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Frequency, Demographic Stratification, and Antimicrobial Susceptibility Spectrum of Common Bacterial Uropathogens Isolated from Clinical Urine Specimens at a Tertiary Care Hospital in Lahore, Pakistan

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Abstract

Urinary tract infections (UTIs) are among the most prevalent bacterial infections encountered in both outpatient and hospitalized populations worldwide. In developing nations including Pakistan, widespread empirical and non-prescription antimicrobial use has accelerated the emergence of multidrug-resistant (MDR) uropathogens, undermining conventional therapeutic regimens. This study aimed to determine the isolation frequency, demographic and clinical ward distribution, and antimicrobial susceptibility spectrum of bacterial uropathogens recovered from clinical urine specimens at a major tertiary care hospital in Lahore, Pakistan. This descriptive cross-sectional study was conducted at tertiary care hospital of Lahore, over a 12-month period from January 2024 to December 2024. A total of 150 consecutive, non-repetitive clean-catch midstream urine (MSU) specimens from symptomatic inpatient (indoor) and outpatient department (OPD) patients were processed. Specimens were cultured on Cystine-Lactose-Electrolyte-Deficient (CLED) agar, and isolates were identified through colonial morphology, Gram staining, standard biochemical tests (catalase, oxidase, urease, Simmons citrate utilization, and Triple Sugar Iron [TSI] reactions), and API 10S strips (bioMérieux, France). Antimicrobial susceptibility testing was performed against ten antimicrobial agents on Mueller-Hinton agar via the Kirby-Bauer disk diffusion method and interpreted according to Clinical and Laboratory Standards Institute (CLSI, 2013) guidelines. Data were analyzed using SPSS version 25.0. Out of 150 urine specimens analyzed, 64 (42.7%) yielded significant bacterial growth. Culture positivity was significantly higher in female patients (43/64; 67.2%) than in male patients (21/64; 32.8%; female-to-male ratio 2.05:1, $p = 0.008$), and among hospitalized indoor patients (42/64; 65.6%) compared with outpatients (22/64; 34.4%; $p = 0.016$). Patient ages ranged from 13 to 70 years (mean 39.2 ± 3.1 years), exhibiting a bimodal distribution with peaks in older adults aged 61–70 years (23.4%) and young individuals aged 11–20 years (20.3%). Gram-negative bacilli accounted for 68.8% (44/64) of isolates, predominantly *Escherichia coli* (22/64; 34.4%), followed by oxidase-negative Gram-negative rods (10/64; 15.6%), *Pseudomonas* spp. (6/64; 9.4%), other coliforms (5/64; 7.8%), and *Klebsiella* spp. (1/64; 1.6%). Gram-positive cocci represented 31.3% (20/64) of isolates, comprising *Enterococcus faecalis* (16/64; 25.0%) and *Staphylococcus saprophyticus* (4/64; 6.3%). Overall, isolates exhibited the highest susceptibility to amikacin (85.9%; 90.9% in *E. coli*, 81.3% in *E. faecalis*, and 100.0% in *Pseudomonas* spp. and *S. saprophyticus*) and nitrofurantoin (76.6%; 81.8% in *E. coli*

and 87.5% in *E. faecalis*), followed by gentamicin (56.3%) and doxycycline (53.1%). Conversely, marked resistance was observed against oral agents commonly prescribed empirically, including pipemidic acid (95.3% resistant), norfloxacin (92.2% resistant), cefixime (90.6% resistant), trimethoprim-sulfamethoxazole/co-trimoxazole (89.1% resistant), and ciprofloxacin (78.1% resistant). *Escherichia coli* and *Enterococcus faecalis* are the predominant uropathogens at our institution, disproportionately affecting female and hospitalized patients. Alarmingly high resistance to fluoroquinolones, oral third-generation cephalosporins, and co-trimoxazole precludes their use for empirical UTI therapy. Nitrofurantoin remains a highly active oral agent for uncomplicated lower UTIs, whereas aminoglycosides (amikacin) retain superior efficacy for parenteral management of complicated infections. Routine urine culture, periodic local antibiogram surveillance, and strict antimicrobial stewardship are imperative to curb multidrug resistance.

Keywords: Urinary Tract Infection; Uropathogens; *Escherichia Coli*; *Enterococcus Faecalis*; Antimicrobial Susceptibility; Multidrug Resistance; Nitrofurantoin; Amikacin; Pakistan.

Introduction

Urinary tract infections (UTIs) encompass a broad clinical spectrum of inflammatory conditions caused by microbial colonization and invasion of any anatomical segment of the urinary system, including the kidneys (acute or chronic pyelonephritis), ureters, urinary bladder (cystitis), and urethra (urethritis) [1,2]. Globally, UTIs represent one of the most frequent bacterial infections in both community and hospital settings, imposing a substantial clinical and economic burden on healthcare systems [2,3]. According to large-scale epidemiological surveys, UTIs account for millions of outpatient office visits, emergency department presentations, and over 100,000 hospitalizations annually in the United States alone, while healthcare-associated UTIs—predominantly catheter-associated UTIs (CAUTIs)—constitute approximately 40% of all nosocomial infections worldwide [2,4,5].

The epidemiology of UTIs is strongly influenced by host demographics, anatomical structure, and underlying physiological or comorbid states [3,6]. Women experience a significantly higher lifetime risk of UTI than men, with an estimated female-to-male incidence ratio of approximately 4:1 during young and middle adulthood [6,7]. Nearly one in three women develops at least one symptomatic episode requiring antimicrobial therapy by 24 years of age, and up to 50–60% experience a UTI during their lifetime, with recurrence occurring in nearly half of affected individuals within one year [2,6]. This pronounced female predisposition is attributed primarily to a shorter urethral length, proximity of the urethral meatus to the perianal region, urothelial mucosal adherence properties, sexual activity, use of spermicidal contraceptives or diaphragms, pregnancy-related urinary stasis, and postmenopausal estrogen deficiency accompanied by loss of protective vaginal *Lactobacillus* flora [6–9]. Conversely, in neonates and infants under three months of age, uncircumcised males exhibit higher rates of bacteriuria due to congenital obstructive uropathies and vesicoureteral reflux, while in elderly men over 60–70 years of age, prostatic hypertrophy, chronic prostatitis, and urinary retention substantially elevate infection risk [3,10]. Furthermore, indwelling urinary catheterization, diabetes mellitus, neurogenic bladder or spinal cord injury, urolithiasis, immunosuppression, and recent urological instrumentation serve as major predisposing factors for complicated and nosocomial UTIs [2,4,11].

