

## Antimicrobial Susceptibility Patterns of *Pseudomonas aeruginosa* Isolated from Surgical Site Infection

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### Abstract

### Original Research Article

**Background:** Surgical site infections (SSIs) remain a significant cause of postoperative morbidity worldwide, with *Pseudomonas aeruginosa* recognized as a major pathogen due to its intrinsic and acquired antimicrobial resistance. The increasing prevalence of multidrug-resistant (MDR), metallo-β-lactamase (MBL)-producing, and blaNDM-1 harboring strains of *Pseudomonas aeruginosa* has further complicated therapeutic management in resource-limited settings.

**Objective:** This study aimed to determine the antimicrobial susceptibility patterns of *Pseudomonas aeruginosa* isolated from SSIs and to assess the prevalence of MBL production and the blaNDM-1 gene among these isolates. **Method:** A cross-sectional study was conducted from January to December 2021 at Sylhet MAG Osmani Medical College. Wound swabs were collected from 185 SSI patients and processed using standard microbiological techniques for organism isolation and identification. Antimicrobial susceptibility testing was performed by the Kirby-Bauer disc diffusion method following CLSI guidelines. Phenotypic MBL detection was conducted using the combined disc test, and blaNDM-1 gene detection was performed by polymerase chain reaction (PCR). Data were analyzed using SPSS version 26. **Results:** Among 185 samples, 130 (70.27%) showed bacterial growth, of which *P. aeruginosa* accounted for 32 (17.30%). MBL production was detected in 23 (71.87%) of the isolates, and among these, 7 (30.41%) carried the blaNDM-1 gene. Overall, *P. aeruginosa* demonstrated high resistance to ceftriaxone (87.50%), ciprofloxacin (81.25%), ceftazidime (75%), and gentamicin (71.88%). Colistin exhibited the highest sensitivity, with 90.62% susceptibility among all isolates, including 91.30% among MBL-positive and 71.43% among blaNDM-1-positive strains.

**Conclusion:** This study reveals a high prevalence of MBL-producing, and blaNDM-1-harboring *P. aeruginosa* in surgical site infections, highlighting a critical public health concern. The extensive resistance to commonly used antibiotics and dependence on colistin as the primary effective treatment underscore the need for robust antimicrobial stewardship, strict infection control measures, routine molecular surveillance, and early detection of resistance mechanisms to limit the spread of highly resistant strains in healthcare facilities.

**Keywords:** Surgical site infection, *Pseudomonas aeruginosa*, antimicrobial resistance, MBL, blaNDM-1, multidrug resistance, colistin.

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## INTRODUCTION

Surgical site infections (SSIs) remain a major cause of postoperative morbidity, prolonged hospital stays, and increased healthcare costs worldwide. Despite advancements in surgical techniques, sterilization methods, and antimicrobial prophylaxis, SSIs continue to burden healthcare systems, particularly in low- and middle-income countries. Among the various pathogens implicated in SSIs, Gram-negative bacteria play a critical

role due to their increasing resistance to multiple classes of antibiotics. [1-3] This growing resistance complicates treatment strategies and significantly impacts patient outcomes. Understanding the epidemiology and antimicrobial susceptibility patterns of these pathogens is therefore essential for effective management and prevention. [4]

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*Pseudomonas aeruginosa* is one of the most frequently isolated non-fermenting Gram- negative bacilli associated with healthcare-associated infections, including SSIs. It is an opportunistic pathogen with intrinsic resistance to several first-line antibiotics and a remarkable ability to acquire additional resistance mechanisms. Its presence in surgical wounds poses a serious challenge due to its capacity to form biofilms, survive in harsh hospital environments, and resist many commonly used antimicrobial agents. [5-6] As a result, infections caused by *P. aeruginosa* are often severe, difficult to treat, and associated with increased mortality.

