

Characteristics and Antibiotic Resistance of Urinary Tract Pathogens Isolated from Indoor Cases of A Tertiary Care Hospital

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ABSTRACT

Objective: To determine the antimicrobial sensitivity of uropathogens among inpatients at a tertiary care hospital in Pakistan.

Study Design: Cross-sectional study

Place and Duration of Study: Pak Emirates Military Hospital, Rawalpindi; Pakistan Apr to Jun 2023.

Methodology: After ethics approval, male and female adult patients from medical wards were included. Non-probability, consecutive sampling was used. Patients with clinical and/ or urinalysis findings of urinary tract infection were included, excluding those unwilling or unable to provide informed consent. Demographic variables and clinical history were collected. 1,339 Urine cultures were sent to the Department of Microbiology, Army Medical College, Rawalpindi. Antimicrobial susceptibility testing was performed via the disc diffusion method for isolates.

Results: The mean age of the participants was 51.00 (34.00-67.00) years with 795(59.37%) females. The most common primary diagnosis was neurological disease (n=177 [13.22%]). Positive growth was identified in 1,294 (96.64%), with 27(2.02%) fungal infections. Of the 1,267 bacterial isolates, 1,055(83.27%) were Gram-negative. *Escherichia coli* was the most frequently isolated pathogen, with 846 cases (66.78%), followed by *Enterococcus faecalis* with 178(14.05%). High sensitivity for *E. coli* was observed for Imipenem (99.44%), Meropenem (98.19%), and Amikacin (97.92%), while Ampicillin (6.73%) and Erythromycin (16.38%) exhibited low sensitivity. *E. faecalis* exhibited susceptibility to Nitrofurantoin (97.19%) and Fosfomycin (91.57%) but showed lower susceptibility to Ampicillin (38.20%) and doxycycline (17.98%).

Conclusion: Antibiotics exhibiting higher efficacy across various uropathogens should be considered for the first-line empirical treatment for UTIs. Local antimicrobial resistance patterns exist and susceptibility testing is recommended for inpatient UTIs.

Keywords: Antimicrobial susceptibility, Urinary tract infections, Urine Cultures

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INTRODUCTION

Urinary tract infections (UTIs) are a widespread challenge in clinical practice, affecting millions worldwide annually. The infections of the urinary tract are estimated to affect 150 million individuals worldwide posing a cumulative burden of 6 billion dollars in lieu of health expenses.^{1,2} These figures easily highlight the impact of UTIs on financial planning and health budgets of different countries. UTIs can affect the kidneys, ureters, bladder, and urethra, often originating from uropathogens in the gastrointestinal tract. These infections range from cystitis to severe conditions like pyelonephritis and sepsis.³ UTIs are classified into uncomplicated and complicated forms. A complicated UTI encompasses all urinary tract infections except for simple ones. This includes infections in immunocompromised

individuals, males, pregnant patients, and those presenting with fevers, stones, sepsis, urinary obstruction, catheters, or involving the kidneys.⁴ Complicated UTIs, if untreated, can progress to involve the kidneys and bloodstream, incurring substantial healthcare costs and societal burdens. Despite their prevalence, UTIs present diagnostic and therapeutic hurdles due to factors like antimicrobial resistance, recurrent infections, and diverse pathogen profiles. The growing threat of antibiotic resistance, particularly against β -lactam antibiotics, fuelled by inappropriate usage, poses a significant challenge.⁵ Mechanisms like β -lactamase production are prevalent among bacteria, rendering conventional antibiotics ineffective.

UTIs pose a significant challenge in hospitalised patients due to various factors inherent to the hospital setting. Bacterial colonisation of indwelling catheters can lead to ascending infections. Hospitalized patients frequently have compromised immune systems, either

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due to underlying medical conditions or immunosuppressive therapies, rendering them more susceptible to UTIs.⁶ Prolonged hospital stays coupled with multiple antibiotic courses commonly prescribed during hospitalization contribute to the emergence of multidrug-resistant UTI pathogens, complicating treatment strategies.⁷ Certain medical conditions commonly seen in hospitalized patients, such as diabetes, chronic kidney disease, or neurogenic bladder dysfunction, predispose individuals to UTIs. The consequences of UTIs in hospitalized patients can be severe, ranging from localized infections to systemic complications such as pyelonephritis, urosepsis, and acute kidney injury. These complications not only increase morbidity and mortality but also impose substantial healthcare costs and resource utilization.⁸

The objective of this study was to ascertain the prevalence of uropathogens accountable for UTIs among hospitalized, non-surgical patients and assess their antibiotic resistance profiles at PEMH, a tertiary care hospital in Rawalpindi, Pakistan. The primary goal was to delineate appropriate empirical antibiotic therapies for hospitalized patients with UTIs based on these findings. Through analysis of antibiotic resistance rates among UTI patients, this study seeks to enhance antibiotic resistance control strategies and advocate for judicious antibiotic use in UTI management.

