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Research Article

ANTIMICROBIAL RESISTANCE PATTERNS AND PHENOTYPIC CARBAPENEMASE DETECTION IN CLINICAL *PSEUDOMONAS AERUGINOSA* ISOLATES

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ABSTRACT

Objectives: *Pseudomonas aeruginosa* is a significant pathogen responsible for hospital-acquired infections. Its capacity for multidrug resistance (MDR) limits therapeutic options and contributes to elevated patient morbidity. This study investigates the frequency of MDR and extensively drug-resistant (XDR) *P. aeruginosa* isolates and evaluates the effectiveness of phenotypic methods for detecting carbapenemase activity.

Methods: A total of 100 *P. aeruginosa* isolates were collected from various clinical samples over 6 months in a cross-sectional study. Antimicrobial susceptibility testing was conducted using the Kirby–Bauer disk diffusion technique, adhering to the Clinical and Laboratory Standards Institute 2024 standards. Carbapenem-resistant isolates were further analyzed for carbapenemase production using three methods: modified Hodge test (MHT), combined double-disk synergy test (CDDT), and modified carbapenem inactivation method (mCIM)/EDTA-modified carbapenem inactivation method.

Results: Out of 100 isolates, 19% were classified as MDR and 24% as XDR. The highest susceptibility was observed with imipenem (79%), followed by meropenem (75%) and piperacillin–tazobactam (67%). Among the 26 carbapenem-resistant isolates, MHT detected carbapenemase production in 46.2%, CDDT in 26.9%, and mCIM in 38.5%. Metallo-β-lactamase production was observed in 34.6% of isolates, whereas 3.8% demonstrated serine-β-lactamase activity.

Conclusion: The presence of MDR and XDR *P. aeruginosa* and substantial carbapenem resistance underscores the importance of consistent antimicrobial surveillance and the implementation of accurate detection techniques such as mCIM. Prompt identification of resistance mechanisms is critical for appropriate therapy and the control of nosocomial infections.

Keywords: *Pseudomonas aeruginosa*, Antimicrobial resistance, Multidrug resistance, Extensively drug-resistant, Carbapenemase detection, Modified carbapenem inactivation method.

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INTRODUCTION

Pseudomonas species are Gram-negative, non-fermenting bacteria known for their metabolic versatility. This adaptability allows them to thrive in a wide range of environments, including health-care settings where moisture facilitates their survival and transmission [1,2]. Among these, *Pseudomonas aeruginosa* stands out due to its intrinsic resistance mechanisms and ability to form biofilms, evade host immune defenses, and produce virulence factors such as pigments and siderophores [3]. These attributes contribute to its persistence and resistance to treatment.

Recognized as a major opportunistic pathogen in hospitals, *P. aeruginosa* is capable of causing a broad spectrum of infections, particularly in immunocompromised patients, where it leads to increased morbidity [4,5]. Globally, it accounts for nearly 10% of all hospital-acquired infections, and its increasing resistance to available antimicrobials poses a serious public health challenge [6].

As part of the ESKAPE group – comprising *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *P. aeruginosa*, and *Enterobacter* species – *P. aeruginosa* is particularly concerning due to its ability to evade conventional antibiotics [7,8]. It exhibits both inherent and acquired resistance, making it difficult to treat infections in both hospital and community settings [9].

The mechanisms responsible for antimicrobial resistance in *P. aeruginosa* include β-lactamase enzyme production, porin loss, efflux pump overexpression, and target site mutations [4,9]. Based on susceptibility profiles, strains are classified into multidrug-resistant (MDR), extensively drug-resistant (XDR), and pandrug-resistant (PDR) categories. MDR isolates are resistant to at least one agent in three or more antimicrobial categories, XDR strains are susceptible to only one or two classes, and PDR isolates are resistant to all available antimicrobial agents [9,10].

Particularly alarming is the resistance to carbapenems, often mediated by carbapenemase enzymes that can hydrolyze a wide range of β-lactams, including penicillins, cephalosporins, monobactams, and carbapenems [11]. These enzymes can spread rapidly in health-care settings, resulting in high-mortality outbreaks. Early identification of carbapenemase-producing *P. aeruginosa* is essential for timely clinical intervention and effective infection control [1].