The emergence of multidrug-resistant (MDR), extensively drug-resistant (XDR), and metallo-β-lactamase (MBL)-producing strains of *Pseudomonas aeruginosa* has further exacerbated this challenge. Among various MBL enzymes, the New Delhi metallo-β-lactamase (NDM) type—especially *bla*NDM-1—has gained global attention due to its ability to confer high-level resistance to carbapenems, one of the last-resort antibiotic groups. [7-8] The rapid dissemination of *bla*NDM-1 among *Pseudomonas* species represents a critical threat to public health, particularly in regions where antibiotic misuse and poor infection control practices are prevalent.

Antimicrobial susceptibility testing (AST) plays a central role in guiding appropriate antibiotic therapy and in monitoring resistance trends. Identifying susceptibility patterns among *Pseudomonas aeruginosa* isolates from surgical site infections is crucial for informing empirical therapy, optimizing patient management, and implementing targeted infection prevention interventions. Surveillance data allow clinicians to anticipate resistance profiles and choose the most effective antimicrobial regimen, thereby reducing the risk of treatment failure. [9-10]

Given the increasing prevalence of drug-resistant *Pseudomonas aeruginosa*, especially MBL- and NDM-1-producing strains, it is essential to understand their local epidemiology and susceptibility patterns. Such information not only contributes to the regional antimicrobial resistance profile but also helps in establishing evidence-based treatment guidelines for surgical infections. Therefore, this study was undertaken to determine the distribution and antimicrobial susceptibility patterns of *Pseudomonas aeruginosa* isolated from surgical site infections and to assess the prevalence of MBL and *bla*NDM-1 gene among these isolates.

## OBJECTIVE

The investigation aims to highlight the magnitude of resistance among *P. aeruginosa* isolates and provide valuable insights for clinicians, microbiologists, and policymakers to strengthen infection control practices, promote antimicrobial

## METHODOLOGY

### Study Design and Setting

This study employed a cross-sectional design and was conducted over a one-year period, from January to December 2021. The research was carried out in the Department of Microbiology at Sylhet MAG Osmani Medical College, with active collaboration from the Departments of Obstetrics and Gynaecology and Department of Surgery to facilitate comprehensive case identification and sample collection.

### Study Population and Sampling

The study included all patients who developed surgical site infections during the designated period in the participating departments. Only individuals meeting the predefined inclusion criteria were enrolled, while patients younger than eighteen years or those receiving immunosuppressive therapy were excluded. Data were documented using a structured data collection sheet. A non-probability convenient sampling technique was used to recruit eligible participants.

### Sample Size Determination

Sample size calculation was performed using Guilford and Frucher's formula based on a reported surgical site infection prevalence of 14.1%, applying a five percent significance level and a five percent margin of error. The estimated minimum required sample size was 185 participants to ensure adequate statistical validity.

### Specimen Collection and Transportation

After confirming eligibility, written informed consent was obtained from each participant. Wound swab specimens were collected from the deeper portion of the infected surgical site using sterile cotton swabs and placed into sterile containers. All samples were properly labeled and transported promptly to the bacteriology laboratory to maintain specimen quality.

### Microbiological Processing and Identification

Collected specimens were inoculated onto blood agar and MacConkey agar, followed by aerobic incubation at 37°C for 24 hours. Presumptive *Pseudomonas aeruginosa* isolates were identified based on colony characteristics, pigment production, motility, staining properties, and standard biochemical reactions. Motility was assessed using the hanging-drop method, Gram staining confirmed gram-negative rod morphology, and biochemical tests—including oxidase, catalase, triple sugar iron agar, and Simmons citrate tests—were used for confirmation.

### Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was conducted using the Kirby–Bauer disk diffusion method

on Mueller–Hinton agar in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines. Antibiotics from multiple therapeutic classes were tested. The bacterial inoculum was adjusted to a 0.5 McFarland standard prior to inoculation, and inhibition zones were measured and interpreted according to CLSI criteria.

### Phenotypic Detection of MBL Production

Metallo- $\beta$ -lactamase (MBL) production was detected using the combined disc assay. Imipenem discs with and without EDTA were placed on the inoculated medium. An increase of six millimeters or more in the inhibition zone around the imipenem–EDTA disc indicated the presence of MBL activity.

