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ANTIBIOTIC SUSCEPTIBILITY PATTERN OF PSEUDOMONAS AERUGINOSA IN UTI PATIENTS

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Abstract

Pseudomonas aeruginosa is a significant opportunistic pathogen responsible for nosocomial and community-acquired infections, including urinary tract infections (UTIs). This study investigates the antibiotic resistance and sensitivity patterns of P. aeruginosa in UTI patients to inform effective therapeutic strategies. A cross-sectional analysis was conducted on 100 clinical isolates collected between August and December 2024. Using

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the Kirby-Bauer disc diffusion method, susceptibility to a range of antibiotics was tested. The findings reveal alarming resistance rates to commonly used antibiotics such as ciprofloxacin, amikacin, and imipenem, underscoring the pathogen's adaptive resistance mechanisms, including biofilm formation and efflux pumps. However, newer antibiotics like colistin and ceftolozane-tazobactam demonstrated significant efficacy. The study highlights a higher prevalence of infections in males (86%) and older patients, particularly those over 60 years of age. These results emphasize the critical need for regular antibiotic susceptibility testing, robust stewardship programs, and targeted prevention strategies, particularly for high-risk populations. This research provides valuable insights for optimizing clinical management and addressing the growing threat of multidrug-resistant P. aeruginosa in UTIs. Further studies are recommended to explore alternative treatments and the genetic basis of resistance.

INTRODUCTION

Pseudomonas aeruginosa is a Gram-negative opportunistic pathogen responsible for a wide range of healthcare-associated infections, including pneumonia, bloodstream infections, urinary tract infections (UTIs), and surgical site infections, particularly among immunocompromised patients and those with chronic lung diseases such as cystic fibrosis. It accounts for approximately 7.1%–7.3% of healthcare-associated infections, with higher prevalence observed in intensive care unit (ICU) settings (1).

The extensive adaptability of P. aeruginosa is attributed to its large genome, which enables survival in diverse environments and promotes phenotypic differentiation in biofilms and infected tissues, including the urinary tract (2). UTIs are among the most common bacterial infections worldwide, affecting individuals of all ages and accounting for millions of healthcare visits annually. Urinary catheterization represents a major risk factor for P. aeruginosa-associated UTIs (2). According to a European point-prevalence survey, UTIs constitute 19% of healthcare-associated infections, ranking third after surgical site infections and pneumonia (3).

Catheter-associated UTIs (CAUTIs) caused by P. aeruginosa contribute to prolonged hospitalization and increased healthcare costs. Experimental models have demonstrated a biphasic course of infection, comprising an acute phase with systemic symptoms followed by chronic asymptomatic colonization (3). Treatment of P. aeruginosa infections is challenging due to its intrinsic resistance to multiple antibiotics and the rapid emergence of acquired resistance. Unlike uropathogenic Escherichia coli, which forms stable intracellular reservoirs, the persistence mechanisms of P. aeruginosa in UTIs remain poorly understood (3).

The increasing antibiotic resistance of P. aeruginosa is associated with significant morbidity and mortality. The pathogen is classified among the ESKAPE organisms by the Infectious Diseases Society of America, reflecting its clinical importance and limited treatment options (4). Resistance mechanisms are categorized as intrinsic, acquired, or adaptive, involving reduced membrane permeability, efflux pumps, β-lactamase production, horizontal gene transfer, and environmentally induced phenotypic changes such as biofilm formation and persister cell development (5).

Outer membrane porins, particularly OprD, play a crucial role in carbapenem uptake. Loss of OprD is strongly associated with resistance to imipenem and meropenem (5). In addition, P. aeruginosa produces diverse β-lactamases belonging to Ambler Classes A–D, including ESBLs, metallo-β-lactamases, AmpC, and OXA-type enzymes, which further compromise antimicrobial efficacy (5). Quorum sensing–regulated biofilm formation enhances adaptive resistance by limiting antibiotic penetration and promoting chronic infection (6)

In this study, we investigated antimicrobial resistance in P. aeruginosa isolated from 100 patients with confirmed UTIs across a broad age range. Clinical isolates were tested against 32 antibiotics to identify the most effective therapeutic options and provide evidence-based guidance for managing P. aeruginosa UTIs in the context of increasing antimicrobial resistance.

