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Original Research Article

Prevalence of MBL Producing Pseudomonas Aeruginosa in Hospital-Acquired Infections at a Tertiary Care Centre in Darbhanga, Bihar

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Abstract

Background: Pseudomonas aeruginosa is a leading cause of hospital-acquired infections (HAIs), particularly in intensive care settings. The emergence of Metallo-β-lactamase (MBL) producing strains has significantly limited therapeutic options and increased morbidity and mortality worldwide.

Aim: To determine the prevalence of MBL-producing Pseudomonas aeruginosa among hospital-acquired isolates at Darbhanga Medical College & Hospital (DMCH), Darbhanga, Bihar, and to analyze antimicrobial resistance patterns and associated risk factors.

Methods: A prospective cross-sectional study was conducted from 05th February 2025 to 25th November 2025at the Department of Microbiology, Darbhanga Medical College & Hospital, Bihar, India. Clinical samples from patients with hospital-acquired infections were processed as per standard microbiological procedures. Identification of P. aeruginosa was performed by conventional biochemical methods. Antimicrobial susceptibility testing (AST) was carried out using the Kirby–Bauer disc diffusion method according to CLSI guidelines. Carbapenem-resistant isolates were screened and phenotypically confirmed for MBL production using the Imipenem-EDTA combined disc test. Statistical analysis was performed using chi-square test with $p < 0.05$ considered significant.

Results: Out of 278 non-duplicate hospital-acquired isolates of P. aeruginosa, 82 (29.5%) were carbapenem resistant. Among them, 49 (17.6% overall; 59.8% of carbapenem-resistant isolates) were confirmed as MBL producers. Highest prevalence was observed in ICU (38.7%). MBL production showed significant association with prolonged hospitalization (>10 days), mechanical ventilation, and prior carbapenem exposure ($p<0.05$). MBL isolates demonstrated high resistance to ceftazidime (91.8%), piperacillin-tazobactam (87.7%), and ciprofloxacin (83.6%), while colistin and polymyxin B retained >95% susceptibility.

Conclusion: The study highlights a concerning prevalence of MBL-producing P. aeruginosa in hospital settings of Bihar. Strict antimicrobial stewardship, early detection, and infection control strategies are urgently required to curb further dissemination.

Keywords: Pseudomonas aeruginosa, MBL, carbapenem resistance, hospital-acquired infection, Bihar, antimicrobial resistance.

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Introduction

Pseudomonas aeruginosa is an opportunistic Gram-negative bacillus widely recognized as a major cause of hospital-acquired infections (HAIs), particularly among immunocompromised patients and those admitted to intensive care units (ICUs) [1]. It is intrinsically resistant to multiple antibiotics due to low outer membrane permeability, efflux pumps, and production of β-lactamases [2]. Over the past decade, the emergence of carbapenem-resistant P. aeruginosa (CRPA) has become a significant global health concern [3].Carbapenems, including imipenem and meropenem, were previously considered last-resort agents for severe pseudomonal infections [4]. However, resistance mediated by carbapenem-hydrolyzing enzymes, particularly Metallo-β-lactamases (MBLs), has severely compromised their efficacy [5]. MBLs belong to Ambler class B β-lactamases and require zinc ions for enzymatic activity. These enzymes hydrolyze nearly all β-lactam antibiotics, including carbapenems, but are inhibited by metal chelators such as EDTA

[6].Globally, the prevalence of MBL-producing P. aeruginosa varies widely, ranging from 10% to 70% depending on geographical region and hospital setting [7,8]. High prevalence rates have been reported from South Asia, particularly India, where antimicrobial misuse and inadequate infection control practices contribute significantly to resistance development [9]. Studies from various tertiary care centers in India have documented MBL prevalence between 15% and 45% among P. aeruginosa isolates [10–12].

The spread of MBL genes such as blaVIM, blaIMP, and blaNDM is facilitated by integrons and plasmids, enabling rapid horizontal gene transfer among Gram-negative organisms [13]. The presence of MBL-producing strains is associated with increased morbidity, prolonged hospital stay, higher treatment cost, and increased mortality rates [14].

Hospital-acquired infections due to P. aeruginosa commonly involve ventilator-associated pneumonia, catheter-associated urinary tract infections, surgical site infections, and bloodstream infections [15]. Risk factors include prolonged ICU stay, mechanical ventilation, invasive procedures, prior exposure to broad-spectrum antibiotics, and underlying comorbidities [16].

