Antimicrobial Susceptibility Pattern of E. Coli, P. Aeruginosa, K. Pneumoniae and E.faecalis isolates in UTI infection "

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Abstract

Background: Antimicrobial Susceptibility Pattern: The ability of microorganisms to resist the effects of antimicrobial agents, such as antibiotics. Urinary Tract Infection: An infection that occurs in the urinary system, including the kidneys, bladder, and urethra. Escherichia coli: A gram-negative bacterium that commonly causes UTIs, particularly in women. Pseudomonas aeruginosa: An opportunistic pathogen that causes UTIs, especially in patients with compromised immune systems or urinary tract devices. Klebsiella pneumoniae: A gram-negative bacterium known for causing UTIs and producing ESBLs, which confer antibiotic resistance. Enterococcus faecalis: A gram-positive bacterium that can cause UTIs, particularly in people with urinary tract abnormalities or compromised immune systems.

Objective(s): The research aimed to explore both the antibiotic resistance patterns among isolated microorganisms in addition to determining which antibiotics provide the best treatment for UTI sufferers. It also investigated both the antibiotic resistance information of isolated microorganisms and the connection between antibiotic prescriptions and UTI patient resistance patterns.

Methodology: In this study, bacterial isolates from UTI infections were collected in a clinical setting, and species were identified using common microbiological protocols. Using the disk diffusion method, antibiotic susceptibility and resistance was evaluated by measuring the zone of inhibition. Bacterial species and resistance patterns were compared using statistical techniques such as Chi-Square testing. The therapeutic effect of antibiotics for various isolates was evaluated using descriptive statistics.

Results: This study analyzed 200 UTI samples, showing a higher prevalence in females (67.5%) compared to males (32.5%). E. coli was the most common pathogen, with significant antibiotic resistance observed, particularly to FF and Cefipime. Resistance patterns varied across bacterial species, with Pseudomonas showing high resistance to AMC, and Enterococcus having no resistance to SXT and Cipro. Chi-square tests confirmed significant associations between bacterial species and antibiotic resistance, highlighting the need for improved antimicrobial management.

Conclusion(s): E. coli was the most prevalent UTI pathogen, especially among females, showing high resistance to key antibiotics like Ciprofloxacin and SXT. The findings stress the importance of improved antimicrobial stewardship and exploring alternative therapies.

Key words: Urinary Tract Infections (UTIs), Bacterial Prevalence, Antibiotic Resistance, Escherichia coli, Ciprofloxacin (CIP), Trimethoprim-Sulfamethoxazole (SXT), Antimicrobial Susceptibility, Chi-Square Test, Infection Control.

Introduction

The urinary system consists of several components which Urinary Tract Infection (UTI) refers to as a harmful process that targets kidneys together with ureters and bladder and urethra. The urinary tract infection focuses on the infection of bladder and urethra. UTI stands as a common hospital and community infection which results in severe side effects and high treatment expenses.¹ The general population faces urinary tract infection (UTI) as one of their main health threats and this bacterial condition ranks as the second reason for hospital admissions.² Acute UTI presents three pivotal infection symptoms: frequency, urgency, painful urination alongside sensation of needing to pee after finishing or dysuria, cloudy urine or pyuria, spine discomfort and abdominal pain. However, bacteria may be present in the urinary tract without any apparent symptoms.³

Urinary tract infection affects every age group of population yet numerous demographic factors like race and genetics together with age and gender and sexual activity and night-time wetting and penile circumcision in males determine how likely an individual will develop this infection.⁴ The incidence of UTI grows more common in elderly men because of prostate enlargement and neurogenic bladder conditions.⁵ The condition makes expectant mothers more vulnerable to developing infections during pregnancy.⁶ The initial UTI experience in women develops before age 5, showing maximum frequency when girls learn to use the toilet and during their infancy period. Between 60 to 80 percent of females will develop a second UTI after experiencing their first UTI during an 18-month period.⁷ The bacterial urinary tract infections impose the most dangerous and common uropathogenic threats which present regularly throughout the community and hospital environments.⁸