In this context, the present study aimed to assess the antimicrobial resistance profiles of *P. aeruginosa* isolates collected from a tertiary care hospital. In addition, it sought to determine the prevalence of MDR and XDR strains and to evaluate the diagnostic accuracy of three phenotypic methods for detecting carbapenemase production: modified carbapenem inactivation method (mCIM), modified Hodge test (MHT), and combined double-disk synergy test (CDDT).

METHODS

This prospective, cross-sectional investigation was carried out over 6 months (September 2024–February 2025) following ethical approval (Ref No MDC/JNMCIEC/505, dated October 01, 2024). A total of 100 clinical isolates of *P. aeruginosa* were obtained from hospitalized patients. These samples were derived from a range of clinical specimens, including urine, pus, blood, and tracheal aspirates. Identification of isolates was achieved through standard biochemical procedures. Antimicrobial susceptibility testing (AST) was conducted in accordance with the 2024 Clinical and Laboratory Standards Institute (CLSI) guidelines.

AST

The Kirby–Bauer disk diffusion technique was used to assess the antimicrobial susceptibility of the *P. aeruginosa* isolates. The antibiotics tested included tobramycin (TN, 10 μg), amikacin (AK, 30 μg), imipenem (IMI, 10 μg), meropenem (MEM, 10 μg), ceftazidime (CAZ, 30 μg), cefepime (CPM, 30 μg), ciprofloxacin (CIP, 5 μg), levofloxacin (LEV, 5 μg), piperacillin-tazobactam (PTZ, 110 μg), aztreonam (ATM, 30 μg), and norfloxacin (NO, 10 μg). *P. aeruginosa* American Type Culture Collection 27853 was utilized as the quality control strain.

Classification of MDR and XDR isolates

Isolates were classified as MDR if they exhibited non-susceptibility to at least one drug in three or more antimicrobial classes. Those resistant to at least one agent in six or more antimicrobial categories were defined as XDR.

Phenotypic detection of carbapenemase production

All isolates were screened for carbapenem resistance using the Kirby–Bauer method with IMI and MEM (10 μg) disks, as per CLSI 2024 criteria. A zone of inhibition ≤18 mm indicated possible carbapenemase production. Suspected isolates were further analyzed using the MHT, CDDT with ethylenediaminetetraacetic acid (EDTA), and the mCIM in combination with EDTA-enhanced testing, EDTA-modified carbapenem inactivation method (eCIM).

Statistical analysis

Statistical analysis was performed using the Chi-square test to evaluate the association between resistance patterns and demographic variables such as gender and sample type. A $p<0.05$ was considered statistically significant. All statistical analyses were conducted using MedCalc software (version 22.014).

RESULTS

During the study period, 100 isolates of *Pseudomonas* spp. were obtained from diverse clinical specimens. There were slightly more isolates recovered from male patients (68%) than from female patients (32%). The male-to-female ratio is 2.1:1. The highest frequency of the isolates (41%) was recovered from patients in the old age group (61–100 years), whereas the lowest (7%) was from the young age group (1–20 years). Pus swabs were the predominant specimen source (49%), followed by ear swabs (14%), sputum (14%), urine (13%), ET aspirate (2%), mother's milk (2%), and nasal swabs (2%). The BAL and blood samples yielded the lowest frequency of isolates (1%). The 100 isolates had been obtained among patients hospitalized in different wards (surgery ward, 39%; medicine ward, 23%; ENT ward 14%; and other wards) (Table 1).

As shown in Table 2, IMI was the most effective antibiotic against *Pseudomonas* species, with 79% sensitivity, followed by MEM, with 75% sensitivity. The percentage sensitivity to other antibiotics in the present study was as follows: PTZ (67%), ATM (64%), TN (63%), CAZ (58%), and LEV (57%). Pseudomonas in the present study showed the highest resistance to cefpime (52%), followed by CIP (47%). In urine samples, both AK and NO showed 38.5% resistance.

