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Prevalence, Antibiogram and ESBL Profile of *Pseudomonas aeruginosa* Isolates in a Tertiary Care Hospital

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ABSTRACT

Background: *Pseudomonas aeruginosa* is an important opportunistic pathogen responsible for a wide range of nosocomial infections, particularly in immunocompromised patients. The emergence of multidrug-resistant (MDR) strains and extended-spectrum beta-lactamase (ESBL) producers has significantly limited therapeutic options, posing a major challenge in clinical management. Continuous surveillance of its prevalence and antibiotic susceptibility pattern is essential for guiding empirical therapy and infection control strategies.

Methods: A cross-sectional study was conducted in the Microbiology Department of a tertiary care hospital over two years. A total of 7,143 clinical samples were processed using standard methods. *P. aeruginosa* was identified by conventional techniques. Antibiotic susceptibility was tested by the Kirby-Bauer method, and ESBL production was confirmed phenotypically. Data were analyzed statistically.

Results: Out of 7143 clinical samples, 396 isolates of *P. aeruginosa* were obtained with a prevalence of 8.1%. Most isolates were from indoor patients (78.8%) and males (63.9%). Maximum isolates were observed in the age group of 46–60 years (27.3%). Pus samples (36.9%) were the most common source, followed by respiratory specimens (27.5%). Highest susceptibility was observed to imipenem (83.6%), meropenem (78.0%) and piperacillin-tazobactam (75.2%), while high resistance was noted against ceftazidime (72.5%) and cefepime (63.9%). Multidrug resistance was observed in 77% of isolates and 69.4% were ESBL producers.

Conclusion: The study highlights a high prevalence of multidrug-resistant and ESBL-producing *P. aeruginosa* in hospital settings. Carbapenems and beta-lactam/beta-lactamase inhibitor combinations remain the most effective therapeutic options. Regular antimicrobial surveillance and judicious use of antibiotics are crucial to combat the rising trend of resistance.

Key-words: *Pseudomonas aeruginosa*, Antibiogram, Multidrug resistance, ESBL, Nosocomial infections

INTRODUCTION

P. aeruginosa is a non-fermenting, aerobic, Gram-negative bacillus that has emerged as one of the most significant opportunistic pathogens in both community and hospital settings [1].

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It is particularly notorious for causing healthcare-associated infections such as ventilator-associated pneumonia, urinary tract infections, surgical site infections, bacteremia and infections in burn patients [2]. The organism predominantly affects individuals with compromised immunity, including patients with malignancy, diabetes, chronic illnesses, or those undergoing invasive procedures, thereby contributing substantially to morbidity, mortality and increased healthcare burden [3].

One of the defining characteristics of *P. aeruginosa* is its remarkable adaptability to diverse environmental

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conditions. It is ubiquitously present in soil, water, hospital environments and even on medical devices, where it can persist despite routine cleaning and disinfection measures [4]. Its minimal nutritional requirements and ability to thrive in moist environments enable colonization of hospital surfaces such as sinks, ventilators, catheters and other medical equipment. This environmental resilience facilitates its transmission within healthcare settings, leading to frequent outbreaks and cross-infections, particularly in intensive care units and surgical wards [5].

The pathogenicity of *P. aeruginosa*is attributed to a wide array of virulence factors, including exotoxins, enzymes, biofilm formation, and motility mechanisms. Biofilm formation plays a crucial role in chronic infections and antibiotic resistance, as it protects the bacteria from host immune responses as well as antimicrobial agents. Additionally, the organism can rapidly adapt to host environments, making infections difficult to eradicate and prone to recurrence [6].

A major clinical challenge associated with *P. aeruginosa*is its intrinsic and acquired resistance to multiple antimicrobial agents. Intrinsic resistance is primarily due to low permeability of the outer membrane, efflux pump systems and production of chromosomal β-lactamases. Furthermore, the organism can acquire resistance through plasmid-mediated mechanisms, including the production of extended-spectrum beta-lactamases (ESBL), AmpC β-lactamases and carbapenemases. These mechanisms significantly reduce the efficacy of commonly used antibiotics such as cephalosporins, fluoroquinolones and aminoglycosides [7].

In recent years, the emergence and rapid spread of multidrug-resistant (MDR) strains of *P. aeruginosa* have become a serious global health concern. These MDR strains are resistant to at least three classes of antibiotics, thereby severely limiting therapeutic options. Carbapenems, which were once considered the drugs of choice for resistant infections, are also showing declining efficacy due to the increasing prevalence of carbapenemase-producing strains. This alarming trend has led to increased reliance on last-resort antibiotics, which are often associated with higher toxicity and cost [8].

