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# Antimicrobial Resistance Pattern of Pseudomonas aeruginosa Isolated from Urine Specimen in Peshawar, Pakistan

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## ABSTRACT

*Pseudomonas aeruginosa* can cause many nosocomial infections, especially in the urinary tract, particularly in severe burns, bed ulcers, and immune-compromised patients. **Objective:** To determine the antibiotic resistance pattern and prevalence of *Pseudomonas aeruginosa* isolated from urine specimens. **Methods:** This a cross-sectional study. Urine samples were collected from UTI patients and culture on CLED agar and susceptibility was checked with 7 antimicrobial drugs by Disc Diffusion Method. SPSS software version 25.0 was used for data analysis. **Results:** A total of 243 urine samples collected from patients were tested, out of which *Pseudomonas aeruginosa* was isolated from 132 (54.32%) samples. In patients aged less than 8 years it accounted for 14.4 % of the sample, 19.7 % in those aged between 9 and 30 years, 28.8% in patients aged between 31 and 50, and 37.1 % in patients aged between 51 and 70. 7 different antibiotics were tested on *Pseudomonas aeruginosa* isolated from the urine samples. The resistance of *Pseudomonas aeruginosa* to Imipenem, (29.5%), Cefotaxime (90.2%), Cefoperazone (59.1%), Polymyxin-B (3.0%), Colistin, (10.6%), Aztreonam,(26.5%) and Tobramycin (22.0%). There were no significant differences in antibiotic resistance patterns between males and females. **Conclusions:** The results of this study showed that *Pseudomonas aeruginosa* was more common in females than males. Most of the stains were found to be resistant to Cefotaxime and the most sensitive to polymyxin-B. This study also showed a higher resistance percentage in older (51-70 years).

## INTRODUCTION

Twenty to forty-nine percent of all hospital-related infections are Urinary Tract Infections (UTIs), with Pseudomonas responsible for seven to ten percent of these cases [1]. *Pseudomonas aeruginosa* particularly causes infection in those patients using catheters, and it is responsible for around 10% of all catheter-associated UTIs and almost 16% of UTIs in Intensive Care Unit cases. It commonly causes infection in those patients with immune-compromised systems, and those with lung diseases like cystic fibrosis [2]. In the United States, about $1.6 billion yearly is wasted while fighting against Urinary Tract Infections (UTIs) [3]. World Health Organization (WHO) declares *Pseudomonas aeruginosa* as a significant antibiotic-resistant bacterium. Although, Escherichia coli is the most common pathogen of UTIs, but *Pseudomonas aeruginosa* often showed higher levels of antibiotic resistance than Escherichia coli [3].*Pseudomonas aeruginosa* showed resistance to antibiotics through several methods, like efflux pump, enzyme degradation, gene expression, forming a protective biofilm, a mutation in porin protein, and antibiotics target site modification [4, 5]. Several bacterial pathogens, particularly those belonging to the ESKAPE group (Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumonia, Acinetobacter baumannii, *Pseudomonas aeruginosa,* and Enterobacter), have been identified as being extremely drug-resistance [6, 7]. *Pseudomonas aeruginosa* is one of the most important pathogens of the ESKAPE group [8, 9].

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The rise of antibiotic-resistant bacteria in healthcare is a serious problem. The hospitals, especially the ICU are the primary sources of microbial diversity. A recent study has shown that microbial diversity and drug-resistant microbes mainly populate the ICU [8]. *Pseudomonas aeruginosa* patients have few treatment choices now due to antimicrobial resistance, which has turned into a significant and serious problem that results in 51,000 healthcare infections in the USA annually [9]. As per the World Health Organization (WHO) and the US Centers for Disease Control and Prevention (CDC), the growing antimicrobial resistance is highly alarming and dangerous to human health, and it can potentially return us to time before the arrival of antibiotics [10]. Studies have shown that the sensitivity pattern of bacteria alters with time and varies from place to place [11, 12]. Regular observation and updated antibiotic resistance patterns can guide doctors in selecting the most effective antibiotics for treatment, thereby improving patient satisfaction [13].

