RESEARCH PAPER

Study of AmpC β-Lactamase Producing Pseudomonas Aeruginosa from Clinical Samples in Tertiary Care Centre

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Abstract

In rural Indian hospitals, limited surveillance, inadequate diagnostics, and antibiotic misuse hinder AMR control; this study evaluated Pseudomonas aeruginosa (P. aeruginosa) isolation, antimicrobial susceptibility, and AmpC β-lactamase prevalence. A total of 187 P. aeruginosa isolates obtained from 55 clinical samples were analysed during this study. The overall prevalence of AmpC β-lactamase production was 33.16%. Our study's AmpC prevalence remained within the upper range of Indian reports, higher than earlier low-prevalence reports with resistance burden. Male predominance was observed, with high AmpC prevalence in ICU and urine isolates, and the highest number of isolates from patients aged 41–60 years (38.75%). A high prevalence of AmpC β-lactamase–producing P. aeruginosa was detected, especially in ICU and urine isolates, with widespread resistance to both β-lactam and non-β-lactam antibiotics. Maximum resistance was noted against cefoxitin (100%), followed by piperacillin (52.5%), ciprofloxacin, ceftazidime, and meropenem (each 51.25%). The isolates showed the best response to piperacillin–tazobactam (61.25%), followed by imipenem and amikacin (56.25% each) and aztreonam (51.25%). In contrast, very low sensitivity was observed to ceftazidime (16.1%) and piperacillin–tazobactam (8.5%). AmpC producers were isolated from urine samples (25%) and ICU patients (32.26%). The cefoxitin–cloxacillin double-disk synergy test showed the highest detection rate (63.75%), followed by the disk approximation test (48.75%) and the boronic acid disk test (45%). These findings highlight the need for routine AmpC screening, stronger antimicrobial stewardship, and effective infection control in both tertiary and rural healthcare settings in India to limit the spread of multidrug-resistant P. aeruginosa.

Keywords: Pseudomonas aeruginosa, antibiogram, AmpC-β-lactamases, multidrug-resistant (MDR), antimicrobial resistance (AMR)

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Introduction

A growing concern in recent years is the emergence of multidrug-resistant (MDR) P. aeruginosa strains, particularly those producing AmpC β-lactamases, Metallo-β-lactamases (MBL), and extended-spectrum β-lactamases (ESBL). AmpC β-lactamase confers resistance to a wide spectrum of β-lactam antibiotics, including penicillins, cephalosporins, and monobactams, thereby complicating treatment options and contributing to poor clinical outcomes [1-3]. According to the United States Centre for Disease Control and Prevention (CDC), approximately 51,000 P. aeruginosa-related hospital-acquired infections (HAIs) occur annually, with 13% (around 6,000 cases) attributed to MDR strains. World Health Organization (WHO) and the Centers for Disease Control and Prevention (CDC) have classified P. aeruginosa as a "critical priority pathogen" requiring urgent research and development of new antibiotics and enhanced global surveillance systems [3-5].

In India, the emergence and regional spread of AmpC β-lactamase–producing P. aeruginosa further underscore the escalating challenge of antimicrobial resistance. This situation is compounded by the lack of comprehensive, nationwide surveillance data in many parts of the country to accurately assess the disease burden and resistance patterns associated with P. aeruginosa, including AmpC-producing strains, thereby limiting effective monitoring and targeted intervention strategies. The earliest documented evidence originated in 2003 from Aligarh, North India, where Shahid et al. (2004) conducted phenotypic screening of clinical isolates [6]. Their findings revealed that approximately 20% of P. aeruginosa strains expressed AmpC enzymes, while an additional 10% were categorised as indeterminate. In Central India, a surveillance study from rural Ujjain found P. aeruginosa

comprising 18% of isolates, exhibiting high resistance to ceftazidime (87%), ciprofloxacin (63%), and reduced susceptibility to meropenem (35%). Although AmpC-specific data were not detailed, the elevated resistance to cephalosporins suggests possible AmpC involvement [6]. Many studies identified gender-based risk factors, comorbidities, prolonged stays in the intensive care unit (ICU), and clinical outcomes for the targeted patient group. The retrospective observational studies and clinical outcomes associated with infections showed that male patients admitted to a significant tertiary hospital in a developing country are the main concern [7-10].

