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Original Research Article

Detection of ESBL and MBL among uropathogenic *Escherichia coli* in a tertiary care hospital in Maharashtra

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ABSTRACT

Background: Urinary tract infection (UTI) is one of the most common infections worldwide. Although the spectrum of etiological agents causing UTI has remained relatively constant, their antimicrobial susceptibility patterns have changed over time due to the emergence of resistant strains. *Escherichia coli*, a gram-negative bacillus, is the most common uropathogen. Antimicrobial resistance mediated by extended-spectrum β-lactamases (ESBL) and metallo-β-lactamases (MBL) in *E. coli* contributes significantly to prolonged hospital stay and increased treatment costs in patients with UTI.
Methods: All urine samples received from inpatients and outpatients in the microbiology laboratory were processed for microscopy and cultured on Blood agar and MacConkey agar. Isolates were identified based on colony morphology and standard biochemical tests. Antimicrobial susceptibility testing was performed by the Kirby–Bauer disk diffusion method according to CLSI guidelines. ESBL production in *E. coli* was detected by the cephalosporin/clavulanate disk test and MBL production by the imipenem/EDTA disk test.
Results: Of the 2234 urine samples processed, 845 yielded significant growth. *E. coli* was the most common isolate (41.89%). The uropathogenic *E. coli* (UPEC) strains showed more resistance to ampicillin (96.78%) and cephalosporins (93.55%) and comparatively less resistance to imipenem (11.93%) and nitrofurantoin (13.55%). ESBL and MBL production were observed in 55.81% and 10.65% of *E. coli* isolates, respectively.
Conclusions: The increasing prevalence of ESBL- and MBL-producing *E. coli* among uropathogens is a major concern. Judicious and rational use of antimicrobial agents, guided by local susceptibility patterns, is essential to reduce treatment costs, limit morbidity and curb the further spread of resistance in UTI cases.

Keywords: Antimicrobial resistance, *Escherichia coli*, ESBL, MBL, Urinary tract infection

INTRODUCTION

UTIs are among the most common bacterial infections in both developed and developing countries, including India. They are frequently diagnosed in hospitalized as well as community-based patients, affecting millions of individuals and contributing substantially to morbidity, mortality and healthcare costs.[1,2] UTIs are more common in females due to multiple anatomical and physiological factors, including easier contamination with fecal flora, the shorter female urethra, physiological changes during pregnancy and the absence of antibacterial prostatic secretions. Between 20% and 40% of women experience recurrent episodes of UTI and nearly 50% are estimated to have at least one episode during their lifetime. The strong association between sexual activity and UTIs further contributes to the higher incidence in females, as it increases the risk of bacterial contamination of the urethra.[3-5] The typical clinical manifestations of UTIs include fever with chills (more common in upper UTIs), flank pain suggestive of renal involvement and lower urinary tract symptoms such as increased frequency,

burning micturition, urgency and dysuria. When the same uropathogen is isolated in two consecutive clean-catch midstream urine samples from a patient without signs or symptoms of urinary infection, the condition is termed asymptomatic bacteriuria.[6,7] UPEC is the leading cause of UTIs in both community and hospital settings and is responsible for considerable morbidity and mortality worldwide. UPEC strains are distinct from commensal *E. coli* due to their ability to adhere to uroepithelial cells via adhesins, along with other virulence factors such as fimbriae and hemolysins. Other common uropathogens include *Klebsiella* spp., *Proteus* spp., *Staphylococcus* spp., *Enterococcus* spp., *Citrobacter* spp., *Pseudomonas aeruginosa* and *Candida* spp.[8,9] Commonly used antibiotics for the treatment of UTIs include β-lactam agents (penicillin's and cephalosporins), fluoroquinolones, nitrofurantoin and trimethoprim/ sulfamethoxazole. β-lactam antibiotics are often combined with β-lactamase inhibitors to enhance their efficacy. However, UPEC isolates increasingly show resistance to these commonly prescribed drugs. In most UTI cases, empirical antibiotic therapy is initiated before urine culture and sensitivity reports are available and this widespread, often inappropriate use of β-lactam antibiotics has driven the emergence of various novel β-lactamase enzymes.[10,11] *E. coli* has become a major producer of these enzymes, particularly ESBLs and MBLs, which play a key role in conferring resistance to β-lactam antibiotics.[12] As global and regional data indicate a rising prevalence of β-lactamase–producing UPEC strains, it is essential to monitor their current antimicrobial susceptibility patterns. Such information is critical for formulating effective antibiotic policies and guiding clinicians in the rational selection of therapy, especially in tertiary care settings.