## METHODS

A descriptive cross-sectional study was employed, using a convenience sampling technique [14]. The sample size for this study was 243. It was calculated by using the frequency of multi-drug resistant *Pseudomonas aeruginosa* as 18.6% prevalence from the previous study [15]. Approval was given by the Institutional Ethics Committee of NCS University System Peshawar on 10 August 2023, NCS/AHS/1302/23. This study was conducted at Hayatabad Medical Complex Hospital and Sina Lab in Peshawar from August 2023 to December 2023. Samples were collected in the Department of Microbiology of HMC. All the male and female patients of any age presented with clinical symptoms associated with urinary tract infection and all the patients willing to participate were included. Patients with asymptomatic urinary tract infections and those who used the antibiotic against the UTIs at least one week before the urine sampling collection were excluded. Consent was obtained from patients before urine samples were collected. The urine samples were collected from patients using normal microbiological procedures. Urine bags for infants and the clean catch method for adults were used for urine collection. To avoid bacterial contamination, women were directed to wash their hands first, and then three disposable wipes were provided to them to clean the area around the urethral opening. The midstream urine was collected in sterile containers. The specimens were transported to the HMC microbiology lab and Sina Lab as soon as possible for tests of resistance and sensitivity to culture. To prevent leukocyte decline, all collected samples were examined rapidly after collection [16]. Isolation and Identification of *Pseudomonas aeruginosa:* The samples were cultured on CLED agar to detect the microorganisms involved. On CLED agar, 0.001 ml of urine specimen was inoculated using a standardized wire loop that was free of germs. After that, the culture media were incubated for 24 hours at 42 °C. To confirm, the samples that showed no growth after 24 hours of incubation were further incubated for an additional 48 hours. To estimate the load of bacteria per milliliter (ml) of urine specimen, the numbers of solitary colonies of bacteria were counted and multiplied by the dilution factor. Different biochemical tests were used for the identification of *Pseudomonas aeruginosa* like oxidase test, oxidase fermentation test, motility test, and catalase test [17]. The total number of samples was 243, while 132 samples were found positive for *Pseudomonas aeruginosa.* Oxidase test: This test was used to differentiate the Pseudomonas from the Enterobacteriaceae family, and other oxidase-negative bacteria. Reagents: Tetra methyl-p-phenylenediamine dihydrochloride manufacturer = Oxoid, and catalog no=BR0058B. Procedure: The oxidase test followed the manufacturer's guidelines. The test organism was shifted to a filter paper sprayed with the oxidase reagent. A blue-purple color change within 10 seconds was taken as oxidase-positive, while no color change was interpreted as oxidase-negative. The oxidation-fermentation test: This test distinguishes microorganisms that ferment carbohydrates anaerobically, like any member of the Enterobacteriaceae family, and those that oxidize carbohydrates (aerobic utilization), like Pseudomonas aeruginosa. Reagents: N2Cl (Manufacturer; Merck, catalog No; 108030):5.0g, Peptone (manufacturer: HiMedia, catalog No: MO28-500G): 2.0g, Di-potassium hydrogen phosphate K2HPO4 (Manufacturer: Merck, catalog No: 105970): 0.3g, Bromothymol Blue (1% aqueous solution) (Manufacturer; Merck, catalog No; 116270): 3.0 ml, Agar (manufacturer: Oxoid, catalog No: LP009B): 3.0g, Water: 1.0Litre. Before autoclaving, the pH was brought to 7.1. Then add the carbohydrate to a final concentration of 1%. After that, the medium was inserted into tubes to a depth of roughly 4 cm. Both tube (sealed and non-sealed) turn into yellow fermentative organisms. Non-sealed tube turns into yellow oxidative organisms, Catalase Test Principle: Some microorganisms contain catalase enzyme, when these microorganisms were added to hydrogen peroxide, they liberate oxygen. A small inoculum of microorganisms of a test was added to a tube or on a slide that has a 3 percent solution of hydrogen peroxide (Manufacturer; HiMedia, catalog No;107089) with a sterile wooden or glass rod. Gas bubble produced Positive (*Pseudomonas aeruginosa*),Gas bubble not produced Negative. Motility Test:There were two approaches to performing the test:The Tube Motility Test and the Wet Mount.Tube Motility Test Reagents: 5 ml of Tube Motility Media per tube was needed for the Tube Motility Test Peptone Water containing 0.2% New Zealand Agar (Manufacturer; HiMedia, catalog No; M170). Sterile, Single-use, Disposable Inoculating Needle (1ul) (Manufacturer; HiMedia, catalog No; LA020). Non-motile organisms, like Acinetobacter species and B. anthraces, will form a single

