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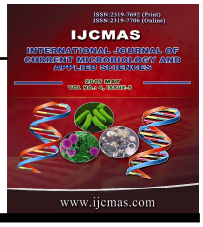


**Original Research Article**

# Prevalence of CTX-M-15 β-Lactamase Producing *Escherichia coli* among Extra-intestinal Infections

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**A B S T R A C T**

CTX-M-15 subtype of extended spectrum beta lactamase (ESBL) has increased in recent years around the world. The management of infections is complicated by the increasing prevalence and spectrum of antimicrobial resistance especially ESBL producing *E. coli*. This study is done to determine the prevalence of ESBL producing *E. coli* from different extra-intestinal infections. Hundred *E. coli* isolates have collected from Krishna Rajendra hospital, Mysore, India over a period of 10 months. The production of ESBLs has investigated using antibiotic disks and by Polymerase chain reaction (PCR) and sequencing to determine the CTX-M types. Out of 100 *E. coli* isolates; 94, 91,75, 69, 68 samples and 52 were resistant to ampicillin, amoxyclav, cefotaxime, ceftazidime, ceftroxime and cefepime respectively while 100, 97,91,90 were sensitive to levofloxacin, doxycycline, imipenime and amikacine respectively. PCR results have shown that 84 of *E. coli* strains were positive to CTX-M-15 while 16 of strains were non CTX-M. The prevalence of ESBL producing *E. coli* was found to be 75% and the CTX-M-15 was the predominant type among extra-intestinal pathogenic *E. coli*. This was significantly higher than the other data available in India which refer to the increased of control of dissemination of antibiotic resistance.

**Keywords**

E. coli,
CTX-M,
ESBLs,
ExPEC,
Antibiotic resistance

## Introduction

The extended-spectrum b-lactamases (ESBLs) enzymes are plasmid-mediated enzymes capable of hydrolyzing and inactivating a wide variety of oxyimino cephalosporins. Their beta-lactamase genes are located on bacterial chromosomes or on transferable elements such as plasmids, transposons or integrons (Machado *et al.*, 2005) which play an important role in a horizontal transmission of resistance genes. The resistance to extended spectrum beta lactams is increasingly reported worldwide. The first β-lactamase was identified in *E. coli* prior to the release penicillin for use in the medical practice (Bradford *et al.*, 2001). The name of *CTX-M* enzymes refers to their ability to hydrolyze cefotaxime and may hydrolyze ceftazidime and cefepime (Paterson *et al.*, 2005). It was designated as *FEC-1* when was isolated from cefotaxime resistant *E. coli* isolates in Japan in 1986 as a non *TEM* and non *SHV* (Bonnet, 2004).

