Volume 3, Number 3, 2026
Pages 2125-2134
Bioscience Insights
ISSN: 3080-3489 (Print)
ISSN: 3080-3497 (Online)
BiosciInsights.com

MOLECULAR CHARACTERIZATION OF CARBAPENEMASE ENCODING GENES IN PSEUDOMONAS AERUGINOSA ISOLATED FROM THE HOSPITALIZED UTI PATIENTS IN D.I. KHAN

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https://doi.org/10.5281/zenodo.24490928

Article Information

Article History

Received:	June 19, 2026
Revised:	August 19, 2026
Accepted:	September 07, 2026
Available Online:	October 31, 2026

Keywords:
Pseudomonas aeruginosa, urinary tract infection, carbapenem resistance, CRPA, blaVIM, blaNDM, blaIMP, PCR, antimicrobial susceptibility.

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Abstract

Urinary tract infections (UTIs) are frequently caused by the opportunistic bacterium Pseudomonas aeruginosa, especially in hospitalized and immunocompromised patients. Because there are few treatment options and carbapenemase genes spread quickly, the rise of carbapenem-resistant Pseudomonas aeruginosa (CRPA) has become a major public health concern. The current work sought to identify and isolate Pseudomonas aeruginosa from UTI patients, ascertain their patterns of antimicrobial sensitivity, and molecularly describe isolates that were resistant to carbapenem by identifying the carbapenemase genes blaVIM, blaIMP, and blaNDM. From January to April 2026, a descriptive cross-sectional laboratory investigation was carried out at Gomal University's Faculty of Veterinary and Animal Sciences in Dera Ismail Khan. One hundred urine samples from patients with clinically suspected UTIs were examined. Gram staining, colony morphology, biochemical testing, and standard culture methods were used to isolate and identify Pseudomonas aeruginosa. The Kirby-Bauer disk diffusion method was used to test for antimicrobial susceptibility in accordance with CLSI 2022 recommendations. PCR was used to detect carbapenemase genes molecularly in isolates that were resistant to carbapenem. Pseudomonas aeruginosa was found in 24% of the 100 urine samples. 62.5% of these isolates were found to be CRPA. 58.3%, 54.2%, and 50.0% of isolates showed resistance to imipenem, meropenem, and doripenem, respectively. Molecular analysis revealed that blaVIM was the predominant gene (53.3%), followed by blaNDM (33.3%) and blaIMP (26.7%). Female patients and individuals aged 41–60 years showed comparatively higher frequencies of CRPA. The study demonstrated a substantial prevalence of carbapenem-resistant Pseudomonas aeruginosa

harboring clinically significant carbapenemase genes among UTI patients, emphasizing the growing threat of multidrug resistance. Continuous molecular surveillance, strict infection control measures, and implementation of antimicrobial stewardship programs are strongly recommended to prevent the emergence and spread of CRPA.

INTRODUCTION

Urinary tract infections (UTIs) are among the most common bacterial infections worldwide and represent a considerable public health concern because of their high incidence, recurrence, and associated healthcare costs. They occur when pathogenic microorganisms colonize and multiply within the urinary system, including the urethra, bladder, ureters, and kidneys. Depending on the anatomical site involved, UTIs are classified as upper urinary tract infections, such as pyelonephritis, and lower urinary tract infections, including cystitis and urethritis. Their clinical manifestations range from asymptomatic bacteriuria and mild urinary discomfort to severe complications, including renal damage, bacteremia, and urosepsis (Foxman, 2014; Flores-Mireles et al., 2015).

The bacterial etiology of UTIs varies according to geographical location, patient characteristics, and healthcare exposure. *Escherichia coli* remains the predominant causative organism, particularly in community-acquired uncomplicated infections. However, complicated and hospital-acquired UTIs are increasingly associated with a broader range of bacterial pathogens, including *Klebsiella pneumoniae*, *Proteus mirabilis*, *Enterobacter* species, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa* (Flores-Mireles et al., 2015). Hospitalization, previous antibiotic exposure, urinary tract abnormalities, underlying diseases, and prolonged catheterization are important factors contributing to infections caused by opportunistic and multidrug-resistant organisms. In particular, catheter-associated urinary tract infections facilitate bacterial colonization and biofilm formation, creating favorable conditions for persistent infections and antimicrobial treatment failure (Donlan, 2001).

