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Title: **Antibacterial Susceptibility Patterns of UTI Pathogens among Different Age Groups in Lahore, Pakistan**

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Antibacterial Susceptibility Patterns of UTI Pathogens among Different Age Groups in Lahore, Pakistan

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ABSTRACT

Background. Urinary tract infections (UTIs) are a significant health problem. They affect individuals across all age groups globally, with a higher prevalence among women and patients with chronic disorders such as diabetes.

Methodology. The current study was performed in the Department of Urology at The University of Lahore to explore the susceptibility of UTIs across different age groups in this region. For this purpose, 157 patients presented with the symptoms of frequent urination and pain during urination were considered. These also included some patients admitted in The University of Lahore Teaching Hospital. Midstream urine samples were collected from patients and cultured. Bacterial colonies of positive cultures were identified through morphological characteristics and serological tests such as Lancefield grouping. Additionally, biochemical tests and API 20E were used to identify members of the Enterobacteriaceae family. The data was analyzed using SPSS (version 25.0).

Results. In this study, the overall prevalence of UTIs was 53.5%, with the highest incidence of UTIs occurring in patients aged 25 to 45 years. The frequently identified isolates comprised *Escherichia coli* which accounted for 46 (54.8%) cases, followed by *Enterococcus* species accounting for 11 (13.1%) cases, *Klebsiella* species accounting for 07 (8.3%) cases, and *Staphylococcus aureus* accounting for another 07 (8.3%) cases. Furthermore, it was found that gram-negative bacteria showed a high level of resistance with 73.3% for cefixime, 73.2% for ceftriaxone, 73.0% for ceftazidime, and a relatively low level of resistance against fosfomycin (15.9%) and nitrofurantoin (16.3%). On the other hand, gram-positive isolates showed a higher level of resistance against penicillin (66.7%). Whereas, all isolates were found to be sensitive to gentamycin (100%).

Conclusion. Routine antimicrobial susceptibility testing prior to antibiotic prescription is recommended. Aminoglycosides, fosfomycin, and nitrofurantoin proved to be the most effective drugs of choice against these bacteria.

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Keywords: antibiotics, antimicrobial resistance, bacterial susceptibility patterns, extensive-drug-resistant (XDR) bacteria, multidrug-resistant (MDR) bacteria, UTI pathogens

Highlights

- UTI susceptibility patterns across age groups in Lahore, Pakistan were assessed, identifying demographic trends in infection rates.
- Diverse methods, including bacterial culture, microscopic examination, and biochemical tests such as API 20E were applied for precise pathogen identification.
- A notably higher UTI prevalence was observed among women with peak incidence in patients aged 25 to 45, underscoring critical demographic findings.

1. INTRODUCTION

Urinary tract infections (UTIs) are the second most common infectious disease (after respiratory tract infections or RTIs) for antibiotic prescriptions, globally. UTIs occur when pathogens invade and proliferate the urinary system, disrupting kidneys and the urinary function and leading to asymptomatic and symptomatic bacteriuria [1]. The presentation of a UTI varies based on the site of infection: urethritis affects the urethra, cystitis primarily impacts the bladder, and pyelonephritis involves the kidneys [2]. With an alarming global incidence of 150 million cases annually, UTIs have become a significant public health issue [3]. In 2019 alone, over 404.6 million individuals were affected by UTIs, which led to 236,786 deaths [4]. It has been estimated that nearly 50% of women suffer from a UTI at least once in their lifetime, with 12% experiencing an initial infection and 48% facing recurrent infections [5, 6]. The increased susceptibility of UTIs among women can be attributed to anatomical factors, such as the shorter length of the female urethra, the absence of bactericidal prostate secretions, pregnancy, and the proximity of the urinary tract to fecal flora, facilitating contamination [7]. Although UTIs affect people of all ages and gender, women are often more vulnerable due to anatomical differences and the risk increases with advancing age [8].

Various pathogenic microorganisms, including bacteria, fungi, and protozoa, contribute to the occurrence of UTIs. Common bacterial pathogens include *Escherichia coli*, *Klebsiella pneumoniae*, *Enterococcus faecalis*, group B *streptococcus*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Candida* species (yeast) [9]. UTIs can be classified into two categories. These include symptomatic cases, which exhibit apparent symptoms of a UTI, as well as asymptomatic cases, where significant bacteriuria occurs without any noticeable symptoms [10]. Clinical manifestations, such as fever, dysuria, hematuria, pyuria, and lower abdominal pain are commonly observed in symptomatic UTIs [11]. If left untreated, UTIs can lead to severe complications, such as irreversible kidney damage and blockage of the urinary tract [12]. The common symptoms in patients with upper UTIs include fever, hematuria (blood in urine), nausea, and vomiting [13].

