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Antibiotic Resistance in Metallo-β-Lactamase-Producing Pseudomonas aeruginosa in Clinical Isolates: Challenges and Phenotypic Detection in a Tertiary Care Setting

Tulika Harsh1, Dr.Harsha V.Patil2, Dr.Satish R. Patil3

1PG Student of Department of Microbiology, Krishna Vishwa Vidyapeeth 'Deemed To Be University" Karad Maharashtra, India.

2Associate Professor of Department of Microbiology, Krishna Vishwa Vidyapeeth 'Deemed To Be University" Karad Maharashtra, India.

3Professor and head of Department of Microbiology, Krishna Vishwa Vidyapeeth 'Deemed To Be University" Karad Maharashtra, India.

[1].tulikasingh4669@gmail.com

[2].harshap_kvv@outlook.com

[3]. patil.drsatish@gmail.com

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Abstract

Background: Pseudomonas aeruginosa is a major cause of hospital infections because of its drug resistance mechanisms and ecological adaptability. It can survive on both living and non-living surfaces, including antiseptic solutions, and can spread resistance in hospitals. Metallo-beta-lactamases (MBLs) are resistant to current β-lactamase inhibitors, with no new inhibitors in development, making their spread a serious clinical concern. Rapid identification of MBLs is vital for patient care and infection control. This study aims to estimate the accuracy of phenotypic tests for detecting MBL-producing Pseudomonas aeruginosa isolates.

Material and methods: This laboratory based study was carried out in the Department of Microbiology throughout the course of two years (November 2021 to November 2023). A total of 410 Pseudomonas aeruginosa isolates were obtained and of which 118 isolates that were resistant to imipenem coming from clinical samples of both outpatients and inpatients were tested for metallo-beta-lactamase (MBL) production using phenotypic methods including the Imipenem-EDTA Combined Disc Test, Ceftazidime- EDTA Combined Disc Test and Double Disc Synergy Test.

Result: In this investigation, it was found that 110 out of 118 imipenem resistant clinical isolates were positive for MBL production. The Imipenem-EDTA combined disc test identified 75.42% of the positive cases, while the Ceftazidime-EDTA combined disc test found 77.11%, and the double disc synergy test detected 50%. Most MBL-producing isolates were from urine samples (50.9%), primarily isolated from the surgical ward (29.10%). Sensitivity to cefepime was observed in 30% of the isolates, followed by amikacin (25.45%). The overall prevalence of MBL-producing Pseudomonas aeruginosa *was 26.82%.*

Conclusion: Acquired MBLs observed in Pseudomonas aeruginosa *are a major concern attributed to their ease of spread and broad antibiotic resistance. In this study, 26.82% of strains were MBL producers, mostly from the surgery ward, with high resistance to Imipenem (100%), Ceftriaxone (98.19%), and Polymyxin B (98.19%).*

Some sensitivity was noted to Cefepime (30%) and Amikacin (25.45%). Imipenem –EDTA combined disc test (CDT) and Imipenem –EDTA double disc synergy test (DDST), which are affordable and simple to perform, must be implemented in each clinical microbiology laboratory to identify MBLs in Pseudomonas aeruginosa and to enhance disease management.

Keywords: *Pseudomonas aruginosa, Imipenem, Metallo beta lactamase, Imipenem- EDTA combined disc test, Ceftazidime- EDTA combined disc test, Imipenem –EDTA double disc synergy test*

I. Introduction

Pseudomonas aeruginosa is a Gram-negative, aerobic bacterium widely recognized as an opportunistic pathogen that poses a severe threat in healthcare settings, especially for immunocompromised patients [1]. The resilience of this microorganism is due to its diverse mechanisms of antibiotic resistance and its adaptability to survive on various surfaces, including medical equipment and antiseptic solutions. This adaptability allows *P. aeruginosa* to become a formidable cause of hospital-acquired infections (HAIs), leading to diseases such as pneumonia, bloodstream infections, urinary tract infections, and surgical site infections. The presence of drug-resistant strains of *P. aeruginosa*, especially those producing metallo-β-lactamase (MBL) enzymes, has significantly complicated the treatment of these infections, raising concerns over patient outcomes and infection control strategies.

One of the critical elements in the antibiotic resistance of *P. aeruginosa* is its production of metallo-β-lactamase enzymes. These enzymes are a subclass of β-lactamases that hydrolyze a broad spectrum of β-lactam antibiotics, including carbapenems, which are often considered last-resort treatments for multidrug-resistant infections. Unlike other β-lactamases, MBLs require zinc ions for their activity and are resistant to traditional β-lactamase inhibitors, making treatment options severely limited [2]. Carbapenem-resistant *P. aeruginosa* (CRPA), particularly MBL-producing strains, are increasingly prevalent worldwide, with multiple hospital outbreaks reported. The spread of these MBL-producing strains within hospitals poses a significant public health risk due to their association with high morbidity and mortality rates, especially among critically ill patients in intensive care units (ICUs).