4. Material and Methods

4.1 Study setting:

Urine samples and antibiotic sensitivity testing were collected from Islamabad Diagnostic Centre (IDC).

4.2 Sample size:

100 affected cases

4.3 Study design:

A cross-sectional study design

4.4 Sampling technique:

Convenient sampling method was used in this study.

4.5 Study period:

20 August 2024 to 19 December 2024.

4.6 Statistical analysis:

- Percentages and frequencies were used to determine the Demographic Data.
- Data were presented in the form of histograms and Pi charts.
- Statistical Package of Social Sciences (SPSS) version 25 was used for the analysis of the data.

4.7 Inclusion criteria:

- Patients with a confirmed UTI diagnosis.
- Patients with a positive urine culture for Pseudomonas aeruginosa

4.8 Exclusion criteria:

- Patients who have negative UTI diagnosis.

4.9 Ethical consideration:

Approval was obtained from the IRC of the NCS Institute of Sciences Capital Campus Islamabad prior to the commencement of the study. All patients gave written informed consent, and all information and data were kept confidential. Subjects were informed that there was no risk associated with the study and that they were free to withdraw at any time during the process.

a. Sample collection:

4.10.1 Sample type:

Midstream clean-catch urine samples (10-20mL) were collected in sterile containers.

4.10.2 Collection process:

Patients were instructed to clean the perineal area before collecting midstream urine to avoid contamination

b. Microbiological Analysis:

4.11.1 Culture Media and Procedure:

Urine samples were inoculated on Cysteine Lactose Electrolyte Deficient (CLED) agar to isolate and identify urinary pathogens, Pseudomonas aeruginosa.

1. Preparation of CLED :

CLED agar plates were prepared according to the manufacturer's instructions. The medium was sterilized, poured into petri dishes, and allowed to solidify.

2. Inoculation:

A calibrated 0.001 mL inoculating loop was used to streak urine samples onto CLED agar. This enabled semi-quantitative analysis of bacterial growth and ensured proper isolation of colonies.

3. Incubation:

Inoculated plates were incubated aerobically at 37◦C for 24 to 48 hours.

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4. **Observation of Growth:**

Pseudomonas aeruginosa appeared as non-lactose fermenting pale or greenish colonies with characteristic pigmentation due to pyocyanin production.

A fruity odor was noted for suspected Pseudomonas aeruginosa colonies.

4.11.2 Identification:

Pseudomonas aeruginosa was performed using:

1. Oxidase Test: Positive result indicated the presence of Pseudomonas species.
2. Pigment production: The presence of green or yellow pigment confirmed the isolate.

4.11.3 Rationale for Using CLED Agar:

CLED Agar was chosen for urine culture due to its unique properties:

1. CLED agar electrolyte-deficient nature inhibited the swarming of Proteus species.
2. It supports the growth of a wide range of urinary pathogens, Gram-positive and Gram-negative bacteria.
3. It differentiates lactose fermenters from non-lactose fermenters, initially identifying Pseudomonas aeruginosa.

4.11.4 Antibiotic Sensitivity and Resistance Testing:

Method:

Antibiotic susceptibility testing was performed using the Kirby-Bauer disc diffusion method. This method was used to determine the sensitivity and resistance patterns of pseudomonas aeruginosa isolates from UTI patients.

Procedure:

The isolates of pseudomonas aeruginosa were cultured on CLED Agar as previously mentioned. After an overnight incubation period, individual colonies were selected and suspended in sterile saline to achieve turbidity. This bacterial suspension was then evenly spread on Mueller-Hinton agar plates using a sterile swab.

Antibiotics Tested:

A panel of commonly prescribed antibiotics for urinary infections caused by Pseudomonas aeruginosa were tested, including:

- Pipratazobactam,Cefepime,Ciprofloxacin,
- Levofloxacin,Ceftazidime,Amikacin,
- Tobramycin,Imipenem,Meropenem,Ceftazidime-Avibactam,Cefiderocol
- Ceftolozane-tazobactam,Colistin,Norfloxacin ,Aztreonam