UTI-causing bacteria from the gram-positive group most frequently include Enterococcus faecalis together with Staphylococcus saprophyticus. Such bacteria remain important for certain groups including sexually active young women and people using urinary catheters.^{9,10} Community studies indicate that uropathogens such Escherichia coli (46.4 - 74.2%), Klebsiella spp (6.0 - 13.45%), Proteus spp (4.7 - 11.9%), and Enterococcus spp (5.3 - 9.54%) are the leading causes of UTIs. E. coli is the most common uropathogen found in community-acquired UTIs due to its normal flora in the human intestine and ease of colonization in the urinary system. ITU can be spread sexually, as evidenced by E. coli strains found in the feces of sexually active patients and their partners.¹¹ A timely diagnosis of UTI needs to be accurate to stop infections from rising into upper urinary areas and causing renal failure. Day by day the number of UTI cases proves resistant to antibiotic treatments. The worldwide increase in antibiotic resistance stems from excessive and improper antibiotic applications during recent times.^{12,13} Public health faces a serious problem due to antibiotic resistance development in UTI treatment. So many fake drugs of unknown quality circulate in regions with substantial poor populations and low literacy rates and poor hygiene standards primarily in developing nations.14,15

Antimicrobial resistance in uropathogens had become a serious challenge in UTI treatment, leading to higher recurrence rates and limited therapeutic options. E. coli, P. aeruginosa, K. pneumoniae, and Enterococcus spp. were among the most common causes of UTIs, yet their resistance patterns varied significantly across regions. Current empirical treatment guidelines were often ineffective due to changing resistance trends. This study aimed to evaluate local antimicrobial susceptibility patterns, ensuring that treatment protocols were based on the latest

evidence and helping healthcare professionals make more effective antibiotic choices for UTI management. To investigate the antibiotic resistance profiles of the isolated microorganisms and identify the most effective antibiotics for UTI treatment, To determine the relationship between antibiotic usage and resistance patterns in UTI patient.

Material and Methods

Study Design: It was a descriptive cross-sectional study.

Settings: This retrospective study was conducted to investigate the "Antimicrobial Susceptibility Pattern of E. coli, P. aeruginosa, K. pneumoniae and E. faecalis isolates in UTI infection" at Nawaz Sharif Social Security Lahore.

Duration of Study: The study was carried out over a period of 4 to 6 months after the synopsis had been approved.

Sample Size: A total of 200 samples were collected.

Formula: $n = (Z^2 * p * (1-p)) / E^2$

Sampling Technique:

Random conventional sampling was used to select participants who met the inclusion criteria and were available during the study period.

Sample Selection:

Inclusion criteria

- Patients of any, age or sex, who have the symptoms of CA-UTI's.
- Frequency, urgency and hesitancy of urination, burning micturition or dysuria Patients diagnosed with Urinary Tract Infection (UTI) based on clinical symptoms and laboratory confirmation (e.g., positive urine culture, pyuria, bacteriuria).
- Patients aged 18 years and above.

Exclusion Criteria

- Pregnant or lactating women
- Recent antibiotic use (past 2 weeks)
- Immunocompromised conditions (e.g., HIV/AIDS, cancer)
- History of kidney transplantation or urinary tract abnormalities

Equipment: Automated urine analyzers were used for initial screening. Culture media (e.g., MacConkey agar, CLED agar) for bacterial isolation. Antibiotic susceptibility testing (AST) equipment, such as the VITEK-2 Compact system or disk diffusion method (Kirby-Bauer test). Incubators, autoclaves, and other standard microbiological laboratory equipment.

Scanning Technique:

- Firstly, urine samples were collected from the middle of the flow through a clean-catch technique.
- Next, the samples were then placed on specific culture media.
- Then be put in an incubator for 24-48 hours. The bacteria responsible for urinary tract infection were identified according to (CLSI).

Data Collection Procedure

This study was done in Lahore. According to the medical records verified by the assigned research assistant, UTI infection patients were selected for the study. The following data was collected from hospital laboratories.

1. Sample Collection

• **Patient Samples**: Samples were collected from patients diagnosed with **urinary tract infections (UTIs)** in a clinical setting. The primary samples collected were **midstream urine samples** or **catheterized urine** from patients suspected or confirmed to have UTIs.

2. Sample Processing and Bacterial Identification

• Culture: All urine samples were inoculated onto appropriate culture media such as blood agar and MacConkey agar and incubated at **37°C** for 24–48 hours.

• Colony Morphology:

- *Escherichia coli*: Colorless or light pink colonies on MacConkey agar
- Pseudomonas aeruginosa: Green or blue colonies due to pyocyanin pigment
- Klebsiella pneumoniae: Colorless or light pink colonies
- *Enterococcus faecalis*: White or cream-colored colonies
- Identification: The isolated bacterial colonies were further identified using:

• **Gram Staining**: E. coli, P. aeruginosa, K. pneumoniae, and E. faecalis were identified as Gram-negative rods or Gram-positive cocci.

