

## Acute Antimicrobial Resistance Patterns of *Pseudomonas aeruginosa* and *E. coli* Clinical Isolates

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**Abstract:** Antimicrobial resistance among Gram-negative bacteria is a growing global health concern, particularly in hospital settings, where opportunistic pathogens contribute to severe infections. *Pseudomonas aeruginosa* and *Escherichia coli* are common causes of healthcare-associated infections and frequently demonstrate multidrug resistance, limiting therapeutic options. **Objective:** To determine the antimicrobial resistance patterns and prevalence of multidrug-resistant strains of *Pseudomonas aeruginosa* and *Escherichia coli* isolated from clinical specimens in a tertiary care hospital. **Methods:** A retrospective observational study was conducted at the University of Veterinary and Animal Sciences, Lahore, Pakistan, from June 2025 to December 2025. A total of 60 clinical isolates (30 *Pseudomonas aeruginosa* and 30 *Escherichia coli*) obtained from various specimens, including urine, sputum, blood, pus, wound swabs, and catheter tips, were analyzed. Bacterial identification was performed using standard microbiological techniques. Antimicrobial susceptibility testing was performed using the Kirby–Bauer disk diffusion method in accordance with established clinical laboratory standards. Antibiotics tested for *Pseudomonas aeruginosa* included ceftazidime, piperacillin-tazobactam, ciprofloxacin, gentamicin, amikacin, imipenem, and colistin, whereas *Escherichia coli* isolates were tested against ampicillin, ceftriaxone, ciprofloxacin, gentamicin, amikacin, meropenem, and nitrofurantoin. Multidrug resistance was defined as resistance to three or more antimicrobial classes. Data were analyzed using SPSS, with descriptive statistics used for frequencies and percentages, and chi-square and independent t-tests applied for comparisons, with  $p < 0.05$  considered statistically significant. **Results:** A total of 60 bacterial isolates were analyzed. The mean age of patients associated with *Pseudomonas aeruginosa* isolates was  $48.97 \pm 16.32$  years, while for *Escherichia coli* it was  $49.53 \pm 15.06$  years; there was no significant difference between groups ( $p = 0.889$ ). *Pseudomonas aeruginosa* demonstrated the highest resistance to ciprofloxacin (56.7%), gentamicin (53.3%), and ceftazidime (50.0%), while the lowest resistance was observed for colistin (13.3%). *Escherichia coli* showed the highest resistance to ampicillin (70.0%) and ceftriaxone (63.3%), with lower resistance to meropenem and nitrofurantoin (20.0%). Overall, 56.7% of isolates were identified as multidrug resistant. No statistically significant difference in the prevalence of multidrug resistance was observed between the two organisms. **Conclusion:** A high prevalence of antimicrobial resistance and multidrug-resistant strains was observed among *Pseudomonas aeruginosa* and *Escherichia coli* isolates, highlighting the urgent need for continuous antimicrobial surveillance, rational antibiotic prescribing, and strengthened antimicrobial stewardship programs to control the spread of resistant pathogens in healthcare settings.

**Keywords:** Antimicrobial Resistance, *Escherichia coli*, Gram-Negative Bacteria, Multidrug Resistance, *Pseudomonas aeruginosa*

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### Introduction

Antimicrobial resistance (AMR) represents one of the most pressing global public health emergencies of the twenty-first century (1). The irrational use of broad-spectrum antibiotics is widely recognized as the primary driver of AMR, contributing substantially to increased morbidity, mortality, and healthcare costs worldwide (1). Among Gram-negative pathogens, *Escherichia coli* and *Pseudomonas aeruginosa* are consistently identified as the most clinically significant and frequently isolated organisms in both community and hospital settings (2, 3). These pathogens are notorious for their capacity to acquire and disseminate multidrug resistance (MDR) determinants, including extended-spectrum

$\beta$ -lactamases (ESBLs), carbapenemases, and plasmid-mediated resistance genes, thereby severely limiting therapeutic options (4, 5).

