Original Article

DOI: 10.7860/JCDR/2019/40684.13055

Microbiology Section

# Prevalence of *bla*$_{CTX\text{-}M}$, *bla*$_{CTX\text{-}M\text{-}2}$, *bla*$_{CTX\text{-}M\text{-}8}$, *bla*$_{CTX\text{-}M\text{-}25}$ and *bla*$_{CTX\text{-}M\text{-}3}$ Genes in *Escherichia coli* Isolated from Urinary Tract Infection in Kermanshah City, Iran

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## ABSTRACT

**Introduction:** Urinary Tract Infection (UTI) is one of the most common bacterial infections and *Escherichia coli* is the most common organism that causes UTI. However, the incidence of community acquired UTI caused by Extended spectrum beta-lactamase (ESBL)-producing strains of *E.coli*, in particular *CTX-M* genes, is on the rise worldwide.

**Aim:** To detect the frequency of *CTX-M* gene subgroups in uropathogenic *E.coli*.

**Materials and Methods:** In this descriptive-analytical study, 240 isolates of *E. coli* were studied. All isolates were isolated from UTIs in Kermanshah University of Medical Sciences, Kermanshah, Iran, in 2014 to 2015. After screening for ESBL, the *CTX-M, CTX-M-2, CTX-M-8, CTX-M-25 and CTX-M-3* genes were detected among ESBL- producing isolates using PCR.

**Results:** Of the 240 *E. coli* isolates, 67 were ESBL-producing isolates. Sixty one isolates (91%) contained *CTX-M* gene, of which 57 (85%), 3 (4.5%), 3 (4.5%) and 1(1.49%) contained *CTX-M-3*, *CTX-M-8*, *CTX- M-25* and *CTX-M-2*, respectively.

**Conclusion:** Due to the high resistance of *E. coli* to beta-lactam drugs in this region, these drugs have limited effects for treatment of UTI in outpatient. The frequency of *CTX-M-2, CTX-M-8, CTX-M-25* beta-lactamases in isolates of *E. coli* is relatively low but the overall prevalence of *CTX-M* and *CTX-M-3* beta-lactamases is high which indicates the spread of drug resistance.

**Keywords:** Antibiotic resistance, Extended-spectrum beta-lactamases, Uropathogen

## INTRODUCTION

UTI is one of the most common bacterial infections [1]. Failure to diagnose and improper treatment of UTI may lead to complications such as kidney damage and hypertension [2]. Among the uropathogenic bacteria in the outpatients and inpatients, *Escherichia coli* (*E. coli*) is the most common organism [1,3]. Given the obvious role of *E. coli* as the main and prevalent cause of UTIs in all ages, it is important to recognise its regional susceptibility pattern to antibiotics [1]. The sensitivity of bacteria to diverse antibiotics varies in different regions, which can be the consequence for the usage of various types and quantity of antibiotics in each region [3]. The first line of antibiotics for the treatment of UTIs is usually determined experientially; therefore, it is essential to obtain accurate and up-to-date information on the antibiotic susceptibility pattern of the regionally circulating strains. Beta-lactam antibiotics are often used for the treatment of bacterial infections [4]. The main resistance mechanism used in gram-negative bacteria against beta-lactam antibiotics is the production of beta-lactamase enzymes to hydrolyze the beta-lactam ring of antibiotics [5]. *E. coli* strains produce ESBLs resistant to beta-lactam antibiotics [4].

The common types of ESBLs in gram negative bacteria are *TEM*, *SHV* and *CTX-M*. In recent years, the *CTX-M* group has been increasingly reported in gram-negative bacteria, especially *E. coli* [6]. Until recently, more than 123 types of *CTX-M* had been identified and reported [7]. These beta-lactamases have no genetic linkage with *TEM* and *SHV* beta-lactamases [8]. In *CTX-M*, the presence of a serine amino acid at position 237 has led to the expansion of its beta-lactamase activity spectrum [9]. The *CTX-M* subgroups are divided into 5 main groups based on the amino acid sequences; Group 1 (*CTX-M-1, 3, 10, 11, 12, 15, 22, 23, 28, 29, 30, UOE- 1*), Group 2 (*CTX-M-2, 4, 5, 6, 7, 20, Toho-1*), Group 3 (*CTX-M-8*), Group 4 (*CTX-M-9, 13, 14, 16, 17, 19, 21, 27, Toho-2*) and Group 5 (*CTX-M-25,26*) [8].

