excel 2016 (Microsoft Corporation, USA). The prevalence rate of Pseudomonal infections, distribution of patients' sex, and frequencies of carbapenemase genes and antimicrobial-resistant patterns were expressed as percentages. The mean and standard deviation of the patients' age were also analyzed.

3. Results

3.1. *Prevalence Rate of P. aeruginosa Respiratory Infection*

Among a total of 150 ventilated patients admitted to ICUs during the study period, 50 (33.3%) had *P. aeruginosa* in their respiratory secretions (Figure 1A). The patients with Pseudomonas infections consisted of 22 (44%) males and 28 (56%) females (Figure 1B). The age of the patients ranged from 31 to 80 (55.5±14.6) years old. In light of the culture characteristics and preliminary biochemical tests, the bacterial isolates exhibited phenotypic characteristics consistent with *P. aeruginosa*. The isolates displayed typical colony morphology on MacConkey agar and Blood agar, revealed as non-lactose-fermenting colonies with characteristic pigmentation and a distinctive odor (Figure 2a**)**. All isolates were positive following Oxidase and Catalase tests (Figure 2b), and were Gram-negative rod-shaped under microscopic examination. The bacterial growth on the selective medium, Cetrimide agar, further supported the presumptive identification of the *P. aeruginosa* isolates (Figure 2c)

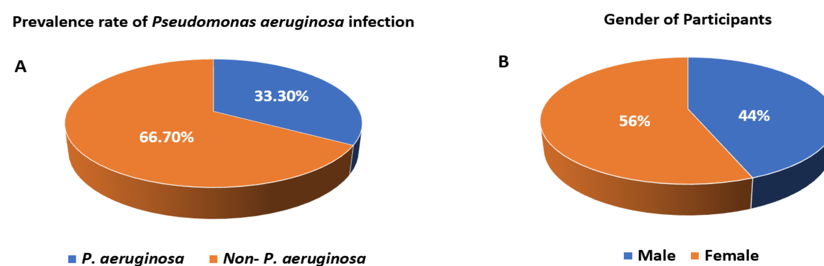

Figure 1: (**A**) Prevalence rate of *P. aeruginosa* infections among ventilated ICU patients (n=150). (**B**) Gender distribution of ventilated patients with *P. aeruginosa* infections (n=50).

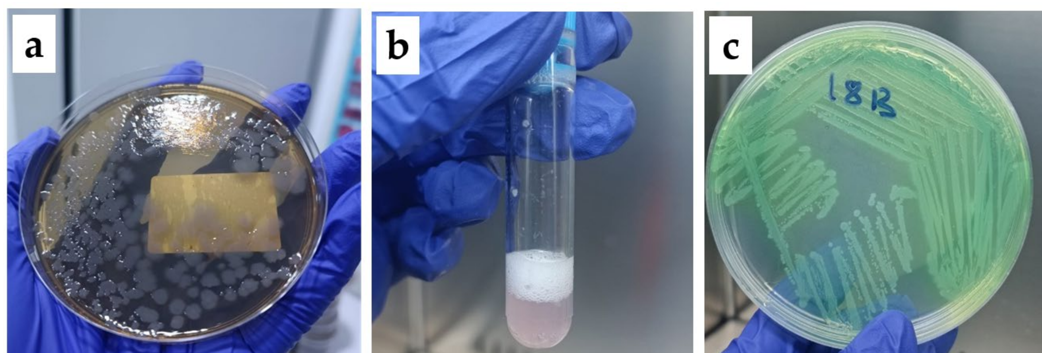

Figure 2: Culture characteristics and biochemical tests: **(a)** Colony morphology of *P. aeruginosa* grown on *MacConkey agar*; **(b)** Tube-based oxidase and catalase tests (+/+); **(c)** Growth of *P. aeruginosa* on *Cetrimide agar*.

In addition, the results of the BD Phoenix™ M50 identification system confirmed that all 50 isolates are *P. aeruginosa* with a confidence value of 99% for each isolate (reports not shown). The molecular identification, using PCR amplification and sequencing of the 16S rRNA gene, emphasized the identity of the carbapenem-resistant *P. aeruginosa* (CRPA). As this study mainly focused on CRPA, the molecular identification was only performed for the 10 carbapenem-resistant isolates (two multidrug-resistant isolates and eight XDR isolates). All isolates produced a band of approximately 1500 bp in the agarose gel electrophoresis (Figure 3).

The blasting of the 16S rDNA sequences showed greater than 99% similarity with reference *P. aeruginosa* sequences, available in the NCBI database. Distinct accession numbers were obtained for the isolates, designated *P. aeruginosa* R-01 (PZ213037), *P. aeruginosa* R-02 (PZ213038), *P. aeruginosa* R-03 (PZ213039), *P. aeruginosa* R-04 (PZ213040), *P. aeruginosa* R-05 (PZ213041), *P. aeruginosa* R-06 (PZ213042), *P. aeruginosa* R-07 (PZ213043), *P. aeruginosa* R-08 (PZ213044), *P. aeruginosa* R-09 (PZ213045), and *P. aeruginosa* R-10 (PZ213046).

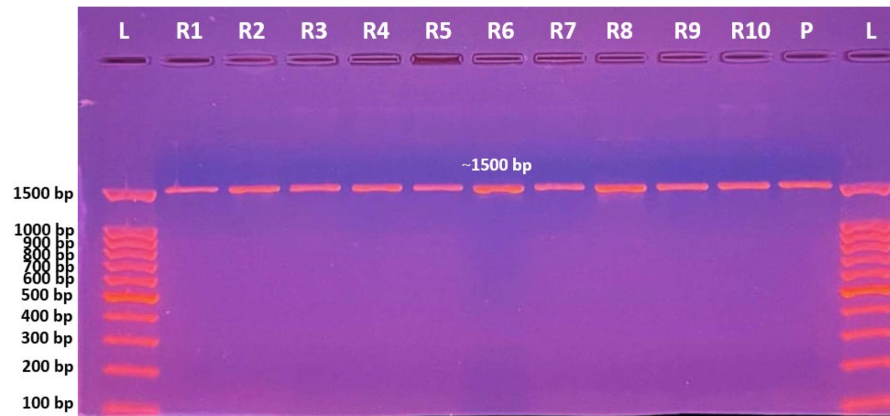

Figure 3: Agarose gel showing the PCR products (~1500 bp) of the 16S rRNA gene in the carbapenem-resistant *P. aeruginosa* isolates (Lane: R1- R10). P: Positive control. L: 100 bp DNA ladder.

