

## Original Article

# Detection of CTX-M gene in extended spectrum beta lactamase (ESBL) producing *Escherichia coli* and *Klebsiella species* of different hospitals.

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### Abstract:

A total of 200, non-duplicate ESBL producing strains (171 *Escherichia coli* and 29 *Klebsiella spp.*) from three tertiary care hospitals were detected using screening test & double disc synergy test. All isolates were screened for the detection of CTX-M type Extended spectrum Beta-lactamase (ESBL) using PCR. Among them 133 (66.5%) were positive for CTX-M type ESBLs which include 114 (66.66%) *E.coli* and 19 (65.51%) *Klebsiella spp.* This is the first report of identifying CTX-M gene in ESBL producing *Escherichia coli* and *Klebsiella species* of different hospitals.

**Keywords:** ESBL, *bla* CTX-M gene, PCR.

### Introduction

Resistance to extended spectrum cephalosporins can occur in *Enterobacteriaceae* via the production of extended spectrum Beta-lactamases (ESBLs) that are capable of hydrolyzing the oxyiminocephalosporins and monobactams.<sup>1</sup> In recent years a new family of plasmid mediated CTX-M extended spectrum  $\beta$ -lactamases (ESBLs) called CTX-M has arisen and reported in the literature with increasing frequency from Europe, Africa, Asia, South America and North America.<sup>2</sup> These ESBLs were named CTX-M type Beta-lactamases, owing to their high activity against cefotaxime.<sup>2</sup>

In a study at Indian hospitals 73% (72% of total *K.pneumoniae* and 73% of total *E.coli*) 3<sup>rd</sup> generation cephalosporin-resistant isolates were found to carry CTX-M gene.<sup>3</sup> In our country isolates producing ESBLs have not

been characterized in earlier studies. Although ESBL phenotypes have been reported from Bangladesh, there is no information on their molecular types. Hence the present study was undertaken to characterize the Beta-lactamases in multidrug resistant clinical isolates of *Enterobacteriaceae* by molecular techniques.

### Materials and Methods

This cross-sectional study was carried out in the department of Microbiology, Sir Salimullah Medical College for a period of one year from January 2009 to December 2009. Total 200 ESBL producing *E.coli* & *Klebsiella spp* were taken as study strain, of which 50 isolated from 308 samples of wound swab, throat swab and urine were collected from in-patient and out-patient department of Sir Salimullah Medical College & Mitford Hospital & 150 were known ESBL producing strains of Bangabandhu Sheikh Mujib Medical University (BSMMU) and Bangladesh Institute of Research & Rehabilitation in Diabetes Endocrine & Metabolic Disorders (BIRDEM) hospital. Sample from patients clinically suspected to have urinary tract infection, wound infection and respiratory tract infection were collected. All ESBL

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producing isolates were phenotypically detected by screening test & double-disc synergy test.

**Culture**

All specimens (wound swabs, urine samples and throat swabs) were inoculated into Blood agar and MacConkey’s agar media and incubated at 37° C aerobically. After over night incubation, bacterial isolates were identified by colony morphology, staining, motility test and appropriate biochemical tests (catalase, coagulase, oxidase and others).<sup>4,5</sup>

**Screening test for ESBL producers: Disc diffusion method**

Screening test was done by disc diffusion method according to the NCCLS. Isolated Gram negative strains were treated as screening positive which showed specific zone diameter to any one of the following antimicrobial discs- ceftazidime (22µg), cefotaxime (27µg), ceftriaxone (25µg), and aztreonam ( 27µg).<sup>6</sup>

**Double disc synergy test**

Isolated Gram negative bacteria were subjected to double disc synergy test as described previously. ESBLs production was considered positive when the inhibition zone around the test antibiotic disc (ceftazidime, ceftriaxone, cefotaxime and aztreonam disc) was increased towards the augmentin disc (20µg amoxicillin and 10µg of clavulanic acid) which was placed in the centre of the plate and 20 mm apart from other discs.<sup>7</sup>

