

Original Article

Antimicrobial Resistance Patterns and Prevalence of tetA/tetB Genes in Uropathogenic Escherichia coli Isolated from Urinary Tract Infections in Swat, Pakistan.

Aftab Ali ¹, Sadaf Anwar Qureshi ², Aamir Sohail ³, Dildar Khan⁴, Muhammad Salahuddin⁵, Sofia Bano ⁶

^{1.} PhD Scholar, Institute of Pathology and Diagnostic Medicine, Khyber Medical University, Peshawar, Pakistan

^{2.} Assistant Professor, Department of Pathology, Ayub Medical College, Abbottabad, Pakistan

^{3.} PhD Scholar, Department of Microbiology, University of Haripur, Haripur

^{4.} M Microbiologist, Public Health Reference Laboratory, Khyber Medical University, Peshawar, Pakistan

^{5.} B5. Microbiology Student, Institute of Pathology and Diagnostic Medicine, Khyber Medical University, Peshawar, Pakistan

^{6.} BS Microbiology Student, Institute of Pathology and Diagnostic Medicine, Khyber Medical University, Peshawar, Pakistan

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Corresponding Author

Sadaf Anwar Qureshi

Email: drsadaf9596@gmail.com

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ABSTRACT

Objective: To characterize uropathogenic Escherichia coli isolated from urinary tract infections in non-hospitalized patients in Swat, Pakistan, by assessing demographic distribution, antimicrobial susceptibility patterns, and the prevalence of tetracycline resistance genes (tetA and tetB).

Methods: We collected 250 midstream urine samples from outpatients with suspected UTI at a tertiary care hospital in Swat, Khyber Pakhtunkhwa, Pakistan. Samples were cultured on Cystine Lactose Electrolyte-Deficient (CLED) and MacConkey agar, and isolates meeting the threshold for significant bacteriuria ($\geq 10^5$ CFU/mL) were identified using Gram staining and standard biochemical tests (indole, urease, lactose fermentation), with identification confirmed by PCR targeting the 16S rRNA gene. We determined antibiotic susceptibility of confirmed isolates by the Kirby-Bauer disk diffusion method on Mueller-Hinton agar and interpreted results according to CLSI M100 (35th edition, 2025) breakpoints for *Enterobacterales*. PCR subsequently screened isolates phenotypically resistant to tetracycline for the *tetA* and *tetB* resistance genes.

Results: Of the 250 samples screened, 150 (60.0%) were presumptively identified as *E. coli*, of which 145 (58%) were confirmed by 16S rRNA PCR. Women accounted for the substantial majority of confirmed isolates (80.7%, 117/145), with infections concentrated in the 20–30-year age group, whereas male isolates peaked later, in the 60–70-year group. Resistance was highest against ampicillin (97.2%), cefixime (87.6%), ciprofloxacin (56.6%), and tetracycline (55.2%), while piperacillin-tazobactam (2.1%), amikacin (4.1%), fosfomycin (4.8%), imipenem 6.9%, and meropenem 13.1% retained the greatest in vitro activity. Among the 80 phenotypically tetracycline-resistant isolates, *tetA* was detected more frequently (61.3%, 49/80) than *tetB* (45.0%, 36/80).

Conclusion: UPEC isolates in this study showed extensive resistance to first-line agents, including ampicillin, cefixime, and fluoroquinolones, while carbapenems, fosfomycin, and piperacillin-tazobactam remained largely reliable. The predominance of *tetA* over *tetB* adds region-specific molecular detail to the local tetracycline resistance landscape. These findings support revising empirical prescribing guidance in this setting and reinforce the need for robust antimicrobial stewardship, ongoing resistance surveillance, and safe antibiotic use.

