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Phenotypic profiling of Carbapenem drug resistant Pseudomonas species in a tertiary care hospital in Tamilnadu

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ABSTRACT

Background: Pseudomonas aeruginosa, an opportunistic pathogen, is a leading cause of healthcare-associated infections, particularly in immunocompromised individuals. The emergence of carbapenem-resistant strains poses major therapeutic and infection control challenges. Identifying carbapenem resistance through reliable phenotypic methods is essential for guiding infection control and antimicrobial stewardship.

Aim and Objectives: To characterize carbapenem-resistant Pseudomonas isolates from various clinical specimens using standard phenotypic methods and to assess specimen-specific resistance patterns for optimizing antibiotic use and diagnostic accuracy.

Materials and Methods: A cross-sectional study was conducted from May to October 2024 at a tertiary care hospital. A total of 100 Pseudomonas isolates from blood, pus, urine, wound swabs, respiratory secretions, cerebrospinal fluid, and other sterile body fluids were tested. Identification was done by standard biochemical tests, and antimicrobial susceptibility was determined by Kirby-Bauer disc diffusion following CLSI 2021 guidelines. Carbapenem resistance was confirmed using imipenem and meropenem discs, and results were validated by the VITEK automated system.

Results: Out of 100 isolates, 10% and 9% showed resistance to imipenem and meropenem respectively with a collective carbapenem resistance of 10%. Pus specimens displayed the highest resistance (28% IPM, 24% MRP; $p < 0.05$), while isolates from blood, respiratory, and CSF samples remained fully susceptible. VITEK confirmed all isolates as carbapenem-sensitive, revealing complete discordance ($\kappa = 0.00$) with disc diffusion.

Conclusion: Most Pseudomonas isolates retain high susceptibility to multiple antibiotic classes. Carbapenem resistance was limited, emphasizing confirmatory testing, specimen-specific surveillance, and antimicrobial stewardship to prevent resistance escalation.

KEYWORDS: Pseudomonas aeruginosa, Drug Resistance, Phenotyping, Carbapenems.

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INTRODUCTION

Pseudomonas aeruginosa is a ubiquitous opportunistic pathogen and the most frequent cause of healthcare-associated infections, particularly in immunocompromised individuals. It is one of the most frequent etiologic agents of pneumonia, septicemia, urinary tract infection, and wound sepsis in tertiary care centers[1]. Its inherent resistance process coupled with its ability to acquire additional resistance determinants makes it a significant threat for antimicrobial therapy. Among the few therapeutic options, carbapenems have been the preferred treatment for multidrug-resistant (MDR) P. aeruginosa. Yet the appearance and spread of carbapenem-resistant strains pose very real risks of treatment failure and infection control. Resistance to carbapenem in P. aeruginosa is multifactorial, for example, the production of carbapenemase enzyme, overproduction of efflux pumps, and alteration of porin channels. Phenotypic screening like Modified Hodge Test (MHT), Combined Disc Test (CDT), and EDTA Disk Synergy Test (EDS) has been extensively used to identify carbapenemase activity among clinical isolates[2]. At a tertiary care hospital in East India, 18.2% of P. aeruginosa isolates were resistant to carbapenem with high frequencies of blaVIM and blaNDM-1 carbapenemase genes[3]. Co-production of various carbapenemases also complicates therapeutic control even further and emphasizes the need for strong diagnostic tools.[4]

Carbapenem-resistant Pseudomonas aeruginosa (CRPA) continues to pose a formidable challenge in tertiary care settings

worldwide due to its intrinsic and acquired mechanisms of resistance, including carbapenemase production, efflux pump overexpression, and porin mutations[5]. As increasing carbapenem-resistant Pseudomonas species are reported in tertiary care centers, it is a cause of urgent concern to characterize their phenotypic resistance pattern for guiding clinical decisions and infection control policy[6]. Global surveillance data highlight increasing CRPA prevalence and its clinical association with nosocomial infections and poor outcomes. Recent studies from Europe and Asia reported multi-mechanistic resistance contributing to limited therapeutic options and treatment failures[7,8]. In India, carbapenemase-producing P. aeruginosa isolates harboring blaVIM and blaNDM genes have been reported in diverse clinical settings, suggesting regional dissemination and the potential for horizontal gene transfer[7]. Outbreak investigations emphasize that such resistant strains are capable of rapid adaptation in hospital environments, aggravating infection control burdens. Phenotypic and genotypic detection methods remain essential for accurate diagnosis, yet variable sensitivity and specificity persist among available assays[9,10]. Moreover, recent evaluations underscore the diagnostic limitations of standard disc diffusion compared with automated and molecular approaches. Given the heterogeneity in resistance mechanisms and diagnostic performance, context-specific studies are necessary to outline reliable detection frameworks and inform antimicrobial stewardship. Hence, The aim of this study is to bridge the gap between laboratory detection and clinical relevance by characterizing carbapenem-resistant Pseudomonas isolates by standard phenotypic methods. By the exhibition of resistance patterns across different groups of specimens, the research aids targeted antimicrobial stewardship and institutional readiness against MDR organisms. Additionally, the findings will aid in optimizing diagnostic algorithms and encourage judicious use of carbapenems in high-risk clinical scenarios