### Molecular Detection of the *blaNDM-1* Gene

Polymerase chain reaction (PCR) was performed to detect the *blaNDM-1* gene. Plasmid DNA was extracted using a commercial extraction kit per manufacturer instructions. The PCR mixture consisted of master mix, gene-specific primers, template DNA, and nuclease- free water. Amplification was carried out under standardized thermal cycling conditions. PCR products were separated by agarose gel electrophoresis

### Ethical Considerations and Data Analysis

All ethical standards were adhered to throughout the study. Written informed consent was obtained from every participant, and confidentiality was strictly maintained. Ethical approval was obtained from the Ethical Review Committee of Sylhet MAG Osmani Medical College. Data were entered and analyzed using the Statistical Package for the Social Sciences (SPSS), version 26.

## RESULTS

In this study, the participants' ages ranged from 18 to 74 years, with a mean age of  $39.02 \pm 14.83$  years. The highest proportion of cases was observed in the 18–27 years age group (29.7%), followed by the 28–37 years group (21.1%). The least number of cases (4.3%) occurred among individuals aged  $\geq 68$  years. Among the 185 patients with surgical site infection, 98 (53%) were male and 87 (47%) were female with a male to female ratio of 1.13:1.

**Table-1: Distribution of Participants by Age Group and Sex (n = 185)**

Variable	Category	Frequency (n)	Percentage (%)
Age Group (Years)	18–27	55	29.7
	28–37	39	21.1
	38–47	32	17.3
	48–57	31	16.8
	58–67	20	10.8
	$\geq 68$	8	4.3
Sex	Male	98	53.0
	Female	87	47.0

The table shows that *Escherichia coli* was the most frequently isolated organism, accounting for 40 isolates (31%), followed by *Pseudomonas aeruginosa* with 32 isolates (25%). *Staphylococcus aureus* constituted 20 isolates (15%), while coagulase-negative *Staphylococci* (CoNS) accounted for 17 isolates (13%).

Less frequently isolated organisms included *Acinetobacter* (9, 7%), *Klebsiella spp.* (4, 3%), *Enterobacter spp.* (3, 2%), and *Proteus spp.* (3, 2%). *Salmonella spp.* and *Serratia spp.* were the least common, each represented by 1 isolate (1%).

**Table 2: Prevalence of Isolated Bacteria (n=130)**

Isolated Bacteria	Frequency (n)	Percentage (%)
<i>Acinetobacter</i>	9	7
<i>Coagulase-negative Staphylococci (CoNS)</i>	17	13
<i>Escherichia coli</i>	40	31
<i>Enterobacter spp.</i>	3	2
<i>Klebsiella spp.</i>	4	3
<i>Pseudomonas aeruginosa</i>	32	25
<i>Proteus spp.</i>	3	2
<i>Staphylococcus aureus</i>	20	15
<i>Salmonella spp.</i>	1	1
<i>Serratia spp.</i>	1	1

Out of 32 participants positive for *Pseudomonas aeruginosa*, 23 (71.87%) were identified as MBL producers, while the remaining 9 (28.13%) were MBL negative. Among the MBL-producing isolates, 7 (30.41%) harbored the *blaNDM-1* gene, and 16 (69.59%) did not carry this gene. These findings

highlight the high prevalence of metallo-β-lactamase production among *Pseudomonas aeruginosa* isolated from surgical site infections and the presence of *blaNDM-1* in a significant subset of these multidrug-resistant strains.

