

PREVALENCE OF ANTIMICROBIAL SUSCEPTIBILITY PROFILE AND SUL1/SUL2 GENES AMONG UROPATHOGENIC ESCHERICHIA COLI (UPEC) ISOLATED FROM UTI PATIENTS IN A TERTIARY CARE HOSPITAL IN PESHAWAR, PAKISTAN

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ABSTRACT

Background: *Escherichia coli* is the most common cause of urinary tract infections (UTIs) which are among the most prevalent bacterial infections in the world. Rising antimicrobial resistance, particularly among Uropathogenic *E. coli* (UPEC), is increasingly compromising empirical treatment in resource-limited settings such as Pakistan, yet region-specific antibiogram and molecular resistance data from Peshawar remain scarce. This study aimed to determine the antimicrobial susceptibility profile of UPEC isolated from UTI patients at a tertiary care hospital in Peshawar and to screen co-trimoxazole-resistant isolates for the *sul1* and *sul2* sulfonamide-resistance genes. **Materials and Methods:** In this descriptive cross-sectional study, 150 non-duplicate *E. coli* samples were recovered from urine cultures of UTI patients over a twelve-month period march 2024 to Feb 2025. Antimicrobial susceptibility to fifteen antibiotics across eight classes was determined by the Kirby Bauer disk diffusion method followed the EUCAST, CLSI, 2025 guidelines. All co-trimoxazole-resistant UPEC isolates were screened by PCR for the presence of *sul1* and *sul2* genes. Resistance rates were compared between male and female patients using Fisher's exact test, with $p < 0.05$ considered significant. **Results:** Of the 150 isolates, 88 (58.7%) were from female and 62 (41.3%) from male patients. Carbapenems remained highly effective, with resistance to imipenem and meropenem of only 2.7% and 3.3%, respectively. In contrast, resistance was high to very high against gentamicin (50.0%), kanamycin (57.3%), the fluoroquinolones (63.3–66.0%), third-generation cephalosporins (63.3–66.7%), cefaclor (71.3%), nalidixic acid (80.7%), co-trimoxazole (81.3%), doxycycline (81.3%), cephradine (88.7%) and ampicillin (88.0%). Statistically no significant differences in resistance rates were observed with respect to gender female, and male patients for any antibiotic tested (all $p > 0.05$). Among the 122 co-trimoxazole-resistant isolates, *sul2* was detected in 46 (37.7%) and *sul1* in 19 (15.6%), with the majority of resistant isolates negative for both genes. **Conclusion:** UPEC isolated in Peshawar show extensive multidrug resistance to conventional empirical agents, while carbapenems remain the most reliable therapeutic option. *sul2* was the predominant sulfonamide-resistance determinant, differing from previously reported regional patterns, and a substantial proportion of resistant isolates carried neither gene, indicating additional undefined resistance mechanisms. These findings underscore the need for local antibiogram-guided empirical therapy, judicious carbapenem stewardship, and continued molecular surveillance.

INTRODUCTION

Urinary tract infections are one among the most common bacterial infections encountered in clinical practice, occurring in all age groups and causing a significant burden of disease in health care systems around the world.^[1] The incidence is estimated to be several hundred million cases per year worldwide, and the disease burden is higher in women due to anatomical and physiological predisposing factors.^[1,2] *E. coli* is the major contributor of both community, and hospital acquired UTI, and has a wide array of virulence factors that allow it to colonize and persist in the urinary tract.^[3] With the worldwide rise of antimicrobial resistance (AMR), the clinical management of UTIs has become more complex. In 2019, a detailed study of the global burden of bacterial AMR estimated that almost 1.27 million people died directly from AMR infections, with *E. coli* being one of the most important pathogens associated with these deaths.^[4] The World Health Organization's Global Antimicrobial Resistance and Use Surveillance System (GLASS) has also reported that over 20% of *E. coli* isolates globally are resistant to both first-line drugs (ampicillin and co-trimoxazole) and second-line fluoroquinolones, limiting the number of effective oral treatment options for uncomplicated UTIs.^[5]

