Identification of extended-spectrum β-lactamase genes in *Escherichia coli* isolated from patients with urinary tract infection

Alireza Jahantabi[1], Farzaneh Hosseini[1*], Mohammad Asgharzadeh[2], Abbas Akhavan Sepehi[1], Hossein Samadi Kafil[2]

1. Department of Biological Sciences, Islamic Azad University, Tehran North Branch, Tehran, Iran
2. Department of Medical Sciences, Tabriz University of Medical Sciences, Tabriz, Iran

***Corresponding author:** Tel: +98 2122641842 Fax:-
Address: Department of Biological Sciences, Islamic Azad University, Tehran North Branch, Tehran, Iran
E-mail: hosseinimicrobiology@gmail.com
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Abstract

Introduction: Recentlly, resistance to antibiotics has increased and antibiotic-resistant strains producing extended-spectrum beta-lactamases (ESBLs) have emerged among Enterobacteriaceae, mainly in *Escherichia coli* (*E. coli*). In this study we aimed to determine phenotypic and genotypic ESBL production in isolated *E. coli* from women with urinary tract infection (UTI).

Materials and Methods: In total, 92 *E. coli* isolates were collected from patients with UTI. The antimicrobial susceptibility of all *E. coli* isolates were investigated. Morover, Mast D68C test and polymerase chain reaction (PCR) were used for phenotypic and genotypic investigation of ESBLs in the studied isolates.

Results: Totally, 92 isolates of *E. coli* were investigated, among which 51 (55.4%) isolates were resistant to cefotaxime/ceftazidime. These resistante isolates were included in the study. Among the resistant isolates, 40 (78.4%) cases were ESBL producers. Moreover, all the 40 isolates were observed with both CTX-M-15 and CTX-M-14 resistance genes.

Conclusion: In general, increasing prevalence of ESBL producer *E. coil* isolates is a serious problem in the investigated region. Therefore, development of a rapid and simple method is essential for the identification of various ESBL producer isolates.

Keywords: Urinary tract infection, *Escherichia coli*, Extended-spectrum β-lactamase, CTX-M

Introduction

Escherichia coli (*E. coli*) is one of the major causes of urinary tract infections (UTIs) among women (1). This pathogen is the cause of more than 80% of UTI in community and 50% in hospitals. Moreover, *E. coli* is one of the major organismsresponsible for high rates of hospitalization and health care costs (2). Recently, prevalence of resistant strains with extended-spectrum beta-lactamases (ESBLs) have increased among Enterobacteriaceae. In particular, prevalence of *E. coli* strains with ESBLs is a great concern in the world (3, 4). ESBLs have undergone substitutions of amino acids in their active sites, which has caused an increase in their affinity and hydrolytic activity against monobactams and third-generation cephalosporins (5, 6).

The new-generation cephalosporins is a important factor to investigation of newe b-lactamases. Beta-lactamases are coded by transferable plasmids, which might contain determinants conferring resistance to other other antibiotics as well. This has caused a

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great concern worldwide (7). The distribution of strains with CTX-M were limited to a particular region during 1990s. However, this has changed during recent years. In this regards, recent epidemiological studies on strains with ESBL have reported a significant increase in the prevalence of strains with CTX-M (8).

The most important categories of ESBLs are in three families, including SHV, TEM, and CTX-M types (9, 10). Previous studies on the prevalence of ESBL in northwest of Iran were performed during 2015 and 2016 (11, 12). Therefore, our study can demonstrate prevalence of ESBL among *E.coli* strains since 2016 until 2020, which can help in prevention and treatment of these isolates. The aim of this study was to investigate the frequency of ESBLs genes among *E. coli* isolates from women with UTI in Imam Reza Hospital, Tabriz, Iran.

Materials and Methods

Selection of the Strains

In this study, 92 Enterobacteriaceae species wereisolated from women with UTI reffered to Imam Reza Hospital, Tabriz, Iran during 2018. The biochemical tests, colony morphology, Gram staining, and motility tests were conducted to identify the studied isolates. A total of 51 isolates were identified as *E. coli* and were included in the study. *E. coli* isolates with resistance to one of the third-generation cephalosporins, ceftriaxone, cefotaxime, and ceftazidime.