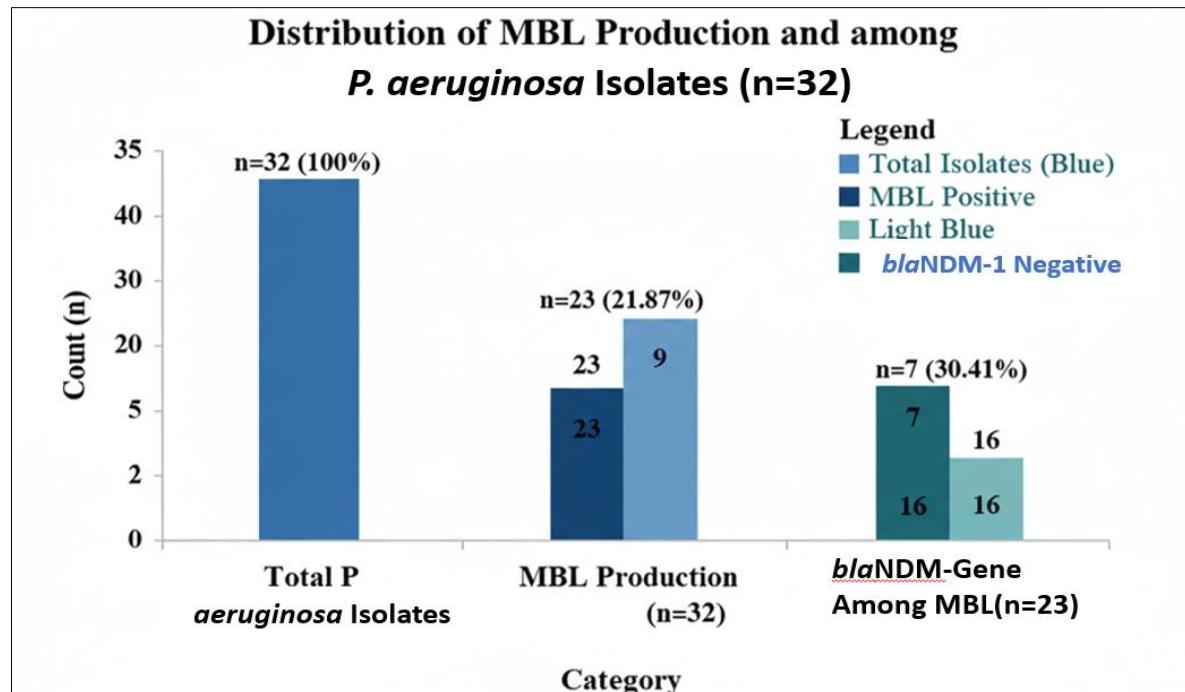


Figure 1: Distribution of *Pseudomonas aeruginosa* Isolates, MBL Production, and *blaNDM-1* Gene (n = 32)

Following table showed the antimicrobial susceptibility patterns of *Pseudomonas aeruginosa*. Out of 32 isolated *Pseudomonas aeruginosa*, 29 (90.62%) were sensitive to colistin, 19 (59.37%) were sensitive to imipenem, 18 (56.25%) were sensitive to meropenem.

On the other hand, it showed maximum resistance to ceftriaxone (28.87.50%). This organism was 81.25% resistant to ciprofloxacin and 75% resistant to ceftazidime.

Table-4: Antimicrobial Susceptibility Patterns of *Pseudomonas aeruginosa* (n = 32)

Antibiotic	Sensitive n (%)	Intermediate n (%)	Resistant n (%)
<b>Amikacin (AK)</b>	11 (34.37%)	0 (0%)	21 (65.63%)
<b>Aztreonam (ATM)</b>	15 (46.87%)	3 (9.38%)	14 (43.75%)
<b>Cefepime (FEP)</b>	14 (43.75%)	0 (0%)	18 (56.25%)
<b>Ceftazidime (CAZ)</b>	7 (21.87%)	1 (3.13%)	24 (75%)
<b>Ceftriaxone (CRO)</b>	4 (12.50%)	0 (0%)	28 (87.50%)
<b>Ciprofloxacin (CIP)</b>	6 (18.75%)	0 (0%)	26 (81.25%)
<b>Colistin (CT)</b>	29 (90.62%)	0 (0%)	3 (9.38%)
<b>Gentamicin (CN)</b>	9 (28.12%)	0 (0%)	23 (71.88%)
<b>Imipenem (IPM)</b>	19 (59.37%)	1 (3.13%)	12 (37.50%)
<b>Meropenem (MRP)</b>	18 (56.25%)	0 (0%)	14 (43.75%)
<b>Piperacillin (PRL)</b>	11 (34.37%)	0 (0%)	21 (65.63%)
<b>Piperacillin-Tazobactam (TPZ)</b>	13 (40.62%)	0 (0%)	19 (59.38%)

In this study MBL positive isolates showed excellent sensitivity (91.31%) to colistin. On the other hand, it showed maximum resistance (91.30%) to

amikacin, ceftriaxone, ceftazidime, gentamicin and piperacillin.