Globally, resistance trends among *E. coli* and *P. aeruginosa* have demonstrated alarming trajectories, with increasing resistance to fluoroquinolones, third-generation cephalosporins, and carbapenems documented across diverse clinical settings (4, 5). A systematic analysis of AMR trends in blood and cerebrospinal fluid cultures in Pakistan between 2011 and 2015 demonstrated a sharp increase in carbapenem resistance. It highlighted the escalating burden of resistance among major invasive pathogens (5). Similarly, surveillance data from Punjab, Pakistan, identified *E. coli* and *P. aeruginosa* as the second- and third-most frequently isolated pathogens, respectively, with severely restricted antimicrobial susceptibility profiles (3).

Pakistan presents a uniquely challenging epidemiological landscape for AMR. The country lacks a robust national AMR surveillance network, and existing data are largely fragmented and institution-specific (2, 4). Contributing factors include widespread over-the-counter availability of antibiotics, self-medication practices, inappropriate prescription habits, and the absence of enforced antimicrobial stewardship programs (ASPs), Afridi et al. (6, 1). Studies have documented that up to 96.9% of pharmacy outlets in Pakistan dispense antibiotics without a valid prescription (6). Antibiotic consumption increased by 65% between 2000 and 2010 (6). Furthermore, in over 75% of hospitalized cases, the rationale for antibiotic prescribing is not documented, with 96.2% of antibiotics prescribed empirically (1).

Retrospective analyses from major Pakistani institutions have consistently identified *E. coli* and *P. aeruginosa* among the top five most commonly isolated bacteria in both adult and pediatric populations (2, 7). High rates of ESBL production, metallo-β-lactamase (MBL) carriage up to 71%, and carbapenem resistance have been reported in clinical isolates (4). Despite this burden, comprehensive, up-to-date local antibiograms remain scarce, impeding evidence-based empirical therapy (1, 3). This study is therefore designed to characterize the acute AMR patterns of *P. aeruginosa* and *E. coli* clinical isolates, thereby contributing critical surveillance data to inform rational antibiotic prescribing and stewardship initiatives in Pakistan.

**Methodology**

This retrospective observational study was conducted at the University of Veterinary and Animal Sciences, Lahore, Punjab, Pakistan, over seven months from June 2025 to December 2025. The study was designed to evaluate the antimicrobial resistance profiles of clinical isolates of *Pseudomonas aeruginosa* and *Escherichia coli* obtained from patients receiving care at a tertiary care hospital. The primary objectives were to determine the frequency of resistance to commonly used antimicrobial agents and to assess the prevalence of multidrug-resistant (MDR) strains among these two clinically important bacterial pathogens.

The study used routinely recorded clinical microbiology laboratory data. Bacterial isolates were recovered from diagnostic specimens submitted as part of standard patient care from different hospital departments during the study period. Specimen sources included urine, sputum, wound swabs, pus, blood, and catheter tips. Only isolates identified as *Pseudomonas aeruginosa* or *Escherichia coli* were eligible for inclusion. A total of 60 non-duplicate clinical isolates were included in the final analysis, comprising 30 *Pseudomonas aeruginosa* and 30 *Escherichia coli* isolates. Isolates were obtained from patients of both sexes and various age groups. For each isolate, demographic and clinical information were extracted from laboratory and hospital records, including patient age, sex, specimen type, and hospital unit of origin. Bacterial identification was performed using routine microbiological procedures employed in the clinical microbiology laboratory. Antimicrobial susceptibility testing (AST) was carried out using the Kirby-Bauer disk diffusion method in accordance with established clinical laboratory standards. Zone diameters were interpreted using accepted clinical breakpoints recommended by standard reference guidelines. For *Pseudomonas aeruginosa*, susceptibility was assessed against ceftazidime, piperacillin-tazobactam, ciprofloxacin, gentamicin, amikacin, imipenem, and colistin. For *Escherichia coli*, susceptibility was determined against ampicillin, ceftriaxone, ciprofloxacin, gentamicin, amikacin, meropenem, and nitrofurantoin. Antimicrobial test results were recorded as sensitive or resistant according to the laboratory's interpretation.

