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Original article

Phenotypic and Molecular Detection of Metallo-β-Lactamase Genes and Antimicrobial Resistance Profiles among Clinical *Pseudomonas aeruginosa* Isolates from Intensive Care Unit Patients in Misurata, Libya

Mohanned Alwashaish[1]*, Marwa Attayeb[2], Fauzia Abuhtna[2], Mansor Wafi[2], Awatif Almaqrahi[3], Budour Elmihub[2]

[1]*Biomedical Science Department, Faculty of Pharmacy, Misurata University, Misurata, Libya.*
[2]*Department of Microbiology, Libyan Biotechnology Research Center, Tripoli, Libya*
[3]*Department of Environmental, Food and Biological Applications, Libyan Biotechnology Research Center, Tripoli, Libya*
Corresponding Email. *Mohanad.aloshesh@phar.misuratau.edu.ly*

Abstract
Pseudomonas aeruginosa is an important cause of healthcare-associated infections in intensive care units (ICUs), where invasive devices and antimicrobial exposure promote multidrug resistance. This prospective cross-sectional study assessed antimicrobial resistance, phenotypic metallo-β-lactamase (MBL) production, and MBL-encoding genes among 83 non-duplicate clinical *P. aeruginosa* isolates recovered from ICU patients at Misurata Medical Centre, Libya, from September 2025 to March 2026. Antimicrobial susceptibility testing was performed by disk diffusion. Carbapenem-resistant isolates were screened using the imipenem–EDTA combined-disk method, while all isolates were tested by PCR for *blaVIM*, *blaIMP*, *blaNDM*, and *blaSPM*. Carbapenem resistance and multidrug resistance were detected in 50.6% and 59.0% of isolates, respectively. Respiratory specimens were the predominant source (56.6%). Phenotypic MBL production was detected in 71.4% of carbapenem-resistant isolates, whereas targeted MBL genes were identified in 32.5% of all isolates. *blaVIM* was the predominant gene (19.3%), followed by *blaIMP* (10.8%), *blaNDM* (8.4%), and *blaSPM* (1.2%). MBL gene-positive isolates showed significantly greater resistance to carbapenems and other tested antimicrobial agents. Previous carbapenem exposure and mechanical ventilation were independently associated with MBL gene carriage. The phenotypic test showed 92.6% sensitivity and 66.7% specificity compared with PCR. These findings highlight a substantial burden of MBL-associated resistance among ICU-derived *P. aeruginosa* in Misurata and support enhanced carbapenem stewardship, molecular surveillance, and infection-prevention measures.
Keywords. *Pseudomonas aeruginosa*, Metallo-β-lactamase, Carbapenem Resistance, Multidrug Resistance.

Introduction

Pseudomonas aeruginosa is a major opportunistic Gram-negative pathogen responsible for a substantial proportion of healthcare-associated infections, particularly among critically ill patients. It is frequently implicated in ventilator-associated pneumonia, bloodstream infections, urinary tract infections, surgical-site infections, and infections associated with invasive medical devices. Patients admitted to intensive care units (ICUs) are especially vulnerable because of prolonged hospitalization, severe underlying illness, frequent exposure to broad-spectrum antimicrobials, and the extensive use of mechanical ventilation, central venous catheters, and urinary catheters [1,2].

The clinical importance of *P. aeruginosa* is amplified by its remarkable ability to survive under adverse conditions and to develop resistance to multiple antimicrobial agents. This organism possesses intrinsic resistance mechanisms, including low outer-membrane permeability, multidrug efflux systems, and chromosomally encoded β-lactamase activity. In addition, it can acquire further resistance through chromosomal mutations and horizontal transfer of mobile genetic elements carrying antimicrobial-resistance determinants. Consequently, *P. aeruginosa* may exhibit reduced susceptibility to several important therapeutic classes, including antipseudomonal penicillins, cephalosporins, fluoroquinolones, aminoglycosides, and carbapenems [3–5].

Carbapenems have historically represented important therapeutic options for severe infections caused by multidrug-resistant *P. aeruginosa*. However, the increasing emergence of carbapenem-resistant *P. aeruginosa* has substantially narrowed available treatment choices and is associated with adverse clinical outcomes, prolonged hospitalization, and increased healthcare burden. Carbapenem resistance in this organism is multifactorial and may arise through alterations in the OprD porin, overexpression of efflux pumps, increased AmpC β-lactamase activity, and acquisition of carbapenem-hydrolyzing enzymes [4,6]. In recognition of its global public-health importance, *P. aeruginosa* is included in the World Health Organization bacterial priority pathogens list for antimicrobial-resistance research, surveillance, and intervention strategies [7].

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Among acquired mechanisms of carbapenem resistance, metallo-β-lactamases (MBLs) are of particular concern. MBLs are zinc-dependent enzymes belonging to Ambler class B β-lactamases and are capable of hydrolyzing a broad spectrum of β-lactam antibiotics, including carbapenems. The most frequently reported transferable MBL determinants among *P. aeruginosa* isolates include *blaVIM*, *blaIMP*, and *blaNDM*, whereas other genes, such as *blaSPM*, *blaGIM*, and *blaSIM*, have also been described. Their frequent association with integrons, plasmids, and other mobile genetic elements facilitates their dissemination within healthcare settings and contributes to the emergence and persistence of multidrug-resistant and difficult-to-treat strains [8–10].

Molecular surveillance of MBL-encoding genes is important because antimicrobial resistance in *P. aeruginosa* is dynamic and may vary considerably across regions, hospitals, clinical settings, and patient populations. ICU-specific data are particularly valuable because critically ill patients may serve as a high-risk population for colonization and infection with resistant strains, while ICU environments may promote the selection and transmission of antimicrobial-resistant organisms. Although antimicrobial resistance among clinical *P. aeruginosa* isolates has been reported in Misurata [11], evidence focusing specifically on ICU-derived isolates and the distribution of MBL genes remains limited. Therefore, this study aimed to characterize the antimicrobial-resistance profiles of clinical *P. aeruginosa* isolates recovered from ICU patients in Misurata, Libya, and to investigate the occurrence of metallo-β-lactamase production and selected MBL-encoding genes.

