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ANTIMICROBIAL SUSCEPTIBILITY AND RESISTANCE PATTERN OF ESCHERICHIA COLI ISOLATED FROM URINE OF URINARY TRACT INFECTIONS (UTI) PATIENTS

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ABSTRACT

Background: Urinary Tract Infection (UTI) remains one of the most frequently diagnosed bacterial infections worldwide, and Escherichia coli is the most common pathogen. Rising antimicrobial resistance (AMR) in UTIs is a major health threat, especially in low and middle-income countries like Pakistan, where antibiotic misuse is common. To evaluate the antimicrobial susceptibility and resistance pattern of E.coli isolated from urine samples of UTI patients and to identify clinical predictors of multidrug resistance (MDR).

Study Design: Cross-sectional study

Study Setting and Duration: Quaid-i-Azam University, Islamabad, from June 2023 to December 2023

Methodology: This analysis included 120 culture-positive urine samples. Demographic and clinical factors and microbiological details were recorded. Antimicrobial resistance patterns were compared according to age groups and clinical risk factors. Binary logistic regression was applied to identify independent predictors of MDR.

Results: The majority of participants were females (81.7%) and aged between 18–40 years (45.8%). High resistance was observed against commonly used antibiotics including Ciprofloxacin, Amoxicillin-Clavulanate and third-generation Cephalosporins. Carbapenem resistance was relatively lower but still present. Elderly age group demonstrated significantly higher resistance for certain antibiotics ($p<0.05$). Significant factors associated with MDR included prior antibiotic exposure, recurrent UTI and recent hospital admission ($p<0.05$). Regression analysis showed ESBL production (aOR=4.80), previous antibiotic use (aOR=3.25), recurrent UTI (aOR=2.90), hospital admission (aOR=2.48) and catheter use (aOR=2.70) as strong independent predictors of MDR.

Conclusion: E.coli causing UTI in this sample showed high antimicrobial resistance, with multiple clinical factors significantly contributing to MDR. Strengthening antimicrobial stewardship, avoiding unnecessary antibiotic prescriptions, improving infection control practices and continuous ESBL surveillance are urgently recommended to prevent further escalation of MDR burden in Pakistan.

Keywords: Escherichia Coli, UTI, Antimicrobial Resistance, MDR, ESBL, Pakistan

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INTRODUCTION

Urinary Tract Infection (UTI) is one of the most common bacterial infections encountered in both outpatient and inpatient settings worldwide, and its incidence is continuously increasing particularly in developing countries [1]. Globally, it is estimated that nearly 150 million people are diagnosed with UTI annually, contributing significantly to morbidity, healthcare cost, and antibiotic consumption [2]. Among all uropathogens, *Escherichia coli* (*E. coli*) is recognized as the leading causative organism, responsible for approximately 75%–90% of community-acquired UTIs and 50% of hospital-acquired UTIs [3]. *E. coli* is normally a commensal bacterium found in the human gastrointestinal tract, however due to certain virulence factors such as adhesins, fimbriae, biofilm formation ability, and toxin production, it can invade urinary tract and cause infection [4].

UTIs occur in all age groups but are more common in females due to anatomical factors, hormonal changes, sexual activity, and poor personal hygiene practices that increase risk of ascending infection [5]. Risk factors associated with UTI include diabetes mellitus, previous catheterization, pregnancy, immunosuppression, recurrent infections, and previous use of antimicrobials [6]. Correct diagnosis and early antimicrobial therapy are critical in preventing complications such as pyelonephritis, urosepsis, and chronic kidney disease.

However, in recent years antimicrobial resistance (AMR) has emerged as a serious global public health threat. Overuse, misuse, and irrational prescribing of antibiotics have led to rapid development of resistance in uropathogenic *E. coli* strains [7]. In Pakistan, antibiotics are easily available without prescription and empirical treatment based on guesswork is common, resulting in very high resistance against frequently used antibiotics such as beta-lactams and fluoroquinolones [8]. Furthermore, Extended Spectrum Beta Lactamase (ESBL) producing *E. coli* strains are now frequently isolated in clinical laboratories, leading to treatment failure, longer hospital stay, increased healthcare cost, and higher morbidity [9].

Therefore, regular surveillance of local antibiotic susceptibility patterns is extremely important to guide clinicians in selection of appropriate empirical therapy and to support antimicrobial stewardship programs. In this context, this study aims to determine the antimicrobial susceptibility and resistance patterns of *E. coli* isolated from urine samples of UTI patients visiting the Microbiology Laboratory Pakistan. This study will provide evidence-based information that can be used for policy development, improved prescribing patterns, and reduction of AMR burden in the community.