This trend is especially true in low and middle-income countries like Pakistan, where antibiotics are often available over the counter and are often prescribed on a trial-and-error basis before culture and sensitivity results are known. Acquired non-susceptibility to at least one agent in three or more antimicrobial categories,^[6] is now disproportionately common among uropathogenic *E. coli* (UPEC) isolated from various parts of the country. Ampicillin, co-trimoxazole and fluoroquinolones have been reported to have high level of resistance in studies from Khyber Pakhtunkhwa and Balochistan provinces, whereas carbapenems have generally shown the highest level of retained activity.^[7,8] Even this final barrier is now being breached, however, as carbapenem-resistant UPEC clones with extended-spectrum β -lactamase and metallo- β -lactamase genes have been reported from tertiary care hospitals in Lahore and Faisalabad.^[9] Although Khyber Pakhtunkhwa province is a major referral center for the surrounding districts, comparatively little data are available on the antibiogram of UPEC isolated from patients in Khyber Pakhtunkhwa province and Peshawar in particular.

Resistance to co-trimoxazole (sulfamethoxazole-trimethoprim) is of particular concern, as this antibiotic has been a first-line treatment for uncomplicated UTIs in resource-limited settings. Resistance to sulfonamides is primarily mediated by the acquisition of one or more alternative genes encoding dihydropteroate synthase (dihydropteroate II) that have a significantly lower affinity for the drug (sul1, sul2, and, less frequently, sul3).^[10] These genes

are often found on mobile genetic elements like class 1 integrons and conjugative plasmids, which can be horizontally transmitted not only between *E. coli* populations but also between different bacterial species and ecological reservoirs, including humans, animals, and the environment.^[10,11] In Pakistan, molecular surveillance of carbapenem-resistant UPEC has already shown that the distribution of sul1 and sul2^[2] is significantly different between the two groups of sulfonamide-resistant isolates,^[9] highlighting the importance of ongoing local surveillance to understand the genetic basis of resistance in a population.

Given this substantial geographic and institutional variability in both phenotypic resistance patterns and the underlying genetic determinants of resistance, standardized antimicrobial susceptibility surveillance combined with molecular characterization of resistance genes remains essential for guiding rational, evidence-based empirical therapy and for informing local antibiotic stewardship policies. The present study was therefore designed to determine the current antimicrobial susceptibility profile of uropathogenic *E. coli* isolated from urine samples of UTI patients attending a tertiary care hospital in Peshawar, Pakistan, and to screen all co-trimoxazole-resistant isolates for the presence of the sul1^[1] and sul2^[2] sulfonamide-resistance genes, with the aim of providing updated, region-specific data to inform clinical decision-making and future antimicrobial stewardship efforts in this setting.

MATERIALS AND METHODS

Study design, setting and duration

This descriptive, cross-sectional study was carried out in the Department of Microbiology of a tertiary care hospital in Peshawar, Khyber Pakhtunkhwa, Pakistan, over a twelve-month period (March 2024 to Feb 2025). Patients presenting to the outpatient and inpatient departments with clinical features suggestive of urinary tract infection (UTI) and referred for routine urine culture and sensitivity testing were considered for enrolment.

Sample collection and inclusion criteria

Mid-stream urine specimens were collected under aseptic conditions in sterile, wide-mouthed, leak-proof containers from patients of either sex and any age with a clinical suspicion of UTI. Samples were transported to the laboratory and processed within two hours of collection; where immediate processing was not possible, specimens were refrigerated at 4 °C and cultured within twenty-four hours. To avoid duplication, single sample per patient was included in the final analysis. Samples yielding no bacterial growth, mixed growth of two or more organisms (regarded as probable contamination), or growth of an organism other than *E. coli* were excluded.

Bacterial isolation and identification

A calibrated 0.001 mL inoculating loop was used to culture each urine sample onto MacConkey agar and

Cysteine Lactose Electrolyte Deficient (CLED) agar, followed by aerobic incubation at 37 °C for 18–24 hours. Significant bacteriuria was defined as a colony count of $\geq 10^5$ colony-forming units per milliliter (CFU/mL). Lactose-fermenting, pink colonies on MacConkey agar were sub-cultured onto nutrient agar to obtain pure growth, and the identity of *Escherichia coli* was confirmed using standard microbiological techniques, comprising Gram staining and a battery of biochemical tests (indole, methyl red, Voges Proskauer, citrate utilization, triple sugar iron, and motility testing). Confirmed isolates were preserved in nutrient broth supplemented with 20% glycerol and stored at –80 °C for subsequent susceptibility and molecular testing.