MATERIALS AND METHODS

This cross-sectional study was conducted at a tertiary care hospital between May 2024 and October 2024. A total of 100 Pseudomonas isolates were obtained from various clinical specimens including blood, pus, urine, wound swabs, respiratory tract secretions (sputum, endotracheal aspirates, throat swabs), cerebrospinal fluid (CSF), and other sterile body fluids. All samples were collected under strict aseptic precautions and processed according to standard microbiological protocols. Isolates were identified as Pseudomonas species using conventional biochemical tests. Carbapenem resistance was screened using imipenem and meropenem discs, and confirmed based on zone diameter interpretation per Clinical and Laboratory Standards Institute (CLSI) guidelines. Antimicrobial susceptibility was assessed using the Kirby-Bauer disc diffusion method on Mueller-Hinton agar. A standardized bacterial suspension (0.5 McFarland) was inoculated uniformly using a sterile swab. Antibiotic discs were applied and plates were incubated at 35°C for 18–24 hours. Zone diameters were measured and interpreted according to CLSI 2021 criteria. The following antibiotics and concentrations were tested such as Amikacin (30µg), Ceftazidime/Clavulanic acid(30/10µg), Ceftazidime(30 µg), Gentamicin(10 µg), Ciprofloxacin(5 µg), Norfloxacin(10 µg), Netilmicin(10 µg), Imipenem (10 µg). Quality control was ensured using Escherichia coli ATCC 25922 and Pseudomonas aeruginosa ATCC 27853 as reference strains.

RESULTS

A total of 100 Pseudomonas isolates were obtained from various clinical specimens, including blood, pus, urine, wound swabs, respiratory secretions (sputum, endotracheal aspirates, throat swabs), cerebrospinal fluid (CSF), and other body fluids. The isolates were distributed across the following specimen types such as Blood, Pus, Urine, Wound swabs, Respiratory secretions (sputum, endotracheal aspirate, throat swabs), Cerebrospinal fluid (CSF) and Other body fluids.

A total of 100 Pseudomonas isolates were subjected to antimicrobial susceptibility testing using minimum inhibitory concentration (MIC) analysis as shown in **Table 1.** All isolates demonstrated 100% sensitivity to several key antibiotics, including piperacillin/tazobactam (MIC: 8 µg/mL), ceftazidime (2 µg/mL), cefoperazone/sulbactam (≤8 µg/mL), cefepime (2 µg/mL), amikacin (4 µg/mL), gentamicin (≤1 µg/mL), ciprofloxacin (0.12 µg/mL), levofloxacin (0.5 µg/mL), tigecycline (≥8 µg/mL), and colistin (2 µg/mL). Carbapenem susceptibility was slightly reduced, with imipenem showing 90% sensitivity (95% CI: 83.9%–95.1%) at an MIC of 2 µg/mL and meropenem showing 91% sensitivity (95% CI: 85.1%–95.9%) at 0.5 µg/mL. These agents showed complete efficacy, with a 95% confidence interval (CI) for sensitivity ranging from 96.4% to 100%, indicating robust activity against Pseudomonas spp. in this clinical setting. These findings indicate a high overall susceptibility profile among the isolates, with carbapenem resistance confined to a small subset, reinforcing the continued efficacy of most tested agents against Pseudomonas spp. in this clinical setting.