METHODS

This descriptive study was conducted in the Department of Microbiology at Government Medical College, Miraj, Maharashtra over a period of one year, from September 2022 to August 2023.

Inclusion criteria

All *E. coli* isolated from urine sample of patients ≥18 was included in the study.

Exclusion criteria

All urine samples showing polymicrobial and insignificant growth were excluded from study.

A total of 310 urine samples were collected from patients aged 18 years and above of both sexes. All samples were subjected to microscopic examination (including Gram staining) and aerobic bacterial culture using standard microbiological procedures. The urine samples were inoculated onto Blood agar and MacConkey agar and incubated aerobically. *Escherichia coli* isolates were identified based on colony morphology and standard biochemical tests. All confirmed *E. coli* isolates were subjected to antimicrobial susceptibility testing by the modified Kirby–Bauer disc diffusion method in accordance with CLSI guidelines. All *E. coli* isolates were further screened and tested for ESBL and MBL production.

Detection of ESBL[13,14]

Screening for extended-spectrum β-lactamases production

Screening for ESBL production was performed as per CLSI guidelines using the disc diffusion method. Ceftazidime (30 µg) discs were used for initial screening. Isolates showing reduced susceptibility to ceftazidime, with a zone of inhibition diameter ≤22 mm and remaining sensitive to cefoxitin were considered potential ESBL producers.

Phenotypic confirmation by cephalosporin/clavulanate combination disc

Phenotypic confirmation of ESBL production was performed by the combination disc test. Ceftazidime (30 µg) and ceftazidime + clavulanic acid (30 µg/10 µg) discs were placed on a Mueller–Hinton agar plate inoculated with the test organism, ensuring a minimum distance of 30 mm (center to center) between the discs. Plates were incubated overnight at 37°C. ESBL production was confirmed when there was an increase of ≥5 mm in the inhibition zone diameter around the ceftazidime + clavulanic acid (CAC) disc compared with that around the ceftazidime (CAZ) disc alone.

Detection of metallo-β-lactamases production[13,14]

Isolates showing resistance to carbapenems such as imipenem and meropenem by the Kirby–Bauer disc diffusion method were considered potential MBL producers.

Phenotypic confirmation by Imipenem–EDTA combined disc test

For phenotypic confirmation of MBL production, the imipenem–EDTA combined disc test was performed. On a Mueller–Hinton agar plate inoculated with the test organism, one imipenem disc (10 µg) and another imipenem disc supplemented with EDTA (750 µg/ml) were placed at a distance of 20 mm apart (center to center). The plates were incubated at 37°C for 24 hours and the zones of inhibition around both discs were measured. An increase in the zone of inhibition of ≥4 mm around the imipenem+EDTA disc compared with the imipenem disc alone was considered indicative of MBL production.

RESULTS

In the present study, a total of 2,234 urine samples were processed for culture and sensitivity testing. Of these, 845

