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Original Research Article

Prevalence of extended spectrum beta lactamases and Metallo beta lactamases among *Escherichia coli* isolates in a tertiary care hospital, Thiruvananthapuram

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ABSTRACT

Background: Antimicrobial resistance in *Escherichia coli*, particularly due to extended-spectrum β-lactamases (ESBL), Amp C β-lactamases, and Metallo-β-lactamases (MBL), poses a major therapeutic challenge. This study aimed to determine the prevalence of these resistance mechanisms among clinical *E. coli* isolates and to assess their antibiotic susceptibility patterns.
Methods: A hospital-based cross-sectional study was conducted on 130 non-duplicate *E. coli* isolates obtained from urine, blood, sterile body fluids, and aspirates over six months in a tertiary care hospital in south India. Identification was performed using standard microbiological techniques. Antimicrobial susceptibility testing was carried out by Kirby-Bauer disc diffusion following CLSI guidelines. ESBL, Amp C, and MBL production was detected using phenotypic screening and confirmatory disc synergy tests.
Results: ESBL production was detected in 67 (51.5%) isolates, MBL in 9 (7%), and AmpC in 6 (4.6%). Co-production of ESBL and MBL was observed in 2 (1.5%) isolates, while no isolate produced all three enzymes. The highest resistance was observed to ampicillin (96.1%), ceftriaxone (91.5%), and ciprofloxacin (77.6%). Colistin (100%) and tigecycline (98.4%) showed the highest susceptibility. ESBL-producing isolates were most susceptible to colistin (100%), tigecycline (97%), and carbapenems (68-73%), whereas MBL producers retained susceptibility mainly to colistin and tigecycline.
Conclusions: The high prevalence of ESBL-producing *E. coli* and the emergence of MBL producers underscore the urgent need for continuous surveillance, rational antibiotic use, and strengthened antimicrobial stewardship programs to limit the spread of multidrug-resistant organisms.

Keywords: Amp C, Antimicrobial resistance, Beta-lactamase, ESBL, *Escherichia coli*, MBL

INTRODUCTION

Antimicrobial resistance (AMR) has emerged as one of the most serious global public health threats, particularly in low- and middle-income countries where infectious diseases remain a leading cause of morbidity and mortality.[1] Gram-negative bacteria, especially members of the family *Enterobacterales*, have developed multiple resistance mechanisms that significantly compromise therapeutic options.[2] Among them, *Escherichia coli* is a major pathogen responsible for urinary tract infections, bloodstream infections, intra-abdominal infections, and sepsis, both in community and hospital settings.[3]

The production of β-lactamases is the most important mechanism of resistance to β-lactam antibiotics in *E. coli*.

Extended-spectrum β-lactamases (ESBLs) hydrolyze penicillins, third-generation cephalosporins, and aztreonam and are frequently associated with co-resistance to fluoroquinolones, aminoglycosides, and trimethoprim-sulfamethoxazole.[4] AmpC β-lactamases confer resistance to cephamycins and are poorly inhibited by clavulanic acid, often leading to therapeutic failure when β-lactam/β-lactamase inhibitor combinations are used.[5] Carbapenems have traditionally been considered the drugs of last resort for infections caused by ESBL- and AmpC-producing organisms.[6] However, the increasing emergence of carbapenem resistance mediated by metallo-β-lactamases (MBLs) has further limited treatment options.[7]

MBLs hydrolyze almost all β-lactams, including carbapenems, and are not inhibited by available β-lactamase inhibitors.[8] Their rapid dissemination, often through plasmid-mediated gene transfer, has resulted in the global spread of carbapenem-resistant *Enterobacterales*.[9] The coexistence of multiple β-lactamase enzymes within a single isolate further complicates laboratory detection, antimicrobial therapy, and infection control practices.[10] In developing countries, limited access to advanced diagnostic tools and the widespread empirical use of broad-spectrum antibiotics exacerbate this problem.[11]

Surveillance of antimicrobial resistance patterns and the prevalence of ESBL, AmpC, and MBL producers is therefore essential for guiding empirical therapy, optimizing antibiotic stewardship programs, and implementing effective infection control strategies.[12] Data from different geographical regions vary considerably, highlighting the importance of local epidemiological studies.[13]

The present study was undertaken to determine the prevalence of ESBL, AmpC, and MBL production among clinical *E. coli* isolates in a tertiary care hospital in South India and to evaluate their antimicrobial susceptibility profiles.