METHODOLOGY

This cross-sectional study was initiated after approval from the Ethics Committee (Certificate ID: A/28/EC/522/23). Culture and sensitivity testing was done on urine samples collected from UTI patients admitted in various wards in the Department of Medicine, Pak Emirates Military Hospital (PEMH), Rawalpindi Pakistan during the three-month study period (April-June, 2023). The sample was calculated using an OpenEpi (Version.3.01) sample size calculator with a 5% margin of error, 95% confidence interval and 65.1% prevalence of UTI.⁹ A consecutive convenient sampling technique was employed.

Inclusion Criteria: Specimens were obtained from nonsurgical patients, encompassing individuals receiving care across medical subspecialties within general medical wards, as well as from patients in a 20-bedded Intensive Care Unit (ICU) and a 20-bedded Acute Medical Unit (AMU).

Exclusion Criteria: Patients in the ICU, where urine cultures were routinely performed as part of protocol

regardless of clinical presentation were excluded. Moreover, patients with urinary tract structural abnormalities (urethral stricture, recent surgery of urogenital tract, and BPH), renal transplant, and those not willing to participate in the study were also excluded.

After informed consent, patients were interviewed about their symptoms and relevant history. The culture and sensitivity analyses were conducted when clinical assessments or urinalysis results indicated potential infection or abnormalities. Mid-stream urine samples for culture and sensitivity testing were collected using sterile plastic containers and sent to the Department of Microbiology, Army Medical College, Rawalpindi. The samples were subjected to various procedures, including Gram staining, culture, and antibiotic susceptibility testing. Urine specimens were subjected to culture on cysteine lactose electrolyte deficient (CLED) agar to isolate uropathogens, followed by incubation at 37°C for 24 hours. A standardized volume (0.01 mL) of urine was inoculated onto the agar surface for colony quantification. Bacterial growth exceeding 10⁵ organisms/mL of urine was deemed clinically significant. Subsequent biochemical profiling of isolates adhered to established protocols. To ensure pure growth, isolates were subcultured onto MacConkey agar. Antibiotic susceptibility testing of the isolates was performed via the Kirby-Bauer disk diffusion method. Müller-Hinton agar plates were utilized for this purpose, followed by incubation at 37°C for 24 hours. The diameter of the zone of inhibition surrounding antibiotic disks was measured and compared against standard institute guidelines. Isolates exhibiting intermediate susceptibility were classified as resistant. Reference strains for *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Enterococcus faecalis* (*E. faecalis*), and *Staphylococcus aureus* (*S. aureus*) were included as per institutional protocol. Isolates, comprising both gram-negative and gram-positive bacteria, underwent sensitivity testing against 21 antibiotics commonly prescribed for UTI at PEMH. Detection of extended-spectrum Beta-lactamases (ESBL) production was carried out using the Cephalosporin/Clavulanic acid combination discs method. ESBL production was confirmed if the zone diameter for Ceftazidime (30 µg) tested in combination with clavulanic acid exceeded that of Ceftazidime tested alone by ≥ 5 mm. Data was analysed in Statistical Package for the Social Sciences (SPSS) version 23.00. Descriptive statistics (frequency and

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percentages) were computed for categorical variables. Mean $\pm$ Standard Deviation (SD) was presented for quantitative variables.

RESULTS

A total of 1,339 urine specimens were sent to the laboratory for culture and sensitivity testing. Among these, 795 (59.4%) patients were females. The mean age of the participants was 51.00 (34.00-67.00) years, with an age range spanning from 18 to 83 years. The most common primary diagnosis was neurological disease (n=177 [13.22%]) and the largest number of patients were from general wards (n=902 [67.36%]) (Table - I).