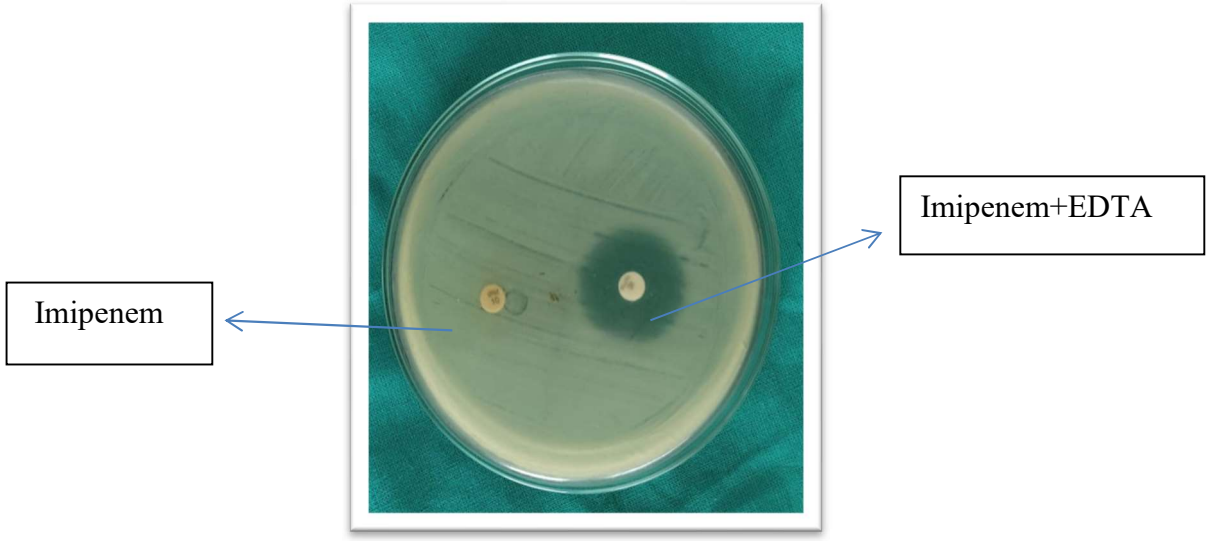


Figure. 1 Imipenem- EDTA Combined Disc Test

The genetic basis of MBL production in *P. aeruginosa* further amplifies its threat in healthcare settings. MBL genes are often located on mobile genetic elements, such as plasmids and transposons, facilitating horizontal gene transfer between bacteria [3]. This genetic mobility allows MBL resistance to spread rapidly within hospital environments, particularly under conditions of antibiotic selection pressure. Moreover, MBL genes are commonly linked with resistance to other antibiotic classes, including aminoglycosides and fluoroquinolones, leading to multidrug-resistant *P. aeruginosa* strains that are challenging to manage and treat. The rapid dissemination of MBL-producing *P. aeruginosa* within healthcare facilities underscores the need for effective surveillance, prompt identification, and strict infection control measures to prevent the spread of these resistant strains.

The emergence of MBL-producing *P. aeruginosa* has spurred an urgent need for reliable and accessible methods to detect these strains, especially in resource-limited settings where molecular testing may not be feasible. Phenotypic methods, such as the Imipenem-EDTA Combined Disc Test (CDT), Ceftazidime-EDTA Combined Disc Test, and the Imipenem-EDTA Double Disc Synergy Test (DDST), offer cost-effective approaches to screen for MBL activity in clinical isolates [4]. These tests exploit the ability of EDTA, a metal chelator, to inhibit the action of MBLs by removing the essential zinc ions required for enzyme activity. Phenotypic tests are thus valuable tools in the early identification of MBL-producing *P. aeruginosa*, aiding in infection control measures and guiding appropriate antimicrobial therapy.

Understanding the epidemiology of MBL-producing *P. aeruginosa* is essential to developing targeted infection prevention and control strategies. The prevalence of these resistant strains varies globally, influenced by factors such as antibiotic usage patterns, infection control practices, and local epidemiological trends [5]. Studies in India and other regions with high rates of antibiotic consumption have reported a particularly high prevalence of MBL-producing *P. aeruginosa* in clinical settings. These findings highlight the need for continuous surveillance to monitor resistance trends and to evaluate the efficacy of phenotypic detection methods across diverse healthcare settings.

In addition to its clinical implications, MBL-producing *P. aeruginosa* presents significant challenges for public health due to the limited treatment options available for infections caused by these strains. Treatment of infections caused by MBL-producing *P. aeruginosa* is often complex, involving combinations of antibiotics that may have limited efficacy [6]. The increasing resistance to carbapenems and other β-lactam antibiotics necessitates the development of alternative treatment strategies and new antibiotics capable of overcoming MBL resistance mechanisms. Moreover, the genetic adaptability of *P. aeruginosa* implies that even newly developed antibiotics may soon encounter resistance, further complicating treatment efforts.

The current study aims to address these challenges by investigating the prevalence of MBL-producing *P. aeruginosa* in a tertiary care hospital and assessing the efficacy of phenotypic tests in detecting MBL activity. By analyzing the prevalence and resistance patterns of MBL-producing strains, this study seeks to provide valuable insights into the spread of carbapenem-resistant *P. aeruginosa* within the hospital environment and inform the development of effective infection control and antibiotic stewardship strategies. Through the use of phenotypic detection methods, this research also aims to establish practical diagnostic approaches for identifying MBL-producing *P. aeruginosa* in clinical microbiology laboratories, particularly in settings where molecular techniques are not readily accessible.