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growth line on the motility-test medium along the original inoculum stab. Around the inoculum stab, motile organisms will create a diffuse growth zone. Incubate the tube aerobically at 35–37°C for 18 to 24 hours. Positive: *Pseudomonas aeruginosa*. Negative: Acinetobacter spp, Determination of Antimicrobial Susceptibility Profile. Antimicrobial susceptibility test was done according to CLSI using the antibiotic discs of (drugs with concentrations given in brackets) Cefoperazone (75ug), Aztreonam (30ug), Imipenem (10ug), Colistin (10ug), Cefotaxime (30ug), Polymyxin-B (25µg), Tobramycin (30µg) from Oxoid Limited Company, United Kingdom on the Muller Hinton agar which were pre- inoculated with each isolate [18, 19]. The study variables comprised both quantitative and qualitative data. Quantitative variables were age and resistance rates. The type of antibiotics tested as well as gender were considered qualitative variables.SPSS software, version 25.0, was used for data analysis, while Microsoft Excel 2010 for data visualization. Frequency and percentage distributions were calculated for both age and gender. Frequency tables and bar charts were used to present the results, including gender-wise and age-wise percentage distributions of *Pseudomonas aeruginosa*. To assess the antibiotic resistance patterns of *Pseudomonas aeruginosa*,cross-tabulations were created to determine the proportion of isolates that were resistant to each antibiotic. The results were presented in tabular format and visually represented through bar charts. The Chi-square test was used to find the relationship between antibiotic resistance patterns and categorical variables like gender and different age groups. A p-value of less than 0.05 was considered statistically meaningful, representing significant associations.

## RESULTS

The below table showed the percentage of UTI due to *Pseudomonas aeruginosa* in males and females. The females have higher percentage of *Pseudomonas aeruginosa* than males. The samples which showed growth of *Pseudomonas aeruginosa* were 132 (54.3%) out of 243; 55 (41.7%) were of male and the remaining 77 (58.3%) were of female patients (Table 1).

**Table 1:** Gender Distribution among *Pseudomonas aeruginosa* Isolates

| Gender | N (%) |
|---|---|
| Male | 55 (41.7%) |
| Female | 77 (58.3%) |
| Total | 132 (100%) |

**Table 2:** Age Distribution among *Pseudomonas aeruginosa* Isolates

| Age | N (%) |
|---|---|
| <8 Years | 19 (14.4%) |
| 9-30 Years | 26 (19.7%) |
| 31-50 Years | 38 (28.8%) |
| 51-70 Years | 49 (37.1%) |
| Total | 132 (100%) |

Antimicrobial resistance pattern of *Pseudomonas aeruginosa*:7 different antibiotics on *Pseudomonas aeruginosa* isolated from the urine sample were tested. Cefotaxime was found to be the most resistant drug, while polymyxin-B was the most sensitive drug to *Pseudomonas aeruginosa* (Table 3).

**Table 3:** Gender Distribution among Pseudomonas aeruginosa Isolates

| Antibiotics | Resistance N (%) |
|---|---|
| Imipenem | 39 (29.5 %) |
| Cefotaxime | 119 (90.2%) |
| Cefoperazone | 78 (59.1%) |
| Polymyxin-B | 4 (3.0 %) |
| Colistin | 14 (10.6 %) |
| Aztreonam | 35 (26.5 %) |
| Tobramycin | 29 (22.0 %) |

The total *Pseudomonas aeruginosa isolates* were 132, out of them 77 were females and 55 were males. The table showed the frequency (%) of male and female resistance to each antibiotic. The chi-square test was applied and p values were calculated for each drug, indicating that there was no association between antibiotic resistance patterns of *Pseudomonas aeruginosa* and gender (Table 4).

**Table 4:** Association of Antimicrobial Resistance Patterns of Pseudomonas aeruginosa by Gender (n=132)

| Antibiotics | Male N (%) | Female N (%) | p-value |
|---|---|---|---|
| Imipenem | 16 (29.09%) | 23 (29.87%) | 0.84 |
| Cefotaxime | 49 (89.09%) | 70 (90.90%) | 0.71 |
| Cefoperazone | 33 (60.00%) | 45 (58.44%) | 0.80 |
| Polymyxin-B | 2 (3.64%) | 2 (2.60%) | 0.62 |
| Colistin | 6 (10.91%) | 8 (10.39%) | 0.79 |
| Aztreonam | 15 (27.27%) | 20 (25.97%) | 0.81 |
| Tobramycin | 12 (21.82%) | 17 (22.08%) | 0.86 |

The following table showed the resistance pattern of Pseudomonas aeruginosa in different age groups. The frequency and percentage of resistance of each antibiotic were mentioned. Overall there was a higher resistance trend to various antibiotics in older ages (51-70 years). The chi-square test was applied, and the p-value was significant (0.04), indicating an association between the resistance pattern of Pseudomonas aeruginosa and age groups (Table 5).