- **Biochemical Tests**: Standard biochemical tests, such as:
- Oxidase Test: (P. aeruginosa is oxidase positive)
- Catalase Test: (E. coli, P. aeruginosa, and K. pneumoniae are catalase positive)
- Citrate Utilization: (E. faecalis is citrate positive)
- Indole Test: (E. coli is indole positive)
- 3. Antimicrobial Susceptibility Testing (AST)

• **Kirby-Bauer Disk Diffusion Method**: The antibiotic susceptibility of the isolates was tested using the **Kirby-Bauer Disk Diffusion Method** on **Mueller-Hinton agar**:

• Bacterial suspensions were prepared to match the **0.5 McFarland standard**.

• Disks containing antibiotics commonly used to treat UTIs, such as amikacin, gentamicin, imipenem, meropenem, ciprofloxacin, piperacillin/tazobactam, cefepime, nitrofurantoin, trimethoprim-sulfamethoxazole, were placed on the inoculated plates.

• The plates were incubated at **37°C** for 18–24 hours.

• Zone of inhibition was measured in millimeters, and the results were interpreted according to CLSI guidelines to categorize the isolates as sensitive, intermediate, or resistant.

4. Data Recording

Patient information, including age, sex, underlying conditions, and clinical symptoms, was recorded. Details of the sample, such as the collection date, infection source, and specimen type, were also documented. The antibiotic susceptibility results, including zone sizes or MIC values, were carefully noted for further analysis.

Data Analysis Procedure

For the data analysis, SPSS (Statistical Package for the Social Sciences), version 28.0, was used. The analysis included both descriptive statistics and inferential tests. Descriptive statistics, particularly frequency distribution, were applied to summarize categorical variables such as gender, types of bacteria, and their antibiotic resistance profiles. To examine relationships between variables, the Chi-Square test of significance was performed. This test was used to determine whether there was a statistically significant association between different bacterial species and their resistance to various antibiotics.

Results

A total of 200 UTI patients were evaluated and 4 bacteria were isolated from them, such as Gram-negitive bacteria (E. coli, Pseudomonas aeruginosa, and Klebsiella pneumonia) and Gram-positive bacteria (Enterococcus aeruginosa). The Gram staining results showed that all were positive.

1-Gender Distribution of Bacterial Isolates in UTIs

	Frequency	Percentage
Female	135	67.5
Male	65	32.5
Total	200	100.0

Table 1: Gender distribution of UTIs

A total 200 samples were investigated in this study. The positive cultures were predominantly found in female patients (n= 135), while male patients (n=65). This suggests a higher occurrence of UTI infections in females in hospitalized settings. The gender distribution of UTI infection cases is presented in Table 1.

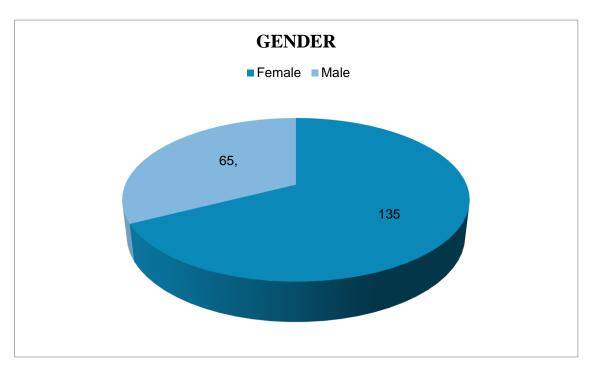


Figure 1: Gender distribution

Bacteria	Frequency	Female	Male
E.coli	80	54	26
Entero	29	20	09
Klebsiella	41	26	15
Pseudo	50	35	15

Table 2: Distribution of different bacteria across Gender

This table shows the distribution of different bacterial types (e.g., E. coli, Entero, Pseudo, etc.) across genders (Male and Female).

The data showed that E. coli had the highest frequency, with a significantly higher number of females (67.5%) than males. Entero also affected more females (69%). Klebsiella and Pseudo had more balanced distributions, with a slight female majority. Overall, females tended to have a higher occurrence of E. coli and Entero, while Klebsiella and Pseudo showed less pronounced gender differences.