Many part of India still lacks comprehensive, nationwide surveillance data on the burden of disease and antimicrobial resistance specifically attributable to P. aeruginosa, including strains producing AmpC β-lactamases. This hinders effective monitoring and response strategies against the P. aeruginosa, a critical healthcare-associated pathogen. For example, P. aeruginosa remains a significant, though less frequent, uropathogen in female urinary tract infections (UTIs) compared to E. coli, but its association with chronic and recurrent infections underscores clinical relevance. Dominance and incidence of P. aeruginosa in urinary samples from women aged between 35 and 45 with chronic UTIs were reported by Rana and Sharma (2025) [7]. A tertiary care teaching hospital study from rural Gujarat, India, reported a high prevalence of P. aeruginosa, with 73.8% of culture-positive cases occurring in male patients [9]. Not only are chronically ill or immunocompromised and hospitalized male patients at significant risk, but the young age group of 30-45 is showing growing MDR [10-11].

The objective of the present study is to determine the prevalence of P. aeruginosa among culture-positive infections and assess its distribution among various patients in rural health care in Maharashtra, India. The presented evidence-based data will contribute to filling the research gap and advancing the efforts for antibiotic management in infections. Our study also sheds light on the strong need for gender-based studies in the future for clinical practices based on evidences particular in the Indian scenario. Additionally, our findings will be helpful for national and international efforts to combat MDR and educate prudent antibiotic use.

Material and Methods

Cross-sectional Study Design in a Rural Setting

The study was conducted for a period of 2 years (August 2022 to August 2024). Ethical approval has been obtained from the Ethical Committee of Krishna Hospital and Medical Research Center, Karad (KIMSDU/IEC/02/2023, dated 15.03.2023). Written informed concerned were taken from the patient and/or relatives. A Convenience sampling was done. Bacterial isolates recovered on culture media were identified using standard microbiological procedures. All timely received clinical specimens were inoculated and incubated at 37°C for 48 hours and identified by standard microbiological techniques. Point estimate and 95% confidence interval were calculated.

Sample Size

The sample size was calculated using the previous study from India [1]. The prevalence of AmpC ß- lactamase produced by P. aeruginosa was 16.4%. Thus, referring to their prevalence rate, the sample size was calculated by the formula, n = 4pq/ l2 [Where, p = prevalence (16.4); q = 100 – Prevalence (100 –16.4= 83.6), l.0 = allowable error (10%)]. The sample size was calculated to be 54 samples. Thus, to fit the sample size, a minimum of 55 samples were studied.

Inclusion & Exclusion Criteria

P. aeruginosa from various clinical samples received in the pathological laboratory. Patients of both sexes and all age groups were included. P. aeruginosa isolated from the same specimen of the same patient was excluded from the study to avoid duplication.

Sample Collection

A total of 62 AmpC ß-lactamase producing P. aeruginosa isolates from various samples like pus, sputum, urine, ETT, blood, body fluids, wound swabs, CSF, and suction tips from both genders and all age groups of patients were included. The samples were collected using aseptic techniques before the administration of any antibiotics. Once obtained, they were placed in sterile containers and transported to the microbiology laboratory for subsequent processing.

Sample Processing & Identification of Isolates

All samples were subjected to Gram staining for microscopic examination of the culture, following standard guidelines. The clinical specimen received was smeared onto a clean, grease-free glass slide and stained with Gram stain. After staining, the smear was examined microscopically under oil immersion to identify the presence of Gram-negative Bacilli. The specimens were inoculated onto appropriate culture media, including nutrient agar, blood agar, MacConkey agar, and Cetrimide Agar. The inoculated plates were then incubated overnight at 37°C. Identification of the bacterial isolates grown on the culture media was carried out following standard microbiological guidelines. P. aeruginosa was identified based on its characteristic colony morphology, Gram staining results, motility, and positive reactions in oxidase and catalase tests, along with a series of biochemical

reactions, following the protocols described by Mackie and McCartney (2006) [2].

Antimicrobial Susceptibility Testing

Inoculum Preparation

Four to five colonies exhibiting similar morphology were selected from the agar culture plate. Using a sterile bacteriological loop, the selected colonies were inoculated into a broth medium and incubated for a minimum of three to four hours to achieve a turbid suspension. The turbidity of the resulting culture was then adjusted to match the 0.5 McFarland standard.

