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PREVALENCE, ANTIMICROBIAL RESISTANCE, AND RISK FACTORS OF ESBL-PRODUCING ENTEROBACTERIACEAE IN PATIENTS WITH URINARY TRACT INFECTIONS

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ABSTRACT

Urinary tract infections (UTIs) are among the most common bacterial infections, frequently caused by Enterobacteriaceae. The emergence of extended-spectrum beta-lactamase (ESBL)-producing strains poses a significant challenge due to multidrug resistance, complicating empiric therapy. This study aimed to determine the prevalence of ESBL-producing Enterobacteriaceae, their antimicrobial susceptibility patterns, and associated risk factors in patients with suspected UTIs. A total of 200 urine samples were collected from patients with suspected UTIs. Bacterial isolates were identified, and antimicrobial susceptibility testing was performed. ESBL production was confirmed using standard phenotypic methods. Patient demographics, clinical characteristics, and risk factors were recorded. Data were analyzed to evaluate the prevalence, species distribution, resistance patterns, and associated risk factors for ESBL-producing isolates. Of 200 samples, 150 (75%) showed significant bacterial growth, with Enterobacteriaceae comprising 130 isolates (86.7%). *Escherichia coli* was the most frequently isolated species (n = 35 ESBL isolates, 70%), followed by *Klebsiella pneumoniae* (n = 12, 24%), *Proteus mirabilis* (n = 2, 4%), and *Enterobacter cloacae* (n = 1, 2%). Overall, 50 isolates (38.5%) were confirmed as ESBL producers. Resistance was highest against ampicillin (84.6%), ceftriaxone (55.4%), and cefotaxime (53.8%), whereas carbapenem resistance remained low (3.8%). Multidrug resistance (MDR) was observed in 60% of Enterobacteriaceae isolates. ESBL prevalence was higher in females (64%) and hospitalized patients (56%). Significant risk factors for ESBL infection included prior antibiotic use ($p < 0.01$), indwelling urinary catheters ($p < 0.01$), and recurrent UTIs ($p = 0.02$). ESBL-producing Enterobacteriaceae are highly prevalent among UTI patients, particularly in hospitalized individuals and those with prior antibiotic exposure or indwelling catheters. High rates of multidrug resistance emphasize the need for routine antimicrobial susceptibility testing, careful antibiotic stewardship, and stringent infection control measures to mitigate the spread of resistant uropathogens.