The problem is further compounded in developing countries like India, where irrational use of antibiotics, lack of strict infection control practices and limited antimicrobial stewardship programs contribute to the emergence and dissemination of resistant strains. Several studies from different regions of India have reported considerable variation in the prevalence and antibiogram patterns of *P. aeruginosa*, highlighting the importance of region-specific data for guiding empirical therapy [7,8]. Continuous surveillance of antimicrobial susceptibility patterns is therefore essential for early detection of resistance trends and formulation of effective treatment guidelines.

Understanding the local epidemiological profile of *P. aeruginosa*, including its distribution across clinical samples, patient demographics and resistance mechanisms, is crucial for improving patient outcomes. It not only aids clinicians in selecting appropriate empirical antibiotics but also helps in implementing targeted infection control measures to prevent the spread of resistant strains within healthcare facilities.

In this context, the present study was undertaken to assess the prevalence, antibiogram and ESBL profile *of P. aeruginosa* isolates in a tertiary care hospital, aiming to support effective antimicrobial therapy and infection control practices.

MATERIALS AND METHODS

Study setting and design- This cross-sectional observational study was conducted in the Department of Microbiology at Veer Surendra Sai Institute of Medical Sciences and Research (VSSIMSAR), Burla, Odisha, India, over a period of two years from November 2019 to October 2021.

Study population- All clinical samples received from various departments of the hospital for culture and sensitivity testing during the study period were included.

Sample size and sampling technique- Based on a previous study reporting a prevalence of 10%, with 95% confidence interval and 3% precision, the minimum sample size was calculated to be 384 using the formula

$$\mathbf{n = Z^2p(1\text{-}p)/d^2\ (Z = 1.96)}.$$

The systematic random sampling technique was adopted for the selection of study samples.

Inclusion criteria- All clinical samples, such as pus, urine, sputum, blood and body fluids received for microbiological analysis.

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Exclusion criteria- Contaminated samples (leaked or soiled) and patients already receiving anti-pseudomonal antibiotics were excluded.

Study variables- Demographic and clinical variables, including age, sex, duration of hospital stay, immune status, comorbid conditions and history of antibiotic use, were recorded.

Study tools- Pre-tested predesigned proforma, sterile swabs and syringes, culture media (nutrient agar, blood agar, MacConkey agar and cetrimide agar), biochemical reagents, Mueller-Hinton agar and antibiotic discs were used.

Methodology

Sample collection and transport- Clinical samples including pus/wound swab, urine, sputum, blood and various body fluids were collected under strict aseptic precautions. Samples were transported immediately to the microbiology laboratory for further processing.

Sample processing- All samples were subjected to direct microscopic examination using Gram staining. Urine samples were additionally examined by wet mount. The samples were cultured aerobically on nutrient agar, blood agar and MacConkey agar and incubated at 37°C for 24 hours. Blood samples were initially inoculated in brain heart infusion broth, followed by subculture.

Identification of isolates- Preliminary identification of *P. aeruginosa* was based on colony morphology, pigment production and characteristic odor. Further confirmation was done using Gram staining, oxidase test, catalase test and motility testing. Biochemical tests such as oxidative-fermentative test, citrate utilization, indole test, urease test and triple sugar iron test were performed for definitive identification.

Antimicrobial susceptibility testing- Antibiotic susceptibility testing was carried out by Kirby-Bauer disc diffusion method on Mueller-Hinton agar. The inoculum was standardized to 0.5 McFarland turbidity and plates were incubated at 37°C for 18–24 hours. Zone diameters were measured and interpreted according to standard Clinical and Laboratory Standards Institute (CLSI) guidelines. The antibiotics tested included ceftazidime, cefepime, gentamicin, ciprofloxacin, piperacillin-tazobactam, meropenem, imipenem and aztreonam.

Detection of antimicrobial resistance- Screening for extended-spectrum beta-lactamase (ESBL) production was done using ceftazidime disc. Phenotypic confirmatory testing was performed by double disc synergy method using ceftazidime and ceftazidime-clavulanic acid discs. A ≥5 mm increase in zone diameter indicated ESBL production.

Statistical Analysis- Data were analyzed using SPSS version 21.0. Results were expressed as frequencies, percentages, mean and standard deviation. Chi-square or Fisher's exact test was used for categorical variables, and Student's t-test for continuous variables. A p-value <0.05 was considered statistically significant, and results were summarized and presented in tables for clear interpretation.

Ethical approval- The study was conducted after obtaining prior approval from the Institutional Ethics Committee, with all necessary documents and informed consent from patients.