However, in 1989 it was designated as *CTX-M-1* when it was isolated from cefotaxime-resistant clinical *E. coli* strains that neither produced *TEM* nor *SHV* (Paterson *et al.*, 2005). Currently, there are more than 120 different CTX-M β-lactamases that are clustered into five groups (CTX-M-1, -2, -8, -9, and-25). The *CTX-M* family shows approximately 40% similarities to *TEM* and *SHV* beta-lactamases at the amino acid level (McCracken 2006). CTX-M depending on their amino acid sequence similarities is sub classified into five groups: (i) *CTX-M-1* group includes six plasmid-mediated enzymes (*CTX-M-1, CTX-M-3, CTX-M-10, CTX M-12, CTX-M-15, CTX-M-22, CTX-M-23, CTX-M-28* and *FEC-1*), (ii) *CTX-M-2* group includes eight plasmid-mediated *CTX-M* enzymes (*CTX-M-2, CTX-M-4, CTX-M-4L, CTX-M-5, CTX-M-6, CTX-M-7, CTX-M-20,* and *Toho-1*), (iii) *CTX M-8* group includes one plasmid-mediated member, (iv) *CTX-M-9* group includes nine plasmid-mediated enzymes (*CTX-M-9, CTX-M-13, CTX-M-14, CTX-M-16, CTX-M-17, CTX-M-19, CTX-M-21, CTX-M-27, CTX-M-24*) and (v) *CTX-M-25* group includes the *CTX-M-25* and *CTX-M-26* enzymes (Bonnet, 2004). Extra-intestinal pathogenic *E. coli* (ExPEC) that can cause infection outside the intestinal tract are split up as uropathogenic *E. coli* (UPEC), sepsis associated *E. coli* (SEPEC), or neonatal meningitis associated *E. coli* (NEMEC) which reflect their ability to cause disease at multiple anatomical sites (Russo *et al.*, 2003 and Dobrindt and Hacker, 2008). Pathogenic *E. coli* as intestinal pathogens and extra-intestinal *E. coli* infections are recognized as a major source of morbidity, mortality, and increased health costs (Hamelin *et al.*, 2006). Enterobacteriaceae with CTX-M enzymes have been associated with both hospital-acquired and community infections, mostly *Escherichia coli* from the urinary tract in the UK (Karisik *et al.*, 2006). In India prevalence of extended-spectrum β-lactamase (ESBL) producing bacteria ranges from 23-86% in earlier reports and 63.6% of *E. coli* in 2009 (Anandan *et al.*, 2009). Prevalence of ESBLs varies greatly around the world and is increasing. The difference of the prevalence of ESBLs in certain parts of the world may relate to differences in cephalosporin usage and methods of detecting ESBLs as well as lack of a sufficient surveillance system. In this study we will determine the prevalence of ESBLs and investigate the type of CTX-M among *E. coli* isolates that were isolated from different Extra-intestinal infections.

## Materials and Methods

### Bacterial isolates

A total of 100 of *E. coli* clinical isolates were collected from the Department of Microbiology, Krishna Rajendra (K. R.) Hospital, Mysore, India. All isolates were obtained from different extra-intestinal specimens including urine, blood, sputum and exudates over a Period of 10 months (June 2013 to March 2014). All isolates were identified as *E. coli* by inoculated them on MacConky Agar (M.A.) and Eosin Methylen Blue (E.M.B.) Agar and by using biochemical identification kit (HiE. coli Identification Kit, HiMedia Laboratories Pvt. Ltd, Mumbai, India).

### Antibiotic sensitivity test

Antibiotic Sensitivity was determined by Kirby-Bauer disk diffusion method as per CLSI recommendations (2012). Antimicrobial disks used were Ampicillin (10 µg), Cefotaxime (30 µg), Cefoxitin (30 µg), Ceftazidime (30 µg), Gentamicin (10 µg), Amikacin (30 µg), Cefepime (30 µg), Cefuroxim (30 µg), Norfloxacin (10 µg), Chloramphenicol (30 µg), Levofloxacin (5

µg), Trimethoprim-sulfamethoxazole (25 µg), Amoxyclav (30µg), Doxycycline Hydrochloride (30 µg), Cefpodoxime (10 µg) and Imipenem (10 µg) (HiMedia, India).

**Screening test for ESBL production**

According to the CLSI guidelines, isolates showing inhibition zone size of  22 mm with Ceftazidime (30 µg), and ≤ 27 mm with Cefotaxime (30 µg) were identified as potential ESBL producers and shortlisted for confirmation of ESBL production.

**Confirmatory test for ESBLs**

Phenotypic confirmatory test of ESBL production was performed by a double disc synergy test using Hexa disc rings (HiMedia, India) that contain three antibiotics and three antibiotics with clavulanic acid that include cefotaxime (30 µg), cefotaxime-clavulanic acid (30/10 µg), ceftazidime (30 µg), ceftazidime-clavulanic acid (30/10 µg) and Cefpodoxim (10µg) and Cefpodoxim-clavulanic acid (10/5 µg). Each ring was placed on a lawn culture of the test isolate on Muller Hinton Agar (MHA) plate and incubated overnight at 37C.