McFarland turbidity standard preparation

To prepare the solution, 0.05 mL of 1% anhydrous barium chloride ($BaCl_2$) was mixed with 9.95 mL of 1.0% sulfuric acid (H_2SO_4) in a test tube. The test tube was then sealed and stored in a refrigerator.

Inoculation and incubation

Antibiotic susceptibility testing was performed using the Kirby-Bauer disk diffusion method on Mueller-Hinton Agar, following the Clinical and Laboratory Standards Institute (CLSI) guidelines-2023 [13]. Pseudomonas aeruginosa ATCC 27853 served as the quality control strain. After sterilization, a sterile swab was dipped into the standardized bacterial suspension and used to inoculate the surface of a Mueller-Hinton agar plate. To ensure uniform distribution of the inoculum, the plate was swabbed in three directions. Fifteen minutes after inoculation, commercially available antibiotic discs (HiMedia, Mumbai, India) were aseptically placed onto the agar surface using sterile forceps. The antibiotics tested in this study included- Amikacin (30 µg), Ciprofloxacin (30 µg), Ceftazidime (30 µg), Piperacillin/Tazobactam (100/10 µg), Aztreonam (30 µg), Cefepime (30 µg), Meropenem (10 µg), Piperacillin (100 µg), Cefoxitin (30 µg), and Imipenem (10 µg). Each antibiotic disc was gently pressed to ensure complete contact with the agar surface. To prevent moisture accumulation, which can affect test interpretation, the plates were incubated in an inverted position. After 24 hours of incubation at 37°C, the diameters of the zones of inhibition were measured and evaluated using a zone measurement scale.

Phenotypic Methods for Detection of AmpC ß-lactamase Production

Cefoxitin – Cloxacillin Double Disk Synergy Test

The test organism was inoculated on the Mueller-Hinton Agar plates as recommended by CLSI guidelines, 2003 [12]. A 30 µg Cefoxitin and 200 µg Cefoxitin-Cloxacillin disks were placed on Mueller- Hinton Agar on a lawn culture of the test organism at a 20 mm distance between each other. The plate was incubated overnight at 37°C. After incubation, an isolate was considered AmpC-positive if the difference between the inhibition zones of Cefoxitin-Cloxacillin and Cefoxitin alone was ≥ 4.0 mm.

Disk Approximation Test

A 30 µg Ceftazidime disk was placed at the center of the plate of Mueller-Hinton Agar on lawn culture of the isolate organism. Then 10 µg Imipenem, 30 µg Cefoxitin, and 20/10 µg Amoxicillin-Clavulanate disks were placed at a distance of 20 mm from the 30 µg Ceftazidime disk. The plate was incubated overnight at 37°C. After incubation, examine the plate for any obvious blunting or flattening of the zone of inhibition observed between the Ceftazidime disk and the inducing substrates (Imipenem, Cefoxitin, and Amoxicillin-Clavulanate disk). Therefore, it was considered to be AmpC positive [13].

Boronic Acid Disk Test

A 30 µg Cefoxitin disk and a 30 µg Cefoxitin disk supplemented with 300 µg of phenylboronic acid were placed on Mueller-Hinton agar inoculated with a lawn culture of the test organism, maintaining a distance of 20 mm between the disks. The plate was incubated overnight at 37°C. After incubation, the zone diameter of the two disks was noted and compared. Phenyl boronic acid added Cefoxitin disk zone observed was ≥ 5.0 mm. So, it was considered AmpC positive.

Results

Among 80 samples, the maximum number of isolates was obtained from urine, 30 cases (37.5%), followed by pus, 23 cases (28.75%). Other significant sources included endotracheal tube secretions 13 (16.25%), sputum 6 cases (7.5%), and body fluids 5 cases (6.25%). Less common sources were blood-1case (1.25%) and Drain tubes, 2 cases (2.5%). According to the patient's demographics, over two-thirds, i.e., 51 cases (63.75%) of the isolates were from males, while females comprised 29 cases (36.25%). The distribution of P. aeruginosa was not significantly associated with age but varied according to the type of clinical isolates (Chi-square=3.964, p= 0.2654) (Table 1).

Table 1. Distribution of Pseudomonas aeruginosa by age and gender (n=80).