METHODS

Study design and setting

A hospital-based cross-sectional study was conducted over a six-month period (October 2024 to April 2025) in the Clinical Microbiology Laboratory, Department of Microbiology, Sri Avittam Thirunal Hospital and Post graduate microbiology laboratory, Department of MLT, Govt. Medical college, Thiruvananthapuram.

Study samples

A total of 130 non-duplicate clinical isolates of *E. coli* obtained from urine, blood, sterile body fluids, and aspirates were included in the study. Repeat isolates from the same patient and isolates from sputum, pus swabs, and endotracheal aspirates were excluded.

Sample size

The sample size was calculated using a previously reported prevalence of MBL-producing *E.coli* of 31%, with a 95% confidence interval and 8% precision, yielding a minimum sample size of 128.[14] A total of 130 isolates were analyzed.

Identification of isolates

Clinical specimens were processed using standard microbiological procedures. Isolates were identified as *E.coli* based on Gram staining, cultural characteristics, biochemical test and Antibiotic susceptibility testing (as per CLSI guidelines).[15]

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed by the Kirby-Bauer disc diffusion method on Mueller-Hinton agar in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines.[16] The antibiotics tested included ampicillin (10 µg), gentamicin (10 µg), amikacin (30 µg), cefazolin (30 µg), ceftriaxone (30 µg), cefepime (30 µg), ciprofloxacin (5 µg), nalidixic acid (30 µg), norfloxacin (10 µg), trimethoprim-sulfamethoxazole (1.25/23.75 µg), piperacillin-tazobactam (100/10 µg), cefoperazone-sulbactam (75/30 µg), imipenem (10 µg), meropenem (10 µg), tigecycline (15 µg), colistin, and nitrofurantoin (300 µg) for urinary isolates.

Detection of ESBL

Isolates showing reduced susceptibility to third-generation cephalosporins were screened for ESBL production. Confirmation was performed using the combined disc method with ceftazidime (30 µg) and ceftazidime-clavulanic acid (30/10 µg) discs placed 20 mm apart. An increase of ≥5 mm in the zone diameter around the combination disc compared to ceftazidime alone was interpreted as ESBL production.[17]

Detection of Amp C β-Lactamase

Isolates showing reduced susceptibility to cefoxitin (30 µg) were screened as potential AmpC producers. Confirmation was done using the cefoxitin-cloxacillin double-disc synergy test. An increase in zone diameter of ≥4 mm around the cefoxitin-cloxacillin disc compared to cefoxitin alone indicated AmpC production.[18]

Detection of MBL

Isolates showing reduced susceptibility to imipenem were screened for Carbapenemase production. Confirmation was performed using the imipenem-EDTA combined disc method. An increase in zone diameter of >7 mm around the imipenem-EDTA disc compared to imipenem alone was interpreted as MBL production.[19]

Data analysis

Data were entered into SPSS 27 and analyzed descriptively. Results were expressed as frequencies and percentages.

RESULTS

Sample distribution and demographics

Among the 130 *E. coli* isolates, 66 (51%) were obtained from urine samples, 48 (37%) from syringe pus/aspirates, 8 (6%) from blood, 7 (5%) from peritoneal fluid, and 1 (1%) from bile. The highest number of isolates were from patients aged 40-60 years (35%), followed by those aged >60 years (28%), 21-40 years (22%), and <20 years (15%). Females constituted 65% of cases, while males accounted for 35%.

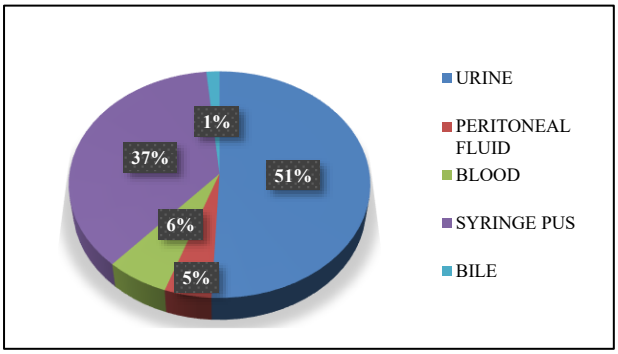


Figure 1: Sample wise distribution of *E. coli* isolates.