3.2. Phylogenetic Analysis

Phylogenetic tree analysis was used to emphasize the species identity of our carbapenem-resistant *P. aeruginosa* (CRPA) isolates. The CRPA isolates formed a separate cluster, with a 100% bootstrap value at the main branch, with three *P. aeruginosa* strains. However, they clustered separately from the other pseudomonal and outgroup bacterial species (Figure 4). All CRPA isolates are clustered with *P. aeruginosa* and ATCC 15692 (AF094715.1) and ATCC27853 (AF094719.1) reference strains at a bootstrap value of 72%. These results support the identification of the *P. aeruginosa* isolates at the species level.

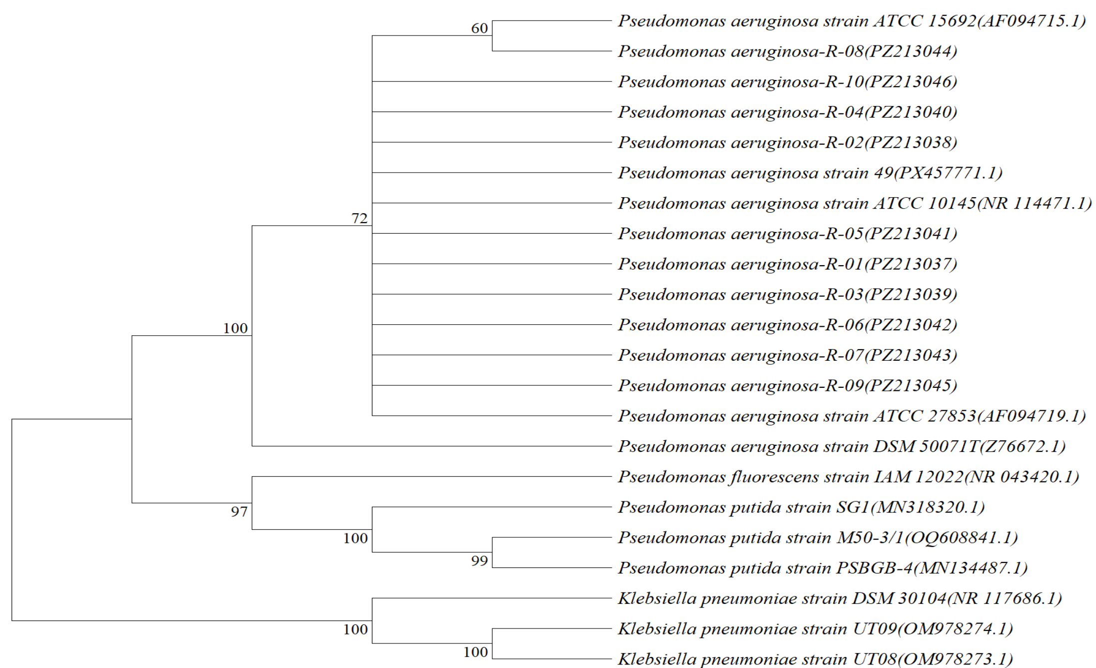

Figure 4: A phylogenetic tree demonstrated the molecular identification of the carbapenem-resistant *P. aeruginosa* (CRPA) isolates (R-01 to R-10), inferred using the Neighbor-Joining method related to the 16S rRNA gene sequences (each has a distinct accession number). It confirmed the species-level identification of the CRPA by comparing it to the reference bacterial species. The numeric values at the nodes represent bootstrap support values (%) based on 1000 replicates, and the scale length value was 0.05. To root the tree, *Klebsiella pneumoniae* was used as the outgroup bacterial species.

3.3. Antimicrobial Profile of the P. aeruginosa Isolates

Antimicrobial susceptibility testing demonstrated variable resistance patterns among the 50 *P. aeruginosa* isolates (Figure 5). The highest resistance rate (100%) was observed for Cefazolin, Cefuroxime, Ceftriaxone, Ampicillin, Amoxicillin, Nitrofurantoin, and Tigecycline, followed by Trimethoprim/Sulfa (82%). Fluoroquinolones (Ciprofloxacin and Levofloxacin) resistance was observed in 26% of isolates, while a lower resistance rate (16%) was recorded for both Amikacin and Ceftolozane/Tazobactam. It was also found that 10 (20%) isolates were resistant to carbapenems (Imipenem and Meropenem), while the rest of the isolates, 40 (80%), were susceptible. All 50 (100%) *P. aeruginosa* isolates were intrinsically resistant to Ertapenem.

The categorization of the isolates as MDR and XDR was implemented based on the standardized criteriaClick or tap here to enter text.. Among the 50 *P. aeruginosa* isolates analyzed, five (10%) isolates were identified as multidrug-resistant (MDR), while eight (16%) isolates exhibited XDR phenotypes. The remaining 37 (74%) isolates did not meet the criteria for multidrug resistance (Table 2). These results also revealed that about a quarter of the *P. aeruginosa* isolates are MDR and XDR. Ten carbapenem-resistant isolates are predominantly involved in two (20%) MDR and eight (80%) XDR categories (Table 2).

