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Comparative Analysis of the Microbiological Spectrum and Antimicrobial Resistance Profiles among Catheter-Associated and Non-Catheter-Associated Urinary Tract Infections in Three Tertiary Care Hospitals of Peshawar, Pakistan

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Abstract

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Keywords: Urinary Tract Infection, Antimicrobial Resistance, Uropathogens, Catheter-associated Urinary Tract Infection, Antimicrobial Susceptibility Testing, Antimicrobial Stewardship

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Abstract

Urinary tract infections (UTIs) are among the most common bacterial infections worldwide, with increasing antimicrobial resistance compromising empirical therapy. This multicenter cross-sectional study evaluated the bacterial spectrum and antimicrobial susceptibility patterns of uropathogens isolated from catheter-associated and non-catheter-associated UTIs in three tertiary care hospitals in Peshawar, Pakistan, between January 2022 and June 2023. Of 1,886 urine specimens, 1,694 (89.8%) yielded significant bacterial growth. *Escherichia coli* (43.0%) was the predominant pathogen, followed by *Citrobacter* spp. (15.6%), *Morganella morganii* (15.1%), *Pseudomonas* spp. (9.7%), and *Klebsiella pneumoniae* (9.3%). Carbapenems, amikacin, tigecycline, nitrofurantoin, fosfomycin, and cefoperazone-sulbactam demonstrated the highest in vitro activity, whereas fluoroquinolones, third-generation cephalosporins, aminopenicillins, and co-trimoxazole showed high resistance rates. These findings underscore the need for continuous antimicrobial resistance surveillance, routine institutional antibiograms, evidence-based revision of empirical treatment guidelines, and strengthened antimicrobial stewardship programs to optimize UTI management.

Keywords: [Urinary Tract Infection](#), [Antimicrobial Resistance](#), [Uropathogens](#), [Catheter-associated Urinary Tract Infection](#), [Antimicrobial Susceptibility Testing](#), [Antimicrobial Stewardship](#)

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Introduction

Urinary tract infections (UTIs) are the commonest bacterial infections that affect humans and are a significant public health problem worldwide due to

their high prevalence, high cost of health care, and growing burden of antimicrobial consumption (Mancuso et al., 2023). UTIs are one of the most prevalent bacterial infections in humans and are a



significant public health problem due to their high prevalence, recurrent nature, significant burden on healthcare resources, and growing burden on antimicrobial use (AMU) (Zeng et al., 2022). It is estimated that more than 150 million individuals develop UTIs each year, leading to millions of outpatient visits, emergency department visits, hospital admissions, and significant economic losses worldwide (Midby & Miesner, 2024).

Among healthcare-associated infections, catheter-associated urinary tract infections (CAUTIs) are the most common healthcare-associated infections (HAIs). CAUTIs are one of the most reported device-associated infections worldwide and are a significant cause of extended hospital stays and higher health care costs. costs, and excess mortality (Werneburg, 2022). The ability of uropathogens to adhere to catheter surfaces, establish mature biofilms, evade host immune responses, and resist antimicrobial therapy significantly complicates the management of these infections (Rubi et al., 2022).

Gram-negative bacteria remain the predominant etiological agents of both community-acquired and healthcare-associated UTIs (Wanke-Rytt et al., 2023). Uropathogenic *Escherichia coli* (UPEC) continues to be the leading pathogen worldwide because of its remarkable capacity to colonize the urinary tract through adhesins, fimbriae, siderophore-mediated iron acquisition systems, toxins, immune evasion strategies, and biofilm formation (Zhou et al., 2023). However, opportunistic Gram-negative organisms including *Klebsiella pneumoniae*, *Proteus mirabilis*, *Citrobacter* spp., *Morganella morganii*, *Providencia* spp., and *Pseudomonas aeruginosa* are increasingly recognized as important causes of complicated and catheter-associated UTIs, particularly in tertiary healthcare settings where prolonged hospitalization, invasive procedures, and prior antimicrobial exposure facilitate the emergence and dissemination of drug-resistant pathogens (Debbarma et al., 2022).

The rapid evolution and global dissemination of antimicrobial resistance (AMR) among uropathogens have become one of the greatest threats to effective clinical management of UTIs (Pothoven, 2023). Increasing resistance to aminopenicillins, fluoroquinolones, trimethoprim-sulfamethoxazole, and third-generation cephalosporins has substantially reduced the effectiveness of empirical treatment regimens that were previously considered highly reliable (Dasgupta-Tsinikas et al., 2022). Moreover, the worldwide emergence of extended-spectrum β -

lactamase (ESBL)-producing Enterobacterales, carbapenem-resistant Enterobacterales (CRE), and multidrug-resistant *Pseudomonas aeruginosa* has further narrowed therapeutic options and contributed to increased treatment failure, prolonged hospitalization, and higher mortality (Gales et al., 2023). Recognizing this growing threat, the World Health Organization (WHO) has recognized AMR as one of the foremost worldwide health priorities, emphasizing the urgent need for organized surveillance, antimicrobial stewardship, and evidence-based prescribing strategies (Ferraz, 2024).