**Polymerase Chain Reaction (PCR) :**

All ESBL producing isolates were screened for the resistance gene CTX-M by PCR assay using universal primers.<sup>8</sup> Bacterial DNA extraction was performed by simple boiling method.<sup>9</sup> PCR amplification reactions were performed in a volume of 375 µl for 15 PCR reaction containing 300 µl PCR Master mix (Invitrogen, USA), 15 µl of each primer, and 3 µl of DNA template. The cycling parameters were as follows: an initial denaturation at 94°C for 7 min; followed by 35 cycles of 94°C for 50s, 54°C for 40s, and 72°C for 60s ; and with a final extension at 72°C for 5 min.<sup>8</sup> The amplified PCR products were subjected to electrophoresis at a 1.5% agarose gel in 1x TBE buffer. Strain with known Beta-lactamase type was included as reference. Here *E.coli* j53 (met pro) pMG 298 used as a positive control for CTX-M gene detection (Kindly donated by George Jacoby, USA).

**Result:**

A total of 50 isolates were phenotypically identified as ESBL producers from Sir Salimullah Medical College Hospital

(SSMCH) and 150 isolates were also taken as known ESBL producing strains from BSMMU and BIRDEM hospital, which were previously phenotypically identified. All those 200 ESBL producers were selected for CTX-M gene detection by PCR, which included 171 *Esch.coli* and 29 *Klebsiella spp.*

Among the 200 ESBL- producing clinical isolates, 133 strains (66.5%) were positive for CTX-M gene by PCR. The amplified product was visualized at 593 bp by agarose gel electrophoresis (Figure 1). These include 114 (66.66%) *E.coli* and 19 (65.51%) *Klebsiella spp.* (Table I).

**Table I: Presence & distribution of CTX-M gene among ESBL producing *E.coli* and *Klebsiella spp.* (n=200)**

Organism	Source of organism	Total ESBL producing isolates tested for CTX-M gene by PCR.	CTX-M gene positive
<i>E.coli</i>	SSMC - 45		
	BSMMU - 45	171	114 (66.66)
	BIRDEM - 81		
<i>Klebsiella spp</i>	SSMC - 5		
	BSMMU - 5	29	19 (65.51)
	BIRDEM - 19		
<b>Total</b>		<b>200</b>	<b>133 (66.5)</b>

Note: Figures in parentheses represent percentage.

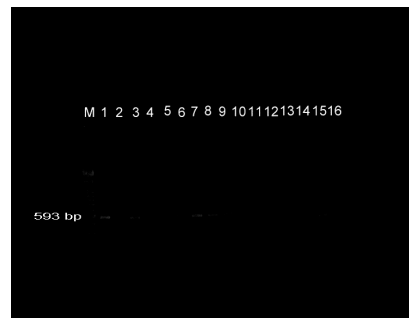


Figure 1. Photograph of electrophoretic bands of CTX-M gene in ESBL producing *E.coli* strains after agarose (1.5%) gel electrophoresis. Lane M: 1 kb plus Ladder, Lane 1: *E.coli* j53 pMG 298 positive control, Lane 2-15: ESBL producing *E.coli*, Lane 2, 3, 6, 7, 8, 9, 14 & 15: positive band of ESBL producing *E.coli*, Lane 16: Negative control (no template DNA added).

The CTX-M gene among ESBL isolates were higher in SSMCH, which were 39 (78%) out of 50 ESBL positive isolates and the lower rate was found in BIRDEM which were 57 (57%) out of 100 ESBL positive isolates. In BSMMU, 37 (74%) out of 50 ESBL positive isolates were found to be CTX-M gene positive (Table II). Statistically the difference among various hospitals is significant ( $p < 0.05$ ).