INTRODUCTION

Urinary tract infections are one of the most common bacterial infections diagnosed in the community and in hospital settings globally. The magnitude of the problem is highlighted by recent estimates of the Global Burden of Disease: between 1990 and 2021, the number of UTI cases rose by 66.5% to an estimated 4.49 billion cases worldwide, with women, older men, and populations in low- and middle-sociodemographic-index regions bearing a disproportionate burden (1). However, the diagnostic and conceptual approach to defining and managing UTI has remained relatively unchanged over the decades, despite the increasing complexity of the microbial and epidemiological picture (2). However, the main culprit remains the same: UPEC still causes most uncomplicated and complicated UTIs in almost all populations studied. UPEC's success as a uropathogen is no coincidence (3). Over time, these strains have evolved a unique repertoire of virulence factors, including type 1 and P fimbriae for adhesion to uroepithelial cells, iron-scavenging siderophore systems, capsular polysaccharides for immune evasion, and a variety of toxins and outer membrane proteins. These factors allow UPEC to colonize, invade, and survive in the otherwise hostile urinary tract environment (4). UPEC's adaptability and virulence have enabled it to remain the most common cause of UTI despite evolving clinical management strategies and sustained, genotype-informed surveillance to guide antimicrobial stewardship (4, 5). However, the antibiotic environment in which UPEC is operating has undergone a dramatic shift. Antimicrobial resistance (AMR) has become a crisis, and recent global modeling estimates that bacterial AMR will cause more than 39 million deaths from 2025 to 2050 if current resistance trends continue (6). Uropathogenic *E. coli* is a major contributor to this crisis. In Pakistan, UPEC isolates have high resistance to ampicillin and third-generation cephalosporins, with significant variation between community-associated and hospital-associated isolates (7, 8). Similarly, extensive multidrug resistance has been reported among UPEC recovered from Pakistani women with uncomplicated UTIs (7). These trends are no longer a side issue in clinical medicine, but are increasingly reducing the number of antibiotics that can be used with confidence in an empirical fashion. Tetracyclines are a special class of antibiotics that are particularly important for these patients. In pooled international data, resistance to tetracycline-class agents is one of the most common AMR phenotypes among UPEC isolates. It has been reported at higher rates than for many β -lactams (9). This resistance is usually conferred by efflux-pump systems that are encoded by the *tetA* and *tetB* genes, which actively extrude the drug from the bacterial cell before it reaches an inhibitory intracellular concentration. However, the relative contribution of *tetA* and *tetB* varies across settings. Recent studies found *tetB* much more prevalent than *tetA* in Lahore and Faisalabad for carbapenem-resistant UPEC (10). It is unclear whether the same pattern is seen in other parts of the country, and tetracycline resistance mechanisms cannot be assumed to be the same between localities. Despite the volume of AMR surveillance emerging from Pakistan in recent years, much of it has focused on hospital-based or provincial cohorts, with no major study conducted in Swat. Relatively few studies have combined phenotypic antibiotic susceptibility testing with molecular screening of specific tetracycline resistance determinants in an exclusively outpatient, non-hospitalized population (7, 8, 11). That gap is not a minor technical omission. Outpatient prescribing decisions are made without the safety net of a hospital antibiogram, relying instead on exactly the kind of regional, guideline-level data that studies like this one are meant to generate. It was this gap that the present study set out to address, by characterizing UPEC isolated from non-hospitalized patients presenting with suspected UTI in Swat Area of Khyber Pakhtunkhwa, Pakistan, with four specific aims: to confirm isolate identity through combined phenotypic and molecular methods; to describe the demographic distribution of infection by age and sex; to determine the antibiotic susceptibility profile of confirmed isolates against a panel of clinically relevant agents; and to screen tetracycline-resistant isolates for the *tetA* and *tetB* genes to establish their relative prevalence in this specific, and so far underrepresented, patient population.

METHODOLOGY

2.1 Study design and sample collection

We collected 250 midstream urine samples from non-hospitalized patients presenting with suspected urinary tract infection at the outpatient department of a tertiary care hospital in Swat, Khyber Pakhtunkhwa, Pakistan. Samples were collected into sterile containers and transported to the microbiology laboratory within 40 minutes of collection to minimize pre-analytical degradation.

2.2 Bacterial isolation and culture

A calibrated 0.01 mL (1 μ L) wire loop was used to inoculate each specimen onto Cystine Lactose Electrolyte-Deficient (CLED) agar, and the plates were incubated aerobically at 37 $^{\circ}$ C for 24 hours. Growth exceeding 10⁵ colony-forming units per milliliter was regarded as clinically significant bacteriuria. Presumptive colonies were sub-cultured onto MacConkey agar to obtain pure isolates, which were preserved in 15% glycerol at low temperature for long-term storage.