RESULTS

A total of 396 *P. aeruginosa* isolates were included in the study. Most isolates were obtained from indoor patients (78.8%) with a male predominance (63.9%). Age-wise distribution showed that the highest number of isolates was observed in the 46–60 years age group (27.3%). The detailed demographic distribution is presented in Table 1.

Table 1: Demographic distribution of isolates

Variable	Category	Number (n=396)	Percentage (%)
Patient type	IPD	312	78.8
	OPD	84	21.2
Gender	Male	253	63.9
	Female	143	36.1
Age group	0–15 yrs	38	9.6
	16–30 yrs	72	18.2
	31–45 yrs	98	24.7
	46–60 yrs	108	27.3
	>60 yrs	80	20.2

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The clinical distribution of isolates revealed that pus samples were the most common source (36.9%), followed by respiratory samples (27.5%) and urine samples (17.2%). Most of the isolates were obtained from patients with prolonged hospital stay (>7 days) (74.2%). A considerable proportion of isolates were from immunocompromised patients (37.4%). These findings are summarized in Table 2.

Table 2: Clinical profile of isolates

Variable	Category	Number	Percentage (%)
Sample type	Pus	146	36.9
	Respiratory	109	27.5
	Urine	68	17.2
	Blood	32	8.1
	Others	41	10.3
Hospital stays	<7 days	102	25.8
	>7 days	294	74.2
Immune status	Immunocompetent	248	62.6
	Immunocompromised	148	37.4

Antimicrobial susceptibility testing showed that carbapenems and beta-lactam/beta-lactamase inhibitor combinations exhibited the highest sensitivity, with imipenem (83.6%) and meropenem (78.0%) being the most effective drugs. In contrast, high resistance was observed against cephalosporins, particularly ceftazidime (72.5%) and cefepime (63.9%). The detailed antibiogram is shown in Table 3.

Table 3: Antibiotic susceptibility pattern of isolates

Antibiotic	Sensitive (%)	Resistant (%)
Imipenem	83.6	16.4
Meropenem	78.0	22.0
Piperacillin-Tazobactam	75.2	24.8
Gentamicin	61.4	38.6
Ciprofloxacin	54.8	45.2
Cefepime	36.1	63.9
Ceftazidime	27.5	72.5
Aztreonam	41.2	58.8

A high prevalence of multidrug resistance was observed, with 77% of isolates classified as MDR. Additionally, 69.4% of isolates were ESBL producers, indicating a significant burden of antimicrobial resistance. The distribution of MDR and ESBL is presented in Table 4.

Table 4: Multidrug resistance and ESBL pattern

Parameter	Category	Number	Percentage (%)
MDR	Present	305	77
	Absent	91	23
ESBL	Positive	275	69.4
	Negative	121	30.6

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DISCUSSION

The present study was undertaken to analyze the clinicomicrobiological characteristics and antimicrobial susceptibility profile of *P. aeruginosa* isolates in a tertiary care setting. In this study, a total of 396 isolates were included, with a clear predominance among hospitalized patients. This observation is in line with previous studies, where *P. aeruginosa* has been identified as a major cause of hospital-acquired infections, largely due to its ability to persist in the hospital environment and colonize medical equipment [9,10]. The higher proportion of isolates from indoor patients in the present study may be attributed to prolonged hospital stay, frequent use of invasive devices and increased exposure to broad-spectrum antibiotics.

A male predominance was noted in the present study, which has also been reported in earlier literature [11,12]. This difference may be related to varying exposure patterns, healthcare access and associated risk factors. Age-wise analysis revealed that the highest number of isolates belonged to the 46–60 years age group. This finding supports previous observations that middle-aged and elderly individuals are more susceptible to infections due to declining immune function and the presence of comorbid conditions such as diabetes and chronic illnesses [11].

With respect to clinical distribution, pus samples accounted for the majority of isolates, followed by respiratory and urinary samples. Similar trends have been documented in other studies, where wound infections and respiratory tract infections are the predominant clinical presentations associated with *P. aeruginosa* [13]. The organism's preference for moist environments and damaged tissues may explain its frequent isolation from wound-related specimens. Additionally, its role in respiratory infections, particularly in hospitalized and ventilated patients, further emphasizes its clinical significance.

An important finding of the present study was the association between isolation of *P. aeruginosa* and prolonged hospital stay. A large proportion of isolates were obtained from patients admitted for more than 7 days, suggesting a strong link with nosocomial acquisition. This is consistent with earlier reports highlighting the role of hospital environment and invasive procedures in facilitating transmission of this organism [9]. Furthermore, a considerable number of isolates were obtained from immunocompromised individuals, reinforcing the opportunistic nature of *P. aeruginosa*.