Zones of Inhibition (Approximate)			
Antibiotic	**Sensitive (S)**	**Intermediate (I)**	**Resistant (R)**
Piperacillin-Tazobactam	≥21 mm	15–20 mm	≤14 mm
Cefepime	≥18 mm	15–17 mm	≤14 mm
Ciprofloxacin	≥21 mm	16–20 mm	≤15 mm
Levofloxacin	≥17 mm	14–16 mm	≤13 mm
Ceftazidime	≥18 mm	15–17 mm	≤14 mm
Amikacin	≥17 mm	15–16 mm	≤14 mm
Tobramycin	≥15 mm	13–14 mm	≤12 mm
Imipenem	≥23 mm	20–22 mm	≤19 mm
Meropenem	≥23 mm	20–22 mm	≤19 mm
Ceftazidime-Avibactam	≥18 mm	15–17 mm	≤14 mm
Cefiderocol	≥19 mm	16–18 mm	≤15 mm
Ceftolozane-	≥21 mm	17–20 mm	≤16 mm

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Tazobactam			
Colistin	Disk diffusion not recommended (MIC method preferred).		
Norfloxacin	≥17 mm	13–16 mm	≤12 mm
Aztreonam	≥21 mm	16–20 mm	≤15 mm

Results

This study investigated the antibiotic sensitivity and resistance pattern of *pseudomonas aeruginosa* in UTI Patients.

Urinary tract infections (UTIs) are among the most prevalent bacterial infections worldwide, affecting millions of individuals annually. *Pseudomonas aeruginosa* is a common causative agent, particularly in hospital-acquired infections and immunocompromised patients. This opportunistic pathogen is known for its ability to develop resistance to a wide range of antibiotics, leading to significant therapeutic challenges.

By understanding the resistance profile of *Pseudomonas aeruginosa* in UTI cases, clinicians can make informed decisions about antibiotic prescriptions, reducing the misuse of antibiotics and slowing the emergence of resistance. This research also emphasizes the need for regular surveillance and updates to local antibiotic stewardship policies to improve patient outcomes and combat the growing threat of antimicrobial resistance.