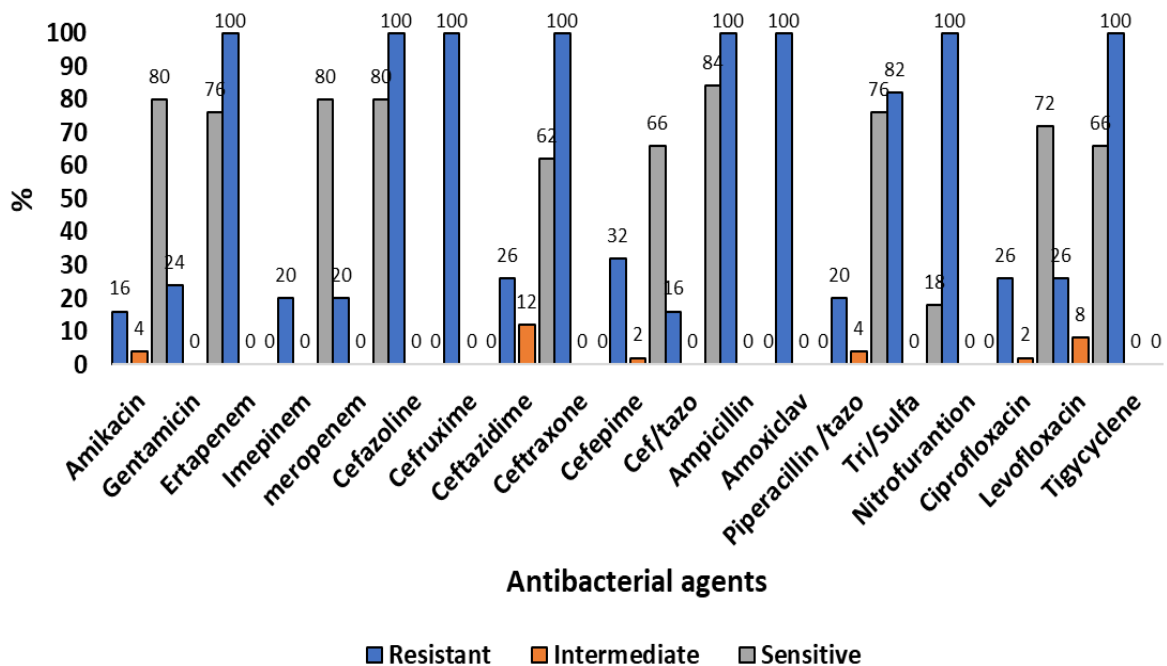

Figure 5: Antimicrobial susceptibility profile of isolated *P. aeruginosa* (n = 50).

Table 2: Multidrug-resistant patterns of isolated *P. aeruginosa*.

Category	*P. aeruginosa* No. (%)	CRPA No. (%)
None-MDR	37 (74)	0 (0)
MDR	5 (10)	2 (20)
XDR	8 (16)	8 (80)
Total	50 (100)	10 (100)

CRPA: Carbapenem-resistant *P. aeruginosa*, MDR: Multi-drug resistance, XDR: Extensively-drug-resistance

3.4. Prevalence Rate of Carbapenem- resistant P. aeruginosa

It has already been shown that 10 (20%) isolates of *P. aeruginosa* are carbapenem-resistant (Figure 5). Genotypic analysis was performed to detect the carbapenemase genes (*bla*$_{IMP-1}$ and *bla*$_{VIM-2}$) in both genomic and plasmid DNA among the 10 CRPA isolates using PCR. The screening for the *bla*$_{VIM-2}$ gene revealed no bands for all isolates, indicating the absence of this carbapenemase determinant in both genomic and plasmid DNA. In contrast, the *bla*$_{IMP-1}$ gene was detected in two (20%) isolates, including isolate no. 3 in the genomic DNA (Figure 6a) and isolate no. 5 in the plasmid DNA **(**Figure 6b**).**

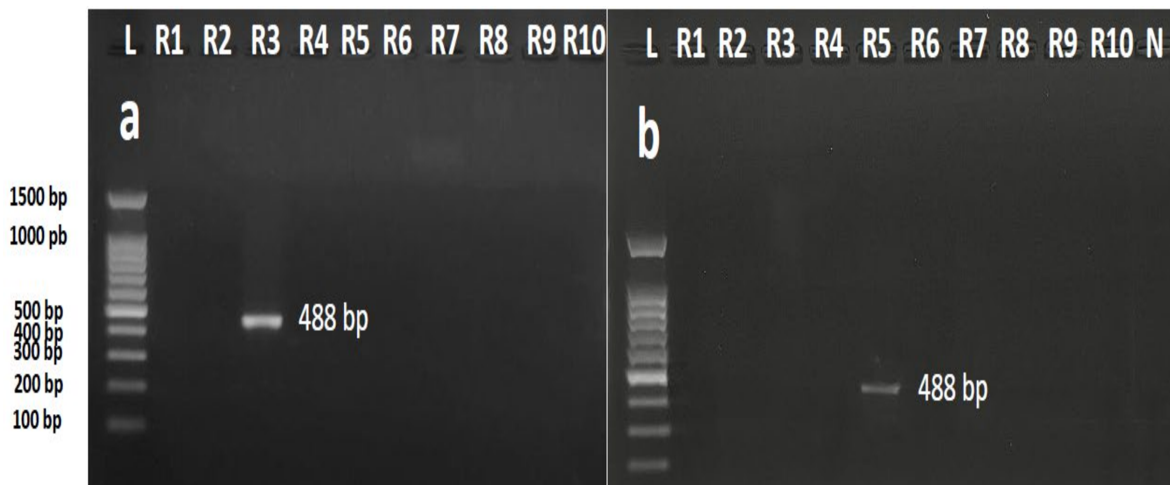

Figure 6: Agarose gel electrophoresis of *bla*$_{IMP-1}$ and *bla*$_{VIM-2}$ genes in the 10 carbapenem-resistant *P. aeruginosa* isolates (Lane: R1 - R10). The PCR band (488 bp) of the *bla*$_{IMP-1}$ gene was detected in isolate no. R 3 in genomic DNA (**a**) and isolate no. R5 in plasmid DNA (**b**). Gel electrophoresis was carried out on 1.5% agarose gels in 1× Tris-acetate-EDTA (TAE) buffer at 100 volts for 40 minutes. N: negative control. L: 100 bp DNA ladder.

To determine whether the other carbapenemase gene candidates (blaKPC, blaNDM, vblaOXA-48, blaGES) are not involved in carbapenem resistance, the mCIM assay was conducted. Out of the 10 CRPA isolates, two (20%) isolates were identified as carbapenemase producers, and the remaining eight (80%) were deemed to be non-carbapenemase producers in the mCIM assay (Figure 7). The mCIM-positive isolates (3 and 5) showed markedly reduced meropenem inhibition zones (≤15 mm, with inner colonies), corresponding to carbapenemase activity. However, the mCIM-negative isolates all produced clear zones ≥19 mm, indicating the absence of detectable carbapenemase activity (Figure 7). The combined phenotypic and genotypic properties of carbapenem-resistant *P. aeruginosa* are summarized in table 3. Based on these findings, the prevalence of CRPA is 20% (10/50) among *P. aeruginosa*-positive ICU patients and 6.6% (10/150) among all ICU patients.