2.3 Identification of isolates

Pure isolates were identified using standard morphological, Gram-staining, and biochemical criteria, and confirmed by molecular testing. We assessed colonial morphology on CLED and MacConkey agar and performed Gram staining on fixed smears using crystal violet, Gram's iodine, an acid-alcohol decolorizer, and a safranin

counterstain. Biochemical identification relied on the indole test (detection of tryptophan decomposition with Kovac's reagent), the urease test (detection of urea hydrolysis on a phenol red-containing slant), and lactose fermentation on differential media. Isolates that were Gram-negative rods, indole-positive, urease-negative, and lactose-fermenting, with colonial morphology characteristic of *E. coli* on both media, were provisionally identified as *E. coli*; this identification was subsequently confirmed by PCR targeting the proximal and distal conserved flanking regions of 16S ribosomal RNA using *E. coli* primers, yielding a 450 bp product.

2.4 Antibiotic susceptibility testing

Antibiotic susceptibility testing of the 145 confirmed UPEC isolates was performed using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar (MHA), in accordance with the Clinical and Laboratory Standards Institute (CLSI) M100, 35th Edition (2025) guidelines (12). Bacterial suspensions were adjusted to a turbidity equivalent to a 0.5 McFarland standard and inoculated uniformly onto MHA plates using a sterile swab. Antibiotic discs of the following potencies were placed on the inoculated plates: amikacin (AK, 30 μ g), gentamicin (CN, 10 μ g), tetracycline (TE, 30 μ g), doxycycline (DO, 30 μ g), levofloxacin (LEV, 5 μ g), ciprofloxacin (CIP, 5 μ g), ampicillin (AMP, 10 μ g), cefixime (CFM, 5 μ g), fosfomycin (FOS, 200 μ g), imipenem (IPM, 10 μ g), meropenem (MEM, 10 μ g), and piperacillin-tazobactam (TZP, 100/10 μ g) provided by Oxoid Turkey. Plates were incubated aerobically at 35 $\pm$ 2 $^{\circ}$ C for 16–18 hours, after which zones of inhibition were measured in millimeters using a digital caliper and interpreted as sensitive (S), intermediate (I), or resistant (R) according to the CLSI M100 (35th edition, 2025) interpretive breakpoint criteria for *Enterobacterales*. *E. coli* ATCC 25922 was used as the quality control strain in each testing batch.

2.5 Molecular confirmation and screening of tetracycline resistance genes *tetA* and *tetB*

Isolates identified as *E. coli* by PCR were subsequently screened for the presence of tetracycline resistance genes (*tetA* and *tetB*), particularly in *E. coli* strains that showed resistance to tetracycline by the disc diffusion method, using *tetA*- and *tetB*-gene-specific primers described previously for UPEC (13). We assessed gene amplification by agarose gel electrophoresis. We scored a product as present when we observed a band of the expected size for that gene relative to a molecular-weight marker.

Table 1: list of primers used in the study

S.NO	Gene name	Primers sequence	Product size	References
1	16s rRNA	F: AGAGTTTGATCMTGGCTCAG R: TACGGYTACCTGTGTACG ACTT	410bp	(14)
2	<i>tetA</i>	F: GGCGGTCTTCTTCTTCATCATGC R: CGGCAGGCAGAGCAAGTAGA	575bp	(13)
3	<i>TetB</i>	F: CATTAAATAGGCCCATCGCTG R: TGAAGGTCATCGATAGCAGG	635bp	(13)

2.6 Data analysis

Results were expressed as frequencies and percentages for categorical variables, including sex distribution, age-group distribution, biochemical test outcomes, and antibiotic susceptibility categories.

Results

3.1 Identification of bacterial isolates

Of the 250 urine samples screened, 150 (60%) were identified as *E. coli* by colony morphology, Gram staining, and biochemical testing; of these, 145 (58% of the original 250) were subsequently confirmed as *E. coli* by PCR targeting the 16S rRNA gene. All confirmed isolates were Gram-negative rods that produced moderate-to-large, lactose-fermenting pink colonies on MacConkey agar and moderate, round, yellow colonies on CLED agar. On biochemical testing, confirmed isolates were uniformly indole-positive, urease-negative, and lactose-fermenting on both media (Table 1).