Methods

Study Design, Setting, and Study Period

A prospective cross-sectional study was conducted at Misurata Medical Centre, Misurata, Libya, from September 2025 to March 2026. The study included clinical *Pseudomonas aeruginosa* isolates recovered from patients admitted to intensive care units (ICUs). All eligible isolates obtained during the study period were included consecutively.

Study Population and Isolate Selection

Clinical specimens submitted for routine microbiological investigation were included when they yielded *P. aeruginosa* from ICU patients. Specimens included respiratory samples, blood, urine, wound or pus swabs, catheter tips, and other clinically relevant specimens.

Only the first *Pseudomonas aeruginosa* isolate recovered from each patient during the study period was included. Any subsequent isolates recovered from the same patient were excluded regardless of specimen type or antimicrobial susceptibility profile. Environmental isolates, surveillance cultures without clinical indication, and isolates with incomplete clinical or laboratory data were excluded.

Clinical Data Collection

Clinical and demographic data were obtained from patients' medical records using a structured data-collection form. Variables included age, sex, ICU stay before culture collection, mechanical ventilation, central venous catheterization, urinary catheterization, previous antibiotic exposure, previous carbapenem exposure, diabetes mellitus, chronic kidney disease, malignancy or immunosuppression, and in-hospital mortality.

Previous antibiotic exposure was defined as receipt of systemic antimicrobial therapy within 90 days before culture collection. Previous carbapenem exposure was defined as receipt of imipenem, meropenem, or another systemic carbapenem within the same period. Mechanical ventilation, central venous catheterization, and urinary catheterization were recorded when present before or at the time of specimen collection.

Isolation and Identification of Pseudomonas aeruginosa

Clinical specimens were processed according to routine microbiological procedures. Specimens were inoculated onto blood agar and MacConkey agar plates and incubated aerobically at 37 ± 2°C for 24 h.

Presumptive identification of *P. aeruginosa* was based on colony morphology, characteristic pigment production, oxidase positivity and Gram-stain morphology. Final identification was confirmed using the API 20 NE identification system (bioMérieux, Marcy-l'Étoile, France), according to the manufacturer's instructions.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was performed by the Kirby–Bauer disk diffusion method on Mueller–Hinton agar using a standardized 0.5 McFarland bacterial suspension. Plates were incubated aerobically at 37 ± 2°C for 18–24 h. Zone diameters were measured in millimetres and interpreted as susceptible, intermediate, or resistant according to the Clinical and Laboratory Standards Institute (CLSI) M100 criteria [12]. For the classification of multidrug resistance, isolates categorized as intermediate or resistant

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were considered non-susceptible. Disk diffusion procedures were performed in accordance with CLSI M02 guidelines [13].
The antimicrobial agents tested were piperacillin–tazobactam (100/10 µg), ceftazidime (30 µg), cefepime (30 µg), aztreonam (30 µg), imipenem (10 µg), meropenem (10 µg), amikacin (30 µg), gentamicin (10 µg), ciprofloxacin (5 µg), and levofloxacin (5 µg).
Pseudomonas aeruginosa ATCC 27853 was used as the quality-control strain for antimicrobial susceptibility testing.
Carbapenem resistance was defined as resistance to imipenem and/or meropenem according to CLSI interpretive criteria [12]. Multidrug resistance was defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories, according to the international criteria proposed by Magiorakos et al. [14].

Phenotypic Detection of Metallo-ß-Lactamase Production
Phenotypic screening for MBL production was performed only among carbapenem-resistant isolates using the imipenem–EDTA combined-disk method described by Yong et al. [15]. An increase of ≥7 mm in the inhibition-zone diameter around the imipenem–EDTA disk compared with imipenem alone was interpreted as phenotypic evidence of MBL production [15].

DNA Extraction and Molecular Detection of MBL-Encoding Genes
Genomic DNA extraction, PCR amplification, and gel electrophoresis were performed. Genomic DNA was extracted from overnight cultures of confirmed *Pseudomonas aeruginosa* isolates using the DNeasy Blood & Tissue Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions for Gram-negative bacteria. Extracted DNA was stored at −20°C until PCR amplification.
All included isolates were screened by conventional PCR for the presence of *blaVIM*, *blaIMP*, *blaNDM*, and *blaSPM*. Primer sequences for detection of the targeted MBL-encoding genes were obtained from previously published studies [16,17].
Each PCR reaction was prepared in a final volume of 25 µL containing 12.5 µL of 2× GoTaq® Green Master Mix (Promega, Madison, WI, USA), 1 µL of each forward and reverse primer (10 pmol/µL), 2 µL of template DNA, and nuclease-free water to complete the final volume. Amplification was performed with an initial denaturation step at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 30 s, annealing at the gene-specific temperatures shown in (Table 1) for 30 s, and extension at 72°C for 45 s, followed by a final extension at 72°C for 5 min.
PCR products were separated on 1.5% agarose gel prepared in 1× TBE buffer and stained with GelRed® Nucleic Acid Gel Stain (Biotium, Fremont, CA, USA). Bands were visualized under ultraviolet illumination, and a 100-bp DNA ladder was used to estimate amplicon sizes. A no-template control containing nuclease-free water was included in each PCR run to monitor potential contamination.

