

Phenotypic Characterization and Antimicrobial Susceptibility Profiling of *Pseudomonas aeruginosa* Clinical isolates at a Tertiary Care Hospital: A Detailed Assessment to Guide Tailored Therapeutic Interventions and Combat Nosocomial Infections

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ABSTRACT

Pseudomonas aeruginosa is a Gram-negative aerobic bacillus ubiquitously distributed across environmental and healthcare niches. It functions as a highly effective opportunistic pathogen, particularly posing severe risks in hospital settings, intensive care units, and through hospital-acquired (nosocomial) infections. This study was conducted on *P. aeruginosa* strains isolated from various clinical specimens to evaluate the institutional prevalence of infection and determine their current antibiotic drug susceptibility and multidrug resistance patterns. Out of 100 clinical diagnostic samples processed over a six-month period, 30 samples returned positive microbial cultures, from which 9 isolates were definitively identified as *P. aeruginosa*. The highest infection rates were recorded in patients aged 70–79 years (33.33%), with sputum (33.33%) and pus aspirates (26.60%) being the predominant isolation sites. Antimicrobial susceptibility testing via the Kirby-Bauer disc diffusion method demonstrated that Imipenem exhibited the strongest therapeutic profile with the lowest resistance rate (11.25%), whereas high resistance levels were observed for Aztreonam (72.50%), Ceftazidime (71.25%), Gentamicin (70.00%), and Ciprofloxacin (65.00%). Continuous local epidemiological monitoring, routine screening, and strict institutional antibiotic stewardship policies are imperative to preserve carbapenem efficacy and mitigate nosocomial transmission.

Keywords: *Pseudomonas aeruginosa*; Antibiotic Susceptibility; Nosocomial Infection; Multidrug Resistance; Tertiary Care Centre; Carbapenems; Opportunistic Pathogen; Phenotypic Identification; Antimicrobial Stewardship; Intensive Care Unit; Microscopic Profiling; Clinical Microbiology.

1.0. Introduction

Pseudomonas aeruginosa is a classic opportunistic pathogen characterized by innate resistance to multiple structural classes of antibiotics and common disinfectants. Due to its physiological versatility, it flourishes as a saprophyte in warm, moist environments, including sinks, drains, respirators, humidifiers, and disinfectant solutions within healthcare facilities.

While the fecal carrier rate of *P. aeruginosa* in healthy adults is typically less than 10%, this figure climbs dramatically up to 30% after three weeks of hospitalization, and can exceed 60% across prolonged stays. This introduces a distinct risk of endogenous and iatrogenic infections linked to clinical procedures like catheterization or tracheostomy.

P. aeruginosa can cause severe localized and systemic conditions, including:

- **Acute and Chronic Respiratory Infections:** Notably, ventilator-associated pneumonia (VAP) in intensive care units (ICUs) and chronic pulmonary infections in patients with cystic fibrosis.
- **Ophthalmic Infections:** A leading cause of aggressive corneal ulceration and panophthalmitis, which can result in rapid vision loss.
- **Bacteremia and Endocarditis:** Carrying high mortality rates (exceeding 50%–70%) in immunocompromised individuals or burns patients.

• **Dermatological, Otic, and Urinary Tract Infections:** Ranging from malignant otitis externa in elderly diabetic individuals to catheter-associated urinary tract infections (UTIs).

The global emergence of multidrug-resistant (MDR) strains displaying decreased susceptibility to β -lactams, fluoroquinolones, and aminoglycosides creates major therapeutic barriers, necessitating continuous local epidemiological monitoring.

2.0. Aims and Objectives

- 1) To determine the institutional prevalence of infections caused by *Pseudomonas aeruginosa*.
- 2) To systematically investigate potential reservoirs and sources of nosocomial pseudomonal infections.
- 3) To analyze the epidemiological distribution of *P. aeruginosa* across age, gender, and clinical specimen types.
- 4) To profile the current antimicrobial drug resistance patterns of isolated strains.

3.0. Materials and Methods

3.1. Study Setting and Sample Collection

Consecutive non-repetitive isolates of *P. aeruginosa* were gathered from diverse clinical samples—including urine, sputum, pus, endotracheal (ET) secretions, semen, blood cultures, and body fluids—submitted to the clinical microbiology laboratory. Comprehensive demographic profile variables (age, gender, admission history) and critical treatment tracking points (prior invasive procedures, pre-existing mechanical ventilation, catheterization status, and history of surgical intervention) were recorded. Samples were processed in strict accordance with standard operating procedures (SOPs).