Antimicrobial susceptibility testing

Antimicrobial susceptibility of all confirmed *E. coli* isolates was determined by the Kirby–Bauer disk diffusion method on Mueller–Hinton agar, performed and interpreted in accordance with Clinical and Laboratory Standards Institute (CLSI), and guidelines.^[12,13] Briefly, a bacterial suspension adjusted to a turbidity equivalent to the 0.5 McFarland standard was prepared from an 18-hour culture and swabbed uniformly across the surface of Mueller–Hinton agar plates. Antibiotic-impregnated discs were applied and the plates incubated aerobically at 37 °C for 18–24 hours, after which the diameter of the zone of inhibition around each disc was measured in millimetres using a digital calliper and classified as susceptible, intermediate, or resistant according to current CLSI breakpoints.^[13]

A panel of fifteen antibiotics spanning eight antimicrobial classes was tested: the carbapenems imipenem and meropenem; the aminoglycosides gentamicin and kanamycin; the fluoroquinolones ciprofloxacin, ofloxacin and enoxacin; the quinolone nalidixic acid; the third-generation cephalosporins cefotaxime and ceftazidime; the second-generation cephalosporin cefaclor; the first-generation cephalosporin cephradine; the amino penicillin ampicillin; the tetracycline doxycycline; and the folate pathway inhibitor co-trimoxazole (sulfamethoxazole–trimethoprim).

The bacterial strains were classification is MDR if they showed resistance to at least one of the antibiotic among three different classes of antibiotics, using the standardized international expert-consensus definition.^[6] For quality control we used *E. coli* ATCC 25922 as a reference strain in every batch of testing to ensure the validity of the results.

Extraction of bacterial genomic DNA

The genomic DNA was extracted from every isolate found to be phenotypically resistant to co-trimoxazole, using the boiling-lysis method. Briefly, two to three pure bacterial colonies from fresh culture were suspended in 200 μ L of nuclease-free water, heated at 95–100 °C for 10 minutes, and immediately chilled on ice for 5 minutes to lyse the bacterial cells. The lysate was then centrifuged at 10,000 rpm for 5 minutes, and the clear supernatant containing the template DNA was carefully transferred to a fresh tube and stored at –20 °C until use. DNA concentration and purity (A260/A280 ratio) were verified spectrophotometrically prior to amplification.

PCR detection of *sul1* and *sul2* genes

All co-trimoxazole-resistant isolates were screened by conventional polymerase chain reaction (PCR) for the two most common sulfonamide-resistance determinants, *sul1* and *sul2*, using previously described gene-specific primer pairs generating amplicons of 430 bp and 290 bp, respectively.^[14,15] Each 25 μ L reaction contained template DNA, PCR master mix, forward and reverse primers at working concentration, and the nuclease-free water was added to adjust the final volume to 25 μ L each tube. Amplification was carried out in Bio Rad thermocycler with pre-specified condition which was: initial denaturation at 94 °C for 5 minutes; 30 cycles of denaturation at 94 °C for 30 seconds, primer annealing at 55–57 °C for 30 seconds, and extension at 72 °C for 45 seconds; followed by a final extension at 72 °C for 7 minutes. Amplified products quality and quantity on 1.55 agarose gel via electrophoresis, and visualized under ultraviolet transillumination, and sized against a 100 bp DNA ladder.

Primer lists

Primers	Sequences (5'-3')	Length	Annealing T	References
<i>Sul1</i>	F: 5'-CGGCGTGGGCTACCTGAACG-3' R: 5'-GCCGATCGCGTGAAGTTCCG-3'	450bp	57°C	(14, 15)
<i>Sul2</i>	F: 5'-GCGCTCAAGGCAGATGGCATT-3' R: 5'-GCGTTTGATACCGGCACCCGT-3'	290bp	55°C	(14, 15)

Statistical Analysis

Data were entered into Microsoft Excel and analyzed using IBM SPSS Statistics, version 28.0 (16). Categorical variables, including the resistance rate for each antibiotic tested, were summarized as frequencies and percentages. Differences in resistance rates between male and female patients were compared using Fisher's exact test, selected over the chi-square test on account of the relatively small stratified sample sizes for several comparisons.