Age (Years)	Male (n) %	Female (n) %	Total (n)	Percentage

0-20	1.0 (1.25)	2.0 (2.50)	3 0	03.75
21-40	14 (17.50)	13 (16.25)	2 7	33.75
41-60	23 (28.75)	8.0 (10.0)	3 1	38.75
>60	13 (16.25)	6.0 (7.50)	1 9	23.75
Total (n) %	**51 (63.75)**	**29 (36.25)**	**80**	**100.0**

n: number; %: percentage

Ward-wise distribution of AmpC-positive P. aeruginosa

Distribution of P. aeruginosa obtained from different hospital wards showed that the ICU accounts for the maximum, i.e., 35% (28 cases) of the total. This was followed by the Surgery wards, which were 18 isolates, representing 22.5%. Isolates from the neurosurgery ward showed 11 cases (13.75%), while from the neonatal intensive care unit (NICU)-8 isolates (10%), the orthopaedics ward, 6 isolates (7.5%), the medicine ward, 5 isolates (6.25%), and the obstetrics and gynaecology (OBGY) ward, 3 isolates (3.75%) were obtained. The oncology ward had the lowest isolates with only 1 (1.25%).

Antibiotic Sensitivity Profile of P. aeruginosa

Out of 80 P. aeruginosa isolates, 49 isolates (61.25%) were highly sensitive to piperacillin/ tazobactam followed by Amikacin 45 isolates (56.25%), Imipenem 45 isolates (56.25%), Cefepime 44 isolates (55%) and Aztreonam 41 isolates (51.25%) (Fig. 1). However, the maximum resistance was observed to Cefoxitin 80 (100%) and Piperacillin 42 isolates (52.50%). Out of 80 isolates, 62 isolates (77.5%) were AmpC-β-lactamase-producing P. aeruginosa, and 18 isolates (22.5%) were AmpC-β-lactamase-non-producing P. aeruginosa. Among 6 isolates from sputum specimens, 5 isolates (6.25%) were AmpC producing and one (1.25%) was non-producing. In body fluid samples (n=5), four isolates (5.0%) were AmpC β-lactamase producers and one (1.25%) was a non-producer. From drain tube samples (n= 2), one isolate (1.25%) was AmpC-producing and another one (1.25%) was non-AmpC-producing. A single blood isolate (1.25%) was obtained, which was identified as an AmpC-β-lactamase producer. Out of the 62 AmpC-positive P. aeruginosa isolates identified in the study, the maximum AmpC-producing isolates were from the ICU, accounting for 20 isolates (32.26%), followed by surgical ward-14 isolates (22.58%), and neurosurgery ward-10 isolates (16.13%). The NICU accounted for 8 isolates (12.9%), while the oncology ward-1 isolate (1.61%). Three isolates each (4.84%) were obtained from the medicine, OBGY, and orthopaedics (Ortho) wards.

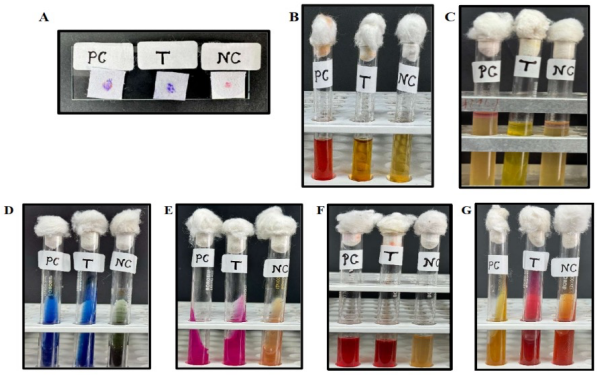

Fig.1. Antibiotic sensitivity profile of P. aeruginosa from isolates

P. aeruginosa Identification Using Biochemical Footprints

The oxidase and catalase tests were positive for aerobic respiration and are shown in Fig. 2a-2c. The non-fermentative target isolate was also confirmed by the traditional IMViC series (ethyl red test, indole test, and citrate utilization test). These biochemical fingerprint test results were negative for Methyl Red (MR) Test and Indole (Fig. 2e-2f) and summarised in Table 2.