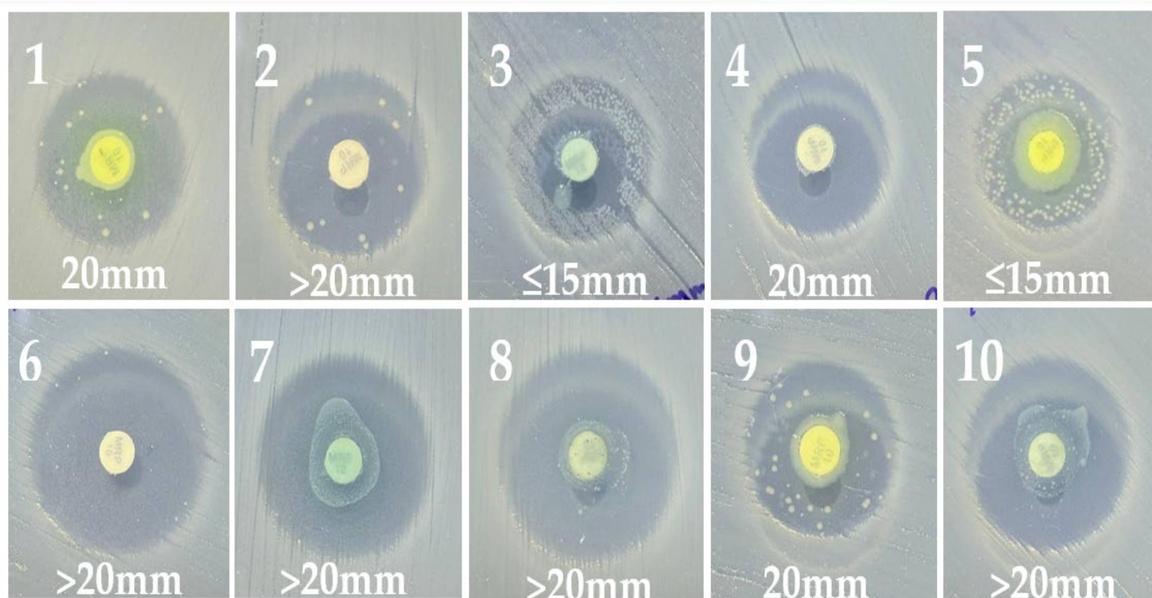

Figure 7: Modified carbapenem inactivation method assay shows the different diameters of the inhibition zone around the meropenem disks. No. 3 and no. 5 isolates of *P. aeruginosa* reduced the efficacy of the meropenem disk (indicating carbapenemase production by said isolates). The rest of the isolates (1, 2, 4, 6, 7, 8, 9, and 10) showed no effect on meropenem activity (indicating no carbapenemase production).

Table 3: Summary of the phenotypic (mCIM assay) and genotypic ($bla_{\text{IMP-1}}$ and $bla_{\text{VIM-2}}$ genes) characteristics of carbapenem-resistant *P. aeruginosa* (n=10).

Isolate No.	Phenotypic analysis	Genotypic analysis			
	mCIM assay (Carbapenemase)	$bla_{\text{VIM-2}}$		$bla_{\text{IMP-1}}$	
		Genomic	Plasmid	Genomic	Plasmid
1	–	–	–	–	–
2	-	–	–	-	–
3	+	–	–	+	–
4	–	–		–	–
5	+	–	–	–	+
6	–	–	–	–	–
7	–	–	–	–	–
8	–	–	–	–	–
9	–	–	–	–	–
10	–	–	–	–	–

(-): Negative, (+): Positive

4. Discussion

In this study, a concerning prevalence rate (33.3%) of *P. aeruginosa* respiratory infections suggests a hyperendemic establishment of *P. aeruginosa* in clinical settings, increasing the risk of hospital-acquired respiratory infections among ventilated ICU patients [23, 24]. Global studies have almost consistently demonstrated different prevalence rates of *P. aeruginosa* respiratory colonization among ICU patients, including 31.8% [25], 17.6% [26], 27.4% [27], 15%-20% [28], and 11.6% [29]. The key risk factors related to acquiring *P. aeruginosa* are immunosuppression, chronic respiratory diseases, prolonged mechanical ventilation, hyperglycemia, tracheostomy, empirical prescription of broad-spectrum antibiotics, and contamination of the hospital environment [30, 31]. Patients admitted for respiratory diseases are more liable to be colonized with *P. aeruginosa* [32]. *P. aeruginosa*-negative patients may have been admitted for other health conditions (e.g., cardiologic or neurological diseases).

The phenotypic and molecular methods were concordant with definitively identifying the *P. aeruginosa* isolates. Routine microbiological and biochemical examinations consistently matched established species characteristics [13, 32]. Subsequent genetic sequencing confirmed these identities, showing high sequence alignment with reference strains in the NCBI database. Phylogenetic analysis further validated these results, yielding robust results with strong support from the main branch (100%) and moderate support for the subgroup (72%). Interestingly, all local isolates clustered closely with *P. aeruginosa* strain PX457771.1, a clinical variant originally isolated from nasal discharge in Pakistan. Taken together, the congruent phenotypic, genotypic, and phylogenetic data provides unequivocal confirmation of *P. aeruginosa* within the target population.

The findings of antimicrobial susceptibility testing highlight a concerning epidemiological trend in the prevalence of drug-resistant *P. aeruginosa*. The emergence of an XDR phenotype alongside MDR strains greatly restricts the clinical armamentarium [33]. In the present study, the high prevalence of resistance among critical antimicrobial classes highlights a significant therapeutic challenge. The isolates demonstrated pronounced nonsusceptibility to several conventional agents, with universal resistance (100%) observed against both nitrofurantoin and tigecycline, and high resistance rates (82%) noted for trimethoprim/sulfamethoxazole. This aligns with the pathogen's notoriously resilient nature, which is often driven by a combination of low outer membrane permeability, the expression of efflux pumps, and chromosomal mutations [34]. Despite these high resistance rates, targeted therapeutic options remain. Favorable susceptibility profiles were observed for aminoglycosides such as amikacin (only 16% resistance), carbapenems including meropenem and imipenem (20% resistance), and certain fluoroquinolones like levofloxacin and ciprofloxacin (26% resistance). While these lower resistance rates point to avenues remaining for targeted empirical therapy, the broad-spectrum attrition observed across other β-lactams indicates a constrained capacity for managing empirical pseudomonal infections [35].