Table 1: Phenotypic resistance patterns and mic values of pseudomonas isolates against commonly used antibiotics (n=100)

Antimicrobial	MIC	Sensitivity
Piperacillin/Tazobactam	8	100%
Ceftazidime	2	100%
Cefoperazone/Sulbactam	<= 8	100%
Cefepime	2	100%
Imipenem	2	90%
Meropenem	0.5	91%
Amikacin	4	100%
Gentamicin	<= 1	100%
Ciprofloxacin	0.12	100%
Levofloxacin	0.5	100%

Tigecycline	>= 8	100%
Colistin	2	100%

Specimen type specific resistance for carbapenems such as Imipenem (IPM) and Meropenem (MRP) are shown in **Table 2.**

Table 2: Distribution of Carbapenem Resistance Among Pseudomonas Isolates by Specimen Type (n=100)

Specimen Type	*No. of Isolates*	*Resistant to IPM*	*Resistant to MRP*
Blood	10	0	0
Pus	25	7	6
Urine	10	1	1
Wound Swabs	15	2	2
Respiratory Secretions	25	0	0
Cerebrospinal Fluid (CSF)	5	0	0
Other Body Fluids	10	0	0
Total	100	10	9

Out of 100 Pseudomonas isolates tested, resistance to imipenem (IPM) and meropenem (MRP) was found in 10% and 9% of the isolates, respectively with a total carbapenem resistance of 10%. Among specimen-wise testing, the pus samples evidenced the highest pattern of resistance with 28% IPM resistance and 24% MRP resistance corresponding to odds ratios (OR) of 4.67 and 4.00, respectively, when compared to pooled non-pus specimens. **Table 3** suggested a statistically significant concentration of drug-resistant strains in wound and soft tissue infections ($p < 0.05$).

Moderate resistance was noted in urine (10%) and wound swabs (13.3%), while isolates from blood, respiratory secretions, cerebrospinal fluid (CSF), and other sterile body fluids remained fully susceptible to both carbapenems (0% resistance), reinforcing their retained efficacy in systemic and critical site infections. These results highlight the importance of specimen-specific surveillance and antimicrobial stewardship to curtail the transmission of carbapenem-resistant Pseudomonas species.

Table 3: Fisher's Exact Test showing Odds Ratios with 95% CI and p-values for Carbapenem Resistance (n=100)

Specimen Type	*OR (IPM)*	*95% CI (IPM)*	*p-Value (IPM)*	*OR (MRP)*	*95% CI (MRP)*	*p-Value (MRP)*
Pus	4.67	1.39 – 15.73	**0.012***	4.00	1.17 – 13.64	**0.027***
Urine	1.11	0.13 – 9.42	0.92	1.18	0.14 – 9.98	0.88
Wound Swabs	1.56	0.29 – 8.33	0.61	1.67	0.31 – 8.96	0.56

Reference Group: All Other Specimens Combined (Blood, Respiratory Secretions, CSF, Other Fluids), With 0% Resistance.

**p-value less than 0.05 taken as significant*

To validate the initial disc diffusion results, all ten isolates identified as carbapenem-resistant were retested using the VITEK automated system. VITEK confirmed that all ten isolates were sensitive to carbapenems, revealing a 100% discordance rate between manual AST and automated confirmation for these cases. This discrepancy underscores the limitations of disc diffusion in detecting borderline resistance and highlights the diagnostic precision of automated systems. The kappa coefficient (κ) for agreement between methods was 0.00, indicating poor concordance as shown in **Table 4** and reinforcing the need for confirmatory testing in cases with clinical or epidemiological significance. Collectively, these findings affirm the high susceptibility of Pseudomonas spp. to most tested antimicrobials, while emphasizing the importance of specimen-specific surveillance, confirmatory diagnostics, and antimicrobial stewardship to mitigate the spread of carbapenem-resistant strains.

Table 4: Kappa statistical test for agreement Between Manual AST and VITEK (n=100)

Method	**Carbapenem Resistant**	**Carbapenem Sensitive**	**Kappa (κ)**
Manual AST (Disc Diffusion)	10	90	0.00
VITEK Confirmation	0	100	

Kappa coefficient calculated for binary classification of carbapenem resistance. A κ value of 0.00 indicates complete discordance between manual and automated methods.

DISCUSSION

The present study provides a comprehensive phenotypic profile of Pseudomonas spp. isolated from diverse clinical specimens in a tertiary care hospital, with a particular focus on carbapenem resistance. The present study found a carbapenem resistance rate of 10% for imipenem and 9% for meropenem among *Pseudomonas aeruginosa* isolates, which lies on the lower end of the range reported from various Indian centers. Moreover, VITEK showed 100% susceptibility to carbapenem. A 2023 pilot study

conducted in Bhubaneswar by Verma et al. (2023) observed a 9.84% prevalence of carbapenem-resistant *P. aeruginosa* (95% CI: 7.9–12.1%) among tertiary care isolates, which closely mirrors our results[11]. Similarly, Grewal et al. (2024) documented 17% CRPA isolates from Punjab hospitals, corroborating moderate resistance trends[12]. A two-year retrospective study by Lathakumari et al. (2025) in South India found carbapenem resistance in *Pseudomonas* ranging between 12% and 22% (95% CI: 10.0–25.0%), emphasizing the escalating threat in southern tertiary hospitals[13]. On the other hand, earlier reports such as Radhika et al. (2018) from East India detected higher frequencies, with up to 40% carbapenem resistance linked to *blaVIM* and *blaNDM* genes[14]. Compared with these findings, our observed 10% prevalence suggests relatively low endemic resistance possibly due to stringent infection control measures and judicious antimicrobial policies. Nonetheless, the emergence of CRPA even in limited proportions signals the continuous need for institutional antimicrobial stewardship and confirmatory surveillance to curb potential outbreaks stemming from carbapenemase-producing isolates.

