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Molecular typing of bla $_{CTX\ M}$ β-lactamase producing *Escherichia coli* from clinical isolates

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Abstract- Objective: Production of Extended Spectrum β- Lactamase is an important mechanism of antimicrobial resistance exhibited by Enterobacteriaceae. There are many types of ESBLs among which CTX M is a latest emerged one which carries the bla $_{CTX\ M}$ gene. CTX M lactamase producing Enterobacteriaceae are present in the intestinal flora without harming the host causing infection in extra intestinal sites. Transferring the gene through plasmids is responsible for the dissemination of antimicrobial resistant strains in the community. This study is designed to detect *bla* $_{CTX\text{-}M}$ gene in Escherichia coli isolates.
Method: A total of 532 Escherichia coli isolates were screened for susceptibility testing. Isolates with decreased resistance to third generation Cephalosporins (3GC) were subjected to double disk synergy test for phenotypic confirmation of ESBL in which 178 were positive. Multiplex PCR was done for these 178 isolates to detect the four groups I, II, III & IV of CTX M.
Results: Multiplex PCR yielded the products with predicted size for group 1 CTX M in 152 (28.57%) isolates. None of the isolates were positive for other groups of CTX M (Group II, III & IV)
Conclusion: The presence of CTX M ESBL producing *E. coli* cause enormous problems in therapeutic interventions of infections which can be prevented only by continuous surveillance and prudent use of antibiotics.
Key Words: *bla* $_{CTX\text{-}M}$, Extended-Spectrum β-Lactamase, Escherichia coli

Introduction

Widespread mechanisms of antimicrobial resistance are posing a continuous problem in therapeutic interventions of infectious diseases. CTX M is relatively a new enzyme of Extended Spectrum β-Lactamases (ESBL) frequently isolated from Enterobacteriaceae [1]. CTX M producing Escherichia coli are present in the normal intestinal flora which disseminates in the community through fecal spread [2,3]. There are five groups of CTX M type ESBL in which Group I (CTX M 15) is more prevalent in Enterobacteriaceae which constitutes the intestinal flora [4]. This study describes the detection of bla $_{CTX\ M}$ gene coding for CTX M group I in *E. coli* isolates.

Materials and Methods

A total of 532 Non-repetitive clinical isolates of *E. coli* from patients in Community as well as Hospital set up recovered over a period of 10 months (Nov. 2009 to Aug 2010) from a variety of clinical specimens, i.e., Urine, Pus, Sputum, Blood etc, were subjected to the study. Identification of these isolates was done based on colony morphology on Blood agar, MacConkey agar and by standard biochemical reactions. The antibiotic susceptibility testing was done on Muller Hinton agar (MHA) after standardizing the suspension to 0.5 McFarland's standards. The results were interpreted as recommended by CLSI guidelines [5].Escherichia coli ATCC 25922 strain was used for quality control. Isolates with resistance or with lesser susceptibility to any of the three 3 GC's were selected for the study.

In Vitro Susceptibility Testing for Detection of ESBL

In vitro susceptibility was determined using double disk synergy test (DDST). In the DDST, synergy was determined between a disc of Augumentin (20 mcg of Amoxicillin & 10 mcg of Clavulanic Acid) and 30 mcg disc of each of the 3 GC antibiotics placed at a distance of 30 mm apart on a lawn culture of the resistant isolate under test on MHA [6]. The test organism was considered to produce ESBL if the zone size around the test antibiotic increased towards the Augumentin disc. This increase occurs because of the Clavulanic acid present in the Augumentin disc inactivates the ESBL produced by the test organisms [7].

PCR Screening

Multiplex PCR of $bla_{CTX\text{-}M}$ gene for groups I,II, III, & IV was carried out as quoted by *Pitout JDD et al., 2004* [8]. PCR amplification was carried out with specific primers using specific reaction parameters (Table1). DNA was prepared by emulsifying 2 - 5 Colonies in 100 µL of PCR grade water. 1 µL of the DNA template was added to 20 µL of the PCR reaction mixture. The cycling conditions were: initial DNA release and denaturation at 94^{o}C for 3 min, followed by 25 cycles of 94^{o}C for 30 s, 57^{o}C for 30 s and 72^{o}C for 30 s, followed by final elongation step at 72^{o} C for 5min. The PCR products were analyzed by gel electrophoresis with 2 % agarose in TBE with ethidium bromide (5 µg/ml) and visualized by UV-trans-illumination. A 100- bp DNA ladder (Invitrogen) was used as a marker.

Results

Phenotypic confirmation is done based on the CLSI guidelines [5]. Totally 532 *E. coli* isolates were screened for the production of ESBL out of which 178 were positive in phenotypic confirmation (Table 2). Isolates with increased zone size towards the Augmentin disc in DDST were considered positive by phenotypic confirmation. These 178 isolates were subjected to Multiplex PCR for the presence of the four groups (I, II, III & IV) of CTX M. Multiplex PCR yielded the products with predicted size for group 1 CTX-M enzyme in 152 (28.57%) isolates and they are grouped under Group I CTX M β-lactamases. None of the isolates were positive for other groups of CTX M (Group II, III & IV).