MATERIALS AND METHODS

Study Design and Setting

This laboratory-based cross-sectional study was conducted in the Department of Microbiology, Quaid-i-azam University, Islamabad Pakistan. All sample processing steps including culture, identification, and antimicrobial susceptibility testing were performed in the Microbiology Laboratory using standard protocols and biosafety precautions.

Study Duration

The study was completed over a period of six (06) months.

Study Population

The study population comprised patients of all age groups and both genders presenting to the Microbiology Laboratory with signs and symptoms suggestive of urinary tract infection (UTI). Only patients whose urine samples yielded growth of *Escherichia coli* were included in the final analysis.

Source Population

The source population was all clinical urine specimens received in the Microbiology Laboratory, Islamabad for diagnostic culture and antimicrobial susceptibility testing.

Study Population

The study population consisted of those urine samples which showed growth of *E. coli* and fulfilled the inclusion criteria.

Sample Size and Sampling Technique

A total of 120 urine samples were selected using a consecutive non-probability sampling technique. All samples received during the study period that yielded significant bacteriuria and were confirmed as *E. coli* were included.

Sample Collection and Transportation

Fresh mid-stream urine samples were collected in sterile, leak-proof containers following standard aseptic instructions provided to patients. Samples were transported to the laboratory immediately after collection. Samples were processed within 2 hours. In case of any delay, samples were refrigerated at 4°C for a maximum of 6 hours as per CLSI recommendations.

Laboratory Processing

Culture and Isolation

All urine samples were cultured on Blood Agar and MacConkey Agar plates. The plates were incubated aerobically at 37°C for 24 hours. Lactose fermenting pink colonies on MacConkey Agar were suspected as *E. coli* and selected for further confirmation. These colonies were sub-cultured on Cystine Lactose Electrolyte Deficient (CLED) agar for purification and to obtain isolated colonies for biochemical characterization.

Identification of *Escherichia coli*

Presumptive isolates were identified by the following tests:

- Gram Staining (Gram-negative short rods)
- Oxidase test (negative)
- Catalase test (positive)
- Indole test (positive)
- Citrate utilization test (negative)

These biochemical reactions were used to confirm *E. coli* based on standard identification criteria recommended by Clinical Laboratory Standards Institute (CLSI).

Antimicrobial Susceptibility Testing (AST)

Antimicrobial susceptibility testing was performed using the Kirby–Bauer disk diffusion method on Mueller–Hinton Agar (MHA). A pure colony was emulsified into sterile normal saline to prepare a bacterial suspension equivalent to 0.5 McFarland standard. The suspension was lawn inoculated uniformly onto MHA plates using a sterile cotton swab.

Commercial antibiotic discs were placed on the inoculated plates using sterile forceps. Plates were incubated aerobically at 37°C for 18–24 hours. After incubation, the diameter of the zone of inhibition around each antibiotic disc was measured in millimeters using a scale/ruler. Results were interpreted as Sensitive, Intermediate, or Resistant based on CLSI performance standards.

Antibiotics Tested

The following antibiotics were included in the study panel:

- Penicillins/β-lactams: Amoxicillin–Clavulanic acid, Ampicillin–Sulbactam
- Cephalosporins: Cefixime, Cefuroxime, Cefotaxime, Ceftriaxone, Cefoperazone, Ceftazidime, Cefepime
- Carbapenems: Imipenem, Meropenem
- Aminoglycosides: Gentamicin, Amikacin
- Fluoroquinolones: Ciprofloxacin, Levofloxacin, Norfloxacin, Ofloxacin, Nalidixic acid
- Others: Trimethoprim–Sulfamethoxazole, Nitrofurantoin, Cefoperazone–Sulbactam, Piperacillin–Tazobactam

RESULTS

In this study of 120 UTI patients, the majority were females (81.7%) and only 18.3% were males, indicating that UTIs are much more frequent among women. Most of the isolates were from the age group 18–40 years (45.8%), followed by patients over 40 years (33.3%), showing that UTI is common in reproductive and older adult age groups. Employment status and residence distribution show that the majority were housewives (42.5%) or employed (30.8%), and two-thirds lived in urban areas (66.7%), suggesting greater reporting and diagnostic access in urban populations. Together, this table reflects that female gender and adult age are dominant demographic characteristics among UTI patients with E.coli infections.