Table 1. Primer sequences and PCR conditions used for detection of MBL-encoding

Target gene	Primer	Sequence (5′–3′)	Expected amplicon size	Annealing temperature
blaVIM	Forward	GTTTGGTCGCATATCGCAAC	382 bp	55°C
	Reverse	AATGCGCAGCACCAGGATAG		
blaIMP	Forward	GGAATAGAGTGGCTTAAYTCTC	232 bp	52°C
	Reverse	GGTTTAAYAAAACAACCACC		
blaNDM	Forward	GGTTTGGCGATCTGGTTTTC	621 bp	52°C
	Reverse	CGGAATGGCTCATCACGATC		
blaSPM	Forward	AAAATCTGGGTACGCAAACG	271 bp	52°C
	Reverse	ACATTATCCGCTGGAACAGG		

Statistical analysis
Data were analyzed using IBM SPSS Statistics version 26. Continuous variables were assessed for distributional characteristics before analysis. Normally distributed continuous variables were summarized as mean ± standard deviation and compared using the independent-samples t-test. Non-normally distributed continuous variables were summarized as median and interquartile range and compared using the Mann–Whitney *U* test.
Categorical variables were presented as frequencies and percentages. Associations between molecular MBL gene carriage and categorical clinical variables or antimicrobial-resistance phenotypes were assessed using Fisher's exact test. Crude odds ratios with 95% confidence intervals were calculated for relevant binary associations.
The association between specimen category and molecular MBL gene carriage was evaluated using the Fisher–Freeman–Halton exact test. For comparisons involving multiple antimicrobial-resistance outcomes, *P*-values were adjusted using the Benjamini–Hochberg procedure to control the false discovery rate.

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The diagnostic performance of the phenotypic MBL test was assessed against PCR detection of at least one targeted MBL gene as the molecular reference standard. Sensitivity, specificity, positive predictive value, negative predictive value, overall accuracy, and Cohen's kappa coefficient with 95% confidence intervals were calculated. McNemar's exact test was used to assess discordance between phenotypic and molecular results.

Firth penalized logistic regression was performed to identify clinical factors independently associated with molecular MBL gene carriage. Variables with P < 0.20 in univariable analyses and variables considered clinically relevant were evaluated for multivariable modelling. Previous carbapenem exposure, mechanical ventilation, and ICU stay of ≥7 days before culture collection were included in the final model. The 7-day cutoff was selected based on the median ICU stay before culture collection in the study cohort. Carbapenem resistance and MDR phenotype were excluded because they were microbiological outcomes closely related to MBL gene carriage. Two-sided P values <0.05 were considered statistically significant.

Ethical Considerations

Permission to conduct the study was obtained from Misurata Medical Centre. Verbal informed consent was obtained from participants or their legally authorized representatives. Data were anonymized and analyzed without personal identifiers.

Results

Patient Characteristics and Clinical Factors Associated with Molecular MBL Gene Carriage

A total of 83 non-duplicate clinical *Pseudomonas aeruginosa* isolates obtained from ICU patients were included in the analysis. Molecular detection identified at least one targeted MBL-encoding gene in 27 isolates (32.5%), whereas 56 isolates (67.5%) were negative for the targeted genes.

The mean age of the patients was 58.2 ± 16.9 years. Patients with MBL gene-positive isolates had a higher mean age than those with MBL gene-negative isolates; however, the difference was not statistically significant (61.6 ± 15.5 vs. 56.6 ± 17.4 years, $P = 0.214$). Male patients accounted for 62.7% of the study population, with a higher proportion among patients with MBL gene-positive isolates than among those with MBL gene-negative isolates (74.1% vs. 57.1%), although this difference was not statistically significant ($P = 0.154$).

The median ICU stay before culture collection was significantly longer among patients with MBL gene-positive isolates than among those with MBL gene-negative isolates [12 (IQR, 8–18) vs. 7 (IQR, 4–12) days, $P = 0.006$]. Mechanical ventilation was more frequent among patients with MBL gene-positive isolates than among those with MBL gene-negative isolates (85.2% vs. 50.0%; crude OR = 5.75, 95% CI: 1.76–18.79; $P = 0.002$). Previous carbapenem exposure was also significantly more common in the MBL gene-positive group (74.1% vs. 33.9%; crude OR = 5.56, 95% CI: 2.00–15.48; $P = 0.001$). No statistically significant differences were observed for central venous catheterization, urinary catheterization, previous antibiotic exposure, diabetes mellitus, chronic kidney disease, malignancy/immunosuppression, or in-hospital mortality (Table 2).

Table 2. Demographic and Clinical Characteristics of ICU Patients According to Molecular MBL Gene Carriage

Variable		Overall, N = 83	MBL gene-positive, n = 27	MBL gene-negative, n = 56	Crude OR (95% CI)	P-value
Age, years, mean ± SD		58.2 ± 16.9	61.6 ± 15.5	56.6 ± 17.4	—	0.214
Sex	Male	52 (62.7)	20 (74.1)	32 (57.1)	2.14 (0.78–5.89)	0.154
	Female	31 (37.3)	7 (25.9)	24 (42.9)	Reference	—
ICU stay before culture, days, median (IQR)		8 (5–14)	12 (8–18)	7 (4–12)	—	0.006
Mechanical ventilation		51 (61.4)	23 (85.2)	28 (50.0)	5.75 (1.76–18.79)	0.002
Central venous catheter		49 (59.0)	20 (74.1)	29 (51.8)	2.66 (0.97–7.29)	0.061
Urinary catheter		51 (61.4)	18 (66.7)	33 (58.9)	1.39 (0.53–3.64)	0.631
Previous antibiotic exposure		63 (75.9)	24 (88.9)	39 (69.6)	3.49 (0.92–13.17)	0.061
Previous carbapenem exposure		39 (47.0)	20 (74.1)	19 (33.9)	5.56 (2.00–	0.001

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Variable	Overall, N = 83	MBL gene-positive, n = 27	MBL gene-negative, n = 56	Crude OR (95% CI)	P-value
				15.48)	
Diabetes mellitus	26 (31.3)	10 (37.0)	16 (28.6)	1.47 (0.56–3.89)	0.458
Chronic kidney disease	18 (21.7)	7 (25.9)	11 (19.6)	1.43 (0.48–4.23)	0.575
Malignancy/immunosuppression	13 (15.7)	5 (18.5)	8 (14.3)	1.36 (0.40–4.65)	0.749
In-hospital mortality	20 (24.1)	10 (37.0)	10 (17.9)	2.71 (0.96–7.64)	0.098

CI, confidence interval; ICU, intensive care unit; IQR, interquartile range; MBL, metallo-β-lactamase; OR, odds ratio; SD, standard deviation.