Of particular clinical gravity is the escalating crisis of CRPA. Although only 20% of the overall isolates exhibited resistance to carbapenems, the data reveal a dangerous cascading effect: within this CRPA subset, the phenotypic expression is highly concentrated toward extreme drug resistance, comprised of 20% MDR and 80% XDR isolates. The disproportionate clustering of MDR and XDR profiles within the CRPA group acts as an early sentinel event. It serves as an alarming epidemiological alert for the potential dissemination of pan-resistant, nosocomial strains within the healthcare setting, which may ultimately become virtually untreatable by currently available therapeutics [36]. Similarly, a study in Iran reported a high prevalence (70%) of carbapenem resistance, including 40% MDR and 50% XDR among *P. aeruginosa* isolates [37]. Other studies have reported a low prevalence (2.2% and 3.5%) of CRPA isolates in Turkey [38, 39]. It could be difficult to compare the results of wide-scale studies with one another because of the variations in patient management procedures and infection control measures. The most recent studies exhibited that the prevalence of CRPA ranged from 18% to 35% in ICUs, exceeding 50% in certain regions or units [8, 40-44]. Thus, the antibiotic selective pressure is a key factor behind the emergence of drug-resistant bacteria. This limits the available treatment options and proposes the need for novel or combination therapies.

In addition, several studies from the Middle East demonstrated the high prevalence of metallo β-lactamase encoding genes, predominantly *bla*$_{IMP-1}$ and *bla*$_{VIM-2}$, among CRPA in ICUs where the CRPA strains are highly related to ventilator-associated pneumonia [40, 45-47]. Notably, out of the 10 CRPA isolates, only two exhibited phenotypical (mCIM assay +ve) and genotypical (*bla*$_{IMP-1}$ gene +ve) evidence of carbapenemase production, suggesting that other CRPA isolates likely harbor alternative resistance mechanisms. For example, downregulated OprD porin, overexpressed efflux pumps, and AmpC β-lactamase activity [48]. Other studies also observed that the majority of CRPA lacked carbapenemase genes and instead exhibited *oprD* gene downregulation as the principal mechanism [49, 50]. Similarly, a recent study concluded that there was a low occurrence (22%) of carbapenem-resistant genes in CRPA isolates in Turkey [51]. Carbapenemase-encoding genes were infrequently detected in *P. aeruginosa* isolates in Riyadh, Saudi Arabia, and it was reported that carbapenem resistance is predominantly mediated by intrinsic resistance mechanisms, such as AmpC *β*-lactamases, efflux pump overexpression, and permeability defects [52].

This study reports that the resistance of the CRPA isolates is directly attributable to metallo-β-lactamase, encoded by the *bla*$_{IMP-1}$ gene, located on both chromosomal and plasmid DNA. The *bla*$_{IMP-1}$ gene is a globally prominent carbapenemase-encoding gene, providing resistance against carbapenem in *P. aeruginosa*. The gene is usually a part of class-1 integron in plasmids [6]. From an epidemiologic perspective, the presence of plasmid-borne *bla*$_{IMP-1}$ suggests a risk of rapid horizontal dissemination, warranting stringent infection control. The absence of the *bla*$_{VIM-2}$ gene among our CRPA isolates suggests the lack of this genotype in Sulaymaniyah ICU settings. A study in Baghdad was alarmed by the high prevalence of CRPA isolates (26.6%) harboring the *bla*$_{IMP}$ gene in respiratory samples from ICU patients [53]. In contrast, studies across Turkey have reported the detection of different variants of the *bla*$_{VIM}$ gene, including *bla*$_{VIM-1}$, *bla*$_{VIM-2}$, and *bla*$_{VIM-5}$, among carbapenemase-producing *P. aeruginosa* [38, 51, 54]. Although the results of the mCIM assay confirmed the lack of carbapenemase activities in 8 of the CRPA isolates, the roles of other carbapenemase-encoding genes (*bla*$_{KPC}$, *bla*$_{NDM}$, v*bla*$_{OXA-48}$, *bla*$_{GES}$) [54, 55] cannot be ruled out and have been suggested for future studies. Each CRPA isolate requires comprehensive genetic characterization to understand the precise mechanisms of drug resistance. Collectively, these findings demonstrated a 20% prevalence rate of CRPA among *P. aeruginosa* positive patients and 6.6% among the total patients at the ICU over the study period. Of these, only 20% of the CRPA depended on IMP carbapenemase (metallo-β-lactamase), while 80% possibly utilized other mechanisms of resistance. The limitations of the study include the small sample size due to limited access to ICUs, screening for only two carbapenemase-encoding genes, and the unreliability of the automated system used for determining colistin susceptibility.

5. Conclusions

This study presents evidence of the concerning prevalence rate of *P. aeruginosa* and its carbapenem-resistant strains among ventilated patients in Sulaymaniyah ICU settings. The *P. aeruginosa* isolates exhibited different patterns of MDR and XDR, including carbapenem-resistant strains. Carbapenem resistance is mediated through a carbapenemase, encoded by the bla_{IMP-1} gene, while other resistance mechanisms (reduced OprD porin, enhanced efflux pump, and AmpC production) may be exploited by CRPA isolates. Intensive epidemiological investigations, including routine carbenemase screening, antibiotic stewardship, contact precautions, and environmental control measures, are fundamental for guiding therapeutic management and controlling the circulation of high-risk Pseudomonal infections in clinical settings.

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References

[1] Z. Pang, R. Raudonis, B. R. Glick, T. J. Lin, and Z. Cheng, "Antibiotic resistance in *Pseudomonas aeruginosa*: Mechanisms and alternative therapeutic strategies," *Biotechnology Advances,* vol. 37, no. 1, pp. 177–192, Jan. 2019, doi: 10.1016/j.biotechadv.2018.11.013.

