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IP International Journal of Medical Microbiology and Tropical Diseases

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Original Research Article

Phenotypic detection of carbapenemase producing *Pseudomonas aeruginosa* isolated from clinical specimen in a tertiary care hospital

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Abstract

Aim and Objectives: The significant nosocomial pathogen *Pseudomonas aeruginosa* is inherently resistant to several medications. Metallo-beta-lactamases (MBLs) are a specific type of carbapenemase, belonging to class B enzymes. MBL producing *Pseudomonas aeruginosa* are a significant concern due to their ability to hydrolyze a wide range of beta-lactams, including carbapenems and has far higher mortality rates. Aim of the present study is to detect and evaluate the presence of carbapenemase-producing *Pseudomonas aeruginosa* isolates from clinical specimens using phenotypic methods in a tertiary care hospital setting. Also to assess the prevalence of carbapenemase-producing *P. aeruginosa* in the hospital's clinical setting.

Materials and Methods: In the current investigation, *Pseudomonas aeruginosa* isolates have been identified using standard biochemical reactions and an antibiotic susceptibility test. The imipenem disk diffusion method was employed as a screening test to select suspected MBL producers. The strains were further confirmed by imipenem-(Ethylene diamine tetra acetic acid) EDTA combined disc method and imipenem- EDTA double disc synergy test.

Result: Out all 50 isolates *Pseudomonas aeruginosa*, 14 (28%) produced MBL, while 36 (72%) did not. The DDST (Double Disk Synergy Test) and CDST (Combined Disc Synergy Test) techniques had respective sensitivity levels of 78.5% & 92.8%. Compared to isolates of *Pseudomonas aeruginosa* that do not produce MBL, those that exhibit high levels of resistance to ceftazidime and all other antibiotics. The isolates that produced MBL are 100% resistant to ciprofloxacin, tobramycin and meropenem, followed by imipenem (92.8%) & gentamicin (85.7%).

Conclusion: According our study, *P. aeruginosa* that produced MBL exhibited the greatest degrees of fluoroquinolone, aminoglycoside, and carbapenem resistance. This implies that the gene that produces MBL have co-resistance to quinolones, aminoglycosides, and other antibiotic families. Prompt evidence that rules out carbapenemases guides clinicians to more optimal therapeutic selections based on local phenotypic profiling of non-carbapenemase-producing, carbapenem-resistant *P. aeruginosa*. Also, rapid confirmation and differentiation among the various classes of carbapenemases is key to the initiation of early effective therapy.

Keywords: Carbapenemase, Drug Resistance, *Pseudomonas aeruginosa*

Received: 27-03-2025; **Accepted:** 01-05-2025; **Available Online**: 28-05-2025

1. Introduction

Pseudomonas aeruginosa is commonly linked to nosocomial outbreaks among hospitalized or vulnerable individuals and is a prevalent isolate in hospital settings. These hospital isolates frequently exhibit a variety of multidrug resistance pathways as a survival strategy due to their persistence in the hospital setting.[1,2] Consequently, the quantity of antibiotics that are available for treatment is reduced. The recommended medication for severe *Pseudomonas aeruginosa* infections is carbapenems.

Pseudomonas aeruginosa is among several nosocomial pathogens which is resistant to several antibiotics. Since it can hydrolyze all βlactams except monobactams, genetically transportable Thus far, the most adaptable β-lactamases discovered are metallo-β-lactamases (MBL).[3]

MBLs are the primary reason why people develop resistance to carbapenem in *Pseudomonas aeruginosa*.[4] Due to the common beta-lactamase inhibitors, the majority of beta lactam antibiotics sulbactam or clavulanic acid do not inhibit MBLs, which are broad-spectrum enzymes that are broken down through all but monobactams.[5] In Intensive care units (ICUs) of the hospital, the overuse of carbapenem has caused

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http://doi.org/10.18231/j.ijmmtd.2025.023

P. aeruginosa to produces MBL alarmingly high.[6] The widespread use of broad-spectrum antibiotics within the ICU creates great selection pressure. This results in the elimination of competing flora, which favors the selection of drug-resistant strains.[6]