1. INTRODUCTION

Urinary tract infections (UTIs) are among the most prevalent bacterial infections globally, affecting an estimated 150–250 million people each year (Søraas, Sundsfjord, Sandven, Brunborg, & Jenum, 2013). They account for a substantial proportion of outpatient visits and hospital admissions, with women being disproportionately affected—studies suggest that more than 50% of women will experience at least one UTI during their lifetime (Nakai et al., 2016). In addition to their high incidence, UTIs represent a major public health burden due to recurrent infections, the need for repeated antibiotic courses, and associated healthcare costs. The risk is particularly high in elderly patients, individuals with diabetes, pregnant women, and hospitalized patients with indwelling urinary catheters (Kurtaran1ABDEFG et al., 2010). Members of the family *Enterobacteriaceae* are the predominant pathogens responsible for UTIs. *Escherichia coli* is the most common, responsible for 80–85% of community-acquired UTIs, while *Klebsiella pneumoniae* accounts for a significant proportion of both community- and hospital-acquired cases (Junaid Ahmad & Ahmad, 2023; Rehman et al., 2023). Other species, such as *Proteus*, *Enterobacter*, and *Citrobacter*, are also implicated but less frequently. The pathogenicity of *E. coli* and *K. pneumoniae* is attributed to their virulence factors, including adhesins, fimbriae, and toxin production, which facilitate colonization and infection of the urinary tract. Over the past two decades, the treatment of UTIs has become increasingly complicated due to the alarming rise of antimicrobial resistance. Among the most concerning mechanisms is the production of extended-spectrum beta-lactamases (ESBLs). ESBLs are enzymes that hydrolyze a wide range of β-lactam antibiotics, including third-generation cephalosporins and monobactams, which are widely used in the management of UTIs. First described in the mid-1980s, ESBL-producing Enterobacteriaceae have now spread globally, with prevalence rates ranging from 2% in northern Europe to over 70% in parts of the Middle East and Africa (Abdullah, 2022; A Aziz et al., 2022). These organisms are often resistant not only to β-lactams but also to fluoroquinolones, aminoglycosides, and trimethoprim-sulfamethoxazole, leaving carbapenems as one of the few remaining effective options (Javed et al., 2023; Shah et al., 2023). However, the growing reports of carbapenem-resistant Enterobacteriaceae (CRE) highlight the threat of dwindling treatment alternatives (Ali Syed et al., 2024; S. Khan et al., 2023; Munir et al., 2023). The emergence of ESBL-producing Enterobacteriaceae has significant clinical and public health consequences. Infections caused by these organisms are associated with delayed initiation of effective therapy, prolonged hospital stays, higher treatment costs, and increased mortality (Aamir Aziz et al., 2022; Jabeen et al., 2020). In the United States, ESBL prevalence among UTI isolates rose from 7.8% in 2010 to 18.3% in 2014, while in Egypt, pooled data suggest a prevalence as high as 60% among urinary isolates. Similarly, studies in Asia have reported prevalence rates above 50%, particularly in India and Pakistan (Ahamd et al., 2022; Ullah et al., 2019). Beyond the hospital, community carriage of ESBL producers has been detected in healthy individuals—9% in Qatar and 18.8% in Kuwait—suggesting widespread dissemination outside healthcare facilities. These trends complicate empirical therapy, strain healthcare systems, and underscore the urgent need for surveillance and stewardship. Despite growing global concern, prevalence rates of ESBL-producing Enterobacteriaceae vary greatly depending on region, healthcare infrastructure, and antibiotic use practices (M. K. Khan et al., 2021; Munawar et al., 2021). Many regions still lack up-to-date data, making it difficult for clinicians to select appropriate empirical therapy. Furthermore, differences in diagnostic methods contribute to variability in reporting. Continuous monitoring of ESBL prevalence is therefore essential to improve treatment outcomes and inform infection control policies. The present study aims to determine the prevalence of ESBL-producing Enterobacteriaceae among patients with urinary tract infections. By generating local data and identifying resistance patterns, this research seeks to fill existing knowledge gaps, guide empirical antibiotic selection, and contribute to antimicrobial stewardship and public health strategies

2. Materials and Methods

2.1. Study Design and Setting

This was a cross-sectional study conducted in the Microbiology Department of a tertiary care center. The study aimed to determine the prevalence of extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae among patients clinically suspected of urinary tract infections.

2.2. Study Population

Urine samples were collected from patients of all age groups and both genders who presented with symptoms suggestive of UTIs, including dysuria, frequency, urgency, suprapubic pain, or fever with urinary complaints. Both outpatients and inpatients were included. Patients who had received antibiotics within the past 48 hours were excluded to minimize false-negative culture results.

2.3. Sample Collection and Transport

Clean-catch midstream urine specimens were obtained in sterile, wide-mouthed containers after providing proper instructions to patients. For catheterized patients, urine samples were collected aseptically from the catheter port using a sterile syringe. All samples were labeled, transported immediately to the microbiology laboratory, and processed within two hours of collection.

2.4. Culture and Identification of Isolates

Urine samples were inoculated onto Cysteine Lactose Electrolyte Deficient (CLED) agar and MacConkey agar plates using a calibrated loop delivering 0.001 mL of urine. Plates were incubated aerobically at 37°C for 18–24 hours. Significant bacteriuria was defined as a colony count of $\geq 10^5$ CFU/mL. Isolates were identified based on colony morphology, Gram staining, and standard biochemical tests, including indole, citrate utilization, urease, triple sugar iron, and motility tests. Further identification was confirmed using automated systems such as VITEK-2 (if available).

2.5. Antimicrobial Susceptibility Testing

Antibiotic susceptibility testing was performed using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar, following Clinical and Laboratory Standards Institute (CLSI) guidelines. The antibiotics tested included ampicillin, cefotaxime, ceftazidime, ceftriaxone, ciprofloxacin, gentamicin, amikacin, nitrofurantoin, trimethoprim-sulfamethoxazole, and meropenem. *Escherichia coli* ATCC 25922 was used as a quality control strain (J Ahmad & Pervez, 2021; Hayat et al., 2022; Robina et al., 2021).