Antibiotic Susceptibility Testing

Antimicrobial resistance was investigated by disk diffusion method on Muller-Hinton agar. The antibiotic discs used were as follows: ciprofloxacin (5 µg), meropenem (5 µg), colistin (10 µg), amikacin (30 µg), cefotaxime (30 µg), imipenem (5 µg), gentamicin (10 µg), piperacillin-tazobactam (100/10 µg), ceftazidime (30 µg), cotrimoxazol (25 µg), and nitrofurantoin (300 µg). *E. coli* ATCC 25922 was used as control strain.

Phenotypic Detection of ESBL β-Lactamases

The isolates of *E. coli* with resistance to cefotaxime or ceftazidime were investigated for the production of ESBLs by combination disk test (CDT). For this purpose, cefotaxime/clavulanic acid (30/10 µg), ceftazidime/clavulanic acid (30/10 µg), ceftazidime (30 µg), and cefotaxime (30 µg) disks were used. The isolates which had >5 mm difference in the size of inhibition zone between single antibiotic disk (cefotaxime or ceftazidime alone) and the combination disk (cefotaxime/clavulanic acid or ceftazidime/clavulanic acid) were concidered as ESBL-producers. The isolates with zone diameter difference <5 mm were considered as negative for ESBL production.

DNA Extraction and Polymerase Chain Reaction (PCR)

The ESBL-producer isolates were selected for DNA extraction and molecular investigation. The used primers are presented in Table 1. The polymerase chain reaction (PCR) was performed in a 20 µL total valume reaction which indluded the extracted DNA, Master PCR mixture, and the primers.

Table 1. Sequences of the primers used for the detection of ESBL-producing isolates.

Gene	Primer	Sequence	Product Size	Reference
CTX-M-14	CTX-M-14-F	TACCGCAGATAATACGCAGGTG	355 bp	12
	CTX-M-14-R	CAGCGTAGGTTCAGTGCGATCC		
CTX-M-15	CTX-M-15-F	GAT TCC TTG GAC TCT TCAG	499 bp	13
	CTX-M-15-R	TAAAACCAG GTTCCCAGATAGC		

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The amplification was performed in a DNA thermal cycler. The products of PCR were electrophoresed on 2% agarose gell and visualized using a gel document instrument. *E. coli* ATCC 25922 were used as the standard strain.

Statistical Analysis

SPSS (ver. 20) software (IBM SPSS Statistics, USA) were used to analyze the data. The Fisher's exact or chi-square tests were used for the descriptive statistics and presence of ESBL genes. P value <0.05 was considered as statistically significant.

Results

Bacterial Isolates

Totally, 92 *E. coli* isolates were identified from 289 sample reffered to Laboratory. Among these 92 *E. coli* isolates, 51 (55.4%) were resistant to the tested third-generation cephalosporins (cefotaxime and ceftazidime).

Antibiotic Resistance Patterns

The resistance patterns of ESBL-producer isolates indicated a high sensitivity to nitrofurantoin, amikacin, and imipenem (Table 2).

Phenotypic Detected ESBL-producing *E. coli*

40 out of 51 isolates of *E. coli* with cephalosporin-resistant were ESBL producers. These 40 ESBL-producer *E. coli* isolates were obtained from 28 women with UTI.

Frequency of ESBL Genes

All 40 ESBL producing *E. coli* isolates were positive for both *CTX-M-15* and *CTX-M-14* genes (Figure 1).

Table 2. Antibiotic resistance patterns of *E. coli* isolates.

Antibiotic	Dose (µg)	Resistance Patterns		
		Sensitive	Semi-sensitive	Resistant
Amikacin	30	29	0	11
Gentamicin	10	20	2	18
Ciprofloxacin	5	10	0	30
Ceftazidime	30	0	0	40
Cefotaxime	30	0	1	29
Piperacillin-Tazobactam	100/10	28	3	11
Nitrofurantoin	300	35	0	5
Imipenem	5	29	8	3
Meropenem	5	32	2	6
Cotrimoxazol	25	9	5	26
Colistin	10	30	0	10

Discussion

Urinary tract infection (UTI) is a prevalent bacterial infections, and *E. coli* is the major cause. The extended-spectrum cephalosporins have been used for the treatment of UTI (14). The treatment and management of UTI has become a problem due to the increased production of ESBLs (15), TEM and SHV being detected as the major ESBLs genes. Recently, CTX-M gene has become the main and prevalent β-lactamases among the clinical isolates of *E. coli* (16). In the present study, we have provided the molecular-epidemiological data on ESBL produsing *E. coli* isolates obtained from women with UTI in Imam Reza Hospital, Tabriz, Iran during 2018.