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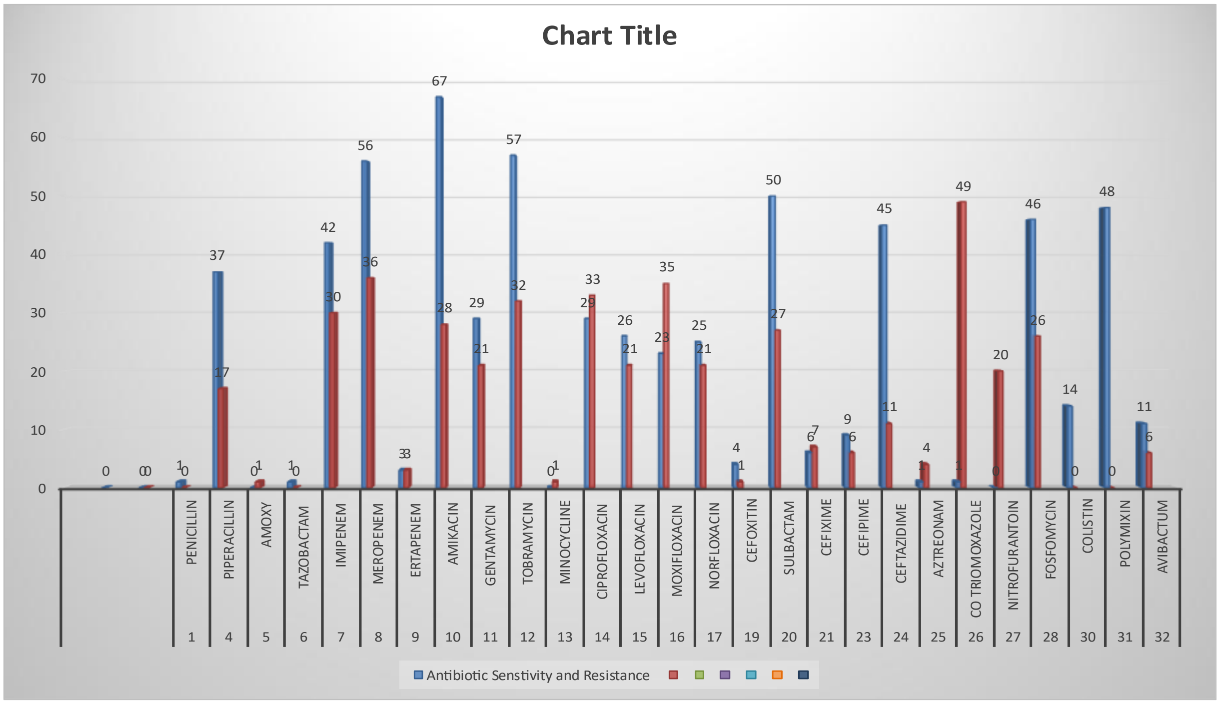
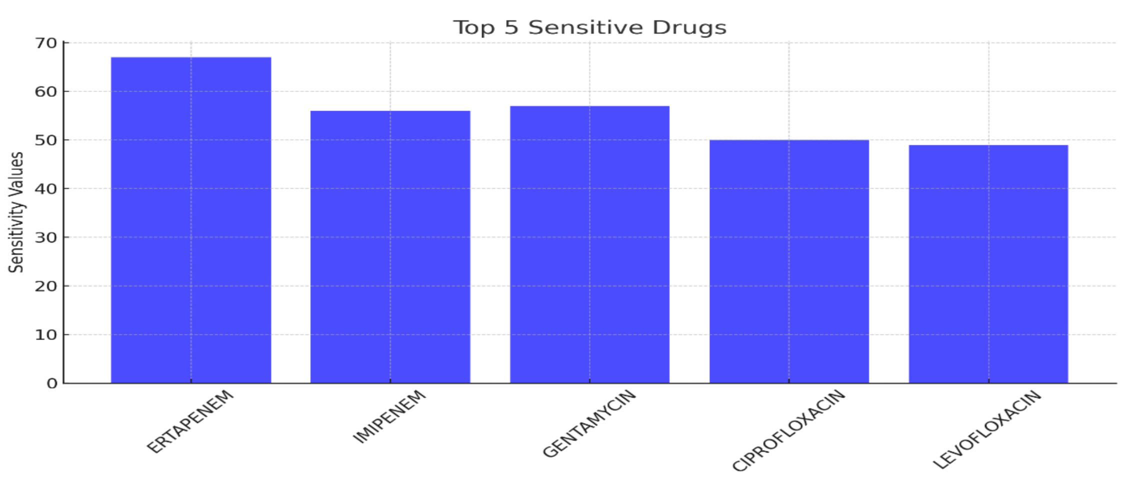
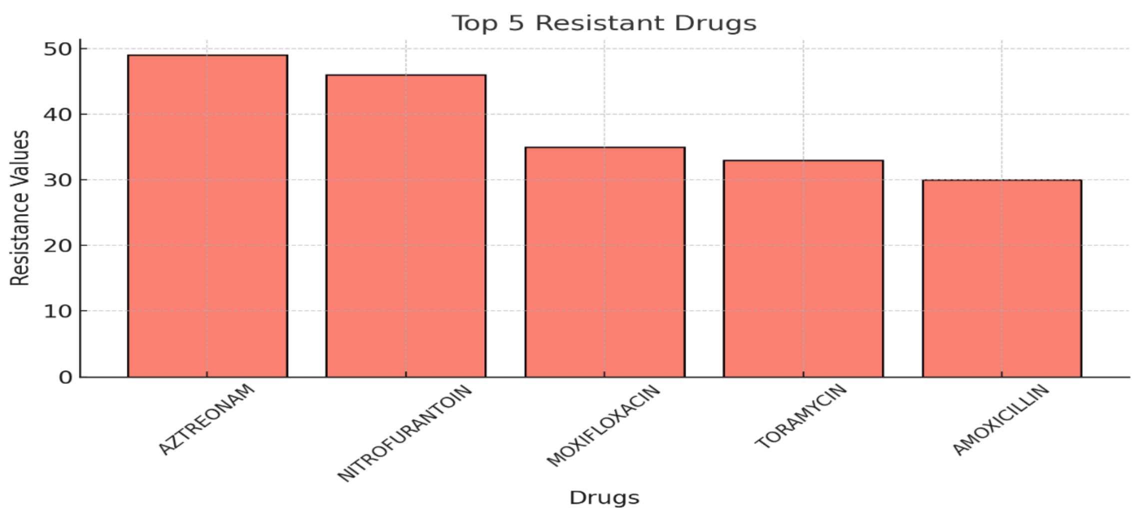


Chart of Antibiotic sensitivity and resistance of Pseudomonas Aureginosa Fig 5.1.