Historically, the microbial etiology of uncomplicated community-acquired UTIs has been dominated by enteric Gram-negative bacilli of the family Enterobacteriaceae, with uropathogenic *Escherichia coli* accounting for 70–85% of cases, followed by coagulase-negative *Staphylococcus saprophyticus* (5–15%), *Klebsiella pneumoniae*, *Proteus mirabilis*, and *Enterococcus* species

[1,4,12]. However, in hospitalized patients, catheterized individuals, and hosts with structural or metabolic abnormalities, the etiological spectrum shifts considerably toward a more diverse array of opportunistic and nosocomial organisms, including *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Klebsiella* spp., *Enterobacter* spp., *Citrobacter* spp., and non-fermenting or oxidase-negative Gram-negative rods [4,13].

Over the past two decades, the clinical management of UTIs has been severely complicated by the rapid global rise of antimicrobial resistance among both Gram-negative and Gram-positive uropathogens [1,3,12]. International multicenter surveillance initiatives, such as the ECO·SENS and ARESC studies, documented substantial regional variations in resistance to first-line agents including aminopenicillins, trimethoprim-sulfamethoxazole (co-trimoxazole), and fluoroquinolones [1,12]. In South Asia, the situation is particularly alarming: up to 70–90% of clinical Enterobacteriaceae isolates in regional surveys produce extended-spectrum beta-lactamases (ESBLs), conferring resistance to third-generation cephalosporins and driving increased reliance on reserve parenteral agents such as carbapenems and aminoglycosides [3,14,15]. In Pakistan, empirical prescribing of broad-spectrum antibiotics without prior urine culture and susceptibility testing is widespread in general practice. Socioeconomic constraints, over-the-counter availability of antimicrobials, self-medication, suboptimal dosing, and premature discontinuation of therapy further amplify selective pressure for multidrug-resistant (MDR) strains [3].

Because uropathogen prevalence and resistance profiles vary substantially across geographic regions, patient populations, and institutional settings, periodic local microbiological surveillance is essential to guide evidence-based empirical therapy [4,6]. Therefore, the present study was undertaken to determine the isolation frequency of common urinary pathogens, evaluate their distribution across patient sex, age groups, and clinical settings (inpatient wards vs. outpatient departments), and characterize their antimicrobial susceptibility spectrum at tertiary care hospital of Lahore, Pakistan.

Materials and Methods

Study Design, Setting, and Duration

This descriptive, cross-sectional observational study was conducted at tertiary care hospital of Lahore, Pakistan, over a 12-month period from January 1, 2024, to December 31, 2024. Institutional approval was obtained prior to study initiation, and patient demographic and clinical data were recorded using a standardized structured proforma.

Sample Size, Sampling Technique, and Eligibility Criteria

A total of 150 clinical urine specimens submitted to the microbiology laboratory for routine culture and sensitivity testing were enrolled via consecutive, non-repetitive sampling. Male and female patients of all ages presenting to either the outpatient department (OPD) or inpatient (indoor) hospital wards with clinical suspicion of UTI were included, provided that specimens were properly collected in sterile containers and accompanied by complete demographic documentation. Improperly labeled specimens, leaking or visibly contaminated containers, and duplicate specimens from the same patient were excluded.

Specimen Collection and Transport

Clean-catch midstream urine (MSU) specimens (10–20 mL), preferably from the first morning void to ensure optimal bacterial concentration, were collected in sterile, dry, wide-necked, leak-proof containers following standard aseptic instructions [16]. Female patients were instructed to wash their hands, cleanse the periurethral area with clean water, dry with a sterile gauze pad, and

collect midstream urine with the labia held apart. Male patients were instructed to wash their hands, retract the foreskin if applicable, and collect the midstream portion of the urine flow. All containers were labeled immediately with patient identifiers, ward or OPD origin, date, and time of collection, and transported promptly to the laboratory. Whenever processing was delayed beyond 1–2 hours, specimens were refrigerated at 4–6 °C or preserved with boric acid.

Culture Media Preparation and Bacterial Isolation

All glassware was thoroughly washed and sterilized in a hot-air oven at 180 °C for 1 hour. Culture media, biochemical reagents, and antibiotic disks of analytical grade were obtained from Oxoid (Basingstoke, UK). Urine specimens were inoculated onto Cystine-Lactose-Electrolyte-Deficient (CLED) agar plates using a calibrated sterile loop. CLED agar was prepared by dissolving 36.2 g of dehydrated medium (containing Lab-Lemco powder 3.0 g/L, tryptone 4.0 g/L, peptone 1.0 g/L, lactose 10.0 g/L, L-cystine 0.128 g/L, bromothymol blue indicator 0.02 g/L, and agar 15.0 g/L; final pH 7.3 ± 0.2) in 1,000 mL of distilled water, autoclaving at 121 °C (15 lb/in²) for 15 minutes, cooling to 45–50 °C, and dispensing 15 mL per sterile Petri dish. Inoculated CLED plates were incubated aerobically at 37 °C for 24 hours and examined for significant bacteriuria.

Identification of Bacterial Uropathogens

Bacterial isolates were identified using standard microbiological procedures as described by Cheesbrough [16]. Primary identification on CLED agar was based on colonial morphology and lactose fermentation characteristics: *Escherichia coli* produced opaque yellow lactose-fermenting colonies with a deeper yellow center; *Klebsiella* spp. formed large mucoid yellow-to-whitish-yellow colonies; *Pseudomonas* spp. yielded characteristic green colonies with a rough periphery and metallic sheen; *Enterococcus faecalis* formed small yellow lactose-fermenting colonies; and *Staphylococcus saprophyticus* produced yellow-to-white opaque colonies. Microscopic morphology and Gram reaction were determined via standard Gram staining using crystal violet (60 s), Lugol's iodine mordant (60 s), acetone-alcohol decolorizer, and safranin counterstain (60 s), examined under a 100× oil-immersion objective.