Specimen Distribution, Carbapenem Resistance, and Antimicrobial Susceptibility

Respiratory specimens represented the largest proportion of isolates [47/83 (56.6%)], followed by blood [13/83 (15.7%)], wound or other specimens [12/83 (14.5%)], and urine [11/83 (13.3%)]. Carbapenem-resistant isolates accounted for 42/83 (50.6%) of all isolates. The highest proportion of carbapenem-resistant isolates was observed among respiratory specimens [30/47 (63.8%)], followed by blood [5/13 (38.5%)], wound or other specimens [4/12 (33.3%)], and urine specimens [3/11 (27.3%)].

Among the 42 carbapenem-resistant isolates, 30 (71.4%) were phenotypically positive for MBL production. Molecular MBL gene carriage was detected in 21/47 respiratory isolates (44.7%), 3/13 blood isolates (23.1%), 2/12 wound or other isolates (16.7%), and 1/11 urine isolates (9.1%). The association between specimen category and molecular MBL gene carriage was not statistically significant according to the Fisher–Freeman–Halton exact test ($P = 0.058$) (Figure 1).

The overall resistance rate was highest for aztreonam [49/83 (59.0%)], followed by ceftazidime [48/83 (57.8%)], piperacillin–tazobactam [47/83 (56.6%)], cefepime [46/83 (55.4%)], ciprofloxacin [44/83 (53.0%)], and levofloxacin [42/83 (50.6%)]. Resistance to imipenem and meropenem was observed in 38/83 (45.8%) and 36/83 (43.4%) isolates, respectively. Amikacin showed the lowest resistance rate [22/83 (26.5%)], followed by gentamicin [30/83 (36.1%)]. Overall, multidrug-resistant isolates accounted for 49/83 (59.0%) of the study isolates (Figure 2).

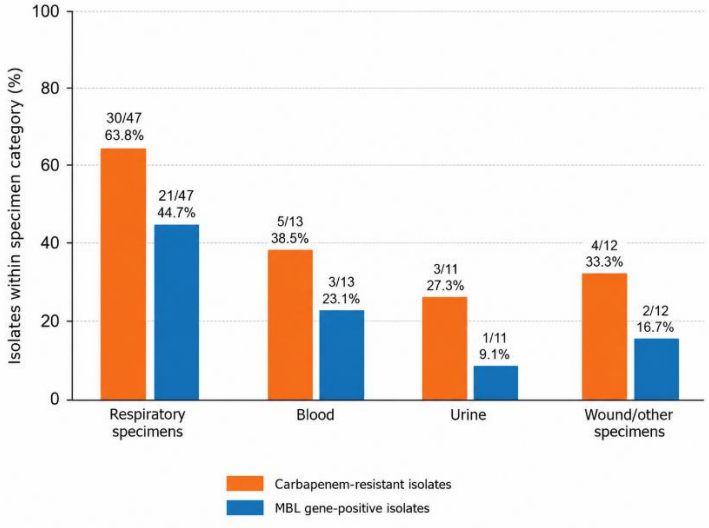

Figure 1. Distribution of carbapenem resistance and molecular MBL gene carriage among clinical Pseudomonas aeruginosa isolates according to specimen category.

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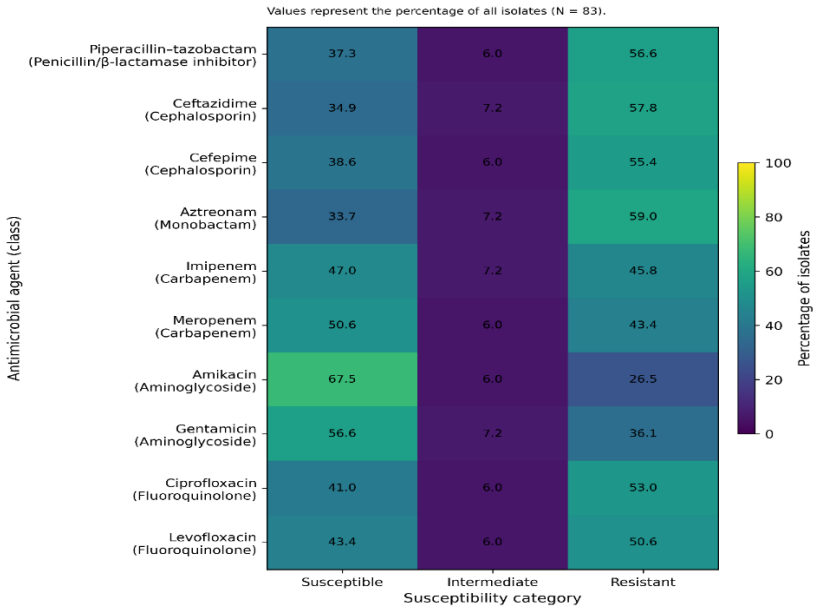

Figure 2. Heatmap showing antimicrobial susceptibility profiles of clinical Pseudomonas aeruginosa isolates recovered from ICU patients. Values represent the percentage of isolates in each susceptibility category.

Association Between Molecular MBL Gene Carriage and Antimicrobial Resistance

MBL gene-positive isolates demonstrated significantly higher resistance rates to the antimicrobial agents included in (Table 3) compared with MBL gene-negative isolates.

Resistance to imipenem was detected in 26/27 (96.3%) MBL gene-positive isolates compared with 12/56 (21.4%) MBL gene-negative isolates (crude OR = 95.33, 95% CI: 11.71–776.06; adjusted $q < 0.001$). Similarly, meropenem resistance was observed in 24/27 (88.9%) MBL gene-positive isolates and 12/56 (21.4%) MBL gene-negative isolates (crude OR = 29.33, 95% CI: 7.53–114.22; adjusted $q < 0.001$).

