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EVALUATION OF ANTIBIOTIC RESISTANCE PATTERNS OF *PSEUDOMONAS AERUGINOSA* ACROSS DIFFERENT INFECTIONS AND HOSPITAL SETTINGS IN DERA GHAZI KHAN

Sania Hanif[1], Hasnain Javed[*2], Anwar Ul Hassan[*3]

[1]*Superior University, Lahore, Pakistan*
[*2] *Faculty of Allied Health Sciences, Superior University, Lahore, Pakistan*
[*3]*Riphah International University, Islamabad, Pakistan*

[1]ksaniaa975@gmail.com, [*2]hasnain.javed@superior.edu.pk, [*3]anwarhassan419@gmail.com

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Corresponding Author: *
Hasnain Javed
Anwar Ul Hassan

Abstract

Background: *Antimicrobial resistance in Pseudomonas aeruginosa is an important challenge in the management of healthcare-associated and community-associated infections. Local surveillance of resistance patterns is essential for guiding antimicrobial therapy and stewardship.*

Objective: *To determine the antibiotic susceptibility and resistance profiles of Pseudomonas aeruginosa isolated from various clinical samples and to compare antibiotic resistance patterns between hospital-acquired (ICU and non-ICU) and community-acquired infections.*

Methods: *A cross-sectional descriptive study was conducted at Allama Iqbal Teaching Hospital, Dera Ghazi Khan. A total of 156 non-duplicate P. aeruginosa isolates recovered from clinical specimens were included. Isolates were obtained from outpatient, non-ICU inpatient, and ICU settings and were identified using conventional microbiological and biochemical methods. Antimicrobial susceptibility testing was performed by the Kirby-Bauer disk diffusion method according to applicable CLSI criteria. Susceptibility patterns were assessed for 13 antibiotics, and resistance was compared according to specimen type, infection acquisition, and healthcare setting. Statistical analysis was performed using IBM SPSS Statistics version 26.0, with $p<0.05$ considered statistically significant.*

Results: *Among 156 isolates, urinary tract specimens were the most frequent source (52, 33.3%), followed by wound/burn (43, 27.6%) and bloodstream infections (31, 19.9%). The highest resistance rates were observed for imipenem (75.0%), ciprofloxacin (71.2%), levofloxacin (70.5%), and meropenem (66.7%). Resistance to piperacillin, amikacin, ceftazidime, cefepime, aztreonam, and tobramycin was 57.7%, 57.1%, 53.8%, 51.9%, 51.9%, and 49.4%, respectively. Colistin showed the highest susceptibility, with 122 (78.2%) isolates susceptible and 9 (5.8%) resistant. MDR was detected in 155 (99.4%) isolates. Resistance was generally higher among ICU isolates for several antibiotics; however, differences according to hospital setting were not statistically significant ($p>0.05$). Similarly, differences*

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between hospital-acquired and community-acquired isolates were not statistically significant for any tested antibiotic ($p>0.05$).

Conclusion: *P. aeruginosa isolates in this study demonstrated a very high burden of antimicrobial resistance and MDR, with particularly high resistance to carbapenems and fluoroquinolones. Colistin retained the greatest in-vitro activity, although resistant isolates were identified. The findings support routine culture-based susceptibility testing, antimicrobial stewardship, infection-prevention measures, and continued local surveillance of P. aeruginosa resistance patterns.*

INTRODUCTION

Antimicrobial resistance (AMR) is a major threat to effective treatment of bacterial infections and is particularly challenging for healthcare systems because resistant organisms can prolong illness, increase treatment failure, and restrict therapeutic options. The World Health Organization (WHO) identifies carbapenem-resistant *Pseudomonas aeruginosa* as a high-priority bacterial pathogen because of its clinical burden, difficult treatment, and importance in healthcare settings [1]. AMR is especially concerning in low- and middle-income countries, where antibiotic exposure, infection-prevention practices, laboratory capacity, and access to newer antimicrobial agents vary considerably, making local surveillance essential for appropriate treatment and control strategies [2].

Pseudomonas aeruginosa is an opportunistic, non-fermenting Gram-negative bacillus that can cause a broad spectrum of infections, including respiratory tract infections, urinary tract infections (UTIs), wound and surgical-site infections, bloodstream infections, and burn-associated infections. It is particularly important among hospitalized and critically ill patients, especially those with prolonged hospitalization, mechanical ventilation, invasive devices, severe underlying disease, or previous antimicrobial exposure [3,4]. Its ability to survive in healthcare environments and persist on moist surfaces and medical equipment further facilitates healthcare-associated transmission. In Pakistan, clinical investigations have documented multidrug-resistant (MDR) *P. aeruginosa* in different patient populations, emphasizing the continuing local burden of this pathogen [5,6].

The treatment of *P. aeruginosa* infection is complicated by its intrinsic and acquired resistance mechanisms. The organism has a relatively impermeable outer membrane, multiple efflux pumps, chromosomal AmpC β-lactamase activity, and the capacity to acquire additional resistance determinants. Mutations affecting porins, antibiotic targets, and regulatory pathways can further reduce antimicrobial activity, while horizontal acquisition of resistance genes may produce additional resistance phenotypes. Biofilm formation also contributes to persistence and reduced antimicrobial penetration. Consequently, *P. aeruginosa* can develop resistance to several important antimicrobial classes, including antipseudomonal β-lactams, carbapenems, fluoroquinolones, and aminoglycosides, resulting in MDR, extensively drug-resistant, and difficult-to-treat infections [1,3,7].

Determining antibiotic susceptibility is therefore essential for understanding the local resistance profile of *P. aeruginosa* and supporting appropriate antimicrobial selection. Resistance, however, may not be uniform across clinical specimens. Isolates recovered from urine, respiratory specimens, wounds, blood, and other samples may differ in their susceptibility profiles because infection sites are associated with different patient characteristics, antimicrobial exposures, devices, and patterns of healthcare contact. A single overall resistance estimate may consequently mask clinically important differences among infection types. Recent evidence from Pakistan has also demonstrated resistant *P. aeruginosa* among wound isolates, including colistin-resistant strains, illustrating the continuing need for specimen-specific surveillance [6].

The healthcare environment is another important determinant of antimicrobial resistance. Intensive care units (ICUs) generally contain patients with greater disease severity, more frequent invasive procedures, longer hospital stays, and greater exposure to broad-spectrum antibiotics than non-ICU wards. These conditions can create strong selection pressure and facilitate acquisition and transmission of resistant *P. aeruginosa*. Nevertheless, resistance is not restricted to ICUs. A multinational study of carbapenem-resistant *P. aeruginosa* found that a substantial proportion of isolates originated from non-ICU patients, demonstrating that resistant organisms can be widely distributed across hospital settings [8]. Similarly, a recent comparative study of hospitalized patients reported differences in resistance to several antimicrobial agents between ICU and non-ICU isolates, supporting the value of setting-specific susceptibility surveillance [9].