Table 1. Morphological and biochemical characteristics used to identify *Escherichia coli*

Test	Result
Gram stain	Gram-negative rods
Colony morphology – MacConkey agar	Moderate–large, round, convex, lactose-fermenting pink colonies
Colony morphology – CLED agar	Moderate, round, convex yellow colonies
Indole test	Positive
Urease test	Negative
Lactose fermentation	Positive

3.2 Sex and age distribution

Among the 145 confirmed UPEC isolates, women accounted for the clear majority (80.7%, 117/145), consistent with the well-documented female predominance in urinary tract infections. Isolates were most frequently recovered from patients aged 20–30 years (17.9%, 26/145), followed closely by the 0–10 and 60–70-year groups (15.2% each, 22/145). This age distribution was significantly different between the sexes. Infections were highest in the reproductive age group in females (20–30 years, 19.7%, 23/117), but in males, the burden was shifted towards older age (60–70 years, 32.1%, 9/28), which may be due to age-related prostatic and urological changes that increase the risk of UTIs in older men. The 10–20-year group had the lowest number of isolates (5.5%, 8/145), indicating a relatively low infection burden in adolescence in both sexes. (Table 2)

Table 2: Age- and sex-wise distribution of confirmed UPEC isolates

Age group (years)	Female n (%)	Male n (%)
0–10	20 (17.1%)	2 (7.1%)
10–20	7 (6.0%)	1 (3.6%)
20–30	23 (19.7%)	3 (10.7%)
30–40	13 (11.1%)	2 (7.1%)
40–50	11 (9.4%)	2 (7.1%)
50–60	14 (12.0%)	1 (3.6%)
60–70	13 (11.1%)	9 (32.1%)
70–80	9 (7.7%)	3 (10.7%)
80–90	7 (6.0%)	5 (17.9%)
Total	117 (80.7%)	28 (19.3%)

3.3 Antibiotic susceptibility profile of UPEC

A highest resistance was reported against ampicillin (97.2%, 141/145), followed by cefixime (87.6%, 127/145), ciprofloxacin (56.6%, 82/145), tetracycline (55.2%, 80/145), and levofloxacin (46.9%, 68/145). Doxycycline and gentamicin showed moderate resistance (33.8% and 29.0%, respectively), while carbapenems showed comparatively lower resistance (15.2% for meropenem and 6.9% for imipenem). Fosfomycin (4.8%, 7/145), amikacin (4.1%, 6/145), and piperacillin-tazobactam (2.1%, 3/145) had the lowest resistance rates and the highest in vitro activity against the UPEC isolates. Intermediate susceptibility was rare for all antibiotics tested, from 0% (imipenem, meropenem, piperacillin-tazobactam) to 3.4% (levofloxacin). (Figure 1, Table 3)

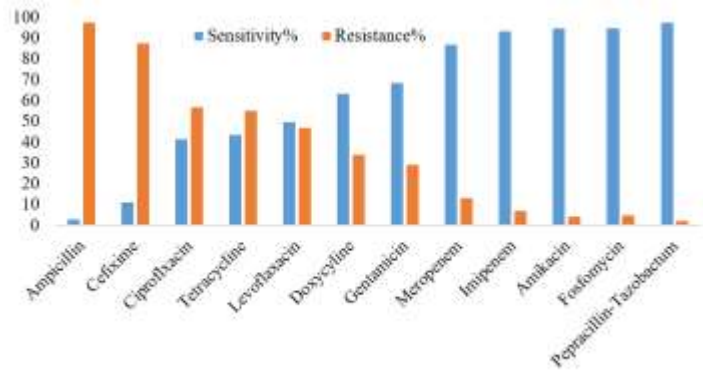


Figure 1. Antibiotic resistance patterns of UPEC

Table 3. The antibiotic susceptibility profile of confirmed UPEC isolates (145)

Antibiotic	Sensitive n (%)	Intermediate n (%)	Resistant n (%)
Amikacin	137 (94.5%)	2 (1.4%)	6 (4.1%)
Gentamicin	99 (68.3%)	4 (2.8%)	42 (29.0%)
Tetracycline	63 (43.4%)	2 (1.4%)	80 (55.2%)
Doxycycline	92 (63.4%)	4 (2.8%)	49 (33.8%)
Levofloxacin	72 (49.7%)	5 (3.4%)	68 (46.9%)
Ciprofloxacin	60 (41.4%)	3 (2.1%)	82 (56.6%)
Ampicillin	4 (2.8%)	0 (0%)	141 (97.2%)
Cefixime	16 (11.0%)	2 (1.4%)	127 (87.6%)