In resource-limited settings such as Bihar, surveillance data on MBL-producing P. aeruginosa remain limited. Regional antimicrobial resistance patterns may differ significantly due to local prescribing practices and infection control measures [17]. Therefore, continuous monitoring of resistance trends is crucial for guiding empirical therapy and formulating antibiotic policies.

Darbhanga Medical College & Hospital (DMCH) is a major tertiary referral center catering to North Bihar and neighboring regions. Given the increasing burden of HAIs and the limited regional data on MBL prevalence, this study was undertaken to determine the prevalence of MBL-producing P. aeruginosa among hospital-acquired isolates and to evaluate associated risk factors and antimicrobial susceptibility patterns.

Materials and Methods

This prospective cross-sectional study was conducted in the Department of Microbiology at Darbhanga Medical College & Hospital (DMCH), Darbhanga, Bihar, India, over a period of eleven months from 05th February 2025 to 25th November 2025. DMCH is a tertiary care referral center catering to North Bihar and adjoining regions, with multidisciplinary inpatient services including intensive care units (ICUs), surgical wards, medical wards, and specialty units.

Clinical specimens were collected from patients who developed signs and symptoms of infection after 48 hours of hospital admission, fulfilling the criteria for hospital-acquired infection. Only non-duplicate isolates of Pseudomonas aeruginosa from individual patients were included in the study. Repeat isolates from the same patient and isolates from community-acquired infections were excluded. Specimens processed during the study period included tracheal aspirates, sputum, bronchoalveolar lavage, pus, wound swabs, urine, blood, and other sterile body fluids. All samples were transported promptly to the microbiology laboratory and processed according to standard microbiological procedures.

Isolation and identification of Pseudomonas aeruginosa were performed using conventional bacteriological methods. Specimens were cultured on Blood agar and MacConkey agar plates and incubated aerobically at 37°C for 18–24 hours. Identification was based on colony morphology, characteristic pigment production (pyocyanin), grape-like odor, oxidase positivity, motility testing, and the ability to grow at 42°C. Additional biochemical tests were performed where required to confirm the organism.

Antimicrobial susceptibility testing (AST) was carried out by the Kirby–Bauer disc diffusion method on Mueller–Hinton agar plates following Clinical and Laboratory Standards Institute (CLSI) 2025 guidelines. The antibiotics tested included piperacillin-tazobactam, ceftazidime, cefepime, ciprofloxacin, levofloxacin, amikacin, gentamicin, imipenem, meropenem, colistin, and polymyxin B. After incubation at 37°C for 16–18 hours, zone diameters were measured and interpreted as susceptible, intermediate, or resistant according to CLSI breakpoints. Quality control was ensured using Pseudomonas aeruginosa ATCC 27853 reference strain.

Isolates showing resistance to either imipenem or meropenem were considered carbapenem-resistant and were further screened for Metallo-β-lactamase (MBL) production. Phenotypic detection of MBL was performed using the Imipenem-EDTA combined disc test. Two imipenem discs (10 µg) were placed on Mueller–Hinton agar inoculated with the test organism; one disc was supplemented with 0.5 M EDTA. After overnight incubation, an increase of ≥7 mm in zone diameter around the imipenem-EDTA disc compared to imipenem alone was interpreted as positive for MBL production. All tests were performed in duplicate to ensure reproducibility. Demographic and clinical data including age, gender, duration of hospitalization, ICU admission, use of mechanical ventilation, presence of invasive devices, and prior exposure to antibiotics (particularly carbapenems) were collected from patient records. Patient confidentiality was strictly maintained throughout the study. Data were entered into Microsoft Excel

and analyzed using Statistical Package for the Social Sciences (SPSS) version 26.0. Categorical variables were expressed as frequencies and percentages. Associations between risk factors and MBL production were assessed using the Chi-square test or Fisher's exact test where appropriate. A p-value of less than 0.05 was considered statistically significant.