**Table-5: Antimicrobial Susceptibility Patterns of MBL-Positive *Pseudomonas aeruginosa* (n = 23)**

Antibiotic	Sensitive n (%)	Intermediate n (%)	Resistant n (%)
<b>Amikacin (AK)</b>	2 (8.70%)	0 (0%)	21 (91.30%)
<b>Aztreonam (ATM)</b>	9 (39.13%)	0 (0%)	14 (60.87%)
<b>Cefepime (FEP)</b>	8 (34.78%)	0 (0%)	15 (65.22%)
<b>Ceftazidime (CAZ)</b>	2 (8.70%)	0 (0%)	21 (91.30%)
<b>Ceftriaxone (CRO)</b>	2 (8.70%)	0 (0%)	21 (91.30%)
<b>Ciprofloxacin (CIP)</b>	3 (13.04%)	0 (0%)	20 (86.96%)
<b>Colistin (CT)</b>	21 (91.30%)	0 (0%)	2 (8.70%)
<b>Gentamicin (CN)</b>	2 (8.70%)	0 (0%)	21 (91.30%)
<b>Imipenem (IPM)</b>	11 (47.83%)	0 (0%)	12 (52.17%)
<b>Meropenem (MRP)</b>	9 (39.13%)	0 (0%)	14 (60.87%)
<b>Piperacillin (PRL)</b>	2 (8.70%)	0 (0%)	21 (91.30%)
<b>Piperacillin-Tazobactam (TPZ)</b>	4 (17.39%)	0 (0%)	19 (82.61%)

Following table showed the antimicrobial susceptibility patterns of *bla*NDM-1 positive *Pseudomonas aeruginosa*. Out of seven *bla*NDM-1 gene positive isolates, five (71.43%) were sensitive to colistin.

On the other hand, all the isolates (100%) were resistant to amikacin, ceftriaxone, ceftazidime, ciprofloxacin, gentamicin, meropenem, piperacillin and piperacillin-tazobactam combination.

**Table-6: Antimicrobial Susceptibility Patterns of *bla*NDM-1 Positive *Pseudomonas aeruginosa* (n = 7)**

Antibiotic	Sensitive n (%)	Intermediate n (%)	Resistant n (%)
<b>Amikacin (AK)</b>	0 (0%)	0 (0%)	7 (100%)
<b>Aztreonam (ATM)</b>	1 (14.29%)	0 (0%)	6 (85.71%)
<b>Cefepime (FEP)</b>	1 (14.29%)	0 (0%)	6 (85.71%)
<b>Ceftazidime (CAZ)</b>	0 (0%)	0 (0%)	7 (100%)
<b>Ceftriaxone (CRO)</b>	0 (0%)	0 (0%)	7 (100%)
<b>Ciprofloxacin (CIP)</b>	0 (0%)	0 (0%)	7 (100%)
<b>Colistin (CT)</b>	5 (71.43%)	0 (0%)	2 (28.57%)
<b>Gentamicin (CN)</b>	0 (0%)	0 (0%)	7 (100%)
<b>Imipenem (IPM)</b>	1 (14.29%)	0 (0%)	6 (85.71%)
<b>Meropenem (MRP)</b>	0 (0%)	0 (0%)	7 (100%)
<b>Piperacillin (PRL)</b>	0 (0%)	0 (0%)	7 (100%)
<b>Piperacillin-Tazobactam (TPZ)</b>	0 (0%)	0 (0%)	7 (100%)