2.6. Screening for ESBL Production

Isolates resistant to third-generation cephalosporins (ceftazidime 30 µg, cefotaxime 30 µg, or ceftriaxone 30 µg) were considered potential ESBL producers.

2.7. Phenotypic Confirmation of ESBL

Confirmation of ESBL production was performed by the combined disk method (CLSI guidelines). Disks containing ceftazidime (30 µg) and cefotaxime (30 µg) were placed 25 mm apart from disks containing ceftazidime-clavulanic acid (30/10 µg) and cefotaxime-clavulanic acid (30/10 µg). A ≥5 mm increase in the zone diameter around the antibiotic-clavulanic acid disk compared to the antibiotic disk alone was interpreted as ESBL positive.

2.8. Data Collection and Analysis

Demographic and clinical details of patients (age, sex, outpatient/inpatient status, risk factors) were recorded using a structured proforma. Data were analyzed using [SPSS version XX / R software]. The prevalence of ESBL-producing Enterobacteriaceae was calculated as a percentage of the total isolates. Associations between ESBL production and patient characteristics were assessed using chi-square or Fisher's exact test, with a p-value <0.05 considered statistically significant.

3. Results

3.1. Patient Demographics

A total of 200 urine samples were collected from patients suspected of urinary tract infections (UTIs), including 120 (60%) females and 80 (40%) males. The mean age of patients was 35.6 ± 14.2 years, with most patients aged 21–40 years (110, 55%), followed by 41–60 years (50, 25%), ≤20 years (20, 10%), and >60 years (20, 10%). Outpatients accounted for 130 (65%) and hospitalized patients 70 (35%). Recurrent UTIs were reported in 60 patients (30%), prior antibiotic use in 80 (40%), indwelling catheters in 30 (15%), and urinary instrumentation in 12 (6%). Comorbidities included diabetes mellitus (40, 20%), hypertension (35, 17.5%), chronic kidney disease (10, 5%), and immunocompromised conditions (8, 4%). Dysuria was present in 150 patients (75%), urinary frequency and urgency in 140 (70%), suprapubic pain in 90 (45%), fever in 50 (25%), and hematuria in 30 (15%). Clean-catch midstream urine was collected in 150 cases (75%) and catheterized samples in 50 (25%). Among females, 80 (40%) were outpatients and 40 (20%) inpatients, while among males, 50 (25%) were outpatients and 30 (15%) inpatients. Most patients (70%) presented within 3–5 days of symptom onset, while 20% reported symptoms for over 7 days (Table 1).

Table 1: Demographic and clinical characteristics of patients with suspected urinary tract infections (n = 200), including age, gender, comorbidities, risk factors, symptoms, sample type, and duration of presentation.

Variable	Category / Data	Number of Patients	Percentage (%)
Gender	Female	120	60
	Male	80	40
Age (years)	Mean ± SD	35.6 ± 14.2	-
	≤20	20	10
	21–40	110	55
	41–60	50	25
	>60	20	10
Patient Setting	Outpatient	130	65
	Hospitalized	70	35
History / Risk Factors	Recurrent UTI	60	30
	Prior antibiotic use (last 3 months)	80	40
	Indwelling catheter	30	15
	Urinary tract instrumentation	12	6
Comorbidities	Diabetes mellitus	40	20
	Hypertension	35	17.5
	Chronic kidney disease	10	5
	Immunocompromised	8	4
Symptoms	Dysuria	150	75
	Frequency/urgency	140	70
	Suprapubic pain	90	45
	Fever >38°C	50	25
	Hematuria	30	15
Sample Type	Clean-catch midstream urine	150	75
	Catheterized urine	50	25
Presentation Duration	3–5 days	140	70
	>7 days	40	20

3.2. Bacterial Growth and Isolate Distribution

Out of 200 urine samples collected from patients with suspected urinary tract infections (UTIs), 150 samples (75%) showed significant bacterial growth. Among these positive cultures, Enterobacteriaceae were the predominant pathogens, accounting for 130 isolates (86.7%), while the remaining 20 isolates (13.3%) included *Staphylococcus saprophyticus*, *Enterococcus spp.*, and other Gram-positive bacteria. The mean number of Enterobacteriaceae isolates per positive sample was 1.13 ± 0.34, reflecting occasional polymicrobial infections. Among

Enterobacteriaceae, *Escherichia coli* was the most frequently isolated species, followed by *Klebsiella pneumoniae*, *Proteus mirabilis*, and *Enterobacter cloacae*. The species-wise distribution is summarized in Figure 1.