Results

Table 1 presents the distribution of Pseudomonas aeruginosa isolates obtained from various clinical specimens of hospital-acquired infections during the study period (n = 278). The majority of isolates were recovered from tracheal aspirates (34.5%), indicating a high burden of ventilator-associated and lower respiratory tract infections in hospitalized patients. This was followed by pus and wound swab samples (26.6%), reflecting the significant contribution of surgical site and wound infections. Urine samples accounted for 18.7% of isolates, suggesting the role of catheter-associated urinary tract infections, while bloodstream infections constituted 12.9% of cases. Other sterile body fluids contributed a smaller proportion (7.1%).Among the total isolates, MBL-producing strains were most frequently identified in tracheal aspirates (23.9%), highlighting the predominance of carbapenem resistance in respiratory infections, particularly in ICU settings.

Bloodstream isolates also showed notable MBL positivity (19.4%), emphasizing the clinical seriousness of these infections.

Overall, the data indicate that respiratory and invasive device-associated infections serve as major reservoirs for MBL-producing P. aeruginosa in the hospital environment.

Table 1: Distribution of P. aeruginosa Isolates by Clinical Specimen (n=278)

Specimen Type	Total Isolates n (%)	MBL Positive n (%)
Tracheal aspirate	96 (34.5%)	23 (23.9%)
Pus/Wound swab	74 (26.6%)	11 (14.8%)
Urine	52 (18.7%)	6 (11.5%)
Blood	36 (12.9%)	7 (19.4%)
Others	20 (7.1%)	2 (10%)

Table 2 summarizes the prevalence of carbapenem resistance and Metallo-β-lactamase (MBL) production among the 278 hospital-acquired Pseudomonas aeruginosa isolates. Carbapenem resistance was observed in 82 isolates (29.5%), indicating a substantial level of resistance to last-resort β-lactam antibiotics.

Among the carbapenem-resistant isolates, 49 (59.8%) were confirmed as MBL producers. Overall, MBL-producing strains constituted 17.6% of the total isolates. These findings suggest that MBL production is a major mechanism contributing to carbapenem resistance in the study setting.

Table 2: Carbapenem Resistance and MBL Prevalence

Parameter	Number (%)
Total isolates	278
Carbapenem-resistant	82 (29.5%)
MBL positive (overall)	49 (17.6%)
MBL among CRPA	49/82 (59.8%)

Table 3 depicts the association between clinical risk factors and MBL-producing Pseudomonas aeruginosa. A significantly higher proportion of MBL-positive isolates was observed among patients admitted to the ICU, those on mechanical ventilation, individuals with prolonged hospital stay (>10 days), and patients with prior exposure to carbapenems ($p < 0.05$).Among these factors, prior carbapenem use showed the strongest statistical association with MBL production. The findings indicate that invasive interventions, extended hospitalization, and antibiotic pressure play a critical role in the emergence and spread of MBL-producing strains in the hospital setting.

Table 3: Risk Factors Associated with MBL Production

Risk Factor	MBL+ (n=49)	MBL- (n=229)	p-value
ICU admission	19 (38.7%)	41 (17.9%)	0.002
Ventilation	22 (44.8%)	48 (21%)	0.001
Hospital stay >10 days	27 (55.1%)	64 (27.9%)	0.0004
Prior carbapenem use	31 (63.2%)	52 (22.7%)	<0.001

Table 4 illustrates the antimicrobial resistance pattern of MBL-producing Pseudomonas aeruginosa isolates (n = 49). The isolates demonstrated very high resistance to ceftazidime (91.8%), piperacillin–tazobactam (87.7%), and ciprofloxacin (83.6%), indicating limited effectiveness of commonly used antipseudomonal agents.

Resistance to aminoglycosides such as gentamicin and amikacin was also notably high.In contrast, colistin and polymyxin B retained excellent activity, with susceptibility rates exceeding 95%. These findings emphasize the multidrug-resistant nature of MBL-producing strains and highlight polymyxins as the remaining effective therapeutic options in severe infections.

Table 4: Antibiotic Resistance Pattern of MBL Isolates (n=49)

Antibiotic	Resistant n (%)
Ceftazidime	45 (91.8%)
Piperacillin-Tazobactam	43 (87.7%)
Ciprofloxacin	41 (83.6%)
Amikacin	37 (75.5%)
Gentamicin	39 (79.5%)
Colistin	2 (4.0%)
Polymyxin B	1 (2.0%)

Figure 1 illustrates the proportion of carbapenem resistance and MBL production among hospital-acquired Pseudomonas aeruginosa isolates. Nearly one-third of the isolates (29.5%) were resistant to carbapenems, indicating a significant resistance burden. Among these carbapenem-resistant isolates, 59.8% were confirmed as MBL producers. Overall, MBL-producing strains constituted 17.6% of the total isolates.The figure clearly demonstrates that MBL production represents a major mechanism contributing to carbapenem resistance in the study setting.