Multidrug resistance was defined as resistance to three or more classes of antimicrobial agents relevant to the treatment of infections caused by the study organisms. Isolates fulfilling this definition were classified as MDR and included in the MDR prevalence analysis. A structured data collection form was used to compile study variables for each isolate, including isolate identification number, bacterial species, patient demographics, specimen type, hospital location, and AST results for all tested antibiotics.

Data were entered and analyzed using IBM SPSS software. Continuous variables, particularly age, were summarized as mean and standard deviation, while categorical variables were presented as frequencies and percentages. Comparative analyses were performed between the *Pseudomonas aeruginosa* and *Escherichia coli* groups. An independent samples t-test was used to compare the means of the two organism groups. Associations between categorical variables, including sex, specimen type, hospital unit, antimicrobial resistance patterns, and MDR status, were evaluated using the chi-square test or Fisher's exact test where appropriate. A two-sided *P* value of less than 0.05 was considered statistically significant.

**Results**

This study included 60 bacterial isolates, comprising 30% *Pseudomonas aeruginosa* (*P. aeruginosa*) and 30% *Escherichia coli* (*E. coli*), derived from various clinical specimens. The mean age of patients associated with *P. aeruginosa* isolates was 48.97 ± 16.32 years, and that for *E. coli* isolates was 49.53 ± 15.06 years. An independent-samples t-test showed that age did not differ significantly between the groups of organisms (*p* = 0.889). As shown in Table 1, these results suggest that patients' demographic characteristics were similar across groups.

**Table 1: Demographic Characteristics of Patients from Whom Bacterial Isolates Were Obtained**

Organism	Number of isolates (n)	Mean age (years) ± SD
<i>Pseudomonas aeruginosa</i>	30	48.97 ± 16.32
<i>Escherichia coli</i>	30	49.53 ± 15.06

In terms of the distribution of isolates by sex, for *Pseudomonas aeruginosa*, isolates were recovered from 19 male and 11 female patients. In contrast, for *Escherichia coli*, isolates were recovered from 11 male and 19 female patients. More isolates were from male patients; however, the difference between the groups was not statistically significant ( $\chi^2 = 3.27$ , *p* = 0.071). The distribution of isolates by sex is shown in Table 2.

**Table 2: Sex Distribution of Patients According to Bacterial Isolates**

Organism	Male	Female	Total
<i>Pseudomonas aeruginosa</i>	19	11	30
<i>Escherichia coli</i>	11	19	30

Bacterial isolates were collected from various clinical samples, including urine, sputum, pus, blood, wound swabs, and catheter tips. Most *Escherichia coli* isolates were identified from urine samples, and a significant proportion of *Pseudomonas aeruginosa* isolates were obtained from sputum, pus, and blood. Specimen types were unevenly distributed across the two organisms ( $\chi^2 = 17.63$ , *p* = 0.0035). The distribution of isolates by specimen type is presented in Table 3.

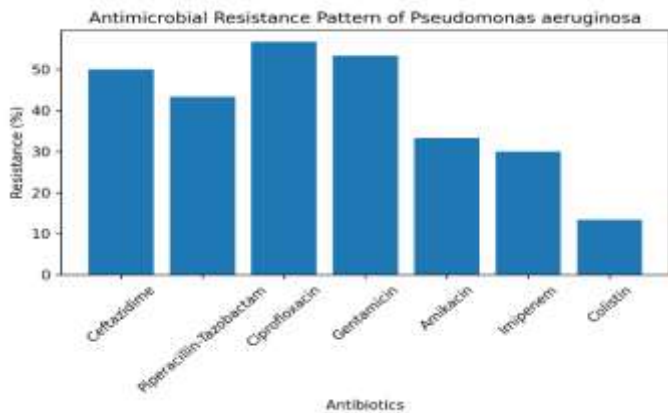
**Table 3: Distribution of Bacterial Isolates According to Specimen Type**

Specimen type	<i>Pseudomonas aeruginosa</i>	<i>Escherichia coli</i>
Blood	4	1
Catheter tip	4	3
Pus	6	3
Sputum	10	3
Urine	3	18
Wound swab	3	2

Isolates were further classified according to the hospital unit from which the specimens were obtained. Predominant *Pseudomonas aeruginosa*

isolates were isolated from surgical wards and intensive care units, whereas *Escherichia coli* isolates were recovered most frequently in the medicine department. However, the distribution across hospital units did not reach statistical significance ( $\chi^2 = 7.87, p = 0.096$ ). The distribution of isolates per hospital department is presented in Table 4.