Table 1: Demographic profile of patients with *Pseudomonas* spp. infections (n=100)

Parameters	No of *Pseudomonas* spp. isolated (n=100)
Gender	
Male	68
Female	32
Age distribution	
1–20 years	7
21–40 years	27
41–60 years	25
61–80 years	37
81–100 years	4
Type of clinical specimen	
Pus swab	49
Ear Swab	14
Sputum	14
Broncho alveolar lavage	1
Blood	1
ET aspirate	2
Mother milk	2
Nasal swab	2
Tissue	2
Urine	13
Hospital ward	
Dermatology	1
ENT	14
OBGYN	7
Medicine	23
Surgery	39
Pediatric	4
Respiratory	12

Table 2: Antibiotic susceptibility patterns of *Pseudomonas* spp. (n=100)

Antibiotic class	Antibiotics	Susceptible isolates (%)
Beta-lactam drugs	Piperacillin–tazobactam	67
Cephalosporin	Ceftazidime	58
	Cefipime	48
Monobactam	Aztreonam	64
Carbapenem	Imipenum	79
	Meropenem	75
Aminoglycosides	Tobramycin	63
	Amikacin (urine only)	61.5
Floroquinolones	Ciprofloxacin	52
	Levofloxacin	57
	Norfloxacin (urine only)	61.5

According to the susceptibility test results, 19% of *P. aeruginosa* isolates were categorized as MDR strains. The prevalence of XDR in *P. aeruginosa* strains was 24% (Fig. 1).

26 carbapenem-resistant isolates (resistant to IMI or MEM), as detected using the Kirby–Bauer disc diffusion method, were subjected to three phenotypic methods for carbapenemase detection. Among the 26 carbapenem-resistant isolates, 9 (34.6%) were obtained from females and 17 (65.4%) from male patients. The distribution of carbapenemase-producing Pseudomonas isolates among the clinical samples was as follows: Wound/pus swabs (n=14, 53.8%), sputum (n=5, 19.2%), urine (n=4, 15.4%), ear swabs (n=1, 3.8%), nasal swabs (n=1, 3.8%), and tissue (n=1, 3.8%). Carbapenemase was detected using the MHT, CDDT with EDTA (CDDT, IMP [10 μg], IMI + EDTA discs), and mCIM in conjunction with EDTA mCIM/eCIM. Of the 26 Pseudomonas isolates, 12 (46.2%) were MHT-positive and 14 (53.8%) were MHT-negative. The CDDT test was positive in 7 (26.9%) and negative in 19 (37.1%) isolates. The mCIM test results were positive for 10 (38.5%) and

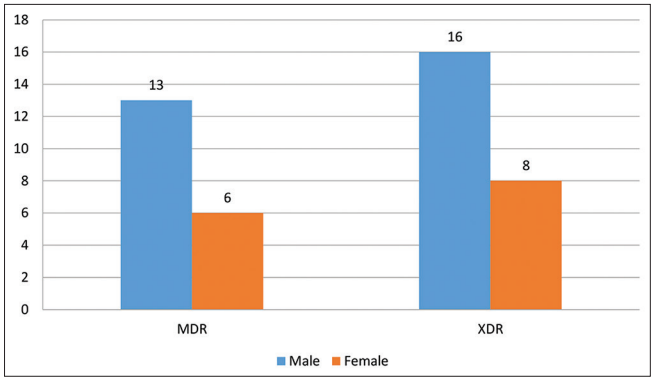

Fig. 1: Prevalence of multidrug resistance and extensively drug-resistant isolated strains according to gender

negative for 16 (61.5%) isolates. One (3.8%) of the total isolates tested was a serine-β-lactamasese producer, and 9 (34.6%) were a metallo-β-lactamase (MBL) producer (Table 3).

The performance of three phenotypic methods – the MHT, CDDT, and mCIM/eCIM – was evaluated in 26 carbapenem-resistant *P. aeruginosa* isolates. This study showed that mCIM has a better balance of sensitivity and specificity than MHT or CDDT.

Statistical analysis showed no significant association between gender and MDR/XDR status (p=0.987) or between clinical sample type and carbapenem resistance pattern (p=0.985). These results indicate that demographic and specimen-related variables did not significantly influence resistance distribution in the study population.

DISCUSSION

In this study, 100 clinical isolates of *P. aeruginosa* were obtained from various specimens. The male-to-female ratio of affected individuals was approximately 2.1:1, aligning with findings from previous studies by Sau *et al*. and Dash *et al*., who reported male predominance in their patient populations [12,13]. The highest prevalence was noted in elderly individuals (61–100 years), which may be attributed to weakened immunity, chronic illnesses, and prolonged hospital stays [14].