## DISCUSSION

In the present study, the majority of participants belonged to the younger age groups, with 29.7% aged between 18–27 years and 21.1% between 28–37 years. This is consistent with the findings who also reported a higher incidence of surgical site infections (SSI) among young and middle-aged adults.<sup>11</sup> The slightly higher proportion of male participants (53%) compared to females (47%) aligns with similar studies conducted in Bangladesh and India, where male predominance has been attributed to greater outdoor exposure and higher rates of trauma-related surgeries. However, some international studies, reported female dominance, suggesting that gender distribution may vary based on hospital settings and surgical case types. [12]

Bacterial growth was detected in 70.27% of samples, which is comparable to the culture- positivity rates reported by study indicating that postoperative infections remain a major concern in surgical units across South Asia. In this study, *Escherichia coli* was the most frequently isolated organism (21.62%), followed by *Pseudomonas aeruginosa* (17.30%) and *Staphylococcus*

*aureus* (10.81%).[13] Similar pathogen distribution was reported by one study who also observed Gram-negative bacteria as the predominant isolates in SSIs.[14] The high prevalence of *Pseudomonas aeruginosa* further supports findings from studies conducted in tertiary hospitals in Dhaka and Chennai, emphasizing its emerging role as a major nosocomial pathogen.

A notable finding of the present study is the high prevalence (71.87%) of MBL-producing *Pseudomonas aeruginosa*, which is higher than the rates reported in earlier studies from Bangladesh, who found MBL prevalence of 50–60%.[15] International studies also documented increasing MBL production, but the prevalence in the current study appears notably higher. Furthermore, 30.41% of the MBL-positive isolates were confirmed to carry the *bla*NDM-1 gene. This prevalence is similar to that reported by Islam et al. (2019), who detected *bla*NDM-1 in 28–35% of MBL-producing isolates, suggesting the continued dissemination of *bla*NDM-1 in the hospital environment.

Antibiotic susceptibility patterns observed in this study demonstrate extensive multidrug resistance

among *Pseudomonas aeruginosa* isolates. High resistance to ceftriaxone (87.5%), ceftazidime (75%), ciprofloxacin (81.25%), and gentamicin (71.88%) are aligned with the resistance patterns reported who similarly highlighted  $\beta$ -lactam and fluoroquinolone resistance in clinical isolates. Colistin exhibited the highest sensitivity (90.62%) among the total isolates, which is consistent with global trends that identify colistin as one of the last- resort antibiotics effective against MDR *Pseudomonas*. Among MBL-positive isolates, resistance remained extremely high (up to 91.30% for multiple antibiotics), which is in agreement with the studies indicating that MBL production severely limits therapeutic options.[12]

In *blaNDM-1*-positive isolates, resistance was even more alarming, with all isolates (100%) showing resistance to amikacin, ceftriaxone, ceftazidime, ciprofloxacin, gentamicin, meropenem, piperacillin, and piperacillin-tazobactam. Only colistin retained a relatively good activity (71.43% sensitivity). Similar resistance patterns were reported by one study following the initial identification of *blaNDM-1*, emphasizing that *blaNDM-1*-harboring isolates are among the most difficult pathogens to treat.[11] The findings of this study underscore the urgent need for effective antibiotic stewardship, strict infection control practices, and continuous surveillance to prevent further spread of carbapenemase-producing organisms in hospital settings.

## CONCLUSION

Based on the findings of this study, *Pseudomonas aeruginosa* emerged as a major pathogen in surgical site infections, with a high prevalence of multidrug-resistant and MBL-producing strains. Notably, 71.87% of *P. aeruginosa* isolates were MBL positive, and among them, 30.41% carried the *blaNDM-1* gene, indicating a substantial burden of highly resistant organisms. The antimicrobial susceptibility patterns demonstrated widespread resistance to commonly used antibiotics—including amikacin, ceftazidime, ceftriaxone, gentamicin, ciprofloxacin, and piperacillin—while colistin remained the only consistently effective therapeutic option, showing high sensitivity across both MBL-positive and *blaNDM-1*-positive isolates. These findings highlight an alarming trend in antimicrobial resistance and emphasize the urgent need for strengthened infection control practices, judicious antibiotic use, routine detection of resistance mechanisms, and continuous surveillance to prevent further spread of *blaNDM-1* and other MBL-producing strains in healthcare settings.

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