Because antimicrobial resistance patterns exhibit considerable geographical and temporal variation, empirical treatment recommendations should be informed by contemporary local epidemiological data rather than extrapolated from international studies. Institutional antibiograms and regional surveillance programs therefore play a pivotal role in guiding empirical antimicrobial therapy, optimizing antimicrobial stewardship interventions, reducing inappropriate antibiotic use, and improving patient outcomes (Cantón et al., 2023). Despite the increasing burden of antimicrobial resistance in Pakistan, comprehensive multicenter investigations evaluating the bacterial spectrum and antimicrobial susceptibility patterns of uropathogens from both catheter-associated and non-catheter-associated UTI remain limited, creating an important knowledge gap for clinicians and healthcare policymakers.

Therefore, the present multicenter cross-sectional study was undertaken to characterize the bacterial etiology and antimicrobial susceptibility profiles of clinically significant uropathogens isolated from catheter-associated and non-catheter-associated UTIs in three major tertiary care hospitals in Peshawar, Pakistan. By providing comprehensive and contemporary regional surveillance data, this study seeks to support evidence-based empirical antimicrobial therapy, inform institutional antibiogram development, strengthen antimicrobial stewardship programs, and contribute to national efforts aimed at combating the escalating burden of antimicrobial resistance.

Materials and Methods

Study Design and Setting

A multicenter laboratory-based cross-sectional study was conducted over an 18-months period started from January 2022 to June 2023 in the clinical microbiology laboratories of three major tertiary care teaching hospitals in Peshawar, Khyber Pakhtunkhwa, Pakistan,

including the Khyber Teaching Hospital (KTH), Lady Reading Hospital (LRH), and Hayatabad Medical Complex (HMC). These hospitals are among the largest tertiary referral centers in the province and provide comprehensive inpatient and outpatient healthcare services to patients from Peshawar and surrounding districts. The current study was designed to reveal the bacterial etiology of UTIs and evaluate the AST profiles of uropathogens isolated from catheter-associated and non-catheter-associated urine specimens.

Study Population

Patients of all age groups and both sexes presenting with clinical features suggestive of urinary tract infection were eligible for inclusion. Consecutive urine specimens submitted to the participating microbiology laboratories for routine diagnostic evaluation during the study period were included.

Specimen Collection and Transportation

A total of 1,886 urine specimens were collected during the study period. For non-catheterized patients, clean-catch midstream urine specimens were collected in sterile, wide-mouthed, leak-proof containers after providing standardized instructions regarding perineal hygiene to minimize contamination. Patients were instructed to discard the initial urine stream before collecting the midstream portion.

For catheterized patients, urine specimens were collected aseptically from the sampling port of the indwelling urinary catheter after disinfecting the port with 70% isopropyl alcohol. Urine was aspirated using sterile disposable syringes. Samples were never collected from catheter drainage bags to avoid contamination. All specimens were transported promptly to the microbiology laboratory and processed within two hours of collection. Specimen requiring delayed processing were refrigerated at 4°C and cultured within the recommended holding period.

Isolation and Identification of Uropathogens

Urine specimens were cultured using a calibrated 1-μL sterile inoculating loop onto 'Cystine Lactose Electrolyte Deficient (CLED)' agar, MacConkey agar, and 5% sheep blood agar. The inoculated plates were then incubated aerobically at $35 \pm 2^\circ\text{C}$ for 18–24 hours.

Following incubation, colony counts were determined, and significant bacteriuria was interpreted according to established clinical microbiology criteria. The mixed

bacterial growth indicative of specimen contamination was excluded from further analysis. Bacterial isolates revealing clinically significant growth were confirmed based on Gram staining characteristics, colony morphology, and conventional biochemical methods routinely used in diagnostic microbiology laboratories.

Antimicrobial Susceptibility Testing

The AST was done using the Kirby Bauer disk diffusion method on Mueller Hinton agar (MHA) according to the Clinical and Laboratory Standards Institute (CLSI-2022) (Yang et al., 2022). In brief, bacterial inocula were prepared from freshly isolated colonies and were evenly inoculated onto MHA plates using sterile cotton swabs, which were equivalent to a 0.5 McFarland turbidity standard.