Comparison with community-acquired infections is also important. Patients attending outpatient or community settings may have different antibiotic exposure histories, healthcare contacts, and risk factors from hospitalized patients. Although healthcare-associated environments are important reservoirs of resistant *P. aeruginosa*, resistant strains can also occur among community-associated infections. Comparing isolates from outdoor/community patients with those recovered from ICU and non-ICU/inpatient settings can therefore help determine whether resistance is concentrated within healthcare environments or is also evident among community-associated infections. Such comparisons are clinically valuable because empirical treatment based solely on hospital-wide or pooled resistance estimates may not accurately reflect the susceptibility profile of isolates encountered in different patient populations [10].

Despite increasing evidence of *P. aeruginosa* resistance in Pakistan, available studies have often focused on individual hospitals, specific specimen types, or selected clinical populations. Consequently, comparative information integrating clinical specimen type with ICU, non-ICU, and community/outpatient settings remains limited, particularly for Dera Ghazi Khan. Locally generated data are important because antimicrobial resistance patterns can vary geographically and between healthcare institutions. Institution-specific susceptibility surveillance can help clinicians select more appropriate empirical therapy, support antimicrobial stewardship, identify emerging resistance, and strengthen infection-prevention measures [1,2].

Therefore, the present study was designed to evaluate the antibiotic susceptibility and resistance profiles of *P. aeruginosa* isolated from various clinical samples and to compare antibiotic resistance patterns between hospital-acquired infections, including ICU and non-ICU/inpatient settings, and community-acquired infections represented by outdoor/outpatient patients in Dera Ghazi Khan. By examining both clinical sample type and healthcare setting, this study aims to provide locally relevant evidence on the distribution of *P. aeruginosa* resistance and contribute to improved antimicrobial selection, stewardship, and infection-control strategies.

MATERIALS AND METHODS

Study Design and Setting

A cross-sectional descriptive study was conducted to evaluate the antibiotic susceptibility and resistance patterns of *Pseudomonas aeruginosa* isolated from different clinical specimens and to compare resistance profiles across different healthcare settings. The study was conducted at Allama Iqbal Teaching Hospital, Dera Ghazi Khan, a tertiary-care healthcare facility serving a large population of Southern Punjab, Pakistan. The study included specimens received from both outpatient and inpatient departments. Inpatient specimens were obtained from medical, surgical, pediatric, gynecology, and intensive care units (ICUs), while outpatient specimens were collected from patients attending the outpatient departments (OPDs). All microbiological processing, bacterial isolation, identification, and antimicrobial susceptibility testing were performed in the hospital's clinical microbiology laboratory using standard microbiological procedures.

Study Duration and Population

The study was conducted over a four-month period from March 2026 to July 2026. The study population comprised patients of all age groups and both sexes who were clinically suspected of having bacterial infections and whose submitted clinical specimens yielded *P. aeruginosa*. Both hospitalized and non-hospitalized patients were included. Hospitalized patients were further categorized according to their place of admission as ICU and non-ICU patients, whereas patients attending OPDs were considered community/outpatient cases according to the study classification.

Sampling and Sample Size

Consecutive sampling was used. All eligible clinical specimens submitted to the microbiology laboratory during the study period that yielded *P. aeruginosa* and fulfilled the predefined eligibility criteria were included. Only the first non-duplicate *P. aeruginosa* isolate from each patient during the study period was considered for analysis.

Because the study aimed to include all eligible *P. aeruginosa* isolates recovered during the defined study period, a census of eligible isolates was performed rather than restricting enrollment to a predetermined number. The conventional sample-size formula,

$$n = Z^2p(1-p)/d^2$$

was considered during study planning, where n represents the required sample size, Z represents the standard normal deviate at a 95% confidence level (1.96), p represents the estimated prevalence, and d represents the desired margin of error. However, all eligible non-duplicate isolates available during the study period were included to maximize the representation of resistance patterns across clinical specimens and hospital settings.

Eligibility Criteria

Clinical specimens yielding confirmed *P. aeruginosa* from patients of any age and either sex were eligible. Specimens obtained from both outpatient and inpatient settings, including ICU and non-ICU wards, were included. The clinical specimens included urine, blood, pus, wound swabs, cerebrospinal fluid (CSF), sputum, tracheal aspirates, and other clinically relevant specimens submitted for routine microbiological investigation.

Specimens that yielded organisms other than *P. aeruginosa*, duplicate isolates from the same patient, contaminated specimens, improperly collected or inadequately transported specimens, and mixed cultures that could not be reliably interpreted were excluded. Cases with insufficient demographic or clinical information required for the study analysis were also excluded.

Specimen Collection and Processing

Clinical specimens were collected by trained healthcare personnel using appropriate aseptic techniques according to the suspected site of infection and routine hospital procedures. Specimens were appropriately labeled and transported promptly to the clinical microbiology laboratory for processing.

Specimens were inoculated onto appropriate culture media, including blood agar and MacConkey agar. Cetrimide agar was used when required for selective isolation of *P. aeruginosa*. Inoculated media were incubated aerobically at 37°C for approximately 18–24 hours. Following incubation, plates were examined for bacterial growth and colonies suggestive of *P. aeruginosa*.

Isolation and Identification of *Pseudomonas aeruginosa*

Presumptive identification was based on colony morphology, Gram staining, and conventional biochemical characteristics. Suspected *P. aeruginosa* colonies typically demonstrated large, flat colonies with irregular margins and, where produced, blue-green pigmentation associated with pyocyanin production and a characteristic odor.

Gram staining was performed to assess cellular morphology. Suspected isolates were identified as Gram-negative bacilli occurring singly or in pairs. Further identification was performed using conventional biochemical tests, including oxidase, catalase, citrate utilization, triple sugar iron (TSI), motility, and growth at 42°C. Isolates

demonstrating characteristic phenotypic and biochemical features consistent with *P. aeruginosa* were included in the study.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing (AST) was performed using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar in accordance with the applicable Clinical and Laboratory Standards Institute (CLSI) recommendations available during the study period. A fresh, well-isolated colony of each confirmed *P. aeruginosa* isolate was suspended in sterile normal saline, and the turbidity was adjusted to a 0.5 McFarland standard, corresponding approximately to 1.5×10^8 CFU/mL.

The standardized suspension was evenly inoculated onto the surface of Mueller-Hinton agar using a sterile cotton swab to produce a confluent bacterial lawn. Antibiotic disks were subsequently placed aseptically on the agar surface at appropriate distances. The antimicrobial agents tested included piperacillin, piperacillin/tazobactam, ceftazidime, cefepime, aztreonam, imipenem, meropenem, ciprofloxacin, levofloxacin, gentamicin, amikacin, tobramycin, and colistin, where applicable according to laboratory testing practices and available interpretive criteria.

The inoculated plates were incubated aerobically at 35–37°C for approximately 16–18 hours, according to the applicable CLSI methodology. After incubation, inhibition-zone diameters were measured in millimeters. Results were categorized as susceptible (S), intermediate (I), or resistant (R) according to the CLSI interpretive criteria applicable during the study period. For analysis, isolates categorized as intermediate were retained as a separate category where appropriate and were not automatically classified as resistant unless justified by the applicable guideline.