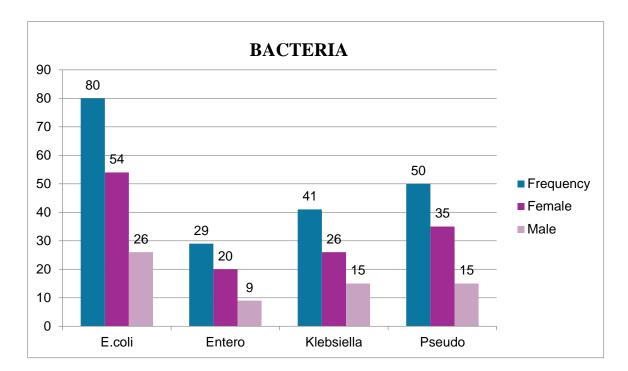


Figure 2: Distribution of different bacteria across Gender

2. Antibiotic Susceptibility Testing (AST) Result

E. coli, Entero, Pseudo, klebsiella isolates were tested against various antibiotics to determine their susceptibility patterns.

	MBM	CEF	AMC	SXT	AK	AUG	CN	Cipro	Cefipime	Imipenem
E.coli	19	43	30	6	32	10	21	53	47	20
Entero	16	12	15	0	2	24	7	22	0	7
Klebsiella	8	37	28	8	2	21	17	11	18	2
Pseudo	8	36	40	10	9	40	18	8	20	11

Table3: Antibiotic resistance rates of E. coli, Entero, Klebsiella, Pseudo isolated from various drugs

Highest Resistance Rates:

- Cefixime (CEF): E. coli (43), Klebsiella (37), Pseudomonas (36)
- Amoxicillin-Clavulanic Acid (AMC): Pseudomonas (40), Klebsiella (28), E. coli (30)
- **Co-trimoxazole (SXT):** E. coli (32), Pseudomonas (10)
- Augmentin (AUG): Pseudomonas (40), Klebsiella (21)

* *Interpretation:* These antibiotics show the **highest resistance**, particularly in **Pseudomonas**, **E. coli**, and **Klebsiella**, indicating they are less effective for treatment.

Moderate Resistance Rates:

- **Ciprofloxacin (Cipro)**: E. coli (21), Klebsiella (11), Pseudomonas (8)
- Gentamicin (CN): E. coli (10), Klebsiella (17), Pseudomonas (18)
- **AK (Amikacin)**: E. coli (10), Klebsiella (2), Pseudomonas (9)

✤ Interpretation: These antibiotics show moderate resistance, suggesting selective effectiveness, depending on the bacterial species.

Lowest Resistance Rates:

- Imipenem: E. coli (20), Enterococcus (7), Klebsiella (2), Pseudomonas (11)
- MBM (possibly Meropenem or another β-lactam): Klebsiella (8), Pseudomonas (8)

• **Cefipime**: Klebsiella (18), Pseudomonas (20), E. coli (47) (depending on context, could also be moderate)

Interpretation: Imipenem and MBM show the lowest resistance, making them the most effective options across all studied organisms.

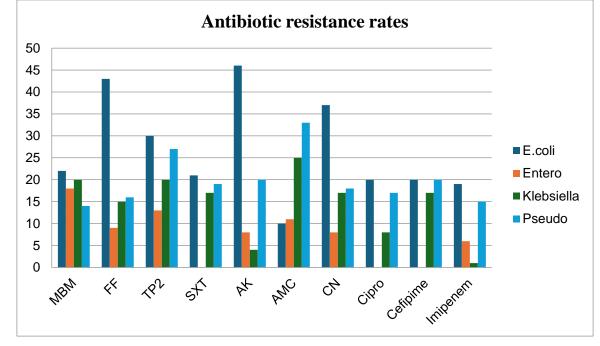


Figure3: Chart show Antibiotic resistance rates of E. coli, Entero, Klebsiella, Pseudo species

CEF Resistance in Various Bacteria

		Cefixime		
	0	1	2	Total
E.coli	43	26	11	80
Entero	12	15	2	29
Klebsiella	37	4	0	41
Pseudo	36	14	0	50
otal	128	59	13	200
	Entero Klebsiella Pseudo	Entero12Klebsiella37Pseudo36	0 1 E.coli 43 26 Entero 12 15 Klebsiella 37 4 Pseudo 36 14	0 1 2 E.coli 43 26 11 Entero 12 15 2 Klebsiella 37 4 0 Pseudo 36 14 0

Table 4: Anti-microbial resistance of CEF in different bacteria

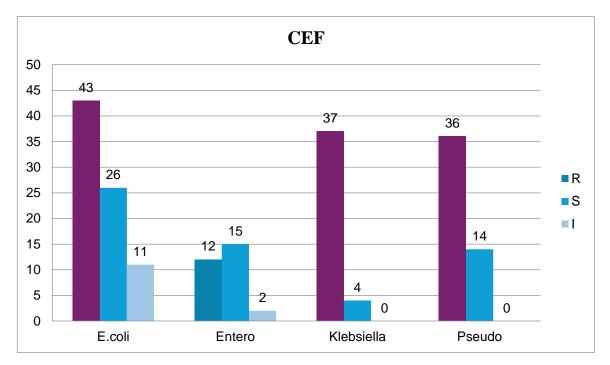
✤ This table presents the counts for each bacterial type's response to the antibiotic CEF categorized as "Resistant," "Sensitive," "Intermediate".