A two-tailed p-value of less than 0.05 was considered statistically significant.

RESULTS

Study population and isolate characteristics

A total of 150 non-duplicate *E. coli* isolates were recovered from urine cultures of patients with clinically and microbiologically confirmed urinary tract infection (UTI) at a tertiary care hospital in

Peshawar, Pakistan. Of these, 88 isolates (58.7%) were obtained from female patients and 62 (41.3%) from male patients, consistent with the higher burden of UTI generally reported among women.

Overall antimicrobial susceptibility pattern

Antimicrobial susceptibility testing revealed a marked divergence in efficacy between antibiotic classes. The carbapenems, imipenem and meropenem, remained the most reliably active agents, with resistance detected in only 2.7% and 3.3% of isolates, respectively, indicating that carbapenem resistance has not yet become widespread in this uropathogenic *E. coli* (UPEC) population.

In contrast, resistance to most other agents tested was high to very high. More than half of the isolates were resistant to gentamicin (50.0%) and kanamycin (57.3%), and resistance exceeded 60% for the fluoroquinolones ofloxacin (64.0%), enoxacin (63.3%) and ciprofloxacin (66.0%), as well as for the third-generation cephalosporins cefotaxime (63.3%) and ceftazidime (66.7%). Resistance was even higher against cefaclor (71.3%), nalidixic acid (80.7%), co-trimoxazole (81.3%) and doxycycline (81.3%), and the greatest levels of resistance were recorded for the

first-generation cephalosporin cephradine (88.7%) and the aminopenicillin ampicillin (88.0%). Overall, the results depict a UPEC population with extensive resistance to the older, commonly prescribed empirical agents (penicillins, first-/second-generation cephalosporins, folate pathway inhibitors and tetracycline), while carbapenems remain the most dependable therapeutic option.

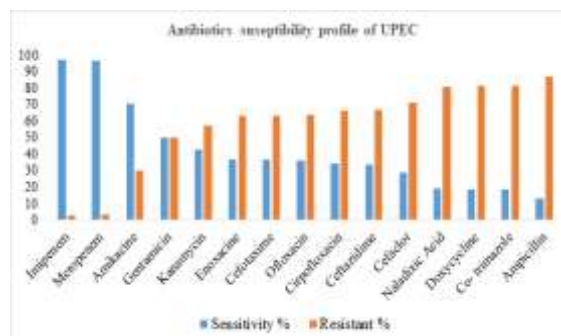


Figure 1: Antimicrobial susceptibility profile of uropathogenic *E. coli* isolates (n = 150) recovered from UTI patients. Bars represent the percentage of isolates susceptible (blue) and resistant (red) to each antibiotic tested

Table 1: Antimicrobial susceptibility pattern of uropathogenic *E. coli* isolates (n = 150), overall and stratified by patient sex

Antibiotic	Class	Overall R (%)	Female R (%) (n = 88)	Male R (%) (n = 62)	Fisher's exact p
Imipenem	Carbapenem	4 (2.7)	2 (2.3)	2 (3.2)	1.000
Meropenem	Carbapenem	5 (3.3)	3 (3.4)	2 (3.2)	1.000
Gentamicin	Aminoglycoside	75 (50.0)	49 (55.7)	26 (41.9)	0.135
Kanamycin	Aminoglycoside	86 (57.3)	50 (56.8)	36 (58.1)	1.000
Ofloxacin	Fluoroquinolone	96 (64.0)	53 (60.2)	43 (69.4)	0.301
Enoxacin	Fluoroquinolone	95 (63.3)	54 (61.4)	41 (66.1)	0.608
Cefotaxime	3rd-gen cephalosporin	95 (63.3)	54 (61.4)	41 (66.1)	0.608
Ciprofloxacin	Fluoroquinolone	99 (66.0)	56 (63.6)	43 (69.4)	0.489
Ceftazidime	3rd-gen cephalosporin	100 (66.7)	57 (64.8)	43 (69.4)	0.601
Cefaclor	2nd-gen cephalosporin	107 (71.3)	60 (68.2)	47 (75.8)	0.361
Co-trimoxazole	Folate pathway inhibitor	122 (81.3)	75 (85.2)	47 (75.8)	0.201
Doxycycline	Tetracycline	122 (81.3)	73 (83.0)	49 (79.0)	0.671
Nalidixic acid	Quinolone	121 (80.7)	70 (79.5)	51 (82.3)	0.834
Cephradine	1st-gen cephalosporin	133 (88.7)	77 (87.5)	56 (90.3)	0.794
Ampicillin	Aminopenicillin	132 (88.0)	79 (89.8)	53 (85.5)	0.453