[2] D. H. Halat and C. A. Moubareck, "The intriguing carbapenemases of *Pseudomonas aeruginosa*: current status, genetic profile, and global epidemiology," *Yale Journal of Biology and Medicine*, vol. 95, no. 4, pp. 507–515, Dec. 2022. [Online]: Available: https://pdfs.semanticscholar.org/8946/7bb013439c3fe971250d65235cdf303dd393.pdf. [Accessed Feb. 2, 2026].

[3] D. M. Livermore, "Multiple mechanisms of antimicrobial Resistance in *Pseudomonas aeruginosa*: Our worst nightmare?," *Clinical Infectious Diseases*, vol. 34, no. 5, pp. 634–640, Mar. 2002, doi: 10.1086/338782.

[4] E.-J. Yoon and S. H. Jeong, "Mobile carbapenemase genes in *Pseudomonas aeruginosa*," *Frontiers in Microbiology*, vol. 12, Feb. 2021, doi: 10.3389/fmicb. 2021.614058.

[5] M. S. Walters *et al.*, "Carbapenem-resistant *Pseudomonas aeruginosa* at US emerging infections program sites, 2015," *Emerging Infectious Diseases*, vol. 25, no. 7, pp. 1281–1288, Jul. 2019, doi: 10.3201/eid2507.181200.

[6] A. Elfadadny *et al.,* ''Antimicrobial resistance of *Pseudomonas aeruginosa*: navigating clinical impacts, current resistance trends, and innovations in breaking therapies,'' *Frontiers in Microbiology*, vol. 15:1374466, Apr. 2024, doi: 10.3389/fmicb.2024.1374466.

[7] M. R. Rao, P. Chandrashaker, R. P. Mahale, S. G. Shivappa, R. S. Gowda, and V. B. Chitharagi, "Detection of carbapenemase production in *Enterobacteriaceae* and Pseudomonas species by carbapenemase Nordmann–Poirel test," *Journal of Laboratory Physicians*, vol. 11, no. 02, pp. 107–110, Apr. 2019, doi: 10.4103/JLP.JLP_132_18.

[8] T. Ramatla *et al.*, "Global prevalence and antibiotic resistance profiles of carbapenem-resistant *Pseudomonas aeruginosa* reported from 2014 to 2024: a systematic review and meta-analysis," *Frontiers in Microbiology*, vol. 16, Jul. 2025, doi: 10.3389/fmicb.2025.1599070.

[9] M. T. Arowolo *et al.*, "Prevalence of carbapenem resistance in *Acinetobacter baumannii* and *Pseudomonas aeruginosa* in sub-Saharan Africa: A systematic review and meta-analysis," *PLOS ONE*, vol. 18, no. 11, Article e0287762, Nov. 2023, doi: 10.1371/journal.pone.0287762.

[10] M. G. Wang *et al.*, "Retrospective data insight into the global distribution of carbapenemase-producing *Pseudomonas aeruginosa*," *Antibiotics*, vol. 10, no. 5, p. 548, May 2021, doi: 10.3390/antibiotics10050548.

[11] N. Verma, A. Prahraj, B. Mishra, B. Behera, and K. Gupta, "Detection of carbapenemase-producing *Pseudomonas aeruginosa* by phenotypic and genotypic methods in a tertiary care hospital of East India," *Journal of Laboratory Physicians*, vol. 11, no. 4, pp. 287–291, Oct. 2019, doi: 10.4103/JLP.JLP_136_19.

[12] G. Asokan, T. Ramadhan, E. Ahmed, and H. Sanad, "WHO global priority pathogens list: A bibliometric analysis of medline-pubMed for knowledge mobilization to infection prevention and control practices in Bahrain," *Oman Medical Journal*, vol. 34, no. 3, pp. 184–193, May 2019, doi: 10.5001/omj.2019.37.

[13] Patricia M. Tille, *Bailey & Scott's Diagnostic Microbiology*, 14th ed. St. Louis, MO, USA: Elsevier, 2017. [Online]. Available: https://shop.elsevier.com/books/bailey-and-scotts-diagnostic-microbiology/tille/978-0-323-35482-0. Accessed: Feb. 3, 2026.

[14] R. M. Satokari, E. E. Vaughan, A. D. L. Akkermans, M. Saarela, and W. M. de Vos, "Bifidobacterial diversity in human feces detected by genus-specific PCR and denaturing gradient gel electrophoresis," *Applied and Environmental Microbiology*, vol. 67, no. 2, pp. 504–513, Feb. 2001, doi: 10.1128/AEM.67.2.504-513.2001.

[15] S. Kumar, G. Stecher, M. Suleski, M. D. Sanderford, S. Sharma, and K. Tamura, "MEGA12: Molecular evolutionary genetics analysis version 12 for adaptive and green computing," *Molecular Biology and Evolution*, vol. 41, no. 12, msae263, Dec. 2024, doi: 10.1093/molbev/msae263.

[16] F. Cosentino, P. Viale, and M. Giannella, "MDR/XDR/PDR or DTR? Which definition best fits the resistance profile of *Pseudomonas aeruginosa*?" *Current Opinion in Infectious Diseases*, vol. 36, no. 6, pp. 564–571, Dec. 2023, doi: 10.1097/QCO.0000000000000966.

[17] V. M. Pierce *et al.*, "Modified carbapenem inactivation method for phenotypic detection of carbapenemase production among *Enterobacteriaceae*," *Journal of Clinical Microbiology*, vol. 55, no. 8, pp. 2321–2333, Aug. 2017, doi: 10.1128/JCM.00193-17.

[18] M. Beig, M. Taheri, and M. R. Arabestani, "Comparison of different phenotypic tests versus PCR in the detection of carbapenemase-producing *Pseudomonas aeruginosa* isolates in Hamadan, Iran," *International Journal of Microbiology*, vol. 2021, pp. 1–8, Jul. 2021, doi: 10.1155/2021/5582615.

[19] Y. M. Tsai, S. Wang, H. C. Chiu, C. Y. Kao, and L. L. Wen, "Combination of mCIM and eCIM for detection of carbapenemase-producing *Enterobacteriaceae*," *BMC Microbiology*, vol. 20, no. 1, p. 315, Dec. 2020, doi: 10.1186/s12866-020-02010-3.