Commercial antibiotic discs belonging to the main therapeutic classes employed for the treatment of UTIs were applied, such as β -lactams, β -lactam or β -lactamase inhibitor combinations, cephalosporins, carbapenems, fluoroquinolones, aminoglycosides, tetracyclines, folate pathway inhibitors, nitrofurantoin, fosfomycin, tigecycline, macrolides, and chloramphenicol. Plates were incubated aerobically at $35 \pm 3^\circ\text{C}$ for 16–18 hours and the diameters of the inhibition zones were measured to the nearest millimeter. Susceptibility results were interpreted as susceptible, intermediate, or resistant based on CLSI 2022 interpretive breakpoints. Antibiotics that are intrinsically resistant to certain organisms or not routinely tested were reported as intrinsically resistant (R) or not tested (NT), respectively.

Quality Assurance

Quality control was performed throughout the study as recommended by the CLSI. Standard operating procedures were used for culture media performance, incubation conditions, inoculum preparation and AST were periodically checked for accuracy, precision and reproducibility using reference quality-control strains, such as *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853.

Statistical Analysis

Data on clinical, demographic, microbiological and AST were recorded in Microsoft Excel and analyzed using IBM SPSS Statistics (Version 22). Categorical variables were displayed as frequencies and percentages.

Results

Demographic characteristics and distribution of study participants

A total of 1,886 urine samples of patients with clinically suspected UTIs were collected and processed in the microbiology laboratories of three tertiary care hospitals in Peshawar, Pakistan, during the 18-month study period. KTH contributed the largest proportion of specimens (924/1,886; 49.0%), followed by HMC (528/1,886; 28.0%) and LRH (434/1,886; 23.0%) (Table 1).

Of all specimens processed, 1,694 (89.8%) had clinically significant bacterial growth and 192 (10.2%) had mixed bacterial growth, indicative of contamination, and were not included in further microbiological analysis. Hospital-specific culture positivity was highest at KTH (846/924; 91.6%), followed by HMC (472/528; 89.4%) and LRH (376/434; 86.6%) (Table 1).

Of the study population, 1,137 (60.3%) were females and 749 (39.7%) were males, with a female to male ratio of 1.52:1. The female predominance was observed in all the participating hospitals with 53.8% at KTH, 67.4% at HMC and 65.4% at LRH (Table 1).

Table 1

Demographic characteristics and hospital-wise distribution of urine specimens (n = 1,886)

Variable	KTH n (%)	HMC n (%)	LRH n (%)	Total n (%)
Total specimens	924 (49.0)	528 (28.0)	434 (23.0)	1,886 (100)
Culture positive	846 (91.6)	472 (89.4)	376 (86.6)	1,694 (89.8)
Culture negative/contaminated	78 (8.4)	56 (10.6)	58 (13.4)	192 (10.2)
Male	427 (46.2)	172 (32.6)	150 (34.6)	749 (39.7)
Female	497 (53.8)	356 (67.4)	284 (65.4)	1,137 (60.3)

Age-stratified analysis demonstrated that individuals aged 21–40 years represented the largest proportion of the study population (826/1,886; 43.8%), followed by those aged 41–60 years (538; 28.5%), 61–80 years (234; 12.4%), 0–20

years (209; 11.0%), and >80 years (79; 4.2%). The age distribution across the participating hospitals is summarized in Table 2, while the overall demographic profile is illustrated in Figure 1.

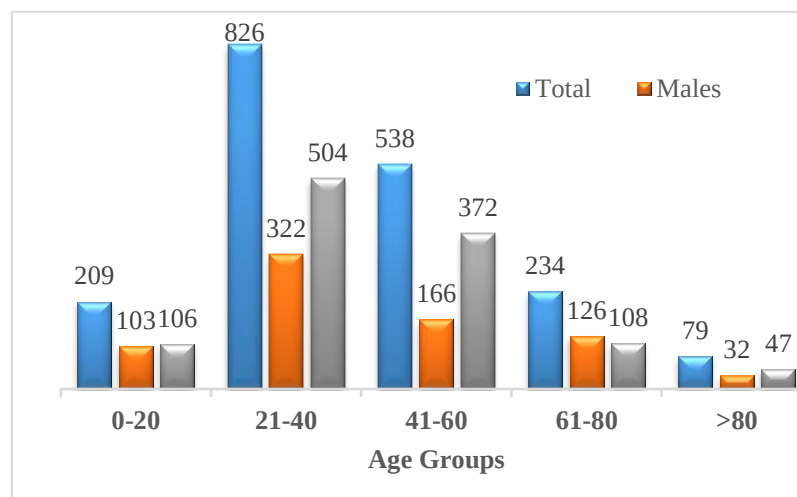
Table 2.