Fosfomycin	137 (94.5%)	1 (0.7%)	7 (4.8%)
Imipenem	135 (93.1%)	0 (0%)	10 (6.9%)
Meropenem	126 (86.8%)	0 (0%)	19 (13.1%)
Piperacillin-	141 (97.2%)	1 (0.7%)	3 (2.1%)
Tazobactam			

3.4 Screening of Tetracycline resistance genes

All 80 phenotypically tetracycline-resistant UPEC isolates were screened for *tetA* and *tetB* by PCR. *tetA* was the more prevalent determinant, detected in 61.3% (49/80) of isolates, while *tetB* was identified in 45.0% (36/80), indicating that efflux-pump-mediated resistance via *tetA* predominates over *tetB* in this collection. Amplicon sizes for both genes matched the expected fragment lengths (*tetA*, 575 bp; *tetB*, 635 bp), confirming specific amplification (Figure 2; Table 4).

Table 4. Detection frequency of Tetracycline resistance genes among confirmed isolates

Gene	Isolates tested	Positive isolates	Positive (%)
<i>TetA</i>	80	49	61.3%
<i>TetB</i>	80	36	45.0%

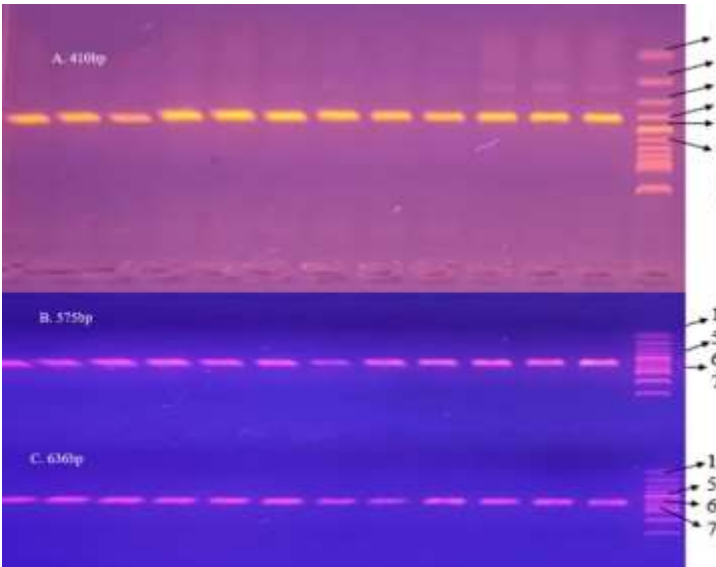


Figure 2. Gel-based identification of (A) the 16S rRNA gene of UPEC (410 bp) with 100 bp DNA markers (ladder), (B) the *tetA* gene (575 bp), and (C) the *tetB* gene of UPEC (635 bp).

Discussion

Urinary tract infections remain among the most common bacterial infections encountered in both community and healthcare settings, with a substantial and increasing global disease burden. Recent estimates indicate a 66.5% increase in the number of UTI cases between 1990 and 2021, reaching approximately 4.49 billion cases, with women, older men, and populations in lower socioeconomic settings disproportionately affected (1). The increasing burden of UTI is occurring alongside the global expansion of antimicrobial resistance (AMR), which poses a major threat to the effective management of bacterial infections. Recent global estimates project that, if current resistance trajectories persist, bacterial AMR could contribute to more than 39 million deaths between 2025 and 2050 (6). Within this context, the present study provides region-specific data on the demographic distribution, antimicrobial susceptibility profile, and selected tetracycline resistance determinants among UPEC isolates recovered from non-hospitalized patients in Swat, Khyber Pakhtunkhwa, Pakistan. The identification of 145 molecularly confirmed *E. coli* isolates from 250 urine specimens shows that UPEC contributed substantially to the clinically suspected UTI population investigated. Combining conventional microbiological characterization with 16S rRNA-targeted PCR provided complementary phenotypic and molecular evidence for isolate identification. This approach is particularly relevant for regional surveillance studies in settings where conventional biochemical identification remains widely used. The predominance of female patients among confirmed isolates (80.7%) aligns with the established epidemiology of UTI. This may be explained by anatomical and physiological factors, including the shorter female urethra and its