**Table 5:** Comparison of Antimicrobial Resistance Patterns of *Pseudomonas aeruginosa* by Age Group

| Age | IMI N (%) | CEF N (%) | CEFO N (%) | POL N (%) | COL N (%) | AZT N (%) | TOB N (%) |
|---|---|---|---|---|---|---|---|
| <8 Year | 1 (2.5%) | 12 (10%) | 3 (3.8%) | 0 (0.0%) | 0 (0.0%) | 1 (2.8%) | 1(3.4%) |

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| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 9-30 Year | 4 (10.2%) | 23 (19.3%) | 10 (12.8%) | 0 (0.0%) | 1 (7.1%) | 2 (5.7%) | 3 (10.3%) |
| 31-50 Year | 12 (30.7%) | 36 (46.1%) | 29 (37.1%) | 1 (25%) | 3 (21.4%) | 7 (20%) | 4 (13.7%) |
| 51-70 Year | 22 (56.4%) | 48 (40.3%) | 36 (30.2%) | 3 (75%) | 10 (71.4%) | 25 (71.4%) | 21 (72.4%) |

IMI=imipenem, CEF= cefotaxime, CEFO= cefoperazone, POL= plymyxin B, COL= colistin, AZT= aztreonam, TOB= tobramycin

## DISCUSSION

*Pseudomonas aeruginosa* was a significant human pathogen responsible for many types of infectious diseases, particularly in individuals with weakened immunity and specifically in patients with burns, wounds, and respiratory and urinary tract infections [9]. This study investigates 132 samples for antibiotic susceptibility patterns of *Pseudomonas aeruginosa* in UTI. The patients aged less than 8 years have 14.4 % of *Pseudomonas aeruginosa* in UTI and the patients aged between 9 to 30 years have 19.7 %.The patients aged between 31 and 50 were 28.8%, and those aged between 51 and 70 were 37.1% of the total sample. The percentage of *Pseudomonas aeruginosa* in this study was found to be female (58.3%) and male (41.7%). The overall prevalence was found to be 54.32%. These results were related to the reports of another study which showed prevalence higher in females (64.71%)than males (35.29%) and the highest incidence was seen in the age of 61 to 80 [13]. A study done in India showed contrasting results, which found the incidence higher in males (55%) than females (45%), the resistant pattern of 7 different antibiotics on *Pseudomonas aeruginosa* isolated from the urine sample was tested [20]. The most sensitive drug was polymyxin-B, while the most resistant drug was found to be Cefotaxime. This study found resistance to Polymyxin-B (3.0%), and cefotaxime (90.1%). The resistance of polymyxin-B was reported (2% and 00.0%) by studies done in Suzhou district, China [21], and Nepal [19] respectively. A contrasting result was reported by a study in Minia, Egypt which showed resistance to polymyxin-b was (49.8%) another study in Khyber Teaching Hospital, Peshawar reported resistance to Cefotaxime (30.5%), while a study in Nepal (56.5%), in another study, the reported resistance was found to Cefotaxime (34.0%) [22, 14, 19, 23]. This study found resistance to Imipenem (29.5%), which was almost similar to another study done in Saudi Arabia, which reported resistance to imipenem (36.7%). Other studies done in Iran and China reported resistance to imipenem (19.2%), and (16.2%) respectively [24-25]. Studies done in Karachi, and at Nishtar Hospital, Multan reported contrasting results showing resistance to Imipenem (80.0%), (10.4%), and (50.0%) respectively [26, 27]. According to this study, resistance to Tobramycin was (22.0%), which was almost the same as the study done in Nepal (28.2%) and in India (16.2%) [19, 25]. A study done in Karachi, Pakistan found resistance to Tobramycin (58.4%) [15], while another study reported (60.2%), which was a contrast to this study's finding [28]. This study found resistance of Colistin and Aztreonam to *Pseudomonas aeruginosa:* (10.6%) and (26.5%), respectively. Another study in Pakistan reported resistance to Colistin (00.0%) and aztreonam (80.0%, 56.7%, and 13.5%) [29]. Resistance to Cefoperazone was (59.0%), which was almost similar to the previous study which reported (60.1%), this study showed that there was no significant difference in antibiotic resistance patterns between males and females. This was true for another study that reported no difference in gender resistance patterns [28, 30]. This study found overall higher resistance in older ages and these findings were aligning with other study [31].

## CONCLUSIONS

The results of this study showed that urinary tract infection due to Pseudomonas aeruginosa was more common in females than males. Pseudomonas aeruginosa showed different percentages of resistance to various drugs used in UTI. Polymyxin-B was found to be the most sensitive drug, while Cefotaxime is found to be the resistant drug. The result showed that there was no significant difference in resistance pattern of Pseudomonas aeruginosa between females and males. This study also showed, the higher resistance percentage in older age group (51-70 year).

### Authors Contribution

Conceptualization: AU
Methodology: AJ[2], FM, SU, MK, UUR
Formal analysis: AU, AJ[2], FM, SU
Writing, review and editing: AJ[1], AU, TJ, MK, UUR, AA, AB

All authors have read and agreed to the published version of the manuscript.

### Conflicts of Interest

The authors declare no conflict of interest.

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