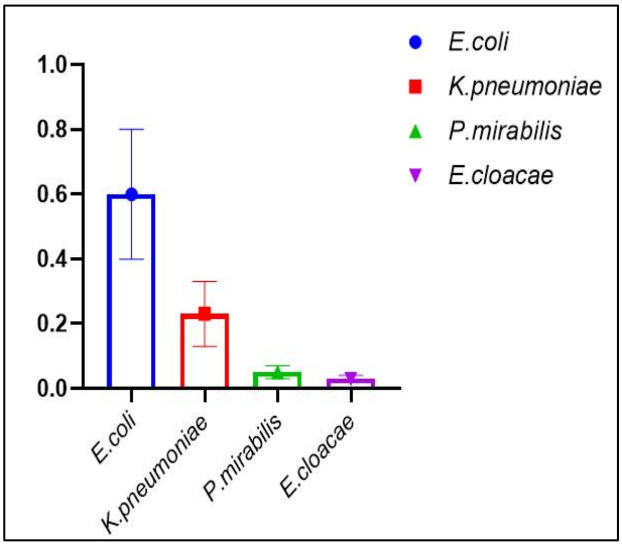

Figure 1: Distribution of bacterial isolates from urine samples of patients with suspected urinary tract infections (n = 200), highlighting the prevalence of Enterobacteriaceae and other Gram-positive organisms.

3.3. Antimicrobial Susceptibility Patterns

Among the 130 Enterobacteriaceae isolates, resistance rates varied considerably across different antibiotics. Ampicillin resistance was observed in 110 isolates (84.6%), followed by ceftriaxone resistance in 72 isolates (55.4%), cefotaxime resistance in 70 isolates (53.8%), and ceftazidime resistance in 65 isolates (50%). Resistance to fluoroquinolones was also notable, with ciprofloxacin resistance in 52 isolates (40%) and levofloxacin resistance in 50 isolates (38.5%). Aminoglycoside resistance was comparatively lower, with gentamicin resistance in 32 isolates (24.6%) and amikacin resistance in 20 isolates (15.4%). Carbapenem resistance remained minimal, with only 5 isolates (3.8%) showing resistance to imipenem or meropenem. The mean number of resistant isolates per antibiotic was 57.0 ± 33.2, highlighting the variability of resistance among different drugs. Multidrug resistance (MDR), defined as resistance to three or more antibiotic classes, was detected in 78 isolates (60%). Species-wise, *Escherichia coli* had 51 MDR isolates, *Klebsiella pneumoniae* had 21 MDR isolates, *Proteus mirabilis* had 4 MDR isolates, and *Enterobacter cloacae* had 2 MDR isolates. The mean number of MDR isolates per species was 19.5 ± 20.1, indicating a substantial burden of multidrug resistance. Notably, *K. pneumoniae* showed a higher MDR proportion (70%) compared to *E. coli* (57%), reflecting its potential as a more resistant pathogen in urinary tract infections(Figure 2).

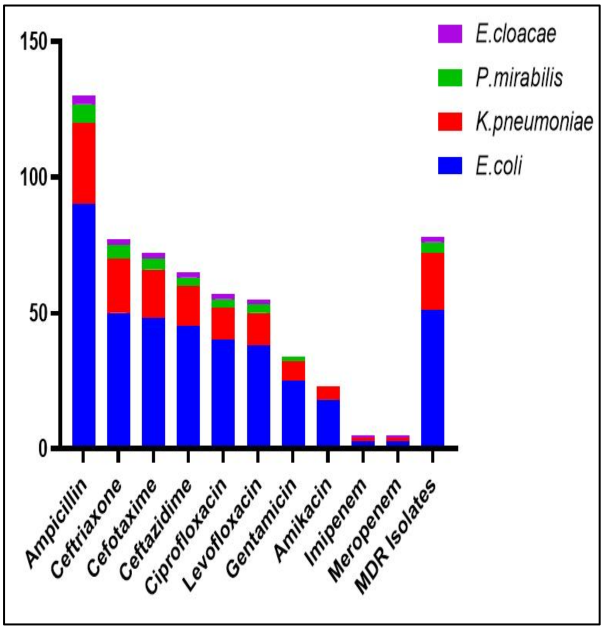

Figure 2: Antimicrobial susceptibility patterns of Enterobacteriaceae isolates (n = 130) from urinary tract infection patients, showing resistance rates across commonly used antibiotics and the prevalence of multidrug resistance (MDR).