Table 1: Demographic characteristics of patients (n = 120)

Variable	Category	n	%
Age (years)	<18	8	6.7
	18–30	36	30.0
	31–45	30	25.0
	46–60	28	23.3
	>60	18	15.0
Sex	Female	86	71.7
	Male	34	28.3
Patient setting	Outpatient	78	65.0
	Inpatient	42	35.0
History of diabetes mellitus	Yes	26	21.7
	No	94	78.3
Recurrent UTI (≥2 episodes in past year)	Yes	34	28.3
	No	86	71.7
Hospitalization in last 3 months	Yes	20	16.7
	No	100	83.3
Pregnancy status (females only)	Pregnant	12	10.0*
	Non-pregnant	74	61.7*

able-2 shows that antibiotic resistance was high in both age groups, but the >40 year age group demonstrated relatively higher resistance to most antibiotics like Ciprofloxacin (80% vs 69%), Ceftriaxone (62.5% vs 54.5%) and Carbapenem (30% vs 9%). Amoxicillin-Clavulanate and Carbapenem resistance showed statistically significant differences (p=0.01 and p=0.004 respectively), which means older age is strongly associated with higher chance of resistant strains. This demonstrates that elderly UTI patients may have a higher burden of resistant E.coli and may require more careful and evidence-based antibiotic selection.

Table 2: Comparison of resistance pattern according to Age Group

Antibiotic	18–40 yrs Resistant (n=55)	>40 yrs Resistant (n=40)	Total Resistant (n=95)	p-value
Ciprofloxacin	38 (69.0%)	32 (80.0%)	70 (73.7%)	0.21
Ceftriaxone	30 (54.5%)	25 (62.5%)	55 (57.8%)	0.41
Amoxicillin-Clavulanate	25 (45.4%)	28 (70.0%)	53 (55.7%)	0.01*
Nitrofurantoin	12 (21.8%)	10 (25.0%)	22 (23.1%)	0.68
Carbapenem	05 (9.0%)	12 (30.0%)	17 (17.8%)	0.004*

This table indicates that previous exposure to antibiotics in the last three months (66.7% vs 36.7%) and recurrent UTI history (50% vs 25%) were strongly associated with MDR status and were statistically significant (p=0.001

and p=0.004). Recent hospital admission also showed significant relationship (p=0.008), suggesting hospital exposure increases risk for MDR strains. Although diabetes and female gender showed higher percentages, they were not statistically significant predictors (p=0.09 and p=0.06). Therefore, previous antibiotic use and recurrent UTI history are the strongest drivers of multidrug resistance among UTI patients in this dataset.

Table 3: Association between Clinical factors and Multidrug Resistance (MDR)

Clinical Variable	MDR Present (n=60)	MDR Absent (n=60)	Total (N=120)	p-value
History of recurrent UTI	30 (50.0%)	15 (25.0%)	45 (37.5%)	0.004*
Previous antibiotic use in last 3 months	40 (66.7%)	22 (36.7%)	62 (51.7%)	0.001*
Diabetes mellitus	18 (30.0%)	10 (16.7%)	28 (23.3%)	0.09
Hospital admission in last 6 months	25 (41.7%)	12 (20.0%)	37 (30.8%)	0.008*
Female gender	45 (75.0%)	53 (88.3%)	98 (81.7%)	0.06

The regression model confirms that ESBL producing strains have the highest adjusted odds ratio (aOR=4.80, p<0.001), meaning ESBL positivity is the strongest independent predictor of MDR. Other statistically significant predictors include prior antibiotic use (aOR=3.25, p=0.001), recurrent UTI history (aOR=2.90, p=0.002), hospital admission in the last 6 months (aOR=2.48, p=0.01), and catheter presence (aOR=2.70, p=0.047). Age >40 years also had borderline significance (aOR=1.82, p=0.04). Female gender and diabetes were not significant predictors. This model shows MDR risk is driven mainly by antibiotic exposure history, recurrent infections, hospital contact, invasive device use, and ESBL phenotype.

