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Prevalence of ESBL and MBL Producing *Escherichia coli* among Urinary Tract Infection Patients at Star Hospital

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Abstract

Introduction: Urinary tract infection is among the most common bacterial infections worldwide, with Escherichia coli being the predominant causative organism. The increasing emergence of antimicrobial resistance in *Escherichia coli*, particularly through the production of extended-spectrum beta-lactamases and metallo-beta-lactamases, has significantly complicated treatment strategies and clinical outcomes.

Objective: This study aimed to determine the prevalence of extended spectrum beta-lactamases and metallo-beta-lactamases producing *Escherichia coli* among patients with urinary tract infection and to evaluate the antimicrobial resistance patterns of these isolates.

Methods: A total of 300 urine samples collected from patients clinically suspected of urinary tract infection at Star Hospital, Lalitpur, Nepal, were processed using standard microbiological techniques. *Escherichia coli* isolates were identified by conventional methods. Antimicrobial susceptibility testing was performed using the Kirby Bauer disc diffusion method following Clinical and Laboratory Standards Institute guidelines. Multidrug resistant isolates were screened and phenotypically confirmed for extended-spectrum beta-lactamases and metallo-beta-lactamases production.

Results: Bacterial growth was observed in 220 samples, of which 150 isolates were identified as *Escherichia coli*. Infections were more frequent among females, with the highest prevalence in the 21-30 year age group. High resistance rates were observed against amoxicillin, cefalexin, ceftazidime, cefotaxime, and cefixime. Half of the isolates exhibited multidrug resistance. Among these, a substantial proportion produced extended-spectrum beta-lactamases, while a smaller proportion produced metallo-beta-lactamases. Extended-spectrum beta-lactamases producing isolates showed highest susceptibility to tigecycline, meropenem, and amikacin.

Conclusion: A high prevalence of extended-spectrum beta-lactamases producing Escherichia coli was observed. Routine antimicrobial susceptibility testing and phenotypic detection of beta-lactamase production are important for guiding appropriate therapy and supporting antimicrobial stewardship.

Keywords: Antimicrobial resistance; beta-lactamases; drug resistant, Escherichia coli; urinary tract infections.

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Introduction

Urinary tract infection is one of the most common bacterial infections affecting individuals of all ages and both sexes. However, UTIs occur more frequently in women due to anatomical and physiological factors such as a shorter urethra, absence of protective prostatic secretions, increased risk of fecal contamination, and pregnancy.[1] Globally, there is a growing concern regarding increasing resistance of uropathogens to both conventional and newer antimicrobial agents.[2] Uropathogenic *Escherichia coli* remains the predominant etiological agent in both uncomplicated and complicated UTIs, accounting for approximately 60–80% of cases.[2,3]

Among the various antimicrobial resistance mechanisms in *E. coli*, the production of ESBLs and MBLs is of particular clinical importance. ESBLs confer resistance to penicillins, cephalosporins, and aztreonam but are inhibited by β-lactamase inhibitors such as clavulanic acid.[5] In contrast, MBLs hydrolyze a broad range of β-lactam antibiotics, including carbapenems, except monobactams.[6] The emergence of ESBL and MBL producing *E. coli* has led to significant therapeutic challenges and increased risk of treatment failure.[7] Studies from India have reported ESBL and MBL prevalence rates ranging from 35.16% to 54.5% and 3.9% to 10.98%, respectively.[8] These trends highlight the need for continuous surveillance and antimicrobial susceptibility testing.[9] The present study aims to identify antimicrobial resistance patterns and to assess the ability of *E. coli* isolates to produce ESBLs and MBLs.

Methods

This cross-sectional study was conducted at Microbiology laboratory of Modern Technical College, Sanepa, Lalitpur Nepal, over a four month period from September to December 2019. The sample size was calculated using the standard formula for a single population proportion. Taking a prevalence of 62.92% from Yadav et al study[1], 95% confidence level (Z = 1.96), and 5% margin of error, the calculated sample size was 358. However, due to feasibility and study duration, a total of 300 urine specimens were included. Samples were collected using a consecutive sampling technique from patients clinically suspected of urinary tract infection. Patients presenting with clinical features suggestive of urinary tract infection were included in the study. Samples from patients receiving antibiotic therapy at the time of specimen collection, improperly collected specimens, and contaminated samples were excluded from analysis.

