PREVALENCE OF BLA CTX-M EXTENDED SPECTRUM BETA LACTAMASE GENE IN UROPATHOGENIC ESCHERICHIA COLI

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ABSTRACT

Purpose: Extended spectrum beta lactamases (ESBLs) have been observed in virtually all the species of family Enterobacteriaceae. The enzymes are predominantly plasmid mediated and are derived from broad-spectrum beta lactamase by a limited number of mutations. The aim of this study was to determine the prevalence of the bla CTX-M gene in Uropathogenic Escherichia coli isolates obtained from tertiary care hospitals in and around Coimbatore, South India. Methods: Ten Uropathogenic E. coli (UPEC) isolates were analyzed regarding the presence of the bla CTX-M gene, using PCR which were initially screened by phenotypic methods. CTX-M gene was detected in only 8 UPEC isolates from tertiary care hospitals. Results: The study concludes that there was a high prevalence of the bla CTX-M gene (80%) among ESBL positive E. coli isolates from the UTI patients using CTX-M primer.

KEY WORDS: ESBL, Urinary tract Infections, E.coli, CTX-M, Multidrug resistance.

INTRODUCTION

CTX-M type enzymes are rapidly spreading among Enterobacteriaceae worldwide and now being the most prevalent ESBLs in many parts of the world [1]. There are currently 90 different CTX-M β-lactamases recognized [2] and the first CTX-M beta-lactamase (CTX-M-1/MEN-1) was characterized in Escherichia coli strains isolated from German and Italian patients [3, 4].

According to available data, developing countries (Europe, Africa, South America and Asia) have also been affected by the prevalence of Enterobacteriaceae producing CTX-M type
ESBL [5, 6]. CTX-M is closely related to chromosomally encoded beta lactamases in the genes *Kluyvera* *spp.* [7]. CTX-M type β-lactamases are plasmid borne which have been classified under Amber class A and also they confer high level of resistance to cefotaxime, ceftazidime and aztreonam [8].

Several Indian studies have reported the prevalence rate of ESBLs around 50% and higher [9, 10]. The prevalence of CTX-M and its groups have been reported from both North [11, 12] and South India [13, 14, 15]. Moreover, these research reports represent a large reservoir of resistant genes [12] in a country like India with more than 1.2 billion population.

Molecular characterization of Uropathogenic *E. coli* (UPEC) has become important objective in the epidemiological investigation of infectious agents. CTX-M-producing *Escherichia coli* and *Klebsiella pneumoniae* are becoming increasingly involved in urinary tract infections, especially among outpatients. Furthermore, these bacteria seem to have been imported from the community into the hospital setting [16]. Recent studies that used genotypic methods to differentiate uropathogenic *E. coli* suggest that community and hospital acquired UTI's could also occur as an outbreak [17] and only bla CTX-M gene was included in the study. The aim of this study is to determine the prevalence of CTX-M among ESBL producing Uropathogenic *E.coli* isolated from UTI patients attending tertiary care hospitals by molecular techniques and examine their antibiotic resistant pattern against 17 antibiotics which can provide useful epidemiological information and an aid in the antimicrobial therapy.

**METHODOLOGY**

**Bacterial isolates and ESBL detection**

A total of 512 (73.9%) *E. coli* strains were isolated from 830 urine specimens collected from urinary tract infection patients attending tertiary care hospitals in and around Coimbatore, South India. *E. coli* was identified by standard microbiological methods. All the 512 *E. coli* strains were screened for ESBL production as recommended by the Clinical and Laboratory Standards Institute (CLSI, 2009) guidelines. ESBL production was confirmed by using ceftazidime (30 µg) and cefotaxime (30 µg) antibiotic disks with and without clavulanic acid (10 µg) and by Double Disk Synergy Test (DDST) [18].
Antibiotic susceptibility test
Antimicrobial susceptibility testing was performed on Mueller-Hinton agar (Hi-Media, India) by Kirby Bauer disk diffusion technique as per the CLSI guidelines (CLSI, 2009). The following antibiotic disks (drug concentration in µg); amikacin (30), piperacillin (100), gentamicin (15), tobramycin (10), ampicillin (10), cefixime (5), rifampcin (5), norfloxacin (10), cephalothin (30), carbenicillin (100), cefepime (30), imipenem (10), meropenem (10), piperacillin/tazobactam (100/10), cefotaxime (30), ceftazidime (30) and cefoxitin (30) were used. The antibiotic disks used were obtained from Hi-Media Pvt. Ltd, Mumbai, India.

Preparation of Genomic DNA extraction
Genomic DNA was isolated from bacterial cells using DNA purification kit (PureFast® Bacterial Genomic DNA purification kit, HELINI Biomolecules, Chennai). The purified DNA was stored at -20°C. The samples were run on agarose gel and stained with ethidium bromide. The stained gel was examined for presence of bands under UV-light using molecular weight marker (HELINI Biomolecules, Chennai).