Table - I: Baseline Patient Characteristics (n=1,339)

Baseline Characteristics	Values
Age (Years)	51.00 (34.00-67.00)
Gender n (%)	
Male	544(40.63%)
Female	795(59.37%)
Ward n (%)	
General	902(67.36%)
ICU	199(14.86%)
AMU	238(17.77%)
Diagnosis n (%)	
Neurological disease	177(13.22%)
Renal disease	168(12.55%)
Endocrine disease	163(12.17%)
Other	163(12.17%)
Gastrointestinal disease	162(12.10%)
Pulmonary disease	152(11.35%)
Liver disease	141(10.53%)
Infectious disease	123(9.19%)
MODS	90(6.72%)

ICU: Intensive care unit; AMU: Acute Medical Unit; MODS: Multi-organ Dysfunction Syndrome

Of the 1,339 patients, positive pathogen growth was detected in urine samples of 1,294 (96.64%) patients. Fungal infections were detected in 27 (2.02%) cases. Of the 1,267 bacterial isolates, the majority were Gram-negative (n=1,055 [83.27%]). Among the bacteria identified, *E. coli* was the most frequently isolated, accounting for 846 cases (66.78%), followed by *E. faecalis* with 178(14.05%), and *Klebsiella pneumoniae* (*K. pneumoniae*) with 109 cases (8.60%). Of the positive bacterial cultures, the majority (n=757

[59.75%]) were associated with female patients. Notably, irrespective of gender, *E. coli* remained the predominant infectious strain. Among the Enterobacteriaceae (n=996), ESBL production was detected in 346(40.90%) *E. coli*, 30(27.52%) *K. pneumoniae*, and 12(29.27%) of *Proteus mirabilis* (*P. mirabilis*) isolates. The comprehensive antimicrobial susceptibility profile of Gram-negative isolates to the tested antibiotics revealed notable findings (Table - II). The highest sensitivity rates for *E. coli* were observed for Imipenem (n=841 [99.44%]), followed by Meropenem (n=831 [98.19%]) and Amikacin (n=828 [97.92%]). Conversely, Ampicillin (n=57 [6.73%]) and Erythromycin (n=139 [16.38%]) exhibited the lowest sensitivity rates against the *E. coli* isolates. *K. pneumoniae* was found to be highly sensitive to Imipenem (n=108 [99.25%]), Piperacillin-tazobactam (n=100 [91.82%]) and Meropenem (n=98 [90.83%]) but exhibited uniform resistance and low sensitivity to Ampicillin (n=5 [4.52%]) and Amoxicillin-clavulanic acid (n=11 [10.53%]). *P. mirabilis* isolates showed high sensitivity to Imipenem (n=41 [100.00%]), Meropenem (n=40 [97.56%]), and Amikacin (n=39 [95.12%]). *P. aeruginosa* isolates demonstrated the greatest sensitivity to Meropenem (n=48 [87.28%]), Imipenem (n=46 [83.64%]), and Piperacillin-tazobactam (n=45 [81.82%]). Of note, 4 Gram-negative isolates, all from ICU patients, were identified as *Acinetobacter baumannii* (*A. baumannii*) which were found to be resistant to all the 21 antibiotics in the study. These were further tested against Polymyxin B and all were found to be sensitive.

Noteworthy trends emerged regarding antibiotic susceptibility patterns of two prevalent Gram-positive pathogens (Table - III), *E. faecalis* (n=178) and *S. aureus* (n=34). *E. faecalis* displayed high susceptibility to Nitrofurantoin 173(97.19%) and Fosfomycin 163(91.57%) while exhibiting lower susceptibility to agents like Ampicillin 68(38.20%) and Doxycycline 32(17.98%). In contrast, *S. aureus* exhibited susceptibility to Nitrofurantoin 33(97.06%), Piperacillin-tazobactam 33(97.06%), and Imipenem 33(97.06%), with lower susceptibility observed for Tetracycline 23 (67.65%) and Cefoxitin 25(73.53%).