Age distribution of study participants according to hospital

Age group (years)	KTH	HMC	LRH	Total (%)
0–20	106	53	50	209 (11.0)
21–40	528	209	89	826 (43.8)
41–60	221	159	158	538 (28.5)
61–80	45	89	100	234 (12.4)
>80	24	18	37	79 (4.2)
Total	924	528	434	1,886 (100)

Figure 1

Age group distribution of UTI patients



Distribution of catheter-associated and non-catheter-associated urinary tract infections

Of the 1,886 urine specimens, 736 (39.0%) were obtained from patients with CAUTIs, whereas 1,150 (61.0%) were collected from patients with non-catheter-associated urinary tract infections (non-CAUTIs). KTH contributed the largest proportion of catheter-associated specimens (335/736; 45.5%), followed by HMC (231/736; 31.4%) and LRH (170/736; 23.1%). Likewise, KTH accounted for the

greatest number of non-catheter-associated specimens (589/1,150; 51.2%), followed by HMC (297/1,150; 25.8%) and LRH (264/1,150; 23.0%) (Table 3).

Among catheterized patients, 650 (88.3%) urine specimens yielded significant bacterial growth, whereas 1,044 (90.8%) culture-positive isolates were recovered from non-catheterized patients. The distribution of CAUTIs according to participating hospitals is presented in Table 3.

Table 3

Distribution of catheter-associated and non-catheter-associated urinary tract infections according to hospital

Hospital	CAUTI n (%)	Non-CAUTI n (%)	Total
KTH	335 (45.5)	589 (51.2)	924
HMC	231 (31.4)	297 (25.8)	528
LRH	170 (23.1)	264 (23.0)	434
Total	736 (39.0)	1,150 (61.0)	1,886

Distribution of bacterial uropathogens

A total of 1,694 bacterial isolates were obtained from culture-positive urine specimens. Gram-negative bacteria constitute the predominant etiological agents of UTI. *E. coli* was the most frequently isolated

pathogen (728 isolates; 43.0%), followed by *Citrobacter spp.* (264; 15.6%), *Morganella morganii* (256; 15.1%), *Pseudomonas spp.* (164; 9.7%), *Klebsiella pneumoniae* (158; 9.3%), *Providencia spp.* (78; 4.6%), and *Proteus mirabilis* (46; 2.7%) (Table 4).

Bacterial Isolate	Frequency	Percentage
<i>Escherichia coli</i>	728	43
' <i>Citrobacter spp.</i> '	264	15.6
' <i>Morganella morganii</i> '	256	15.1
<i>Pseudomonas spp.</i>	164	9.68
<i>Klebsiella pneumoniae</i>	158	9.33
<i>Providencia spp.</i>	78	4.6
<i>Proteus mirabilis</i> ,	46	2.72
Total	1694	100

Antimicrobial susceptibility profiles of Gram-negative uropathogens

The susceptibility patterns varied considerably among bacterial species and antimicrobial classes. Carbapenems remained the most active agents against the majority of Enterobacterales, whereas resistance to β -lactam or β -lactamase inhibitor combinations, cephalosporins, and fluoroquinolones was frequently observed as mentioned in table 4.

E. coli demonstrated excellent susceptibility to tigecycline (99.8%), nitrofurantoin (95.6%), fosfomycin (95.1%), amikacin (94.3%), meropenem (92.6%), imipenem (91.1%), and cefoperazone/sulbactam (88.5%). Moderate susceptibility was observed for

gentamicin (75.3%) and chloramphenicol (68.9%), whereas relatively poor activity was noted for third- and fourth-generation cephalosporins (ceftriaxone 33.5%, ceftazidime 43.2%, cefepime 49.6%), fluoroquinolones (ciprofloxacin 39.3% and levofloxacin 43.4%), doxycycline (44.2%), tetracycline (40.6%), and co-trimoxazole (34.3%).

Citrobacter spp. exhibited high susceptibility to tigecycline (99.4%), amikacin (92.7%), meropenem (93.2%), imipenem (83.6%), gentamicin (80.5%), and chloramphenicol (60.7%). Susceptibility to cephalosporins ranged from 35.9% to 54.7%, while ciprofloxacin susceptibility was 44.0%.

Morganella morganii remained highly susceptible to carbapenems (imipenem 100%, meropenem 97.5%) and amikacin (97.4%), with excellent activity also observed for cefoperazone/sulbactam (94.5%), piperacillin/tazobactam (95.3%), and fosfomycin (92.0%). However, reduced susceptibility was noted for fluoroquinolones (43.2–47.9%), cephalosporins (34.1–53.4%), doxycycline (58.2%), tetracycline (63.5%), and co-trimoxazole (29.6%).

Among *Pseudomonas spp.*, amikacin demonstrated the highest activity (90.5%), followed by cefotaxime (100%; limited testing), gentamicin (79.2%), meropenem (75.0%), and piperacillin/tazobactam (75.0%). Susceptibility to cefepime (52.8%), ceftazidime (50.0%), ciprofloxacin (45.5%), and levofloxacin (52.9%) remained relatively low.