Over the past decades, *CTX$_{-M}$* subgroups have been more frequently reported than *TEM* and *SHV* in Europe, North America and Asia [10,11]. Similarly, the reports for *CTX-M* type have also been in rise in Iran [12]. Given the diversity of *CTX-M* beta-lactamases and their different effects on susceptibility to various antibiotics, it is epidemiologically essential to determine the frequency of subgroups of these resistant genes in *E. coli*. Previous studies in Iran primarly have focused on the main ESBL groups in clinical strains of *E. coli*, while various *CTX-M* subtypes have been less investigated in different regions of Iran [2,3,12]. The first part of data derived from this work has already been published which indicate the high frequency of *blaCTX-M1*, *blaCTX-M14* and *blaCTX-M15* among isolates in our region [13]. Therefore, the present study aimed to evaluate the frequency of other *blaCTX-M* subtyped genes including *CTX-M-2*, *CTX-M-8*, *CTX-M-25* and *CTX-M-3* in *E. coli* isolates.

## MATERIALS AND METHODS

### Isolation of Bacteria and Collection of Sample Data

The present descriptive-analytic study was conducted on 240 isolates of *E. coli* obtained from outpatients with UTI referred to the Clinic of Kermanshah University of Medical Sciences and the Central laboratory. The study was approved by the Kermanshah University Ethics Committee (Approval number: IR.KUMS.REC.1393.519). All patients agreed to participate in the study and signed the informed consent form. The UTI was defined as the presence of $10^5$ or more *E. coli* bacteria per ml of midstream urine sample in the patients who were clinically suspected to UTI [14]. The patients' data including age, gender and type of samples were also collected. *E. coli* isolates from urine samples were isolated and identified using Gram staining, morphology, culture characteristics and conventional biochemical tests for verification, including oxidase, simmons' citrate, urease, phenylalanine deaminase, lysine decarboxylase, Sulfur Indole

Motility Medium (SIM), Triple Suger Iron Agar (TSI), Methyl Red/ Voges-Proskauer (MR/VP) [15].

### Screening Isolates for ESBL and their Resistance Pattern

The verification tests for analysing the phenotypes were used to screen ESBL production in bacteria. This method requires combination discs including ceftazidime (30 µg) + clavulanic acid (10 µg) and cefotaxime (30 µg) + clavulanic acid (10 µg) (MAST, England). The diameter of the bacterial inhibition zone around the combination disc about 5 mm or more than the inhibition zone diameter of the single disc relating to the same antibiotic was considered as the ESBL-producing isolates. The standard strain of *E. coli* ATCC 35218 was used for qualitative control of ESBL-producing isolates. The ESBL-positive isolates were further tested using PCR for evaluating the frequency of *CTX-M, CTX-M-2, CTX-M-8, CTX-M-25*, and *CTX-M-3* genes. The antibiotic susceptibility of isolates to beta-lactam antibiotics was assessed using Disk diffusion test according to Clinical and Laboratory Standards Institute (CLSI 2015) guidelines and antibacterial discs, including ceftriaxone (30 µg), ceftazidime (30 µg), cefotaxime (30 µg), aztreonam (30 µg), ampicillin (10 µg) and imipenem (10 µg) (MAST, England). The results were interpreted based on standard tables of CLSI [16]. Meanwhile, the standard strain of *E. coli* ATCC 25922 and ATCC 700603 was used as qualitative control.