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Table - II: Antibiotic sensitivity profile of Gram-negative bacterial isolates (n=1,055)

Antibiotic	E. coli (n=846)	K. pneumoniae (n=109)	P. mirabilis (n=41)	P. aeruginosa (n=55)	A. baumannii* (n=4)
Amikacin	828 (97.92%)	97 (88.82%)	39 (95.12%)	43 (78.18%)	0 (0.00%)
Amoxicillin-Clavulanic acid	367 (43.33%)	11 (10.53%)	31 (76.58%)	15 (27.27%)	0 (0.00%)
Ampicillin	57 (6.73%)	5 (4.52%)	18 (43.29%)	1 (18.18%)	0 (0.00%)
Cefazolin	537 (63.44%)	61 (55.87%)	27 (66.37%)	18 (32.73%)	0 (0.00%)
Cefoxitin	622 (73.56%)	76 (69.44%)	33 (79.52%)	4 (7.27%)	0 (0.00%)
Ceftazidime	493 (58.24%)	78 (71.63%)	32 (77.48%)	42 (76.36%)	0 (0.00%)
Ceftriaxone	292 (34.55%)	82 (75.67%)	26 (63.25%)	17 (30.90%)	0 (0.00%)
Ciprofloxacin	534 (63.08%)	91 (83.81%)	22 (53.33%)	39 (70.90%)	0 (0.00%)
Clarithromycin	219 (25.85%)	34 (31.44%)	24 (59.23%)	18 (32.73%)	0 (0.00%)
Co-Trimoxazole	311 (36.72%)	55 (50.00%)	21 (51.22%)	5 (9.09%)	0 (0.00%)
Doxycycline	423 (50.00%)	43 (39.58%)	6 (13.82%)	2 (3.64%)	0 (0.00%)
Erythromycin	139 (16.38%)	32 (28.98%)	21 (51.22%)	11 (20.00%)	0 (0.00%)
Fosfomycin	774 (91.44%)	71 (64.90%)	30 (73.17%)	19 (34.55%)	0 (0.00%)
Gentamycin	670 (79.24%)	97 (88.82%)	35 (85.36%)	36 (65.45%)	0 (0.00%)
Imipenem	841 (99.44%)	108 (99.25%)	41 (100.00%)	46 (83.64%)	0 (0.00%)
Levofloxacin	564 (66.62%)	80 (73.35%)	24 (58.54%)	41 (74.55%)	0 (0.00%)
Meropenem	831 (98.19%)	99 (90.83%)	40 (97.56%)	48 (87.27%)	0 (0.00%)
Nalidixic-acid	336 (39.67%)	76 (70.14%)	6 (14.63%)	5 (9.09%)	0 (0.00%)
Nitrofurantoin	785 (92.79%)	51 (46.53%)	1 (2.44%)	24 (43.64%)	0 (0.00%)
Piperacillin-tazobactam	705 (83.33%)	100 (91.82%)	36 (87.80%)	45 (81.82%)	0 (0.00%)
Tetracycline	150 (17.73%)	59 (53.77%)	1 (2.44%)	17 (30.91%)	0 (0.00%)

*Isolates were subsequently tested against Polymyxin B and found to be sensitive.

The members of the Enterobacteriaceae family, particularly *E. coli* and *Klebsiella* spp., are recognized as key causative agents of UTIs due to their possession of various virulence factors, including adhesion molecules, pili, and fimbriae. These factors facilitate the attachment of bacteria to the urothelium, promoting colonization and subsequent infection.¹⁰ In our study, the predominant urinary isolates were *E. coli*, with 846 isolates (66.77%). However, we identified the Gram-positive *E. faecalis* as the second most frequently isolated organism with 178(14.05%) isolates, followed by *K. pneumoniae*, with 109(8.60%).

Table - III: Antibiotic sensitivity profile of Gram-positive bacterial isolates (n=212)

Antibiotic	E. faecalis (n=178)	S. aureus (n=34)
Amikacin	105 (58.99%)	32 (94.12%)
Amoxicillin-Clavulanic acid	128 (71.91%)	24 (70.59%)
Ampicillin	68 (38.20%)	13 (38.24%)
Cefazolin	0 (0.00%)	19 (55.88%)
Cefoxitin	0 (0.00%)	25 (73.53%)
Ceftazidime	0 (0.00%)	29 (85.29%)
Ceftriaxone	0 (0.00%)	30 (88.24%)
Ciprofloxacin	64 (35.96%)	26 (76.47%)
Clarithromycin	155 (87.08%)	30 (88.24%)
Co-Trimoxazole	37 (20.79%)	15(44.12%)
Doxycycline	32 (17.98%)	8 (23.53%)
Erythromycin	35 (19.66%)	28 (82.35%)
Fosfomycin	163 (91.57%)	33 (97.06%)
Gentamycin	41 (23.03%)	30 (88.24%)
Imipenem	159 (89.33%)	33 (97.06%)
Levofloxacin	122 (68.54%)	27 (79.41%)
Meropenem	81 (45.51%)	32 (94.12%)
Nalidixic-acid	0 (0.00%)	30 (88.24%)
Nitrofurantoin	173 (97.19%)	33 (97.06%)
Piperacillin-tazobactam	130 (73.03%)	33 (97.06%)
Tetracycline	119 (66.85%)	23 (67.65%)

