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HIGH BURDEN OF MULTIDRUG-RESISTANT UROPATHOGENIC *ESCHERICHIA COLI* AND PRESERVED SUSCEPTIBILITY TO NITROFURANTOIN AND FOSFOMYCIN: A CROSS-SECTIONAL STUDY FROM PESHAWAR, PAKISTAN

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Abstract

Background: *Urinary tract infections (UTIs) are among the most prevalent bacterial infections globally and are increasingly complicated by antimicrobial resistance (AMR). Uropathogenic Escherichia coli (UPEC) remain the principal causative agent, with rising multidrug resistance (MDR) compromising empirical treatment strategies. This study investigated the prevalence, antimicrobial susceptibility patterns, of UPEC isolates from UTI patients in Peshawar, Pakistan, with supplementary in silico analysis of β-lactamase enzymes.*

Methods: *A laboratory-based cross-sectional study was conducted between October and December 2025. Midstream urine samples (n = 70) from clinically suspected UTI patients were processed using standard microbiological methods. Identification of E. coli was confirmed via colony morphology, Gram staining, and biochemical assays. Antimicrobial susceptibility testing (AST) was performed using the Kirby–Bauer disk diffusion method following Clinical and Laboratory Standards Institute (CLSI 2024) guidelines. MDR was defined as resistance to at least one agent in three or more antimicrobial classes.*

Results: *E. coli was the predominant uropathogen, accounting for 35.7% of isolates. Antimicrobial susceptibility testing demonstrated markedly high resistance to commonly used antibiotics, including ampicillin (96%), cefotaxime (80%), azithromycin (80%), and amoxicillin–clavulanate and ceftriaxone (64% each). In contrast, nitrofurantoin, fosfomycin, and meropenem exhibited high efficacy, with 96% susceptibility rates. MDR was observed in 84% of isolates, with a small proportion (4%) classified as extensively drug-resistant (XDR).*

Conclusion: *The high prevalence of MDR E. coli limits the utility of commonly prescribed β-lactams for empirical therapy in this setting. Nitrofurantoin and*

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fosfomycin remain effective first-line options. These findings underscore the necessity for routine AST-guided treatment and strengthened antimicrobial stewardship to mitigate the escalating AMR burden.

Introduction

Urinary tract infections (UTIs) are among the most prevalent bacterial infections globally and constitute a significant clinical and economic burden on healthcare systems [1]. UPEC is the principal etiological agent, accounting for approximately 70–90% of community-acquired UTIs [2]. The management of UTIs has become increasingly challenging due to the rapid emergence and dissemination of antimicrobial resistance (AMR), which compromises the efficacy of standard empirical treatment regimens [3].

UPEC exhibits a pronounced ability to acquire resistance to multiple antimicrobial classes, particularly β-lactam antibiotics, including penicillins and third-generation cephalosporins [4]. This resistance is frequently associated with enzymatic mechanisms such as the production of β-lactamases, which significantly limit therapeutic options. In Pakistan, persistently high resistance rates to commonly prescribed first-line agents, including ampicillin and cephalosporins, have been reported, further complicating empirical treatment approaches and increasing the risk of treatment failure [5,6].

Given the dynamic nature of antimicrobial resistance, continuous local surveillance of pathogen prevalence and susceptibility patterns is essential for guiding evidence-based therapy [7]. However, region-specific data on the antimicrobial susceptibility profiles of UPEC in areas such as Peshawar remain limited. Therefore, the present study was undertaken to determine the prevalence and antimicrobial susceptibility patterns of *E. coli* isolates from patients with urinary tract infections in Peshawar, Pakistan, with the aim of informing local treatment strategies and supporting antimicrobial stewardship efforts.

Methodology

Study Design and Setting

A laboratory-based cross-sectional study was conducted between October to December 2025 at the Microbiology Laboratory, Institute of Pathology and Diagnostic Medicine (IPDM), Khyber Medical University, Peshawar.

Sample Collection and Selection Criteria

A total of 70 midstream urine samples demonstrating significant bacteriuria ($\geq 10^5$ CFU/mL) were obtained from the Public Health Reference Laboratory (PHRL), Khyber Medical University. Samples were collected from patients of all age groups and both sexes presenting with clinical features suggestive of urinary tract infection (e.g., dysuria, urinary frequency, and urgency). Patients who were receiving antibiotic therapy at the time of sample collection were excluded to minimize confounding effects on microbial growth and susceptibility patterns.