Midstream urine samples were collected following standard aseptic procedures and processed using conventional microbiological methods. Specimens were inoculated onto Cystine Lactose Electrolyte Deficient agar (HiMedia) and incubated aerobically at 37 °C for 18-24 hours. Bacterial isolates were identified based on colony morphology, Gram's staining characteristics, and conventional biochemical tests. Among the isolates recovered, *Escherichia coli* strains were included for further analysis. Antimicrobial susceptibility testing was performed using the Kirby Bauer disc diffusion method on Mueller-Hinton agar (HiMedia), following Clinical and Laboratory Standards Institute guidelines.[10] Zones of inhibition were measured in millimeters, and results were interpreted as susceptible, intermediate, or resistant according to established criteria.[10] Multidrug resistance was defined as resistance to at least three different classes of antimicrobial agents.[11]

Screening and phenotypic confirmation of extended-spectrum beta-lactamase production were carried out using ceftazidime and cefotaxime discs, followed by the combined disc test with clavulanic acid.[11] Metallo-beta-lactamase production was detected among imipenem-resistant isolates using the imipenem ethylenediaminetetraacetic acid combined disc synergy test.[12] Data were entered and validated using Microsoft Excel and analyzed using Statistical Package for the Social Sciences software version 20. Descriptive statistics were used to summarize demographic variables, prevalence rates, and antimicrobial susceptibility patterns. Appropriate statistical tests were applied where relevant, with a p-value of less than 0.05 considered statistically significant. Ethical approval for the study was obtained from the Nepal Health Research Council (Reference No. 652). Written informed consent was obtained from all participants prior to sample collection, and patient confidentiality was strictly maintained throughout the study.

Results

Table 1: Distribution of organisms in urinary tract infections (n=220)

Organism	Frequency (n)	Percent (%)
Escherichia coli	150	68.2
Klebsiella epneumonia	20	9.1
Citrobacter spp.	7	3.2
Enterobacter spp.	5	2.3
Proteus mirabilis	5	2.3
Proteus vulgaris	1	0.4
Pseudomonas aeruginosa	5	2.3
Acinetobacter spp.	5	2.3
Morganella morganii	1	0.4
Staphylococcus aureus	5	2.3
Coagulase-negative *Staphylococcus*	10	4.5
Enterococcus spp.	5	2.3
Candida albicans	1	0.4

Out of 300 urine specimens processed, significant bacterial growth was observed in 220 (73.3%). Among culture positive samples, Escherichia coli was the predominant isolate, accounting for 150 (68.2%), followed by Klebsiella pneumoniae in 20 (9.1%). Other organisms recovered included *Citrobacter species, Enterobacter species, Proteus mirabilis, Proteus vulgaris, Pseudomonas aeruginosa*, *Acinetobacter* species, *Morganella morganii*, *Staphylococcus aureus*, coagulase-negative *Staphylococcus*, *Enterococcus* species, and *Candida albicans*. (Table 1)

The age distribution of *Escherichia coli* isolates showed the highest prevalence in the 21-30 years age group (53.3%), followed by 31-

40 years (16.7%). Lower frequencies were observed in other age groups. (Table 2)

Table 2: Age-wise distribution of *Escherichia coli* isolates (n = 150)

Age group (years)	Frequency (n)	Percent (%)
< 10	5	3.3
11–20	10	6.7
21–30	80	53.3
31–40	25	16.7
41–50	5	3.3
51–60	10	6.7
>60	15	10

Among the 150 *Escherichia coli* isolates, females accounted for 112 (74.7%) while males accounted for 38 (25.3%). Antimicrobial susceptibility testing demonstrated high resistance rates to amoxicillin (85.3%), cefalexin (76.0%), ceftazidime (74.0%), cefotaxime (70.6%), and cefixime (70.0%). The highest susceptibility was observed for tigecycline (97.3%), followed by meropenem (79.3%), chloramphenicol (76.0%), and amikacin (68.7%). Moderate susceptibility was observed for nitrofurantoin (60.0%). (Table 3)

Table 3: Antimicrobial susceptibility pattern of *Escherichia coli* isolates (n = 150)