Among healthcare-associated uropathogens, *Pseudomonas aeruginosa* is an important opportunistic Gram-negative bacterium associated with complicated UTIs, particularly in catheterized and hospitalized patients. It is widely distributed in natural and healthcare environments and is characterized by considerable metabolic adaptability, environmental persistence, and pathogenic potential. Its ability to cause infections is associated with several virulence factors, including flagella, pili, lipopolysaccharides, extracellular enzymes, and pigments that contribute to adhesion, colonization, tissue damage, and evasion of host immune responses (Gellatly & Hancock, 2013). Furthermore, *P. aeruginosa* possesses a remarkable ability to form biofilms on urinary catheters and other medical devices. These biofilms protect antimicrobial agents and host immune responses, contributing to persistent colonization, recurrent UTIs, and treatment failure (Donlan, 2001; Gellatly & Hancock, 2013).

Antimicrobial resistance in *P. aeruginosa* has become a major challenge in the treatment of healthcare-associated infections. This organism exhibits intrinsic resistance to several antimicrobial agents and can acquire additional resistance mechanisms through chromosomal mutations and horizontal gene transfer. Important mechanisms include reduced outer membrane permeability, loss or modification of the OprD porin, overexpression of multidrug efflux pumps, and increased production of AmpC β-lactamase (Lister et al., 2009). These mechanisms frequently occur in combination, resulting in multidrug-resistant (MDR) and extensively drug-resistant (XDR) phenotypes. Consequently, infections caused by resistant *P. aeruginosa* are associated with limited therapeutic options, prolonged hospitalization, increased healthcare expenditure, and unfavorable clinical outcomes (Pang et al., 2019).

Carbapenems, including imipenem, meropenem, doripenem, and ertapenem, are broad-spectrum β-lactam antibiotics that play an important role in the treatment of severe infections caused by multidrug-resistant Gram-negative bacteria. Their antibacterial activity involves binding to penicillin-binding proteins and inhibiting bacterial cell wall synthesis (Papp-Wallace et al., 2011). However, the increasing emergence of carbapenem-resistant *P. aeruginosa* (CRPA) has significantly reduced the effectiveness of these important therapeutic agents. Carbapenem resistance may result from several mechanisms, including OprD porin deficiency, efflux pump overexpression, AmpC β-lactamase overproduction, and

the acquisition of carbapenemase-encoding genes (Lister et al., 2009; Halat & Moubareck, 2022). The coexistence of these mechanisms can further increase resistance and substantially restrict available treatment options.

Carbapenemase production represents a particularly important mechanism of acquired carbapenem resistance in *P. aeruginosa*. These enzymes hydrolyze carbapenem antibiotics and belong to different molecular classes, including class A, class B metallo-β-lactamases (MBLs), and class D β-lactamases. Important carbapenemase families reported in *P. aeruginosa* include Guiana extended-spectrum β-lactamases (GES), imipenemases (IMP), Verona integron-encoded metallo-β-lactamases (VIM), New Delhi metallo-β-lactamases (NDM), and certain oxacillinases (OXA) (Halat & Moubareck, 2022). The genes encoding these enzymes may be associated with mobile genetic elements, including integrons and plasmids, which facilitate the dissemination of resistance determinants among bacterial populations. The increasing global distribution of carbapenemase-producing *P. aeruginosa* represents a significant threat to healthcare systems because these organisms can spread within healthcare facilities and compromise the effectiveness of available antimicrobial treatments (Tenover et al., 2022).

Molecular characterization provides additional information by detecting carbapenemase-encoding genes and identifying the genetic determinants associated with antimicrobial resistance. Recent investigations have highlighted the diversity and changing epidemiology of acquired carbapenemase genes in *P. aeruginosa*, particularly VIM- and GES-type enzymes, emphasizing the importance of combining phenotypic susceptibility testing with molecular surveillance (Halat & Moubareck, 2022; Martínez-Zavaleta et al., 2023). However, geographically representative molecular data on carbapenem-resistant *P. aeruginosa* isolated specifically from UTI patients remain important for understanding local resistance patterns and developing effective infection prevention strategies.