Susceptibility pattern according to patient sex

When resistance rates were compared between male and female patients, some numerical differences were noted, but none reached statistical significance for any of the 15 antibiotics tested (Fisher's exact test, all $p > 0.05$; Table 1). Susceptibility to the carbapenems was uniformly high in both sexes, with resistance to imipenem and meropenem each detected in only two isolates per sex group. Female isolates tended to show somewhat higher resistance to gentamicin (55.7% vs 41.9%) and co-trimoxazole (85.2% vs 75.8%), while male isolates showed slightly higher resistance to several fluoroquinolones and cephalosporins, including ofloxacin (69.4% vs 60.2%), ciprofloxacin (69.4% vs 63.6%), ceftazidime (69.4% vs 64.8%) and cefaclor (75.8% vs 68.2%). These findings suggest that patient sex was not an

independent determinant of the resistance phenotype in this UPEC cohort.

Detection of *sul1* and *sul2* genes among co-trimoxazole-resistant isolates

Given the high prevalence of co-trimoxazole (SXT) resistance observed (122/150 isolates, 81.3%), all SXT-resistant isolates were screened by PCR for the two most common sulfonamide-resistance determinants, *sul1* and *sul2*. The *sul1* gene was detected in 19 of the 122 resistant isolates (15.6%), while the *sul2* gene was detected in 46 isolates (37.7%), making *sul2* the more prevalent determinant, occurring at more than double the frequency of *sul1* in this collection. Together, these two genes accounted for the resistance mechanism in a substantial proportion of the phenotypically SXT-resistant isolates, although the majority of resistant strains that were negative for both genes points to the

likely contribution of additional or alternative resistance mechanisms—such as *sul3*, chromosomal folP mutations, or mobile genetic elements not targeted in this screen—warranting further molecular characterization in future work.

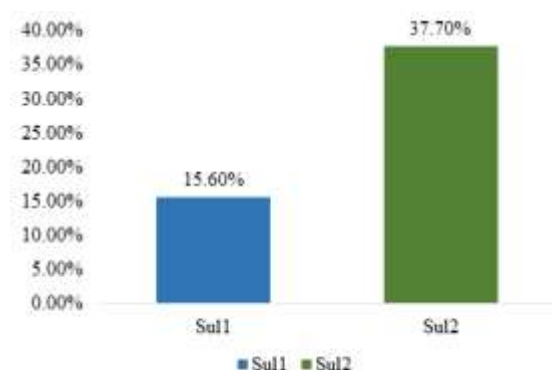


Figure 2: Prevalence of *sul1* and *sul2* genes among co-trimoxazole-resistant *E. coli* isolates (n = 122)

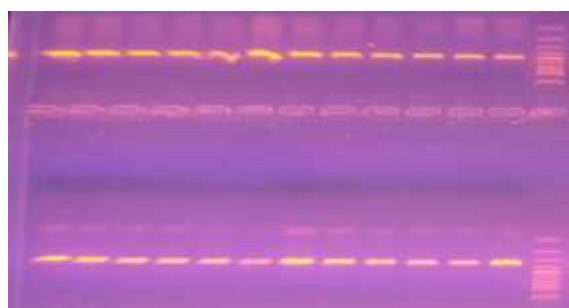


Figure 3: PCR, and Gel based confirmation of *Sul1*, and *sul2* genes

DISCUSSION

The present study demonstrates that uropathogenic *Escherichia coli* (UPEC) isolated from UTI patients attending a tertiary care hospital in Peshawar exhibit extensive resistance to most of the antibiotics traditionally relied upon for empirical therapy, while retaining high susceptibility to the carbapenems. This pattern is broadly consistent with regional and global surveillance data, which have repeatedly documented that older, inexpensive, and widely available agents, penicillins, co-trimoxazole, first-/second-generation cephalosporins and tetracycline's, are now unreliable for empirical UTI management in many low- and middle-income settings, largely as a consequence of unregulated antibiotic availability, widespread self-medication, and inconsistent adherence to prescribing guidelines.^[5,17,18]