Klebsiella pneumoniae exhibited substantial multidrug resistance. Tigecycline retained complete activity (100%), while susceptibility to fosfomycin (82.0%), nitrofurantoin (79.4%), amikacin (78.4%), piperacillin/tazobactam (76.9%), cefoperazone/sulbactam (74.3%), meropenem (71.1%), and imipenem (70.5%) remained comparatively preserved. Markedly reduced susceptibility was observed for ceftriaxone (34.7%), cefepime (29.7%), ceftazidime (26.9%), ciprofloxacin (54.3%), levofloxacin (54.3%), doxycycline (42.9%), tetracycline (43.4%), co-trimoxazole (15.3%), and co-amoxiclav (20.5%).

Providencia spp. demonstrated excellent susceptibility to carbapenems (imipenem 100%, meropenem 97.2%), amikacin (94.4%), cefoperazone/sulbactam (91.7%), cefepime (76.5%), doxycycline (77.8%), and gentamicin (70.0%). In contrast, poor susceptibility was observed for piperacillin/tazobactam (12.1%), ceftriaxone (48.1%), ciprofloxacin (58.3%), levofloxacin (53.3%), ceftazidime (65.5%), chloramphenicol (67.7%), and co-trimoxazole (37.9%).

Proteus mirabilis remained highly susceptible to carbapenems (imipenem 99.1%, meropenem 98.7%), amikacin (98.4%), cefoperazone/sulbactam (96.7%), gentamicin (94.2%), ceftazidime (87.6%), piperacillin/tazobactam (87.4%), nitrofurantoin (84.8%), cefepime (84.0%), chloramphenicol (79.5%), cefotaxime (79.7%), and doxycycline (71.9%). Lower susceptibility was observed for ciprofloxacin (33.4%), levofloxacin (56.8%), tetracycline (53.4%), ceftriaxone (61.1%), and co-amoxiclav (31.3%).

Collectively, carbapenems, amikacin, tigecycline, and cefoperazone/sulbactam exhibited the highest overall in vitro activity against urinary Gram-negative pathogens, whereas fluoroquinolones, co-trimoxazole, and third-generation cephalosporins demonstrated substantially reduced effectiveness across multiple bacterial species, highlighting the ongoing burden of antimicrobial resistance among uropathogens.

Table 4

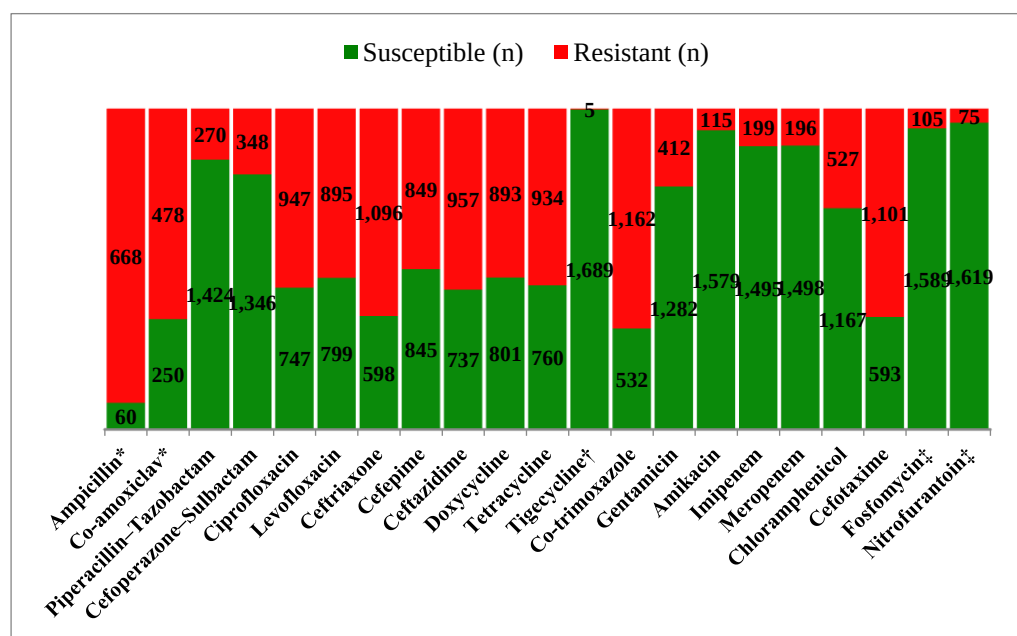
AST Profiles in (%) of Gram-negative bacteria isolated from urine specimens.