### Detection of *CTX-M* Genes

The specific primers using PCR were applied to detect the *CTX-M* genes and related subgroups of *CTX-M*, *CTX-M-2*, *CTX-M-8*, *CTX-M-25* and *CTX-M-3* [17-19]. The sequences of used primers and the size of the PCR products have been listed in [Table/Fig-1]. Initially, the isolates were cultured on nutrient agar medium and DNA was extracted using boiling method. The PCR was performed using the reaction solution with final volume of 25 µL, including 12.5µl of Master Mix 2X, 3 µL of DNA template, 10 picomoles (1µL) of each of paired primers, and 7.5 µL of sterilized double-distilled water. The agarose gels were immersed in 0.5 to 1 mg/L of ethidium bromide for 10 minutes, rinsed with distilled water and then examined by Gel-Documentation (BioRad, USA). The DNA sequencing was performed using an ABI 3730XL DNA analyser apparatus (Macrogen Inc., Korea). The DNA sequence data were analysed for homology with genetic data using the National Center for Biotechnology Information GenBank database (http:/www.ncbi.nlm.nih.gov/BLAST/).

| Gene | Forward and reverse primers (5'-3') | Amplicon size (bp) |
|---|---|---|
| $bla_{CTX-M-2}$ | F:ATGATGACTCAGAGCATTCG<br>R:TTATTGCATCAGAAACCGTG | 884 |
| $bla_{CTX-M-8}$ | F:ATGATGAGACATCGCGTTAAG<br>R:CGGTGACGATTTTCGCGGCAG | 924 |
| $bla_{CTX-M-25}$ | F:CACACGAATTGAATGTTCAG<br>R: TCACTCCACATGGTGAG | 864 |
| $bla_{CTX-M-3}$ | F:AATCACTGCGCCAGTTCACGCTGAACGT<br>R: TTCGTCTCCCAGCTGT | 540-600 |
| $bla_{CTX-M}$ | F:TTTGCGATGTGCAGTACCAGTAA<br>R:CGATATCGTTGGTGGTGCCAT A | 544 |

**[Table/Fig-1]:** Primers.

## STATISTICAL ANALYSIS

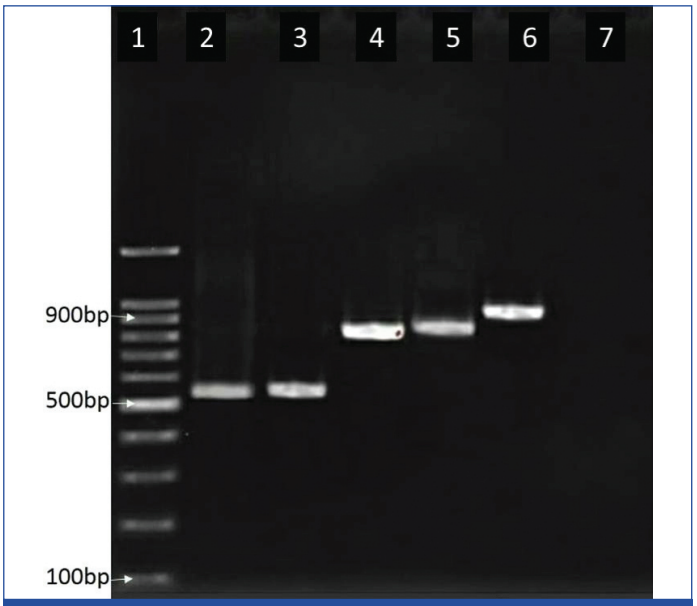
The collected data were analysed by SPSS 19 using descriptive and analytical statistical indexes.

## RESULTS

### Data of Samples and Patients

Out of 67(27.9%) ESBL-producing *E. coli* isolates, 34 (50.7%) belonged to the Clinic of University and 33 (49.3%) to the Central Laboratory. Of these 67 samples, 57 belonged to females (85.1%) and 10 to males (14.1%). The mean age of patients was 43.1±22.1 years with the maximum age of 97 years and the minimum age of 1 year.

The amplification results of target genes are displayed in [Table/Fig-2].