The misuse and overuse of common antibiotics lead to the evolution of drug-resistant pathogens and increase the prevalence of microbial infections, including UTIs. Drug-resistant pathogens

are defined as pathogens that could have been inhibited previously by antibiotics but have now become insensitive to them [14]. The World Health Organization (WHO) reported recently that approximately 700,000 deaths each year occur due to infections caused by multi-drug-resistant (MDR) pathogens. This figure may reach 2 million deaths and could potentially burden the global economy with over 2.9 trillion dollars in 2050, if the same trend continues [15]. Antimicrobial resistance is a critical health issue that leads to prolonged hospitalization, increases healthcare costs, and limits the treatment options for clinicians, ultimately resulting in higher mortality rates [16, 17]. According to WHO, *Acinetobacter baumannii, Escherichia coli, Klebsiella pneumoniae,* non-typhoidal *Salmonella, Staphylococcus aureus, streptococcus pneumoniae,* and *Shigella* species are gaining resistance to various antibiotics and complicating infection control [18].

During the past six decades, empirical antibiotic therapy has been employed to treat several UTIs, often initiated before the availability of antimicrobial susceptibility test results. However, increased infection caused by resistant pathogens limits and complicates treatment options [19, 20]. In several developing countries including Pakistan, the empirical approach for treating UTIs continues to transmit resistance genes among pathogens, increasing the prevalence of MDR gram-negative-bacilli-associated UTIs [21, 22]. UTI-causing pathogens show increased resistance to antibiotics, such as quinolones, carbapenems, and third-generation cephalosporines through different mechanisms including the production of various enzymes. These enzymes include carbapenemase, extended-spectrum-beta lactamases (ESBL), and biofilm formation. These resistance mechanisms allow the bacteria to survive under non-favorable conditions and increase the incidence of chronic infections through genetic mutations or horizontal gene transfer, hence bacteria acquire resistance-carrying genes [23]. MDR bacteria are pathogens that exhibit resistance to at least one antibiotic among three or more antibiotic classes, whereas extensive-drug-resistant or XDR bacteria are susceptible to only one or two antimicrobial classes and are also resistant to the most commonly available antibiotics [24]. The rising prevalence of MDR and XDR pathogens, particularly resistance to last-resort antibiotics including carbapenemase, complicates UTI treatment [25].

UTIs pose a significant health concern in Pakistan, particularly due to the rising prevalence of drug-resistant bacteria. Recent studies reported high rates of MDR *E. coli* [26] and XDR *Salmonella* that showed 100% resistance to fluoroquinolones [27]. The absence of regional surveillance data makes treatment difficult. So, there is a need to focus on more local studies of drug resistance patterns among uropathogens because such pathogens make it challenging to manage UTIs [28]. Routine antimicrobial susceptibility test (AST) is a benchmark to guide clinicians in selecting antibiotics, managing empirical therapy, and aiding in effective antimicrobial stewardship [25, 29]. However, only a few studies in Pakistan have identified common uropathogens and their AST profiles [30].

This study was designed to investigate the antimicrobial susceptibility patterns of uropathogens isolated from patients in Lahore, Pakistan. The objectives included identifying the etiological agents causing UTIs across various age groups and gender,

determining the prevalence of antimicrobial resistance among these pathogens, and exploring the relationship between age, gender, and susceptibility patterns. By achieving these objectives, the current study aims to offer valuable insights into resistance trends and pathogen distribution, which can inform better treatment and management strategies for UTIs.

2. MATERIALS AND METHODS

This hospital-based, descriptive, cross-sectional study was conducted in the Pathology Laboratory of the Department of Urology at the University Institute of Medical Laboratory Technology (UIMLT), University of Lahore. The institutional ethics committee approved this study. All the participants were voluntary and each participant supplied informed consent.

2.1. Sample Collection

From 4013 patients who visited the pathology laboratory, 157 midstream urine samples were collected aseptically from individuals presented with symptoms such as frequent urination and harboring pain during urination. The participants included patients from various locations across Lahore, with several admitted to The University of Lahore Teaching Hospital. The samples were collected from March 2024 to August 2024. The patients with a history of antibiotic therapy (within the last 72 hours) were excluded from the study population.

2.2. Culture and Identification

Following the manufacturer's instructions (Merk, Germany), powdered agar media was dissolved in water and mixed thoroughly to ensure homogenization. The media flasks were autoclaved for 15 minutes at 121°C and 15 PSI to achieve sterilization. When the media cooled to 50°C, 10 ml of sterile blood was added per 100 ml of media. Once sufficiently cooled, the media was poured into sterile petri dishes labeled with the date and batch information.

The urine samples were inoculated using a standardized inculcation wire loop (0.001 ml) onto Cystine Lactose Electrolyte Deficient (CLED) and 5% blood agar plates via the streak plate method, adhering to the standard microbial techniques [31]. The inoculated plates were incubated aerobically for 24 hours at 37°C. After incubation, the plates were examined for bacterial growth. If growth was present, the colonies were counted and multiplied with the reciprocal of the loop's volume. Bacterial counts exceeding 10^5 colony-forming units per milliliter (CFU/ml) indicated a significant infection, whereas counts below 10^3 CFU/ml were considered non-pathogenic. Counts between 10^4 and 10^5 CFU/ml suggested potential infection, whereas counts between 10^3 and 10^4 CFU/ml were generally considered to indicate contamination [32].