Interpretation: when there is an increase of ≥5 mm in inhibition zone diameter around combination disk of Cefotaxime+Clavulanic acid versus the inhibition zone diameter around Cefotaxime disk alone, it confirms ESBL production.

**Determination of CTX-M types By Polymerase Chain Reaction (PCR)**

Template DNA was prepared by boiling method as described by Ruppe *et al.*, 2009.The presence of *bla*CTX-M β-lactamase genes will be screened as described previously (Paterson *et al.*, 2003) by using primers blaCTX-Mf 5'-CGCTTTGCGATGTGCAG-3' and blaCTX-Mr 5'-ACCGCGATATCGTTGGT-3' with using the following reaction parameters: 35 cycles of amplification at a denaturation temperature of 94°C for 30 s, an annealing temperature of 60°C for 1 min and an extension temperature of 72°C for 1 min. This step was followed by a final extension at 72°C for 10 min. PCR products were resolved on 1% agarose gels, stained with ethidium bromide, and photographed with UV illumination. All positive PCR products will send for sequencing to investigate the specific types of CTX-M by blasting on BLAST website (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

**Statistical analysis**

Chi square was used to see the significance of difference between the production of CTX-M in ESBL and non ESBL producer strains.

## Result and Discussion

Hundred *E. coli* isolates were collected from different samples, urine (55%), exudates (30%), sputum (8%) and blood (7%).

**Antibiotic sensitivity test**

Out of 100 *E. coli* isolates; 94, 91,75, 69, 68 samples and 52 are resistant to ampicillin, amoxyclav, cefotaxime, ceftazidime, ceftroxime and cefepime respectively which are considered as multidrug resistant *E. coli* strains. While 100, 97, 91 and 90 still sensitive to levofloxacin, doxycycline, imipenime and amikacine respectively as shown below in table 1.

The isolates that showed resistance to cefotaxime, ceftazidime, cefepime and ceftroxime are considered as ESBL producers and it confirmed by double disk

synergy test using combined antibiotics (cefotaxime, ceftazidime and cefpodoxim) with clavulanic acid (Fig. 1). The antibiotic sensitivity results are explored in the graph (Fig. 2).

**Determination of CTX-M types**

Out of 100 *E. coli* strains 84 are positive for CTX-M gene subtype CTX-M15 while 16 of strains are non CTX-M producers as figure 3.

Compression of the strains related to its resistance to cefotaxime and production of CTX-M enzyme we found that 81 of *E. coli* strains are resistant and intermediate to cefotaxime and producing to CTX-M while 6 strains are resistant to cefotaxime but not producing CTX-M. However, 9 strains of *E. coli* are sensitive to cefotaxime are CTX-M producers. The difference between the production of CTX-M of ESBLs and non ESBLs producing *E. coli* was significant (P=0.00 □0.05).