3.2. Processing of Clinical Specimens

- **Urine:** Collected via mid-stream catch or sterile catheter port evacuation and inoculated onto CLED and MacConkey agar.
- **Sputum/Respiratory Secretions:** Collected early morning following an aseptic saline rinse and inoculated onto blood, chocolate, and MacConkey agar.
- **Pus/Wound Aspirates:** Obtained deeply via sterile syringes from previously undrained abscesses or deep wound layers to prevent superficial microflora contamination, then cultured on blood and MacConkey agar.
- **Blood Culture:** Drawn under thorough skin antisepsis (using Chloraprep/70% alcohol friction scrub) into Brain Heart Infusion (BHI) / BacT-Alert bottles, followed by routine subcultures over a 7-day incubation cycle.

3.3. Phenotypic and Biochemical Identification

Primary cultures were incubated aerobically at 35°C–37°C for 16–18 hours. Strains were initially segregated based on colony morphology (large, flat, opaque colonies with an irregular margin, metallic sheen, and a characteristic sweet, grape-like or alcohol-like fruity odor) and the production of typical diffusible pigments (pyocyanin, pyoverdinin). Definitive identification was confirmed using standard microbiological assays:

- Gram Stain: Identification of slender, Gram-negative bacilli.
- Motility Test: Active swarming behavior in semi-solid agar stabs.
- Biochemical Profiling: Oxidase positive, Catalase positive, Indole negative, Methyl Red (MR) negative, Voges-Proskauer (VP) negative, Urease negative, Citrate utilization positive, and Triple Sugar Iron (TSI) utilization showing an alkaline slant and alkaline butt (Alk/Alk, non-fermentative tracking).

3.4. Antimicrobial Susceptibility Testing (AST)

Routine AST was performed using the standard Kirby-Bauer Disc Diffusion Method on Mueller-Hinton Agar (MHA) according to Clinical and Laboratory Standards Institute (CLSI) guidelines. Overlapping test controls utilized the standard reference strain *P. aeruginosa* ATCC 27853. Zone diameter breakpoints were checked to categorize isolates into Susceptible (S), Intermediate (I), or Resistant (R). The antimicrobial panel included: Ceftazidime (30 µg), Gentamicin (10 µg), Tobramycin (10 µg), Piperacillin (100 µg), Amikacin (30 µg), Cefepime (30 µg), Ciprofloxacin (5 µg), Levofloxacin (5 µg), Meropenem (10 µg), Aztreonam (10 µg), Imipenem (10 µg), and Piperacillin/Tazobactam (100/10 µg).

For pan-resistant pan-bacterial isolates, a secondary Colistin Broth Microdilution (CBMD) method was executed using 96-well round-bottom microtiter plates filled with Cation-Adjusted Mueller-Hinton Broth (CaMHB) to establish precise Minimum Inhibitory Concentration (MIC) end points (ranging from 0.03 µg/mL to 16 µg/mL) relative to *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 quality controls.

4.0. Observations and Results

Out of a total of 100 clinical diagnostic samples processed over a span of six months, 30 samples returned culture-positive results. From these 30 positive cultures, 9 isolates were definitively identified and confirmed as *Pseudomonas aeruginosa*.

4.1. Demographic and Specimen Distribution

Table 1. Age, Gender, and Site-Wise Distribution of Culture-Positive Cases (\$N=30\$).

Age Group (Yrs)	Total Cases	Total %	Urine (M/F)	Sputum (M/F)	Pus (M/F)	ET (M/F)	Semen (M/F)	Blood C/S (M/F)	Fluid (M/F)
20–29	1	3.33	0 / 1	3 / 0	0 / 1	0 / 0	0 / 0	0 / 0	0 / 0
30–39	4	13.30	0 / 1	1 / 2	1 / 1	1 / 0	0 / 0	0 / 1	0 / 0
40–49	2	6.66	0 / 0	0 / 2	1 / 1	0 / 0	1 / 0	0 / 0	0 / 1
50–59	5	16.66	1 / 0	0 / 0	2 / 0	0 / 1	0 / 0	1 / 0	0 / 0
60–69	8	26.66	0 / 2	1 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0
70–79	10	33.33	1 / 0	1 / 0	1 / 0	0 / 0	0 / 0	0 / 0	0 / 0
Total	30	100%	2 / 4	6 / 4	5 / 3	1 / 1	1 / 0	1 / 1	0 / 1

Table 2. Prevalence Rates Across Specific Specimen Locations.

Specimen Site	Number of Specimens	Percentage of Total Positive (%)
Urine	6	20.00%
Sputum	10	33.33%
Pus	8	26.60%
Endotracheal (ET) Secretion	2	6.66%
Semen	1	3.33%
Blood Culture (C/S)	2	6.66%
Body Fluid	1	3.33%
Total	30	100.00%

Table 3. Temporal Isolation Trends of *Pseudomonas aeruginosa*.