All positive urine cultures showing significant bacterial growth were initially identified based on their morphological characters through Gram staining. Based on Gram staining and colony morphology, biochemical tests were selected for further bacterial identification. For gram-positive bacteria, catalase and coagulase tests were performed [31]. Landfield grouping was used to classify the *streptococcus* species further. On the other hand, for the identification of gram-negative bacteria, different biochemical tests including Kligler's iron agar (KIA), citrate utilization, urease hydrolysis, and sulfur indole motility (SIM) were conducted. For quality control, reference strains *S. aureus* (ATCC 25923) and *E. coli* (ATCC 25922) were used. As per the recommendations by

the Clinical and Laboratory Standards Institute (CLSI, 2024) [33], Kirby-Bauer (KB) disk diffusion method was followed for antibiotic susceptibility testing using Muller-Hinton (MH) agar (Merk, Germany). Two to three colonies with similar morphology were emulsified in distilled water and the solution's optical density was adjusted to 0.5% MacFarland standard. The inoculated plates were homogenously swabbed and allowed to dry at room temperature for 3-5 minutes.

Antibiotics from Oxoid Ltd. were applied at their respective concentrations to treat UTIs. These included ampicillin (AMP, 10 µg), ampicillin/sulbactam (AMC, 20/10 µg), piperacillin/tazobactam (TZP, 110 µg), cefotaxime (CTX, 30 µg), ceftriaxone (CRO, 30 µg), ceftazidime (CAZ, 30 µg), cefepime (FEP, 30 µg), meropenem (MEM, 10 µg), imipenem (IPM, 10 µg), ciprofloxacin (CIP, 5 µg), levofloxacin (LEV, 5 µg), tobramycin (TOB, 30 µg), gentamycin (CN, 10 µg), amikacin (AK, 10 µg), trimethoprim/sulfamethoxazole (SXT, 1.25/23.75 µg), colistin (CT, 10 µg), chloramphenicol (C, 30 µg), fosfomycin (F, 30 µg), tetracycline, doxycycline (DO, 30 µg), penicillin (PEN, 10 µg), cefoxitin (FOX, 30 µg), clindamycin (DA, 10 µg), erythromycin (E, 15 µg), and clarithromycin. Following the CLSI guidelines, the clearance zone around each antibiotic disc was measured with a ruler after 18-20 hours of incubation at 37°C and pathogens were categorized as sensitive (showed recommended cleared zone around antibiotics disc), resistant, and intermediate.

2.3. Statistical Analysis

Categorical variables were summarized, with numerical and percentage data presented in tables created using Microsoft Excel (version 2021). Data analysis was performed using IBM's Statistical Package for Social Sciences (SPSS) software (version 25.0). Statistical differences between antibiotic susceptibility results were assessed using the chi-square statistical test and the Fisher-Freeman-Halton test. The confidence limit for statistical tests was considered 95% with a significance level of $p < 0.05$.

3. RESULTS

Table 1. Prevalence of Gram-Positive Cocci and Gram-Negative Rods in UTIs

UTI Isolates		Frequency	Percentage
Gram Negative Rods (GNRs), *n* = 63/84 (75.0%)	*Escherichia coli (E. coli)*	46	54.8% (46/84)
	Klebsiella species	7	8.3% (07/84)
	Proteus vulgaris	3	3.6% (03/84)
	Pseudomonas aeruginosa	3	3.6% (03/84)
	Proteus mirabilis	2	2.4% (02/84)
	Enterobacter	2	2.4% (02/84)
Gram Positive Cocci (GPCs), *n* = 21/84 (25.0%)	*Enterococcus faecalis*	11	13.1% (11/84)
	Staphylococcus aureus	7	8.3% (0784)
	Streptococcus agalactiae	3	3.6% (03/84)
Total	Total Positive cultures	84	53.5% (84/157)
	Total Negative cultures	73	46.5% (73/157)
	Total Cultures	157	

Table 2. Distribution of Infection among Genders and Different Age Groups

		Gender				Total	
		Male		Female			
		n	%	*n*	%	*N*	%
Age Groups (years)	1–12	4	44.4	5	55.6	9	10.7
	13–24	5	38.5	8	61.5	13	15.5
	25–45	7	28.0	18	72.0	25	29.8
	46–60	8	47.1	9	52.9	17	20.2
	> 60	9	45.0	11	55.0	20	23.8
Microbial Growth		33	39.3	51	60.7	84	53.5
No Growth		38	52.1	35	47.9	73	46.5

A total of 157 patients symptomatic of UTIs were included in the current study. The mean age of the study population was 41.73 ± 19.4 and the age range of patients was 7-75 years. Moreover, 85 (54.2%) samples were collected from women, while the remaining 72 (45.8%) were collected from men. The results of the Pearson chi-square and Fisher-Freeman-Halton exact test showed that there was no statistical association between gender and the distributions of bacterial isolates or between gender and the antibacterial susceptibility patterns of the pathogens. Patients' age distribution showed that 26 (17%) were children, 20 (13%) were adolescents and young adults, 47 (30%) were adults, 26 (17%) were middle-aged adults, and 37 (24%) were from the elderly population. The study found that out of 157 urine samples, 84 (53.5%) tested positive for urine cultures. Of the total 84 isolates, the majority (63, 75%) were gram-negative organisms, while the remaining (21, 25%) were gram-positive bacteria. *Escherichia coli* was found to be the most frequent isolate collected from 46 patients, accounting for 73.0% of gram-negative and 54.8% of all pathogens. This was followed by *Enterococcus faecalis* (13.1%), *Klebsiella* species (8.3%), and *Staphylococcus aureus* (8.3%). Additionally, the other isolated organisms included *Pseudomonas aeruginosa* (3.6%), *Proteus vulgaris* (3.6%), *Streptococcus agalactiae* (3.6%), *Proteus mirabilis* (2.4%), and *Enterobacter* species (2.4%).