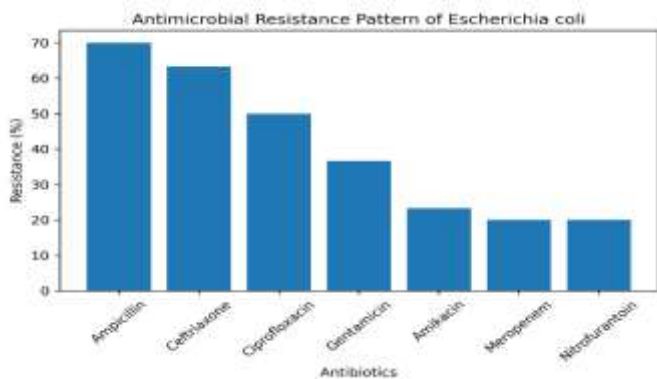
*Pseudomonas aeruginosa* susceptibility testing revealed significant resistance. The highest rates of resistance were found to ciprofloxacin (56.7%), gentamicin (53.3%), and ceftazidime (50.0%). Moderate resistance was observed to piperacillin–tazobactam (43.3%), and low resistance to both amikacin (33.3%) and imipenem (30.0%). Colistin was the drug with the lowest resistance (13.3%). The detailed resistance profile is shown in Table 5, and the graphical pattern is shown in Figure 1.



**Figure 1:** Antimicrobial resistance pattern of *Pseudomonas aeruginosa* clinical isolates. The highest resistance rates were observed for ciprofloxacin and gentamicin, whereas the lowest resistance was detected for colistin.

**Table 4: Distribution of Bacterial Isolates by Hospital Unit**

Hospital unit	<i>Pseudomonas aeruginosa</i>	<i>Escherichia coli</i>
ICU	7	1
Medicine	6	13
Outpatient	4	6
Pediatrics	4	3
Surgery	9	7



**Figure 2:** Antimicrobial resistance pattern of *Escherichia coli* clinical isolates. High resistance was observed for ampicillin and ceftriaxone, whereas lower resistance was detected for meropenem and nitrofurantoin

Antimicrobial susceptibility testing of the *E. coli* isolates demonstrated high resistance to ampicillin (70.0%) and ceftriaxone (63.3%). Mean resistance rates of ciprofloxacin (50.0%) were higher than gentamicin

(36.7%) and amikacin (23.3%). Sensitivity to meropenem and nitrofurantoin was relatively low (20.0%). The resistance patterns are tabulated in Table 6 and visualized in Figure 2.

**Table 5: Antimicrobial Resistance Pattern of *Pseudomonas Aeruginosa***

Antibiotic	Resistant (n)	Sensitive (n)	Resistance (%)
Ceftazidime	15	15	50.0
Piperacillin–Tazobactam	13	17	43.3
Ciprofloxacin	17	13	56.7
Gentamicin	16	14	53.3
Amikacin	10	20	33.3
Imipenem	9	21	30.0
Colistin	4	26	13.3

Antimicrobial susceptibility testing of the *E. coli* isolates demonstrated high resistance to ampicillin (70.0%) and ceftriaxone (63.3%). Mean resistance rates of ciprofloxacin (50.0%) were higher than gentamicin (36.7%) and amikacin (23.3%). Sensitivity to meropenem and nitrofurantoin was relatively low (20.0%). The resistance patterns are tabulated in Table 6 and visualized in Figure 2.