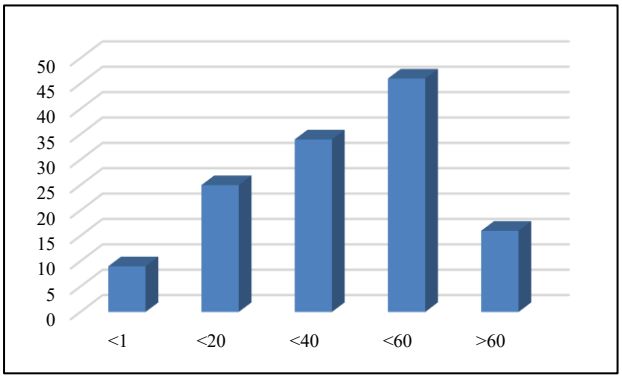


Figure 2: Age wise distribution of *E. coli*.

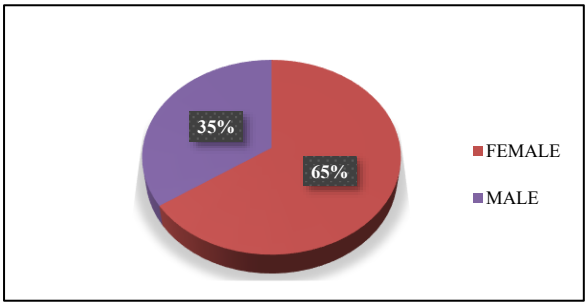


Figure 3: Gender wise distribution of *E. coli* isolates.

Antimicrobial susceptibility pattern

Overall susceptibility was highest to colistin (100%), tigecycline (98.4%), imipenem (82.3%), meropenem (78.4%), cefoperazone-sulbactam (75.3%), and piperacillin-tazobactam (68.4%). High resistance was observed to ampicillin (96.1%), cefazolin (94.6%), ceftriaxone (91.5%), ciprofloxacin (77.6%), and nalidixic acid (82.3%). Nitrofurantoin showed good activity against urinary isolates (72%).

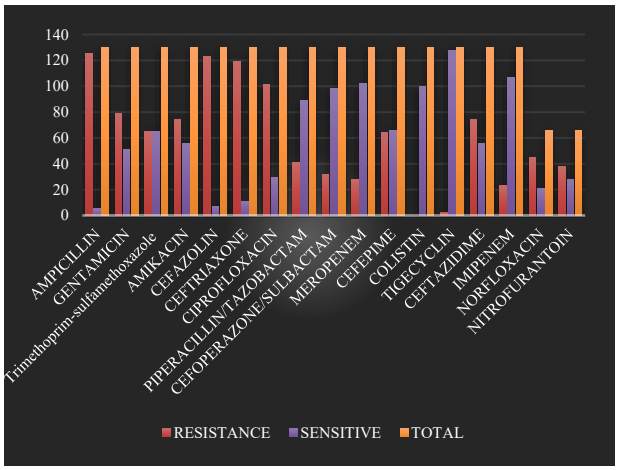


Figure 4: Antibiotic susceptibility pattern of *E. coli* isolates.

Prevalence of β-lactamase production

Of the 130 isolates, ESBL production was detected in 67 (51.5%), MBL in 9 (7%), and AmpC in 6 (4.6%). Co-production of ESBL and MBL was observed in 2 (1.5%) isolates, while no isolate produced all three enzymes. Forty-six isolates (35.3%) did not produce any of the tested β-lactamases.

