**Original Article**

# CTX-M ESBL enzyme in Escherichia coli from urology patients in Rawalpindi, Pakistan

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## Abstract

**Objective:** To detect CTX-M phenotype utilizing disc diffusion and MIC testing in Escherichia coli isolated from a tertiary care urology setting.

**Methods:** Fifty single, non duplicate ESBL producing isolates from a tertiary care urology hospital were evaluated for the presence of CTX-M phenotype. Initially all the urinary isolates were tested for ESBL production. The isolates were identified by using API 20E galleries and screened for ESBL production by combination disc methods. Representative 4 ESBL isolates were sent to Antibiotic Resistance Monitoring and Reference Laboratory (ARMRL), Health Protection Agency, Colindale, London, UK where those were further subjected to MIC testing by agar dilution and E-test strips.

**Results:** A total of 4 ESBL producing E. coli isolates were characterized to be CTX-M on phenotypic characterization. The overall yield of CTX-M phenotypes was 75%.

**Conclusion:** The emergence of CTX-M from Pakistan is alarming; however, further studies are required to study the epidemiology and genetic characterization of CTX-M types of ESBLs (JPMA 56:576;2006).

## Introduction

Extended spectrum beta lactamases (ESBLs) have become a challenge both from the diagnostic as well as on the management point of view. Beta lactam antibiotics are the most common treatment for bacterial infections. Concurrently the beta lactamases are the major defense of gram negative bacteria against beta lactam antibiotics. These enzymes cleave the amide bond in the beta lactam ring, rendering beta lactam antibiotics harmless to bacteria.[1] Oxyimino-cephelosporins were introduced in the early 1980's and it was hoped that the resistance problem would be overcome. But the euphoria proved short lived and resistance to the newer class of drugs developed soon with the first incidence of resistance to these compounds reported in 1983. Ever since then, the resistance has been on the increase with modifications in the detection occurring simultaneously.[2,3]

These enzymes which now number more than 150 were initially limited to Escherichia coli and Klebsiella species. Lately many have been spreading and are engulfing other genera specially Enterobacter and Proteus. ESBL phenotypes and detection have become more complex due to the diversity of the enzymes produced, emergence of inhibitor resistant ESBL variants plasmid borne resistance genes, Concurrent Amp-C production enzyme hyperproduction and porin loss. During the last decade a number of ESBL phenotype has been reported. The production of multiple enzymes, inhibitor resistant ESBL variant, emergence of CTX-M types of ESBLs, plasmid borne AmpC and production of ESBLs in AmpC producing strain, has rendered more complexity to the ESBL phenotypes.[4] During the late 1990s and early 2000s CTX-M producing enterobacteriacea has emerged as the most common ESBL type in many parts of the world including Africa, South America, Asia and some parts of Europe.[5,6]

In Pakistan, a number of studies have reported a high incidence of ESBL producing gram negative bacteria[7,8], however phenotypic characterization of these enzymes to our knowledge have not been reported so far. We report our findings of CTX-M ESBL producing E. coli isolates.

## Methods

A total of 4 ESBL producing E. coli isolates from hospitalized urology patients were studied for phenotypic characterization at the Microbiology Department of the Armed Forces Institute of Pathology, Rawalpindi. Urine specimen were cultured onto CLED medium (Oxoid). The isolates were identified by using API 20E galleries (Biomerieux, France) and screened for ESBL phenotype in light of CLSI recommendations. This was checked by initial screening by Ceftazidime(30 g) and Cefotaxime(30g), and inhibition zones of equal to or lesser than 22mm and 27mm respectively were taken as breakpoints for further confirmation by phenotypic method. This was done by ceftazidime(30 g) and combination discs of Ceftazidime-clavulanate (30/10g) and cefotaxime(30 g) and Cefotaxime-clavulanate (30/10g) discs(obtained from Becton Dickinson, Cockeysville, MD, USA) and interpreted as per

recommendations of NCCLS.[9] In the phenotypic confirmation method an increase of 5mm or more of the zone diameter for either antimicrobial agent when tested in combination with clavulanic acid was taken to be positive. All the isolates were sensitive to imipenem, ertapenem and meropenem and resistant to quinolones and aminoglycosides. These isolates were sent to Antibiotic Resistance Monitoring and Reference Laboratory (ARMRL), Health Protection Agency, London for further analysis. Each isolate was subjected to MIC testing by agar dilution and E-test strips and were interpreted according to British Society of Antimicrobial Chemotherapy (BSAC) guidelines.[9] The resistance breakpoints for the antibiotics used were as Amikacin >16µg/ml, Ampicillin >32µg/ml, Aztreonam >1µg/ml Ceftriaxone >1µg/ml, Cefotaxime >1µg/ml, ceftazidime >2µg/ml, Cefpirome >1µg/ml, cefoxitin >8µg/ml, Ciprofloxacin >4µg/ml, Co-amoxiclav >32µg/ml, Co-trimoxazole >32µg/ml, Colistin >4µg/ml, Doxycycline >1µg/ml, Ertapenam >2µg/ml, Gentamicin >4µg/ml, Imipenam >4µg/ml, Levofloxacin >2µg/ml, Meropenam >4µg/ml, ofloxacin >1µg/ml , Piperacillin/ Tazobactam >16µg/ml, Piperacillin >16µg/ml and Toberamycin >4µg/ml. In the E-test method the ratio of MICs for the cephalosporin alone in relation to the combined discs was more than 8 when Ceftriaxone was used and in three out of the four isolates it was less than 8 when Ceftazidime was used.