**Table 6: Antimicrobial Resistance Pattern of *Escherichia Coli***

Antibiotic	Resistant (n)	Sensitive (n)	Resistance (%)
Ampicillin	21	9	70.0
Ceftriaxone	19	11	63.3
Ciprofloxacin	15	15	50.0
Gentamicin	11	19	36.7
Amikacin	7	23	23.3
Meropenem	6	24	20.0
Nitrofurantoin	6	24	20.0

Resistance to commonly tested antibiotics—ciprofloxacin, gentamicin, and amikacin—was compared between the two groups. Although *Pseudomonas aeruginosa* demonstrated slightly higher resistance rates than *Escherichia coli*, the differences were not statistically significant. These comparisons are summarized in Table 7.

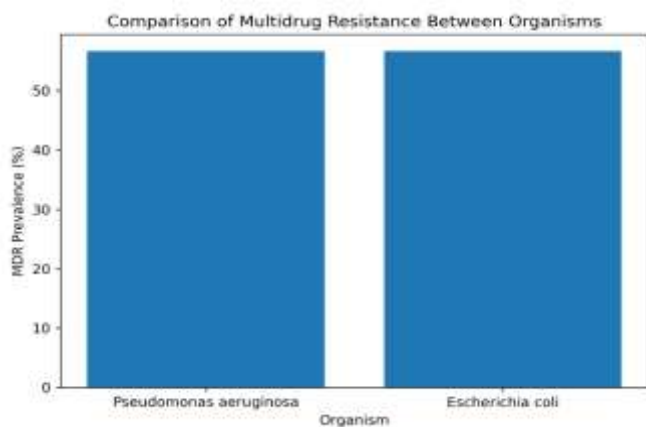
**Table 7: Comparison of Resistance to Commonly Tested Antibiotics**

Antibiotic	<i>P. aeruginosa</i> (%)	<i>E. coli</i> (%)	p-value
Ciprofloxacin	56.7	50.0	0.796
Gentamicin	53.3	36.7	0.299
Amikacin	33.3	23.3	0.567

A total of 17 multidrug-resistant (MDR) isolates (56.7%) were identified, defined as exhibiting resistance to three or more antimicrobial classes in either *Pseudomonas aeruginosa* or *Escherichia coli*. Statistical analysis indicated that the difference in MDR prevalence of the two organisms was not significant ( $p = 1.000$ ). Distribution of MDR isolates is given in Table 8, and the prevalence of MDR is illustrated in Figure 3.

**Table 8: Prevalence of Multidrug Resistance Among Isolates**

Organism	MDR (n)	Non-MDR (n)	MDR (%)
<i>Pseudomonas aeruginosa</i>	17	13	56.7
<i>Escherichia coli</i>	17	13	56.7



**Figure 3:** Comparison of multidrug resistance (MDR) prevalence between *Pseudomonas aeruginosa* and *Escherichia coli* isolates.

## Discussion

The present study characterized the antimicrobial resistance profiles of *P. aeruginosa* and *E. coli* clinical isolates, revealing high rates of resistance and a high prevalence of multidrug resistance (MDR) in both pathogens. Regarding *P. aeruginosa*, the highest resistance was observed against ciprofloxacin (56.7%), gentamicin (53.3%), and ceftazidime (50.0%), with relatively preserved susceptibility to colistin (13.3% resistance). Arman et al. reported comparable findings in Palestine, where *P. aeruginosa* demonstrated high resistance to ciprofloxacin (60%) and imipenem (59.3%). Arman et al. (8). Similarly, Mohamed et al. documented increasing *P. aeruginosa* resistance to ceftazidime (53.8%) and ciprofloxacin in catheter-associated UTI patients, closely mirroring our findings (9). Maina et al. reported that 100% of *P. aeruginosa* ICU isolates in Kenya were MDR (10), which is notably higher than the 56.7% MDR prevalence observed in our study. In the Pakistani context, Saleem et al. confirmed severely restricted antimicrobial options for *P. aeruginosa* across Punjab laboratories (11). At the same time, Arif et al. identified MDR in 59.5% of *P. aeruginosa* uropathogenic isolates from Peshawar (12), consistent with our findings.

