MOLECULAR CHARACTERIZATION OF NOSOCOMIAL CTX-M TYPE β-LACTAMASE PRODUCING ENTEROBACTERIACEAE FROM A TERTIARY CARE HOSPITAL IN SOUTH INDIA

*SA Jemima, S Verghese

Abstract

CTX-M group of extended spectrum β lactamases (ESBLs) represents a rapidly emerging problem in many countries. The prevalence of nosocomial blaCTX-M-1 producing Enterobacteriaceae strains has not been reported earlier in Indian hospitals. This study describes molecular subtyping of nosocomial blaCTX-M producing strains of Enterobacteriaceae. Polymerase chain reaction with primers specific for blaCTX-M-1 coding genes was used to identify 95 Enterobacteriaceae strains producing blaCTX-M positive isolates. Of the 95 blaCTX-M producing isolates, 45 strains were positive for blaCTX-M-1. blaCTX-M-1 was found to be most prevalent in Klebsiella strains.

Key words: blaCTX-M, blaCTX-M-1, extended spectrum β lactamase, Enterobacteriaceae.

During the past decade, ESBLs of blaCTX-M type emerged in many countries of the world.[1-4] The blaCTX-M type enzymes are a group of molecular class A extended spectrum beta lactamases that exhibit an overall preference for cefotaxime and ceftriaxone and a higher susceptibility to tazobactum than to clavulanate.[5] They were initially reported in the second half of 1980s, and their rate of dissemination among bacteria and in most parts of the world has increased dramatically since 1995.[6] Several different variants of blaCTX-M type enzymes have been identified till date.

The blaCTX-M genes are often carried on transferable plasmids.[7] We report the detection of blaCTX-M type enzyme CTX-M-1 gene in clinical isolates of Enterobacteriaceae isolates from a South Indian tertiary care hospital.

Materials and Methods

Bacterial strains

A total of 600 phenotypically ESBL positive isolates were checked for the presence of blaCTX-M gene by PCR and 95 (15.83%) strains were found positive for the same. The 95 non repetitive Enterobacteriaceae strains included 25-E. coli, 22-Enterobacter spp. and 48-Klebsiella spp. These CTX-M positive isolates included, 30-isolates from urine samples, 26- from endotracheal secretions, 13 from pus aspirates, 10 from blood cultures, 8-sputum samples, 5-βuids and 3-central line tip cultures. Identification of these isolates was done based on colony morphology on blood agar, MacConkey agar and by standard biochemical reactions. All strains were collected from a tertiary care hospital in South India.

In vitro susceptibility testing for detection of ESBL production

In vitro susceptibility was determined using double disk synergy test (DDST), phenotypic confirmatory double disk test (PCDDT) as recommended by CLSI guidelines.[8] E. coli (ATCC-25922) and K. pneumoniae (ATCC-700603) were used as reference strains for quality control of in vitro susceptibility testing.

Molecular analysis

PCR amplification of blaCTX-M and blaCTX-M-1 gene was carried out with specific primers using specific reaction parameters (Table). Bacterial strain producing the known β-lactamase- E. coli J53-pMG 267 (CTX-M-14) was used for quality control.

PCR was carried out in 50 μL volume with 20 pmol of each primer, 200 μM deoxynucleosidetriphosphates, 1.5 mM MgCl2, and 0.5U Taq DNA polymerase (RBC-Bioscience) in the reaction buffer provided by the enzyme manufacturer using 1 μL of plasmid DNA as a template DNA.

Restriction fragment length polymorphism (RFLP)

The amplified CTX-M product (10μL) was directly subjected to digestion with 9U Pst-I and 4U Pvu-II enzymes (Medox) in One-Phor-All Plus Buffer (10mM Tris acetate (pH 7.5), 10 mM magnesium acetate, 50 mM potassium acetate) for four hours at 37°C. The restriction fragments of the PCR products were analysed by electrophoresis in 3.5% agarose containing ethidium bromide (Bio gene, USA).

*Corresponding author: (email: <sam_jemi@yahoo.co.in>)

Department of Microbiology, International Centre for Cardio Thoracic and Vascular Diseases, A Unit of Frontier Lifeline, R-30-C, Ambattur Industrial Estate Road, Mogappair, Chennai-600 101, Tamilnadu, India

Received: 25-11-07
Accepted: 31-12-07
Conjugation assays

Conjugation assays were carried out by the filter mating procedure using the E. coli K-12 Nal’Rif’ mutant as the recipient. Transconjugants were selected on MH agar containing rifampicin (100 µg/mL) plus ceftazidime (2 µg/mL). Plasmid DNA was extracted by boiling-lysis method and analysed by electrophoresis on 0.8% (wt/vol) agarose gels. PCR was carried out for the transconjugants with plasmid as template for blaCTX-M.

Results

Phenotypic detection of ESBLs was carried out according to CLSI guidelines. Out of 1010 isolates screened for ESBL production, out of which 600 gave positive result. According to the guidelines mentioned a difference of 3-5 mm increase in zone diameter on either agent tested in combination with clavulanic acid versus its zone diameter when tested alone confirms the presence of ESBLs.

Among the 600 ESBL positive isolates, blaCTX-M was demonstrated (Fig. 1) in 95 (15.83%) clinical strains. All 95 CTX-M positive isolates were subjected to RFLP. All isolates showed similar restriction patterns (Fig. 2) and they all grouped under CTX-M-1 group as previously described by Edelstein.[3]

Among the 95 CTX-M producing isolates, CTX-M-1 gene was positive (Fig. 3) in 45 (47.3%) isolates. It included 9/25 E. coli isolates (36%), 8/22 Enterobacter species (36.3%) and 28/48 Klebsiella species (58.3%).