 \clubsuit It helps to visualize the distribution of antibiotic responses among different types of bacteria.

			Asymptotic Significance
	Value	Df	(2-sided)
Pearson Chi-	31.491 ^a	6	.000
Square			

✤ The chi-square value is 31.491, with a p-value of 0.000 Interpretation

The Chi-Square test revealed a significant association between bacterial species and their resistance patterns (p = .000), indicating that resistance varies meaningfully by bacteria type. The analysis is reliable based on a sufficient sample size of 200 valid cases.





The table shows that Cefixime (CEF) has a high resistance rate among most bacterial isolates. Overall, the table highlights the limited effectiveness of Cefixime against Gram-negative bacteria in this sample.

AUG Resistance in Various Bacteria

			Aug		
		0	1	2	Total
Bacteria	E.coli	10	59	11	80
	Entero	24	5	0	29
	Klebsiell	21	12	8	41
	а				
	Pseudo	40	10	0	50

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Table 5: Anti-microbial resistance of AUG in different bacteria

✤ This table presents the counts for each bacterial type's response to the antibiotic AUG, categorized as "Resistant," "Sensitive".

 \clubsuit It helps to visualize the distribution of antibiotic responses among different types of bacteria.

Chi-Square Tests

			Asymptotic Significance
	Value	Df	(2-sided)
Pearson Chi-	82.827 ^a	6	.000
Square			

The chi square value is 82.827 and P value is 0.000 Interpretation

The Chi-Square test shows a significant association between bacterial species and their resistance patterns (p = 0.000). The Pearson Chi-Square value of 82.827 indicates a strong relationship across variables. This confirms that antibiotic resistance differs notably among the bacteria studied.

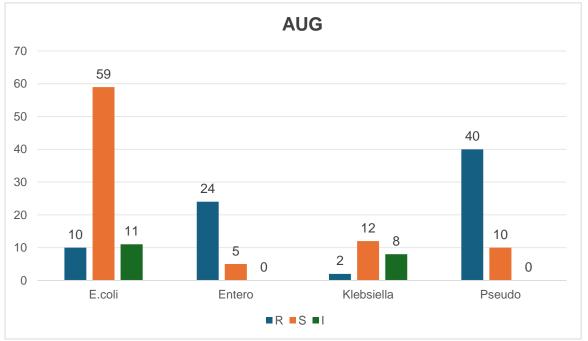


Figure 5: Chart showing Resistivity and Sensitivity patterns of AUG in different bacteria

It highlights that Pseudomonas and Enterobacter species exhibit the highest resistance, making Augmentin largely ineffective against them. Klebsiella shows moderate resistance, while E. coli demonstrates the lowest resistance and highest sensitivity, indicating Augmentin is more effective in treating E. coli infections.

AMC Resistance in Various Bacteria

			Ampicillin	I	
		0	1	2	Total
Bacteria	E.coli	30	38	12	80

Entero	15	12	2	29
Klebsiella	28	13	0	41
Pseudo	40	10	0	50
Total	113	73	14	200

 Table 6: Anti-microbial resistance of CEF in different bacteria

✤ This table presents the counts of different bacterial types with responses to AMC (e.g., Intermediate, Resistant, Sensitive).

The objective is to analyze resistance or sensitivity to AMC across bacterial types.
Chi-Square Tests

		10	Asymptotic Significance
	Value	df	(2-sided)
Pearson Chi-Square	31.638 ^a	6	.000

The chi-square test is 31.638 and P value is 0.000. Interpretation

The Chi-Square test shows a significant association between bacterial species and their resistance to Augmentin (p = 0.000). The Pearson Chi-Square value (31.638) indicates the resistance pattern is not random. This suggests different bacteria respond differently to Augmentin, requiring targeted treatment decisions.