Therefore, the present study aimed to investigate carbapenem-resistant *Pseudomonas aeruginosa* isolated from patients with urinary tract infections by determining its occurrence and antimicrobial susceptibility profile, identifying carbapenem-resistant isolates, and molecularly detecting selected carbapenemase-encoding genes. The study further aimed to characterize the distribution of these resistance determinants and provide baseline molecular epidemiological information to support antimicrobial surveillance, infection prevention, and appropriate therapeutic management of UTIs caused by carbapenem-resistant *P. aeruginosa*.

Materials and Methods

The present study was designed as a descriptive cross-sectional, laboratory-based investigation to characterize carbapenem-resistant *Pseudomonas aeruginosa* (CRPA) isolated from patients with urinary tract infections (UTIs). The study was conducted over four months, from January 1 to April 30, 2026, at the Microbiology Laboratory, Faculty of Veterinary and Animal Sciences, Gomal University, Dera Ismail Khan, Pakistan. The investigation involved the collection of urine samples, bacterial isolation and identification, antimicrobial susceptibility testing, and molecular detection of selected carbapenemase-encoding genes. Both phenotypic and molecular approaches were employed to investigate antimicrobial resistance and identify the genetic determinants associated with carbapenem resistance (Lister et al., 2009; Breidenstein et al., 2011).

A total of 100 urine samples were collected from patients presenting with clinical signs and symptoms suggestive of UTIs, including dysuria, increased urinary frequency, urgency, hematuria, and suprapubic pain. Both male and female patients of different age groups were included in the study. Midstream urine samples were collected in sterile, properly labelled containers following appropriate genital cleaning procedures. For catheterized patients, urine specimens were aseptically collected from the catheter sampling port using a sterile syringe. Each sample was labelled with the patient's identification number, collection date, and time. The collected specimens were transported to the microbiology laboratory in an icebox maintained at 4–8°C and processed within two hours of collection to minimize bacterial multiplication and contamination.

All laboratory procedures were performed under appropriate aseptic conditions and in accordance with established microbiological practices. Glassware, including Petri dishes, test tubes, and flasks, was sterilized using dry heat at 160°C for two hours. Culture media and laboratory solutions were prepared

according to the manufacturer's instructions or standard microbiological protocols. The prepared media were adjusted to the required pH and sterilized by autoclaving at 121°C under 15 psi pressure for 15 minutes before use. Appropriate quality control and biosafety procedures were followed throughout the investigation to minimize contamination and ensure the reliability of laboratory findings.

For the isolation of *Pseudomonas aeruginosa*, urine samples were inoculated onto cystine lactose electrolyte-deficient (CLED) agar plates using the streak plate technique. The inoculated plates were incubated aerobically at 37°C for 18–24 hours. Presumptive colonies were evaluated based on their cultural characteristics, including colony appearance and lactose fermentation. Suspected non-lactose-fermenting colonies were subsequently subcultured onto cetrimide agar, a selective medium for *P. aeruginosa*, and incubated at 37°C for 24 hours. Characteristic growth and greenish pigmentation associated with pyocyanin production were considered presumptive evidence of *P. aeruginosa*. Suspected colonies were further purified through repeated quadrant streaking onto nutrient agar plates. The purification process was repeated until pure cultures with uniform colony characteristics were obtained (Collee et al., 1996).

Identification of the bacterial isolates was performed using cultural, microscopic, and biochemical characteristics. Colony morphology was assessed based on the appearance, pigmentation, and characteristic fruity or grape-like odour of the colonies. Gram staining was performed using fresh 24-hour bacterial cultures to determine the Gram reaction and cellular morphology. The isolates were examined under a light microscope, and Gram-negative rod-shaped bacteria were considered presumptive *P. aeruginosa*. Biochemical identification included catalase and oxidase testing. For the catalase test, a small quantity of a fresh bacterial colony was transferred onto a clean glass slide and mixed with a drop of 3% hydrogen peroxide (H_2O_2). The immediate formation of gas bubbles indicated a positive catalase reaction. For the oxidase test, a fresh bacterial colony was exposed to an appropriate oxidase reagent containing tetramethyl-p-phenylenediamine dihydrochloride. Development of a dark purple colour within the specified reaction time indicated a positive oxidase reaction. The combined cultural, microscopic, and biochemical characteristics were used to confirm the identity of the suspected isolates