Resistance to both imipenem and meropenem was reported in 23/27 (85.2%) MBL gene-positive isolates compared with 9/56 (16.1%) MBL gene-negative isolates (crude OR = 30.03, 95% CI: 8.36–107.90; adjusted $q < 0.001$). MBL gene-positive isolates also had significantly higher resistance to ceftazidime, cefepime, piperacillin–tazobactam, ciprofloxacin, amikacin, and gentamicin after Benjamini–Hochberg adjustment for multiple comparisons. The multidrug-resistant phenotype was detected in 26/27 (96.3%) MBL gene-positive isolates and in 23/56 (41.1%) MBL gene-negative isolates (crude OR = 37.30, 95% CI: 4.72–294.77; adjusted $q < 0.001$) (Table 3).

Table 3. Association Between Molecular MBL Gene Carriage and Antimicrobial Resistance Phenotypes

Resistance phenotype	Overall, N = 83 n (%)	MBL gene-positive, n = 27	MBL gene-negative, n = 56	Crude OR (95% CI)	P-value	FDR-adjusted q-value
Imipenem resistant	38 (45.8)	26 (96.3)	12 (21.4)	95.33 (11.71–776.06)	<0.001	<0.001
Meropenem resistant	36 (43.4)	24 (88.9)	12 (21.4)	29.33 (7.53–114.22)	<0.001	<0.001
Resistant to both imipenem and meropenem	32 (38.6)	23 (85.2)	9 (16.1)	30.03 (8.36–107.90)	<0.001	<0.001
Ceftazidime resistant	48 (57.8)	24 (88.9)	24 (42.9)	10.67 (2.87–39.60)	<0.001	<0.001
Cefepime resistant	46 (55.4)	22 (81.5)	24 (42.9)	5.87 (1.94–17.73)	0.001	0.001
Piperacillin–tazobactam resistant	47 (56.6)	24 (88.9)	23 (41.1)	11.48 (3.09–42.67)	<0.001	<0.001

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Resistance phenotype	Overall, N = 83 n (%)	MBL gene-positive, n = 27	MBL gene-negative, n = 56	Crude OR (95% CI)	P-value	FDR-adjusted q-value
Ciprofloxacin resistant	44 (53.0)	23 (85.2)	21 (37.5)	9.58 (2.91–31.55)	<0.001	<0.001
Amikacin resistant	22 (26.5)	12 (44.4)	10 (17.9)	3.68 (1.32–10.22)	0.016	0.016
Gentamicin resistant	30 (36.1)	17 (63.0)	13 (23.2)	5.62 (2.07–15.25)	<0.001	0.001
Multidrug-resistant phenotype	49 (59.0)	26 (96.3)	23 (41.1)	37.30 (4.72–294.77)	<0.001	<0.001

CI, confidence interval; FDR, false discovery rate; MBL, metallo-ß-lactamase; MDR, multidrug-resistant; OR, odds ratio.

Molecular Distribution of MBL-Encoding Genes

As revealed in (Table 4 and Figure 4), mechanistic perturbations revealed distinct antibacterial drivers across. At least one targeted MBL-encoding gene was detected in 27/83 isolates (32.5%). The most frequently identified gene was *blaVIM*, detected in 16/83 isolates (19.3%), representing 59.3% of PCR-positive isolates. This was followed by *blaIMP* in 9/83 isolates (10.8%), *blaNDM* in 7/83 isolates (8.4%), and *blaSPM* in 1/83 isolate (1.2%) (Table 4).

Single-gene carriage was observed in 21 isolates, including 11 isolates carrying *blaVIM* alone, five carrying *blaIMP* alone, four carrying *blaNDM* alone, and one carrying *blaSPM* alone. Co-carriage of MBL genes was identified in six isolates. The *blaVIM* + *blaIMP* combination was detected in three isolates, while *blaVIM* + *blaNDM* and *blaIMP* + *blaNDM* were detected in two and one isolates, respectively (Table 5).

Table 4. Frequency of Individual MBL-Encoding Genes among Clinical Pseudomonas aeruginosa Isolates

Gene detected by PCR	Number of isolates, n	Percentage of all isolates, N = 83	Percentage of PCR-positive isolates, n = 27
blaVIM	16	19.3	59.3
blaIMP	9	10.8	33.3
blaNDM	7	8.4	25.9
blaSPM	1	1.2	3.7
At least one MBL gene detected	27	32.5	100.0
No targeted MBL gene detected	56	67.5	—

MBL, metallo-ß-lactamase; PCR, polymerase chain reaction

Table 5. MBL Gene Co-Occurrence Patterns among Clinical Pseudomonas aeruginosa Isolates

Molecular pattern	Number of isolates, n	Percentage of all isolates, N = 83
blaVIM only	11	13.3
blaIMP only	5	6.0
blaNDM only	4	4.8
blaSPM only	1	1.2
blaVIM + *blaIMP*	3	3.6
blaVIM + *blaNDM*	2	2.4
blaIMP + *blaNDM*	1	1.2
No targeted MBL gene detected	56	67.5
Total	83	100.0

MBL, metallo-ß-lactamase; PCR, polymerase chain reaction

Performance of Phenotypic MBL Detection

Among the 42 carbapenem-resistant isolates, phenotypic MBL detection was positive in 30 isolates and negative in 12 isolates. PCR detected at least one targeted MBL gene in 27 isolates. Twenty-five isolates were positive by both phenotypic testing and PCR, whereas 10 isolates were negative by both methods.

Using PCR detection of the selected targeted MBL genes as the reference standard, the phenotypic test demonstrated a sensitivity of 92.6% (95% CI: 76.6–97.9%), specificity of 66.7% (95% CI: 41.7–84.8%), positive predictive value of 83.3% (95% CI: 66.4–92.7%), negative predictive value of 83.3% (95% CI: 55.2–

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95.3%), and overall accuracy of 83.3% (95% CI: 69.4–91.7%). The Cohen's kappa coefficient was 0.62 (95% CI: 0.34–0.85). McNemar's exact test showed no statistically significant discordance between phenotypic and molecular results (P = 0.453) (Table 6).