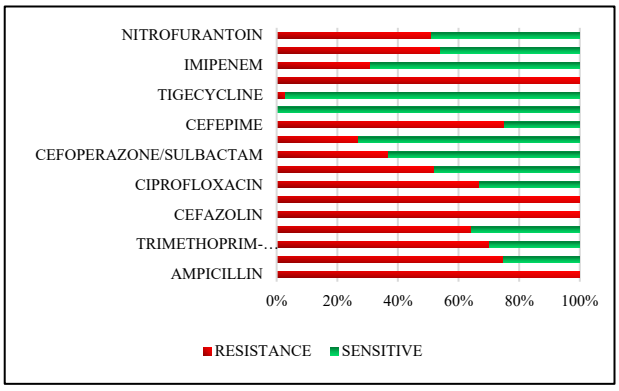
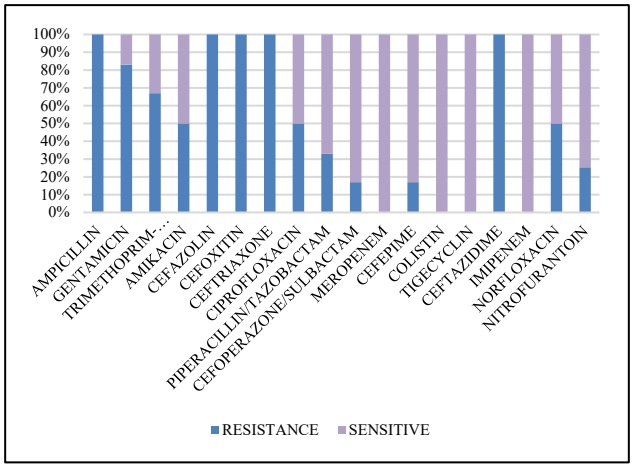


Figure 5: Antibiotic susceptibility pattern of ESBL producers.

Antibiotic susceptibility of ESBL producers

ESBL-producing isolates showed the highest susceptibility to colistin (100%), tigecycline (97%),

meropenem (73%), and imipenem (68.6%). Moderate susceptibility was observed to cefoperazone-sulbactam (62.6%) and piperacillin-tazobactam (47.7%). Resistance was universal to ampicillin, cefazolin, ceftriaxone, and ceftazidime, and high to ciprofloxacin (67.2%), gentamicin (74.6%), and amikacin (64%).



Antibiotic susceptibility pattern of AmpC β lactamase producers

Antibiotic susceptibility of AmpC producers

AmpC producing isolates demonstrated 100% susceptibility to colistin, tigecycline, imipenem, and meropenem, followed by cefoperazone-sulbactam (83%), cefepime (83%), and piperacillin-tazobactam (67%). High resistance was observed to cefoxitin, ceftriaxone, and fluoroquinolones.

Antibiotic susceptibility of MBL producers

Among MBL producers, susceptibility was highest to colistin (100%) and tigecycline (89%). Resistance was universal to imipenem, ceftriaxone, cefazolin, and ceftazidime, with high resistance to aminoglycosides and fluoroquinolones.

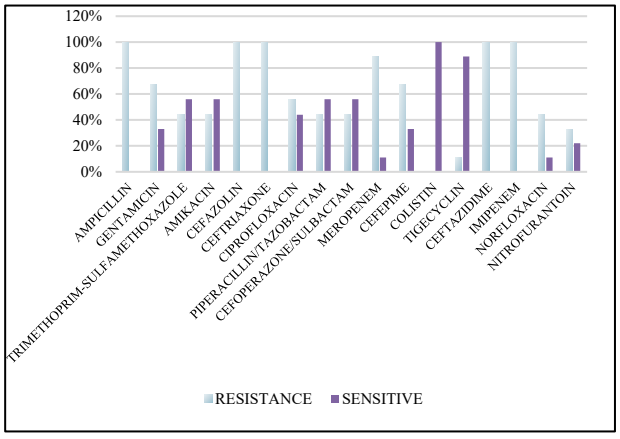


Figure 7: Antibiotic susceptibility pattern of MBL producers.

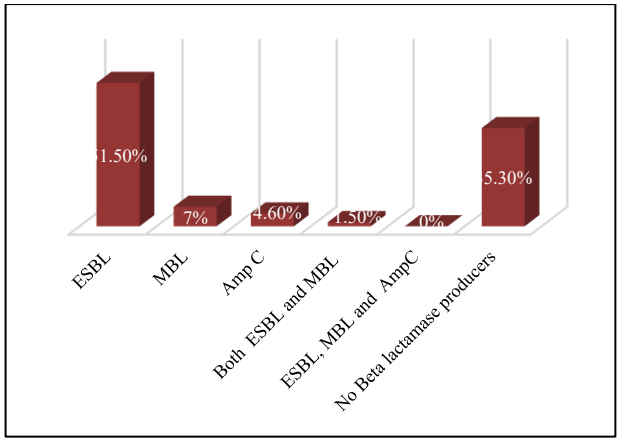


Figure 8: Proportion of beta lactamases among *E. coli* isolates.