II. Literature Review

Global and Regional Prevalence of MBL-Producing *Pseudomonas aeruginosa*

The emergence and spread of metallo-β-lactamase (MBL)-producing *Pseudomonas aeruginosa* have become a global health concern due to their impact on patient outcomes and the effectiveness of available antibiotic treatments. Reports from various regions highlight a concerning prevalence of MBL-producing *P. aeruginosa*, especially in countries with high antibiotic consumption and limited resources for infection control. Studies from Europe, Asia, the Middle East, and North America demonstrate significant regional differences in the prevalence of these resistant strains, influenced by factors such as healthcare practices, antibiotic stewardship, and local epidemiological patterns [7].

In European countries, surveillance programs have identified a steady rise in MBL-producing *P. aeruginosa* isolates, with notable increases in hospital-acquired infections among critically ill patients. Countries such as Greece, Italy, and Turkey have reported some of the highest prevalence rates, attributed to widespread use of carbapenem antibiotics in intensive care units (ICUs) [8]. For instance, a multi-center study conducted in Greece found that nearly 40% of *P. aeruginosa* isolates in ICUs exhibited MBL production, which underscores the critical need for stringent infection control measures.

The prevalence of MBL-producing *P. aeruginosa* is particularly high in Asia, where countries like India, China, and South Korea report a growing burden of these resistant strains. Studies from India have demonstrated a prevalence ranging between 20-45% in various tertiary care hospitals, making it one of the most affected regions globally. This high prevalence is associated with frequent and often unregulated antibiotic use, which creates a selection pressure for resistant organisms [9]. Similar trends are observed in China and South Korea, where studies indicate a rise in MBL-producing *P. aeruginosa* as a result of the extensive use of broad-spectrum antibiotics in healthcare settings.

In the Middle East, reports indicate that MBL-producing *P. aeruginosa* poses a substantial challenge for healthcare systems. Studies in Saudi Arabia and Iran reveal high prevalence rates, particularly in ICUs where carbapenem usage is common. In North America, while the prevalence of MBL-producing *P. aeruginosa* is generally lower than in Asia or the Middle East, isolated outbreaks have been reported in healthcare facilities, emphasizing the need for vigilant monitoring and infection control.

The regional disparities in the prevalence of MBL-producing *P. aeruginosa* highlight the influence of antibiotic stewardship practices and healthcare infrastructure on resistance patterns. These findings underscore the importance of regional surveillance programs to monitor the spread of MBL-producing *P. aeruginosa*, inform treatment guidelines, and establish effective infection prevention strategies tailored to local epidemiological contexts [10].

The antibiotic resistance mechanisms of *Pseudomonas aeruginosa*, particularly the production of MBL enzymes, make it a formidable pathogen in healthcare settings. MBLs are a subclass of β-lactamases that hydrolyze a wide array of β-lactam antibiotics, including carbapenems, which are often last-line treatments for resistant infections [11]. Unlike other β-lactamases, MBLs rely on zinc ions at their active site, allowing them to resist inhibition by traditional β-lactamase inhibitors. This zinc dependency poses a significant challenge, as it requires the development of alternative inhibitors for effective treatment.

The genetic basis of MBL production in *P. aeruginosa* further complicates the issue. MBL genes, such as *blaVIM*, *blaIMP*, and *blaNDM*, are often located on mobile genetic elements, including plasmids and transposons, which facilitate horizontal gene transfer. This genetic mobility allows MBL resistance to spread rapidly within healthcare environments, especially under conditions of antibiotic selection pressure. The

presence of these genes on mobile elements enables MBL-producing *P. aeruginosa* to acquire and transfer resistance to other bacterial species, amplifying the threat of multidrug resistance (MDR) in healthcare settings. In addition to MBL production, *P. aeruginosa* exhibits several other resistance mechanisms that contribute to its persistence in hospitals. For instance, it can develop alterations in membrane permeability by downregulating the production of outer membrane proteins like OprD, which limits antibiotic entry into the bacterial cell. Efflux pumps, such as MexAB-OprM, are another significant mechanism that pumps out antibiotics from the cell, reducing the effectiveness of multiple drug classes. These resistance mechanisms, combined with MBL production, result in a multidrug-resistant phenotype that is difficult to treat with conventional antibiotics.

The resistance profile of MBL-producing *P. aeruginosa* underscores the urgent need for alternative therapeutic strategies and robust infection control practices. Due to the absence of effective MBL inhibitors, treatment of infections caused by these strains is limited to a narrow range of antibiotics, often requiring combination therapies that may have variable success. New drug development targeting MBLs or alternative resistance mechanisms is crucial, as is the implementation of antibiotic stewardship programs to reduce selective pressure and slow the spread of resistance.

Research on MBL enzymes and their genetic determinants continues to advance our understanding of *P. aeruginosa* resistance, yet the clinical management of MBL-producing strains remains a global challenge. Enhanced surveillance, combined with molecular diagnostics and new antibiotic options, are critical steps toward controlling the spread of these resistant pathogens and improving outcomes for affected patients.