Quality Control

Quality assurance procedures were implemented throughout the microbiological investigation. The reference strain *P. aeruginosa* ATCC 27853 was used for quality control of antimicrobial susceptibility testing. Culture media, antibiotic disks, incubation conditions, and laboratory equipment were monitored according to standard laboratory procedures. Laboratory investigations were performed by trained personnel under appropriate supervision.

Data Collection

Data were recorded using a structured data collection proforma. Demographic variables included age and sex. Clinical and epidemiological variables included outpatient/inpatient status, ICU or non-ICU status, hospital department, type of clinical specimen, and infection site where available. Microbiological variables included culture findings, identification of *P. aeruginosa*, and antimicrobial susceptibility results for each tested antibiotic.

For the purpose of comparative analysis, isolates were categorized according to healthcare setting as outpatient/community-associated and inpatient/hospital-associated. Hospital-associated isolates were further classified into ICU and non-ICU groups.

Statistical Analysis

Data were entered into Microsoft Excel and subsequently analyzed using IBM SPSS Statistics version 26.0. Categorical variables were summarized as frequencies and percentages, while continuous variables such as age were summarized using mean and standard deviation where appropriate.

Antibiotic susceptibility and resistance patterns were evaluated according to clinical specimen type, infection category, age group, sex, and healthcare setting. Resistance profiles were specifically compared between ICU and non-ICU/inpatient isolates and between inpatient/hospital-associated and outpatient/community-associated isolates. The frequency and percentage of susceptible, intermediate, and resistant isolates were calculated for each antimicrobial agent. Appropriate statistical tests were applied for comparison of categorical variables, and a two-sided p value <0.05 was considered statistically significant.

Ethical Considerations

Ethical approval was obtained from the Institutional Ethical Review Committee before commencement of the study, and administrative permission was obtained from Allama Iqbal Teaching Hospital, Dera Ghazi Khan. Patient confidentiality was maintained throughout the study. Personal identifiers, including names, addresses, and hospital registration numbers, were not included in the study database or publication. Data were used exclusively for research purposes and were handled in accordance with applicable ethical requirements for biomedical research involving human clinical specimens.

RESULTS

The demographic details of the 156 patients from whom Pseudomonas aeruginosa isolates were recovered are given in Table 1. The highest number of patients were in the age group 19-40 years (48, 30.8%) and followed by age group 41-60 years (44, 28.2%). Patients aged 61–80 years accounted for 37 (23.7%), while 16 (10.3%) were aged 0–18 years. The youngest age group (70–79 years) and the oldest age group (>80 years) had the lowest proportion of patients at 11 (7.1%) and 11 (7.1%) respectively. As for gender distribution, 78 (50.0%) were male and 78 (50.0%) were female, meaning that gender distribution was equal.

Table 1: Demographic Characteristics of *Pseudomonas aeruginosa* Isolates

Characteristic	Category	Frequency (n)	Percentage (%)
Age Group (years)	0–18	16	10.3
	19–40	48	30.8
	41–60	44	28.2
	61–80	37	23.7
	>80	11	7.1
Gender	Male	78	50.0
	Female	78	50.0

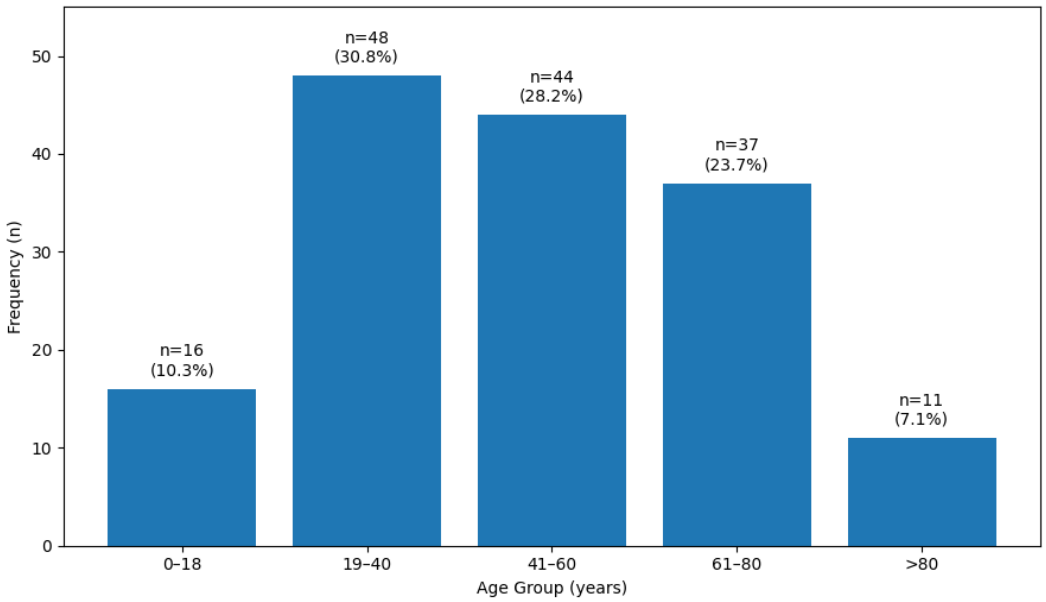


Figure 1: Age Group of Distribution of *Pseudomonas aeruginosa* Isolates

The distribution of Pseudomonas aeruginosa isolates based on the site of infection and hospital setting among the 156 participants in the study is given in Table 4.2. Most of the isolates (52, 33.3%)

were from the urinary tract, followed by wound/burn infections (43, 27.6%) and bloodstream infections (31, 19.9%). Isolates from respiratory tract infections accounted for 17 (10.9%) and central nervous system (CNS) infections 10 (6.4%). The third infection category was the lowest (3, 1.9%).

In the hospital setting, most Pseudomonas aeruginosa isolates (65, 41.7%) were obtained from patients admitted to the non-ICU wards, while 49 (31.4%) were obtained from patients admitted to the ICU. 42 (26.9%) of the isolates were outpatients. These results showed that urinary tract infections (UTIs) were the most common infection associated with Pseudomonas aeruginosa, and Pseudomonas was more commonly encountered in hospitalized patients, especially those in non-ICU wards.