DISCUSSION

The present study evaluated the prevalence of ESBL, AmpC, and MBL production among *E. coli* isolates in a tertiary care hospital in south India. Urine was the most common source of isolates (51%), consistent with the established role of *E. coli* as the leading cause of urinary tract infections.[3] The female predominance observed aligns with known anatomical and physiological risk factors for urinary tract infections.[20]

The overall antimicrobial susceptibility pattern demonstrated high resistance to commonly used antibiotics such as ampicillin, third-generation cephalosporins, and fluoroquinolones, reflecting their widespread and often empirical use.[21] Similar resistance trends have been reported in several Indian studies.[22,23] In contrast, colistin and tigecycline retained excellent in vitro activity, consistent with findings from previous studies, emphasizing their importance as reserve agents.[24]

ESBL production was detected in 51.5% of isolates, comparable to reports from various Indian centres showing prevalence rates between 40% and 60%.[22,25] However, higher rates exceeding 70% have also been documented.[26] These variations likely reflect differences in antibiotic prescribing practices, infection control measures, and laboratory detection methods. The high prevalence of ESBL producers in this study indicates limited utility of third-generation cephalosporins for empirical therapy in this setting.

ESBL-producing isolates demonstrated high resistance to fluoroquinolones, aminoglycosides, and trimethoprim-sulfamethoxazole, indicating multidrug resistance, consistent with previous studies.[25,27] Carbapenems and β-lactam/β-lactamase inhibitor combinations such as cefoperazone-sulbactam and piperacillin-tazobactam retained moderate to good activity, similar to findings reported elsewhere.[28] However, declining susceptibility to carbapenems among ESBL producers is concerning and necessitates continued surveillance.

AmpC β-lactamase production was observed in 4.6% of isolates, which is comparable to several studies reporting prevalence rates between 5% and 10%, though lower than those reported in certain regions.[29-31] AmpC producers demonstrated excellent susceptibility to carbapenems, tigecycline, and colistin, consistent with previous findings.[30] Their resistance to cephalosporins and cephamycins highlights the importance of routine laboratory detection to avoid therapeutic failure.

MBL production was detected in 7% of isolates, indicating the emergence of carbapenem resistance in this setting. Although lower than rates reported in some Indian centres.[31,32] This finding is clinically significant given the limited therapeutic options available for infections caused by MBL-producing organisms.[33] These isolates retained susceptibility mainly to colistin and tigecycline, consistent with global trends.[34]

Co-production of ESBL and MBL was observed in 1.5% of isolates, similar to low but clinically important rates reported in previous studies.[35,36] Such isolates pose significant therapeutic challenges due to extensive drug resistance. The absence of triple enzyme producers in this study is reassuring but does not preclude their future emergence.

Phenotypic detection methods such as disc synergy tests remain practical and cost-effective tools for routine laboratory use, particularly in resource-limited settings.[37] Routine screening and confirmation of β-lactamase production are essential to guide appropriate antimicrobial therapy, optimize stewardship initiatives, and prevent nosocomial transmission.[38]

CONCLUSION

This study demonstrates a high prevalence of ESBL-producing *Escherichia coli* (51.5%) and the emergence of MBL producers (7%) in a tertiary care hospital in South India, with a smaller proportion of AmpC producers (4.6%). Resistance to commonly used antibiotics such as ampicillin, third-generation cephalosporins, and fluoroquinolones was alarmingly high, while colistin, tigecycline, and carbapenems retained good in vitro activity.

These findings underscore the urgent need for continuous surveillance, rational antibiotic prescribing, and strengthened antimicrobial stewardship and infection control programs. Early detection of β-lactamase-producing organisms using simple phenotypic methods remains essential for optimizing patient management and limiting the spread of antimicrobial resistance.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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