Table 6. Performance of Phenotypic MBL Detection Compared with Molecular Detection of Targeted MBL Genes among Carbapenem-Resistant Isolates

Phenotypic MBL result	PCR MBL-positive	PCR MBL-negative	Total
Positive	25	5	30
Negative	2	10	12
Total	27	15	42

Diagnostic performance measure	Estimate %	95% CI
Sensitivity	92.6	76.6–97.9
Specificity	66.7	41.7–84.8
Positive predictive value	83.3	66.4–92.7
Negative predictive value	83.3	55.2–95.3
Overall accuracy	83.3	69.4–91.7
Cohen's kappa coefficient	0.62	0.34–0.85

CI, confidence interval; MBL, metallo-β-lactamase; NPV, negative predictive value; PCR, polymerase chain reaction; PPV, positive predictive value.

Multivariable Analysis of Clinical Factors Associated with MBL Gene Carriage

In the Firth penalized logistic regression model, previous carbapenem exposure remained independently associated with molecular MBL gene carriage (adjusted OR = 3.76, 95% CI: 1.23–11.52; P = 0.020). Mechanical ventilation was also independently associated with MBL gene carriage (adjusted OR = 3.29, 95% CI: 1.02–10.59; P = 0.046). ICU stay of at least 7 days before culture collection was not statistically significant in the adjusted model (adjusted OR = 2.74, 95% CI: 0.87–8.65; P = 0.085) (Figure 3).

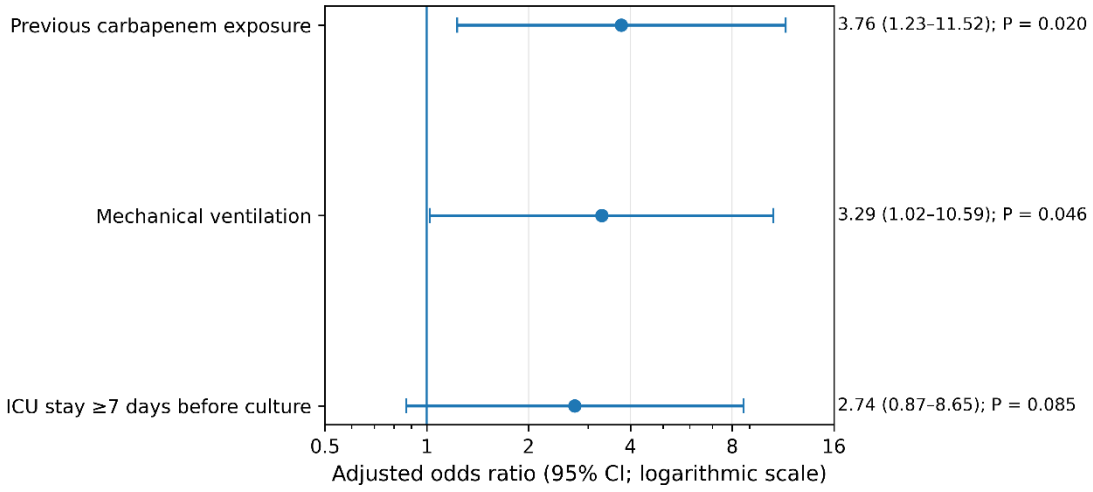

Figure 3. Clinical factors associated with molecular MBL gene carriage among ICU-derived Pseudomonas aeruginosa isolates. Adjusted odds ratios and 95% confidence intervals were estimated using Firth penalized logistic regression.

Discussion

This study provides ICU-specific evidence on the clinical context, antimicrobial-resistance profile, and targeted MBL gene distribution of *Pseudomonas aeruginosa* in Misurata, Libya.

Demographic and Clinical Factors Associated with MBL Gene Carriage

The absence of a statistically significant association between molecular MBL gene carriage and age or sex suggests that demographic variables alone may have been less influential than healthcare exposure within this ICU population. This is plausible because acquisition of resistant *P. aeruginosa* in critical-care settings is primarily shaped by antimicrobial pressure, environmental exposure, severity of illness, invasive devices, and duration of healthcare contact rather than by age or sex in isolation [18,19].

Mechanical ventilation remained independently associated with MBL gene carriage. This relationship is biologically plausible because endotracheal tubes provide a surface for bacterial attachment and biofilm formation, while repeated airway manipulation and prolonged exposure to the ICU environment may increase opportunities for colonization or infection with resistant organisms. Ventilated patients also

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commonly have more severe illness and greater exposure to broad-spectrum antimicrobials, which may amplify selection pressure for resistant strains [18].

Previous carbapenem exposure also remained independently associated with molecular MBL gene carriage. Carbapenem treatment may suppress susceptible bacterial populations while selecting organisms carrying carbapenem-resistance mechanisms, including MBL genes, porin alterations, efflux-pump activity, or combinations of these mechanisms. This interpretation is consistent with Libyan data showing that antimicrobial exposure and MDR Gram-negative infections are closely linked within hospitalized populations, although the available local study was not limited to *P. aeruginosa* or ICU isolates [19].

Central venous catheterization, previous antibiotic exposure, and in-hospital mortality showed non-significant trends toward greater frequency among patients with MBL gene-positive isolates. These findings may still be clinically meaningful because non-significance does not establish the absence of an effect; rather, it may reflect the limited number of molecularly positive isolates, wide confidence intervals, and correlation among variables such as device use, prolonged ICU admission, mechanical ventilation, and antimicrobial exposure. This possibility is particularly relevant in ICU epidemiology, where exposures often occur together rather than independently [18,19].