In the analysis of antimicrobial sensitivity patterns among the gram-negative bacteria, fosfomycin exhibited the highest antibiotic sensitivity rate at 84.1%, followed closely by nitrofurantoin (83.7%), and amikacin (78.3%). Doxycycline and gentamycin also demonstrated significant effectiveness, with sensitivity rates of 78.1% and 76.2%, respectively. Among carbapenems, meropenem showed a sensitivity rate of 69.8%, whereas imipenem showed a slightly lower

sensitivity rate of 61.9%. Other antibiotics, such as tobramycin (64.4%), also demonstrated moderate effectiveness. In contrast, ceftriaxone and cefixime proved to be the least effective, since both showed a sensitivity rate of 26.7%.

Regarding specific bacterial isolates, the antibiotic profile of *E. coli* revealed that nitrofurantoin and fosfomycin were the most effective antibiotics against it, with sensitivity rates of 89.7% and 89.5%, respectively. Gentamycin (82.6%), amikacin (80.4%), meropenem (65.2%), tobramycin (62.5%), and imipenem (58.7%) also showed high sensitivity against *E. coli*. However, ceftriaxone and amoxicillin-clavulanate proved to be the least sensitive antibiotics, both showing a low sensitivity rate of 19.6%. In contrast, *Klebsiella pneumoniae* exhibited 100% sensitivity to cefepime, cefotaxime, imipenem, meropenem, fosfomycin, and tigecycline, highlighting the efficacy of these drugs in treating UTIs caused by this pathogen. Norfloxacin was the least effective antibiotic, with a sensitivity rate of 50% (Table 5). The results of chi-square and Fisher-Freeman-Halton exact test proved that among gram-negative bacteria, there was found a significant association between age groups and antibiotic resistance patterns of co-amoxiclav, ceftriaxone, ceftazidime, cefixime, cefepime, cefoperazone-sulbactam, levofloxacin, and ciprofloxacin (Table 3). Furthermore, there was also observed a strong association between isolate types and antibiotic resistance patterns among antibiotics such as co-amoxiclav, ceftriaxone, ceftazidime, cefixime, cefepime, fosfomycin, nitrofurantoin, and tigecycline (Table 5).

With respect to gram-positive bacterial isolates, gentamycin showed the highest antibacterial susceptibility with 100% sensitivity; however, it was only tested against *Staphylococcus aureus.* Overall, doxycycline demonstrated the highest level of sensitivity towards all gram-positive bacteria with a susceptibility rate of 95.2%. Only one isolate of *S. aureus* showed resistance, maintaining its role as a critical antibiotic in treating gram-positive infections. In contrast, penicillin, levofloxacin, and ciprofloxacin presented lower efficacy, with a sensitivity rate of 33.3%, 47.6%, and 47.6%, respectively. In terms of specific isolates, *Streptococcus agalactiae* displayed the highest antimicrobial susceptibility, being 100% sensitive to tetracycline, doxycycline, ampicillin, and penicillin, with a single isolate showing resistance to ciprofloxacin and levofloxacin. *S. aureus* was found to be completely sensitive to gentamycin (100%) and highly sensitive to doxycycline and tetracycline (85.7%). However, none of *S. aureus* isolates were sensitive to penicillin. For *Enterococcus faecalis,* doxycycline showed the highest sensitivity (100%), followed by teicoplanin (90.9%), with similar susceptibility to fosfomycin and tetracycline (81.8%) (Table 6). The results of the chi-square and Fisher-Freeman-Halton exact test showed that among gram-positive bacteria there was a significant association between age groups and antibiotic resistance patterns of co-amoxiclav, ciprofloxacin, and levofloxacin. Moreover, the results also suggested a significant association between bacterial isolates and penicillin resistance (Table 3 and Table 6).

Table 3. Antibiogram of Gram-Positive Bacteria: Association between Age Groups and Antibiotic Susceptibility Pattern