Antimicrobial susceptibility testing (AST) was performed on confirmed *P. aeruginosa* isolates using the Kirby–Bauer disk diffusion method on Mueller–Hinton agar. A bacterial suspension was prepared and adjusted to a turbidity equivalent to the 0.5 McFarland standard. The standardized inoculum was uniformly spread over the surface of Mueller–Hinton agar plates, followed by the application of appropriate antibiotic discs. The antimicrobial agents tested included the carbapenems imipenem, meropenem, and doripenem, the third-generation cephalosporin ceftazidime, and the β-lactam/β-lactamase inhibitor combination piperacillin–tazobactam. The inoculated plates were incubated at 37°C for 16–18 hours. Following incubation, the diameters of the inhibition zones were measured in millimetres and interpreted according to the Clinical and Laboratory Standards Institute (CLSI, 2022) guidelines. Isolates demonstrating resistance to at least one tested carbapenem were classified as carbapenem-resistant *P. aeruginosa* and selected for further molecular characterization.

Confirmed bacterial isolates were preserved for subsequent antimicrobial susceptibility testing and molecular analysis. For long-term preservation, pure bacterial cultures were suspended in 15–20% glycerol stocks and stored at −80°C. In addition, isolates were maintained on nutrient agar slants at 4°C for short-term storage and routine laboratory use.

Genomic DNA was extracted from selected carbapenem-resistant *P. aeruginosa* isolates using the boiling method. Briefly, a fresh bacterial colony was suspended in sterile distilled water and subjected to boiling at 100°C for 10 minutes. The suspension was subsequently centrifuged to remove cellular debris, and the supernatant containing the extracted DNA was collected and used as the PCR template. Extracted DNA samples were stored at −20°C until further analysis. Polymerase chain reaction (PCR) was performed to detect selected carbapenemase-encoding genes, including *blaVIM*, *blaIMP*, and *blaNDM*. Gene-specific primers were selected for amplification of the target sequences.

Each PCR reaction was prepared in a final volume of 20 µL, consisting of 10.5 µL of PCR master mix, 1 µL of forward primer, 1 µL of reverse primer, 2 µL of DNA template, and 5.5 µL of nuclease-free water. The amplification protocol consisted of initial denaturation at 95°C for 5 minutes, followed by repeated cycles of denaturation at 95°C for 30 seconds, primer annealing at 52–58°C for 30 seconds, and extension

at 72°C for 1 minute. A final extension step was performed at 72°C for 7 minutes. The annealing temperature was selected according to the specific primer set used for each target gene.

The amplified PCR products were analyzed using agarose gel electrophoresis. A 1.5% agarose gel was prepared, and the amplified DNA fragments were separated according to their molecular sizes. Ethidium bromide was used for DNA staining, and the separated bands were visualized under ultraviolet (UV) illumination. A 100-bp DNA ladder was used as a molecular size marker to estimate the sizes of the amplified products. Gel images were documented for subsequent examination and confirmation of the presence or absence of the targeted carbapenemase genes

All collected data were organized and entered into Microsoft Excel before statistical analysis using the Statistical Package for the Social Sciences (SPSS). Descriptive statistics, including frequencies and percentages, were calculated to summarize the distribution of *P. aeruginosa* isolates, antimicrobial resistance patterns, and detected carbapenemase-encoding genes. Where appropriate, associations between the occurrence of resistance genes and demographic or clinical characteristics, including age, sex, and hospital ward, were evaluated using suitable statistical tests. Statistical significance was considered at an appropriate significance level (Field et al., 2012; Kirkwood & Sterne, 2003).

RESULTS

Distribution of Urine Samples and Isolation of *Pseudomonas aeruginosa*

Overall, 100 samples of urine were collected from patients who came to the hospital with clinical symptoms of UTI as shown in Table 1. After culture on MacConkey agar and selective culture on Cetrimide agar, 24 (24.0%) isolates were identified as *Pseudomonas aeruginosa* and 76 (76.0%) samples either produced other bacterial isolates or did not produce any growth that fulfils the culture criteria of the study. The isolation rate found in the present study is similar to those reported in previous studies, which showed that *Pseudomonas aeruginosa* is responsible for around 10-25% of complicated UTIs.