Top Five Sensitive drugs Fig 5.2

This chart (figure 4.2) shows the drugs with the highest sensitivity, meaning these antibiotics were most effective in inhibiting or eliminating the targeted bacteria. ERTAPENEM has the highest sensitivity score (67), indicating it is the most effective among the tested antibiotics. IMIPENEM (56) and GENTAMYCIN (57) follow closely as highly effective antibiotics. CIPROFLOXACIN (50) and LEVOFLOXACIN (49) also demonstrate strong effectiveness, making them suitable options for treatment.



Top Five Resistant Drugs Fig 5.3

This chart (Figure 4.3) shows the least effective antibiotics due to resistance. AZTREONAM (49) and NITROFURANTOIN (46) have the highest resistance. MOXIFLOXACIN (35), TORAMYCIN (33), and AMOXICILLIN (30) also perform poorly. These drugs should be avoided if resistance is an issue.

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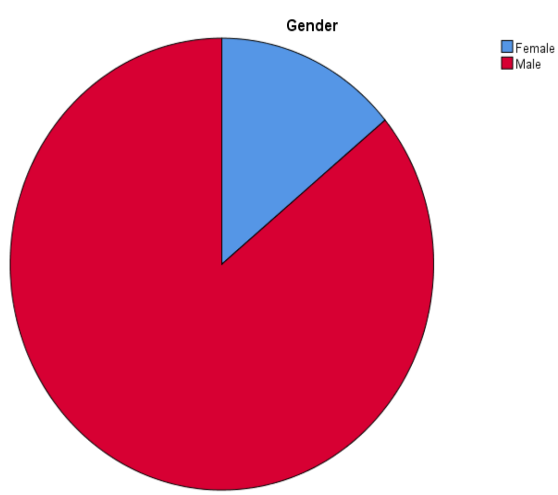
5.1 Gender Wise Distribution: Male Vs Female Frequency

The observed distribution of *Pseudomonas aeruginosa* infections in UTI patients, with a significantly higher frequency in males (86 cases) compared to females (14 cases), may be influenced by several clinical and demographic factors. The gender distribution emphasizes the importance of targeting high-risk groups, such as male patients with catheters or underlying conditions, in infection prevention strategies. It suggests evaluating gender-specific risk factors and adapting management protocols accordingly (Table 5.1).

Valid	Frequency	Percentage	Valid Percentage	Cumulative Percentage
Female	14	14.0	14.0	14.0
Male	86	86.0	86.0	100.0
Total	100	100.0	100.0	

Gender Wise Distribution of UTI Patients Table 5.1



Male and Female frequency in UTI Patients Fig. 5.4

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5.2 Age Group Distribution:

Age Group (Years)	Number of Cases
1-20	6
20-29	9
30- 39	11
40- 49	13
50- 59	10
60-69	21
70+	29

Age Group Distribution of UTI Patients with Pseudomonas Aeruginosa Table 5.2

Urinary tract infection caused by *Pseudomonas aeruginosa* are more common in people over 60, with the highest number (29 cases) in those aged 70 and above. Younger age groups have fewer cases, with only 6 cases in the 1–20 age group. Healthcare providers should pay special attention to preventing and treating these UTIs in older adults.

4.3 Statistics

Mean	46
Median	60
Mode	65
Std. Deviation	20.80

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Statistics of dataset Table 5.3

The dataset has a **mean** of 46, a **median** of 60, a **mode** of 65, and a **standard deviation** of 20.80. **Mean (46):** The average value of the dataset. **Median (60):** The middle value when the data is ordered, indicating that half the values are below 60 and half are above. **Mode (65):** The most frequently occurring value in the dataset. **Standard Deviation (20.80):** Measures how spread out the values are around the mean; a higher value indicates more variability (Table 4.4).

1.4 Age of the Participants Gender Wise (Cross Tabulation)

Age	**Female**	**Male**	**Total**
1-20	2	5	7
21-29	2	7	9
30-39	1	11	12
40-49	1	12	13
50-59	2	7	9
60-69	2	22	24
70+	4	22	26

The table shows the number of participants by age and gender Table 5.4

Out of 100 participants, 14 are female, and 86 are male, showing more males in the group. Younger Participants (1–20 years): There are 7 participants, with 2 females and 5 males. Middle-Aged Participants (21–60 years): This group has 47 participants, including 5 females and 42 males. Older Participants (61+ years): There are 46 participants, with 7 females and 39 males. There are more male participants across all age groups. The number of participants increases with age, especially among males (Table 4.5).