Organism (n)	Most active antibiotics (≥ 90% susceptible)	Moderately active antibiotics (70–89%)	Poor activity (<70%)
<i>E. coli</i> (728)	Tigecycline (99.8), Nitrofurantoin (95.6), Fosfomycin (95.1), Amikacin (94.3), Meropenem (92.6), Imipenem (91.1)	Cefoperazone/Sulbactam (88.5), Gentamicin (75.3)	Fluoroquinolones, cephalosporins, doxycycline, tetracycline, chloramphenicol, co-trimoxazole
<i>Citrobacter spp.</i> (264)	Tigecycline (99.4), Meropenem (93.2), Amikacin (92.7), Imipenem (100), Meropenem (97.5), Amikacin (97.4), Piperacillin/Tazobactam (95.3), Cefoperazone/Sulbactam (94.5), Fosfomycin (92.0)	Imipenem (83.6), Gentamicin (80.5)	Cephalosporins, ciprofloxacin, doxycycline, co-trimoxazole
<i>Morganella morganii</i> (256)		Chloramphenicol (72.2)	Fluoroquinolones, cephalosporins, doxycycline, tetracycline, co-trimoxazole
<i>Pseudomonas spp.</i> (164)	Amikacin (90.5), Cefotaxime (100*)	Gentamicin (79.2), Piperacillin/Tazobactam (75), Meropenem (75)	Fluoroquinolones, cefepime, ceftazidime, imipenem

Organism (n)	Most active antibiotics (≥ 90% susceptible)	Moderately active antibiotics (70–89%)	Poor activity (<70%)
<i>Klebsiella pneumoniae</i> (158)	Tigecycline (100)	Fosfomycin (82), Nitrofurantoin (79.4), Amikacin (78.4), Piperacillin/Tazobactam (76.9), Cefoperazone/Sulbactam (74.3), Meropenem (71.1), Imipenem (70.5)	Cephalosporins, fluoroquinolones, co-amoxiclav, co-trimoxazole, tetracycline, doxycycline
<i>Providencia</i> spp. (78)	Imipenem (100), Meropenem (97.2), Amikacin (94.4), Cefoperazone/Sulbactam (91.7)	Cefepime (76.5), Doxycycline (77.8), Gentamicin (70)	Piperacillin/Tazobactam, cephalosporins, fluoroquinolones, chloramphenicol, co-trimoxazole
<i>Proteus mirabilis</i> (46)	Imipenem (99.1), Meropenem (98.7), Amikacin (98.4), Cefoperazone/Sulbactam (96.7), Gentamicin (94.2)	Piperacillin/Tazobactam (87.4), Ceftazidime (87.6), Cefepime (84), Nitrofurantoin (84.8), Chloramphenicol (79.5), Cefotaxime (79.7), Doxycycline (71.9)	Fluoroquinolones, co-amoxiclav, ceftriaxone, tetracycline

Figure 2

Overall AST profile of Gram-negative bacteria collected from urine specimens (n = 1,694).



* Interpret with caution because several species are intrinsically resistant or were not routinely tested.

† Tigecycline was tested only against organisms for which CLSI/EUCAST interpretation is applicable.

‡ Fosfomycin and nitrofurantoin are recommended primarily for urinary tract isolates and should be interpreted accordingly.

Discussion

UTIs remain the most common bacterial infections affecting both community and hospitalized populations, accounting for a sizable proportion of antimicrobial prescriptions worldwide (Yang et al., 2022). The continued emergence and dissemination of antimicrobial-resistant uropathogens have increasingly compromised empirical treatment strategies, particularly in low and middle income countries where unrestricted antibiotic use, limited antimicrobial stewardship, and inadequate microbiological surveillance accelerate the evolution of resistance (Sulis et al., 2022). The present multicenter study provides comprehensive contemporary data on the epidemiology and AST patterns of bacterial pathogens isolated from catheter-associated and non-catheter-associated UTIs across three major tertiary care hospitals in Peshawar, Pakistan. Collectively, these findings contribute valuable regional evidence that may support evidence-based empirical therapy and strengthen institutional antimicrobial stewardship programs.

A major strength of this investigation is its multicenter design encompassing three tertiary referral hospitals that collectively serve a large and diverse patient population. Approximately half of all specimens collected from KTH, while HMC and LRH aided the remaining cases, providing broad representation of UTI managed within tertiary healthcare settings. Furthermore, the high overall culture positivity (89.8%) imitates the inclusion of patients with clinically suspected UTIs undergoing microbiological evaluation and demonstrates the considerable burden of culture-confirmed urinary tract infections within the study population. Even though this positivity rate is higher than that reported in many community-based studies, it is similar to reports from tertiary referral centers where patients regularly present with complicated infections, prior antimicrobial exposure, and various underlying comorbidities (Setiawan et al., 2022).