proximity to the perianal region, which facilitates ascending colonization by enteric *E. coli* (15). The age distribution also showed distinct patterns by sex. Among females, the greatest proportion of isolates occurred in the 20–30-year age group, whereas among males, isolates were most frequent in the 60–70-year age group. The concentration of UTI among younger adult women is consistent with the recognized epidemiology of uncomplicated UTI, whereas the increasing occurrence among older men may reflect age-associated urological factors. In particular, benign prostatic enlargement, bladder outlet obstruction, impaired urinary clearance, and other age-related urological abnormalities may increase susceptibility to urinary infection in older males (17–19). These findings emphasize the importance of considering age and sex when interpreting local UTI surveillance data rather than relying exclusively on aggregate prevalence estimates. The antimicrobial susceptibility profile observed in this study demonstrates a substantial burden of resistance among UPEC isolates. Ampicillin showed the highest resistance rate, with 97.2% of isolates classified as resistant, followed by cefixime (87.6%), ciprofloxacin (56.6%), tetracycline (55.2%), and levofloxacin (46.9%). The particularly high resistance to ampicillin and cefixime is consistent with the broader antimicrobial resistance pattern reported among UPEC in Pakistan. Studies involving Pakistani women with uncomplicated UTI have similarly documented extensive resistance among UPEC isolates (20). At the same time, national data have demonstrated high levels of third-generation cephalosporin resistance, particularly among hospital-associated isolates (21). Together, these observations suggest that commonly used β -lactam agents may have substantially reduced utility for empirical UTI management in some Pakistani settings. The high prevalence of fluoroquinolone resistance is also clinically relevant. More than half of the isolates were resistant to ciprofloxacin, while almost half were resistant to levofloxacin. Fluoroquinolone resistance among UPEC is particularly concerning because these agents have been used extensively to treat urinary tract infections. The observed resistance profile therefore reinforces the importance of local susceptibility data when selecting empirical therapy. It suggests that continued reliance on historical prescribing practices without contemporaneous antibiogram data may increase the likelihood of treatment failure. These findings also align with the broader multidrug-resistant phenotype described among UPEC populations in Pakistan. Resistance to carbapenems was comparatively lower than resistance to penicillins, cephalosporins, and fluoroquinolones, with meropenem and imipenem resistance observed in 13.1% and 6.9% of isolates, respectively. These proportions fall between community- and hospital-associated resistance estimates previously reported at the national level, where substantially higher resistance has been described among hospital-associated isolates (22). Similar resistance patterns have also been reported from Dera Ismail Khan, another region of Khyber Pakhtunkhwa (19). Nevertheless, the presence of carbapenem-resistant isolates in an outpatient-derived collection remains epidemiologically important because carbapenems are generally reserved for infections caused by organisms resistant to multiple other antimicrobial classes. The emergence and dissemination of carbapenem-resistant UPEC carrying clinically important determinants such as *bla*CTX-M-15 and *bla*NDM-5 have already been documented in Pakistan (23). Accordingly, the relatively lower carbapenem resistance observed in the present study should not be interpreted as evidence that these agents can be used without surveillance; rather, it highlights the importance of preserving their effectiveness through appropriate antimicrobial stewardship and resistance monitoring. Fosfomycin, amikacin, and piperacillin-tazobactam demonstrated the highest *in vitro* activity against the isolates examined, with resistance rates of 4.8%, 4.1%, and 2.1%, respectively. The high activity observed for fosfomycin is consistent with its continued importance as a therapeutic option for susceptible UPEC, particularly in the context of resistance to commonly used oral agents (24). Piperacillin-tazobactam also demonstrated substantial *in vitro* activity in the present collection. However, *in vitro* susceptibility should not be interpreted independently of infection site, patient characteristics, pharmacokinetic and pharmacodynamic considerations, and the underlying resistance mechanisms of individual isolates. Furthermore, the relatively favorable activity of these agents is a cross-sectional observation. Local surveillance should reassess it rather than treating it as a stable characteristic of the regional UPEC population. A major component of the present study was the molecular investigation of tetracycline resistance. Among the phenotypically tetracycline-resistant isolates, *tetA* was reported more frequently than *tetB* in the primary study results, suggesting that *tetA*-associated resistance may be an important determinant within this UPEC population. Both genes encode resistance mechanisms associated with tetracycline efflux, although their distribution can vary considerably according to bacterial lineage, mobile genetic elements, antimicrobial exposure, and geographical setting. The predominance of *tetA* observed here differs from findings reported among carbapenem-resistant UPEC isolates from Lahore and Faisalabad, where *tetB* was more frequently detected than *tetA* (23). This geographic variation is biologically plausible because tetracycline resistance determinants can be associated with distinct plasmid backbones and other mobile genetic elements, resulting in heterogeneous distributions among bacterial populations. Evidence from multinational studies also supports the difference between the present findings and those reported elsewhere in Pakistan: tetracycline resistance is common among UPEC, but the relative prevalence of individual tetracycline resistance determinants is not uniform across populations (25, 26). Therefore, the distribution of *tetA* and *tetB* should not be assumed to follow a single national or global pattern. Instead, the findings