Out of 100 *E. coli* isolates, 94, 91, 75, 69, 68 samples and 52 are resistant to ampecillin, amoxyclav, cefotaxime, ceftazidime, ceftroxime and cefepime, respectively. While 100, 97, 91 and 90 still sensitive to levofloxacin, doxycycline, imipenime and amikacine respectively. This result is agree with Datta *et al.* (2004) and Anandan *et al.* (2009) with the exception for amikacine and gentamicine which last report showed the isolates were resistant for it. 35 % of isolates are resistant to gentamicine while another study (Umadevi *et al.*, 2011) showed 100% of *E. coli* were sensitive to gentamicine. Recently, prevalence of ESBL producing *E. coli* has been increasing over the past years, resulting in limitation options. In current study 75% of *E. coli* strains are ESBL producers related to their resistance to cefotaxime and the increase of zone inhibition of cefotaxime with clavulanic acid. It is a high percentage of ESBLs regarding to another study which reported that the prevalence of ESBL-*E. coli* was 18.5%, 22%, 38.4% and 60% among UTI infections (Agrawal *et al.*, 2008; Rajan and Prabavathy, 2012) and (Narayanaswamy and Mallika, 2010) respectively, but it compatible with another study (Freeman *et al.*, 2008) which reported that 90% of patients who travelled from New Zealand to India are infecting by ESBLs *E. coli.* The differences in the prevalence of ESBLs producing *E. coli* between the regions may refer either to the randomly consuming of antibiotics or to the different endemic strains. Genotypic analysis of CTX-M enzyme showed all CTX-M producing *E. coli* are carrying CTX-M-15. It is a high percent in comparison with previous study which found CTX-M-15 in different countries in different percent 45% and 96.77% (Kingsley *et. al.*, 2008 and Al-Agamy *et al.*, 2014). The travelling between different countries may play an important role in dissemination of ESBLs among different pathogens as what was reported by Yaita (2014) who reported that the travel to India was a risk factor (Odds Ratio 13.6, 95% Confidence Interval 3.0–75.0, p,0.0001) and the dissemination of CTX-M-15 was 77%. Another study (Freeman *et al.*, 2008) reported that 90% of patient who travelled to India was infected by ESBL-E. coli and 88.88% of them were carrying CTX-M-15. In comparison the *E. coli* isolates related to their resistance to cefotaxime and production of CTX-M enzyme, we found that not all cefotaxime-resistant isolates producing of CTX-M, which 6 isolates were not producing of CTX-M. These isolates may carry another beta-lactamase gene which are not including in this study. On contrary 6 isolates that were sensitive to cefotaxime showed positive to CTX-M gene which can be

interpreted by these isolates contain CTX-M gene but still not express it or may exposed to external effects that disrupted the gene. Related to the previous and recent studies, the prevalence of CTX-M-15 has increased so fast around the world. This finding may refers to the randomly using of antibiotics or the combination of antibiotic resistance with specific epidemiological strains which make the control of dissemination of antibiotics resistance is difficult and the infections cannot be treatment.

Prevalence of CTX-M-15 has increased among *E. coli* infections in compression to previous studies which may refer to some epidemiological strains may disseminate that was harboring CTX-M-15. Many studies should be done to investigate the epidemiological *E. coli* strains with their phylogenetic groups which may refer to the source of infection to control the dissemination of ESBLs.

**Table.1** Antibiotics sensitivity test

| Antibiotics | R | I | S |
|---|---|---|---|
| **CTX** | 75 | 6 | 19 |
| **AMP** | 94 | 0 | 6 |
| **CAZ** | 69 | 7 | 24 |
| **GEN** | 35 | 8 | 57 |
| **AK** | 10 | 0 | 90 |
| **CPM** | 52 | 12 | 36 |
| **IPM** | 8 | 1 | 91 |
| **AMC** | 91 | 6 | 3 |
| **CX** | 29 | 8 | 63 |
| **C** | 12 | 14 | 74 |
| **COT** | 46 | 17 | 37 |
| **LE** | 0 | 0 | 100 |
| **CXM** | 68 | 6 | 26 |
| **NX** | 43 | 14 | 43 |
| **DO** | 0 | 3 | 97 |

AMP; Ampicillin, CTX; Cefotaxime, CX; Cefoxitin, CAZ; Ceftazidime, GEN; Gentamicin, AK; Amikacin, CPM; Cefepime, CXM; Cefuroxim, NX; Norfloxacin, C; Chloramphenicol, LE; Levofloxacin, COT; Trimethoprim-sulfamethoxazole, AMC; Amoxyclav, DO; Doxycycline Hydrochloride and IPM; Imipenem