Table 2: Distribution of *Pseudomonas aeruginosa* Isolates According to Infection Site and Hospital Setting (n = 156)

Variable	Category	Frequency (n)	Percentage (%)
Infection Site	Bloodstream	31	19.9
	CNS	10	6.4
	Other	3	1.9
	Respiratory Tract	17	10.9
	Urinary Tract	52	33.3
	Wound/Burn	43	27.6
Hospital Setting	Outpatient	42	26.9
	ICU	49	31.4
	Non-ICU Ward	65	41.7

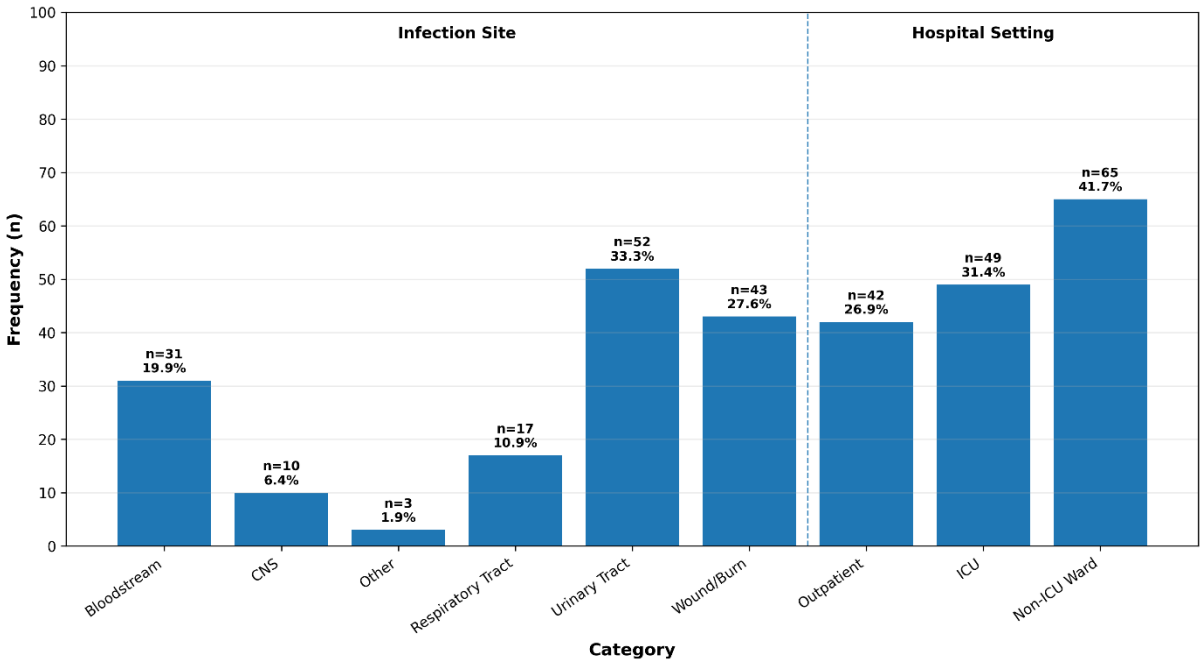


Figure 3: Distribution of *Pseudomonas aeruginosa* Isolates According to Infection Site and Hospital Setting (n = 156)

The antibiotic susceptibility data of Pseudomonas aeruginosa isolates for 13 selected antibiotics is presented in Table 3 Colistin was the most sensitive, with 122 (78.2%) isolates being sensitive

and 9 (5.8%) resistant. Of the remaining antibiotics, gentamicin was most sensitive (47.4%) with piperacillin/tazobactam (42.9%) and cefepime (41.7%). The highest resistance was found for imipenem (75.0%), ciprofloxacin (71.2%), levofloxacin (70.5%) and meropenem (66.7%). High levels of resistance in the strains were also observed against piperacillin (57.7%), amikacin (57.1%), ceftazidime (53.8%), cefepime (51.9%), aztreonam (51.9%) and tobramycin (49.4%).

Table 3: Antibiotic Susceptibility Profile of *Pseudomonas aeruginosa* to 13 Selected Antibiotics

Antibiotic	Sensitive (S) n (%)	Intermediate (I) n (%)	Resistant (R) n (%)
Piperacillin	56 (35.9%)	10 (6.4%)	90 (57.7%)
Piperacillin/Tazobactam	67 (42.9%)	15 (9.6%)	74 (47.4%)
Ceftazidime	63 (40.4%)	9 (5.8%)	84 (53.8%)
Cefepime	65 (41.7%)	10 (6.4%)	81 (51.9%)
Aztreonam	64 (41.0%)	11 (7.1%)	81 (51.9%)
Imipenem	33 (21.2%)	6 (3.8%)	117 (75.0%)
Meropenem	44 (28.2%)	8 (5.1%)	104 (66.7%)
Ciprofloxacin	39 (25.0%)	6 (3.8%)	111 (71.2%)
Levofloxacin	38 (24.4%)	8 (5.1%)	110 (70.5%)
Gentamicin	74 (47.4%)	11 (7.1%)	71 (45.5%)
Amikacin	54 (34.6%)	13 (8.3%)	89 (57.1%)
Tobramycin	64 (41.0%)	15 (9.6%)	77 (49.4%)
Colistin	122 (78.2%)	25 (16.0%)	9 (5.8%)

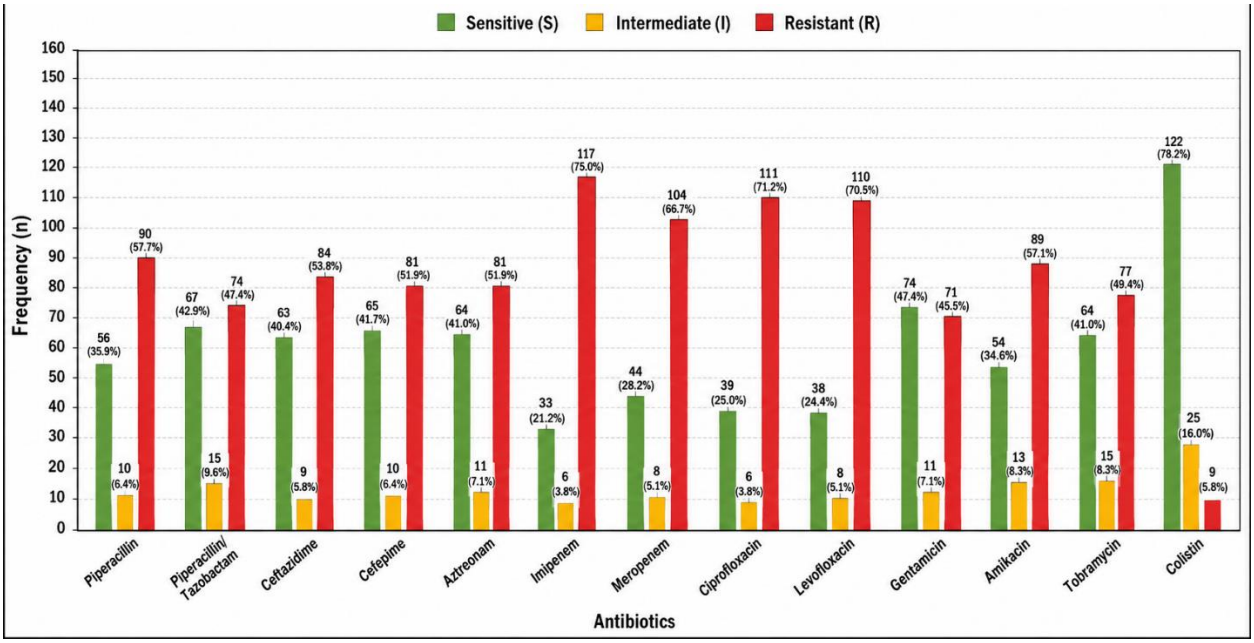


Figure 4: Antibiotic Susceptibility Profile of *Pseudomonas aeruginosa* to 13 Selected Antibiotics

The antibiotic resistance patterns of the Pseudomonas aeruginosa infections in hospitals and community settings are compared in Table 4.4. Hospital-acquired isolates demonstrated higher resistance to cefepime (56.1% vs. 40.5%), ceftazidime (57.9% vs. 42.9%), levofloxacin (73.7% vs. 61.9%), piperacillin (59.6% vs. 52.4%), and tobramycin (51.8% vs. 42.9%). community-acquired isolates showed higher resistance against mainly ciprofloxacin (78.6% vs 68.4%), imipenem (76.2% vs 74.6%) and meropenem (69.0% vs 65.8%). The resistance to amikacin and aztreonam was almost equal in the two groups, and the resistance to colistin was the lowest in both the hospital-acquired (7.0%) and community-acquired (2.4%) isolates.