support the value of geographically resolved molecular surveillance for identifying locally prevalent resistance determinants and understanding their potential contribution to antimicrobial resistance in specific UPEC populations (26). Importantly, detection of *tetA* or *tetB* provides evidence for the presence of recognized tetracycline resistance determinants but does not, by itself, establish that either gene is solely responsible for the observed phenotype. Tetracycline resistance can involve multiple mechanisms, including additional efflux systems, ribosomal protection proteins, enzymatic mechanisms, regulatory changes, and combinations of resistance determinants. Moreover, the PCR approach used in this study was restricted to two predefined genes. Consequently, the molecular findings should be interpreted as evidence of the distribution of selected tetracycline resistance determinants rather than as a comprehensive characterization of the genetic basis of tetracycline resistance. The high resistance observed to cefixime and the measurable resistance to carbapenems also warrant further molecular investigation. The present study did not characterize ESBL, AmpC, or carbapenemase determinants; therefore, the mechanisms underlying these phenotypes cannot be established from the available data. This represents an important knowledge gap because resistance to third-generation cephalosporins and carbapenems in UPEC may arise through diverse combinations of β -lactamases, porin alterations, efflux mechanisms, and other genomic determinants. Previous investigations in Pakistan have identified ESBL-producing and carbapenem-resistant UPEC carrying clinically important resistance genes, including *bla*CTX-M-15 and *bla*NDM-5 (23). Future molecular surveillance should therefore extend beyond *tetA* and *tetB* to include major β -lactamase and carbapenemase determinants, as well as plasmid-associated resistance mechanisms.

CONCLUSION

This study characterized 145 confirmed uropathogenic *E. coli* isolates and identified two distinct epidemiological patterns: infections clustered among women of reproductive age, likely reflecting anatomical vulnerability, and a separate increase among older men, more consistent with age-related urological obstruction. The resistance profile is equally important: ampicillin and cefixime have become largely unreliable as empirical choices, while fluoroquinolone and tetracycline resistance is sufficiently high to warrant susceptibility testing before use.

Carbapenems, fosfomycin, and piperacillin-tazobactam were generally effective, but the modest level of resistance already observed should be seen as a warning sign, not a reassuring one. The tetracycline resistance gene profile (*tetA* more common than *tetB*) differed from that reported elsewhere in Pakistan, highlighting that resistance mechanisms are local and should not be extrapolated from national or global data. Overall, these results suggest a need to re-evaluate empirical prescribing guidelines in this region, given the diminished value of first-line agents, and to preserve the antibiotics that remain effective through careful antimicrobial stewardship and ongoing genotype-based surveillance to detect new resistance before it becomes a larger clinical issue.

Limitations and recommendations

This study was limited by its single-center, cross-sectional design and recruitment from a tertiary-care hospital, which may restrict the generalizability of the findings and preclude assessment of temporal trends in antimicrobial resistance. Molecular characterization was limited to *tetA* and *tetB*, and we did not investigate ESBL, AmpC, carbapenemase, plasmid, or clonal determinants. Future multicenter and longitudinal surveillance studies should therefore incorporate broader molecular characterization, including whole-genome sequencing and plasmid profiling, to elucidate the mechanisms and dissemination of antimicrobial resistance. Strengthening routine culture-based susceptibility testing and local antimicrobial stewardship programs is also recommended to support evidence-based empirical therapy and preserve the effectiveness of currently active antibiotics.

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AUTHORS' CONTRIBUTION

Concept & Design of Study: Aftab Ali1

Drafting: Aftab Ali, Muhammad Salahuddin, Sofia Bano

Sample Collection & Processing: Aftab Ali1, Aamir Sohail, Dildar Khan

Critical Review & Supervision: Sadaf Anwar Qureshi2

Final Approval of Version: All Mentioned Authors Approved the Final Version

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