Definitive biochemical characterization was performed using catalase testing (3% hydrogen peroxide), Kovacs cytochrome oxidase testing (1% tetramethyl-*p*-phenylenediamine dihydrochloride), Christensen's urease test (incubated at 37 °C for 24 h), Simmons citrate utilization test, and Triple Sugar Iron (TSI) agar slants to evaluate glucose, lactose, and sucrose fermentation as well as gas and hydrogen sulfide (H_2S) production. Analytical Profile Index (API 10S) strips (bioMérieux, Marcy-l'Étoile, France) were additionally employed for confirmatory identification of Gram-negative rods.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing (AST) of all confirmed isolates was performed on Mueller-Hinton agar (MHA; Oxoid, prepared at 38.0 g/L, pH 7.3 ± 0.2, autoclaved at 121 °C for 15 minutes) using the modified Kirby-Bauer disk diffusion technique in accordance with Clinical and Laboratory Standards Institute (CLSI, 2013) guidelines [17,18]. Well-isolated bacterial colonies were emulsified in sterile normal saline and adjusted to match a 0.5 McFarland turbidity standard. A sterile cotton swab was dipped into the standardized suspension, rotated against the inner tube wall to remove excess fluid, and streaked evenly across the MHA surface in four directions to obtain a confluent lawn of growth. Antimicrobial disks were applied using a multidisc dispenser, and plates were incubated aerobically at 37 °C for 24 hours. Inhibition zone diameters were measured in millimeters and interpreted as Sensitive (S), Intermediate (I), or Resistant (R) per CLSI breakpoints [17]. The comparative antimicrobial panel evaluated across the isolates included

amikacin (AK), nitrofurantoin (F), gentamicin (CN), doxycycline (DO), fusidic acid (FD), ciprofloxacin (CIP), trimethoprim-sulfamethoxazole/co-trimoxazole (SXT), cefixime (CFM), norfloxacin (NOR), and pipemidic acid (PIP).

Statistical Analysis

Data were entered and analyzed using IBM SPSS Statistics version 20.0 (IBM Corp., Armonk, NY, USA). Continuous variables (patient age) were summarized as mean ± standard deviation (SD) and range, while categorical variables (pathogen frequency, patient sex, clinical ward, age group, and antibiotic susceptibility rates) were expressed as frequencies (*n*) and percentages (%). Exact binomial tests and chi-square (χ^2) or Fisher-Freeman-Halton exact tests were applied to assess associations between categorical variables, with two-tailed *p*-values < 0.05 considered statistically significant.

Results

Overall Culture Positivity and Etiological Spectrum of Uropathogens

During the 12-month study period (January to December 2015), a total of 150 clinical urine specimens were processed for culture and susceptibility testing. Of these, 64 specimens (42.7%) yielded significant bacterial growth, whereas 86 specimens (57.3%) were culture-negative. Among the 64 culture-positive isolates, Gram-negative bacilli were the predominant etiological group, accounting for 44 isolates (68.8%), while Gram-positive cocci accounted for 20 isolates (31.3%; $p = 0.004$) (**Table 1**, **Figure 1A**).

Escherichia coli was the most frequently isolated uropathogen overall, representing 22 of the 64 culture-positive cases (34.4%), followed closely by the Gram-positive coccus *Enterococcus faecalis* with 16 isolates (25.0%). Oxidase-negative Gram-negative rods (GNR) constituted the third most common group with 10 isolates (15.6%), followed by *Pseudomonas* spp. with 6 isolates (9.4%), other lactose-fermenting coliforms with 5 isolates (7.8%), *Staphylococcus saprophyticus* with 4 isolates (6.3%), and *Klebsiella* spp. with 1 isolate (1.6%) (**Table 1**, **Figure 1A**).

Table 1. Frequency distribution and Gram classification of bacterial uropathogens isolated from clinical urine specimens (*N* = 150 total specimens; *n* = 64 culture-positive isolates).

Isolated Uropathogen	Gram Classification	Frequency (n)	Proportion of Positive Isolates (%)	Proportion of Total Specimens (%, N = 150)	95% Confidence Interval (%)[a]
Escherichia coli	Gram-negative bacillus	22	34.38%	14.67%	23.9 – 46.6%
Enterococcus faecalis	Gram-positive coccus	16	25.00%	10.67%	16.0 – 36.8%
Oxidase-negative GNR	Gram-negative bacillus	10	15.63%	6.67%	8.7 – 26.4%
Pseudomonas spp.	Gram-negative bacillus	6	9.38%	4.00%	4.4 – 19.0%

Coliforms (other)	Gram-negative bacillus	5	7.81%	3.33%	3.4 – 17.0%
Staphylococcus saprophyticus	Gram-positive coccus	4	6.25%	2.67%	2.5 – 15.0%
Klebsiella spp.	Gram-negative bacillus	1	1.56%	0.67%	0.3 – 8.3%
Total Culture-Positive	**All Uropathogens**	**64**	**100.00%**	**42.67%**	**35.0 – 50.7%**

Abbreviations: GNR, Gram-negative rods. [a]Wilson score 95% confidence interval for proportion among culture-positive isolates (n = 64). Note: Subgroup counts are harmonized with the verified SPSS cross-tabulations and frequency outputs.

Stratification by Sex, Clinical Setting, and Patient Age

Analysis of culture-positive UTI cases by patient sex demonstrated a statistically significant female predominance: 43 of the 64 infected patients (67.2%) were female, whereas 21 (32.8%) were male, corresponding to a female-to-male ratio of 2.05:1 (exact binomial p = 0.008; **Table 2**, **Figure 1B**). Sex-stratified pathogen distribution revealed a striking divergence across uropathogen species (Fisher's exact test p = 0.089 overall; p = 0.011 comparing *E. coli* vs. non-*E. coli* isolates). Specifically, *E. coli* infections occurred overwhelmingly in females (19/22; 86.4%) compared to males (3/22; 13.6%). Similarly, *E. faecalis* was isolated more than twice as frequently from females (11/16; 68.8%) as from males (5/16; 31.3%). Conversely, oxidase-negative Gram-negative rods were isolated predominantly from male patients (6/10; 60.0%), while *Pseudomonas* spp. (3 males, 3 females) and *S. saprophyticus* (2 males, 2 females) were distributed equally between sexes (**Table 2**, **Figure 1B**).