Members of Enterobacteriaceae have acquired MBL genes from *P. aeruginosa* in recent years.[7] Notably in patients who are very sick, substantial rates of morbidity and mortality (between 27% and 48%) have been noted. Additionally, mortality rates of MBL-producing *P. aeruginosa* (MBL-PA) are significantly higher than those of non-MBLPA. Although molecular methods like Polymerase Chain Reaction (PCR) is a very reliable also accurate method, it is sometimes only available in reference laboratories, and as of now, there is no established technique for detecting MBL.[4]

1.1. Importance of the study

1. *Pseudomonas* species' high levels of antibiotic resistance are a serious risk to hospitalized patients. Antimicrobial stewardship and a rigorous surveillance program to identify and manage environmental pollution are the two key strategies most likely to stop the spread of this bacterium in hospitals.
2. Metallo beta lactamase (MBL) and other carbapenemases must be detected in order to decide on the most effective treatment plan for *Pseudomonas* species that are resistant to carbapenem.
3. In addition to assisting in application for particular hospital infection control protocols, epidemiological research on resistant strains helps to restrict the spread of these strains both within and outside of hospital settings.

2. Objectives

1. To determine the antibiograms of *Pseudomonas aeruginosa* isolated from clinical specimen.
2. Detection of Metallo-Beta Lactamase producing *Pseudomonas aeruginosa* among isolated strains.
3. Evaluation of different phenotypic methods in detecting carbapenemase-producing *Pseudomonas aeruginosa.*

3. Materials and Methods

3.1. Study design

This Laboratory Based Cross section study was performed in the "Department of Microbiology at Navodaya Medical College, Hospital and Research Center, Raichur, Karnataka", after obtaining the approval from the Institutional Ethics Committee.

3.2. Sample size

In the current investigation, we examined 50 *Pseudomonas aeruginosa* isolates for drug resistance and phenotypic metallo-beta lactamase (MBL) production for a period of three months.

3.3. Inclusion criteria

A number of clinical specimens from patients admitted to ICUs and medical along with surgical wards were sent into a microbiology laboratory for bacterial cultures as well as sensitivity tests. These samples contained consecutive *P. aeruginosa* isolates that are not duplicates.

3.4. Exclusion criteria

1. Patients from outpatient clinics were not included.
2. The patients tested positive for other microbes in their cultures.

P. aeruginosa patients were monitored throughout their hospital stay in order to ascertain risk variables, treatment response, and fatality rates.

3.3.1. Identification of P. aeruginosa

Standard laboratory procedures will be followed. Mueller-Hinton agar plates are subjected to antimicrobial sensitivity testing applying the Kirby-Bauer disk diffusion approach following the principles set forth by the "Clinical Laboratory Standards Institute (CLSI)".[8] The disk diffusion technique has been applied in research following CLSI guidelines and using antibiotics (Hi-Media, Mumbai, India): "ciprofloxacin (5µg), ceftazidime (30µg), amikacin (30µg), piperacillin/tazobactam (100µg/10µg), imipenem (10µg), gentamicin (10µg), netilmycin (30µg), polymyxin-B (300 units), and colistin (10µg).(**Figure 1**)

The imipenem-resistant MBL-PA strains found using the imipenem disk diffusion method had been verified includes screening tests utilizing the imipenem-EDTA double disk synergy test or the imipenem-EDTA combination disk method and MBL-E-strip method (**Figure 3**). Following its creation with distilled water, 0.5M EDTA (Hi-Media, Mumbai, India) being autoclave sterilized. To add EDTA, ten microliters from this solution have been applied to each imipenem disk.

3.5. Imipenem-EDTA combined disk method (CDT)

The procedure outlined by Yong et al. was followed.[9] The test isolates were cultivated by lawn culture method. Across the surface of agar plates, two imipenem discs—one containing 0.5 M EDTA along with the other containing a simple imipenem disc—were positioned roughly 30 mm apart after being allowed to dry for five minutes. The plates had been incubated at 37^{O}C all night. Compared to the imipenem disk alone, a zone diameter increase of almost 7 mm around the imipenem + EDTA disk suggested the establishment of MBL.

3.6. Imipenem-EDTA double disk synergy test (DDST)

This procedure was carried out as Lee et al.[10] As advised by CLSI, test organisms were introduced into Mueller Hinton agar plates. An imipenem (10µg) disk had been placed 20 mm in the center of a blank disk that contained 10µL of 0.5 M EDTA (750µg). A favorable outcome for MBL production was found by comparing the zone with inhibition along the drug's far side against the area of inhibition between the EDTA disk and imipenem.(**Figure 2**)

3.7. Statistical analysis

Each finding was documented in a data sheet in MS Excel, and descriptive statistics will be used as needed to analyze the data after the study is complete. The statistical significance associated with the discrepancies in the results will be evaluated utilizing Fisher's precise tests and the chi-square. If a p-value is less than 0.05, it is considered statistically significant. SPSS version 16.0, a statistical analysis software program, will be used to do the statistical analysis.