[20] K. Hu *et al.*, "Antibiotic susceptibility and molecular analyses of clinical *Enterobacter cloacae* isolates in Eastern Heilongjiang Province, China," *Annals of Palliative Medicine*, vol. 9, no. 3, pp. 1211–1219, May 2020, doi: 10.21037/apm-20-1089.

[21] F. Meimani, R. Nazari, and M. N. Rostami, "Detection of metallo-beta-lactamase genes among *Pseudomonas aeruginosa*," *Zahedan Journal of Research in Medical Sciences*, vol. 20, no. 9, Article e59559, 2018, doi: 10.5812/zjrms.59559.

[22] B. C. Jeon *et al.*, ''Investigation of a nosocomial outbreak of imipenem-resistant *Acinetobacter baumannii* producing the OXA-23 beta-lactamase in Korea,'' *Journal of Clinical Microbiology*, vol. 43, no. 5, pp. 2241-2245, May 2005, doi: 10.1128/JCM.43.5.2241-2245.2005.

[23] Y. Migiyama *et al.*, "Airway Pseudomonas aeruginosa density in mechanically ventilated patients: clinical impact and relation to therapeutic efficacy of antibiotics," *Critical Care*, vol. 25, no. 1, Article 59, Feb. 2021, doi: 10.1186/s13054-021-03488-7.

[24] Ritu, S. S. Prasad, and A. Lal, "Clinical and microbiological profile of ventilator-associated pneumonia in ICU patients," *International Journal of Pharmaceutical Quality Assurance*, vol. 16, no. 11, pp. 416–421, 2025, doi: 10.25258/ijpqa.16.11.66.

[25] H. Hu, H. Huang, Q. Peng, J. Lu, and X. Lei, "Prospective study of colonization and infection because of *Pseudomonas aeruginosa* in mechanically ventilated patients at a neonatal intensive care unit in China," *American Journal of Infection Control*, vol. 38, no. 9, pp. 746–750, Nov. 2010, doi: 10.1016/j.ajic.2010.02.012.

[26] R. Sobala *et al.*, "An observational study of *Pseudomonas aeruginosa* in adult long-term ventilation," *European Respiratory Journal Open Research*, vol. 8, no. 2, pp. 00687–02021, Apr. 2022, doi: 10.1183/23120541.00687-2021.

[27] P. Mergulhão *et al.*, "Epidemiology and burden of ventilator-associated pneumonia among adult intensive care unit patients: A Portuguese, multicenter, retrospective study (eVAP-PT study)," *Antibiotics*, vol. 13, no. 4, p. 290, Mar. 2024, doi: 10.3390/antibiotics13040290.

[28] F. Howroyd *et al.*, "Ventilator-associated pneumonia: pathobiological heterogeneity and diagnostic challenges," *Nature Communications*, vol. 15, Article 6447, 2024, doi: 10.1038/s41467-024-50805-z.

[29] A. D. Harris *et al.*, "*Pseudomonas aeruginosa* colonization in the intensive care unit: Prevalence, risk factors, and clinical outcomes," *Infection Control and Hospital Epidemiology,* vol. 37, no. 5, pp. 544–548, May 2016, doi: 10.1017/ice.2015.346.

[30] S. Hoang *et al.*, ''Risk factors for colonization and infection by *Pseudomonas aeruginosa* in patients hospitalized in intensive care units in France,'' *PLOS ONE*, Vol. 13, no. 3, Article e0193300, Mar. 2018, doi: 10.1371/journal.pone.0193300.

[31] A. Boyer *et al.*, "*Pseudomonas aeruginosa* acquisition on an intensive care unit: relationship between antibiotic selective pressure and patients' environment," *Critical Care*, vol. 15, no. 1, p. R55, Feb. 2011, doi: 10.1186/cc10026.

[32] P. R. Murray, K. S. Rosenthal, and M. A. Pfaller, *Medical Microbiology*, 9th ed. Philadelphia, PA, USA: Elsevier, 2020.

[33] M. Bassetti, A. Vena, A. Croxatto, E. Righi, and B. Guery, "How to manage Pseudomonas aeruginosa infections," *Drugs in Context*, vol. 7, pp. 1–18, May 2018, doi: 10.7573/dic.212527.

[34] N. G. Naga, D. E. El-Badan, M. E. M. Mabrouk, H. S. Rateb, K. M. Ghanem, and M. I. Shaaban, "Innovative application of ceftriaxone as a quorum-sensing inhibitor in *Pseudomonas aeruginosa*," *Scientific Reports*, vol. 15, Article 5022, 2025, doi: 10.1038/s41598-025-87609-0.

[35] A. Karruli *et al.*, "Evidence-based treatment of *Pseudomonas aeruginosa* infections: a critical reappraisal," *Antibiotics*, vol. 12, no. 2, Article 399, 2023, doi: 10.3390/antibiotics12020399.

[36] R. Itani *et al.*, "Difficult-to-treat resistant *Pseudomonas aeruginosa* infections in Lebanese hospitals: impact on mortality and the role of initial antibiotic therapy," *PLOS ONE*, vol. 20, no. 5, Article e0321935, May 2025, doi: 10.1371/journal.pone.0321935.

[37] A. D. Khosravi, S. Taee, A. A. Dezfuli, H. Meghdadi, and F. Shafie, "Investigation of the prevalence of genes conferring resistance to carbapenems in *Pseudomonas aeruginosa* isolates from burn patients," *Infection and Drug Resistance*, vol. 12, pp. 1153–1159, May 2019, doi: 10.2147/IDR.S197752.

[38] Z. K. Çekin *et al.*, "Carbapenemase -producing *Pseudomonas aeruginosa* isolates from Turkey: first report of *P. aeruginosa* high-risk clones with VIM-5– and IMP-7–type carbapenemases in a tertiary hospital," *Diagnostic Microbiology and Infectious Disease*, vol. 99, no. 1, p. 115174, Jan. 2021, doi: 10.1016/j.diagmicrobio.2020.115174.