Antibiotic	Age Groups						*p* value
	1-12 years	13-24 years	25-45 years	46-60 years	>60 years	Total	Pearson chi-square/ Fisher-Freeman-Halton exact test
Penicillin	3 (S), 1 (R)	2 (S), 2 (R)	2 (S), 3 (R)	0 (S), 3 (R)	0 (S), 5 (R)	7 (S), 14 (R)	0.114
Ampicillin	3 (S), 1 (R)	2 (S), 2 (R)	2 (S), 3 (R)	0 (S), 3 (R)	4 (S), 1 (R)	11 (S), 10 (R)	0.288
Co-Amoxiclav	3 (S), 1 (R)	3 (S), 1 (R)	4 (S), 1 (R)	0 (S), 3 (R)	5 (S), 0 (R)	15 (S), 6 (R)	0.044[a]
Ciprofloxacin	3 (S), 1 (R)	4 (S), 0 (R)	1 (S), 4 (R)	0 (S), 3 (R)	2 (S), 3 (R)	10 (S), 11 (R)	0.047[a]
Levofloxacin	3 (S), 1 (R)	4 (S), 0 (R)	1 (S), 4 (R)	0 (S), 3 (R)	2 (S), 3 (R)	10 (S), 11 (R)	0.047[a]
Doxycycline	4 (S), 0 (R)	4 (S), 0 (R)	5 (S), 0 (R)	3 (S), 0 (R)	4 (S), 1 (R)	20 (S), 1 (R)	1.00
Teicoplanin	4 (S), 0 (R)	3 (S), 0 (R)	2 (S), 1 (R)	1 (S), 2 (R)	5 (S), 0 (R)	15 (S), 3 (R)	0.075
Nitrofurantoin	4 (S), 0 (R)	3 (S), 0 (R)	2 (S), 1 (R)	2 (S), 1 (R)	4 (S), 1 (R)	15 (S), 3 (R)	0.779
Co-Trimoxazole	2 (S), 2 (R)	3 (S), 1 (R)	4 (S), 1 (R)	0 (S), 3 (R)	3 (S), 2 (R)	12 (S), 9 (R)	0.324
Fosfomycin	4 (S), 0 (R)	1 (S), 0 (R)	1 (S), 2 (R)	1 (S), 0 (R)	2 (S), 0 (R)	9 (S), 2 (R)	0.273
Tetracycline	3 (S), 1 (R)	4 (S), 0 (R)	4 (S), 1 (R)	2 (S), 1 (R)	5 (S), 0 (R)	18 (S), 3 (R)	0.673
Gentamycin		2 (S), 0 (R)		2 (S), 0 (R)	3 (S), 0 (R)		b

Note. *S:* Sensitive, *R*: Resistance, *p* value: Statistical significance for the antibiotic against each age group
a: Represent statistically significant values.
b: No statistics are computed because Gentamycin is a constant.

Table 4. Antibiogram of Gram-Negative Bacteria: Association between Age Groups and Antibiotic Susceptibility Pattern

Antibiotic	Age Groups						*p* value
	1-12 years	13-24 years	25-45 years	46-60 years	>60 years	Total	Pearson chi-square/ Fisher-Freeman-Halton exact test
AMC	5 (S), 0 (R)	3 (S), 6 (R)	5 (S), 14 (R)	3 (S), 10 (R)	1 (S), 13 (R)	17 (S), 43 (R)	0.004[a]
CRO	4 (S), 0 (R)	3 (S), 6 (R)	3 (S), 15 (R)	3 (S), 9 (R)	2 (S), 11 (R)	15 (S), 41 (R)	0.019[a]

Antibiotic	Age Groups						*p* value
	1-12 years	13-24 years	25-45 years	46-60 years	>60 years	Total	Pearson chi-square/ Fisher-Freeman-Halton exact test
CAZ	5 (S), 0 (R)	3 (S), 6 (R)	3 (S), 17 (R)	4 (S), 10 (R)	2 (S), 13 (R)	17 (S), 46 (R)	0.003[a]
CFM	5 (S), 0 (R)	3 (S), 5 (R)	3 (S), 17 (R)	3 (S), 10 (R)	2 (S), 12 (R)	16 (S), 44 (R)	0.003[a]
FEP	5 (S), 0 (R)	5 (S), 4 (R)	4 (S), 16 (R)	4 (S), 10 (R)	3 (S), 12 (R)	39 (S), 24 (R)	0.005[a]
SCF	4 (S), 0 (R)	6 (S), 3 (R)	10 (S), 6 (R)	5 (S), 6 (R)	4 (S), 9 (R)	29 (S), 24 (R)	0.115
TZP	3 (S), 2 (R)	3 (S), 4 (R)	8 (S), 8 (R)	5 (S), 8 (R)	5 (S), 8 (R)	24 (S), 30 (R)	0.91
LEV	5 (S), 0 (R)	2 (S), 7 (R)	5 (S), 15 (R)	5 (S), 9 (R)	3 (S), 12 (R)	20 (S), 43 (R)	0.019[a]
CIP	5 (S), 0 (R)	2 (S), 7 (R)	6 (S), 14 (R)	5 (S), 9 (R)	4 (S), 11 (R)	22 (S), 41 (R)	0.039[a]
CN	5 (S), 0 (R)	6 (S), 3 (R)	16 (S), 4 (R)	12 (S), 2 (R)	9 (S), 6 (R)	48 (S), 15 (R)	0.232
AK	5 (S), 0 (R)	7 (S), 2 (R)	14 (S), 5 (R)	10 (S), 4 (R)	11 (S), 2 (R)	47 (S), 13 (R)	0.799
TOB	2 (S), 0 (R)	6 (S), 3 (R)	6 (S), 7 (R)	8 (S), 2 (R)	7 (S), 4 (R)	29 (S), 16 (R)	0.482
IPM	5 (S), 0 (R)	8 (S), 1 (R)	12 (S), 8 (R)	8 (S), 6 (R)	6 (S), 9 (R)	16 (S), 44 (R)	0.062
MEM	5 (S), 0 (R)	8 (S), 1 (R)	13 (S), 7 (R)	9 (S), 5 (R)	9 (S), 6 (R)	44 (S), 19 (R)	0.342
FOS	3 (S), 0 (R)	4 (S), 2 (R)	10 (S), 3 (R)	9 (S), 1 (R)	11 (S), 1 (R)	37 (S), 7 (R)	0.592
F	5 (S), 0 (R)	6 (S), 1 (R)	11 (S), 3 (R)	11 (S), 1 (R)	11 (S), 1 (R)	8 (S), 3 (R)	0.705
NOR	5 (S), 0 (R)	3 (S), 4 (R)	7 (S), 5 (R)	5 (S), 6 (R)	2 (S), 8 (R)	22 (S), 24 (R)	0.066
TGC	1 (S), 0 (R)	3 (S), 2 (R)	1 (S), 3 (R)	1 (S), 2 (R)	2 (S), 0 (R)	8 (S), 7 (R)	0.543
DOX	4 (S), 0 (R)	3 (S), 1 (R)	7 (S), 3 (R)	5 (S), 1 (R)	6 (S), 2 (R)	25 (S), 7 (R)	0.911