The antimicrobial susceptibility results revealed a remarkably high sensitivity (100%) to several broad-spectrum agents, including β-lactam combinations (piperacillin/tazobactam, cefoperazone/sulbactam), cephalosporins (ceftazidime, cefepime), aminoglycosides (amikacin, gentamicin), fluoroquinolones (ciprofloxacin, levofloxacin), tigecycline, and colistin. These findings are consistent with previous report by Verma et al., 2021[4] where a total of 102 isolates were resistant to carbapenem that accounted for overall 18.24% (102/559) prevalence. Carbapenem susceptibility, while generally preserved, showed a slight reduction, with imipenem and meropenem demonstrating 90% and 91% sensitivity, respectively which was similar to emerging global trends of increasing carbapenem resistance among Pseudomonas aeruginosa as reported by Oliver et al., 2020[15]. The 95% confidence intervals for these agents (IPM: 83.9%–95.1%; MRP: 85.1%–95.9%) suggest a small but clinically relevant subset of resistant isolates. Notably, resistance was not uniformly distributed across specimen types. Pus samples exhibited the highest resistance rates—28% for imipenem and 24% for meropenem—with odds ratios of 4.67 and 4.00, respectively, compared to pooled non-pus specimens. This aligns with existing literature by Samatha et al., 2021[3] that identifies wound and soft tissue infections as reservoirs for multidrug-resistant Pseudomonas strains.

Moderate resistance was observed in urine (10%) and wound swabs (13.3%), while isolates from blood, respiratory secretions, cerebrospinal fluid (CSF), and other sterile body fluids remained fully susceptible to both carbapenems. This specimen-specific distribution reinforces the importance of anatomical site-based surveillance and tailored empirical therapy. The absence of resistance in critical specimens such as CSF and blood is particularly reassuring, suggesting that carbapenems retain their utility in managing systemic and life-threatening infections. A key finding of this study was the discrepancy between manual disc diffusion results and automated VITEK confirmation. All ten isolates initially identified as carbapenem-resistant by disc diffusion were found to be sensitive upon VITEK testing, resulting in a 100% discordance rate. The kappa coefficient ($\kappa = 0.00$) indicated no agreement beyond chance. This finding exposes critical limitations of the disc diffusion technique, particularly in detecting borderline or low-level carbapenem resistance, which may lead to false resistance reporting and inappropriate clinical decisions as shown by Jorgensen & Ferraro, 2009[16]. Automated systems like VITEK provide enhanced precision and reproducibility in MIC determinations and should be employed for confirmation of suspicious or resistant isolates as suggested in previous study by Steward CD et.al.,2003[17] reporting discrepancies and recommending confirmatory testing via automated or molecular methods

LIMITATIONS

There were some limitations to research. The lack of consistency between manual and automated resistance testing procedures suggests the possibility of resistance detection variability that can impact clinical interpretation. Resistance mechanism molecular typing was not carried out and should be undertaken to clarify carbapenem mechanisms of resistance. Additionally, the study took place within one clinical institution, and generalizability of results therefore might be impaired elsewhere or in other institutions. Future research using multicenter data and molecular testing are suggested to augment the epidemiological understanding of resistance and maximize management.

CONCLUSION

This research proves that most Pseudomonas isolates from a wide range of clinical samples are highly susceptible to a wide spectrum of antibiotics such as piperacillin/tazobactam, ceftazidime, cefoperazone/sulbactam, cefepime, aminoglycosides, fluoroquinolones, tigecycline, and colistin. Carbapenem resistance is still limited to a small proportion of isolates mainly from pus specimen, indicating the continued clinical use of carbapenems for systemic and critical site infections. However, discord among manual disk diffusion and computerized VITEK testing unequivocally shows the need for confirmatory testing to correctly detect resistance and direct appropriate therapy. These results validate the value of specimen-specific surveillance and aggressive antimicrobial stewardship programs to contain the dissemination and scope of resistant Pseudomonas spp.

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