

**Detection of *ctx-M* gene in ESBL-producing *E. coli* strains isolated from urinary tract infection in Semnan, Iran**Mahbobeh Mohammad Tabar<sup>1</sup>, Shiva Mirkalantari<sup>2</sup>, Rabeeh Izadi Amoli<sup>3</sup><sup>1</sup>M.Sc., Department of Microbiology, Ayatollah Amoli Branch, Islamic Azad University, Amol, Iran<sup>2</sup> Ph.D., Assistant Professor, Department of Microbiology, Faculty of Medicine, Student Research Committee, Semnan University of Medical Sciences, Semnan, Iran<sup>3</sup>M.Sc., Instructor, Department of Microbiology, Ayatollah Amoli Branch, Islamic Azad University, Amol, Iran**Type of article:** Original**Abstract**

**Introduction:** The incidence of urinary tract infections caused by Extended-Spectrum Beta Lactamase (ESBL) producing Escherichia coli (*E. coli*) strains due to long term and overuse of broad-spectrum cephalosporine is on the rise. CTX beta-lactamase type, a broad-spectrum beta-lactamase, has been expanding in many countries. The *ctx* gene is harbored on a plasmid that is spread between Enterobacteriaceae family, especially in *E. coli*. The aim of this study was to determine the pattern of antimicrobial resistance and investigate the prevalent ESBL phenotype and the *ctx-M* gene in *E. coli* isolated from patients with urinary tract infections (UTI) in Semnan.

**Methods:** A cross sectional study was performed on 109 strains of *E. coli* isolated from the urine culture of patient suffering from a UTI referred to Shafa hospital (Semnan, Iran) during March-July 2015. Antimicrobial susceptibility testing was applied and the prevalence of the ESBL phenotype was confirmed using combination disk. PCR methods were completed for amplification of the *bla ctx* gene. Data were analyzed using SPSS version 18 software.

**Results:** One hundred ninety samples (4.16%) were identified as *E. coli*. Twenty one (26.6%) of *E. coli* were ESBL positive and 73.4% were ESBL negative. There was 100% susceptibility to imipeneme. Twenty (68.97%) out of 29 isolates were positive for the *ctx-M* gene, as detected by PCR.

**Conclusion:** In urinary tract infections, antibiotic treatment was experimental and detailed information regarding the sensitivity of bacteria in the area can be useful to achieve the best treatment.

**Keywords:** *E. coli*, *ctx-M* gene, urinary tract infection, ESBL

**1. Introduction**

Escherichia coli (*E. coli*) is an important and common pathogen of urinary tract infections (UTIs) (1, 2). Treatment of UTIs caused by *E. coli* is becoming difficult due to antibiotic resistance (3). Resistance by the various mechanisms, such as altered target sites, enzymatic inactivation of antibiotics, active efflux pump, and decreased permeability by the porins, are known in Gram negative bacteria (4). One of the most common resistance mechanisms is the production of Extended-Spectrum Beta-Lactamase enzymes (ESBL) that hydrolyze all penicillins, early cephalosporins, oximino-cephalosporins, and monobactames, but they cannot hydrolyze carbapenemes or cephamycins (5-7). These enzymes are susceptible to inhibitors, such as clavulanic acid, sulbactam, and tazobactam (8, 9). The prevalence of ECBLs in pathogens continues to be associated with higher rates of healthcare costs and mortality (10). Until now, 400 types of enzymes are known to be seen in the Enterobacteriaceae family (11). The CTX-M beta lactamase types have been expanding in many countries and are currently the most widespread enzymes (12).

**Corresponding author:**

Assistant Professor Dr. Shiva Mirkalantari, Semnan University of Medical Sciences, Semnan, Iran.

Tel: +98.2144846137, Email: [Sh\\_mirkalantari@yahoo.com](mailto:Sh_mirkalantari@yahoo.com)

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At present, CTX-M-Beta Lactamases are encoded in a plasmid and can hydrolyze both ceftazidime and cefotaxime, but give a high level of resistance to cefotaxime and a low level of activity against ceftazidime (13, 14). These preplasmic enzymes were reported for the first time in the late 1980s (7). The CTX-M enzymes are a group of class A ESBL that have disseminated among a wide range of clinical bacteria within and across the species in the world since 1995 (12). Nowadays, based on >130 amino acid sequences, CTX-M allelic variant have been categorized in five major phylogenetic groups: CTX-M1, CTX-M2, CTX-M8, CTX-M9, and CTX-M25 (15). The incidence of urinary tract infections caused by ESBL producing *E. coli* strains that result from long term and overuse of broad-spectrum cephalosporine is on the rise. Prevalence of CTX-M Beta Lactamase in commonly isolated organisms, such as *E. coli*, is now a serious public health problem worldwide. In urinary tract infections, antibiotic treatment was experimental and few details exist about the sensitivity and molecular epidemiology of *E. coli* in Semnan. Current knowledge of antibiotic resistance patterns and the frequency of CTX-M B-Lactamase between *E. coli* isolates from patients with UTI in every region could help with rapid treatment. With this background, the aim of this study was to determine the pattern of antimicrobial resistance and investigate the prevalence of the ESBL phenotype and *ctx-M* gene in *E. coli* isolated from patients with UTIs in Semnan.