3.4. Prevalence of ESBL-Producing Isolates

Among the 130 Enterobacteriaceae isolates obtained from patients with urinary tract infections, 50 isolates (38.5%) were confirmed as extended-spectrum beta-lactamase (ESBL) producers. ESBL production was predominantly observed in *Escherichia coli*, which accounted for 35 of the 50 ESBL isolates (70%). *Klebsiella pneumoniae* contributed 12 isolates (24%), while *Proteus mirabilis* and *Enterobacter cloacae* were less frequently identified as ESBL producers, with 2 (4%) and 1 (2%) isolates, respectively. The mean number of ESBL-producing isolates per species was 12.5 ± 14.0, reflecting a notable prevalence of ESBL-mediated resistance across different Enterobacteriaceae species. These findings underscore the clinical significance of ESBL production, which can complicate treatment due to resistance to multiple β-lactam antibiotics (Figure .3).

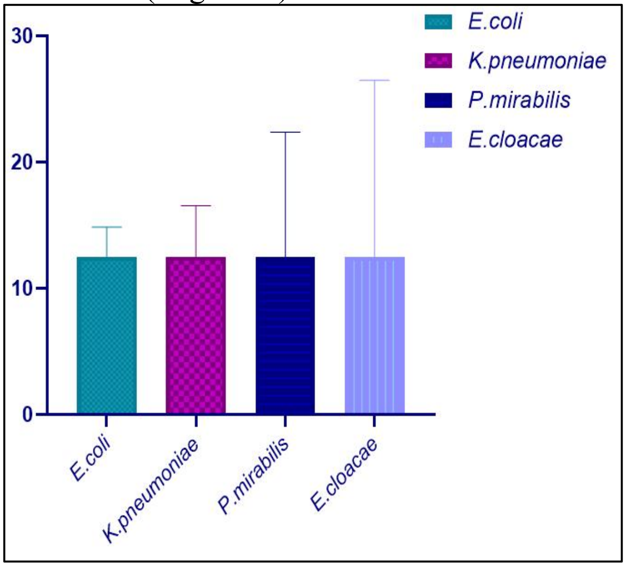

Figure 3: Prevalence of extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae isolates (n = 130) among urinary tract infection patients, with species-wise distribution.

3.5. ESBL Prevalence by Patient Demographics

Among the 50 ESBL-producing Enterobacteriaceae isolates, females accounted for 32 isolates (64%), whereas males contributed 18 isolates (36%), indicating a higher prevalence of ESBL production in female patients. Age-wise, the highest prevalence was observed in the 21–40 years age group with 25 isolates (50%), followed by 41–60 years with 15 isolates (30%), and patients older than 60 years with 10 isolates (20%). No ESBL-producing isolates were identified in patients aged ≤20 years. Hospitalization status also influenced ESBL prevalence. Hospitalized patients accounted for 28 isolates (56%), while outpatients had 22 isolates (44%), suggesting that inpatients are more likely to harbor ESBL-producing Enterobacteriaceae. The mean number of ESBL isolates per demographic category was 12.5 ± 8.7, reflecting variability across gender, age, and patient setting. These findings emphasize the need for targeted infection control and empiric therapy strategies based on patient demographics (Figure. 4).

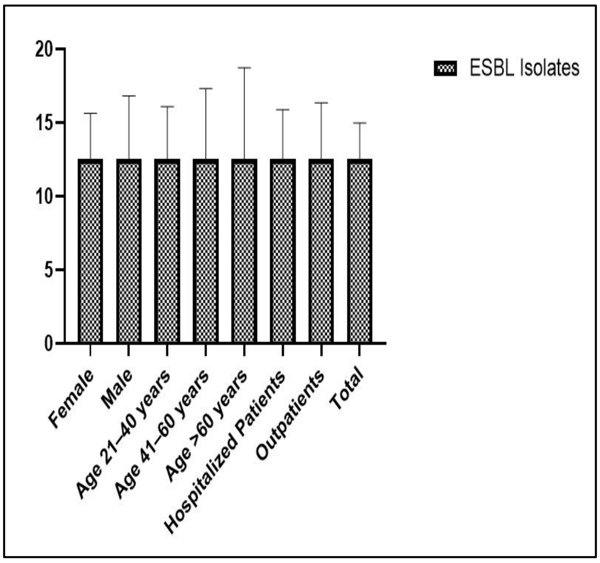

Figure.4: Distribution of ESBL-producing Enterobacteriaceae isolates according to patient demographics, including gender, age groups, and hospitalization status.