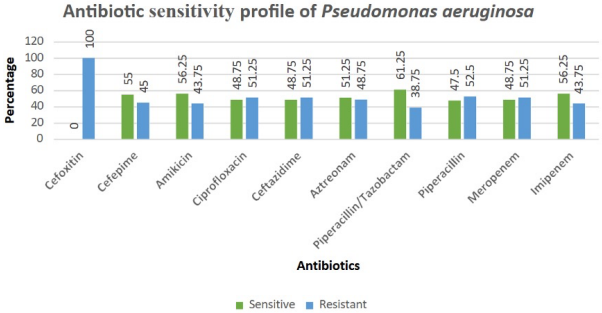

Fig. 2. Prevalence of AmpC producing P. aeruginosa by different Phenotypic methods: Oxidase Test (A); Methyl Red Test (B); Indole Test (C); Citrate Utilization Test (D); Urea hydrolysis test (E); Nitrate Reduction Test (F); and TSI (Triple Sugar Iron) Test (K/K/No gas/No H_2S) (G).

Table 2. Biochemical reactions for the identification of P. aeruginosa.

Sr. No.	Biochemical tests	Results
1	Gram staining	Gram-negative Bacilli
2	Motility (Hanging drop)	Motile
3	Catalase	Positive
4	Oxidase	Positive
5	Methyl red	Negative
6	Indole	Negative
7	Citrate utilization	Positive
8	Urease hydrolysis	Positive
9	Triple Sugar Iron	K/K, No gas/No H_2S
10	Nitrate Reduction test	Positive
11	Pigment Production	Positive

Phenotypic confirmatory tests for P. aeruginosa

Mueller-Hinton Agar plates showed the inhibition zones of Cefoxitin-Cloxacillin and Cefoxitin. Fig. 3A showed that the diameter of the Cefoxitin was ≥ 4.0 mm. Subsequently, 62 (33.16%) Cefoxitin-resistant isolates were confirmed to produce AmpC β- lactamase through phenotypic characterization. Several phenotypic methods have been employed across various studies to detect AmpC β-lactamase production in Pseudomonas aeruginosa, with varying detection rates. Confirmation was carried out using three established phenotypic methods: the Cefoxitin-Cloxacillin Double Disk Synergy Test (CC-DDS), the Disk Approximation Test (DAT), and the Boronic Acid Disk Test (BADT) (Fig. 3A-3F). Subsequently, 62 (33.16%) Cefoxitin-resistant isolates were confirmed to produce AmpC β- lactamase through phenotypic characterization. The CC-DDS detected AmpC production in 63.75% of isolates, while the DAT and BADT showed positivity rates of 48.75% and 45%, respectively.

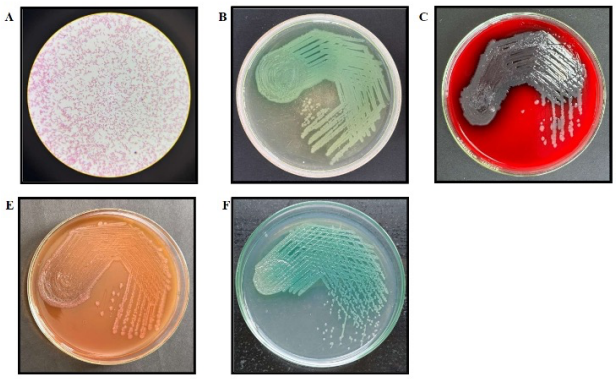

Fig. 3. Gram-negative bacilli (A), nutrient agar-bluish- green pigment (B), blood agar-metallic sheen (C), MacConkey agar-non-lactose fermenting colonies (D), and cetrimide agar-blueish pigment (E).

Antimicrobial Susceptibility Testing (AST)

Various antimicrobial drugs listed in Table 3 were tested against the isolated bacterial strains of P. aeruginosato check their sensitivity. Antimicrobial susceptibility testing revealed higher resistance trends.

Cefoxitin showed absolute resistance in all isolates (100%), indicating its limited clinical utility. Moderate to high resistance rates were observed against several antibiotics (piperacillin, ciprofloxacin, ceftazidime, meropenem, aztreonam, cefepime, amikacin, and imipenem) (Fig. 4).

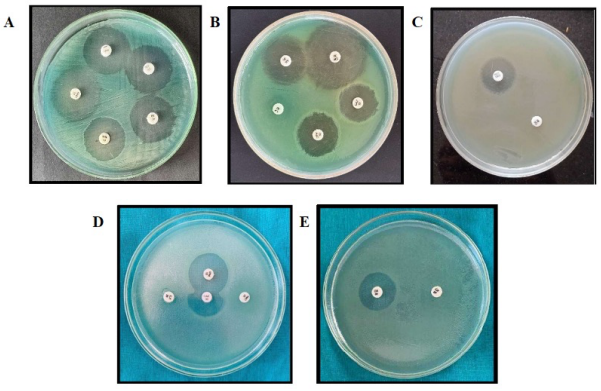

Fig. 4. Antibiotic sensitivity test (A &B), Cefoxitin-Cloxacillin double disk synergy test (C), Disk approximation test (D), and Boronic Acid disk test (E).