III. Materials and Methods

a. Description of Sample Collection

This study was conducted in a tertiary care hospital setting to examine the prevalence of metallo-β-lactamase (MBL)-producing Pseudomonas aeruginosa among clinical isolates and evaluate phenotypic detection methods for MBL activity. The sample collection process followed a systematic approach to ensure that isolates were representative of various infection sites within the hospital. Over a two-year period, from November 2021 to November 2023, clinical specimens were obtained from patients in different hospital wards, including the Intensive Care Unit (ICU), surgery, neurology, orthopedic, and neonatal intensive care units (NICU), as well as from outpatient departments (OPD). The selection included specimens from both genders and a wide age range, ensuring that data captured a broad demographic spectrum.

The samples collected for this study included bodily fluids, urine, pus, sputum, and endotracheal tube (ETT) samples. All clinical samples were collected by trained healthcare professionals following standard aseptic techniques to prevent contamination. Samples were immediately transported to the microbiology laboratory for processing. Upon arrival, each specimen was carefully examined, stained using Gram stain for preliminary identification, and then cultured on selective media, including Blood Agar and MacConkey Agar. The plates were incubated overnight at 37°C to allow bacterial growth. Isolates showing characteristic Pseudomonas aeruginosa colony morphology (e.g., greenish pigmentation, fruity odor) were further identified through oxidase tests and biochemical analyses to confirm their identity as Pseudomonas aeruginosa.

b. Phenotypic Detection of MBL Production

The primary objective of this study was to assess MBL production among imipenem-resistant Pseudomonas aeruginosa isolates. To determine MBL activity, three phenotypic detection methods were employed: the Imipenem-EDTA Combined Disc Test (CDT), the Ceftazidime-EDTA Combined Disc Test (CDT), and the Imipenem-EDTA Double Disc Synergy Test (DDST). These methods were selected for their reliability, cost-effectiveness, and ease of implementation, especially in settings where molecular testing may not be available.

1. Imipenem-EDTA Combined Disc Test (CDT)

The Imipenem-EDTA CDT was performed to detect the inhibition effect of EDTA, a metal chelator, on MBL-producing isolates. For this test, a Mueller Hinton Agar (MHA) plate was prepared with a lawn culture of the Pseudomonas aeruginosa isolate resistant to imipenem. A 10 µg imipenem disc was placed on the agar surface, with a second imipenem disc containing EDTA placed 20 mm apart from the first disc. After incubating the plate overnight at 37°C, the zone of inhibition around each disc was measured. If the inhibition zone around the imipenem-EDTA disc was at least 7 mm greater than that around the imipenem disc alone, the isolate was considered MBL-positive. This increase in zone size indicates that EDTA inhibited the MBL enzyme, supporting the presence of MBL activity in the isolate.

2. Ceftazidime-EDTA Combined Disc Test (CDT)

The Ceftazidime-EDTA CDT was used as an alternative phenotypic method to detect MBL production. On a Mueller Hinton Agar plate with a lawn culture of the imipenem-resistant isolate, two 30 µg ceftazidime discs were placed. One disc was supplemented with 10 µl of 0.5 M EDTA solution to reach the necessary concentration for MBL inhibition. After incubating the plates overnight at 37°C, the zone diameters around the ceftazidime and ceftazidime-EDTA discs were compared. An increase in the zone diameter by 7 mm or more around the ceftazidime-EDTA disc, relative to the ceftazidime disc alone, was interpreted as positive for MBL production, as the EDTA was presumed to chelate zinc ions required by MBL enzymes.

3. Imipenem-EDTA Double Disc Synergy Test (DDST)

The Imipenem-EDTA DDST involved placing a blank disc containing 10 µl of 0.5 M EDTA solution 20 mm away from a 10 µg imipenem disc on an MHA plate inoculated with the P. aeruginosa isolate. Following overnight incubation at 37°C, the inhibition zones around the imipenem disc and the EDTA disc were observed. A clear expansion of the inhibition zone in the direction of the EDTA disc indicated synergy between imipenem and EDTA, suggesting the presence of MBL production. This method provided an additional verification for MBL activity in isolates.

All three phenotypic tests were performed in duplicate to ensure consistency, and positive controls were included for quality assurance. The combined use of these methods allowed for cross-verification and minimized the chances of false-negative or false-positive results, thereby enhancing the reliability of MBL detection.

c. Antibiotic Susceptibility Testing Based on CLSI Standards

To determine the antibiotic susceptibility of Pseudomonas aeruginosa isolates, the Kirby-Bauer disc diffusion method was employed, following the Clinical and Laboratory Standards Institute (CLSI) guidelines (2022). Mueller Hinton Agar was prepared for each test, and the standard protocol was followed to create a uniform bacterial lawn on the agar surface. Commercially available antibiotic discs (HiMedia, Mumbai) were used, and the selection included a range of antibiotics commonly used to treat P. aeruginosa infections. These antibiotics included Gentamicin (10 µg), Amikacin (30 µg), Ciprofloxacin (30 µg), Ceftazidime (30 µg), Ceftriaxone (30 µg), Imipenem (10 µg), Polymyxin B (300 µg), Piperacillin-Tazobactam (100/10 µg), Cefepime (30 µg), Meropenem (10 µg), Piperacillin (100 µg), and Levofloxacin (5 µg).