The prevalence of ESBL-producing Enterobacteriaceae represents a pressing global challenge due to heightened antibiotic resistance and limited treatment options. In this study, the Gram-negative Enterobacteriaceae isolates (n=996) were tested for ESBL production. Overall, ESBL production was identified in 388(38.96%) isolates. Reported rates of ESBL production among UTI-causing Enterobacteriaceae vary widely in the literature, influenced by geographic location, healthcare settings, patient populations, and study methodologies, with rates frequently higher in healthcare-associated infections than in community-acquired infections. In a study aimed to characterize ESBL producing Enterobacteriaceae isolated from community-acquired UTIs in three teaching hospitals in Egypt, 70.68% of isolates were identified as ESBL-producers through phenotypic testing. ESBL production rates were 69.6% in *E. coli*, 74.6% in *K. pneumoniae*, and 46.6% in *P. mirabilis* isolates.¹¹ Whereas in a study aimed to assess the prevalence of ESBL-producing *E. coli* and *K. pneumoniae* at a tertiary hospital in Nepal, 91.7% of *E. coli* but only 8.3% of *K. pneumoniae* were found to be ESBL-producers.¹² Similarly, a retrospective case-control study aimed to assess the prevalence of ESBL-positive isolates among adults with UTI in Qatar which included 1100 subjects found that 25.2% of isolates were ESBL-producing strains.¹³ The predominant pathogens were *E. coli* and *K. pneumoniae*, and 46.4% of *E. coli* and 16.6% of *K. pneumoniae* were ESBL-producers. In another cross-sectional study conducted at various hospitals in Sudan, ESBL-producing bacteria isolated from UTIs in hospitalized pregnant women were investigated.¹⁴ *E.*

coli was the predominant pathogen and ESBL was detected in 44.4% of the Gram-negative isolates. The variation in findings across these studies underscores the significance of local surveillance and systematic reviews to provide valuable insights into the prevalence of ESBL-producing Gram-negative bacteria in different regions and settings.

Earlier investigations conducted in Pakistan on uropathogen antimicrobial resistance have yielded varying results. In a meta-analysis examining the prevalence of ESBL-producing Enterobacteriaceae in Pakistan, the pooled proportion of ESBL-producers across Pakistan was determined to be around 40% depicting a high ESBL burden in Pakistan.¹⁵ These findings are very close to our finding as 38.96% of the isolated Enterobacteriaceae were found to be ESBL-producing. Another study which looked at 5-year data to assess the prevalence of ESBL-producing *E. coli* found that among 173 *E. coli* isolates, 82 (47.40%) were phenotypically ESBL-producers, indicating the rapid emergence of ESBL-producing *E. coli* in Pakistan.¹⁶ In our study, of the 846 *E. coli* isolates, 346 were found to be ESBL-producing, showing a slightly lower (40.90%) proportion.

Antibiotic resistance in UTIs among hospitalized patients presents a critical concern. In our study, we found high rates of resistance against commonly prescribed beta-lactam antibiotics. Overall, among the *E. coli* isolates, Amikacin, Imipenem, Meropenem, Nitrofurantoin, and Fosfomycin demonstrated notable efficacy, while substantial resistance was observed across the spectrum of other antibiotics evaluated in this study. Similarly, besides Imipenem, Piperacillin/tazobactam, Meropenem, Amikacin, Gentamycin, and Ciprofloxacin, *K. pneumoniae* isolates exhibited very high rates of resistance against a wide spectrum of antibiotics. These findings are consistent with studies done in various parts of the world. In a study conducted in Iran aimed to investigate uropathogen drug susceptibility, *E. coli* was the most common bacterial isolate, followed by *K. pneumoniae*, and *S. agalactiae*. In assessing the antibiotic susceptibility patterns of *E. coli*, a notable degree of sensitivity was observed towards Imipenem, Amikacin, Meropenem, and Nitrofurantoin, while Piperacillin and Ampicillin displayed the least sensitivity.¹⁷ These findings align with similar trends observed in other investigations. For instance, a study reported *E. coli*'s heightened resistance to Ampicillin and Piperacillin, coupled with low resistance rates

