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CTX-M type extended-spectrum β-lactamase in *Escherichia coli* isolated from extra-intestinal infections in a tertiary care hospital in south India

Anusha Rohit[1], Vijaya Kumar Deekshit[3], Malathi Balaraj[1], Veena Shetty Alandur[4], Georgi Abraham[2], Iddya Karunasagar[†] & Indrani Karunasagar[††]

Departments of [1]Microbiology & [2]Nephrology, The Madras Medical Mission, Chennai, [3]Division of Infectious Diseases, [††]Nitte University Centre for Science Education & Research, [4]Department of Microbiology, K.S. Hegde Medical Academy, [†]Nitte (Deemed to be University), Mangaluru, India

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***Background & objectives*: Infections caused by extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* carrying bla_{CTX-M} genes have been spreading globally, but there are geographical variations in the type of bla_{CTX-M} genes prevalent and there are scanty data from India. This study was conducted to determine the CTX-M type ESBLs in *E. coli* isolates obtained from clinical specimens from patients with extra-intestinal infections attending a tertiary care hospital in south India.**

***Methods*: ESBL-producing *E. coli* isolated from patients with extra-intestinal infections were subjected to PCR using CTX-M group-specific primers. From a representative isolate, full-length *CTX-M-15* gene was amplified and sequenced. An internal fragment of this gene was sequenced in 10 representative isolates.**

***Results*: Of the 300 isolates of *E. coli* tested, 88 per cent carried *CTX-M* genes and $bla_{CTX-M-15}$ was the most dominant gene present in 90 per cent of the positive isolates. Most (91%) of the isolates positive for bla_{CTX-M} were sensitive to meropenem.**

***Interpretation & conclusions*: Our findings showed $bla_{CTX-M-15}$ to be the dominant gene. Based on the data on antimicrobial susceptibility, cefoperazone-sulbactum could be an antimicrobial of choice.**

Key words CTX-M - *Escherichia coli* - extended-spectrum β-lactamase - molecular epidemiology

Extended-spectrum β-lactamases (ESBLs) are the most prevalent type of antimicrobial resistance mechanisms among *Enterobacteriaceae* such as *Escherichia coli* and *Klebsiella pneumoniae*[1]. There are over 200 enzymes characterized to be in the ESBL spectrum, which are encoded by plasmid or chromosomally carried genes[1]. Several studies indicate that CTX-M-type ESBLs are spreading globally and becoming dominant types in *Enterobacteriaceae* in many countries[2,3]. However, the diversity of *CTX-M* types occurring in India is yet to be fully understood. There are only a few reports of molecular identification

of β-lactamases. For example, a few studies indicated that CTX-M-15 could be the predominant ESBL in northern India[4,5]. Report from south India[5,6] has indicated the prevalence of *CTX-M-1*-type gene in 58.3 per cent of *Klebsiella* spp. and in 36 per cent of *E. coli*. This study was undertaken to estimate the presence of CTX-M type ESBLs in *E. coli* isolated from samples of extra-intestinal infections in patients attending a tertiary care hospital in south India.

Material & Methods

Three hundred isolates of *E. coli* obtained from urine, wound swab, sputum, pus, endotracheal secretions, bronchoalveolar lavage, bile fluids and other fluids from sterile body sites such as pleural fluid, bile, peritoneal fluid and blood cultures were collected over two and a half years (2013-2016) in the Microbiology department of The Madras Medical Mission, a tertiary care hospital in Chennai, India. Standard clinical microbiological procedures were used for the isolation of *E. coli*[7] and identification was done through VITEK II compact (BioMérieux, France). Antimicrobial susceptibility testing was done in Mueller-Hinton agar according to the current Clinical and Laboratory Standards Institute (CLSI) guidelines[8-11].

Phenotypic testing for ESBLs: ESBL phenotypic screening was performed for all the isolates by the double-disk diffusion test using ceftazidime (30 µg) and ceftazidime/clavulanic acid[8-11]. *E. coli* ATCC 25922 and *E. coli* ATCC BAA-2326 were used as the negative and positive controls and the zone diameter interpreted as per the CLSI M-100 recommended guidelines[8-11]. ESBL phenotypic confirmatory test was performed by the double-disk diffusion method using antibiotic disks containing a combination of cephalosporin plus clavulanic acid in combination with a corresponding cephalosporin disk alone[8-11].

Storage of isolates: Isolates were transferred to semisolid agar and stored at −20°C. For long-term storage, cultures were suspended in Luria-Bertani (LB) broth (Himedia, India) with 30 per cent glycerol and stored at −80°C.

Molecular testing for CTX-M: As per the antibiogram analyzed over 2013 to 2016 the rate of ESBL-positive organisms was 70-80 per cent among various isolates from The Madras Medical Mission. All isolates were subjected to (PCR) using four sets of primers and PCR conditions described earlier for CTX-M groups[12].

Sequencing of β-lactamase gene from a typical strain: To amplify the entire β-lactamase gene of Group I (CTX-M-15), the sequence from *E. coli* strain KS127 (accession number AB976567.1) was accessed from GenBank (*https://www.ncbi.nlm.nih.gov/nuccore/AB976567*). Primer design was based on primer search using NCBI Primer BLAST (*https://www.ncbi.nlm.nih.gov/tools/primer-blast/*). The annealing temperature used for amplification was 55°C. An isolate of *E. coli*, IRU1638, recovered from a patient with urinary tract infection was used to sequence the β-lactamase gene.