The antibiotic discs were placed on the agar surface at predetermined intervals, and the plates were incubated overnight at 37°C. After incubation, the diameter of each inhibition zone was measured in millimeters, and the results were interpreted according to CLSI M100 guidelines. An isolate was classified as resistant, intermediate, or sensitive to each antibiotic based on these measurements. Pseudomonas aeruginosa ATCC 27853 was used as a control strain to validate the accuracy of the susceptibility testing.

This susceptibility testing provided critical insights into the resistance patterns of MBL-producing Pseudomonas aeruginosa isolates. Special attention was given to carbapenem antibiotics (imipenem and meropenem) and other β-lactams, as these are the primary classes of antibiotics impacted by MBL enzymes. Additionally, susceptibility to aminoglycosides (amikacin and gentamicin), fluoroquinolones (ciprofloxacin and levofloxacin), and polymyxins was evaluated to explore potential alternative treatments for MBL-producing infections.

d. Data Analysis

Following the completion of phenotypic tests and susceptibility assays, data from each isolate were recorded and analyzed. The prevalence of MBL-producing Pseudomonas aeruginosa was calculated by determining the percentage of isolates positive for MBL production out of the total imipenem-resistant isolates. The sensitivity and specificity of each phenotypic detection method were compared, and the most reliable method was identified. Furthermore, antibiotic resistance patterns were analyzed to assess the prevalence of multidrug resistance among MBL-producing isolates. Statistical software was used to perform data analysis, and significance testing was conducted to compare resistance rates between different antibiotics and detection methods.

This study's methodology allowed for comprehensive detection and analysis of MBL-producing Pseudomonas aeruginosa in a tertiary care setting. By employing multiple phenotypic tests and adhering to CLSI standards for susceptibility testing, the study provided a robust framework for identifying MBL activity and assessing resistance patterns. The use of accessible, phenotypic methods for MBL detection can serve as a valuable diagnostic approach in resource-limited settings, where advanced molecular testing may not be feasible. This methodological approach also facilitates effective antibiotic stewardship and informs infection control strategies in healthcare facilities.

IV. Results

This section presents the findings on MBL prevalence among Pseudomonas aeruginosa isolates, the distribution of these isolates by sample source, antibiotic susceptibility patterns, and a comparative analysis of the three phenotypic detection methods (Imipenem-EDTA Combined Disc Test (CDT), Ceftazidime-EDTA Combined Disc Test (CDT), and Imipenem-EDTA Double Disc Synergy Test (DDST)).

1. Prevalence of MBL-Producing Pseudomonas aeruginosa

Out of 410 Pseudomonas aeruginosa isolates collected, 118 (28.78%) were found to be resistant to imipenem and screened for MBL production. Among these, 110 isolates (26.82% of the total collected isolates) tested positive for MBL production using at least one of the phenotypic tests.

Parameter	Value
Total Pseudomonas aeruginosa isolates	410
Imipenem-resistant isolates	118 (28.78%)
MBL-producing isolates	110 (26.82%)

Table 1: Prevalence of MBL-producing Pseudomonas aeruginosa among clinical isolates.

This finding aligns with studies from regions with high antibiotic use, reflecting the growing concern over MBL-producing pathogens in hospital environments.

2. Sample Source Distribution of MBL-Producing Isolates

The distribution of MBL-producing Pseudomonas aeruginosa varied significantly by sample source. The highest proportion of MBL producers was found in urine samples, followed by pus, endotracheal tube (ETT) samples, body fluids, and sputum.

Sample Source	Total Isolates	MBL Producers (%)	Non-MBL Producers (%)
Urine	59	56 (50.90%)	3 (37.5%)
Pus	34	29 (26.36%)	5 (62.5%)
ETT	17	17 (15.46%)	0
Body Fluids	5	5 (4.55%)	0
Sputum	3	3 (2.73%)	0

Table 2: Distribution of MBL-producing and non-MBL-producing Pseudomonas aeruginosa isolates by sample source.