Table 1 Isolation frequency of Pseudomonas aeruginosa from urine samples

Parameter	Number (n)	Percentage (%)
Total urine samples collected	100	100.0
Confirmed Pseudomonas aeruginosa isolates	24	24.0
Non-Pseudomonas aeruginosa isolates/no growth	76	76.0

Morphological and Biochemical Characteristics of the Isolaes

The 24 isolates had typical colony morphology of Pseudomonas aeruginosa. Colonies grown on CLED media were non-lactose fermenting and on Cetrimide media they were producing greenish pigmentation (pyocyanin) as shown in Table 2. Gram-negative rod-shaped bacteria are seen by Gram staining. All isolates were biochemicaly confirmed to be Pseudomonas aeruginosa based on their catalase and oxidase positive.

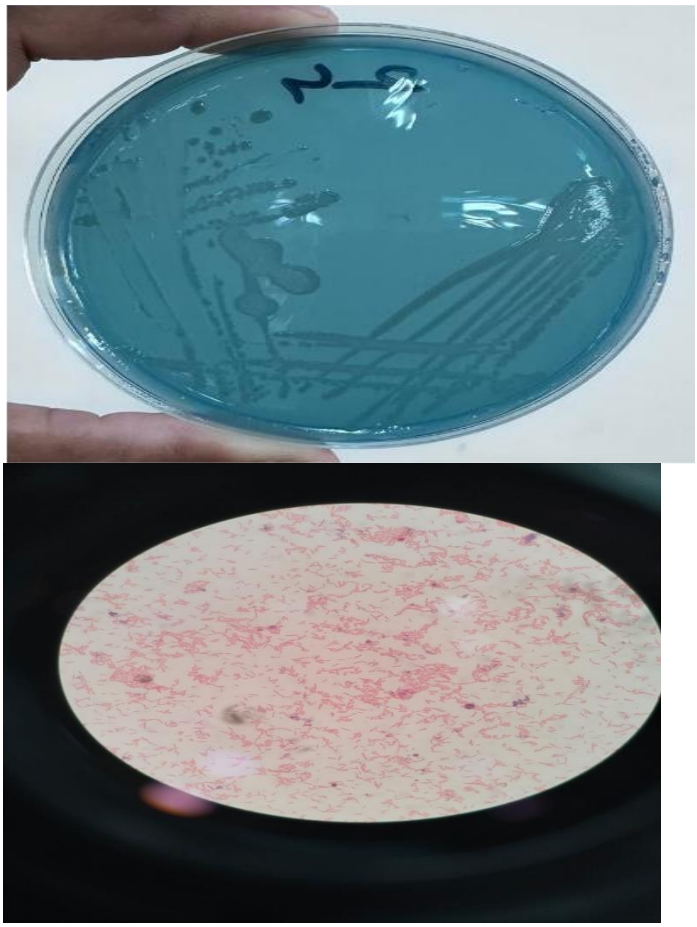

Figure 1 Morphological and biochemical characteristics of the isolates

Table 2 Morphological and Biochemical Characteristics of the Isolates

Characteristic	Observation	Positive isolates n (%)
CLED agar	Non-lactose fermenting colonies	24 (100)
Cetrimide agar	Green pigmented growth	24 (100)
Gram staining	Gram-negative rods	24 (100)
Catalase test	Positive	24 (100)
Oxidase test	Positive	24 (100)

Kirby – Bauer disk diffusion method was used to test antibiotic susceptibility as per CLSI (2022) Guidelines as shown in Tab. 2 and Fig.1. Carbapenem antibiotic resistance was seen at a high level. Resistance to imipenem, meropenem and doripenem was found in 58.3%, 54.2% and 50.0% of the isolates, respectively. The percentages of ceftazidime and piperacillin-tazobactam resistant isolates were 45.8% and 37.5%, respectively.