The antimicrobial susceptibility pattern observed in this study demonstrated that carbapenems, particularly imipenem and meropenem, were the most effective agents against *P. aeruginosa*. These findings are comparable to those reported in other studies, where carbapenems have retained relatively high efficacy due to their stability against many β-lactamases [14]. Piperacillin-tazobactam also showed good sensitivity, suggesting its usefulness as a therapeutic option. However, a significant level of resistance was observed against cephalosporins, including ceftazidime and cefepime. This resistance pattern may be attributed to the production of β-lactamases and other resistance mechanisms.

A major concern highlighted by the present study is the high prevalence of multidrug-resistant (MDR) isolates, accounting for 77.0% of the total. This finding is comparable to other studies from similar settings, which have reported increasing trends of MDR *P. aeruginosa* [15,16]. The emergence of MDR strains significantly complicates treatment, as it limits the available antibiotic options and often necessitates the use of more toxic or expensive drugs. The high MDR rate observed in this study may reflect the widespread and often inappropriate use of antibiotics, along with insufficient infection control measures.

In addition to MDR, a high proportion of isolates were identified as ESBL producers (69.4%). This observation is consistent with previous reports indicating a rising trend of ESBL production among non-fermenting Gram-negative bacteria [17]. ESBL production contributes to resistance against third-generation cephalosporins and poses a challenge in selecting appropriate antimicrobial therapy. The coexistence of MDR and ESBL production further aggravates the issue, making management of infections more difficult.

The findings of the present study underscore the need for continuous monitoring of antimicrobial resistance patterns. Regular surveillance of antibiogram data is essential for guiding empirical therapy and updating institutional antibiotic policies. In addition, strict implementation of infection control practices, including hand hygiene and sterilization protocols, is crucial in preventing the spread of resistant organisms within

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healthcare settings [18]. Antimicrobial stewardship programs should be strengthened to promote rational use of antibiotics and minimize the emergence of resistance.

Overall, the study highlights the growing challenge of antimicrobial resistance in *P. aeruginosa* and emphasizes the importance of a coordinated approach involving surveillance, infection control and rational antibiotic use to improve patient outcomes.

SUMMARY

The present study evaluated the clinicomicrobiological profile and antibiotic susceptibility pattern of *P. aeruginosa* isolates in a tertiary care hospital. A total of 396 isolates were analyzed, with a higher prevalence among hospitalized patients and males. Pus and respiratory samples were the most common sources of isolation. The antibiogram revealed higher sensitivity to carbapenems and beta-lactam/beta-lactamase inhibitor combinations, while significant resistance was observed against cephalosporins. A notably high proportion of isolates were found to be multidrug-resistant and ESBL producers. The findings highlight the growing burden of antimicrobial resistance and the need for regular surveillance and rational antibiotic use.

LIMITATIONS

The study has certain limitations. Being a single-center study, the findings may not be generalizable to other regions. Molecular characterization of resistance mechanisms was not performed, which could have provided deeper insights into the genetic basis of resistance. Additionally, patient outcome data and risk factor analysis were not included. Future multicentric studies with molecular analysis are recommended.

CONCLUSIONS

The present study demonstrates a high burden of multidrug-resistant and ESBL-producing *P. aeruginosa* isolates in a tertiary care hospital, with a predominance among hospitalized patients. Pus and respiratory samples were identified as the most common sources of infection. The antibiogram revealed that carbapenems and beta-lactam/beta-lactamase inhibitor combinations remain the most effective therapeutic options, whereas significant resistance was observed against cephalosporins. The high prevalence of MDR and ESBL strains highlights the growing challenge of antimicrobial resistance, likely driven by irrational antibiotic use and inadequate infection control practices. These findings emphasize the need for continuous surveillance of resistance patterns, strict adherence to infection control measures and implementation of robust antimicrobial stewardship programs. Future research should focus on exploring novel antimicrobial agents, understanding emerging resistance mechanisms and developing region-specific antibiotic policies to guide empirical therapy and improve patient outcomes.

CONTRIBUTION OF AUTHORS

Research concept- Lipsa Patra, Abhipsa Patra, Rabindra Naik
Research design- Abhipsa Patra, Rabindra Naik
Supervision- Sulin Kumar Behera, Kumar Prabhas Chandra Padhan
Materials- Lipsa Patra, Abhipsa Patra, Rabindra Naik
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Data analysis and interpretation- Sulin Kumar Behera
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Critical review- Sulin Kumar Behera
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Final approval- Sulin Kumar Behera, Kumar Prabhas Chandra Padhan

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