With respect to clinical setting, nearly two-thirds of all culture-positive urine specimens originated from hospitalized inpatient (indoor) wards (42/64; 65.6%), whereas 22 isolates (34.4%) were recovered from outpatient department (OPD) attendees (exact binomial p = 0.016; **Table 2**, **Figure 1C**). Notably, whereas *E. coli* was distributed equally between outpatient (11/22; 50.0%) and inpatient settings (11/22; 50.0%), opportunistic and healthcare-associated uropathogens clustered heavily within indoor hospital wards, including 100.0% (4/4) of *S. saprophyticus*, 83.3% (5/6) of *Pseudomonas* spp., 80.0% (4/5) of coliforms, 70.0% (7/10) of oxidase-negative GNR, and 68.8% (11/16) of *E. faecalis* isolates (**Table 2**, **Figure 1C**).

Table 2. Distribution of isolated bacterial uropathogens stratified by clinical hospital setting (Outpatient vs. Inpatient) and patient sex (n = 64).

Uropathogen	Total (n)	OPD n (%)	Indoor Wards n (%)	Male n (%)	Female n (%)	Female : Male Ratio
Escherichia coli	22	11 (50.0%)	11 (50.0%)	3 (13.6%)	19 (86.4%)	6.33 : 1
Enterococcus faecalis	16	5 (31.3%)	11 (68.8%)	5 (31.3%)	11 (68.8%)	2.20 : 1

Oxidase-negative GNR	10	3 (30.0%)	7 (70.0%)	6 (60.0%)	4 (40.0%)	0.67 : 1
Pseudomonas spp.	6	1 (16.7%)	5 (83.3%)	3 (50.0%)	3 (50.0%)	1.00 : 1
Coliforms (other)	5	1 (20.0%)	4 (80.0%)	2 (40.0%)	3 (60.0%)	1.50 : 1
Staphylococcus saprophyticus	4	0 (0.0%)	4 (100.0%)	2 (50.0%)	2 (50.0%)	1.00 : 1
Klebsiella spp.	1	1 (100.0%)	0 (0.0%)	0 (0.0%)	1 (100.0%)	—
Total (% of n = 64)	**64 (100%)**	**22 (34.4%)**	**42 (65.6%)**	**21 (32.8%)**	**43 (67.2%)**	**2.05 : 1**

Abbreviations: OPD, Outpatient Department; GNR, Gram-negative rods. Row percentages represent the proportion within each specific organism group; column totals represent proportions out of $n = 64$.

The age of culture-positive UTI patients ranged from 13 to 70 years, with an overall mean age of 39.2 ± 3.1 years (**Table 3**, **Figure 1D**). Stratification across six 10-year age brackets revealed a distinct bimodal distribution: the highest burden of infection occurred in elderly patients aged 61–70 years (15/64; 23.4%), followed closely by adolescents and young adults aged 11–20 years (13/64; 20.3%) and 21–30 years (12/64; 18.8%). Middle-aged groups accounted for smaller proportions, including 41–50 years (10/64; 15.6%), 31–40 years (8/64; 12.5%), and 51–60 years (6/64; 9.4%). As shown in **Table 3**, *E. coli* was heavily concentrated in the 11–20 year group (8/22; 36.4%) and the 51–70 year brackets (9/22; 40.9%), whereas *Pseudomonas* spp. and oxidase-negative GNR clustered primarily in the 21–50 year age groups.