samples (37.82%) showed microbial growth, while the remaining 1,389 samples (62.18%) showed insignificant growth, polymicrobial growth or no growth. Among the culture-positive samples, 740 (33.12% of the total samples) yielded bacterial isolates and 105 (4.70%) grew *Candida* species (Table 1). *Escherichia coli* was the most commonly isolated bacterium, accounting for 41.89% (310/740) of the total bacterial isolates. *Klebsiella* species were the second most common, comprising 20.81% (154 isolates). Among Gram-positive cocci, *Staphylococcus* spp. represented 10% (74 isolates) of the bacterial isolates. Antimicrobial susceptibility testing of the 310 UPEC isolates revealed high levels of resistance to several commonly used antibiotics. Most UPEC isolates were resistant to Ampicillin (96.78%). Similarly, resistance to Cefazolin (CZ) and Cefuroxime (CXM) was 93.55% each. Other antibiotics such as Ceftazidime (CAZ), Ceftriaxone (CTR) and Ciprofloxacin (CIP) also showed high resistance rates, ranging from 67.10% to 79.35%. In contrast, lower resistance rates were observed for Imipenem (IPM) and Nitrofurantoin (NIT), at 11.93% and 13.55%, respectively (Table 2).

Screening for ESBL production identified 185 (59.68%) of the 310 UPEC isolates as potential ESBL producers. On phenotypic confirmation by the cephalosporin/clavulanate combination disc test, 173 isolates (55.81%) were confirmed as ESBL producers, while 12 isolates (3.87%) were negative on confirmation. Similarly, 37 isolates (11.94%) screened positive as potential MBL producers based on carbapenem resistance. Phenotypic confirmation using the Imipenem–EDTA combined disc test identified 33 isolates (10.65%) as MBL producers, while 4 isolates (1.29%) did not show MBL production on confirmation (Table 3).

Table 1: Number of urine samples showing significant growth and no growth.

Parameter	Number (%)
Total samples	2234
Culture positive	845 (37.82)
Number of bacterial isolates	740 (33.12)
Number of *Candida* spp.	105 (4.70)
No growth/insignificant/polymicrobial	1389 (62.18)

Table 2: Antimicrobial susceptibility pattern of uropathogenic *E. coli* isolates (n=310).

Antibiotic	Abbreviation	Strength	Sensitivity N (%)	Resistance N (%)
Ampicillin	Amp	10 µg	10 (3.22)	300 (96.78)
Cefazolin	CZ	30 µg	20 (6.45)	290 (93.55)
Cefuroxime	CXM	30 µg	20 (6.45)	290 (93.55)
Ceftazidime	CAZ	30 µg	64 (20.65)	246 (79.35)
Ceftriaxone	CTR	30 µg	76 (24.51)	234 (75.49)
Cefepime	CPM	30 µg	95 (30.65)	215 (69.35)
Piperacillin–Tazobactam	PTZ	100/10 µg	226 (72.90)	84 (27.10)
Ciprofloxacin	CIP	5 µg	102 (32.90)	208 (67.10)
Cotrimoxazole	COT	1.25/23.75 µg	148 (47.74)	162 (52.26)
Imipenem	IPM	10 µg	273 (88.07)	37 (11.93)
Amikacin	AK	30 µg	245 (79.03)	65 (20.97)
Gentamicin	GEN	5 µg	191 (61.61)	119 (38.39)
Norfloxacin	NX	10 µg	96 (30.97)	214 (69.03)
Nitrofurantoin	NIT	300 µg	268 (86.45)	42 (13.55)

Table 3: Screening and confirmation of ESBL and MBL production in UPEC (n=310).

Parameter	ESBL N (%)	MBL N (%)
Screening test positive	185 (59.68)	37 (11.94)
Confirmed positive	173 (55.81)	33 (10.65)
Screen positive but negative on confirmation	12 (3.87)	4 (1.29)

DISCUSSION

UTIs are among the most common infections seen in both community and hospital settings and represent an important cause of morbidity. In this study, 2,234 urine samples were processed, of which 33.12% showed significant bacteriuria, 4.70% yielded *Candida* species and 62.18% showed non-significant bacteriuria, polymicrobial flora or no growth. This overall isolation rate is comparable to that reported from many tertiary care