Bacterial Isolation and Identification

Urine specimens were inoculated onto Blood agar, Cystine Lactose Electrolyte-Deficient (CLED) agar, and nutrient agar using standard microbiological techniques. Plates were incubated aerobically at 37 °C for 24 hours. Presumptive identification of *UPEC* was based on the presence of lactose-fermenting colonies, followed by confirmatory testing using Gram staining (Gram-negative bacilli) and a panel of biochemical assays. These included indole production (positive), citrate utilization (negative), and Triple Sugar Iron (TSI) agar reaction (acid slant/acid butt without hydrogen sulfide production) [9].

Antimicrobial Susceptibility Testing (AST)

Antimicrobial susceptibility testing was performed using the Kirby–Bauer disk diffusion method on Mueller–Hinton agar, in accordance with CLSI 2024 guidelines [10]. The antibiotics tested included ampicillin (10 µg), amoxicillin–

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clavulanate (20/10 µg), ceftriaxone (30 µg), cefotaxime (30 µg), azithromycin (15 µg), nitrofurantoin (300 µg), fosfomycin (200 µg), and meropenem (10 µg). *Escherichia coli* ATCC 25922 was used as the quality control strain. Multidrug resistance was defined as non-susceptibility to at least one agent in three or more antimicrobial classes [11].

Data Analysis

Demographic and antimicrobial susceptibility data were analyzed using Microsoft Excel (Microsoft Corporation, USA). Descriptive statistical analysis was performed, and results were expressed as frequencies and percentages.

Results

A total of 70 urine specimens from patients with clinically suspected urinary tract infections yielded significant monomicrobial growth. Among these, Gram-negative bacteria predominated, accounting for 82.9% (n = 58) of isolates, while Gram-positive organisms comprised 17.1% (n = 12). *Escherichia coli* was the most frequently isolated uropathogen, representing 35.7% (n = 25) of all isolates, followed by *Klebsiella pneumoniae* (17.1%) and *Pseudomonas aeruginosa* (11.4%). The complete distribution of identified pathogens is presented in Table 1.

Table 1. Etiological distribution of bacterial pathogens isolated from 70 urine samples of presumptive UTI patients.

Pathogen	Number of Isolates	Percentage (%)
Gram-Negative Bacteria	**58**	**82.9**
Escherichia coli	25	35.7
Klebsiella pneumoniae	12	17.1
Pseudomonas aeruginosa	8	11.4
Proteus mirabilis	6	8.6
Enterobacter cloacae	4	5.7
Acinetobacter baumannii	3	4.3
Gram-Positive Bacteria	**12**	**17.1**
Enterococcus faecalis	5	7.1
Enterococcus faecium	3	4.3
Staphylococcus saprophyticus	2	2.9
Staphylococcus aureus	2	2.9
Total Isolates	70	100.0

Demographic Characteristics of the Study Participants

Among the 25 patients with *E. coli*-associated UTIs, females constituted the majority (60%, n = 15), whereas males accounted for 40% (n = 10). The age distribution demonstrated a bimodal pattern, with the highest frequency observed in the 10–20 years and >80 years age groups, each comprising 28% (n = 7) of cases. The lowest frequency was recorded in the 41–60 years age

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range. Detailed age- and sex-specific distributions are summarized in Table 2. Geographically, the highest proportion of isolates originated from Waziristan (28%) and Swat (24%).

Table 2. Age and gender distribution of patients with urinary tract infection of *E. coli* (n=25).

Age Group (Years)	Total, n (%)	Male, n (% of Age Group)	Female, n (% of Age Group)
10 – 20	7 (28.0%)	3 (42.9%)	4 (57.1%)
21 – 30	3 (12.0%)	1 (33.3%)	2 (66.7%)
31 – 40	2 (8.0%)	1 (50.0%)	1 (50.0%)
41 – 50	1 (4.0%)	0 (0.0%)	1 (100.0%)
51 – 60	1 (4.0%)	0 (0.0%)	1 (100.0%)
61 – 70	2 (8.0%)	1 (50.0%)	1 (50.0%)
71 – 80	2 (8.0%)	1 (50.0%)	1 (50.0%)
> 80	7 (28.0%)	3 (42.9%)	4 (57.1%)
Total	25 (100%)	10 (40.0%)	15 (60.0%)

Antimicrobial Susceptibility Profile

Antimicrobial susceptibility testing revealed a high level of resistance to commonly prescribed antibiotics (Table 3). The highest resistance was observed against ampicillin (96%, n = 24), followed by cefotaxime (80%, n = 20) and azithromycin (80%, n = 20). Resistance to amoxicillin–clavulanate and ceftriaxone was 64% (n = 16 each). In contrast, nitrofurantoin, fosfomycin, and meropenem demonstrated high efficacy, with susceptibility rates of 96% (n = 24) each, and only 4% resistance observed.

Table 3: Antibiotic susceptibility of isolates of *E. coli* in UTI patients (n = 25).