Regarding *E. coli*, the highest resistance rates were observed to ampicillin (70.0%) and ceftriaxone (63.3%), with relatively preserved susceptibility to meropenem and nitrofurantoin (20.0% resistance each). Arman et al. similarly reported ampicillin as the most resistant antibiotic among Gram-negative bacteria (79.3%). Arman et al. (8). Saleem et al. documented high resistance of *E. coli* to beta-lactams and fluoroquinolones in Pakistan, Saleem et al. <sup>3</sup>, corroborating our ceftriaxone and ciprofloxacin resistance data. Arif et al. further reported that 86.6% of *E. coli* isolates from Pakistani UTI patients were MDR (11), somewhat higher than the 56.7% observed here, potentially reflecting differences in specimen source and patient population.

The MDR prevalence of 56.7% in both organisms is consistent with regional and international literature. Maina et al. reported 90% MDR in *E. coli* ICU isolates (10), while Mohamed et al. documented 68% MDR in *P. aeruginosa* from Somali hospitals (9). The relatively lower MDR rates in our study may reflect the inclusion of outpatient and community-acquired isolates alongside hospital-acquired specimens. Morris and Cerceo emphasized that MDR Gram-negative pathogens, particularly *E. coli* and *P. aeruginosa*, are increasingly prevalent in hospitalized settings globally, necessitating robust stewardship programs (12).

The specimen distribution in our study, with *E. coli* predominantly isolated from urine and *P. aeruginosa* from sputum and pus, aligns with established epidemiological patterns. Saleem et al. similarly identified urine as a predominant source for *E. coli* in Punjab, Pakistan Saleem et al. (3), and Cheema et al. confirmed *P. aeruginosa* as a leading respiratory pathogen in Pakistani ICU patients (13). The predominance of *P. aeruginosa* in surgical wards and ICUs in our study is consistent with

findings by Kadivar et al., who reported the ICU as the most frequent source of Gram-negative isolates (14), and with Saharman et al.'s scoping review, which identified *P. aeruginosa* as a dominant ICU pathogen in lower-middle-income countries (15).

Colistin retained the highest activity against *P. aeruginosa* (86.7% susceptibility), consistent with findings by Arman et al. Arman et al. (8) and Mohamed et al. (9), reinforcing its role as a last-resort agent. However, the observed 13.3% colistin resistance is concerning and warrants ongoing surveillance, particularly given reports of emerging colistin resistance in Pakistani uropathogens documented by Arif et al. (16).

Collectively, these findings underscore the urgent need for institution-specific antibiograms, antimicrobial stewardship programs, and rational prescribing practices in Pakistan, as consistently advocated by Saleem et al. (3) and Morris and Cerceo (12).

These findings underscore the critical importance of continued antimicrobial surveillance and judicious antibiotic use, along with appropriate infection control measures, to mitigate the transmission of multidrug-resistant strains in clinical settings.

## Conclusion

This study assessed the antimicrobial resistance profiles of *Pseudomonas aeruginosa* and *Escherichia coli* from various hospital specimens. Both organisms displayed high resistance rates to common antibiotics, particularly *P. aeruginosa* to ciprofloxacin, gentamicin, and ceftazidime, and *E. coli* to ampicillin and ceftriaxone. These results align with global trends, as many gram-negative bacteria have developed resistance to  $\beta$ -lactams and fluoroquinolones. Notably, over 50% of the isolates for each organism were multidrug-resistant. This underscores the urgent need for ongoing antimicrobial surveillance, prudent antibiotic use, and rigorous infection-control policies. Antimicrobial stewardship programs are vital for promoting the responsible use of antimicrobials and improving patient outcomes.

## Declarations

### Data Availability statement

All data generated or analysed during the study are included in the manuscript.

### Ethics approval and consent to participate

Approved

### Consent for publication

Approved

### Funding

Not applicable

## Conflict of interest

The authors declared no conflict of interest.

## Author Contribution

All author Contributed equally

All authors reviewed the results and approved the final version of the manuscript. They are also accountable for the study's integrity.

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