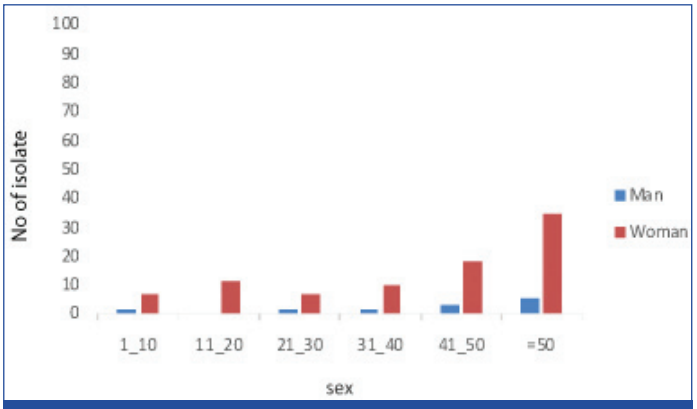

**[Table/Fig-2]:** Electrophoresis of PCR products for *CTX-M* genes.
Row 1: Marker (DNA ladder, 100bp); Row 2: *CTX-M-3* (540 bp); Row 3: *CTX-M* (544 bp); Row 4: *CTX-M-8* (924 bp); Row 5: *CTX-M-25* (846 bp); Row 6: *CTX-M-2* (884 bp); Row 7: Negative control

Of the 67 ESBL-producing *E. coli* isolates, 61 isolates (91%) contained the *CTX-M* gene, of which 57 (85%) had *CTX-M-3* and three (4.5%) had *CTX-M-8*, three (4.5%) had *CTX-M-25* and one (1.49%) contained *CTX-M-2*.

The susceptibility to beta-lactam antibiotics in *CTX-M*-producing and non *CTX-M* isolates are listed in [Table/Fig-3]. [Table/Fig-4] shows the frequency distribution of *CTX-M*-containing isolates based on age and gender.

| Antibiotics | *CTX-M* producer | Non-*CTX-M* | Chi-square | p-value |
|---|---|---|---|---|
| Ampicillin | 1 (1.6%) | 34 (19%) | 11 | 0.001 |
| Ceftriaxone | 1 (1.6%) | 164 (91.6%) | 171.5 | 0.001< |
| Cefotaxime | 2 (3.3%) | 164 (91.6%) | 166.5 | 0.001< |
| Ceftazidime | 13 (21.3%) | 167 (93.3%) | 125.7 | 0.001< |
| Aztreonam | 6 (9.8%) | 166 (92.7%) | 154 | 0.001< |
| Imipenem | 61 (100%) | 179 (100%) | - | - |

**[Table/Fig-3]:** Comparison the susceptibility to beta-lactam antibiotics among the *CTX-M* producing and non *CTX-M* isolates of *E. coli*.




**[Table/Fig-4]:** Distribution of *CTX-M* isolates by age and sex.

## DISCUSSION

The outpatients reffering from UTI are primarily treated empirically which requires the awareness of antibiotic susceptibility and the

frequency of antibiotic resistance genes in each country or region [2]. Beta-lactams are usually used against gram-negative bacteria such as *E. coli* [5]. The results of the present study indicated a high resistance to beta lactam antibiotics among *E. coli* isolates. Accordingly, ampicillin resistance was observed to be very high in these isolates, which is consistent with the results of other studies conducted in Iran and other countries [2,20,21]. These findings indicate the spread of resistance to this drug. On the other hand, the present results revealed all *E. coli* isolates were sensitive to imipenem that is consistent with the results of other studies [2,20-23].

Comparison of the resistance pattern of isolates showed that the antibiotic resistance of *CTX-M*-producing isolates was much higher than non *CTX-M* isolates, emphasizing the important role of *CTX-Ms* for spread of antibiotic resistance in *E. coli* strains, which needs to be considered in the treatment of UTIs. Compared to the previous studies from other countries, our results indicate an increase in the frequency rate of ESBL-producing *E. coli* isolates [2,5,10].

Given the results of other studies and the fact that most previous researches in Iran were basically focused on clinical isolates of inpatient which as expected to be more resistant to antibiotics. The high frequency of *CTX-M* isolates among outpatients in our region suggest the dissemination of this gene group. The beta-lactamase genes in this bacterium, especially the *CTX-M* genes, are major factors involved in the increasing resistance to beta-lactam antibiotics. Organisms containing these genes can also exacerbate the pathogenicity and increase the mortality in the patients [24].