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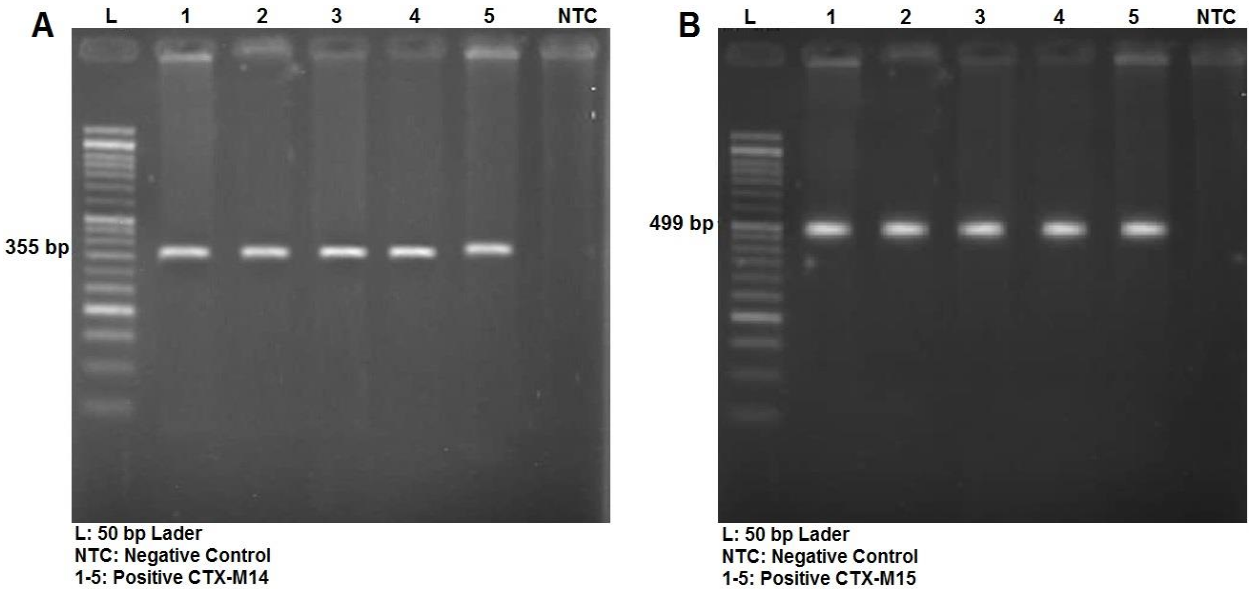

Figure 1. The electrophoresis of PCR products of CTX-M14 (A) and CTX-M15 (B) on 2% agarose gel.

In the present study, ESBL genes? were? identified in 51 E. coli isolates (55.4%), which was had a higher rate compared to reports from other regions in Iran (17)and Middle East (18, 19).

In this study, prevelence of both CTX-M-15 and CTX-M-14 genes among ESBL produsing *E. coli* isolates was higher than that reported by Sadeghi et al. (12) from Azerbaijan, and Feizabadi et al. (20) from Tehran. This result indicated that the distribution of CTX-M-15 and CTX-M-14 genes have increased in the past years. Distribution of ESBL producing isolates is reported from 7-61% in Turkey, and 66.7% in India (21, 22). Middle East is on of the region in which the distribution of ESBLs has increased during the recents years (23). However, prevalence of ESBLs isolates is a concerning problem in the world, and prevalence of these isolates can vary in different countries (24).

In this study, all CTX-M-15 and CTX-M-14 *E. coli* strains showed high resistance to ceftazidime. These genes have an high catalytic activity against ceftazidime, which is in agrement with the previous studies (25). In a study, Ye et al. reported that the prevalence of CTX-M genes is higher than other β-lactamases (26). Our study is in agreement with the study of Kim et al. (27) in which the CTX-M genes? were positive in 23 (45%) isolates of *E. coli*. The extensive use of broad-spectrum antibiotics can cause the emergence of resistant isolates in our hospitals.

Conclusion

In this study a high prevalence of ESBL-producing *E. coli* strains was demonstrated in the studied region. Identification of ESBLs producing isolates is important to therapeutic process and epidemiological aspect.

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