Note. *AMC* Co-Amoxiclav, *CRO* ceftriaxone, *CAZ* ceftazidime, *CFM* cefixime, *FEP* cefepime, *SCF* cefoperazone-sulbactam, *TZP* piperacillin-tazobactam, *LEV* levofloxacin, *CIP* ciprofloxacin, *CN* gentamycin, *AK* amikacin, *TOB* tobramycin, *IPM* imipenem, *MEM* meropenem, *FOS* fosfomycin, *F* nitrofurantoin, *NOR* norfloxacin, *TGC* tigecycline, *DO* doxycycline. *S:* Sensitive, *R:* Resistance, *p* value: Statistical significance for the antibiotic against each age group

a: Represent statistically significant values.

Table 5. Effectiveness of Antibiotics against Gram-Negative Rods (GNR): Association between isolate types and antibiotic susceptibility pattern.

Pattern	*Escherichia coli*		*Klebsiella pneumoniae*		*Pseudomonas aeruginosa*		*Proteus mirbals*		*Proteus vulgaris*		*Enterobacter* spp.		Total		*p* value
	S	R	S	R	S	R	S	R	S	R	S	R	S	R	Pearson χ^2/ Fisher-Freeman-Halton exact test
AMC	9	37	4	2			1	1	1	2	1	1	17(28%)	43(72%)	0.019[a]
CRO	9	37	3	2			0	2	0	3	0	2	15(27%)	41(73%)	0.008[a]
CAZ	13	33	4	2	1	2	1	1	1	2	0	2	17(27%)	46(73%)	0.044[a]
CFM	11	35	6	0	0	3	1	1	1	2	0	2	16(27%)	44(73%)	0.001[a]
FEP	11	35	6	0	1	2	1	1	1	2	1	1	21(33%)	42(67%)	0.014[a]
SCF	22	24	5	1	0	2	1	1	3	0	1	1	29(55%)	24(45%)	0.216
TZP	18	28	4	2	1	2	2	0	1	2	1	1	24(44%)	30(56%)	0.568
LEV	12	34	4	2	1	2	1	1	0	3	1	1	20(32%)	43(68%)	0.102
CIP	14	32	4	2	1	2	1	1	0	3	1	1	22(35%)	41(65%)	0.190
CN	38	8	4	2	1	2	1	1	1	2	2	0	48(76%)	15(24%)	0.078
AK	37	9	5	1	1	2	1	1	1	2	2	0	47(78%)	13(22%)	0.206
TOB	20	12	4	2	1	2	1	1	2	1	2	0	29(64%)	16(36%)	0.964
IPM	27	19	6	0	1	2	2	0	2	1	2	0	39(62%)	24(38%)	0.521
MEM	30	16	6	0	1	2	2	0	3	0	2	0	44(70%)	19(30%)	0.373
FOS	34	4	1	0			2	0	0	3			37(84%)	7(16%)	0.007[a]
F	35	4	4	2	0	2					2	0	41(84%)	8(16%)	0.023[a]
NOR	16	20	3	3	1	1							22(48%)	24(52%)	0.696
TGC	1	7	6	0			1	0					8(53.%)	7(47%)	0.003[a]
DO	21	6	3	1							1	0	25(78%)	7(22%)	1.00

Note. *AMC* Co-Amoxiclav, *CRO* ceftriaxone, *CAZ* ceftazidime, *CFM* cefixime, *FEP* cefepime, *SCF* cefoperazone-sulbactam, *TZP* piperacillin-tazobactam, *LEV* levofloxacin, *CIP* ciprofloxacin, *CN* gentamycin, *AK* amikacin, *TOB* tobramycin, *IPM* imipenem, *MEM* meropenem, *FOS* fosfomycin, *F* nitrofurantoin, *NOR* norfloxacin, *TGC* tigecycline, *DO* doxycycline.
a: Represent statistically significant values.