The resistance rate for ampicillin (88.0%) and cephadrine (88.7%) observed here closely mirrors, and in some respects exceeds, rates previously reported from Khyber Pakhtunkhwa and Balochistan (7, 8), reinforcing the view that aminopenicillins and first-generation cephalosporins have largely lost their clinical utility against UPEC in this region. Similarly, the high resistance to co-trimoxazole (81.3%) confirms the continued erosion of a drug that was

once a mainstay of first-line UTI therapy in resource-limited settings, a trend attributed globally to the ease with which sulfonamide-resistance determinants disseminate on mobile genetic elements across clinical, veterinary, and environmental *E. coli* populations.^[10,11]

Resistance to the fluoroquinolones (63.3–66.0%) and third-generation cephalosporins (63.3–66.7%) was similarly high, a pattern consistent with the co-selection that frequently occurs between these two drug classes. Fluoroquinolone resistance in *E. coli* typically arises through the stepwise accumulation of chromosomal mutations in the *gyrA* and *parC* genes together with plasmid-mediated quinolone-resistance determinants, while resistance to expanded-spectrum cephalosporins is most often attributable to acquisition of extended-spectrum β -lactamase (ESBL)-encoding genes, frequently carried on the same conjugative plasmids that harbor fluoroquinolone- and sulfonamide-resistance genes.^[19–21] Although ESBL production was not phenotypically confirmed in the present study, the concurrently high resistance to third-generation cephalosporins strongly suggests that ESBL-producing UPEC clones are now widespread within this patient population, a concern echoed by recent reports of carbapenem-resistant, ESBL- and metallo- β -lactamase-co-harboring UPEC clones emerging from tertiary care centers elsewhere in Pakistan.^[9]

Encouragingly, resistance to imipenem (2.7%) and meropenem (3.3%) remained low, confirming that carbapenems continue to represent a dependable last-line therapeutic option for complicated or MDR UPEC infections in this setting. However, this finding should not be interpreted as grounds for complacency. The same molecular surveillance that first detected *sul1*-predominant, carbapenem-resistant UPEC clones in Lahore and Faisalabad,^[9] demonstrates the speed at which a once reliable drug class can be undermined when resistance genes are added to a locally circulating, already multidrug-resistant population. However, indiscriminate or prophylactic use of carbapenems in response to high resistance to first- and second-line agents may also contribute to this process, highlighting the importance of carbapenem-sparing stewardship strategies instead of routine empirical use.^[18,22]

No statistically significant difference in resistance rates was seen between male and female patients for any of the 15 antibiotics tested, which is similar to the results of previous Pakistani studies that also found no independent association between patient sex and the UPEC resistance phenotype.^[7,8] This is biologically plausible because the resistance phenotype of the infecting strain is more likely to be determined by the antibiotic selection pressure at the community and healthcare-system level (e.g., prior antibiotic exposure, antibiotic prescribing, circulating clonal lineages) than by host sex. This difference is clinically significant, and means that empirical antibiotic selection in this context should

be based on local antibiogram data and not on patient sex.

Molecular screening of the co-trimoxazole-resistant subset revealed *sul2* to be the predominant sulfonamide-resistance determinant (37.7%), occurring at more than double the frequency of *sul1* (15.6%). This finding is notable because it contrasts with an earlier Pakistani report of carbapenem-resistant UPEC, in which *sul1* was found to predominate over *sul2* among sulfonamide-resistant isolates.^[9] Such divergence is not unexpected: *sul1* is characteristically embedded within the 3'-conserved segment of class 1 integrons and is therefore co-selected alongside other integron-borne resistance cassettes, whereas *sul2* is more commonly carried on small, broad-host-range plasmids (such as those of the IncQ and related incompatibility groups) that can disseminate independently of integron structures.^[10,22] The relative predominance of one determinant over the other in a given collection is therefore likely to reflect the particular mobile genetic elements and clonal lineages circulating locally, rather than any intrinsic biological advantage of one gene over the other, reinforcing the rationale for continued region-specific molecular surveillance rather than extrapolation from data generated elsewhere.^[10,11]