Antibiotic	Resistant, n (%)	Susceptible, n (%)
Ampicillin (10 µg)	24 (96%)	1 (4%)
Amoxicillin-Clavulanate (20/10 µg)	16 (64%)	9 (36%)
Ceftriaxone (30 µg)	16 (64%)	9 (36%)
Cefotaxime (30 µg)	20 (80%)	5 (20%)
Azithromycin (15 µg)	20 (80%)	5 (20%)
Nitrofurantoin (300 µg)	1 (4%)	24 (96%)
Fosfomycin (200 µg)	1 (4%)	24 (96%)
Meropenem (10 µg)	1 (4%)	24 (96%)

Prevalence of Multidrug Resistance

A substantial proportion of *E. coli* isolates exhibited multidrug resistance. Specifically, 84% (n = 21) of isolates met the criteria for MDR, indicating non-susceptibility to at least one agent in three or more antimicrobial classes. One isolate (4%) was classified as XDR, while no PDR isolates were identified. The distribution of resistance categories is detailed in Table 4

Table 4. Multidrug Resistance, Extensive Drug Resistance and Pan drug Resistance Distribution of Escherichia coli isolates (n=25).

Resistance Category	Definition	Number of Isolates	Percentage
Multidrug-Resistant	Non-susceptible to ≥1 drug in **≥3 classes** (out of 7 tested)	21	84%
Extensively Drug-Resistant	Non-susceptible to ≥1 drug in **≥5 classes** (Susceptible to ≤2 classes)	1	4%

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Pan drug-Resistant (PDR)	Non-susceptible to **all agents in all 7 classes** tested	0	0%
Non-MDR	Does not meet MDR criteria	4	16%

Discussion

This study provides contemporary evidence on the antimicrobial resistance profile of uropathogenic *Escherichia coli* in Peshawar, Pakistan, demonstrating a notably high prevalence of multidrug resistance (84%). This rate exceeds previously reported regional estimates (50–70%) and underscores the accelerating burden of antimicrobial resistance in this setting [6,13]. The observed resistance patterns suggest a substantial compromise in the effectiveness of commonly used empirical therapies.

The extremely high resistance to ampicillin (96%) and substantial resistance to third-generation cephalosporins (64–80%) are consistent with global trends associated with the dissemination of extended-spectrum β-lactamase (ESBL)-producing *E. coli* strains [4,14]. These findings indicate that β-lactam antibiotics, historically considered first-line agents, have limited clinical utility in this population. Additionally, the high resistance to azithromycin (80%) further restricts available oral treatment options, complicating outpatient management.

In contrast, nitrofurantoin and fosfomycin demonstrated sustained efficacy, with 96% susceptibility rates. These findings are aligned with international treatment guidelines recommending these agents as first-line therapies for uncomplicated UTIs, particularly in regions with high resistance to conventional antibiotics [15,16]. Their retained activity may be attributed to distinct mechanisms of action and comparatively lower selective pressure. The detection of carbapenem resistance, although low (4%), is clinically significant, as it may indicate the early emergence of carbapenem-resistant Enterobacterales (CRE), which are associated with limited therapeutic options and increased morbidity [17]. The observed predominance of infections in females (60%) and at age extremes is consistent with established epidemiological patterns [1,18].

This study is limited by its single-center design and relatively small sample size, which may affect the generalizability of the findings. Furthermore, phenotypic confirmation of ESBL production was not performed, and molecular characterization was limited. Larger, multicenter studies incorporating comprehensive genotypic analyses are warranted to validate and expand upon these findings.

5. Conclusion

Escherichia coli, the predominant uropathogen in this study, demonstrated a high prevalence of multidrug resistance, with substantial resistance to commonly used β-lactam antibiotics, indicating that empirical use of ampicillin, cephalosporins, and azithromycin is no longer appropriate in this setting. In contrast, nitrofurantoin and fosfomycin retained high efficacy and should be prioritized as first-line agents for uncomplicated UTIs. These findings, supported by structural insights into β-lactamase-mediated resistance, underscore the urgent need for routine antimicrobial susceptibility testing, strengthened antimicrobial stewardship programs, and stricter regulation of antibiotic use to curb the escalating burden of antimicrobial resistance in Pakistan.

Author Contributions

M.O., T.R., and M.S. contributed to study conception and design. M.O., E.A., B.M., and M.I. performed sample collection, laboratory experimentation, and data acquisition. T.R. and M.S. conducted data analysis and interpretation. M.O. and S.A. drafted the initial manuscript. A.W. provided overall supervision, critical revision, and intellectual input. All authors reviewed, approved the final manuscript, and agree to be accountable for all aspects of the work.

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Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this study.

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Data Availability Statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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