Discussion

Antimicrobial resistance is being the chronic problem in the therapeutic intervention of infections. Production of ESBL is an important and common mechanism of resistance among Gram negative bacilli. The rate and type of ESBL production by Enterobacteriaceae has markedly changed during this decade [9]. The old members (SHV, TEM) of ESBL which were responsible for Nosocomial infections have been now replaced by a new type CTX M which gained prominence among ESBL producing Enterobacteriaceae[9]. CTX M producing Enterobacteriaceae have predominance in the community acquired infections and they are mostly the causative organisms of Urinary Tract infection[2,10,11]. *E. coli* producing CTX M lactamases are present in the common intestinal flora being harmless to the host and they disseminate in the community and hospital setup to initiate infection[2,3]. The increased prevalence of group I bla $_{CTX\ M}$ genes in the community and the hospital settings is understood from our study. The bla $_{CTX\ M}$ gene in *E. coli* has been reported in France and Germany in the year 1989.[10]. The spread of CTX M was reported in France in the year 2002.[12]. In India, bla $_{CTX\ M}$ genes were reported from clinical isolates of *E. coli* and Klebsiella pneumoniae in 2000 [10]. According to a recent review and new data within GenBank, CTX-M-ß-lactamases Group I includes CTX-M-1, 3, 10 to 12, 15, 22, 23, 28, 29, and 30 [13]. One hundred and seventy eight isolates were positive for ESBL production in phenotypic confirmation out of 532 total isolates. These 178 isolates were subjected to PCR amplification in which 152 isolates were found to possess bla $_{CTX\ M}$ genes that belonged to group I CTX M. All the isolates were negative for groups II, III, & IV. This indicates that group I is the predominant form in *E. coli* as emphasized by other studies [4]. Twenty six isolates were not positive for any of the CTX M groups which suggest that they may belong to the other members of ESBL. Seriously ill patients have to be hospitalized and treated with heavy dose of antibiotics which in turn would result in the emergence of antibiotic resistant strains due to selection pressure. The hospital also provides a favorable environment for the resistant organisms to disseminate due to various factors like closeness with other patients, failure to follow aseptic precautions during procedures etc. It is reported that some members of group I CTX M do not respond to the drugs which are normally given for treating infections with ESBL producers such as inhibitor combination antibiotics (such as Piperacillin / Tazobactam and Amoxicillin /Clavulanate). The CTX M type ESBLs which are currently prevalent in the community would become the cause for serious infections in the hospital set up, making the therapeutic options limited. If this situation prevails we would have to face difficulties in therapeutic intervention of infections which could be prevented only by continuous surveillance and proper use of antibiotics.

Key Message

a) CTX M type is the newly emerged ESBL and it is the predominant form in Enterobacteriaceae.
b) They are present in the normal intestinal flora and spread in the community as well as in the hospital.
c) They cause serious infections which do not respond to commonly used antibiotics giving rise to problems in therapeutic intervention

References

[1] Canton R., and Coque T. M. (2006) *Curr. Opin. Microbiol.*, 9:466-475.
[2] Munday C.J., Whitehead G.M., Todd N.J. et al. (2004) *J Antimicrob Chemother*, 54: 628–33.
[3] Valverde A., Coque T.M., Sanchez-Moreno M.P. et al. (2004) *J Clin Microbiol.,* 42: 4769–75.
[4] Livermore D.M., Hawkey P.M. (2005) *J. Antimicrob. Chemother.*, 56 (3): 451-454.
[5] *Clinical and Laboratory Standards Institute (CLSI).20th informational supplement:M100-S20U* (2010) 30(15), 1-16.
[6] Vercauteren E., Descheemaeker P., Ieven M., Sanders C.G., Goossens H. (1997) *J Clin Microbiol.*, 35: 2191 – 2197.
[7] Subha A., Ananthan S. (2002) *IJMM,* 20,(2):92– 95.
[8] Johann D. D. Pitout, Ashfaque Hossain and Nancy D. Hanson (2004) *Journal of Clinical Microbiology*, 42(2), 5715–5721.
[9] Nordmann P., Ronco E., Naas T. et al. (1993) *AntimicrobAgents Chemother.*, 37: 962–9.
[10] Bonnet R. (2004) *Antimicrob Agents Chemother.*, 48: 1–14.
[11] Verma A., Desai N., Shannon K. et al. (2001) *Int J Antimicrob Agents,* 17: 123–9.
[12] Dutour C., Bonnet R., Marchandin H., Boyer M., Chanal C., Sirot D., et al. (2002) *Antimicrob. Agents Chemother.*, 46:534-7.
[13] Baraniak A., Fiett J., Sulikowska A., Hryniewicz W. and Gniadkowski M. (2002) *Antimicrob. Agents Chemother*. 46:151-159.

Table 1- Primers used for amplification

Target	Primer	Sequence	Product size (bp)	GenBank accession no.
CTX-M group I	CTXM1-F3 CTXM1-R2	GAC GAT GTC ACT GGC TGA GC AGC CG C CGA CGC TAA TAC A	499	**X92506**
CTX-M group II	TOHO1-2F TOHO1-1R	GCG ACC TGG TTA ACT ACA ATC C CGG TAG TAT TGC CCT TAA GCC	351	**X92507**
CTX-M group III	CTXM825F CTXM825R	CGC TTT GCC ATG TGC AGC ACC GCT CAG TAC GAT CGA GCC	307	**AF189721**
CTX-M group IV	CTXM914F CTXM914R	GCT GGA GAA AAG CAG CGG AG GTA AGC TGA CGC AAC GTC TG	474	**AF252622**

Table 2- Total number of ESBL positive isolates by phenotypic confirmation from various samples

Sl. No.	**SAMPLE**	**NO. OF ESBL POSITIVE ISOLATES (n = 178)**
1	URINE	69
2	BLOOD	25
3	PUS	43
4	RESPIRATORY SPECIMENS	28
5	MISCELLANEOUS	13