Notably, *sul1* and *sul2* together accounted for only 53.3% of phenotypically co-trimoxazole-resistant isolates, leaving a substantial proportion of resistant strains in which neither determinant was detected. This is consistent with the recognized genetic heterogeneity of sulfonamide resistance, which may also be mediated by the less common *sul3* gene, chromosomal mutations in the native *folP* gene encoding dihydropteroate synthase, or efflux- and permeability-based mechanisms, none of which were captured by the present two-gene screening panel.^[10,11] This gap highlights a clear direction for future work, namely broader molecular characterization—including *sul3* screening, *folP* sequencing, and plasmid replicon typing—to fully elucidate the genetic basis of sulfonamide resistance within this UPEC population.

Taken together, these findings carry direct implications for clinical practice and antimicrobial stewardship in Peshawar and comparable settings. The near-universal loss of activity of ampicillin, cephadrine, co-trimoxazole, doxycycline, and nalidixic acid argues strongly against their continued use as empirical first-line agents for UTI in this population, while the persistently high activity of carbapenems, tempered by the risk of resistance emergence, argues for their preservation as reserve agents guided by culture and sensitivity results rather than default empirical prescribing.^[18,23] Regular, locally generated antibiograms updated at intervals appropriate to the pace of resistance evolution—together with structured antimicrobial stewardship programmes and continued molecular surveillance of resistance determinants, remain essential to

safeguard the limited number of effective treatment options that remain.^[17,22]

Study limitations

This study has several limitations. It was conducted at a single tertiary care center over a twelve-month period, so the findings may not fully generalize to community-acquired UTIs or to other healthcare facilities in the region. Phenotypic confirmation of ESBL and carbapenemase production was not performed, limiting direct mechanistic correlation with the observed cephalosporin and carbapenem susceptibility patterns. Molecular screening was also restricted to the *sul1* and *sul2* genes, so the *sul3* gene and chromosomal *folP* mutations both possible contributors to the sulfonamide-resistance genotype-negative isolates identified here were not investigated. Finally, data on patients' prior antibiotic exposure, hospitalization history, and comorbidities were not systematically collected, which precludes an assessment of the clinical and behavioural determinants underlying the resistance patterns observed.

Future recommendations

Future work should build on these findings in several directions. Larger, multicenter studies incorporating community-acquired isolates would help establish whether the resistance patterns observed here are representative of the wider Peshawar and Khyber Pakhtunkhwa population. Phenotypic ESBL and carbapenemase confirmation, together with whole-genome sequencing and plasmid replicon typing, would clarify the genetic basis of the cephalosporin and carbapenem resistance observed and allow tracking of high-risk clones over time. The high percentage of *sul1* and *sul2* negative co-trimoxazole resistant isolates could be explained by molecular screening for *sul3* and chromosomal *folP* mutations. The association of resistance data with patients' antibiotic-exposure history and clinical outcomes would further elucidate the drivers of resistance and inform targeted stewardship interventions. Lastly, local antibiograms should be created and updated on a regular basis, and incorporated into hospital prescribing guidelines to ensure that empirical therapy remains current with the changing resistance patterns.

CONCLUSION

The present study shows that uropathogenic *E. coli* isolated from UTI patients at a tertiary care hospital in Peshawar are highly multidrug resistant to commonly used empirical drugs such as ampicillin, cephadrine, co-trimoxazole, doxycycline, and nalidixic acid with resistance rates of less than 4% for carbapenems. There was no significant difference in antimicrobial resistance patterns between males and females, suggesting that patient sex should not be used as a primary determinant for empirical treatment decisions, but rather local resistance surveillance data. The molecular screening showed that *sul2* was

the most common sulfonamide-resistance determinant in co-trimoxazole-resistant isolates, with a frequency of more than twice that of *sul1*, which is in contrast to the previous regional reports. Many phenotypically resistant isolates lacked both genes, suggesting that other, uncharacterized resistance mechanisms exist and should be explored at the molecular level. Together, these results highlight the critical need for regular local antibiogram surveillance, careful use of carbapenems, and enhanced antimicrobial stewardship policies to ensure that there are still treatment options available and to guide rational, evidence-based empirical treatment of UTI in this and similar resource-limited environments.

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