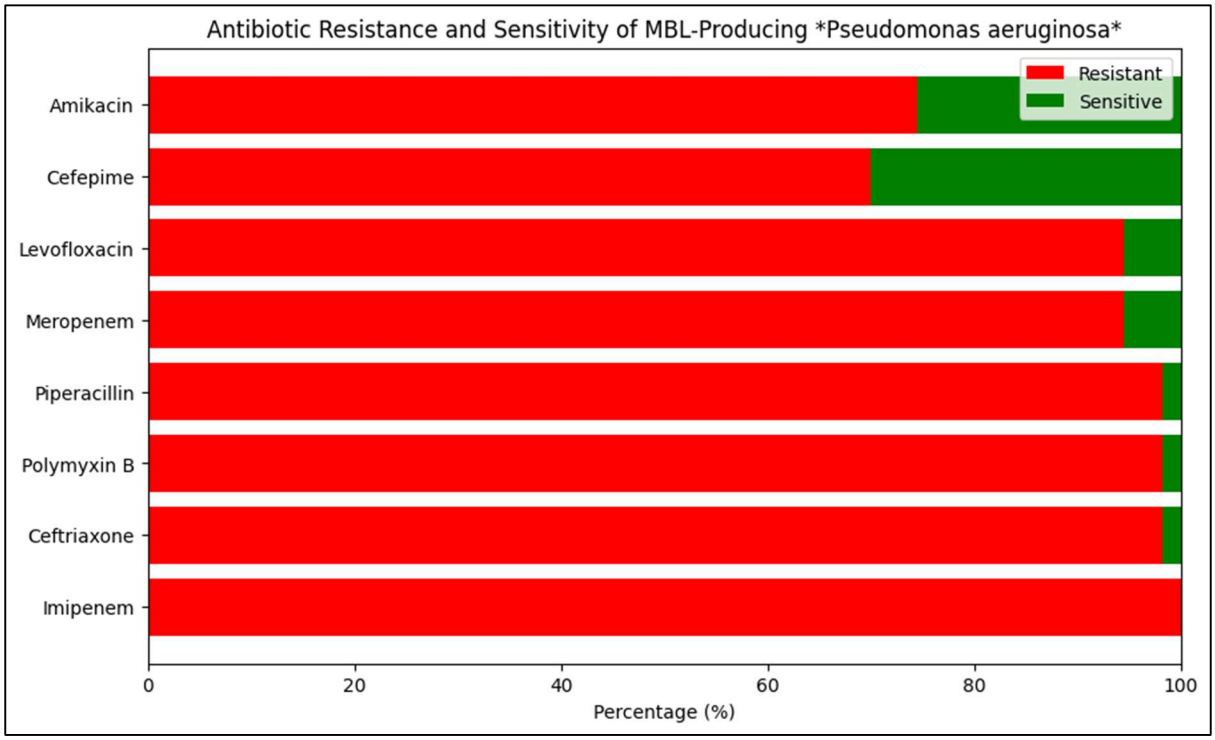
This distribution shows that urine samples were the most common source of MBL-producing isolates, emphasizing the need for vigilance in monitoring infections in urinary tract-related cases.

3. Antibiotic Sensitivity Patterns of MBL-Producing Isolates

Antibiotic susceptibility testing revealed that MBL-producing isolates displayed high resistance rates to multiple antibiotics, particularly imipenem, ceftriaxone, and polymyxin B. Sensitivity was highest to cefepime and amikacin.

Antibiotic	Resistant Isolates (%)	Sensitive Isolates (%)
Imipenem	110 (100%)	0
Ceftriaxone	108 (98.19%)	2 (1.81%)
Polymyxin B	108 (98.19%)	2 (1.81%)
Piperacillin	108 (98.19%)	2 (1.81%)
Meropenem	104 (94.55%)	6 (5.45%)
Levofloxacin	104 (94.55%)	6 (5.45%)
Cefepime	77 (70.00%)	33 (30.00%)
Amikacin	82 (74.55%)	28 (25.45%)

Table 3: Antibiotic susceptibility patterns of MBL-producing Pseudomonas aeruginosa isolates.



Graph 1: Antibiotic Resistance Patterns of MBL-Producing *Pseudomonas aeruginosa*

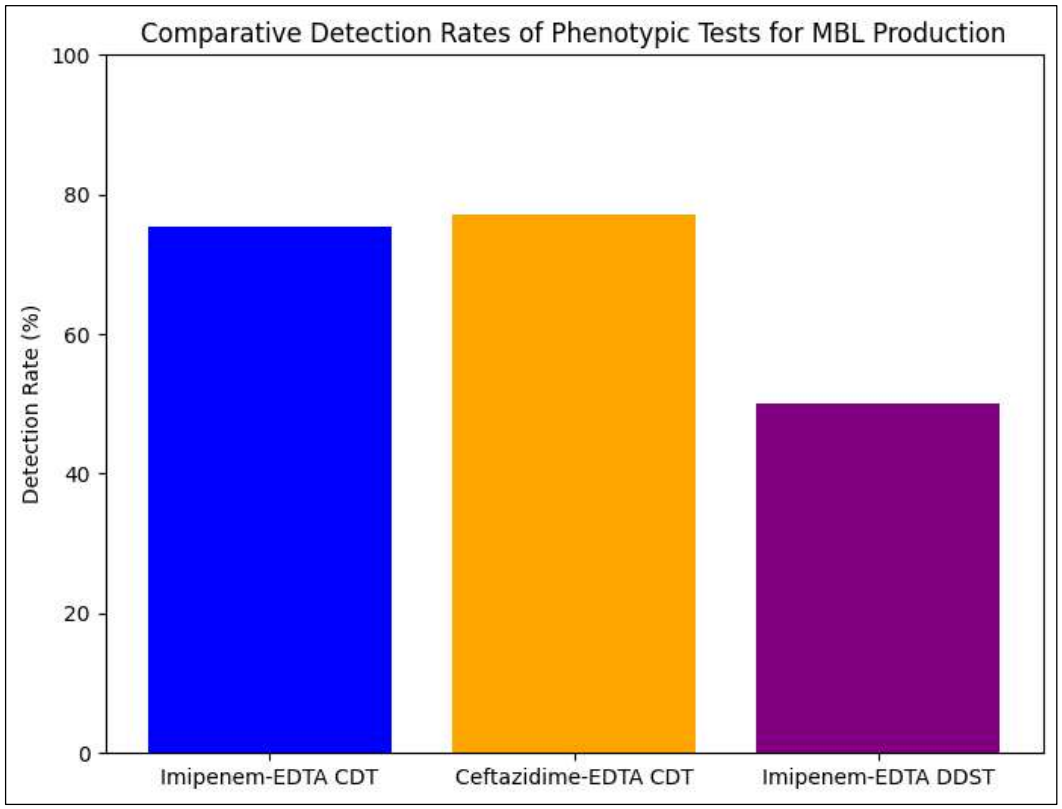
Comparative Performance of Phenotypic Detection Methods

To assess the effectiveness of phenotypic detection methods for MBL production, we compared the performance of the Imipenem-EDTA CDT, Ceftazidime-EDTA CDT, and Imipenem-EDTA DDST.