Table 4: Comparison of Antibiotic Resistance Patterns Between Hospital-Acquired and Community-Acquired *Pseudomonas aeruginosa* Infections

Antibiotic	Hospital-Acquired Resistant (%)	Community-Acquired Resistant (%)	Difference (%)
Piperacillin	59.60%	52.40%	7.30%
Piperacillin/Tazobactam	48.20%	45.20%	3.00%
Ceftazidime	57.90%	42.90%	15.00%
Cefepime	56.10%	40.50%	15.70%
Aztreonam	51.80%	52.40%	-0.60%
Imipenem	74.60%	76.20%	-1.60%
Meropenem	65.80%	69.00%	-3.30%
Ciprofloxacin	68.40%	78.60%	-10.20%
Levofloxacin	73.70%	61.90%	11.80%
Gentamicin	46.50%	42.90%	3.60%
Amikacin	57.00%	57.10%	-0.10%
Tobramycin	51.80%	42.90%	8.90%
Colistin	7.00%	2.40%	4.60%

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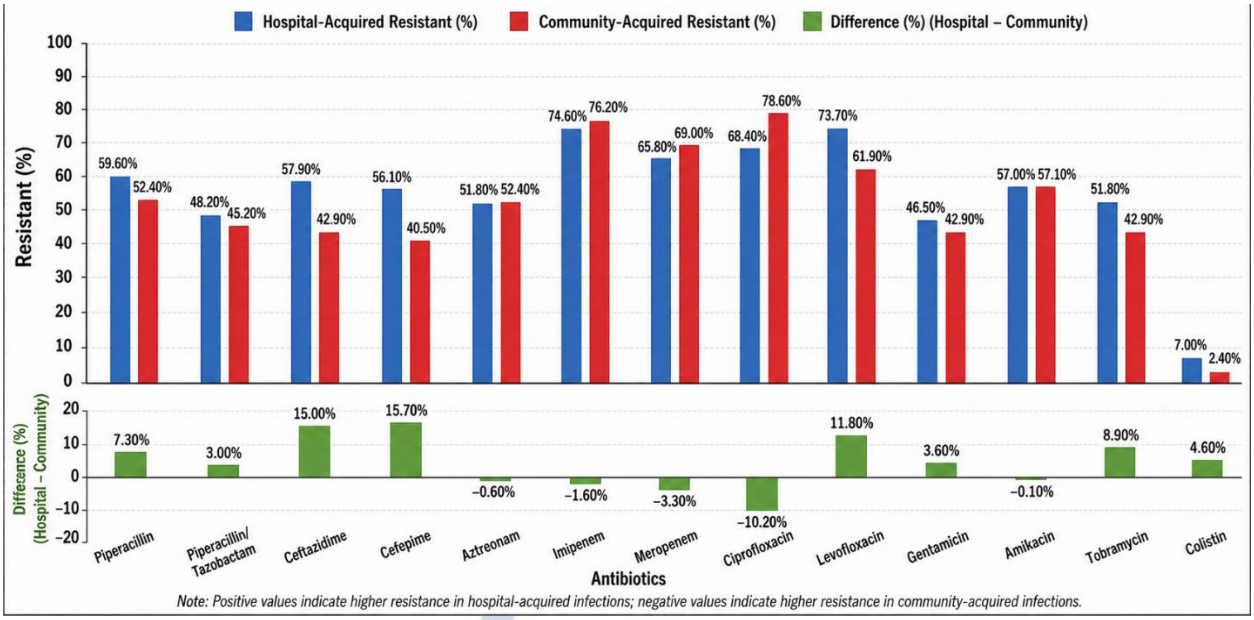


Figure 5: Comparison of Antibiotic Resistance Patterns Between Hospital-Acquired and Community-Acquired *Pseudomonas aeruginosa* Infections

The distribution of antibiotic resistance pattern of Pseudomonas aeruginosa isolates in different setting of hospital (ICU, non-ICU ward and outpatient) is presented in Table 5 In general, the highest resistance was generally seen in the isolates from the ICU for imipenem (83.7%), ciprofloxacin (75.5%), levofloxacin (75.5%), meropenem (75.5%) and piperacillin (69.4%). The non-ICU ward isolates had intermediate resistance rates for most antibiotics while the outpatient isolates had comparatively low resistance for several antibiotics. Colistin had the least resistance with resistance rate being 10.2% in ICU isolate, 4.6% in non-ICU ward isolate, and 2.4% in outpatient isolate. In general, resistance to antibiotics was more seen in isolates from ICU patients than from non-ICU wards and outpatient settings.

Table 5: Distribution of Antibiotic Resistance Patterns by Hospital Setting (ICU vs. Non-ICU vs. Outpatient)

Antibiotic	ICU Setting (%)	Non-ICU Ward (%)	Outpatient (%)
Piperacillin	69.40%	52.30%	52.40%
Piperacillin/Tazobactam	51.00%	46.20%	45.20%
Ceftazidime	67.30%	50.80%	42.90%
Cefepime	61.20%	52.30%	40.50%
Aztreonam	53.10%	50.80%	52.40%
Imipenem	83.70%	67.70%	76.20%
Meropenem	75.50%	58.50%	69.00%
Ciprofloxacin	75.50%	63.10%	78.60%
Levofloxacin	75.50%	72.30%	61.90%
Gentamicin	55.10%	40.00%	42.90%

Amikacin	61.20%	53.80%	57.10%
Tobramycin	51.00%	52.30%	42.90%
Colistin	10.20%	4.60%	2.40%

The Pseudomonas aeruginosa antibiotic resistance profile is by specimen type and infection site as shown in Table 6 Urine accounted for the highest number of isolates (52, 33.3%) followed by pus/wound (43, 27.6%) and blood (31, 19.9%). The average rates of antibiotic resistance were from 48.7% in the other category of specimens to 55.6% in blood isolates. All isolates from blood, urine, pus/wound, respiratory and other specimens were found to be multidrug resistant (MDR), and 90.0% of CSF isolates were MDR. In overall, the presence of high level of antibiotic resistance and MDR observed in all type of specimens.