Table 6. Effectiveness of Antibiotics against Gram-Positive Cocci (GPC): Association between isolate types and antibiotic susceptibility pattern

Antibiotics	Enterococcus faecalis		Staphylococcus aureus		streptococcus agalactiae		Total		*p* value
Abbreviation	S	R	S	R	S	R	S	R	Pearson χ^2/ Fisher-Freeman-Halton exact test
PEN	4 (36.4%)	7 (63.6 %)	0 (0%)	7 (100%)	3 (100%)	0 (0%)	7 (33.3%)	14 (66.7%)	0.007
AMP	6 (54.5%)	5 (45.5%)	2 (28.6%)	5 (71.4%)	3 (100%)	0 (0%)	11 (52.4%)	10 (47.6%)	0.148
AMC	8 (72.7%)	3 (27.3%)	4 (57.1%)	3 (42.9%)	3 (100%)	0 (0%)	15 (71.4%)	6 (28.6%)	0.447
CIP	4 (36.4%)	7 (63.6%)	4 (57.1%)	3 (42.9%)	2 (66.7%)	1 (33.3%)	10 (47.6%)	11 (52.4%)	0.587
LEV	4 (36.4%)	7 (63.6%)	4 (57.1%)	3 (42.9%)	2 (66.7%)	1 (33.3%)	10 (47.6%)	11 (52.4%)	0.587
DOX	11 (100%)	0 (0%)	6 (85.7%)	1 (14.3%)	3 (100%)	0 (0%)	20 (95.2%)	1 (4.8%)	0.476
TEC	10 (90.9%)	1 (9.1%)	5 (71.4%)	2 (28.6%)			15 (83.3%)	3 (16.7%)	0.528
F	8 (72.7%)	3 (27.3%)	7 (100%)	0 (0%)			15 (83.3%)	3 (16.7%)	0.245
SXT	5 (45.5%)	6 (54.5%)	4 (57.1%)	3 (42.9%)	3 (100%)	0 (0%)	12 (57.1%)	9 (42.9%)	0.342
FOS	9 (81.8%)	2 (18.2%)					9 (81.8%)	2 (18.2%)	b
TET	9 (81.8%)	2 (18.2%)	6 (85.7%)	1 (14.3%)	3 (100%)	0 (0%)	18 (85.7%)	3 (14.3%)	1.00
CN			7 (100%)	0 (0%)			7 (100 %)	0 (0%)	c

Note. *PEN* penicillin, *AMP* ampicillin, *AMC* amoxicillin-clavulanate, *CIP* ciprofloxacin, *LEV* levofloxacin, *DO* doxycycline, *TEC* teicoplanin, *F* nitrofurantoin, *SXT* co-trimoxazole, *FOS* fosfomycin, *TET* tetracycline, *CN* gentamycin.
a: Represent statistically significant values.
b: No statistics are computed because Isolates is a constant.
c: No statistics are computed because Isolates and Gentamycin are constants.

4. DISCUSSION

Urinary tract infections (UTIs) are prevalent and clinically significant health concerns, warranting a comprehensive investigation into bacterial prevalence across various age groups [8]. Identifying UTI-causing bacterial pathogens is essential for their effective treatment and for the alignment of clinical practice with laboratory findings [34]. The overall prevalence of significant UTIs among different age groups and gender was 53.6%. This showed a lower prevalence than in Karachi, where the prevalence was 66.5% [35], and a higher prevalence than in Peshawar [36] and Lahore [37], which were 43.2% and 42.5%, respectively. Moreover, the prevalence was lower than in most major cities of other developing countries, such as Dhaka, Bangladesh where the prevalence was 71.0% [38] and Prayagraj, India where it was 77.9% [39]. The inconsistency in UTI prevalence might be due to study populations, sample size, personal hygiene, and the methodology employed. Furthermore, the prevalence of uropathogens in this study was higher than those reported from developed regions, such as France (19.2%) [40] and parts of Central Europe (26.9%) [41]. These differences may be due to variations in antibiotic usage policies, public awareness, improved sanitation infrastructure, and restricted antibiotic access to the public.

In this study, female participants were found to be more affected (male: female, 1:1.20). The data showed similarity with several studies conducted irrespective of geographical locations, including India [40], France [41], Pakistan [42], and the United States [43]. This is probably due to the anatomical differences between both genders, such as a shorter urethra [44], more sensitive skin of the external urethral meatus, and close proximity of the urethra to the rectum [45]. The current study identified a higher prevalence of UTIs in female patients aged 25-45 years. The results resemble a recent study conducted in Lahore which determined the percentage of adult females experiencing bacteriuria as 28.7% [38]. Dadi et al. [46] highlighted that sexual intercourse enhances the bacteria's access to the bladder. The results of the current study also showed that a significant proportion of positive cultures (23.4%) was obtained from individuals aged over 60 years. This finding aligns with the study of Girija et al. [47], who reported that 24% of patients presented with UTIs fell within the 70-90 years age category. A study by Pardeshi [48] found that women are more susceptible to UTIs than men, particularly middle-aged women. The study also observed that men over the age of 45 years have comparable rates of UTIs to women, further emphasizing the age and gender-related vulnerability to these infections.