Studies conducted in different countries including Iran, reported a various frequency rates for the *CTX-M* genes [24-26]. For example, a study from India (2015-2016) on *E. coli* isolated from UTI reported a prevalence rate of 82.6% for *CTX-M* gene in ESBL-producing strains [27]. In another study in Iran (2015), the prevalence of *CTX-M* was 68.9% in 29 strains of ESBL-producing *E. coli* [28]. Our findings along with the aforementioned studies indicate that *CTX-M*-producing *E. coli* have become more frequent and the production of this beta-lactamase is increasing on the rise. The high frequency of *CTX-M* genes could be the consequence of over prescription of cephalosporins in our region followed by the transmission of plasmids among strains of bacteria. On the other hand, this gene has the ability of transmission between animal (poultry) and humans bacteria, which is considered as the another source of resistant genes dissemination [29].

It has been shown that *CTX-M* genes are located upstream to the ISEcp1 conjugated sequences which may explain the expression and transmission of this gene group [30]. In our study, the relatively low percentage of ESBL-producing strains had *CTX-M-2*, *CTX-M-8* and *CTX-M-25* genes, which is similar to other research results. For instance, in a study in Iran (2009), only 0.7% of the strains of *E. coli* had *CTX-M-25* and none of the isolates had *CTX-M-2* [31]. In another Iranian study in 2013, none of the *E. coli* isolates contained *CTX-M-2* [32]. The results of studies in other countries are also in agreement with the findings of research conducted in Iran. For example, the studies in China (2008) and Spain (2011) reported no production of *CTX-M-2*, 8, 25 in *E. coli* [33,34]. Furthermore, in a study in France (2006), none of the isolates had the *CTX-M-8* gene, and only one isolate (2.27%) was detected to have *CTX-M-2* gene among *E. coli* isolates [6]. In Egypt (2014), the frequency of *CTX-M-2* gene was found to be zero in *E. coli* isolates and the frequency of *CTX-M-25* gene was reported to be 1.3% [4]. Considering the results of the above studies, it seems that the prevalence of these genes in *E. coli* isolates is still low and varies in different geographic regions.

The prevalence of *CTX-M-3* subtype varies in different regions. Studies in Iran on Group 1 of *CTX-M* have reported different frequencies which ranged from 35.87% to 87.5% [10]. For instance, a study in Rasht (Iran) showed that only 2.1% of the isolates possessed the *CTX-M-3*. This gene was also observed in 88% of ESBL-positive isolates in France and 84% in India [35,36]. The *CTX-M-1* was found in 60.6% of ESBL-positive isolates in Poland [37], but *CTX-M-3* was reported to have an extremely low prevalence in other countries. For example, studies conducted in Canada reported that the prevalence of *CTX-M-3* was 1% in 2007 and 2% in 2009. The prevalence of this gene in Germany (2009) was reported to be 4.7% [8,38]. In the present study, 85% of ESBL-positive isolates exhibited the *CTX-M-3* gene, which can demonstrate the spread of this gene among *E. coli* strains in our region.

## LIMITATION

As a limitation of our study, it was not possible to collect all relevant data of patients' UTIs including underlined diseases, the history of UTIs and previous treatments.

## CONCLUSION

Due to the high resistance of *E. coli* to beta-lactam drugs in our region, especially cephalosporins and penicillins, these antibiotics cannot effectively treat UTIs in outpatients. Although the prevalence of *CTX-M-2*, *CTX-M-8* and *CTX-M-25* subtypes of beta-lactamases in *E. coli* in Kermanshah region are relatively low, the prevalence of *CTX-M* beta-lactamases and *CTX-M-3* subtype is high, which indicate the spread of drug resistance among the strains of this bacterium.

## ACKNOWLEDGEMENTS

We hereby appreciate and thank the management and staff of the Microbiology Research Laboratory of School of medicine at Kermanshah University of Medical Sciences, Kermanshah, Iran.

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**FINANCIAL OR OTHER COMPETING INTERESTS:** None.

Date of Submission: **Feb 23, 2019**
Date of Peer Review: **Mar 02, 2019**
Date of Acceptance: **Jun 04, 2019**
Date of Publishing: **Aug 01, 2019**