Table 6: Antibiotic Resistance Profile of *Pseudomonas aeruginosa* According to Specimen Type and Infection Site

Specimen Type	Number (%)	Average Resistance Rate (%)	MDR Prevalence (%)
Blood	31 (19.9%)	55.60%	31 (100.0%)
CSF	10 (6.4%)	53.80%	9 (90.0%)
Other	3 (1.9%)	48.70%	3 (100.0%)
Pus/Wound	43 (27.6%)	53.50%	43 (100.0%)
Respiratory	17 (10.9%)	52.90%	17 (100.0%)
Urine	52 (33.3%)	54.60%	52 (100.0%)

The prevalence of multidrug resistant (MDR) Pseudomonas aeruginosa was determined in the study population and is presented in Table 7 Of the 156 isolates, 155 (99.4%) were multidrug resistant (MDR) and 1 (0.6%) was non-MDR. All of the strains (0.0%) were classified as non-carbapenem resistant. The prevalence of MDR among the Pseudomonas aeruginosa isolates in this study is extremely high, thus highlighting the high prevalence of MDR in the collected isolates.

Table 7: Prevalence of Multidrug-Resistant (MDR) *Pseudomonas aeruginosa* Isolates

Resistance Category	Number of Isolates	Percentage (%)
Multidrug-Resistant (MDR)	155	99.40%
Carbapenem-Resistant	0	0.00%
Non-MDR	1	0.60%
TOTAL	156	100.00%

The distribution of multidrug-resistant (MDR) Pseudomonas aeruginosa isolates by place of infection acquisition is shown in Table 7(A). The 114 hospital-acquired isolates included 113 (99.1%) MDR and the 42 community-acquired isolates all (100.0%) were classified as MDR. No CPE were found in either hospital acquired or community acquired isolates. The overall prevalence of MDR Pseudomonas aeruginosa was very high for both acquisition groups.

Table 7(A): Multidrug-Resistant *Pseudomonas aeruginosa* Stratified by Acquisition Type (Hospital-Acquired vs. Community-Acquired)

Acquisition Type	Total Isolates	MDR (n)	MDR (%)	Carbapenem-Resistant (n)	Carbapenem-Resistant (%)
Hospital-Acquired	114	113	99.10%	0	0.00%

Community-Acquired	42	42	100.00%	0	0.00%

The distribution of multidrug-resistant (MDR) Pseudomonas aeruginosa isolates by hospital setting is shown in Table 7(B). All of the 49 (100.0%) isolates recovered from the ICU and 42 (100.0%) from an outpatient setting were found to be MDR. Of the 65 non-ICU ward isolates, 64 (98.5%) were considered MDR. There were no hospital settings that contained any carbapenem-resistant isolates. In general, MDR Pseudomonas aeruginosa was found to be very high in all types of facilities, including the ICU, non-ICU and out-patient departments.

Table 7(B): Multidrug-Resistant *Pseudomonas aeruginosa* Stratified by Hospital Setting (ICU vs. Non-ICU vs. Outpatient)

Hospital Setting	Total Isolates	MDR (n)	MDR (%)	Carbapenem-Resistant (n)	Carbapenem-Resistant (%)
ICU	49	49	100.00%	0	0.00%
Non-ICU Ward	65	64	98.50%	0	0.00%
Outpatient	42	42	100.00%	0	0.00%

A chi-square test was used for the analysis to compare antibiotic resistance among the hospital-acquired and community-acquired Pseudomonas aeruginosa isolates. There were differences in the resistance percentages between the two groups but none of these differences were significant at the 5% level ($p>0.05$).

Table 8: Statistical Comparison of Antibiotic Resistance According to Infection Acquisition

Antibiotic	**χ^2 value**	**p-value**	**Interpretation**
Piperacillin	0.664	0.415	Not significant
Piperacillin/Tazobactam	0.111	0.739	Not significant
Ceftazidime	2.793	0.095	Not significant
Cefepime	3.017	0.082	Not significant
Aztreonam	0.005	0.945	Not significant
Imipenem	0.043	0.835	Not significant
Meropenem	0.147	0.702	Not significant
Ciprofloxacin	1.541	0.215	Not significant
Levofloxacin	2.048	0.152	Not significant
Gentamicin	0.163	0.686	Not significant
Amikacin	<0.001	0.989	Not significant
Tobramycin	0.972	0.324	Not significant
Colistin*	—	0.446	Not significant

*Fisher's Exact Test was used for colistin because of the small number of resistant isolates.

The highest levels of difference in number of isolates by source were found for the antibiotic cefepime, ceftazidime and levofloxacin. These differences were not significant ($p> 0.05$), however. Thus, there was no significant association between the type of infection and the resistance to any of the antibiotics tested.

Pearson's Chi-square test was used to determine the association between the hospital setting (ICU/non-ICU ward/outpatient) and antibiotic resistance. There was a trend toward increased resistance in ICU isolates to most antibiotics, but no association was found between antimicrobial resistance and the type of hospital ($p > 0.05$).

Table 9: Association Between Hospital Setting and Antibiotic Resistance

Antibiotic	χ^2 value	df	p-value	Interpretation
Piperacillin	4.003	2	0.135	Not significant
Piperacillin/Tazobactam	0.377	2	0.828	Not significant
Ceftazidime	5.882	2	0.053	Not significant
Cefepime	3.907	2	0.142	Not significant
Aztreonam	0.064	2	0.969	Not significant
Imipenem	3.849	2	0.146	Not significant
Meropenem	3.801	2	0.150	Not significant
Ciprofloxacin	3.645	2	0.162	Not significant
Levofloxacin	2.186	2	0.335	Not significant
Gentamicin	2.733	2	0.255	Not significant
Amikacin	0.621	2	0.733	Not significant
Tobramycin	0.991	2	0.609	Not significant
Colistin	2.819	2	0.244*	Not significant

*Caution is required in interpreting the Pearson Chi-square result for colistin because of small expected cell frequencies; an exact test may be considered.

Ceftazidime resistance was significant, though not statistically conventional, when compared to non-ICU and outpatient isolates ($\chi^2 = 5.882$, $p = 0.053$), although it was lower in outpatient isolates than in non-ICU isolates. Likewise, no differences were found for imipenem, meropenem, ciprofloxacin or other antibiotics that were tested.

In general, the results suggest that the level of resistance to the studied antibiotics differed by hospital type, but there was not enough evidence in this sample to conclude that the hospital type was significantly related to resistance to the antibiotics studied.

DISCUSSION

The present study evaluated 156 clinical isolates of *Pseudomonas aeruginosa* recovered from patients with different infections at Allama Iqbal Teaching Hospital, Dera Ghazi Khan. The largest proportions of isolates were obtained from patients aged 19–40 years (30.8%), followed by 41–60 years (28.2%) and 61–80 years (23.7%), while males and females were equally represented (50.0% each). The relatively broad age distribution is consistent with the ability of *P. aeruginosa* to cause infections across different clinical populations and infection sites. The age distribution differed from that reported by Farooq et al., who observed the highest number of isolates among patients aged 60–80 years and also reported a substantial MDR burden [11]. Such differences may reflect variation in hospital populations, clinical departments, specimen types, and inclusion criteria rather than a direct effect of age on susceptibility to *P. aeruginosa* infection.