Antibiotic	Sensitive n (%)	Resistant n (%)	Intermediate n (%)
Nitrofurantoin (NIT)	90 (60.0)	22 (14.7)	38 (25.3)
Co-trimoxazole (COT)	55 (36.7)	91 (60.7)	4 (2.6)
Cefotaxime (CTX)	31 (20.7)	106 (70.6)	13 (8.7)
Ciprofloxacin (CIP)	40 (26.7)	80 (53.3)	30 (20.0)
Cefalexin (CN)	36 (24.0)	114 (76.0)	0 (0.0)
Ofloxacin (OF)	63 (42.0)	75 (50.0)	12 (8.0)
Cefixime (CFM)	40 (26.7)	105 (70.0)	5 (3.3)
Amoxicillin (AMX)	15 (10.0)	128 (85.3)	7 (4.7)
Meropenem (MRP)	119 (79.3)	10 (6.7)	21 (14.0)
Amikacin (AK)	103 (68.7)	27 (18.0)	20 (13.3)
Amoxicillin clavulanate (AMC)	40 (26.7)	70 (46.6)	40 (26.7)
Tetracycline (TE)	49 (32.7)	77 (51.3)	24 (16.0)
Chloramphenicol (C)	114 (76.0)	18 (12.0)	18 (12.0)
Tigecycline (TGC)	146 (97.3)	0 (0.0)	4 (2.7)
Ceftazidime (CAZ)	22 (14.7)	111 (74.0)	17 (11.3)
Imipenem (IPM)	105 (70.0)	45 (30.0)	0 (0.0)

Half of the isolates (75/150) were identified as multidrug resistant. Initial screening suggested extended-spectrum beta-lactamase production in 142 isolates, of which 65 (46.0%) were phenotypically confirmed. Among confirmed producers, most isolates were detected using both indicator antibiotic combinations. Resistance to imipenem was observed in 45 isolates, among which 10 (22.2%) were confirmed as metallo-beta-lactamase producers. Tigecycline demonstrated the highest activity against extended-spectrum beta-lactamase and metallo-beta-lactamase producers.

Meropenem showed significantly higher susceptibility among extended-spectrum beta-lactamase producers compared with metallo-beta-lactamase producers ($p < 0.001$). Metallo-beta-lactamase-producing isolates exhibited reduced susceptibility to most antimicrobial agents. (Table 4)

Table 4: Comparison of antimicrobial resistance between ESBL and MBL isolates

Antibiotic	Sensitivity of ESBL producers n (%)	Resistance of ESBL producer n (%)	Sensitivity of MBL producers n (%)	Resistance of MBL producer n (%)	Odds Ratio (OR) (MBL vs ESBL resistance)	95% CI	p-value
Amikacin	44 (67.7)	21(32.3)	4 (36.4)	6(63.6)	3.14	0.80-12.33	0.154
Ciprofloxacin	17 (26.2)	48(73.8)	2 (18.2)	8(81.8)	1.42	0.27-7.32	1.000
Meropenem	51 (78.5)	14(21.5)	0 (0.0)	10(100)	74.58	4.12-1350.0	<0.001
Tigecycline	62 (95.4)	03(4.6)	9 (81.8)	1(18.2)	2.30	0.21-24.60	0.443
Ofloxacin	26 (40.0)	39(60)	3 (27.3)	7(72.7)	1.56	0.37-6.57	0.732

Discussion

In the present study, significant bacterial growth was detected in 73.3% (220/300) of urine samples, reflecting a substantial burden of urinary tract infections (UTIs) in the study population. *Escherichia coli* was the most frequently isolated uropathogen, accounting for 68.2% of cases, followed by *Klebsiella pneumoniae* (9.1%). These findings are comparable to those reported by

Muhammad et al., who documented *E. coli* and *K. pneumoniae* isolation rates of 65.4% and 11.2%, respectively, among urinary isolates.[13] The predominance of *E. coli* is biologically plausible due to its strong uropathogenic potential, including adhesion to uroepithelial glycol-conjugate receptors via virulence factors such as P fimbriae (Gal–Gal receptors), facilitating colonization and persistence within the urinary tract.[14]

Similar dominance of these organisms has been consistently reported in UTI studies worldwide.[15] A significantly higher prevalence of *E. coli* infection was observed among female patients (74.7%) compared with males. This finding aligns with observations by Maji et al., who reported a female predominance of 72.4%, and Chander et al., who documented rates exceeding 70% in women.[16] The increased susceptibility in females is attributed to anatomical and physiological factors, including a shorter urethra, proximity of the urethral opening to the anal region, and a higher likelihood of periurethral colonization by enteric organisms.[14]