**Table II:** Distribution of CTX-M gene positive ESBL producing isolates among three different hospitals.

Hospitals	Number of ESBL positive isolates tested for CTX-M by PCR.	CTX-M gene positive	CTX-M gene negative
SSMC&MH	50	39 (78)	11(22)
BSMMU	50	37 (74)	13 (26)
BIRDEM	100	57 (57)	43 (43)
TOTAL	200	133 (66.5)	67 (33.5)

Note: Figures in parentheses represent percentage.

\* Chi-square = 8.28,  $df = 3$ ,  $P < 0.05$ .

### Discussion

Although several studies addressed the issue of the emergence of ESBL producing *Enterobacteriaceae* worldwide, no reports of genotypic characterization have been published from Bangladesh till now. It has been mostly reported that resistance to Beta-lactam antibiotics is on the rise among clinical isolates in different hospitals of Bangladesh, expressing the need for an extensive research. The present report is the first report of isolation of *bla* CTX-M gene from 133 ESBL strains of *E.coli* & *Klebsiella spp.*, isolated from three different tertiary care hospitals in Dhaka city.

Molecular characterization by PCR revealed that *bla* CTX-M gene was prevalent in 66.5% of these ESBL producers in Bangladesh. A report on ESBL types in *Enterobacteriaceae* in Argentinean public hospitals found that CTX-M accounted for roughly 70% of all ESBLs found, with similar findings being reported in studies conducted in Japan, China, United Kingdom and Spain.<sup>10-14</sup> The prevalence of *bla* CTX-M was also reported by Shahid *et al.*, with 72 (77.4%) of the 93 *E. coli* isolates being found to be CTX-M group -1 positive by

PCR in the north Indian isolates.<sup>15</sup> In a study at Indian hospitals 73% (72% of total *K.pneumoniae* and 73% of total *E.coli*) 3<sup>rd</sup> generation cephalosporin-resistant isolates were found to carry CTX-M gene.<sup>3</sup> In contrast to the findings of above studies the prevalence of CTX-M type ESBL was less in the present study. In India, Sekar *et al.* reported that 44.4% of *E. coli* and 35.29% of *K. pneumoniae* strains were found to be positive for *bla* CTX-M gene by PCR.<sup>16</sup> In this study 66.66% of *E.coli* and 65.51% of *Klebsiella spp.* were found to be positive for *bla* CTX-M gene by PCR. In contrast to the findings of Sekar *et al*, it was higher among different isolates.

The higher rate of CTX-M type ESBL- isolates were detected in SSMC which were 78% (39 out of 50 ESBL positive isolates) and the lower rate was found in BIRDEM which were 57% (57 out of 100 ESBL positive isolates). In case of BSMMU, it was 74% (37 out of 50 ESBL positive isolates). In India, Kingsley & Varghese reported that 45% (85 out of 188 ESBL producing clinical isolates) were found CTX-M type ESBL in their study.<sup>17</sup> In case of SSMC & BSMMU the result was more or less same but in case of BIRDEM the percentage was less than others. The reason of such difference may be due to the fact that ESBLs encoding genes other than CTX-M such as TEM or SHV might be present in these isolates and the primers (CTX-MU1 & CTX-MU2) used in this study are only specific for CTX-M gene. In a study at Indian hospital by Lal *et al.*, TEM and SHV gene was present in 20% and 8.4% among the *Klebsiella spp.* respectively.<sup>18</sup>

Our finding emphasizes the increasing role of the *bla* CTX-M gene in Beta-lactam antibiotic resistance worldwide and leads to consideration of empirical treatment for infections caused by coliforms, especially in patients compromised by underlying disease or immunological status. Identification of *bla* CTX-M gene among *E. coli* and *Klebsiella spp* is uncommon; and this is the first report from Bangladesh. In conclusion, the data presented here illustrates the complexity and extent of the spread of ESBL producing *Enterobacteriaceae* in Bangladesh. This result demonstrates the presence of CTX-M type ESBL in our country.

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