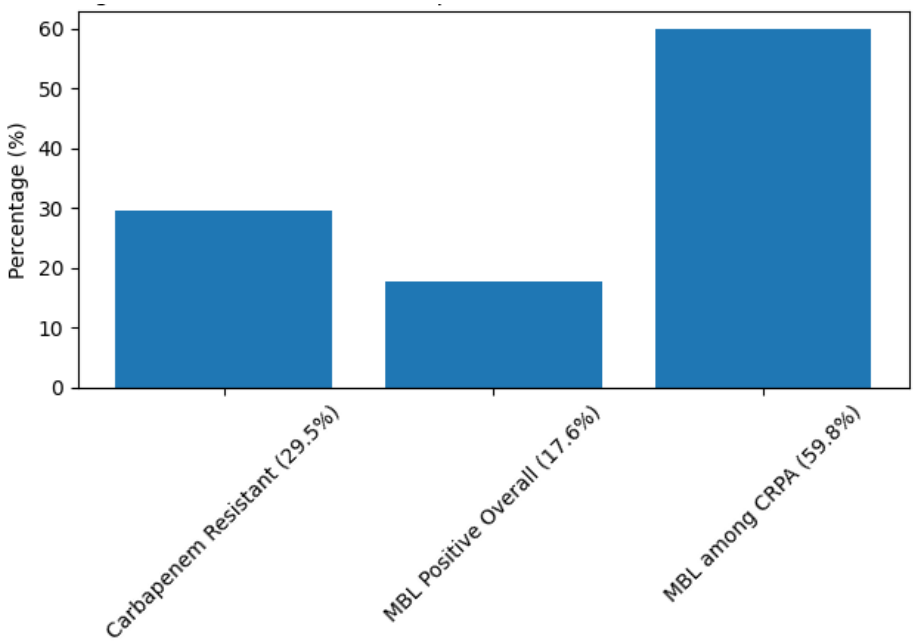

Figure 1: Prevalence of Carbapenem Resistance and MBL production

Discussion

The present study demonstrated an overall MBL prevalence of 17.6% among hospital-acquired P. aeruginosa isolates and 59.8% among carbapenem-resistant strains. These findings align with reports from North India showing MBL rates between 15%–25% [10,11]. However, some tertiary centers in South India have reported higher rates (30–40%) [12].Carbapenem resistance rate (29.5%) observed in our study is comparable to national surveillance data from India [9]. Increasing carbapenem use as empirical therapy likely contributes to selective pressure [3]. The strong association between prior carbapenem exposure and MBL production ($p<0.001$) corroborates findings from earlier Indian studies [10,16].

ICU patients showed significantly higher MBL prevalence (38.7%), consistent with previous research indicating ICU settings as hotspots for multidrug-resistant organisms (14). Mechanical

ventilation and prolonged hospitalization were also significant risk factors, similar to findings reported by global surveillance studies [7,15].

Antimicrobial susceptibility analysis revealed high resistance to cephalosporins, fluoroquinolones, and aminoglycosides. Comparable resistance patterns have been documented in recent Indian studies [11,12]. Fortunately, colistin and polymyxin B retained high efficacy (>95%), consistent with global trends [8]. However, emerging colistin resistance has been reported elsewhere [13], emphasizing the need for cautious use.

The phenotypic detection method used in our study is cost-effective and suitable for resource-limited settings, though molecular characterization would provide deeper epidemiological insights [6].Compared to studies from metropolitan centers, the slightly lower prevalence observed may reflect regional differences in antibiotic usage patterns. Nonetheless, the substantial proportion of MBL among carbapenem-resistant isolates indicates significant therapeutic challenges.Continuous surveillance, strict infection control measures, antibiotic stewardship programs, and periodic resistance audits are essential to prevent further dissemination.

Conclusion

The prevalence of MBL-producing *P. aeruginosa* at DMCH, Darbhanga is alarmingly significant, particularly in ICU settings. Carbapenem resistance and prior antibiotic exposure are major contributing factors. Routine screening for MBL production and implementation of robust antimicrobial stewardship programs are urgently recommended to limit further spread and preserve last-resort antibiotics.

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