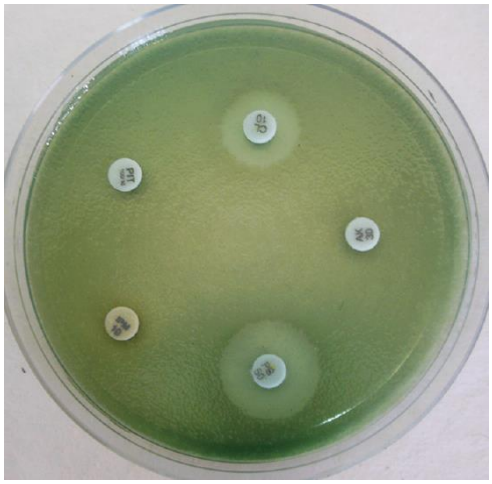


Figure 1: Antibiogram of *Ps. Aeruginosa*

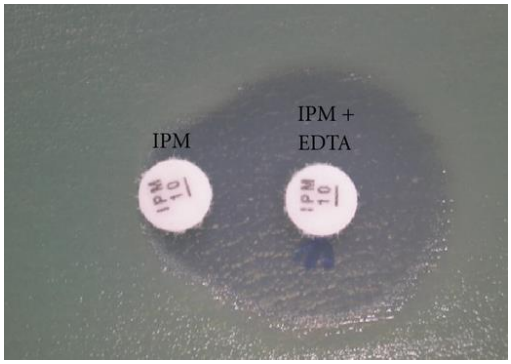


Figure 2: Detection of MBL-production by DDST method

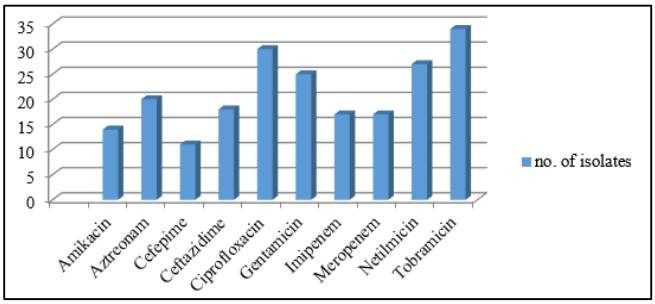
Figure 3: Detection of MBL-production by E-strip method

4. Result

Pseudomonas aeruginosa isolates from 50 consecutive, non-repetitive its antibiotic susceptibility test was used to isolates from various clinical specimens. Furthermore, using phenotypic techniques, the synthesis of MBL was assessed in each isolate of *Pseudomonas aeruginosa*. **Table 1** shows that Pus was the most often isolated organism from (52%), then urine (26%), sputum (16%), blood (4%), or tissue (2%) in that order.

Thirteen (28%) Among the 50 *Pseudomonas aeruginosa* isolates, 36 (72%) did not produce MBL. According to **Table 2**, the DDST and CDST methodologies' respective sensitivity was 78.5% and 92.8%.

When compared to isolates of *Pseudomonas aeruginosa* that do not produce MBL, those that do showed a high degree of resistance to every antibiotic except nitilmicin and ceftazidime. **Table 3** shows which isolates that were responsible for producing MBL were 100% resistant to ciprofloxacin, tobramycin, along with meropenem, with imipenem (92.8%) & gentamicin (85.7%) following closely behind. In contrast, the isolates that did not produce MBL exhibited resistance to these antibiotics at 44.4%, 53.5%, 8%, 1.1%, and 36.1%, respectively.