Phenotypic Test	Positive Cases	Sensitivity (%)
Imipenem-EDTA Combined Disc Test	75 (75.42%)	Moderate
Ceftazidime-EDTA Combined Disc Test	77 (77.11%)	High
Imipenem-EDTA Double Disc Synergy Test	50 (50%)	Moderate

Table 4: Comparative performance of phenotypic detection methods.

The Ceftazidime-EDTA CDT showed the highest detection rate at 77.11%, making it the most effective among the three methods in this study. The Imipenem-EDTA CDT had a slightly lower detection rate (75.42%), while the DDST detected MBL production in only 50% of positive cases. This variation suggests that the Ceftazidime-EDTA CDT may be the most reliable test in resource-limited settings for detecting MBL-producing Pseudomonas aeruginosa.



Graph 2: Detection Rates of Phenotypic Tests for MBL Production

The findings illustrate the widespread prevalence of MBL-producing Pseudomonas aeruginosa, particularly in clinical isolates from urine and other bodily fluids, often collected in ICU and surgical wards. The high resistance to carbapenems and other antibiotics among these isolates poses a significant challenge for clinicians, limiting therapeutic options. The phenotypic tests varied in their detection efficiency, with the Ceftazidime-EDTA CDT showing the highest detection rate. These results suggest that implementing this method in clinical laboratories could improve early detection and help manage MBL-producing infections more effectively.

Overall, the findings underscore the need for enhanced infection control measures, judicious antibiotic use, and consistent screening for MBL production to control the spread of multidrug-resistant Pseudomonas aeruginosa. The data suggest that Ceftazidime-EDTA CDT should be prioritized as a routine phenotypic screening method for MBL production due to its reliability and high detection rate in comparison to the other methods.

V. Discussion

The growing prevalence of metallo-β-lactamase (MBL)-producing Pseudomonas aeruginosa represents a significant challenge in clinical settings, especially in hospitals where the risk of nosocomial infections is high. This study aimed to evaluate the prevalence, distribution, and antibiotic resistance patterns of MBL-producing P. aeruginosa isolates while assessing the effectiveness of phenotypic detection methods. The findings provide valuable insights into the epidemiology of MBL-producing P. aeruginosa and underscore the urgent need for robust infection control measures and effective diagnostic practices.

A. Prevalence and Distribution of MBL-Producing Pseudomonas aeruginosa

The study revealed that 26.82% of the total P. aeruginosa isolates collected over a two-year period were MBL producers. This rate is consistent with findings from other studies conducted in regions with high antibiotic

usage, such as India, which often report MBL prevalence rates between 20% and 45%. The relatively high prevalence in this tertiary care hospital suggests that MBL production is becoming more common, potentially due to selective pressure from the use of broad-spectrum antibiotics, particularly carbapenems.

The sample source distribution shows that MBL-producing isolates were most frequently found in urine samples (50.90%), followed by pus and endotracheal tube (ETT) samples. This pattern aligns with previous studies, which have also reported a high prevalence of MBL-producing P. aeruginosa in urinary tract and wound infections. Urine samples, especially those from catheterized patients, are particularly susceptible to colonization by multidrug-resistant pathogens, highlighting the need for vigilant infection control in such cases. The high rate of MBL-positive isolates from the surgical ward (29.10%) and ICU (19.10%) further emphasizes the importance of these environments in the spread of MBL-producing organisms. Patients in these settings often undergo invasive procedures, increasing the risk of acquiring and transmitting resistant strains.

B. Antibiotic Resistance Patterns of MBL-Producing Isolates

The antibiotic susceptibility testing showed alarming resistance rates, particularly to carbapenems (imipenem and meropenem), ceftriaxone, and polymyxin B. All MBL-producing isolates were resistant to imipenem (100%), and nearly all displayed resistance to ceftriaxone and polymyxin B (98.19%). These results are concerning as they indicate that MBL-producing P. aeruginosa isolates are resistant to many of the last-resort antibiotics typically used to treat severe infections. This resistance profile limits treatment options, often necessitating combination therapy with agents like aminoglycosides or colistin, which can have significant side effects.

Despite this high level of resistance, some sensitivity was observed to cefepime (30%) and amikacin (25.45%), suggesting that these antibiotics may still have some efficacy against certain MBL-producing strains. However, reliance on these agents alone may be inadequate due to the potential for rapid development of resistance. The observed sensitivity rates may also vary across regions due to differences in local antibiotic usage and stewardship practices. The findings underscore the critical need for new therapeutic strategies and alternative antimicrobial agents to combat infections caused by MBL-producing P. aeruginosa.