## 2. Material and Methods

### 2.1. Study design, sampling and bacterial isolates:

A total of 2618 urine samples of early morning mid-stream were collected in sterile, wide mouthed bottles from patients between March and July of 2015. Samples were analyzed within one hour after collection. Samples were inoculated using an inoculating loop with a 10 µl volume calibration on nutrient, blood, and Mac Conkey agar plates and incubated in aerobic condition at 37 °C for 24 hours. Colony counts equal or more than 10<sup>5</sup> per ml were considered as positive UTIs and at less than 10<sup>5</sup> per ml was considered a suspected UTI and the assay repeated. Identification was done based on routine standard biochemical tests, including oxidase, catalase, motility and gas production, reduction of nitrates, Indol, methyl red, voges-proskauer, citrate, lactose fermenter, and lysine decarboxylate tests. Samples that were confirmed as *E. coli* were stored in BHI Broth containing 15% glycerol at -70 °C and were subjected to further molecular tests.

### 2.2. Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was applied using the Kirby Bauer disk diffusion method. *E. coli* ATCC 25922 was used to control the quality of the applied antimicrobial agents. The susceptibility of isolates to the following antibiotics was evaluated; Briefly, a 0.5 McFarland suspension was prepared from pure culture of *E. coli* in a nutrient broth and inoculated on Muller-Hinton agar. The antibiotic discs were applied at distances of 24 mm from center to center. The plates were incubated aerobically for 24h at 37 °C. Based on the diameter of the inhibition zone, the results were interpreted as susceptible, intermediate, or resistant.

### 2.3. Phenotypic detection of ESBLs

The prevalence of the Extended-Spectrum Beta Lactamase (ESBL) phenotype was confirmed with a Cefotaxime (30 µg) or Cefotaxime disk with or without clavulanate (10µg), as per the CLSI 2013 guidelines. A positive ESBL phenotype meant that the zones produced by the disks with clavulanate were ≥5 mm larger than those without inhibitor.

### 2.4. DNA extraction and PCR of *bla-ctx-M* gene

Genomic DNA was extracted by boiling a suspension of ESBL producing bacterial cells in sterile distilled water. Ten µl of DNA template was used in the PCR reaction containing 1X buffer, MgCl<sub>2</sub>, dNTP, Taq polymerase, and primers for detection of the *bla-CTX-M* gene. The primer sequences were as follows: *bla-CTX-M-F* 5'-ACCGCCGATAATTCGAGAT-3' and *bla-CTX-M-R* 5'- GATATCGTTGGTGGTGCCATA -3'. The PCR conditions for amplification of the *bla-CTX-M* gene were done as follows: Initial denaturation at 94 °C for 5 minutes, denaturation at 94 °C for 1 minute, 30 seconds for annealing at 59.2 °C, 1 minute at 72 °C for elongation, and the final extension was conducted at 72 °C for 5 minutes. A negative control was used in every PCR assay. The PCR product was subjected to electrophoresis in a 1% agarose gel and staining with Ethidium bromide (0.5 mg/ml).

### 2.5. Research ethics

This research was conducted in accordance with the ethical principles of clinical specimens.

### 2.6. Statistical analysis

The degree of significance was calculated using the Chi-Square test. P-values less than 0.05% were considered significant. Statistical analysis was carried out using SPSS version 18 (SPSS Inc., Chicago, IL, USA).

### 3. Results

In this study, among 2618 urine samples tested, 109 (4.16%) were identified as *E. coli*. Based on the results of the combination disc method, twenty nine (26.6%) of the *E. coli* samples were ESBL positive and 73.4% were ESBL negative. Twenty six (89.65%) of the positive samples were female and three (10.34%) were male. The average age was 32 years old and patient age ranged from 6 days to 87 years old. According to the results obtained from disc diffusion antimicrobial susceptibility testing, which is represented in Figure 1, the majority of ESBL positive isolates, showed a high level of resistance to Trimetoprim-Sulfametaxazol (46.8%). There was 100% susceptibility to Imipeneme. PCR detection of the *bla<sub>CTX-M</sub>* gene in ESBL producing isolates revealed that 20 (68.97%) out of 29 isolates were positive (Figure 2 and Table 1).