Graph 1: Antibiotic resistance pattern of pseudomonas aeruginosa strains

Table 1: Sample -wise distribution of *Pseudomonas aeruginosa* isolates

Type of sample"	***Pseudomonas aeruginosa* isolates**	
	No.	%
Pus	26	52
Urine	13	26
Sputum	8	16
Blood	2	4
Tissue	1	2

Table 2: MBL-producing *Pseudomonas aeruginosa* by phenotypic method

No. of isolates	**Non-MBL-producing isolates**	**MBL- producing isolates n (%)**		
		DDST method	**CDST method**	**MBL Producing isolates**
50	**36 (72%)**	11	13	**14 (28%)**
"DDST-Double-disk synergy test				
CDST- Combined disk synergy test"				
MBL- Metallo beta lacamase				
n= number of isolates				

Table 3: Comparison of antibiogram of MBL-Positive & MBL-negative *P. aeruginosa* isolates

Antibiotics	**No. of resistance *P. aeruginosa* isolates**		**Statistical analysis ***
	MBL" producers n=14 (%)	**Non-MBL Producers n=36 (%)**	
Amikacin	6 (42.8)	8 (22.2)	p<0.001
Aztreonam	8(57.1)	12 (33.3)	p<0.001
Cefepime	6(42.8)	5 (13.8)	p<0.001
Ceftazidime	5(35.7)	12 (33.3)	p=0.428
Ciprofloxacin	14(100)	16 (44.4)	p<0.001
Gentamicin	12(85.7)	13 (36.1)	p<0.001
Imipenem	13 (92.8)	4 (11.1)	p<0.001
Meropenem	14(100)	3 (8.3)	p<0.001
Netilmicin	8(57.1)	19 (52.7)	p=0.124
Tobramicin	14 (100)	20 (55.5)	p<0.001
MBL-Metallo-beta lactamase			
* Chi square test			

5. Discussion

In our investigation, 28% of the isolates of *Pseudomonas aeruginosa* had carbapenem resistance. Vitkauskiene et al. showed a similar case was found at the prevalence of *P. aeruginosa* that produces MBL was 15.8% in 2003 and 61.9% in 2008, a 46% increase in just five years.[11] 10–30% of clinical specimens in India have been shown to include *P. aeruginosa* that produces MBL.[12] In contrast, a research was conducted in Chennai, India, by Hemalatha V et al., discovered a lower prevalence for *P. aeruginosa* that produces MBL (14%).[13]

In the present study, 25% of the isolated *Pseudomonas aeruginosa* were MBL producers, similar to our study, Anooja and Anu PJ in Kerala state, a southern state of India showed 30% of the *Pseudomonas aeruginosa* isolates were carbapenemase producers.[14] Fred CT et al reported that in United States, 10-30% of *Pseudomonas aeruginosa* isolates were carbapenem resistant.[15]

However, in our research, *P. aeruginosa* that produce MBL are a significant contributor to resistance to widely used antimicrobial drugs. The isolates that produced MBL exhibited most severe resistance to aminoglycosides, carbapenems, and fluoroquinolones. Similar to our results, a number of investigations have shown that MBL-producing bacteria are more resistant to practically all types of antibiotics than MBL-negative strains. [11]. This implies that the gene that produces MBL may have co-resistance to quinolones, aminoglycosides, along with other antibiotic families.

6. Conclusion

In summary, the number of *P. aeruginosa* isolates that produce MBL in hospital settings is rising daily, which is

causing more failures to treat with commonly used antibiotics. Although the epidemiological traits of *P. aeruginosa* that produce MBL and those that do not are same, they differ significantly in their level of antibiotic susceptibility. It is crucial to identify regularly and molecularly characterize *P. aeruginosa* strains which generate MBL are tested in vitro before being treated with antibiotics to prevent and control infections and reduce the negative consequences linked to MBL-producing strains.

Prompt evidence that rules out carbapenemases guides clinicians to more optimal therapeutic selections based on local phenotypic profiling of non-carbapenemase-producing, carbapenem-resistant *P. aeruginosa*.

7. Conflict of Interest

The authors declare that there is no conflict of interest.

8. Source of Funding

None.

9. Acknowledgments

We are grateful to Dr. T. Srinivas, Registrar, Navodaya Education Trust, Raichur and Dr. Arun K N, Medical Superintendent, Navodaya Medical College Hospital & Research Centre, Raichur, Karnataka for their unconditional guidance and support.

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Cite this article: Harwalkar A, Surpur R, Hajare V, Verma S. Phenotypic detection of carbapenemase producing *Pseudomonas aeruginosa* isolated from clinical specimen in a tertiary care hospital. *IP Int J Med Microbiol Trop Dis.* 2025;11(2):146-150.