This finding also showed that pathogens causing UTIs included *E coli., E. faecalis, P. aeruginosa, K. pneumoniae, S. aureus, S. agalactiae, Proteus* species, and *Enterobacter* species. These findings are similar to Ahmad et al. [36] and Odoki et al. [49]. In this study, gram-negative rods (GNR) causing UTIs were found to be more prevalent (75%) than gram-positive bacteria (25%), highlighting the different virulence factors that help them adhere to urogenital epithelial cells, prevent them from being washed away in urine, and aid in tissue invasion [50]. *E. coli* emerged as the predominant etiological agent, responsible for 54.8% of isolated cases. This aligns with the findings of numerous studies conducted across different geographical regions. The results varied from that of a study conducted in Multan, Pakistan, which determined the prevalence of *E. coli* among uropathogens as 33% [51].

Likewise, the majority of previous studies established *E. coli* as the most frequent etiological agent of UTIs in India with 54.95% prevalence [40], in Pakistan with 62.80% prevalence [52], and in Bangladesh with a 48.00% prevalence [53]. A possible explanation is that *E. coli* shows enhanced virulence factors for attachment to the host urogenital tract's epithelial cells, such as Type 1 or Type 2 fimbriae [50].

In this study, among gram-negative bacteria, the highest resistance was shown against third-generation cephalosporines, namely ceftriaxone (73.2%), cefotaxime (73.3%), and ceftazidime (73.0%). These findings align with the study of Bullens et al. [52]. The possible explanation for this is the readily available oral suspension of CFX throughout Pakistan for first-line treatment of UTIs. High levels of resistance against beta-lactam-containing antibiotics could occur due to the production of extended-spectrum beta-lactamase enzymes. Moreover, higher levels of resistance were observed against quinolone antibiotics, that is, levofloxacin (68.3%) and ciprofloxacin (65.1%). These results are consistent with the findings of [54] and highlight the need for updated treatment guidelines. On the other hand, the effectiveness of nitrofurantoin and fosfomycin showed similarity with [55], emphasizing their importance as the first-line option. Additionally, a higher rate of sensitivity (lower resistance) was observed against amikacin, gentamycin, doxycycline, meropenem, tobramycin, and imipenem. A possible justification is the higher cost of carbapenem injections and the infrequent prescription of aminoglycoside antibiotics. Hence, they could be considered as an alternative option for treating UTIs.

Among gram-negative uropathogens, *E. coli* was the predominant isolate (89.7%) sensitive to nitrofurantoin, fosfomycin (89.5%), and amikacin (80.4%). These results are comparable to the findings of Rizvi et al. [56] for Pakistan and Acharjee et al. [53] for Bangladesh. While, high resistance was shown to ceftriaxone (80.4), co-amoxiclav (80.4%), tigecycline (87.5), and cefepime (76.1%). These results are in line with the studies of Hassan conducted in Iraq [57] and Fatima et al., conducted in Pakistan [51]. In the current study, *Klebsiella pnuemoniae* showed complete sensitivity to imipenem (6/6, 100%), meropenem (6/6, 100%), and cefepime (6/6, 100%), Joya et al, France [42]. *Pseudomonas aeruginosa* was isolated in (3/84, 3.6%) specimens. A total of 2 strains were extensive drug resistant (XDRs) and only 1 strain showed sensitivity to carbapenem, quinolones, aminoglycosides, and 3rd and 4th generation cephalosporines. This is in line with a study conducted by Sendra et al. [58] in Spain, which found that *P. aeruginosa* was susceptible to amikacin (42.6%, $n = 43/101$). MDR/XDR *P. aeruginosa* is emerging as a major healthcare issue and infection prevention is gaining utmost importance because it can rapidly develop resistance even to novel drugs [59].

Gram-positive bacteria were found to be relatively sensitive to gentamycin (7/7, 100%), doxycycline (20/21, 95.2%), tetracycline (18/21, 85.7%), and fosfomycin (15/18, 83.3%), similar to a study conducted by [60]. Only penicillin (14/21, 66.7%), ciprofloxacin (11/21, 52.4%), and levofloxacin (11/21, 52.4%) exhibited more than 50% resistance among gram-positive bacteria. This might be due to the easy availability and indiscriminate usage of these drugs, which may lead to an increase in resistance.

4.1. LIMITATIONS

There are several limitations to this study. Firstly, it was conducted at a single hospital with a relatively small sample size of 157 patients. Self-reported clinical symptoms may introduce potential bias. Moreover, sole reliance on phenotypic methods to detect antibiotic resistance without using molecular techniques reduces the depth of antimicrobial resistance analysis. Future studies should include a more diverse patient population and utilize molecular techniques to better understand the resistance mechanisms of UTIs-causing pathogens.

4.2. CONCLUSION

Due to the increasing prevalence of MDR and XDR pathogens and the cost associated with UTIs, precise infection control and careful administration of antibiotics are crucial. Routine antimicrobial susceptibility testing before prescribing antibiotics is recommended. Furthermore, hospitals should have an antibiotic stewardship program to reduce antibiotic resistance and prevent further complications.

CONFLICT OF INTEREST

The authors of this manuscript declare they have no financial and non-financial conflict of interest.

DATA AVAILABILITY STATEMENT

The data associated with this study will be provided by the corresponding author upon request.

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No funding has been received for this research.

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