Table 3 Antimicrobial susceptibility pattern of Pseudomonas aeruginosa isolates (n = 24)

Antibiotic	Sensitive n (%)	Intermediate n (%)	Resistant n (%)
Imipenem	9 (37.5)	1 (4.2)	14 (58.3)
Meropenem	10 (41.7)	1 (4.2)	13 (54.2)
Doripenem	11 (45.8)	1 (4.2)	12 (50.0)
Ceftazidime	12 (50.0)	1 (4.2)	11 (45.8)

Piperacillin-tazobactam	14 (58.3)	1 (4.2)	9 (37.5)

Phenotypic Detection of Carbapenem-Resistant *Pseudomonas aeruginosa* (CRPA)

Isolates that were resistant or intermediate for any one of the carbapenems were considered to be carbapenem-resistant *Pseudomonas aeruginosa* (CRPA) according to CLSI interpretive criteria as shown in Table 4. Of all the isolates, 15 isolates (62.5%) met the criteria of CRPA and were selected for molecular characterization.

Table 4 Frequency of carbapenem-resistant Pseudomonas aeruginosa

Category	Number (n)	Percentage (%)
CRPA isolates	15	62.5
Non-CRPA isolates	9	37.5
Total isolates	24	100

Molecular Detection of Carbapenemase-Encoding Genes

The presence of carbapenemase genes was detected in the 15 CRPA isolates at different frequencies by PCR as shown in Table 5. The blaVIM gene was the predominant and was found in 8 (53.3%) isolates. In 5 (33.3%) isolates blaNDM was found, while blaIMP was found in 4 (26.7%) isolates. Three isolates exhibited co-existence of more than one resistance gene.

Table 5. Distribution of carbapenemase genes among CRPA isolates (n = 15)

Resistance gene	Positive isolates (n)	Frequency (%)
blaVIM	8	53.3
blaNDM	5	33.3
BlaIMP	4	26.7
Multiple genes	3	20.0

Gel Electrophoresis Findings

Amplicons of the desired carbapenemase genes were successfully electrophoresed on a 1.5% agarose gel as shown in Table 6. The distinct bands with expected sizes were obtained against 100 bp DNA ladder confirming successful amplification of the blaVIM gene and blaIMP and blaNDM genes. Representative gel images showed distinct banding patterns among positive isolates with good reproducibility.

Table 6: PCR amplification results of carbapenemase genes

Gene detected	Positive isolates (n)	Expected amplicon size (bp)
blaVIM	8	390
blaIMP	4	232
blaNDM	5	621

Distribution of CRPA According to Gender

The 9 female patients (60.0%) yielded a total of 15 CRPA isolates, while 6 male patients (40.0%) yielded a total of 6 CRPA isolates as shown in Table 4.7. There was no significant difference between the incidence of carbapenem resistance and gender ($P > 0.05$), though females had a greater proportion of CRPA-associated UTIs.

Table 7 Gender-wise distribution of CRPA isolates

Gender	CRPA isolates (n)	Percentage (%)
Male	6	40.0
Female	9	60.0
Total	15	100

Isolates resistant to carbapenems were found in all age groups as shown in Table 8. The most prevalent age group was 41-60 years old (40.0%), with more than 60 years being the 2nd most prevalent (26.7%). The frequencies were comparatively low for younger age groups.

Table 8Age-wise distribution of CRPA isolates

Age group (years)	Number (n)	Percentage (%)
≤20	2	13.3
21–40	3	20.0
41–60	6	40.0
>60	4	26.7
Total	15	100

Association Between Carbapenemase Genes and Demographic Variables

The difference in the presence of blaVIM, blaIMP or blaNDM genes is not significant and demographic data such as age and gender ($P > 0.05$) according to comparative statistical analysis. But there was a higher prevalence of the genes in older people. It is important to note that the blaVIM was the predominant resistance gene found in CRPA, indicating that metallo-β-lactamase-mediated resistance is a major contributor to carbapenem resistance in the present study.

Discussion

Carbapenem-resistant *Pseudomonas aeruginosa* (CRPA) represents a significant public health concern because of its ability to acquire multiple antimicrobial resistance determinants and the limited availability of effective therapeutic options. In the present study, *P. aeruginosa* was isolated from 24% of urine samples collected from patients with clinically suspected urinary tract infections (UTIs). This finding is consistent with previous reports indicating that *P. aeruginosa* accounts for approximately 18–25% of nosocomial urinary infections (Al-Orphaly et al., 2021). Its occurrence may be associated with prolonged hospitalization, inappropriate antibiotic use, and inadequate infection prevention practices.