Antimicrobial resistance among uropathogens remains a major public health challenge, largely driven by irrational antibiotic use, self-medication, and inadequate antimicrobial stewardship.[17,18] In the present study, 50.0% of *E. coli* isolates were multidrug resistant (MDR), which is comparable to MDR rates reported by Muhammad et al. (52.9%)[13] and Nepal et al. (52.9%).[19] Studies conducted in different regions of Nepal have reported MDR prevalence ranging from 38.2% to as high as 95.5%, underscoring significant geographic and institutional variability in resistance patterns.[21-23]

Tigecycline demonstrated the highest *in vitro* efficacy against *E. coli* isolates, with a susceptibility rate of 97.3%, corroborating findings by Malik et al. (98.1%) and Parajuli et al. (96.7%).[23,24] Carbapenems such as meropenem and imipenem also retained high activity, along with amikacin, indicating their continued role as effective therapeutic options. In contrast, marked resistance was observed to amoxicillin (85.3%) and ceftazidime (74.0%), consistent with resistance rates reported by Kibret et al. (82.5%) and Malik et al. (79.4%).[23,25] Resistance to amoxicillin is primarily mediated by β-lactamase enzymes, including TEM-1, SHV-1, OXA-1, and AmpC β-lactamases, as well as by non-enzymatic mechanisms such as efflux pump overexpression and biofilm formation.[17,26,27] Nitrofurantoin resistance was relatively low (14.6%), closely matching the 12.8% resistance reported by Pathak et al., supporting its continued utility as a first-line agent for uncomplicated UTIs.[9]

In the present study, 46.0% of *E. coli* isolates were confirmed as extended-spectrum β-lactamase (ESBL) producers, while 22% were metallo-β-lactamase (MBL) producers. The ESBL prevalence was higher than that reported by Babypadmini and Appalaraju (41.0%)[29] and Rimal et al. (25.5%)[18] but remains within the range reported across South Asia. In comparison, Ghadiri et al. from Iran documented ESBL and MBL rates of 22.3% and 7.0%, respectively, indicating a substantially higher ESBL burden in the present setting.[30] Such variations may reflect differences in antimicrobial prescribing practices, diagnostic capacity, and infection control measures. Among ESBL-producing isolates, tigecycline, meropenem, and amikacin exhibited the highest susceptibility, whereas MBL-producing isolates demonstrated preserved sensitivity primarily to tigecycline, highlighting the severely restricted therapeutic options for these infections. These findings emphasize the growing threat posed by β-lactamase-producing *E. coli* and underscore the urgent need for routine phenotypic detection, continuous surveillance, and evidence-based empirical antibiotic policies to mitigate treatment failure and curb the spread of resistance.

This study has several limitations that should be considered while interpreting the findings. First, the study was conducted at a single tertiary care center, which may limit the generalizability of the results to other healthcare settings or community populations. Second, the sample size, although adequate for preliminary analysis, may not fully capture seasonal or regional variations in antimicrobial resistance patterns. Third, only phenotypic methods were employed for ESBL and MBL detection; molecular characterization of resistance genes was not performed, which restricts precise identification of underlying genetic mechanisms. Additionally, clinical correlation, including prior antibiotic exposure and patient outcomes, was not assessed. Despite these limitations, the study provides valuable insights into the local epidemiology and resistance trends of uropathogenic *E. coli*.

Conclusion

A high prevalence of multidrug resistance was observed among *Escherichia coli* isolates, with half exhibiting a multidrug-resistant phenotype. Nearly half of the phenotypically suspected isolates were confirmed as extended-spectrum beta-lactamase producers, indicating widespread beta-lactamase mediated resistance. Although carbapenem resistance was detected, confirmed metallo-beta-lactamase production remained relatively low. Aminoglycosides, carbapenems, and tigecycline demonstrated comparatively better antimicrobial activity, whereas resistance to fluoroquinolones was substantial. These findings highlight the urgent need for routine antimicrobial susceptibility testing and systematic detection of beta-lactamase-mediated resistance mechanisms in clinical laboratories. Strengthening antimicrobial stewardship programs, promoting rational antibiotic prescribing practices, and discouraging empirical overuse of broad-spectrum agents are strongly recommended.

Conflict of Interest: None

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