3.6. Comparison Between Community- and Hospital-Acquired UTIs

Among the 50 ESBL-producing Enterobacteriaceae isolates, 22 isolates (44%) were from community-acquired urinary tract infections (CA-UTIs), while 28 isolates (56%) were from hospital-acquired urinary tract infections (HA-UTIs). Notably, hospital-acquired isolates exhibited a higher rate of multidrug resistance (75% MDR, n = 21) compared to community isolates (50% MDR, n = 11), indicating that hospitalization is a significant risk factor for resistant infections. The mean number of ESBL isolates per infection type was 25 ± 3.5, and the mean number of MDR isolates per setting was 16 ± 5.3, highlighting the higher burden of resistance in hospitalized patients. These results emphasize the importance of infection control measures in healthcare settings to prevent the spread of ESBL-producing and multidrug-resistant organisms (Table .2).

Table 2: Comparison of ESBL-producing Enterobacteriaceae isolates and multidrug resistance (MDR) between community-acquired and hospital-acquired urinary tract infections.

Infection Type	ESBL Isolates	MDR Isolates	MDR Percentage (%)	Mean ± SD (Isolates)
Community-acquired (CA-UTI)	22	11	50	16.5 ± 7.78
Hospital-acquired (HA-UTI)	28	21	75	24.5 ± 4.95
Total	50	32	64	20.3 ± 7.56

3.7. Risk Factor Analysis

The presence of ESBL-producing Enterobacteriaceae in urinary tract infections was significantly associated with several clinical and behavioral factors. Prior antibiotic use within the last three months was observed in 30 of the 50 ESBL-positive patients (60%), demonstrating a statistically significant association with ESBL infection ($p < 0.01$). Similarly, patients with an indwelling urinary catheter accounted for 18 isolates (36%), which was also significantly associated with ESBL production ($p < 0.01$). Recurrent UTI history was present in 12 patients (24%), showing a moderate but significant association ($p = 0.02$). No statistically significant association was observed with diabetes mellitus or gender alone, although females comprised a higher absolute number of ESBL infections (32/50, 64%) compared to males (18/50, 36%). These findings indicate that antibiotic exposure, catheterization, and recurrent infections are important risk factors for acquiring ESBL-producing organisms, highlighting the need for judicious antibiotic use and careful management of indwelling catheters.

Table 3: Risk factors associated with ESBL-producing Enterobacteriaceae in urinary tract infection patients. NS = Not significant.

Risk Factor	ESBL Positive (n)	Percentage (%)	p-value
Prior antibiotic use (last 3 months)	30	60	<0.01
Indwelling urinary catheter	18	36	<0.01
Recurrent UTI history	12	24	0.02
Diabetes mellitus	8	16	NS
Female gender	32	64	NS
Male gender	18	36	NS