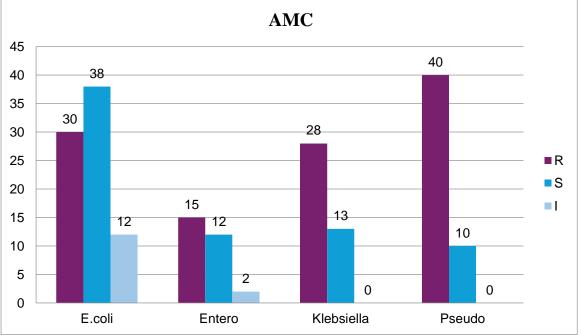


Figure 6: Chart showing Resistivity and Sensitivity patterns of AMC in different bacteria This graph show that Pseudomonas and Klebsiella exhibit the highest resistance (80% and 68.3%, respectively), indicating that Ampicillin is largely ineffective against them. E. coli shows moderate resistance, while Enterobacter presents the lowest resistance, suggesting relatively better sensitivity.

Discussion

To compare the results of the present study on the antimicrobial susceptibility patterns of Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, and Enterococcus faecalis in urinary tract infections (UTIs) with previous studies, it is essential to analyze similarities

and discrepancies across regions and time periods. Such a comparison will highlight evolving resistance trends, diagnostic and treatment challenges, and the global scope of antimicrobial resistance (AMR), which remains a critical issue in healthcare.

In the present study, E. coli was the most commonly isolated pathogen, consistent with the global trend that identifies E. coli as the primary causative agent of community-acquired urinary tract infections (CAUTIs). Research studies around the world demonstrate E. coli to be the primary cause of urinary tract infections however they show different antibiotic susceptibility profiles. In their research of UTI pathogens conducted in India Madapula et al. (2020) discovered E. coli formed 70.3% of all isolates yet most bacteria strains demonstrated significant resistance against ampicillin, ciprofloxacin, trimethoprim, and other antibiotics (Madapula et al., 2020).¹⁶ The current study confirms worldwide resistance trends because its data indicates E. coli bacteria samples demonstrate noticeable resistance to both ampicillin-clavulanic acid (AMC) and ceftriaxone (CEF).

The current research reported a high 63% susceptibility to nitrofurantoin drug detection which matched the findings of Zemer et al. (2021) in South Italy how E. coli showed 10% resistance to nitrofurantoin (Zemer et al., 2021). The effectiveness of nitrofurantoin as a first-line therapy for UTIs remains outstanding because studies demonstrate good treatment results against E. coli bacteria even as beta-lactam resistance keeps rising. ESBLs produced by E. coli strains have evolved into global healthcare concerns because they are frequently found in bacterial populations. The research by Tang et al. (2019) discovered ESBL-producing E. coli strains at high levels which existed across community and hospital locations (Tang et al., 2019).¹⁷ These bacterial strains exhibited 70% resistance to ceftriaxone and other third-generation cephalosporins. The current study documented antibiotic resistance toward these antibiotics which indicates the escalating presence of ESBL-producing germs.

Research findings from this study match international observations which show increasing resistance levels in microorganisms that cause UTIs. The most widespread microorganism in UTIs consists of E. coli exhibiting substantial drug resistance to beta-lactams and fluoroquinolones as investigators across the world observe increasing trends of ESBL-producing E. coli strains. Medical findings support the need for new treatment guidelines because K. pneumoniae showed advanced resistance in third-generation cephalosporins alongside severe P. aeruginosa resistance spanning multiple antibiotics. The challenge caused by E. faecalis extends from its ampicillin and amoxicillin resistance but it remains susceptible to vancomycin and nitrofurantoin treatment. Uropathogenic organisms represent a worldwide antimicrobial resistance challenge which demands joint strategies to track resistance data and develop better therapeutic approaches and antimicrobial stewardship practices for limiting resistance spread according to Zavala-Cerna et al. (2021) and Zemer et al. (2021) and Chidi et al. (2024).^{18,19}

Conclusion

This study highlights the growing concern of antimicrobial resistance in urinary tract infections (UTIs), particularly among bacteria such as E. coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, and Enterococcus faecalis. The findings suggest that females are more affected, with UTIs being more prevalent among female patients (67.5%). Commonly used antibiotics such as Cefixime, Amoxicillin-Clavulanic Acid, and Co-trimoxazole showed high resistance rates among the bacterial isolates. However, Imipenem and MBM (possibly Meropenem) showed the lowest resistance rates, making them potential treatment options. Overall, this study contributes to the growing body of evidence on antimicrobial resistance in UTI.

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