Detection of bla CTX-M genes by PCR
Among 298 (58%) ESBL producing uropathogenic E. coli, only 10 isolates were taken for molecular detection of bla CTX-M which was performed by using uniplex Polymerase Chain Reaction (PCR). The primers used for detection of bla CTX-M gene are shown in Table 1 which is obtained from Helini Biomolecules, Chennai. For PCR amplifications, about 1 ml of DNA was added to 25µl mixture containing 1µl of 10mM dNTPs mixer, 1µl of each primer and 2U of Taq DNA polymerase (Helini Biomolecules, Chennai) in 10X PCR buffer. Amplification was performed in a thermocycler (Corbett Research, Australia) with cycling parameters comprising initial denaturation at 94°C for 3 min followed by 35 cycles each of denaturation at 94°C for 30 sec, annealing at 56°C for 30 sec, amplification at 72°C for 30 sec and final extension at 72°C for 5 min, for the amplification of bla CTX-M. The amplified products were separated using 2% agarose. The gel was visualized by staining with ethidium bromide (0.5 mg/ml) in a dark room for 30 min. A 100 bp ladder molecular weight marker (Helini Biomolecules, Chennai) was used to measure the molecular weight of amplified products. The images of ethidium bromide stained DNA bands were digitized using a gel documentation system (Helini Biomolecules, Chennai). In this study, we did not attempt to detect group specific CTX-M genes.
RESULTS AND DISCUSSION

All the isolates tested in the present study for antibiotic susceptibility were multi drug resistant. The antibiotic resistance patterns of the uropathogenic *E. coli* carrying CTX-M are presented in figure 1. Four CTX-M isolates (50%) showed resistant to 16 of the 17 antibiotics tested, followed by one strain each showing resistant to 14, 12, 11 and 8 antibiotics respectively (Table 3). The non CTX-M strains also exhibited maximum resistance to 15 and 12 antibiotics respectively (Table 3). All the CTX-M producing UPEC strains retained favourable susceptibility to imipenem but not to meropenem and fluoroquinolones. Concomitantly 100% resistance to gentamicin, ciprofloxacin, norfloxacin, cephalothin, meropenem, cefotaxime and ceftazidime was exhibited by CTX-M *E. coli* strains. Similarly, 87.5% of resistance was observed against ampicillin, rifampcin and cefepime. Both CTX-M and non CTX-M (data not shown) in the study were susceptible to imipenem and 50% is the least resistance exhibited by amikacin and piperacillin.

Uniplex PCR detected CTX-M enzymes (Table 2) in 8 out of 10 isolates (80%). In India 44.4, 45 and 77.4 % of ESBL producing *E. coli* strains were found to be positive for bla CTX-M gene by PCR [19, 20, 21]. Similar increases in prevalence (70%) of CTX-M have also been from Argentina, Japan, China, United Kingdom and Spain [22, 23, 24, 25] where as in Korea CTX-M ESBL’s increased from 3.3 to 13.4% [26, 27]. These results indicate the significant increase in ESBL incidents in *E. coli* which may be due to the dissemination of CTX-M enzymes. Among the ESBL genotypes, the CTX-M genotype has been indicated to spread rapidly worldwide, and specific genetic groups have been characterized in different geographical areas [28]. In this study, we have not characterized the different groups of CTX-M (viz 1, 2, 9 and 15).

<table>
<thead>
<tr>
<th>PCR target</th>
<th>Primer sequence/ name</th>
<th>Primer sequence (5’-3’)</th>
<th>Annealing Temperature (°C)</th>
<th>Product size</th>
</tr>
</thead>
<tbody>
<tr>
<td>bla CTX-M</td>
<td>CTX-M-F CTX-M-R</td>
<td>GGTGATGAACGCTTTCCAAT AATGGCCGTATTCAGCGTAG</td>
<td>56</td>
<td>336bp</td>
</tr>
</tbody>
</table>

Table: 1 Primers, Annealing temperature and the expected size for detection of bla CTX-M

<table>
<thead>
<tr>
<th>Organism</th>
<th>Total no. of isolates</th>
<th>CTX-M (+)</th>
<th>CTX-M (-)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td>10</td>
<td>8 (80 %)</td>
<td>2 (20%)</td>
</tr>
</tbody>
</table>
Table 3: Resistance pattern of UPEC carrying ESBL genotypes - CTX-M and non CTX-M