The isolates were identified based on their characteristic morphological, cultural, and biochemical properties. They appeared as Gram-negative rods, produced non-lactose-fermenting colonies on MacConkey agar, and demonstrated characteristic growth on Cetrimide agar. Furthermore, all isolates were catalase- and oxidase-positive, supporting their identification as *P. aeruginosa* (Forbes et al., 2007; Koneman et al., 2017).

Antimicrobial susceptibility testing revealed considerable resistance to carbapenems, with 58.3%, 54.2%, and 50.0% of isolates resistant to imipenem, meropenem, and doripenem, respectively. These findings indicate a substantial burden of carbapenem resistance and are comparable to previous reports from developing countries, where resistance rates of 40–60% have been documented (El Zowalaty et al., 2015). Similarly, Khan et al. (2020) reported approximately 55% imipenem resistance among clinical *P. aeruginosa* isolates in Pakistan. Such resistance may result from reduced membrane permeability, increased efflux pump activity, AmpC β-lactamase production, and acquisition of carbapenemase genes (Lister et al., 2009). The emergence of CRPA is particularly concerning because carbapenems are important treatment options for serious Gram-negative infections.

Molecular characterization demonstrated that *blaVIM* was the most frequently detected gene (53.3%), followed by *blaNDM* (33.3%) and *blaIMP* (26.7%). The predominance of *blaVIM* is consistent with

reports identifying VIM-producing *P. aeruginosa* as an important cause of carbapenem resistance in healthcare settings (Cornaglia et al., 2011; Zafer et al., 2015). Similarly, the detection of *blaNDM* highlights the potential dissemination of this clinically important resistance determinant, which has been associated with extensive antimicrobial resistance (Nordmann et al., 2011). The presence of *blaIMP* further demonstrates the diversity of metallo-β-lactamase genes circulating among CRPA isolates (Walsh et al., 2005). Detection of multiple carbapenemase genes in some isolates suggests the coexistence of different resistance determinants, potentially complicating antimicrobial treatment and infection control (Poirel et al., 2012).

Regarding demographic characteristics, 60% of CRPA isolates were obtained from female patients and 40% from males. This distribution may partly reflect the greater anatomical susceptibility of females to UTIs (Flores-Mireles et al., 2015). Patients aged 41–60 years and those above 60 years represented the most affected groups. However, age and gender were not significantly associated with carbapenemase gene detection ($p > 0.05$), suggesting that demographic characteristics alone may not adequately explain the distribution of resistance.

The findings emphasize the importance of molecular surveillance, antimicrobial stewardship, and effective hospital infection prevention strategies. Routine PCR-based detection of carbapenemase genes can support early identification of resistant isolates and guide appropriate treatment decisions. Rational antibiotic prescribing, restricted carbapenem use, hand hygiene, environmental disinfection, and patient isolation should be strengthened to limit transmission.

This study had limitations, including a relatively small sample size, recruitment from a single institution, and investigation of only three carbapenemase genes. Other resistance determinants, including *blaKPC*, *blaGES*, and OXA-type genes, were not investigated. Furthermore, sequencing and whole-genome analysis were not performed.

Conclusion

The findings of present study revealed a considerable prevalence of *Pseudomonas aeruginosa* among urine samples from patients with suspected UTIs, with 62.5% of the isolates exhibiting carbapenem resistance. Molecular analysis identified *blaVIM* as the predominant carbapenemase gene, followed by *blaNDM* and *blaIMP*. No significant association was observed between carbapenemase gene detection and demographic variables, including age and gender. These findings highlight the importance of routine antimicrobial susceptibility testing, molecular surveillance, rational antibiotic use, and effective infection control measures to limit the spread of carbapenem-resistant *P. aeruginosa*.. This suggests that resistance to carbapenems is predominantly spreading in the region by metallo-β-lactamase–mediated resistance. Routine antimicrobial susceptibility testing should be strictly followed in clinical microbiology laboratories to help administer appropriate antimicrobial therapy. Fast molecular drug detection methods like PCR should be used to identify resistance genes early and correctly to enhance patient outcome.

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