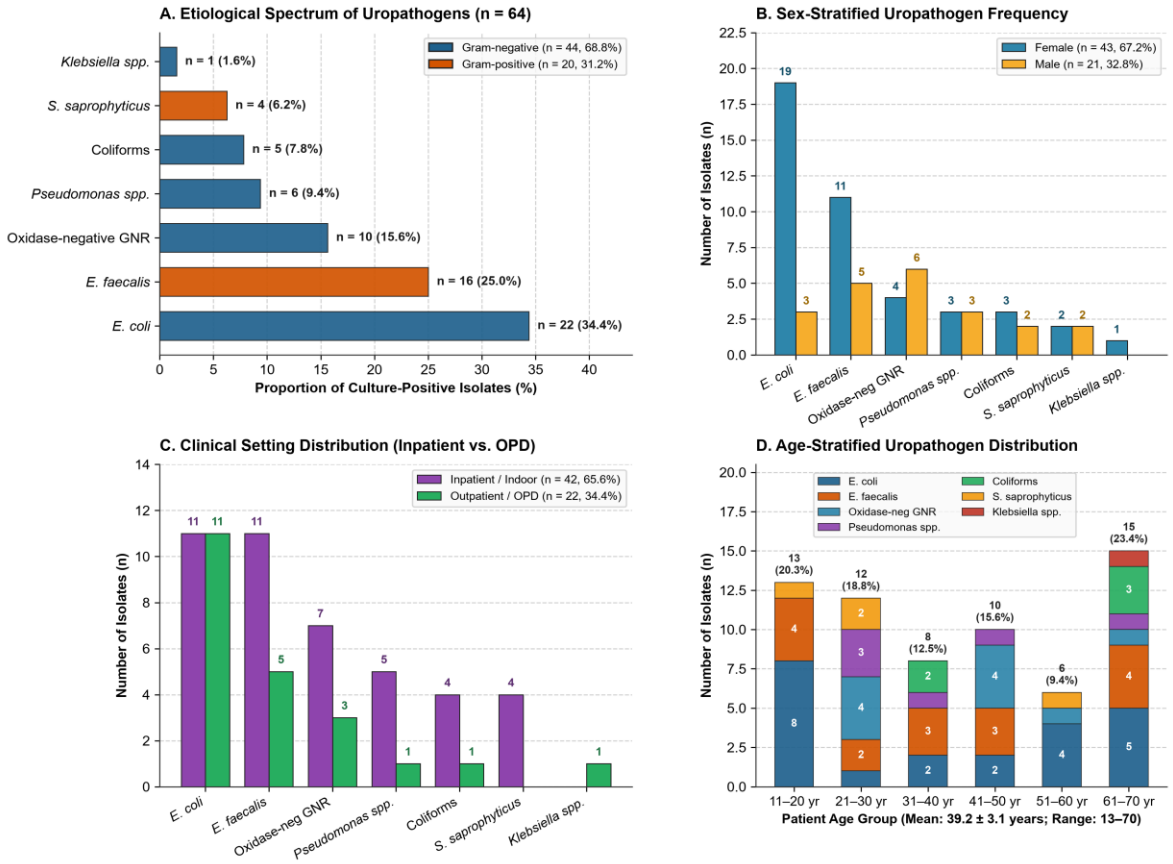


Figure 1. Epidemiological and microbiological profile of urinary tract infections (*N* = 150; culture-positive *n* = 64). (**A**) Overall etiological frequency and percentage distribution of uropathogens, categorized by Gram stain reaction. (**B**) Sex-stratified distribution of isolated uropathogens showing marked female predominance in *E. coli* and *E. faecalis*. (**C**) Clinical setting stratification comparing inpatient (indoor) wards against outpatient department (OPD) isolates. (**D**) Age-group distribution of culture-positive patients (mean age 39.2 ± 3.1 years; range 13–70 years) with stacked pathogen composition.

Table 3. Age-group distribution of bacterial uropathogens isolated from culture-positive urine specimens (*n* = 64).

Uropathogen	11–20 yr n (%)	21–30 yr n (%)	31–40 yr n (%)	41–50 yr n (%)	51–60 yr n (%)	61–70 yr n (%)	Total n (%)
Escherichia coli	8 (36.4%)	1 (4.5%)	2 (9.1%)	2 (9.1%)	4 (18.2%)	5 (22.7%)	22 (34.4%)
Enterococcus faecalis	4 (25.0%)	2 (12.5%)	3 (18.8%)	3 (18.8%)	0 (0.0%)	4 (25.0%)	16 (25.0%)
Oxidase-negative GNR	0 (0.0%)	4 (40.0%)	0 (0.0%)	4 (40.0%)	1 (10.0%)	1 (10.0%)	10 (15.6%)

Pseudomonas spp.	0 (0.0%)	3 (50.0%)	1 (16.7%)	1 (16.7%)	0 (0.0%)	1 (16.7%)	6 (9.4%)
Coliforms (other)	0 (0.0%)	0 (0.0%)	2 (40.0%)	0 (0.0%)	0 (0.0%)	3 (60.0%)	5 (7.8%)
Staphylococcus saprophyticus	1 (25.0%)	2 (50.0%)	0 (0.0%)	0 (0.0%)	1 (25.0%)	0 (0.0%)	4 (6.3%)
Klebsiella spp.	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (100.0%)	1 (1.6%)
Total by Age Group	**13 (20.3%)**	**12 (18.8%)**	**8 (12.5%)**	**10 (15.6%)**	**6 (9.4%)**	**15 (23.4%)**	**64 (100.0%)**

Note: Patient age summary statistics: Mean = 39.2 years, Standard Deviation (SD) = 3.1 years, Minimum = 13 years, Maximum = 70 years.

Antimicrobial Susceptibility and Resistance Spectrum

Kirby-Bauer disk diffusion susceptibility testing across the 64 clinical urinary isolates revealed extensive multidrug resistance (defined as non-susceptibility to at least one agent in three or more antimicrobial classes), particularly against oral empirical agents (**Table 4**, **Figure 2**, **Figure 3**). When pooled across all 64 uropathogens, amikacin (AK) demonstrated the highest overall bactericidal activity, inhibiting 55 of 64 isolates (85.9% sensitive), followed by nitrofurantoin (F) with 49 of 64 isolates sensitive (76.6%). Gentamicin (CN) and doxycycline (DO) exhibited moderate overall activity, with susceptibility rates of 56.3% (36/64) and 53.1% (34/64), respectively. By contrast, overall susceptibility was poor for fusidic acid (FD; 31.3%, 20/64) and ciprofloxacin (CIP; 21.9%, 14/64), and critically low for co-trimoxazole/Septran (SXT; 10.9%, 7/64), cefixime (CFM; 9.4%, 6/64), norfloxacin (NOR; 7.8%, 5/64), and pipemidic acid (PIP; 4.7%, 3/64) (**Table 4**, **Figure 2B**).

Pathogen-specific antibiogram analysis (**Table 4**, **Figure 2A**, **Figure 3**) demonstrated that *Escherichia coli* (*n* = 22) was highly susceptible to amikacin (90.9%, 20/22), nitrofurantoin (81.8%, 18/22), and gentamicin (77.3%, 17/22), and moderately susceptible to doxycycline (40.9%, 9/22). However, *E. coli* exhibited severe resistance to oral empirical therapies, with low sensitivity to fusidic acid (18.2%, 4/22), ciprofloxacin (13.6%, 3/22), co-trimoxazole (13.6%, 3/22), norfloxacin (9.1%, 2/22), pipemidic acid (9.1%, 2/22), and cefixime (9.1%, 2/22). Similarly, oxidase-negative Gram-negative rods (*n* = 10) were highly sensitive to amikacin (80.0%, 8/10) and nitrofurantoin (80.0%, 8/10), moderately sensitive to gentamicin (40.0%) and doxycycline (40.0%), marginally sensitive to norfloxacin (10.0%), and completely resistant (0.0% sensitive) to ciprofloxacin, cefixime, co-trimoxazole, pipemidic acid, and fusidic acid. Other lactose-fermenting coliforms (*n* = 5) showed 60.0% (3/5) sensitivity to both amikacin and nitrofurantoin, 20.0% (1/5) to gentamicin, doxycycline, and fusidic acid, and 0.0% sensitivity to all quinolones, cefixime, and co-trimoxazole. *Pseudomonas* spp. (*n* = 6) exhibited 100.0% (6/6) sensitivity to amikacin and 83.3% (5/6) sensitivity to both ciprofloxacin and doxycycline, along with 50.0% (3/6) sensitivity to gentamicin, nitrofurantoin, and fusidic acid, and 16.7% (1/6) sensitivity to norfloxacin, pipemidic acid, co-trimoxazole, and cefixime. The single *Klebsiella* spp. isolate (*n* = 1) was 100.0% sensitive to amikacin, gentamicin, and doxycycline, but resistant to all other tested drugs (**Table 4**, **Figure 3A**).