Plasmid analysis of the transconjugants showed that the beta lactamase gene was associated with the plasmid. Oxyimino-β-lactam antibiotic resistance was transferred to E. coli K-12. All 95 isolates demonstrated blaCTX-M by PCR.

Discussion

The present study documents the emergence of blaCTX-M gene in clinical isolates from South India for the first time. The blaCTX-M-1 gene has previously been reported in France, Italy and Germany it was reported in the year 1989 in E. coli, P. mirabilis and E. coli respectively.[6] In 2002, Dutour et al reported the spread of CTX-M 1 in France.[9] In Sweden real time PCR assays were developed for the detection of blaCTX-M type beta lactamases.[10] The CTX-M-1 gene was also reported from E. coli strains in healthy pets in Portugal by Daniela et al in the year 2004.[11] In Germany, at the beginning of 1989, Bauernfeind et al reported on a clinical cefotaxime-resistant E. coli strains which produced a non-TEM, non-SHV ESBL, designated CTX-M-1, in reference to its hydrolytic activity against cefotaxime.[4] In Poland, Gniadkowski et al identified a variant of CTX-M-1, designated CTX-M-3, in different members of the family Enterobacteriaceae isolated in 1996.[12] In India, a variant of the CTX-M-3 enzyme, designated CTX-M-15, was reported from six unrelated

---

**Table: Primers and PCR conditions used for blaCTX-M and blaCTX-M-1**

<table>
<thead>
<tr>
<th>PCR target</th>
<th>Primer name</th>
<th>Oligonucleotide sequence. 5’-3’</th>
<th>PCR product size (bp)</th>
<th>Reaction Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTX-M</td>
<td>CTX-M A</td>
<td>5’-ATG TGC AGY ACC AGT AAR GTK ATG GC-3’</td>
<td>543</td>
<td>Initial denaturation at 94ºC-7min; denaturation at 94ºC-50s, annealing at 50ºC-40s, elongation at 72ºC-60s, repeated for 35 cycles; Final extension at 72ºC-5 minutes</td>
</tr>
<tr>
<td></td>
<td>CTX-M B</td>
<td>5’-TGG GTR AAR TAR GTS ACC AGA AYC AGC GC-3’</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTX-M-1</td>
<td>CTX-M-1F</td>
<td>5’-GGT TAA AAA ATC ACT GC GTC-3’</td>
<td>876</td>
<td>Initial denaturation at 94ºC-5min; denaturation at 94ºC-40s, annealing at 50ºC-35s, elongation at 72ºC-50s repeated for 32 cycles; Final extension at 72ºC-7 minutes</td>
</tr>
<tr>
<td></td>
<td>CTX-M-1R</td>
<td>5’-TTA CAA ACC GTC GGT GAC GA-3’</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Figure 1:** Agarose gel showing products of CTX-M PCR amplification. The blaCTX-M positive control (P) is E. coli- pMG 267. N- Negative control with no template DNA. Lane 1 & 2 - E. coli, Lane 3, 4 - Enterobacter spp., Lane 5- Klebsiella spp. The molecular size standard is 1kb DNA ladder
members of the family Enterobacteriaceae (E. coli -4, K. pneumoniae -1, E. aerogenes-1) isolated between April and May 2000. Sekar et al reported that 44.4% of E. coli and 35.29% K. pneumoniae strains were found to be positive for bla_CTX-M gene by PCR. [13]

In this study, of 600 ESBL positive isolates which were analysed for the presence of bla_CTX-M gene by PCR, 95 (15.83%) isolates were positive for bla_CTX-M. These 95 strains were further analysed for the presence of CTX-M-1 gene using primers specific for bla_CTX-M-1. Among the 95 CTX-M producing isolates, CTX-M-1 gene was positive in 45 (47.3%) of the isolates. They were found in 36% of E. coli isolates, 36.3% of Enterobacter spp. and 58.3% of Klebsiella spp. CTX-M.

A high prevalence of CTX-M-1 (58.3%) type gene was recorded in Klebsiella spp. when compared with E. coli (36%) and Enterobacter spp. (36.3%). The restriction patterns of all the CTX-M PCR products was similar, with restriction bands at 267 bp, 156 bp and 120 bp. Therefore, it can be concluded that all the strains belong to CTX-M-1 group as reported by Edelstein. [3] The remaining (55.7%) probably belong to the other CTX-M subtype in CTX-M-1 group. RFLP is a technique which can be used to identify the bla_CTX-M into groups by its restriction patterns. However, only with the specific primer sequence the gene type can be identified.

Cefotaxime is a very commonly used third generation cephalosporin in hospitals in India for community acquired infections like pneumonia, enteric fever and meningitis. This result suggests that the spreading of this plasmid among different E. coli, Klebsiella spp., Enterobacter spp. was important in the dissemination of the CTX-M-1 gene. In a hospital environment, plasmids could be transferred easily between patients through health care workers due to hand carriage and selection pressure. To conclude, we have found a high prevalence of bla_CTX-M-1 β-lactamase in South Indian isolates while the prevalence of CTX-M-15 has been reported the world over. Thus, it indicates the need for a more detailed surveillance and epidemiological survey in this region.

References


Source of Support: Nil, Conflict of Interest: None declared.