Sequencing internal region of β-lactamase of a few representative isolates: Ten representative isolates were selected based on source of isolation (7 urine, 2 blood and 1 bile fluid) and varying antibiogram for sequencing of internal region of $bla_{\text{CTX-M-15}}$. The forward primer 5'ACGTTAAACACCGCCATTCC3' and the reverse primer 5'TCGGTGACGATTTTAGCCGC3' amplified a 356 bp fragment of the *CTX-M-15* β-lactamase gene. PCR products obtained at 56°C were sequenced by Eurofins, Bengaluru, and the sequence was subjected to nucleotide BLAST search.

Results & Discussion

Of the 300 isolates tested, 238 (79.3%) were positive for Group I CTX-M-15 (Table I), and of these, majority 174 (73.1%) were urine isolates. Group IV had eight isolates and Group II and III had only one isolate each. Seventeen isolates showed mixed reactions, nine being positive for both Groups I and II, four with both Groups I and III and three isolates with Groups I and IV (Table I). Urine isolates accounted for 73 per cent of CTX-M Group I, while among CTX-M-negative isolates, urine accounted for 63 per cent (Table I). In a study of 140 *Enterobacteriaceae* ESBL producers from eastern India[13], the most common gene was bla_{TEM} (96.42%) followed by $bla_{\text{CTX-M}}$ (75%) and bla_{SHV} (17.85%). Another study from Central India[14] found that among the 526 urinary isolates of *E. coli*, the most common resistance gene detected was bla_{TEM} (48.7%) followed by $bla_{\text{CTX-M}}$ (7.6%). Our study showed that 88 per cent of the *E. coli* isolated from extra-intestinal infections carried $bla_{\text{CTX-M}}$ (Table I). This finding was similar to a study from Chennai[15] which looked at *E. coli* in HIV patients and detected $bla_{\text{CTX-M}}$ in 70.2 per cent of the isolates.

Our study also demonstrated that $bla_{\text{CTX-M}}$ Group I was the dominant group among extra-intestinal *E. coli* isolates. A study from Korea[16] noted that of the

Table I. Prevalence of different groups of bla_{CTX-M} in different clinical isolates of *Escherichia coli*

CTX-M types	Total number positive	Distribution in various samples (number positive)
Group I	238	Urine - 174 Blood - 22 Pus - 24 Sterile fluids - 9 Respiratory: 9
Group II	1	Urine - 1
Group III	1	Pus - 1
Group IV	8	Urine - 4 Pus - 1 Blood - 3
Group I and II	9	Urine - 6 Bile - 2 Sterile fluid - 1
Group I and III	4	Urine - 3 Blood - 1
Group I and Group IV	3	Urine - 3
Group I and II and IV	1	Blood - 1
Negative for all groups	35	Urine - 22 Pus - 5 Blood - 2 Fluid - 4 Respiratory specimens - 2

80 bla_{CTX-M} carrying extra-intestinal *E. coli* studied, 36 carried $bla_{CTX-M-15}$ (Group I), while 46 carried $bla_{CTX-M-14}$ (Group IV). Predominance of Group IV over Group I has also been reported from China[17]. Among 201 *E. coli* isolates studied from Shandong Province in China, 116 (57.7%) carried $bla_{CTX-M-14}$ (Group IV) and only 31 (15.4%) carried $bla_{CTX-M-15}$ (Group I). In river water in India, $bla_{CTX-M15}$ accounted for 46 per cent of isolates while 32 per cent isolates were positive for bla_{TEM}[18].

The antibiogram pattern of isolates positive and negative for bla_{CTX-M} is indicated in Table II. CTX-M-positive isolates showed lesser sensitivity to clavulanic acid (7%) in comparison to sulbactam (62%) and tazobactam (55%). Majority (91%) of bla_{CTX-M}-positive isolates showed meropenem sensitivity compared to those negative for this gene (72%).

The full-length $bla_{CTX-M-15}$ was amplified and sequenced in one typical isolate and a 356 bp internal fragment was amplified and sequenced in 10 isolates. BLAST analysis showed that these sequences had

Table II. Comparison of sensitivity of isolates positive for bla_{CTX-M} and those negative for this gene

Antibiotic tested	Per cent sensitivity in CTX-M positive	Per cent sensitivity in all negative
Amikacin	94	93
Amoxicillin-clavulanic acid	7	13
Cefepime	4	3.4
Cefoperazone-sulbactam	62	55
Ciprofloxacin	18	13
Ertapenem	81	72
Meropenem	91	72
Imipenem	94	80
Piperacillin-tazobactam	55	54

100 per cent similarity to $bla_{CTX-M-15}$ from *E. coli* and *Klebsiella* spp. in GenBank. These data confirmed the results obtained with group-specific primers. To conclude, the most common type of ESBL in this small single-centre study was identified to be *CTX-M-15*. Cefoperazone-sulbactam may be a better choice than piperacillin-tazobactam in our setting. Further studies need to be done on a large number of isolates in different parts of the country.

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***Conflicts of Interest*:** None.

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For correspondence: Dr Indrani Karunasagar, Nitte Centre for Science Education & Research, Kotekar-Beeri Road, Paneer Campus, Deralakatte, Mangalore 575 018, Karnataka, India
e-mail: indrani.karunasagar@nitte.edu.in