The lack of significant independent associations with diabetes mellitus, chronic kidney disease, malignancy/immunosuppression, urinary catheterization, or sex should therefore be interpreted cautiously. These conditions may increase vulnerability to healthcare-associated infection in general, but may not independently predict carriage of the selected MBL genes after accounting for more proximate ICU-related exposures. In addition, the study was not designed to assess whether individual comorbidities modify the effect of ventilation or prior carbapenem exposure.

The longer ICU stay observed before culture collection is consistent with cumulative exposure to invasive procedures, colonized surfaces, antimicrobial therapy, and cross-transmission opportunities. Its attenuation after multivariable adjustment suggests that prolonged stay may function partly as a marker of other exposures, particularly mechanical ventilation and prior carbapenem treatment, rather than as an entirely independent determinant of MBL gene carriage [18].

Specimen Distribution and Respiratory Isolation Pattern

Respiratory specimens represented the predominant source of isolates, which is expected in an ICU population where mechanically ventilated patients are at increased risk of airway colonization and ventilator-associated lower respiratory tract infection. The respiratory tract can become a reservoir for *P. aeruginosa* because endotracheal devices facilitate adherence and biofilm formation, while repeated suctioning and exposure to contaminated secretions may support persistence and transmission [18].

The higher concentration of carbapenem-resistant and molecularly positive isolates among respiratory specimens may therefore reflect the combined influence of mechanical ventilation, prolonged critical-care exposure, and intensive antibiotic selection pressure. However, respiratory isolation should not automatically be interpreted as infection because endotracheal aspirates and sputum may represent colonization. Clinical interpretation requires integration with radiological findings, signs of systemic infection, and standardized diagnostic criteria for ventilator-associated pneumonia.

The present pattern is broadly consistent with recent Libyan healthcare-associated *P. aeruginosa* data, in which respiratory, urine, wound, and blood specimens all contributed isolates with substantial resistance burdens. The consistency supports the need for ICU-specific surveillance rather than reliance on hospital-wide resistance summaries alone [20].

Antimicrobial Resistance and Multidrug Resistance

The resistance pattern observed across antipseudomonal β-lactams, fluoroquinolones, and carbapenems is compatible with the complex resistance biology of *P. aeruginosa*. Carbapenem resistance may result from multiple interacting mechanisms, including OprD loss or downregulation, efflux-pump overexpression, derepressed AmpC activity, carbapenemase production, and co-selection of additional resistance determinants. Therefore, resistance to a single class should not be interpreted as evidence of one specific molecular mechanism [21].

The MDR burden is particularly concerning because it limits the number of reliably active conventional antipseudomonal agents and may complicate empirical treatment in critically ill patients. A recent global meta-analysis demonstrated substantial geographic heterogeneity in carbapenem-resistant *P. aeruginosa* and resistance to companion antipseudomonal agents, emphasizing that local antibiograms remain essential for empirical-treatment decisions [21].

The comparatively lower resistance to amikacin may reflect lower local selection pressure, differential permeability or efflux effects, or absence of aminoglycoside-resistance determinants in some isolates. However, this finding should not be interpreted as justification for empirical aminoglycoside monotherapy. Aminoglycoside use requires consideration of infection site, pharmacokinetics, nephrotoxicity risk, and isolate-specific susceptibility data.

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Local evidence provides a biologically plausible explanation for the broader MDR phenotype. A Libyan study found that carbapenem resistance in *P. aeruginosa* and *Acinetobacter baumannii* was associated with efflux-pump activity and biofilm formation, both of which may reduce effective antimicrobial exposure and promote survival under treatment pressure [22]. Similarly, Libyan healthcare-associated *P. aeruginosa* isolates have shown a relationship between MDR and virulence-associated traits, particularly biofilm formation, suggesting that resistance and persistence may coexist in the same clinical strains [20].

Distribution of MBL-Encoding Genes

The predominance of *blaVIM* among targeted gene-positive isolates is epidemiologically plausible because VIM-type enzymes are widely distributed among carbapenem-resistant *P. aeruginosa* and are commonly linked to class 1 integrons and mobile genetic elements. Their presence in ICU isolates is especially concerning because mobile resistance determinants can spread through healthcare networks when selection pressure and opportunities for transmission coexist [23,24].

The detection of *blaIMP* and *blaNDM* alongside *blaVIM* indicates that the local problem is not restricted to one MBL lineage. Regional studies have similarly documented concurrent circulation of VIM-, IMP-, and NDM-associated resistance among clinical *P. aeruginosa* isolates. In Erbil, Iraq, MDR and carbapenem resistance were accompanied by molecular detection of *blaVIM* and *blaNDM*, while Egyptian isolates have demonstrated a combination of phenotypic MBL activity and molecularly detectable resistance determinants [23,24].

The detection of *blaSPM*, although infrequent, remains important because low-frequency genes may still signal introduction or emergence of additional carbapenemase lineages. Its presence should not be overinterpreted as evidence of widespread dissemination; however, it supports the value of maintaining broad molecular surveillance rather than restricting PCR testing to one or two common targets.

Co-Carriage of MBL Genes

The occurrence of isolates carrying more than one targeted MBL gene may reflect accumulation of resistance determinants within integrons, transposons, plasmids, or other mobile genetic structures. Such co-carriage may increase the probability that a strain persists under diverse antimicrobial exposures and may facilitate transfer of resistance determinants between bacterial populations. However, the present study cannot establish the genetic location of these genes because sequencing, plasmid analysis, and integron characterization were not performed [25].

Comparable observations have been reported in regional molecular studies. In Sulaimaniyah, Iraq, PCR-based analysis of carbapenemase-producing *P. aeruginosa* identified VIM and IMP-family genes among carbapenem-resistant isolates, whereas a Tunisian study demonstrated that molecular characterization can reveal diverse resistance mechanisms and clinically relevant genetic backgrounds among *P. aeruginosa* isolates [26,27]. These comparisons support the interpretation that gene diversity and co-carriage are plausible in this region, but they do not establish clonal relatedness between the present isolates and those reported elsewhere.