C. Comparative Performance of Phenotypic Detection Methods

The study compared three phenotypic detection methods: the Imipenem-EDTA Combined Disc Test (CDT), Ceftazidime-EDTA CDT, and Imipenem-EDTA Double Disc Synergy Test (DDST). The Ceftazidime-EDTA CDT showed the highest detection rate (77.11%), followed closely by the Imipenem-EDTA CDT (75.42%). The DDST, however, detected MBL production in only 50% of positive cases, indicating a lower sensitivity relative to the other methods.

These results suggest that the Ceftazidime-EDTA CDT may be the most reliable phenotypic test for routine screening of MBL production in resource-limited settings, where molecular diagnostics may not be readily available. The slightly lower detection rate of the Imipenem-EDTA CDT may be due to variations in the affinity of EDTA to different MBL enzymes, as certain MBL variants may not respond as effectively to EDTA inhibition with imipenem. The DDST's lower detection rate may indicate that this test is less sensitive for detecting low-level MBL production, making it potentially less suitable as a standalone screening tool for MBL-producing isolates.

The comparative analysis of these methods reinforces the importance of selecting appropriate phenotypic tests to achieve accurate and early detection of MBL production. Implementing the Ceftazidime-EDTA CDT as a routine screening method may help in identifying MBL-producing P. aeruginosa more effectively, enabling timely infection control interventions and tailored antimicrobial therapy.

D. Clinical and Public Health Implications

The findings of this study have important implications for both clinical practice and public health. The high prevalence and multidrug-resistant nature of MBL-producing P. aeruginosa in the hospital setting pose serious challenges to patient care, as infections caused by these pathogens are often difficult to treat and associated with increased morbidity and mortality. The limited treatment options highlight the importance of antimicrobial stewardship to reduce the selective pressure that drives the spread of MBL-producing organisms. This study underscores the need for stringent infection control measures, such as contact isolation for infected or colonized patients, regular screening in high-risk hospital wards, and adherence to hand hygiene protocols.

Furthermore, the implementation of phenotypic screening methods, particularly the Ceftazidime-EDTA CDT, can enhance the early detection of MBL-producing isolates, facilitating prompt and appropriate infection control responses. Routine screening and reporting of MBL prevalence in healthcare facilities can also inform antibiotic policy development, potentially guiding efforts to limit the use of carbapenems and other broad-spectrum antibiotics, which contribute to the emergence of resistance.

E. Limitations and Future Research Directions

While this study provides valuable insights into the prevalence and detection of MBL-producing P. aeruginosa, it has certain limitations. Firstly, the study relied solely on phenotypic methods, which, although cost-effective, may not detect all MBL variants with the same sensitivity as molecular techniques. Future studies could incorporate molecular methods, such as PCR assays, to confirm the presence of specific MBL genes (blaVIM, blaIMP, blaNDM), providing a more comprehensive understanding of the genetic basis of resistance.

Additionally, research should focus on exploring alternative therapeutic options and developing new antibiotics or MBL inhibitors to counteract the growing threat posed by MBL-producing pathogens. Studies investigating the efficacy of combination therapies and the role of novel antibiotics in treating these infections are critical to expanding the available treatment options. Lastly, longitudinal studies could help monitor changes in MBL prevalence and resistance patterns over time, providing insights into the effectiveness of infection control and antimicrobial stewardship efforts.

This study highlights the high prevalence of MBL-producing Pseudomonas aeruginosa in a tertiary care hospital and identifies the Ceftazidime-EDTA CDT as the most effective phenotypic method for MBL detection. The high rates of antibiotic resistance among these isolates underscore the urgent need for improved infection control practices and the development of alternative therapeutic strategies. By adopting routine phenotypic screening and robust antibiotic stewardship, healthcare facilities can better manage the threat of multidrug-resistant Pseudomonas aeruginosa and improve patient outcomes.

VI. Conclusion

This study investigated the prevalence, distribution, and antibiotic resistance patterns of metallo-β-lactamase (MBL)-producing Pseudomonas aeruginosa in a tertiary care hospital setting, revealing a high prevalence of MBL production among clinical isolates. With 26.82% of isolates testing positive for MBL production, the findings underscore the critical need for vigilant infection control, particularly in high-risk hospital wards. Urine samples and surgical wards emerged as key sources of MBL-producing isolates, indicating areas where targeted infection control efforts may be most impactful. The antibiotic susceptibility results highlighted alarming levels of resistance, particularly to carbapenems and other β-lactam antibiotics, underscoring the limitations of current treatment options. While some sensitivity was noted to cefepime and amikacin, reliance on these antibiotics may not provide a sustainable long-term solution due to the potential for rapid resistance development. Among the phenotypic detection methods assessed, the Ceftazidime-EDTA Combined Disc Test (CDT) showed the

highest detection rate and reliability, making it a practical choice for routine screening of MBL-producing P. aeruginosa in resource-limited settings. Implementing reliable phenotypic methods like the Ceftazidime-EDTA CDT can enhance early detection, helping healthcare providers take prompt infection control measures and select appropriate treatment strategies. The study's findings emphasize the importance of comprehensive antibiotic stewardship and ongoing surveillance for MBL-producing pathogens to manage and mitigate the spread of multidrug-resistant Pseudomonas aeruginosa. Future research should focus on exploring alternative therapeutic options and integrating molecular methods to further refine detection and treatment strategies for these challenging infections.

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