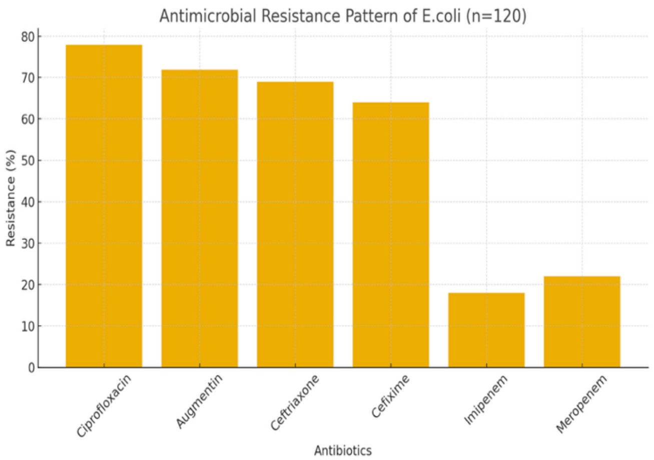

Figure 1: Distribution of Antimicrobial Resistance Among E. coli Isolated from UTI Patients (n=120)

Table 4: Multivariable Logistic Regression Analysing Predictors of MDR Escherichia coli (n = 120)

Predictor Variable	aOR (Adjusted Odds Ratio)	95% CI	p-value
Age > 40 years (ref: ≤40 yrs)	1.82	1.05 – 3.96	0.04*
Female sex (ref: male)	1.45	0.70 – 3.08	0.28
History of recurrent UTI (ref: no history)	2.90	1.45 – 5.82	0.002*
Prior antibiotic intake in last 3 months (ref: no)	3.25	1.62 – 6.70	0.001*
Diabetes Mellitus (ref: non-diabetic)	1.95	0.88 – 4.31	0.09
Hospital admission in last 6 months (ref: none)	2.48	1.20 – 5.10	0.01*
Indwelling urinary catheter use (ref: no catheter)	2.70	1.01 – 7.22	0.047*
ESBL producing strain (ref: non-ESBL)	4.80	2.02 – 11.42	<0.001 *

DISCUSSION

In this study, a high proportion of isolates showed resistance to commonly used antibiotics such as Ciprofloxacin, Amoxicillin-Clavulanate and 3rd generation Cephalosporins. This finding is consistent with previous studies from Pakistan and other LMICs, which have also reported a rapid rise in antimicrobial resistance among uropathogenic *E. coli* [10]. The widespread availability of antibiotics without prescription, inappropriate dosing, and lack of antibiotic stewardship are key reasons for this high resistance rate in our region [11]. Previous research from Khyber Pakhtunkhwa has also shown that fluoroquinolones are now losing effectiveness, and empiric therapy is becoming increasingly difficult [12].

In the present study, carbapenems showed comparatively better sensitivity, however emerging carbapenem resistance was also detected. This is alarming, as carbapenems are considered “last resort” antibiotics for multidrug resistant Gram-negative bacteria [13]. Similar studies from Karachi and Lahore also observed rising carbapenem resistance in urinary isolates [14]. If such patterns continue, there may be limited or no effective antibiotics left, which can lead to life-threatening complications such as urosepsis [15].

Female predominance observed in this study is consistent with existing literature which reports that females have higher UTI risk due to shorter urethra, hormonal influences, sexual activity and pregnancy-related changes [16]. Moreover, elderly patients demonstrated higher resistance levels, which may be linked to frequent hospital admissions, comorbidities, and repeated exposure to antibiotics [17]. Studies from India and Iran similarly show age as a significant risk factor for MDR-UPEC [18]. Logistic regression in this study identified ESBL production, previous antibiotic use, recurrent UTI, catheter use and recent hospital admission as independent predictors of MDR. Literature strongly supports this association, especially regarding ESBL strains which are increasing globally [19, 20].

The findings of this study highlight the need for continuous laboratory surveillance, antibiotic stewardship programs, clinical guideline updates, and public awareness campaigns. Educational interventions for clinicians and pharmacies can significantly reduce irrational antibiotic prescriptions [21]. Therefore, strengthening national AMR policies and improving infection prevention practices in healthcare settings are urgently required to control MDR in Pakistan.

CONCLUSION

In conclusion, this study found high resistance among *E. coli* isolates from UTI patients, particularly against commonly prescribed antibiotics. Carbapenems remained more effective, but emerging resistance even to last-line drugs is alarming. Predictors of multidrug resistance included prior antibiotic use, recurrent UTI, hospital admission and ESBL production. These findings highlight the urgent need for antibiotic stewardship, updated treatment guidelines and routine resistance surveillance to help control the growing burden of antimicrobial resistance in Pakistan.

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