Consistent with the proven epidemiology of UTIs, females represented approximately 2/3 of the study population, with the highest disease burden occurring among patients aged 21- 40 years. These findings are in agreement with several other epidemiological studies demonstrating that women experience a substantially greater lifetime risk of UTI because of anatomical, physiological, and hormonal factors, including a shorter urethra, close-proximity of the urethral opening to the gastrointestinal tract, sexual activity, pregnancy, and recurrent periurethral colonization by enteric bacteria (Thompson et al., 2023). The dominance of young and

middle-aged adults further emphasizes the substantial socioeconomic impact of UTIs during the most productive years of life (Raghupathi, 2011).

CAUTI constituted approximately two-fifths of all the urine samples analyzed, confirming that indwelling urinary catheters remain an important risk factor for HAI. Catheterization promotes bacterial adherence, biofilm formation, and persistent colonization of catheter surfaces, thereby protecting bacteria from host immune defenses and reducing drug penetration. These facilitate chronic infection, increase the likelihood of multidrug resistance, and contribute to recurrent infections and prolonged hospitalization. Consequently, minimizing unnecessary catheterization and implementing catheter-care bundles remain essential components of infection prevention programs (Pelling et al., 2019).

The current study approves that Gram-negative bacteria continue to dominate the etiological spectrum of UTIs. *E. coli* accounted for 43% of all isolates and persisted the prime uropathogen across the participating hospitals. This observation is constant with global surveillance studies identifying UPEC as the main cause of both complicated and uncomplicated UTIs (Zhou et al., 2023). The notable ecological success of UPEC is qualified to an extensive repertoire of virulence determinants, including type I and P fimbriae, toxins, immune evasion mechanisms, iron-acquisition systems, and biofilm-forming capability, which mutually facilitate urinary tract colonization and persistence despite host-immune responses (Zhou et al., 2023; Maurizi, 2024).

Moreover, *E. coli*, the recovery of *K. pneumoniae*, *Citrobacter* spp., *Morganella morganii*, *Providencia* spp., *Pseudomonas* spp., and *Proteus mirabilis* highlights the increasing microbiological diversity of UTIs encountered in tertiary healthcare facilities. These pathogens are often associated with prolonged hospitalization, urinary catheterization, previous antimicrobial exposure, diabetes mellitus, immunosuppression, and other underlying medical conditions (Yang et al., 2020). Their recovery in comparatively high frequencies underscores the changing epidemiology of healthcare-associated UTIs and emphasizes the necessity for continuous microbiological surveillance within referral hospitals (Wagenlehner et al., 2012).

A major clinical implication of the current study is the high prevalence of resistance to several antibiotics that have been used as empirical treatment for UTI. Fluoroquinolones, third generation cephalosporins, aminopenicillins and co-trimoxazole showed poor

activity against multiple bacterial species, suggesting that these drugs may no longer be reliable empirical drugs in our study area. This is in line with recent international surveillance studies that have reported rising resistance to these classes of antimicrobials in Enterobacterales. This progressive decrease in susceptibility is probably due to the accumulation of selective pressure from the misuse of antimicrobials, self-medication, unrestricted access to antimicrobials, prolonged antimicrobial exposure, and poor antimicrobial stewardship programs (Dunne et al., 2022).

Carbapenems were the most active antimicrobial agent overall against Gram-negative uropathogens. Imipenem and meropenem maintained high in-vitro activity against most Enterobacterales and amikacin was also highly susceptible against nearly all bacterial species. These results are consistent with those of several international surveillance programs that have shown that carbapenems and aminoglycosides are among the most effective treatment choices for severe complicated UTI caused by drug resistant bacteria (Rando et al., 2022). However, the use of carbapenems should be used with caution as overuse could drive the emergence and spread of carbapenem-resistant Enterobacterales, which are now identified by the WHO as critical-priority pathogens in need of urgent action (Caliskan-Aydogan & Alocilja, 2023).

One positive finding of the current study was that nitrofurantoin and fosfomycin remained active against *E. coli*, the most common urinary pathogen found in this study. They have excellent susceptibility profiles, which make them suitable for current international guidelines for the treatment of lower urinary tract infection as first-line oral agents. This is likely due to relatively limited clinical use, different mechanisms of antimicrobial activity, and reduced selective pressure relative to fluoroquinolones and broad-spectrum cephalosporins. Maintaining the effectiveness of these agents by judicious prescribing practices will continue to be an important part of antimicrobial stewardship (Thangavelu et al., 2022).