<table>
<thead>
<tr>
<th>E. coli</th>
<th>Resistant to</th>
<th>Resistance patterns</th>
<th>No of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTX-M positive (n=8)</td>
<td>16 AK, PC, G, TB, A, CFX, R, NX, CH, CB, CPM, MRP, PIT/TAZ, CTX, CAZ, CX</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>14 G, TB, A, CFX, R, NX, CH, CB, CPM, MRP, PIT/TAZ, CTX, CAZ, CX</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12 G, A, CFX, R, NX, CH, CB, CPM, MRP, PIT/TAZ, CTX, CAZ</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>11 G, A, CFX, R, NX, CH, CB, CPM, MRP, CTX, CAZ</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8 G, CFX, NX, CH, CB, MRP, CTX, CAZ</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>CTX-M negative (n=2)</td>
<td>15 AK, PC, G, TB, A, CFX, R, NX, CH, CB, CPM, MRP, PIT/TAZ, CTX, CAZ</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12 AK, G, CFX, R, NX, CH, CB, CPM, MRP, PIT/TAZ, CTX, CAZ</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

AK- Amikacin; PC- Piperacillin; G-Gentamicin; TB-Tobramycin; A- Ampicillin; CFX-Cefoxime; R- Rifampcin; NX- Norfloxacin, CH- Cephalothin; CB- Carbenicillin; CPM-Cefepime; MRP-Meropenem; PIT/TAZ- - Piperacillin/ Tazobactam; CTX- Cefotaxime; CAZ-Ceftazidime; CX- Cefoxitin

Figure 1: Antimicrobial Susceptibility pattern of ESBL producing Uropathogenic E. coli carrying CTX-M
Figure: 2 PCR screening test results of ten selected UPEC isolates for bla<sub>CTX-M</sub> presence

PCR products of bla CTX-M: M: 100bp DNA ladder marker, Lanes 1-7, 10: Positive for CTX-M gene, Lanes 8, 9: Negative for CTX-M.

CTX-M <i>E. coli</i> have become widespread in humans since their first appearance in 1989 [29, 30]. According to Canton and Coque (2006) [31], CTX - M genotype among ESBL genotypes were found to spread rapidly worldwide and their genetic groups have been characterized in different geographical areas. The predominant ESBL genotype in <i>E. coli</i> in Europe has changed from TEM / SHV to CTX-M. CTX-M producing <i>E. coli</i> have been found in 80% of ESBL producers collected from 1998 to 2004 in southeast Austria [32]. Even in the current study, CTX-M producing <i>E. coli</i> was predominantly found in UTI patients in South India. <i>E. coli</i> strains harbouring CTX-M genes have been detected [33] which indicates that <i>E. coli</i> isolates producing multiple β-lactamases may be an important characteristic future in South Asia.

The global dissemination of bla CTX-M ESBL genes over the years has been described as a pandemic. CTX-M producing <i>E. coli</i> has emerged as significant cause for both community and hospital acquired infections [34]. We found that CTX-M types accounted for more than 80% of the <i>E. coli</i> isolates in the present study. They also have the ability to infect non compromised host even before prior exposure to anti microbial agents [35, 36].

Nemoy et al. (2005) [37] stated that the presence or absence of beta genes might increase the discriminatory ability among the strains. The results of the current investigation suggest a
possible dissemination of such strains among UTI patients because they were isolated from
different UTI patients. Between 2000 and 2004, three fold increases in the prevalence of the
CTX-M producing *E. coli* was detected and in 2008, over 50% of the ESBL producing
*Enterobacteriaceae* isolates in clinical samples belong to the CTX-M [38]. In India, CTX-M
15 was found to be dominant among the different specific groups of CTX-M [39]. Gaining of
resistance to carbapenems by *E.coli* harbouring CTX-M is due its impermeability of the cell
wall [40, 41]. As per our study, amikacin, piperacillin and to some extent cefoxitin can also
be used as empiric choices to treat UTI caused by ESBL producing *E. coli* isolates.

Therefore, the incidence of serious infection due to CTX-M producing *E. coli* will continue to
increase and it is important that the optimal treatment of UTI caused by *E. coli* is
underpinned by clinically valid susceptibility testing interpretive criteria. The use of
inappropriate empirical antibiotics to treat any infections caused by ESBL producing
*Enterobacteriaceae* has been associated with increased mortality. The high prevalence of
(80%) of CTX-M type ESBLs among UPEC *E. coli* identified in the present study is
consistent with the global trend of CTX-M dominance. This may be one of the reasons for the
spread of ESBL producing bacteria in both hospital and community. A detailed investigation
on the prevalence of ESBL producing UPEC *E. coli* among UTI patients is needed to prevent
UTI infections caused by this type of bacteria.

This is a retrospective study conducted in tertiary care hospitals in and around Coimbatore
and the results may not be applicable to other regions and the sample size was too small to
conduct subgroup analysis. The present results suggest that the incidence of detection of
ESBL’s *E. coli* have increased as a result of the dissemination of CTX-M enzymes in
Coimbatore, South India.

**REFERENCES**

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relationship between the new plasmid-mediated extended-spectrum β-lactamase MEN-1