## Results

All the isolates had MIC of Cefotaxime > 8 fold higher than the MIC of Ceftazidime implying CTX-M type of ESBL. Disc diffusion results tipped in favor of a CTX-M producer which were confirmed on MIC based evaluation.[10] However the MIC of ceftazidime for isolate no 1 was high and exact characterization of the resistant phenotype can only be concluded after genetic studies which were not carried out. Cefoxitin was resistant therefore excluding Amp-C producers. Piperacillin/ Tazobactem combination was resistant in isolate No 1 and sensitive for the others. Piperacillin showed intermediate resistance and isolates were susceptable to carbapenems. Tigecycline was sensitive. There was resistance to Gentamicin and toberamycin and borderline susceptibility to amikacin. Resistance was also observed against Ciprofloxacin, Ampicillin and Augmentin.

## Discussion

This problem started with the detection of transferable resistance in Enterobacteriacae against extended spectum cephelosporins.[2,3] Once detected many reports of its confirmation soon followed. TEM and SHV types, belonging to Class A of the Ambler classification of beta lactamases, were known before the emergence of ESBL.[1] ESBL consist of many classes with TEM and SHV being reported initially. The clinical significance rested in the fact that these resistance genes were carried on the plasmids bearing a direct relationship to the spread of the resistance horizontally across microbial species.[11] On the other hand class B and D beta lactamases have the enzymes for resistance encoded by chromosomal genes therefore the resistance is confined to particular bacterial species. More recently non-TEM and NON-SHV plasmid mediated ESBLs have been reported with VEB, TLA-1 AND CTX-M types being a few. More than 130 types of TEM enzymes, more than 50 variants of SHV- type and more than 40 types of CTX-M enzymes have been described.[12-16] The CTX-M enzymes are the most wide spread and their rate of dissemination among bacteria and in various geographical regions has increased rapidly.[5,6] This shift towards CTX-M types is world wide. ESBLs do not tend to remain confined to hospitals only and have a potential to become the leading community based strains. Animals are also implicated in being a possible source for transfer of ESBL-producing resistance genes to humans. Since the ESBLs belong to the class A beta lactamases, these are plasmid mediated and therefore pose serious risk of crossing generic boundaries, this is exemplified in the increasing genera of bacteria which continue to show ESBL production. ESBL have also been reported in Citrobacter species, Enterobacter cloacae, Enterobacter aerogenes, Providencia, Morganella, Proteus mirabilis, Serratia marcecens and Salmonella species.[21-23] The problem is compounded by the fact that some plasmids also carry resistance genes for aminoglycosides, tetracycline, trimethoprim/sulfamethoxazole, chloramphenicol, Nalidixic acid and flouroquinolones. This results in selective pressure and increases the chances of selecting out the ESBLs. Similarly prior use of 3rd generation cephalosporins, trimethoprim/sulfamethoxazole and flouroquinolones increases the chances to develop ESBL.[17,18]

Appropriate detection protocols need to be devised to readily identify the resistant organism. However even in the developed countries detection is below minimal required standards. In proficiency testing of laboratories outside the United States only 2 of 129 laboratories specifically identified a highly resistant ESBL-producing K. pneumoniae isolate. A study recently published showed that only 8% of clinical laboratories from rural hospitals in the USA routinely screened for ESBL-producing organisms.[19] Amp-C producers, Hyper producers of K1 enzymes and in some organisms inherent resistance to Clavulinic acid further puzzle the diagnostic clinical laboratories. Therefore neither cefotaxime nor ceftazidime alone can be used for the detection of ESBLs, not to mention that a few laboratories have the tendency to use ceftazidime only to screen for ESBL production.

Detection by E-test is useful and time saving but there are cost implications. Cefipime-clavalante combined strip has been used with good results especially in Enterobacter species. Recently a multiplex PCR has been described which can rapidly identify all the five phylogenetic groups of the CTX-M types.[19] It is important to remember that regardless of the method used for detection, it is important to note that none of the methods that rely on phenotypic expression of the beta lactamases will detect every ESBL producing isolate. The presence of other resistance mechanisms also cause confusion and it is better that a holistic view, taking into consideration all other sensitivity results, is adopted before finally labeling any isolate as an ESBL. The emergence of CTX-M from Pakistan is alarming; however, further studies are required to be conducted with the purpose of studying the epidemiology and genetic characterization of CTX-M types of ESBLs.

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