**Figure.1** A, B; Antibiotic sensitivity test using Antibiotic discs, C; ESBL confirmation test using Hexa disc rings

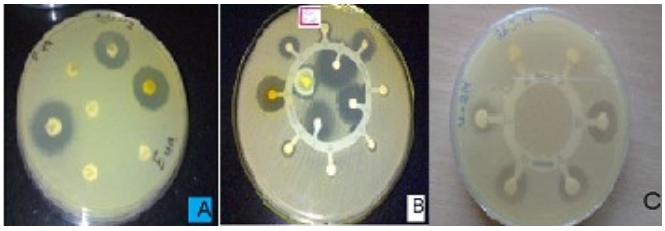


**Figure.2** Antibiotic sensitivity test

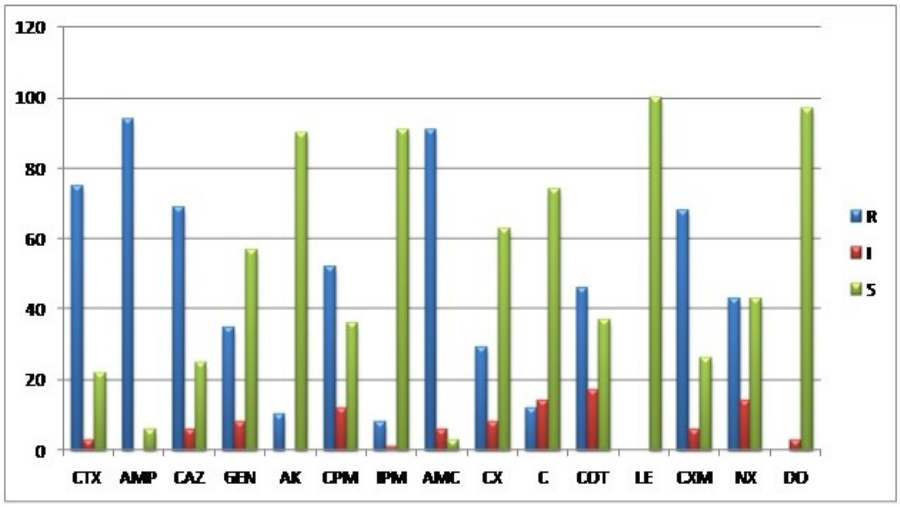
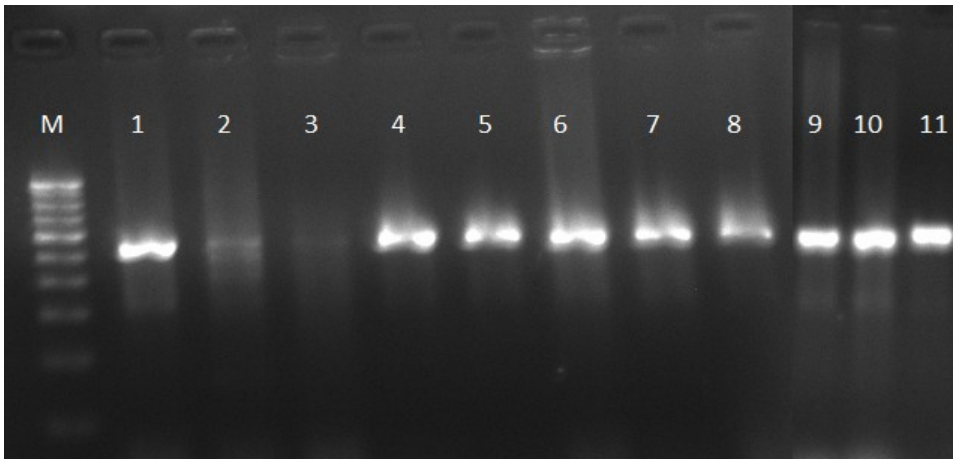



**Figure.3** PCR Result of CTX-M: Lane M; DNA Marker, Lanes 1-11 refer to sample No.; D59, U12, S3, E24, U-5, U-55, U-43, S-10, E56, E-8, and E-62 are positive to CTX-M

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