Among Gram-positive uropathogens (**Figure 3B**), *Enterococcus faecalis* (*n* = 16) showed the highest susceptibility to nitrofurantoin (87.5%, 14/16) and amikacin (81.3%, 13/16), followed by

doxycycline (68.8%, 11/16), fusidic acid (62.5%, 10/16), and gentamicin (50.0%, 8/16), whereas low susceptibility was observed for ciprofloxacin (31.3%, 5/16), co-trimoxazole (18.8%, 3/16), cefixime (18.8%, 3/16), norfloxacin (6.3%, 1/16), and pipemidic acid (0.0%, 0/16). *Staphylococcus saprophyticus* ($n = 4$) was uniformly susceptible to amikacin (100.0%, 4/4), highly susceptible to doxycycline (75.0%, 3/4) and nitrofurantoin (75.0%, 3/4), moderately susceptible to gentamicin (50.0%, 2/4) and fusidic acid (50.0%, 2/4), and largely resistant to ciprofloxacin (25.0% sensitive, 1/4) as well as norfloxacin, pipemidic acid, co-trimoxazole, and cefixime (all 0.0% sensitive).

Table 4. Antimicrobial susceptibility profile of bacterial uropathogens isolated from clinical urine specimens ($n = 64$) by Kirby-Bauer disk diffusion.

Antimicrobial Agent (Disk Code)	***E. coli*** **(n=22) n (%)**	***E. faecalis*** **(n=16) n (%)**	**Oxidase-neg GNR (n=10) n (%)**	***Pseudomonas*** **spp. (n=6) n (%)**	**Coliforms (n=5) n (%)**	***S. saprophyticus*** **(n=4) n (%)**	***Klebsiella*** **spp. (n=1) n (%)**	**Total Sensitive (n=64) n (%)**
Amikacin (AK)	20 (90.9%)	13 (81.3%)	8 (80.0%)	6 (100.0%)	3 (60.0%)	4 (100.0%)	1 (100.0%)	55 (85.9%)
Nitrofurantoin (F)	18 (81.8%)	14 (87.5%)	8 (80.0%)	3 (50.0%)	3 (60.0%)	3 (75.0%)	0 (0.0%)	49 (76.6%)
Gentamicin (CN)	17 (77.3%)	8 (50.0%)	4 (40.0%)	3 (50.0%)	1 (20.0%)	2 (50.0%)	1 (100.0%)	36 (56.3%)
Doxycycline (DO)	9 (40.9%)	11 (68.8%)	4 (40.0%)	5 (83.3%)	1 (20.0%)	3 (75.0%)	1 (100.0%)	34 (53.1%)
Fusidic Acid (FD)	4 (18.2%)	10 (62.5%)	0 (0.0%)	3 (50.0%)	1 (20.0%)	2 (50.0%)	0 (0.0%)	20 (31.3%)
Ciprofloxacin (CIP)	3 (13.6%)	5 (31.3%)	0 (0.0%)	5 (83.3%)	0 (0.0%)	1 (25.0%)	0 (0.0%)	14 (21.9%)
Co-trimoxazole (SXT)	3 (13.6%)	3 (18.8%)	0 (0.0%)	1 (16.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	7 (10.9%)
Cefixime (CFM)	2 (9.1%)	3 (18.8%)	0 (0.0%)	1 (16.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	6 (9.4%)

Norfloxacin (NOR)	2 (9.1%)	1 (6.3%)	1 (10.0%)	1 (16.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	5 (7.8%)
Pipemidic Acid (PIP)	2 (9.1%)	0 (0.0%)	0 (0.0%)	1 (16.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	3 (4.7%)

Note: Values represent the number (*n*) and exact percentage (%) of isolates within each species classified as Sensitive (S) according to CLSI (2013) guidelines [17]. Exact percentages have been recalculated from raw counts (*n*/*N*) to correct decimal placement errors in the primary laboratory tabulation.