[39] G. Malkoçoğlu, E. Aktaş, B. Bayraktar, B. Otlu, and M. E. Bulut, "VIM-1, VIM-2, and GES-5 carbapenemases among *Pseudomonas aeruginosa* isolates at a tertiary hospital in Istanbul, Turkey," *Microbial Drug Resistance*, vol. 23, no. 3, pp. 328–334, Apr. 2017, doi: 10.1089/mdr.2016.0012.

[40] M. Saravanan, M. A. Belete, and J. Arockiaraj, "Carbapenem-resistant *Pseudomonas aeruginosa* in intensive care units increase mortality as an emerging global threat," *International Journal of Surgery*, vol. 109, no. 4, pp. 1034–1036, Apr. 2023, doi: 10.1097/JS9.0000000000000184.

[41] J. P. Horcajada *et al.*, "Current and future options for the treatment of serious infections due to carbapenem-resistant *Pseudomonas aeruginosa*," *Clinical Microbiology Reviews*, vol. 38, no. 4, Dec. 2025, doi: 10.1128/cmr.00233-24.

[42] P. Saengsuwan, N. Kositpantawong, S. Kawila, W. Patugkaro, and C. Romyasamit, "Prevalence of carbapenemase genes among multidrug-resistant *Pseudomonas aeruginosa* isolates from tertiary care centers in Southern Thailand," *Saudi Medical Journal*, vol. 43, no. 9, pp. 991–999, Sep. 2022, doi: 10.15537/smj.2022.43.9.20220219.

[43] G. De Pascale *et al.*, "Incidence of hospital-acquired infections due to carbapenem-resistant *Enterobacterales* and *Pseudomonas aeruginosa* in critically ill patients in Italy: a multicentre prospective cohort study," *Critical Care*, vol. 29, no. 1, p. 32, Jan. 2025, doi: 10.1186/s13054-025-05266-1.

[44] W. Huang, X. Wei, G. Xu, X. Zhang, and X. Wang, "Carbapenem-resistant *Pseudomonas aeruginosa* infections in critically ill children: Prevalence, risk factors, and impact on outcome in a large tertiary pediatric hospital of China," *Frontiers in Public Health,* vol. 11, Article 1088262, Feb. 2023, doi: 10.3389/fpubh.2023.1088262.

[45] M. Al-Orphaly *et al.*, "Epidemiology of multidrug-resistant *Pseudomonas aeruginosa* in the Middle East and North Africa region," *mSphere*, vol. 6, no. 3, Jun. 2021, doi: 10.1128/mSphere.00202-21.

[46] S. Baban, "Molecular detection of carbapenemase-producing *Pseudomonas aeruginosa* isolated from intensive care units of surgical specialty hospital in Erbil city," *Medical Journal of Babylon*, vol. 17, no. 2, p. 185, 2020, doi: 10.4103/MJBL.MJBL_24_20.

[47] M. E. Hassan *et al.*, "Causative bacteria of ventilator-associated pneumonia in intensive care unit in Bahrain: Prevalence and antibiotics susceptibility pattern," *World Journal of Critical Care Medicine*, vol. 12, no. 3, pp. 165–175, Jun. 2023, doi: 10.5492/wjccm.v12.i3.165.

[48] T. Akter, S. Islam, A. M. Haider, K. Fatema, F. Stapleton, and M. Willcox, "Antibiotic resistance mechanisms and global resistance patterns of *Pseudomonas aeruginosa* in microbial keratitis," *European Journal of Clinical Microbiology & Infectious Diseases*, Mar. 2026, doi: 10.1007/s10096-026-05484-7.

[49] M. Wang, Y. Zhang, F. Pei, Y. Liu, and Y. Zheng, "Loss of OprD function is sufficient for carbapenem-resistance-only but insufficient for multidrug resistance in *Pseudomonas aeruginosa*," *BMC Microbiology*, vol. 25, no. 1, Article 218, Apr. 2025, doi: 10.1186/s12866-025-03935-3.

[50] L. Yin *et al.*, "Non-carbapenem-producing carbapenem-resistant *Pseudomonas aeruginosa* in children: Risk factors, molecular epidemiology, and resistance mechanism," *Journal of Infection and Public Health*, vol. 18, no. 2, Article 102634, Feb. 2025, doi: 10.1016/j.jiph.2024.102634.

[51] Z. Kalaycı Çekin, E. S. Tanrıverdi, and B. Otlu, "Investigation of carbapenemase-producing *Pseudomonas aeruginosa* at secondary care hospital in Bolu, Turkey," *Microbial Drug Resistance*, vol. 30, no. 11, pp. 450–457, Nov. 2024, doi: 10.1089/mdr.2024.0067.

[52] H. O. Alshammari, A. M. Albarrag, I. M. Moussa, J. A. Al-Tawfiq, S. A. Aldakeel, and A. M. Somily, "Molecular analysis of carbapenemase-producing *Pseudomonas aeruginosa* strains in a tertiary care hospital in Riyadh," *Journal of Infection and Public Health*, vol. 19, no. 4, p. 103159, Apr. 2026, doi: 10.1016/j.jiph.2026.103159.

[53] H. A. Aal Owaif, M. K. Aldulaimy, and S. A. Abdulateef, ''The Antibiotic resistance genes blaSHV, blaOXA-48, blaTEM and blaIMP in Pseudomonas aeruginosa isolated from respiratory tract infections in Baghdad, Iraq,'' *International Journal of Biomedicine*, vol. 13, no. 4, pp. 341-344, Dec. 2023, doi:10.21103/Article13(4)_OA18.

[54] S. Mohamed *et al.,* "Co-occurrence of carbapenemase genes blaNDM, blaVIM, blaKPC, and blaOXA-48 in *Pseudomonas aeruginosa* clinical isolates," *International Journal of Biomedicine*, vol. 13, no. 3, pp. 123–126, Sep. 2023, doi: 10.21103/Article13(3)_OA12.

[55] K. Zhu, S. Li, Z. Guo, Ch. Xiao, and Q. Wei, "Antimicrobial resistance mechanisms in carbapenem-resistant *Pseudomonas aeruginosa* clinical strains isolated in Shanghai, China," *Polish Journal of Microbiology*, vol. 74, no. 3, pp. 275–288, Sep. 2025, doi: 10.33073/pjm-2025-022.