Nearly half of the isolates (49%) were recovered from pus or wound swabs, with the remaining isolates from ear swabs (14%), sputum (14%), and urine (13%). These findings highlight the pathogen's common association with wound and respiratory infections, consistent with reports from other Indian healthcare settings [15,16].

IMI demonstrated the highest efficacy among the tested antibiotics (79% sensitivity), followed by MEM (75%) and PTZ (67%). These results are in line with previous studies that recognized carbapenems as potent agents against multidrug-resistant *P. aeruginosa* [14,17,18]. On the other hand, CPM showed the highest resistance rate (52%), possibly due to overuse and increased selection pressure, as noted in earlier studies [19,20].

In this study, 19% of isolates were categorized as MDR and 24% as XDR, consistent with data from Mirzaei *et al*., who reported 16.5% MDR and 15.53% XDR prevalence [21]. Variability in local antimicrobial policies and infection control practices may explain differences in resistance patterns across regions [22].

Of the 26 carbapenem-resistant isolates, most (65.4%) were from male patients. The majority were derived from pus/wound swabs (53.8%), followed by sputum and urine. These isolates were further examined using MHT, CDDT, and mCIM/eCIM for carbapenemase detection. The MHT identified 46.2% as positive, CDDT detected 26.9%, and mCIM detected 38.5%. One isolate produced serine-β-lactamase, whereas nine were MBL producers.

Table 3: Comparison of three phenotypic methods for carbapenemase detection in *Pseudomonas aeruginosa* strains (n=26)

MHT positive		CDDT positive		mCIM positive		mCIM+eCIM positive	
Male	Female	Male	Female	Male	Female	Male	Female
6	6	5	2	6	4	6	2
Total 12 (46.2%)		Total 7 (26.9%)		Total 10 (38.5%)		Total 9 (34.6%)	

MHT: Modified Hodge test, CDDT: Combined double-disk synergy test, mCIM: Modified carbapenem inactivation method

Among the detection methods, mCIM showed better performance in identifying carbapenemase-producing strains, corroborating findings from earlier research [23]. The MHT, while widely used, is prone to false positives due to overlapping resistance mechanisms such as ESBL or AmpC combined with porin mutations [24]. The CDDT, which is specific for MBLs, identified 34.6% as MBL producers, similar to observations by Saeed *et al*. and Mirzaei *et al*. [17,21].

Only one isolate in this study produced serine carbapenemase, supporting previous reports that MBLs are more prevalent and more difficult to treat [25]. These patterns highlight the need for routine phenotypic screening to detect resistance mechanisms. As emphasized by Idelevich *et al*. and Bush *et al*., timely detection is crucial for initiating appropriate antimicrobial therapy and limiting hospital transmission [11,26].

CONCLUSION

This study highlights a considerable presence of MDR and XDR *P. aeruginosa*, particularly among elderly patients and those with wound-related infections. The resistance observed against frequently used antibiotics, including cephalosporins and fluoroquinolones, underscores the urgency for stringent antimicrobial stewardship.

Among the three phenotypic tests used for detecting carbapenemase activity, the mCIM demonstrated greater sensitivity and consistency, making it a valuable tool in routine diagnostic workflows. The significant proportion of MBL producers among carbapenem-resistant strains reinforces the need for early and reliable identification of resistance mechanisms.

To curb the spread of MDR and XDR *P. aeruginosa*, ongoing surveillance of antimicrobial resistance trends, timely phenotypic detection, and rational use of antibiotics are essential. Incorporating molecular diagnostic tools in future studies may enhance detection accuracy and complement the phenotypic findings presented here.

Limitation

This study was conducted research within a single tertiary care center with a limited sample size, which may not fully represent the broader epidemiological trends of antimicrobial resistance in other settings. The study concentrates solely on phenotypic methods for the detection of carbepenemase production.

Data are presented as numbers (%). Chi-square test applied for gender versus MDR/XDR status p=0.987 (n=100).

AUTHOR'S CONTRIBUTIONS

Ahuti Pandya: Conceptualization, Investigation, Writing – Original Draft, Project administration. Ria Kotecha: Methodology, Supervision, Writing – Review and Editing. Ujala Sarola: Literature Review, Data Curation, Visualization.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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