4. DISCUSSION

This study investigated the prevalence, antimicrobial resistance patterns, and risk factors associated with urinary tract infections (UTIs) in 200 patients, with a particular focus on extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae. The findings provide important insights into the epidemiology of UTI pathogens and their resistance profiles, which are critical for guiding empiric therapy and infection control measures. In our cohort, females were more frequently affected (60%), consistent with the well-established observation that anatomical and hormonal factors predispose women to UTIs (Briongos-Figuero et al., 2012). The majority of patients were aged 21–40 years (55%), which aligns with previous reports showing a higher incidence of UTIs among sexually active young adults (Medina-Polo et al., 2015). Outpatients represented 65% of the sample, whereas hospitalized patients constituted 35%, reflecting a mixed population of community- and hospital-acquired infections. Dysuria, frequency/urgency, and suprapubic pain were the most common symptoms, supporting prior studies where these symptoms were identified as strong clinical indicators of UTI (Kung et al., 2015). Significant bacterial growth was observed in 75% of urine samples, with Enterobacteriaceae dominating the isolates (86.7%). Among these, *Escherichia coli* was the most prevalent, followed by *Klebsiella pneumoniae*, *Proteus mirabilis*, and *Enterobacter cloacae*. These findings are in agreement with multiple regional and international studies reporting *E. coli* as the primary causative agent of UTIs, responsible for 60–80% of cases (Eshetie et al., 2015; Toner et al., 2016). The presence of other Gram-negative pathogens highlights the need for comprehensive antimicrobial susceptibility testing to guide therapy. The antimicrobial susceptibility profile revealed high resistance rates to commonly used antibiotics, including ampicillin (84.6%), cephalosporins (50–55%), and fluoroquinolones (38–40%). Aminoglycosides showed lower resistance rates (15–25%), and carbapenem resistance was minimal (3.8%), suggesting these agents remain effective options for severe infections.

Multidrug resistance (MDR) was detected in 60% of isolates, with *K. pneumoniae* demonstrating higher MDR rates (70%) compared to *E. coli* (57%). These results are consistent with global trends reporting increasing MDR among Enterobacteriaceae, especially in hospital settings (Abalkhail et al., 2022; Albaramki et al., 2019). The high MDR burden underscores the challenge of empiric therapy and the risk of treatment failure. ESBL production was observed in 38.5% of Enterobacteriaceae, predominantly in *E. coli* (70%), followed by *K. pneumoniae* (24%). This prevalence aligns with studies from South Asia reporting ESBL rates of 30–40% among UTI isolates (Quan et al., 2021; Zhu et al., 2019). Hospital-acquired isolates exhibited a higher rate of MDR (75%) than community-acquired isolates (50%), indicating that nosocomial exposure is a significant risk factor for resistant infections, likely due to antibiotic selection pressure in healthcare settings. Our analysis identified prior antibiotic use (60%), indwelling urinary catheters (36%), and recurrent UTIs (24%) as significant risk factors for ESBL infection, consistent with other studies linking these factors to the acquisition of resistant organisms (Espinar et al., 2015; Lin, Peng, Li, & Chen, 2025). While diabetes mellitus and gender were not statistically significant, females accounted for the majority of ESBL-positive cases (64%), likely reflecting the overall higher incidence of UTIs in women. The findings of our study corroborate previous reports on the dominance of *E. coli* in UTIs and the growing prevalence of ESBL-producing isolates. For instance, a multicenter study in India reported 37% ESBL prevalence among urinary Enterobacteriaceae (Zhou, Long, Liu, Wu, & Xia, 2024), similar to our 38.5%. The observed high MDR rates are consistent with studies from Pakistan and Bangladesh, where hospital-acquired UTIs frequently exhibited resistance to multiple antibiotic classes (Ameshe, Engda, & Gizachew, 2020; Brakemeier et al., 2017). The low carbapenem resistance observed in our study contrasts with reports from regions with high carbapenem use, suggesting that careful stewardship may help preserve the efficacy of these last-line agents. Our findings emphasize the need for targeted antimicrobial stewardship programs and infection control measures, particularly in hospitalized patients. The high prevalence of ESBL-producing and MDR organisms necessitates routine culture and susceptibility testing before initiating empiric therapy. Interventions to reduce prior antibiotic misuse and careful management of indwelling catheters could mitigate the spread of resistant pathogen

5. Conclusion

This study highlights a high prevalence of urinary tract infections among young and middle-aged adults, with females disproportionately affected. *Escherichia coli* was the predominant pathogen, followed by *Klebsiella pneumoniae*, with Enterobacteriaceae accounting for the majority of isolates. Antimicrobial resistance was widespread, particularly against beta-lactams and fluoroquinolones, while aminoglycosides and carbapenems remained largely effective. Extended-spectrum beta-lactamase (ESBL) production was observed in 38.5% of isolates, with higher prevalence among hospitalized patients and females. Prior antibiotic use, indwelling urinary catheters, and recurrent UTIs were significant risk factors for ESBL infections. The study underscores the urgent need for antimicrobial stewardship, judicious use of antibiotics, routine susceptibility testing, and strict infection control measures to combat multidrug-resistant and ESBL-producing uropathogens.

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