Klebsiella pneumoniae, on the other hand, had the most alarming antimicrobial resistance data for all the bacterial species recovered during the study. A significant decrease in susceptibility to cephalosporins, co-amoxiclav, fluoroquinolones and co-trimoxazole indicates the presence of multidrug resistant strains in the tertiary healthcare settings. Molecular characterization was not performed in this study, but the phenotypic resistance profiles suggest ESBL

production and possibly other resistance mechanisms such as AmpC β -lactamases and carbapenemases in some of the isolates. Future studies with molecular characterization of antimicrobial resistance genes would significantly help to understand the epidemiology and transmission dynamics of resistant *K. pneumoniae* in Pakistan (Saravanan & Raveendaran, 2013; Garba et al., 2023).

The antimicrobial susceptibility profile of *Pseudomonas spp.* also exemplifies the therapeutic difficulties of HCAUTI. Moderate susceptibility to piperacillin-tazobactam and carbapenems, combined with decreased activity of fluoroquinolones and cephalosporins, is a reflection of the extraordinary intrinsic and acquired resistance mechanisms of this pathogen. The presence of multidrug efflux pumps, the production of chromosomal AmpC β -lactamase, porin modifications, horizontal gene transfer, and the formation of biofilms significantly reduce therapeutic options and require careful selection of antimicrobials based on susceptibility testing (Glen & Lamont, 2021).

The results of the present study are relevant for empirical antimicrobial therapy. The low efficacy of fluoroquinolones, third generation cephalosporins, aminopenicillins and co-trimoxazole suggests that the current empirical treatment guidelines in many health care facilities may need to be urgently reviewed considering the current local susceptibility data. On the other hand, the retained activity of the carbapenems, amikacin, nitrofurantoin, fosfomycin, tigecycline, and cefoperazone-sulbactam offers valuable therapeutic options for complicated and uncomplicated UTIs, and underscores the need to conserve these agents through comprehensive antimicrobial stewardship programs (Clancy & Nguyen, 2022).

The current study has several noteworthy strengths. The multicenter design, relatively large sample size, inclusion of both CAUTI and non-CAUTI, and detailed AST of the major Gram-negative uropathogens support the clinical relevance and generalizability of the results in the context of tertiary healthcare. Furthermore, the study offers one of the more comprehensive recent reports of urinary antimicrobial resistance patterns from northwestern Pakistan, adding valuable regional data to the literature on antimicrobial resistance surveillance. There are several limitations that need to be recognized. Species identification and antimicrobial susceptibility testing were mostly based on conventional phenotypic methods, with no molecular confirmation of bacterial identification or resistance determinants. Molecular

characterization of ESBL, carbapenemases, plasmid-mediated resistance mechanisms and virulence-associated genes were not performed, which restricted the understanding of the molecular basis of antimicrobial resistance. Furthermore, there was a lack of detailed clinical information, such as previous antimicrobial use, hospitalization status, comorbidities, and treatment outcomes, which prevented detailed risk-factor analyses. Lastly, the study was carried out in tertiary care hospitals only, so the results may not be entirely applicable to primary healthcare or community settings. Future studies should incorporate molecular epidemiology, whole genome sequencing, resistance and virulence profiling and clinical outcome data to further delineate the evolution and transmission of multidrug resistant uropathogens. Longitudinal multicenter surveillance, coupled with antimicrobial consumption data and stewardship interventions, will further strengthen evidence-based infection control and treatment strategies. Overall, the high incidence of AMR among Gram-negative uro-pathogens underscores the urgent need for constant AMR surveillance, routine institutional antibiograms, evidence-based empirical therapy, robust infection prevention practices, and comprehensive antimicrobial stewardship programs to optimize urinary tract infection management in Pakistan and other resource-limited setting

Conclusion

The current multicenter study revealed contemporary epidemiological insights into the distribution and antimicrobial susceptibility of uropathogens associated with catheter associated and non-catheter associated UTIs in tertiary care hospitals of Peshawar, Pakistan. *E. coli* was the predominant pathogen, followed by *Klebsiella pneumoniae*, *Citrobacter* spp., *Morganella morganii*, *Pseudomonas* spp., *Providencia* spp., and *Proteus mirabilis*. High levels of resistance were observed against commonly prescribed empirical agents, including fluoroquinolones, aminopenicillins, co-trimoxazole, and third-generation cephalosporins, highlighting the escalating burden of antimicrobial resistance in both healthcare- and community-associated UTIs. In contrast, carbapenems, amikacin, nitrofurantoin, fosfomycin, tigecycline, and cefoperazone-sulbactam retained high in vitro efficacy against most Gram-negative uropathogens. These findings underscore the need for continuous local AMR surveillance, routine institutional antibiogram development, evidence-based revision of empirical treatment guidelines, and strengthened antimicrobial stewardship and infection prevention strategies. Future multicenter studies incorporating molecular resistance profiling, whole-genome sequencing, and clinical outcome analyses are warranted to elucidate the evolving epidemiology of multidrug-resistant uropathogens and optimize therapeutic and public health interventions.

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