Reporting Period	Total Samples Received	Confirmed Positive Samples	<i>P. aeruginosa</i> Isolates
February–March	10	5	0
March–April	40	15	4
April–May	20	5	3
May–June	30	5	2
Total	100	30	9

4.2. Antimicrobial Drug Resistance Profiling

The resistance landscape for *P. aeruginosa* isolates demonstrated varying degrees of efficacy across drug groups. Carbapenems showed the highest relative potency, while monobactams and third-generation cephalosporins faced high rates of resistance.

Table 4. Antimicrobial Resistance Evaluation of *P. aeruginosa* Strains.

Tested Antibiotic Agent	Overall Resistance Rates (%)
Aztreonam	72.50%
Ceftazidime	71.25%
Gentamicin	70.00%
Ciprofloxacin	65.00%

Amikacin	60.00%
Tobramycin	60.00%
Imipenem	11.25%

Key Observation: Imipenem exhibited the strongest therapeutic profile with the lowest resistance level (11.25%). Conversely, Aztreonam showed the highest resistance level at 72.50%.

5.0. Discussion

Pseudomonas aeruginosa presents severe challenges in infection management due to its minimal nutritional parameters and low baseline outer membrane permeability. In our study, *P. aeruginosa* comprised 30% of the overall culture-positive sample pool (9 out of 30), highlighting its significant presence in tertiary care clinical landscapes.

Demographic profiling showed a prominent vulnerability in older patient groups, with the 70–79 year range representing the highest total positive caseload (33.33%). Sputum (33.33%) and deep pus aspirates (26.6%) were the primary sources of isolation. This aligns with standard clinical observations that *Pseudomonas* often acts as a critical secondary colonizer in respiratory systems and open wounds.

The antibiotic resistance tracking patterns in this study are concerning:

- High resistance profiles were observed for **Aztreonam** (72.50%) and **Ceftazidime** (71.25%).
- **Gentamicin** and **Ciprofloxacin** also showed high resistance levels of 70% and 65%, respectively.
- This points to an increasing trend of multi-drug resistance (MDR), which leaves fewer options for empirical single-agent therapies.

However, carbapenems—specifically Imipenem—maintained strong in-vitro efficacy, with a low resistance rate of 11.25%. While Imipenem remains a highly effective option for targeted therapeutic intervention, its use should be carefully managed to avoid selecting for carbapenem-resistant *Pseudomonas aeruginosa* (CRPA) strains.

5.1. Conclusion

This 6-month study highlights the role of *Pseudomonas aeruginosa* as a major opportunistic pathogen within a tertiary care setting. The findings indicate that:

1. *P. aeruginosa* accounted for 9 out of 30 overall culture-positive isolates.
2. The highest incidence occurred in older age demographics, with a higher frequency observed in male patients.
3. The respiratory tract (sputum) and soft tissue injuries (pus) were the most common isolation sites.
4. **Imipenem** remains the most effective first-line treatment choice, demonstrating a low resistance profile of 11.25%, while **Aztreonam** faced the highest resistance rate at 72.50%.

5.2. Future Suggestions and Recommendations

1. **Molecular Characterization:** Future research should incorporate Polymerase Chain Reaction (PCR) and Whole-Genome Sequencing (WGS) to identify specific carbapenemase genes and efflux pump mutations.
2. **Expansion of Sample Size:** Multi-center prospective studies with larger cohort sizes over multi-year periods should be conducted to monitor temporal resistance dynamics accurately.
3. **Synergy Testing:** In-vitro synergistic evaluation of novel combination therapies (such as Ceftazidime-Avibactam or Imipenem-Relebactam with Colistin) should be investigated for multidrug-resistant strains.
4. **Biofilm Quantification:** Future studies should analyze biofilm-forming capacity in clinical isolates and evaluate anti-biofilm agents to combat catheter- and ventilator-associated infections.
5. **Hospital Environmental Surveillance:** Regular microbiological sampling of hospital water systems, sinks, and respiratory equipment should be institutionalized to map transmission vectors.

Declarations

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Competing Interests Statement

The authors declare that they have no competing interests related to this work.

Consent for publication

The authors declare that they consented to the publication of this study.

Authors' contributions

All the authors made an equal contribution in the Conception and design of the work, Data collection, Drafting the article, and Critical revision of the article.

Availability of data and materials

Authors are willing to share data and material on request.

Ethical Approval

Not applicable for this study.

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