Urinary tract infections were the most frequent infection type in the present study (33.3%), followed by wound/burn infections (27.6%) and bloodstream infections (19.9%). Respiratory, CNS, and other infections accounted for smaller proportions. This distribution differs from that of Ullah et al., who reported pus and wound specimens as the major sources of MDR *P. aeruginosa*, followed by urine, blood, and sputum [12]. Similarly, Farooq et al. identified pus as the most common source of MDR *P. aeruginosa*, followed by tracheal aspirates [11]. These differences likely reflect local variation in infection patterns, hospital populations, specimen distribution, and antimicrobial exposure. Therefore, the predominance of urinary isolates in the current study should be interpreted primarily as a local epidemiological finding.

The considerable proportion of wound/burn and bloodstream isolates is clinically important because *P. aeruginosa* is an important cause of

difficult-to-treat infections in damaged tissues and hospitalized patients. Saleem and Bokhari reported MDR, XDR, and PDR *P. aeruginosa* among isolates recovered from wound infections, bacteremia, and burn wounds in Rawalpindi and Islamabad, supporting the clinical importance of resistant *P. aeruginosa* in these settings [13].

Regarding healthcare setting, most isolates were recovered from non-ICU wards (41.7%), followed by ICU (31.4%) and outpatient settings (26.9%). The larger number of non-ICU isolates primarily reflects the distribution of the study population and should not be interpreted as indicating a greater individual risk than in ICU patients. Nevertheless, ICUs remain important reservoirs of antimicrobial resistance because of severe illness, invasive devices, prolonged hospitalization, and extensive antimicrobial exposure. Sarwari et al. reported substantial resistance to aztreonam, ceftazidime, imipenem, ofloxacin, amikacin, and gentamicin among ICU *P. aeruginosa* isolates in Pakistan [14]. The current findings are broadly consistent with this historical evidence, although differences in study period and patient populations should be considered.

A major finding of the present study was the high level of resistance to most tested antibiotics. Colistin showed the greatest activity, with 78.2% of isolates susceptible and only 5.8% resistant, followed by gentamicin (47.4%), piperacillin/tazobactam (42.9%), and cefepime (41.7%). In contrast, resistance was highest to imipenem (75.0%), ciprofloxacin (71.2%), levofloxacin (70.5%), and meropenem (66.7%). High resistance was also observed to piperacillin, amikacin, ceftazidime, cefepime, aztreonam, and tobramycin. These findings demonstrate a substantial loss of activity of several commonly used antipseudomonal agents in the study population.

The relatively preserved activity of colistin is comparable with findings from Farooq et al., who reported complete colistin susceptibility among their *P. aeruginosa* isolates, while Ullah et al. reported a high MDR burden and resistance to several antimicrobial classes [12,11]. Differences between studies may be related to local antimicrobial prescribing practices, previous antimicrobial exposure, infection sites, resistance mechanisms, and hospital-specific epidemiology. Therefore, susceptibility percentages from different institutions should be interpreted within their local context.

Fluoroquinolone resistance was particularly high in the present study, with resistance rates of 71.2% for ciprofloxacin and 70.5% for levofloxacin. These findings are consistent with the broader pattern of fluoroquinolone resistance reported in Pakistan. Sarwari et al. reported 59% resistance to ofloxacin among ICU *P. aeruginosa* isolates, providing historical evidence of substantial fluoroquinolone resistance in Pakistani ICU populations [14]. The high resistance observed in the present study supports the importance of local susceptibility testing before empirical or definitive treatment and reinforces the need for antimicrobial stewardship.

Resistance to β-lactam antibiotics was also considerable. Piperacillin, ceftazidime, cefepime, and aztreonam showed resistance rates of 57.7%, 53.8%, 51.9%, and 51.9%, respectively. These findings are comparable with those of Sarwari et al., who reported 68% resistance to aztreonam and 67% to ceftazidime among ICU isolates [14]. Recent evidence also indicates continuing antimicrobial resistance among *P. aeruginosa* isolates in Pakistan [15]. Razzak et al. similarly reported resistance to piperacillin, ceftazidime, and cefepime among *P. aeruginosa* isolates from chronic suppurative otitis media, although the magnitude varied from that observed in the current study. Such differences emphasize the importance of infection-site and institution-specific surveillance.

Carbapenem resistance was particularly concerning, with imipenem and meropenem resistance rates of 75.0% and 66.7%, respectively. Sarwari et al. reported approximately 66% imipenem resistance among ICU isolates, which is comparable with the present findings [14]. Carbapenem resistance in *P. aeruginosa* may arise through reduced outer-membrane permeability, alteration or loss of OprD porin, increased efflux activity, and carbapenemase production. Gondal

et al. investigated carbapenem- and aminoglycoside-resistance genes among carbapenem-resistant clinical *P. aeruginosa* isolates in Pakistan, highlighting the potential contribution of acquired resistance mechanisms [16].

An important issue requiring clarification before final submission is the apparent discrepancy between the high phenotypic resistance to imipenem and meropenem and the classification of zero isolates as "carbapenem-resistant" in Table 7. The operational definition used for "carbapenem-resistant" should be clearly specified and checked against the AST data and methodology. This distinction is important because the phenotypic resistance findings should not be interpreted interchangeably with a separately defined carbapenem-resistant category.

Colistin remained the most active agent, with 78.2% susceptibility and 5.8% resistance. Although this comparatively favorable activity is encouraging, detection of colistin-resistant isolates remains clinically important because colistin may be required for infections caused by highly resistant Gram-negative organisms. The current finding agrees with Farooq et al., who reported complete colistin susceptibility [11]. Conversely, Ijaz et al. identified colistin-resistant *P. aeruginosa* and *K. pneumoniae* from infected wounds in Lahore, emphasizing the need for continued molecular and epidemiological surveillance of colistin resistance [17]. Mastoraki et al. also demonstrated the therapeutic relevance of colistin in severe *P. aeruginosa* infections where isolates were resistant to other antimicrobial agents [18]. Thus, preservation of colistin activity should be accompanied by appropriate susceptibility testing and antimicrobial stewardship rather than indiscriminate use.

Comparison according to acquisition type showed numerically higher resistance among hospital-acquired isolates to cefepime, ceftazidime, levofloxacin, piperacillin, and tobramycin, whereas community-acquired isolates showed slightly higher resistance to ciprofloxacin, imipenem, and meropenem. However, none of these differences was statistically significant ($p>0.05$). The findings therefore suggest that resistant *P. aeruginosa* is present in both hospital- and community-associated infections and should not be considered restricted to hospitalized patients. Ullah et al. similarly emphasized the importance of *P. aeruginosa* in both community- and hospital-acquired infections [12].