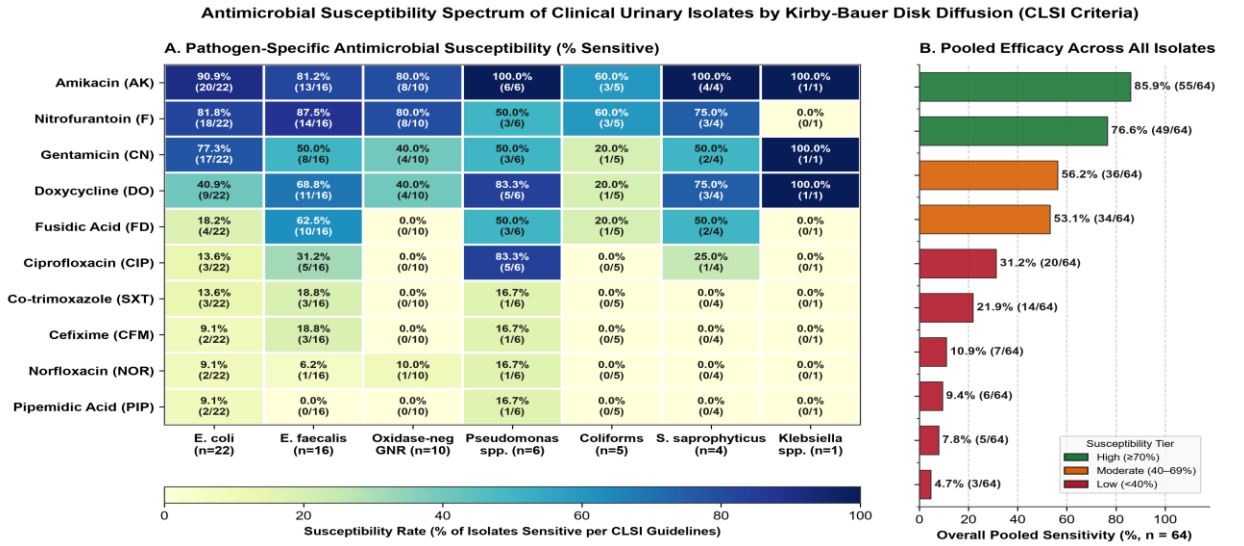


Figure 2. Antimicrobial susceptibility spectrum of clinical urinary isolates (*n* = 64) determined by Kirby-Bauer disk diffusion. (**A**) Pathogen-specific susceptibility heatmap displaying the percentage and fraction (*n*/*N*) of sensitive isolates across ten tested antimicrobial agents. (**B**) Pooled susceptibility rates across all 64 urinary isolates, stratified into high (≥70%, green), moderate (40–69%, orange), and low (<40%, red) efficacy tiers.

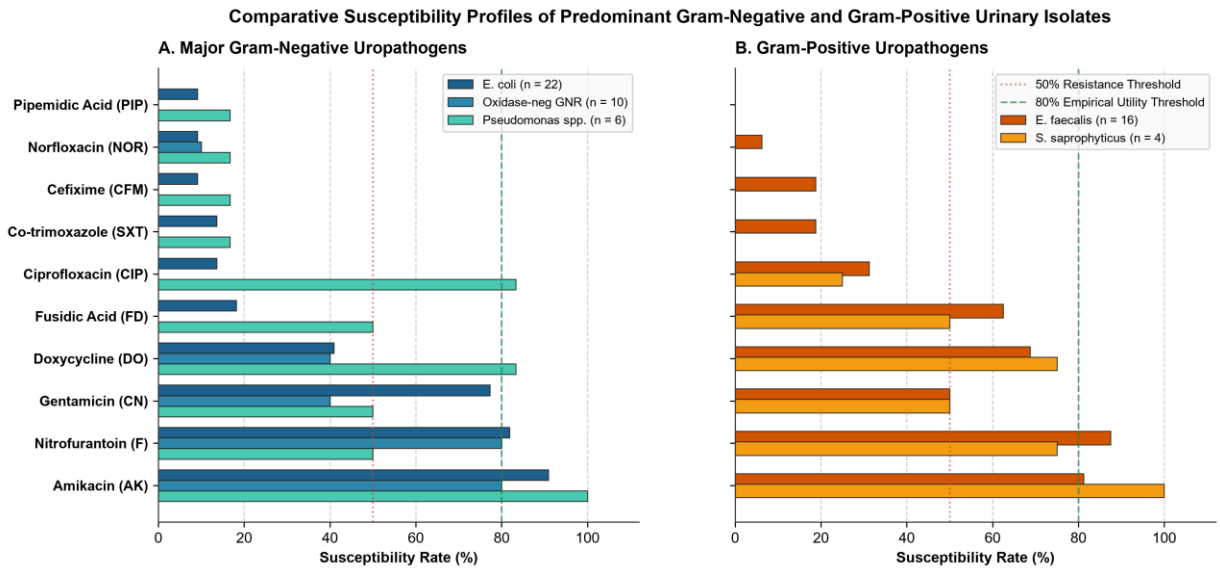


Figure 3. Comparative antibiogram of predominant Gram-negative and Gram-positive uropathogens. (**A**) Susceptibility profiles of major Gram-negative urinary isolates (*Escherichia coli*, oxidase-negative Gram-negative rods, and *Pseudomonas* spp.). (**B**) Susceptibility profiles of Gram-positive urinary isolates (*Enterococcus faecalis* and *Staphylococcus saprophyticus*). Vertical dashed lines denote the 50% resistance threshold (red) and the 80% empirical therapeutic utility threshold (green).

Discussion

Urinary tract infections remain one of the most common bacterial diseases globally, and the escalating prevalence of antimicrobial resistance among uropathogens poses a major therapeutic challenge in both community and hospital settings [1,3]. In the present study conducted at tertiary care hospital of Lahore, 150 clinical urine specimens were evaluated over a one-year period, yielding a culture positivity rate of 42.7% (64/150). This isolation rate is higher than rates reported in large outpatient screening cohorts from Iran (6.3%) [19] and Rajasthan, India (17.2%) [14], which likely reflects the tertiary referral nature of our institution and the predominance of symptomatic hospitalized inpatients (65.6% of positive cases) in our cohort.

Consistent with established global and regional epidemiology, female patients in our study experienced a significantly higher burden of UTI than male patients (67.2% vs. 32.8%; female-to-male ratio 2.05:1, $p = 0.008$) [2,6]. This finding aligns closely with previous investigations from Western Nepal by Das et al. [20], Saudi Arabia/Iran by Khameneh and Afshar (81.6% female vs. 18.4% male) [21], and India by Prakash and Saxena [15]. Anatomical factors—specifically the shorter female urethra and its close proximity to the perirectal bacterial reservoir—facilitate ascending colonization of the bladder urothelium, particularly by enteric flora such as *Escherichia coli* (which occurred in 86.4% female patients in our study) [6,8]. Furthermore, our age-stratified analysis revealed a bimodal distribution peaking in older adults aged 61–70 years (23.4%) and young individuals aged 11–20 years (20.3%). In young females, hormonal changes and early marriage ('honeymoon cystitis') contribute to acute lower UTIs, whereas in older adults of both sexes, declining immunity, postmenopausal urogenital atrophy in women, benign prostatic hyperplasia in men, diabetic neuropathy, and frequent hospitalization with urinary catheterization predispose patients to recurrent and complicated infections [7,9,10].