The lowest resistance was noted for piperacillin/tazobactam. Our study is exclusively based on phenotypic methods for antimicrobial resistance detection due to their practical and affordable option for routine clinical use. The details of the outcome are summarised in Table 3. The prevalence rate of AmpC-β-lactamase-producing P. aeruginosa in the current study was 33.16%. The prevalence of P. aeruginosa infections among male patients in our present study was found to be 63.75%.

Table 3. Antibiotics for Pseudomonas aeruginosa

Antibiotics	Disc Content	Sensitive (mm)	Intermediate (mm)	Resistant (mm)
Amikacin	30 µg	≥ 17	15-16	≤ 14
Ciprofloxacin	30 µg	≥ 25	19-24	≤ 18
Ceftazidime	30 µg	≥ 18	15-17	≤ 14
Piperacillin/Tazobactam	100/10 µg	≥ 22	18-21	≤ 17
Aztreonam	30 µg	≥ 22	16-21	≤ 15
Cefepime	30 µg	≥ 18	15-17	≤ 14
Meropenem	10 µg	≥ 19	16-18	≤ 15
Piperacillin	100 µg	≥ 22	18-21	≤ 17
Imipenem	10 µg	≥ 19	16-18	≤ 15
Cefoxitin	30 µg	≥ 18	15-17	≤ 14

Discussion

A total of 187 Pseudomonas aeruginosa isolates from various clinical specimens were analyzed in this study; the vast majority of isolates were P. aeruginosa, with an overall AmpC β-lactamase production prevalence of 33.16%. This finding closely aligns with the results reported by Bhavana et al. (2024) [14], who observed a prevalence of 33.33%, and Kumar et al. (2012) [15], who reported a prevalence of 32.7%. Further, the lowest prevalence was recorded as only 3.0%. Notably, the highest prevalence of P. aeruginosa infections was observed in the 41–60 years age group, accounting for 38.75% of cases. A higher prevalence was noted in the study conducted by Madhumati et al. (2015) in India, where 41% of P. aeruginosa isolates were found to produce AmpC β-lactamase [12]. Cefoxitin-resistant isolates were confirmed to produce AmpC β-lactamase through phenotypic characterization by using three established phenotypic methods: CC-DDS, DAT, and BADT. Pramodhini et al. (2015), also from India, documented a prevalence of 20.4% [16], while a Nigerian research group reported a rate of 15.23% [17]. Comparatively, Wolska (2008) reported higher detection rates using phenotypic