The analysis by hospital setting demonstrated generally higher resistance among ICU isolates for several antibiotics. ICU resistance was highest for imipenem (83.7%), meropenem (75.5%), ciprofloxacin (75.5%), levofloxacin (75.5%), and piperacillin (69.4%). Sarwari et al. similarly reported substantial resistance among Pakistani ICU isolates [14]. Nevertheless, the differences among ICU, non-ICU, and outpatient groups were not statistically significant for the antibiotics examined. Therefore, the observed ICU-associated increase should be regarded as a descriptive trend rather than evidence of a statistically confirmed association.

Resistance was substantial across all specimen types. Blood isolates had the highest average resistance rate (55.6%), followed by urine (54.6%), pus/wound (53.5%), CSF (53.8%), and respiratory isolates (52.9%). MDR was detected in 100% of blood, urine, pus/wound, respiratory, and other isolates and in 90.0% of CSF isolates. The high resistance among bloodstream isolates is particularly concerning because therapeutic options may be limited in severe systemic infections. Saleem and Bokhari similarly reported high MDR, XDR, and PDR phenotypes among isolates from bacteremia, wounds, and burn infections [13]. The findings from wound specimens are also consistent with Ullah et al. and Saleem and Bokhari, who identified wound and pus specimens as important sources of resistant *P. aeruginosa* [12,13]. Similarly, previous work by Samad et al. reported substantial MDR and resistance among *P. aeruginosa* isolates from respiratory infections, supporting the continuing importance of this pathogen across different infection sites.

The most striking finding was the extremely high prevalence of MDR, with 155 of 156 isolates (99.4%) classified as MDR and only one isolate

(0.6%) classified as non-MDR. This prevalence is considerably higher than that reported in several Pakistani studies. Farooq et al. reported approximately 55% MDR, while Samad et al. reported MDR among approximately half of respiratory isolates. Saleem and Bokhari reported 36.3% MDR, 18.1% XDR, and 4.5% PDR phenotypes, while Ullah et al. also documented a substantial MDR burden among *P. aeruginosa* isolates [13,12] The unusually high MDR prevalence in the current study may reflect differences in patient population, hospital environment, antimicrobial exposure, inclusion criteria, and MDR definitions. Therefore, the 99.4% prevalence should be interpreted as specific to the present study population rather than extrapolated to all *P. aeruginosa* infections in Pakistan.

MDR prevalence was similarly high according to acquisition type: 99.1% among hospital-acquired isolates and 100.0% among community-acquired isolates. Likewise, all ICU and outpatient isolates were MDR, compared with 98.5% of non-ICU isolates. These findings indicate that MDR was not confined to the ICU or hospital-acquired infections. However, the relatively small community-acquired subgroup and the extremely high MDR prevalence across all groups require cautious interpretation. WHO's GLASS framework emphasizes standardized AMR surveillance to support infection-control and antimicrobial-stewardship interventions [19].

The overall findings are consistent with recent evidence from Pakistan showing substantial antimicrobial resistance among *P. aeruginosa*. Farooq et al. reported approximately 55% MDR, while Saleem and Bokhari documented MDR, XDR, and PDR phenotypes and identified colistin as the most active agent tested. Razzak et al. reported clinically important resistance among isolates from chronic suppurative otitis media, while Ijaz et al. documented colistin-resistant isolates from infected wounds [11,13, 15,17]. Collectively, these studies demonstrate that resistance patterns vary between hospitals, regions, specimen types, infection sites, and patient populations.

The present study adds important local evidence from Dera Ghazi Khan, where resistance was high across multiple antimicrobial classes and MDR prevalence reached 99.4%. These findings have important clinical implications. Culture-based identification and antimicrobial susceptibility testing should remain central to treatment decisions, particularly where resistance to commonly used antipseudomonal agents is high. Antimicrobial stewardship is equally important, and the recommendation by Farooq et al. for judicious antibiotic use and continued laboratory surveillance remains relevant.

WHO identifies AMR surveillance as a key component of the global response and emphasizes standardized data generation to guide action [11,19]. In healthcare settings, infection-prevention and control measures should therefore complement antimicrobial stewardship. Because substantial resistance was observed in ICU, non-ICU, and outpatient groups, surveillance and infection-control efforts should extend beyond intensive-care settings and include routine antibiogram review, appropriate isolation precautions, environmental hygiene, and monitoring of antimicrobial use.

Overall, the present study demonstrates a substantial antimicrobial-resistance burden among *P. aeruginosa* isolates from Dera Ghazi Khan. Urinary tract, wound/burn, and bloodstream infections represented the major clinical sources, while resistance was high to several β-lactams, fluoroquinolones, aminoglycosides, and carbapenems. Colistin retained the greatest activity, although resistant isolates were present. The most important finding was the 99.4% MDR prevalence, which highlights the need for routine susceptibility testing, antimicrobial stewardship, infection prevention, and continuous local surveillance. The findings should nevertheless be interpreted within the limitations of the cross-sectional design and study population; numerical differences between acquisition groups and hospital settings did not establish causal or statistically

significant associations. The discrepancy between high imipenem/meropenem resistance and the reported absence of "carbapenem-resistant"

isolates should also be resolved by clearly defining the operational criteria before final submission.

Conclusion

This study demonstrated a high burden of antibiotic resistance among *Pseudomonas aeruginosa* isolates from different clinical specimens and hospital settings in Dera Ghazi Khan. The highest resistance was observed against imipenem, ciprofloxacin, levofloxacin, and meropenem, whereas colistin showed the highest susceptibility. Resistance was generally higher among hospital-acquired and ICU-associated isolates, although the observed differences were not statistically significant. The very high proportion of multidrug-resistant isolates (99.4%) highlights a substantial antimicrobial-resistance burden and emphasizes the need for effective antimicrobial stewardship, infection-control practices, and continuous local surveillance.

Recommendations

1. Strengthen antimicrobial stewardship and rational antibiotic use.
2. Conduct regular surveillance of *P. aeruginosa* resistance patterns.
3. Strengthen infection-prevention and control measures, particularly in ICUs.
4. Perform routine culture and antimicrobial susceptibility testing.
5. Limit unnecessary use of broad-spectrum antibiotics, particularly carbapenems and fluoroquinolones.
6. Provide regular training on infection control and antimicrobial stewardship.
7. Develop and periodically update hospital-specific antibiotic guidelines.
8. Conduct multicenter studies to assess resistance patterns across Pakistan.

Limitations

1. Single-center study, limiting generalizability.
2. Cross-sectional design, limiting assessment of temporal trends and causality.
3. Molecular mechanisms of antibiotic resistance were not investigated.
4. Patient-level clinical outcomes were not assessed.
5. Previous antibiotic exposure was not evaluated.

Conflict of Interest

The authors declare no conflict of interest.

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Ethical Approval

Ethical approval was obtained from the relevant institutional ethics committee.

Data Availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Author Contributions

All authors contributed to the study conception, data collection, analysis, interpretation, and preparation of the manuscript.

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