Genome surveillance studies further demonstrate that carbapenem-resistant *P. aeruginosa* may be associated with successful high-risk lineages and distinct resistance-gene combinations. National genomic surveillance in Japan identified substantial genetic diversity among carbapenem-resistant isolates, emphasizing that phenotypically similar strains may differ in resistance mechanisms and transmission potential [28]. Future studies in Libya should therefore incorporate sequencing or molecular typing to determine whether the observed MBL genes are circulating through clonal expansion, horizontal transfer, or both.

Association Between MBL Gene Carriage and Antimicrobial Resistance

The strong relationship between targeted MBL gene carriage and resistance to carbapenems and other β-lactams is biologically expected because MBLs hydrolyze most β-lactam antibiotics, including carbapenems. By contrast, the associated resistance to fluoroquinolones and aminoglycosides is unlikely to be caused directly by MBL enzymes. It more likely reflects linked resistance determinants, efflux mechanisms, target-site mutations, or selection of multidrug-resistant lineages carrying multiple resistance mechanisms [24,27].

This interpretation is supported by Egyptian molecular work in which MBL-producing *P. aeruginosa* isolates showed extensive resistance beyond carbapenems, including resistance to other antipseudomonal agents [24]. Therefore, targeted MBL gene carriage in the present study should be regarded as a marker of broader resistance complexity rather than as an isolated explanation for carbapenem non-susceptibility.

Phenotypic Detection Compared with Molecular Testing

The high sensitivity of the imipenem–EDTA combined-disk method supports its use as a practical screening tool for possible MBL production, particularly in laboratories where molecular testing is unavailable. However, its lower specificity means that a phenotypically positive result should not be interpreted as definitive proof of carriage of one of the selected MBL genes [29].

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Several mechanisms may explain discordance between phenotypic and molecular findings. EDTA-based methods can produce non-specific enhancement of inhibition zones, whereas carbapenem resistance may be mediated by OprD loss, efflux-pump overexpression, AmpC hyperproduction, serine carbapenemases, or combinations of mechanisms. Conversely, phenotypically positive but PCR-negative isolates may carry MBL genes not included in the assay panel, novel variants not detected by the primers, or resistance mechanisms that mimic an MBL phenotype [29,30].

Previous phenotypic–genotypic comparisons have shown that no single screening method fully captures all carbapenem-resistance mechanisms in *P. aeruginosa.* Studies using both phenotypic and PCR-based approaches have reported variable agreement, supporting a tiered diagnostic strategy in which phenotypic screening is followed by molecular testing when surveillance, outbreak investigation, or therapeutic decision-making requires greater certainty [29,30].

A recent systematic review of carbapenem-resistant *P. aeruginosa* detection further emphasized the variability of available methods and the lack of a single universally optimal approach across clinical contexts. This supports using local laboratory algorithms that balance speed, cost, and diagnostic accuracy, while recognizing the added value of molecular confirmation for epidemiological surveillance [31].

Implications for Infection Prevention and Antimicrobial Stewardship

The association of MBL gene carriage with mechanical ventilation and previous carbapenem exposure supports targeted stewardship and infection-prevention interventions in ICU settings. Carbapenem prescribing should be reviewed against ICU-specific antibiograms, and ventilated patients should receive particular attention regarding device care, hand hygiene, respiratory-equipment handling, and environmental cleaning [18,21].

The presence of multiple targeted MBL genes also supports routine surveillance of carbapenem-resistant isolates. In resource-limited settings, a practical approach may involve phenotypic screening of carbapenem-resistant isolates, followed by selective PCR confirmation for epidemiological monitoring and suspected clusters. Recent Misurata data documenting carbapenem resistance and MBL activity among clinical Gram-negative organisms reinforce the local relevance of this approach [32].

Conclusion

This study found a high burden of carbapenem resistance and multidrug resistance among ICU-derived *Pseudomonas aeruginosa* isolates in Misurata. The *blaVIM* gene was the most common MBL determinant, and MBL gene carriage was strongly associated with resistance to multiple antimicrobial classes. Previous carbapenem exposure and mechanical ventilation were independent risk factors. These findings support stricter carbapenem stewardship, infection-prevention measures for ventilated ICU patients, and routine surveillance of carbapenem-resistant isolates.

Strengths, limitations, and future direction

This study has several strengths, including its ICU-specific design, the inclusion of one clinical *Pseudomonas aeruginosa* isolate per patient, integration of clinical characteristics with antimicrobial susceptibility profiles, and assessment of both phenotypic MBL production and targeted molecular markers. In addition, the use of Firth penalized logistic regression was appropriate given the relatively small number of MBL gene-positive isolates.

Several limitations should be considered when interpreting the findings. First, the single-centre design and modest sample size may limit the generalizability of the results and reduce the statistical power to identify independent associations for some demographic and clinical variables. Second, the cross-sectional design does not allow determination of whether MBL gene-positive isolates were acquired before or during ICU admission. Third, the PCR panel was limited to *blaVIM*, *blaIMP*, *blaNDM*, and *blaSPM*; therefore, PCR-negative isolates may have carried other untested carbapenemase genes or exhibited non-enzymatic mechanisms of carbapenem resistance. In addition, the absence of gene-specific positive controls may have limited the analytical validation of the PCR assays. Finally, sequencing, plasmid characterization, integron analysis, MIC testing, and molecular typing were not performed, preventing definitive identification of gene variants, clonal relatedness, transmission pathways, and the genetic context of the detected resistance genes [25,28].

Future multicentre studies should include larger sample sizes, standardized definitions to distinguish infection from colonization, MIC-based susceptibility testing, expanded carbapenemase gene panels, validated positive controls, sequencing confirmation, and molecular typing or genomic analysis. Such studies would provide a clearer understanding of the epidemiology, transmission dynamics, and clinical implications of MBL-producing *P. aeruginosa* in Libya.

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Conflicts of Interest
The authors declare no conflicts of interest.

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