methods, with 84.9% positivity by the disk approximation test and 98.5% by the boronic acid disk test (BADT) [18], while Mamari et al. (2023) and Wassef et al. (2013) reported positivity rates of 71% and 74.2%, respectively, using the Cefoxitin–Cloxacillin test and 68.5% using BADT [19,20]. The boronic acid test alone showed a lower detection rate (31.48%), consistent with other methods like disk approximation and BADT, which report 20–40% detection. Little variations may reflect differences in methods, sample size, and regional strain diversity; as shown in Table 3, this study reports higher detection rates across all three phenotypic methods than many earlier studies, supporting the use of combined phenotypic approaches for accurate AmpC detection. In Karad, Maharashtra, Yadav et al. (2024) reported AmpC production in 53.7% of 205 isolates, with PCR confirming AmpC genes in 41.95% (bla-PDC 36.1%, bla-CMY 10.7%) [21]. Arora and Bal (2005) found AmpC in 6.7% of Gram-negative bacteria in Eastern India, with P. aeruginosa accounting for 17.3% [22]. Studies from Odisha, Southern, and Central India reported multidrug- and pan-drug-resistant P. aeruginosa, with AmpC positivity up to 38.8% in surgical site infections and prevalence of 10–30% in healthcare-associated infections, highlighting the need for robust surveillance and molecular diagnostics [5,6,23]. Male predominance in P. aeruginosa infections is widely reported and attributed to comorbidities, frequent healthcare exposure, and age-related immune changes. High male (66.7%) prevalence has been documented by Radhika et al. (2022) [24] and Kumari et al. (2020) [25], with even higher rates (70.0–83.89%) in ICU and healthcare-associated infections, consistent with global trends [17]. In the present study (2025), the highest prevalence occurred in the 41–60-year age group (38.75%), aligning with reports by Soni et al. 2024 (49%) [26], Kali et al. 2013 (44.9%) [27], Javiya et al. 2008 (35.71%) [28], Reddy et al. 2018 (32.2%) [29], and Senthamarai et al. 2014 (29.81%) [23]. Overall, evidence supported that the increased risk among middle-aged males, likely due to cumulative healthcare exposure, underlying risk factors, and or behavioural and occupational risks [30-32]. Pseudomonas aeruginosa causes ~10% of hospital-acquired infections, primarily from pus, urine, and blood. Its opportunistic nature allows infection across diverse patients and persistence in hospital settings. In this 2025 study, P. aeruginosa was isolated from 37.5% of urine samples, higher than most earlier reports (2–14.8%) but comparable to Madhumati et al., 2015 (36%) [12], highlighting significant inter-study variability [17, 28, 30]. The catheter-associated urinary tract infections (CAUTIs) were major cause in female patients [26,27]. While it traditionally accounts for 7.0% to 10% of UTIs overall, some recent tertiary care studies reported ~30-50% of positive urine cultures among hospitalised female patients. In our study, ICU P. aeruginosa prevalence was 35%, higher than Javiya et al. (2008) [38] and Dasari et al. (2024) [39] but lower than Soni et al. 2024 (42.4%) [26]. AmpC β-lactamase positivity was 77.5% overall, with 25% in urine isolates, comparable to Bhavana et al. 2024 (22.22%), reflecting geographic, antibiotic use, and diagnostic variations [14]. Higher rates were reported in earlier studies, such as Ogefere et al. (2016) [17] from Nigeria, with 30.77%, and Pramodhini et al. (2015) [16] recorded the prevalence of 38.1%. Other studies have shown moderate to low detection using various methods: Madhumati et al. (2015) [12] reported 41% positivity with the disk approximation test. Aal et al. (2021) [32] found 36.8% positivity with the disk approximation method and only 20% with the boronic acid disk test. Similarly, Mamari et al. (2023) [19] documented 71% positivity using the disk approximation test. Two independent groups reported significantly lower detection rates with the Cefoxitin–Cloxacillin test, at 11.33% and 33.33%, respectively [32,33]. Cefoxitin showed complete resistance in all isolates (100%), indicating its limited clinical utility. Moderate to high resistance rates were observed against several antibiotics, including piperacillin (52.50%), ciprofloxacin (51.25%), ceftazidime (51.25%), meropenem (51.25%), aztreonam (48.75%), cefepime (45%), amikacin (43.75%), and imipenem (43.75%). The lowest resistance was noted for piperacillin/tazobactam at 38.75%, suggesting it may remain a relatively effective option. Compared with Senthamarai et al. (2014), who reported higher resistance to ceftazidime (65.38%), ciprofloxacin (61.53%), and piperacillin (59.61%) but lower resistance to netilmicin (13.46%), our present findings show slight different resistance patterns [23]. As this study used only phenotypic methods, future studies should incorporate molecular diagnostics to better define resistance genes and mechanisms in P. aeruginosa.

Conclusion

The study showed an overall AmpC β-lactamase prevalence of 33.16%. Male predominance was observed in the patients aged 41–60 years, with the highest number of isolates (38.75%). AmpC-producing strains were predominantly isolated from ICU patients and urine samples, which demonstrated extensive resistance to commonly used antibiotics, including universal resistance to cefoxitin (100%). Piperacillin–Tazobactam, Imipenem, and Amikacin showed the highest susceptibility. Overall, 77.5% of isolates were identified as AmpC producers, with the Cefoxitin–Cloxacillin double-disk synergy test (CC-DDS) demonstrating the highest detection rate. The growing MDR trend is observed in the Indian healthcare

setting. Our findings highlight the necessity for routine AmpC β-lactamase screening, increased antimicrobial stewardship, and strengthened infection control measures in both tertiary and rural healthcare settings in India to limit the dissemination of multidrug-resistant P. aeruginosa.

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