centres.[15-17] In the present study, *Escherichia coli* was the most common bacterial isolate causing UTI (41.89%), followed by *Klebsiella* species (20.81%), *Staphylococcus* species (10%), *Pseudomonas aeruginosa* (8.92%), *Enterococcus* species (7.30%), *Citrobacter* species (6.62%) and *Proteus* species (4.46%). The predominance of *E. coli* as a uropathogen agrees with several other studies, such as those by Patel et al (36.11%), Sibi et al and Kumar et al (43.9%), Singhal et al (45.7%) and Kuldeep et al (47.33%). Some authors have reported even higher isolation rates of *E. coli* ranging from about 50.5% up to 80.9%.[17-20] These differences may be related to geographic and population variations, time periods of the studies and differences in sample size and clinical settings.[15,21-25] The antimicrobial susceptibility pattern in the study is of concern. Uropathogenic *E. coli* showed very high resistance to ampicillin and first- and second-generation cephalosporins and high resistance to third-generation cephalosporins and fluoroquinolones. In contrast, better susceptibility was observed to imipenem (88.07%), nitrofurantoin (86.45%) and amikacin (79.03%). Similarly high levels of susceptibility to these drugs have been reported in earlier studies, though some authors have described lower sensitivities to nitrofurantoin and amikacin, while others have reported higher amikacin sensitivity but relatively lower nitrofurantoin sensitivity.[18,25,26] These variations likely reflect local antibiotic usage patterns, differences in empirical therapy practices and regional resistance trends.

Extended-spectrum β-lactamases production in uropathogenic E. coli

In this study, 234 urinary *E. coli* isolates were resistant to third-generation cephalosporins. Of these, 185 isolates that remained sensitive to cefoxitin were considered potential ESBL producers on screening. The remaining cephalosporin-resistant isolates may harbour other mechanisms such as derepressed chromosomal cephalosporinases, plasmid-mediated Amp C β-lactamases or carbapenemases. On phenotypic confirmation, 173 of the 185 screened isolates (93.51%) were confirmed as ESBL producers. This proportion is higher than that reported in a similar study by Upendra Thapa Shrestha et al indicating a substantial burden of ESBL-producing UPEC in the setting and underscoring the limited usefulness of third-generation cephalosporins for empirical therapy.[25]

Metallo-β-lactamases production in uropathogenic E. coli

Among imipenem-resistant *E. coli* isolates, 37 were screened as possible MBL producers and 33 (89.19%) were confirmed MBL producers by the Imipenem–EDTA combined disc test. This confirmation rate is higher than those reported in several other studies, which documented lower proportions of MBL-producing isolates.[27,28] Such differences may reflect variation in diagnostic methods, interpretive criteria, patient populations, antibiotic pressure and clonal spread of resistant strains. Overall, the high prevalence of ESBL-producing and the significant presence of MBL-producing uropathogenic *E. coli* in this tertiary care hospital highlight a serious therapeutic challenge. These findings stress the need for rational antibiotic use, robust antibiotic stewardship programmes, strict infection control measures and regular monitoring of local resistance patterns to guide appropriate empirical therapy and preserve the efficacy of existing antimicrobial agents. ESBL and MBL in this study were identified using phenotypic methods, which do not permit differentiation of specific enzyme subtypes, a distinction that can be achieved using genotypic methods.

CONCLUSION

In this study, Escherichia coli emerged as the predominant uropathogen causing urinary tract infections in a tertiary care hospital, followed by other Enterobacteriaceae, non-fermenters and Gram-positive cocci. Most of the uropathogenic *E. coli* isolates showed resistance to ampicillin and cephalosporins, considerable resistance to fluoroquinolones, while relatively better susceptibility was retained to imipenem, nitrofurantoin and amikacin. More than half of the isolates were ESBL producers and a notable proportion were MBL producers, indicating a substantial burden of multidrug-resistant strains. These findings highlight the growing problem of β-lactam and carbapenem resistance among uropathogenic *E. coli* and underscore the importance of routine detection of ESBL and MBL in clinical laboratories. Judicious, culture-guided use of antibiotics, strict antibiotic stewardship and continuous surveillance of local resistance patterns are essential to optimise empirical therapy, reduce treatment failures, limit healthcare costs and curb further spread of resistant uropathogens.

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