Gram-negative bacilli constituted 68.8% of all isolated uropathogens in our cohort, with *E. coli* representing the single most prevalent species (34.4%). The predominance of *E. coli* as the primary

etiological agent of UTI is universally corroborated across international studies, including the European ECO·SENS project (77.0%) [12], Poland (73.0%) [22], Italy [23], Nepal (59.4%) [20], Iran (68.8–78.6%) [19,21], and India (42.6–61.8%) [14,15]. Notably, however, the relative proportion of *E. coli* in our cohort (34.4%) was lower than in purely community-acquired uncomplicated UTI studies (70–80%) because nearly two-thirds (65.6%) of our culture-positive specimens were derived from hospitalized indoor patients. In hospital settings, catheterization, prior broad-spectrum antibiotic exposure, and underlying comorbidities select for a broader spectrum of nosocomial and opportunistic pathogens [4,22]. Indeed, *Enterococcus faecalis* emerged as the second most frequent isolate in our study (25.0%, with 68.8% originating from indoor wards), followed by oxidase-negative Gram-negative rods (15.6%) and *Pseudomonas* spp. (9.4%, with 83.3% from indoor wards). These findings mirror those of Hryniewicz et al. [22] in Poland and De Francesco et al. [23] in Italy, who similarly observed that Gram-positive cocci (*Enterococcus* spp.) and *Pseudomonas aeruginosa* were substantially enriched among hospitalized patients compared with community outpatients.

A critical finding of our investigation is the alarming rate of resistance among urinary isolates to oral antimicrobials commonly prescribed for empirical UTI treatment in Pakistan [3]. Fluoroquinolones (norfloxacin and ciprofloxacin), oral third-generation cephalosporins (cefixime), first-generation quinolones (pipemidic acid), and folate pathway inhibitors (co-trimoxazole/Septran) exhibited dismal activity against *E. coli*, with susceptibility rates of only 9.1% for norfloxacin, cefixime, and pipemidic acid, and 13.6% for ciprofloxacin and co-trimoxazole. While earlier studies from the 2000s in Poland [22], Nepal [20], and Iran [19,21] reported preserved *E. coli* susceptibility to ciprofloxacin (68.1–83.2%), more recent South Asian reports from Pakistan [3] and India [14,15] have documented a precipitous decline in fluoroquinolone and cephalosporin efficacy driven by the widespread dissemination of plasmid-mediated quinolone resistance determinants and extended-spectrum beta-lactamases (ESBLs). The indiscriminate, empirical prescription of ciprofloxacin, cefixime, and co-trimoxazole without microbiological guidance, coupled with over-the-counter availability and incomplete treatment courses in local communities, has rendered these agents largely ineffective as empirical choices in our setting [3,14]. Interestingly, *Pseudomonas* spp. in our cohort retained 83.3% susceptibility to ciprofloxacin and doxycycline and 100.0% susceptibility to amikacin, underscoring the value of species-level identification and targeted antibiograms.

Conversely, amikacin (85.9% overall susceptibility; 90.9% against *E. coli*, 100.0% against *Pseudomonas* spp., *S. saprophyticus*, and *Klebsiella* spp.) and nitrofurantoin (76.6% overall susceptibility; 81.8% against *E. coli* and 87.5% against *E. faecalis*) demonstrated robust, reliable *in vitro* activity across our clinical isolates. Gentamicin also retained moderate-to-high activity against *E. coli* (77.3%). These results are in strong agreement with Das et al. [20] in Nepal (who reported 87.2% overall susceptibility to amikacin and 98.0% in *E. coli*), De Francesco et al. [23] in Italy, Kashef et al. [19] in Iran (71.3% *E. coli* susceptibility to nitrofurantoin), and Sood and Gupta [14] in India, who emphasized that nitrofurantoin remains the most active oral agent against community- and hospital-acquired *E. coli* despite widespread resistance to other oral classes. Because nitrofurantoin achieves high urinary concentrations, acts via multiple intracellular bacterial targets, and minimally perturbs gut flora, it represents an ideal first-line oral therapy for uncomplicated lower UTIs caused by *E. coli*, *E. faecalis*, and *S. saprophyticus* [6,14]. Meanwhile, parenteral aminoglycosides—particularly amikacin—provide potent therapeutic coverage for hospitalized patients with complicated UTIs or pyelonephritis when guided by renal function monitoring.

Study Limitations and Future Recommendations

This study has several limitations that warrant consideration. First, the sample size (N = 150 total specimens, n = 64 culture-positive isolates) was derived from a single tertiary care center over one year, resulting in small subgroup counts for less frequent species such as *Klebsiella* spp. (n = 1) and *S. saprophyticus* (n = 4). Second, detailed clinical metadata regarding indwelling catheter duration, prior antibiotic exposure within the preceding 90 days, comorbidities (such as diabetes mellitus or obstructive uropathy), and patient treatment outcomes were not captured on the laboratory proforma. Third, phenotypic confirmation of ESBL production (via double-disk synergy testing), minimum inhibitory concentration (MIC) determination, and molecular characterization of resistance genes (e.g., *bla*CTX-M, *bla*NDM, *bla*KPC) were beyond the scope of routine diagnostic processing. Future multicenter prospective studies incorporating larger sample sizes, molecular resistance surveillance, and clinical outcome tracking are strongly recommended.

Conclusion

In conclusion, *Escherichia coli* (34.4%) and *Enterococcus faecalis* (25.0%) are the predominant bacterial uropathogens isolated at tertiary care hospital of Lahore, with a significantly higher infection burden among female patients (67.2%) and hospitalized inpatients (65.6%). Widespread multidrug resistance to commonly prescribed empirical oral antimicrobials—including pipemidic acid, norfloxacin, cefixime, co-trimoxazole, and ciprofloxacin—precludes their empirical clinical use in our setting. Nitrofurantoin retains high *in vitro* efficacy (76.6% overall; >80% against *E. coli* and *E. faecalis*) and should be prioritized for oral management of uncomplicated lower UTIs, whereas amikacin (85.9% overall sensitivity) remains a highly reliable parenteral option for hospitalized and complicated cases. Strengthening hospital infection control practices, mandating urine culture before initiating antimicrobial therapy, and implementing institutional antibiotic stewardship programs are essential to curtail the spread of resistant uropathogens.

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