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Discussion

In total, there were 61,029 patient specimens, of which 5534 were identified as non-duplicated *P. aeruginosa* clinical isolates, most being from males aged over 60 years. The research findings revealed that the maximum antibiotic resistance associated with *P. aeruginosa* isolates was found in colistin (97%), which was followed by piperacillin/tazobactam (75.8%). The maximum resistance rates in *P. aeruginosa* isolates were found in relation to cefepime (42.7%,) which was followed by ciprofloxacin (34.3%) (15).

Pseudomonas aeruginosa was the most frequently occurring species (96.0%); *P. putida* (2.67%) and *P. fluorescens* (0.67%) were also identified as well as an isolate of *Burkholderia pseudomallei* (0.67%). The highest resistance rate among isolates was observed towards gentamicin (35.4%); piperacillin/tazobactam was the most active antibiotic. Multidrug-resistant (MDR) strains constituted 12.8% of the isolates and most MDR strains also displayed a high multiple antibiotic resistance index (MAR) (7).

A total of 700 samples of pus, urine, swab, and other samples from various patients were examined. Based on bacterial growth over routine nutrient agar and MacConkey medium, isolates with positive results on both media were chosen. Using the modified disc-diffusion method (Modified-Kirby Baur method), antimicrobial sensitivity of total isolates was operated by following CLSIs guidelines. In the current study, a large number of isolates of P. aeruginosa obtained from different specimens are resistant to Cefixime (82%), followed by Ampicillin (79%) and Augmentin (61%). However, the antibiogram of P. aeruginosa also showed that most of the isolates (86%) were highly sensitive to Amikacin. The second maximum sensitivity of P. aeruginosa was seen towards Tazocin (80%), followed by Tecarcilline (79%) (4).

In current study, P. aeruginosa isolates obtained from different clinical sources were identified according to traditional biochemical tests. Antibiotic susceptibility testing was performed by the disc diffusion method. Importantly, present results show that 53% out of P. aeruginosa isolates exhibited multi drug resistance (MDR) pattern. P. aeruginosa isolates showed higher resistance to ciprofloxacin, gatifloxacin and meropenem and intermediate resistance to cefoperazone, cefepime, pipracillin, tobramycin, pipracillin-tazobactam, ceftazidime and azetreonem while Low bacterial resistance was noted against colistin only (8).

This study investigated the antibiotic sensitivity and resistance patterns of *Pseudomonas aeruginosa* in urinary tract infection (UTI) patients, emphasizing the growing concern of antimicrobial resistance. The findings revealed that Ertapenem, Imipenem, and Gentamycin were the most effective antibiotics, while Aztreonam and Nitrofurantoin exhibited the highest resistance, necessitating careful antibiotic selection in treatment. A higher prevalence of UTIs caused by *Pseudomonas aeruginosa* was observed in males (86%) compared to females (14%), with older adults, particularly those aged 70 and above, being the most affected age group. The data showed a mean age of 46 years and highlighted significant gender-based differences, with males consistently showing higher infection rates across all age groups. These findings underscore the importance of targeted antibiotic use, regular surveillance, and age- and gender-specific strategies to combat *Pseudomonas aeruginosa* infections effectively. Implementing updated antibiotic stewardship policies and enhancing infection control measures in hospital settings are crucial to improving patient outcomes and mitigating resistance.

7. Conclusion

This study underscores the critical role of ***Pseudomonas aeruginosa*** as a major pathogen in urinary tract infectisssons, particularly in high-risk populations such as males over 60 years old and patients with underlying conditions or catheter use.

The findings reveal alarming resistance patterns to commonly used antibiotics like ciprofloxacin, amikacin, and imipenem, highlighting the pathogen's intrinsic and adaptive mechanisms that complicate treatment. However, the demonstrated effectiveness of colistin and advanced agents like ceftolozane-tazobactam offers valuable options for managing multidrug-resistant strains.

8. **Future Recommendation**

Addressing the growing threat of ***Pseudomonas aeruginosa*** requires a proactive approach, including regular antibiotic susceptibility testing, stricter antibiotic stewardship, and enhanced infection prevention strategies.

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