against Meropenem, Amikacin, and Nitrofurantoin.¹⁸ Similarly, another study found isolated *E. coli* to exhibit high sensitivity to Amikacin, Imipenem, and Meropenem, alongside significant resistance to Ampicillin.¹⁹ Additionally, a study demonstrated that uropathogenic *E. coli* displayed high sensitivity to Imipenem Meropenem and Amikacin, while all isolates exhibited resistance to Ampicillin.²⁰ A low rate of resistance to Imipenem among *E. coli* uropathogens was also consistent with the findings of our study.

Studies looking at the causative agents and antimicrobial resistance of uropathogens among patients in Pakistan also reflect similar trends. In a study conducted at the Armed Forces Institute of Pathology, Rawalpindi aiming to determine the antimicrobial susceptibility pattern of bacterial uropathogens among inpatients, *E. coli* was the most prevalent bacterial isolate followed by *P. aeruginosa* and *K. pneumoniae*.²¹ Similar to our findings, *E. coli* isolates exhibited high sensitivity to Imipenem, Amikacin, Piperacillin-tazobactam, and Nitrofurantoin. For *K. pneumoniae*, Imipenem and Piperacillin-tazobactam demonstrated significant susceptibility, which is also consistent with our findings. The overall findings of the study were in line with our findings that susceptibility to commonly used antibiotics was notably low. Another retrospective study conducted in Islamabad, evaluated the evolving antibiotic sensitivity profile of uropathogenic *E. coli* over 12 years, focusing on ESBL-producing strains.²² 12,000 samples from patients with uncomplicated urinary tract infections were analysed.

RESULTS

indicated decreased susceptibility and high resistance rates of *E. coli* to Amoxicillin-clavulanic acid, Co-trimoxazole, Fluoroquinolones, and Cephalosporins whereas Fosfomycin, Aminoglycosides, and Nitrofurantoin exhibited higher sensitivity rates. These findings are similar to ours in that we also found that uropathogenic *E. coli* are resistant to Amoxicillin/clavulanic acid, Co-trimoxazole, Ceftriaxone, Erythromycin, Clarithromycin, Tetracycline, and Ampicillin. Another study characterized isolates from UTI patients and assessed their susceptibility to commonly used antibiotics in Punjab, Pakistan.²³ Of the 392 positive cultures, *Escherichia coli* was the most frequently identified pathogen which demonstrated high resistance to various antimicrobial drugs, including

Cephalexin and Ampicillin while sensitivity to Imipenem, Meropenem, and Cefoperazone was high. Another laboratory-based cross-sectional study demonstrated the incidence patterns of uropathogens, their antimicrobial susceptibility, and associated risk factors among the general population in Swabi, Pakistan.²⁴ *E. coli* was the predominant isolate, showing notable susceptibility to Cefoperazone and Imipenem while demonstrating resistance to Ampicillin. Overall, for the antibiotics tested, the sensitivity profiles of the detected isolates were consistent with our findings.

LIMITATION OF STUDY

Our study presents antibiotic susceptibility trends over a three-month duration and its scope is limited to UTIs among nonsurgical inpatients rather than encompassing UTI cases in the general population. Thus, the findings may not apply to all patients with UTIs. To obtain a comprehensive understanding of declining sensitivity patterns, large-scale studies involving diverse patient populations are warranted. Such extensive investigations would provide more accurate insights into antibiotic resistance dynamics on a broader scale.

CONCLUSION

Antibiotics exhibiting higher efficacy across various uropathogens should be considered for the first-line empirical treatment for UTIs. Local antimicrobial resistance patterns exist and susceptibility testing is recommended for inpatient UTIs.

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Authors' Contribution

Following authors have made substantial contributions to the manuscript as under:

NK & SS: Data acquisition, data analysis, critical review, approval of the final version to be published.

MI & ARA: Study design, data interpretation, drafting the manuscript, critical review, approval of the final version to be published.

WUR & FS: Conception, data acquisition, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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