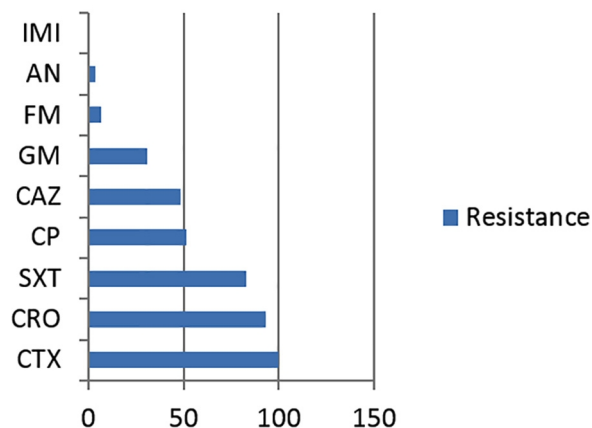


Figure 1. The resistance pattern of 29 ESBL- producing *E. coli* isolates

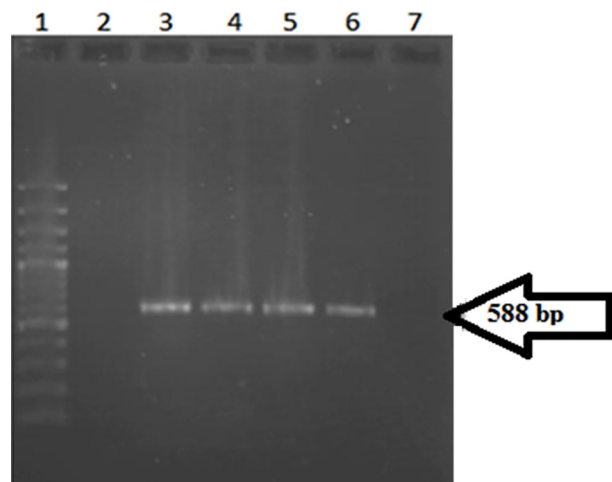


Figure 2. Electrophoresis of PCR products on a 1% agarose gel. Lane 1. Ladder 100 bp. Lane 2. Negative control. Lane 3. Positive control. Lanes 4, 5, 6. Positive samples that indicated a 588 bp PCR product. Lane 7. Negative sample.

**Table 1.** The antibiotic resistance pattern in Positive ESBL strains and prevalence of the *ctx\_M* gene in every pattern.

Pattern	CTX	CRO	SXT	CP	CAZ	GM	FM	AN	IMI	Number of isolates	Number of positive <i>CTX</i> gene
1	R	R								1	1
2	R	R	R							6	2
3	R	R	R	R						3	2
4	R	R			R					2	2
5	R	R	R		R					3	3
6	R		R	R		R				1	0
7	R	R			R		R			1	1
8	R	R	R		R	R				1	1
9	R	R	R	R	R					3	1
10	R	R	R	R		R				3	2
11	R	R		R	R	R				1	1
12	R	R	R	R	R	R				3	3
13	R		R	R		R	R	R		1	1

#### 4. Discussion

UTIs are second only to infections of the respiratory tract as the most common bacterial infections and need for antimicrobial therapy. In most cases, the cause of community and hospital UTI was found to be associated with *E. coli*. Increasingly, resistance to antimicrobials among uropathogens, practically *E. coli*, has limited treatment choices. The worldwide spread of ESBL producing *E. coli* has been strikingly rapid, especially in communities, and is an important case of invasive infection (16, 17). Age over 60, previous antibiotic treatment, long hospitalization, and diabetes were found to be the factors associated with ESBL (18). Identification of betalactamase is essential for a reliable epidemiological investigation of antimicrobial resistance. In the present study, we surveyed antimicrobial drug resistance, the ESBL phenotype, and *ctx* gene detection in *E. coli* strains isolated from urinary tract infections in Semnan. Following the work on 2618 urine samples, we found that 109 of them were infected with *E. coli*. One hundred isolates were from outpatient and the remaining nine were from inpatients. In the present study, the ESBL producing *E. coli* were 26.6% of all *E. coli* strains isolated from urinary tract infections. Our results indicated that females had a higher rate of isolation of ESBL producing *E. coli*, which is consistent with the findings of other studies (19). In our study, the pattern of antimicrobial susceptibilities was determined in all isolates and results showed a 46.8% resistant to Trimetoprim-Sulfamethoxazole and 30.3% were resistant to Cefotaxime and Ciprofloxacin. In accordance with other studies, a 100% sensitivity was seen with Imipenem, which has been found to be the most effective antibiotic (20). This is an important result of this study because many infections are caused by Gram positive and Gram negative that can be treated with carbapenemes. This result may be related to the low use of these antibiotics in this region. The presence of an ESBL phenotype is a good marker of the MDR pattern and resistance to newer beta lactam antibiotics. It is important to note that all of isolates showed an MDR pattern. The resistance rates to Cefotaxime and Ceftazidim were 100% and 48.27%, respectively. Additionally, 68.97% of ESBL producing *E. coli* and 18.35% of all isolates carried the *ctx-M* gene. Edelstein and colleagues reported that CTX-M beta lactamases have a more destructive effect on Cefotaxime and Ceftazidim than Ceftazidim. This is in agreement with our findings (13). In this study another worrisome characteristic of ESBL strains was the resistance rate to non-beta lactam antibiotics.

#### 5. Conclusions

The rate of ESBL- producing *E. coli* isolated from urinary tract infection was 26.6% with multiple drug resistance, resulting in limited treatment options. Therefore, investigating of the molecular and epidemiological traits of bacteria will help prevent and reduce the spread of resistance.

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#### Conflict of Interest:

There is no conflict of interest to be declared.

**Authors' contributions:**

All authors contributed to this project and article equally. All authors read and approved the final manuscript.

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