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Original Article

Evaluation of the Prevalence of *bla*$_{SHV}$, *bla*$_{TEM}$, and *bla*$_{CTX}$ Genes in *Escherichia coli* Isolated From Urinary Tract Infections

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Abstract

Background: Beta-lactamases are the most important factors in the resistance to beta-lactam antibiotics among gram-negative bacteria, especially *Escherichia coli*. Today, the prevalence of infections caused by extended-spectrum β-lactamases (ESBLs)-producing *E. coli* is increasing, as one of the emerging health problems worldwide. This study aimed to investigate the prevalence of bla$_{SHV}$ (sulfhydryl variable β-lactamase), *bla*$_{TEM}$ (temoneira β-lactamase), and *bla*$_{CTX}$ (cefotaximase β-lactamase) genes in *E. coli* isolated from urinary tract infections (UTIs).

Methods: In this study, 3192 midstream urine samples collected from Babol and Qaemshahr counties, Mazandaran province (Iran) were cultured on eosin methylene blue and blood agars. An antibiotic susceptibility test was performed to determine ESBL-producing *E. coli* isolates using the combined disk method. Finally, the ESBLs were evaluated for the presence of *bla*$_{SHV}$, *bla*$_{TEM}$, and *bla*$_{CTX}$ genes by the polymerase chain reaction (PCR) technique.

Results: Of the 3192 cultured urine samples, 192 isolates were identified as *E. coli* by the IMViC and biochemical tests. In addition, the ESBL producers were detected in 45 (28/12 %) out of 192 *E. coli* isolates by the double-blind synergism test. The PCR of the 45 ESBL-producing *E. coli* isolates demonstrated that the bla$_{TEM}$ was the most abundant gene (89%), followed by *bla*$_{CTX-M}$ (27%) and *bla*$_{SHV}$ (20%). Eventually, the co-existence of *blaSHV, bla*$_{CTX-M}$, and *bla*$_{TEM}$ was detected in 3 (7%) isolates.

Conclusions: Due to the high prevalence of ESBL-producing uropathogenic *E. coli* (UPEC) in the studied region, future studies are recommended to perform phenotypic or genotypic tests to detect ESBL-producing isolates in laboratories to select appropriate antibiotics for treating UTIs.

Keywords: Extended-spectrum β-lactamases, Uropathogenic Escherichia coli, Urinary tract infection

Background

Escherichia coli is the most important agent of urinary tract infections (UTIs) which accounts for nearly 75% of the isolates (1, 2). Annually, about 150 million women suffer from UTIs worldwide (3). Uropathogenic *E. coli* (UPEC) strains often produce α-hemolysin which can cause an inflammatory response by the lysis of cells and the release of cytokine (4). β-lactamases are the enzymes that disrupt beta-lactam antibiotics and secret into the periplasmic space in gram-negative bacteria or out of the cell by Gram-positive organisms (5,6). Based on the protein sequence homology (the Ambler classification scheme), beta-lactamases are divided into A, B, C, and D types. According to Tooke et al study (7), the A-, C-, and D-type β-lactamases have serine at their active site (seine-β-lactamases) whereas B-type enzymes are considered as zinc-containing metalloenzymes (metallo-β-lactamases). The extended-spectrum β-lactamases (ESBLs) were first recognized in *Klebsiella pneumoniae* and *Serratia marcescens* in the mid-1980s. These enzymes are produced by gram-negative bacilli and are often classified into temoneira (*bla*$_{TEM}$ β-lactamase), sulfhydryl variable active site (*bla*$_{SHV}$ β-lactamase), and the cefotaxime degrading enzyme (*bla*$_{CTX-M}$ β-lactamase) classes (8). The high prevalence of ESBLs in *E. coli* has raised concerns about the treatment of the infection caused by this bacterium (9). The ESBLs represent resistance to almost all β-lactam antibiotics except for carbapenems and cefamycins and are mainly produced by *E. coli* and *K. pneumoniae* (10). TEM-1, TEM-2, and SHV-1 are named narrow-spectrum β-lactamases because they hydrolyze penicillins and are considered as the first generation of cephalosporins such as cephalothin, cephaloridine, or cefazolin (11). The ESBLs are emanated from the narrow-spectrum

beta-lactamase genes (i.e., TEM-1, TEM-2, or SHV-1) by mutations that change the amino acid sequence at their active site (12). The genes encoding ESBLs are located on the bacterial chromosome or plasmid (13). The bla_{SHV}, bla_{TEM}, and bla_{CTX-M} types are regarded as the most common ESBLs (14). The UPEC strains contain bla_{SHV} and bla_{TEM} genes which cause resistance to beta-lactam antibiotics and the bacterium also possesses a bla_{CTX-M} gene that hydrolyzes cefotaxime and ceftazidime (15). Given the importance of resistance to penicillins, cephalosporins, and carbapenems, mediated by extended-spectrum β-lactamases and carbapenemases, this study was performed to investigate the prevalence of ESBL enzymes in the UPEC strains isolated from patients with UTIs.

Materials and Methods

Isolation and Detection of *Escherichia coli*

This study was conducted on 3192 patients who referred to the therapeutic centers of Babol and Qaemshahr, Mazandaran Province, Iran between March and December 2017. The urine samples were streaked on eosin methylene blue and blood agars (Merk Company, Germany) and placed at 37°C for 24 hours. UPEC strains were identified using the gram-stain, the IMViC test, β-hemolytic activity, and the other conventional biochemical tests. These strains were then stored in Luria-Bertani (LB) broth (Merk Company, Germany) including 20% glycerol at -20°C for future studies (34).

Combined Disc Test for the Phenotypic Detection of Extended-Spectrum β-Lactamases

One hundred and ninety-two *E. coli* isolates were studied to detect the presence of ESBLs by the combined disc method. A 0.5 McFarland suspension of *E. coli* was spread on the Mueller-Hinton agar. In this study, ceftazidime (30 µg) disks (MAST, UK) alone and in the combination of ceftazidime and clavulanic acid (30/10 µg) disks (MAST, UK) were placed on the Mueller-Hinton agar at a distance of 20 mm from each other and were placed at 3 °C for 24 hours. The isolates that displayed an increase of ≥5 mm in the inhibition halo of the combined disk (ceftazidime plus clavulanic acid) were considered as an ESBL producer compared to the ceftazidim disc alone (16,17). The polymerase chain reaction (PCR) detection was carried out on all positive ESBLs. The isolates were confirmed by the PCR and the Basic Local Alignment Search Tool (BLAST) sequence analysis was used as a positive control.

Extraction of DNA by Boiling Lysis Method

A single colony was used for inoculating 5 mL of LB broth and then was incubated at 37°C for 24 hours. Next, 1 mL of bacterial suspension was transferred to a 1.5 mL microcentrifuge tube and was centrifuged at 6000 rounds per minute (rpm) for 5 minutes. The supernatant fluid was discarded as well. The pellet was resuspended in 200 µL nuclease-free distilled water and boiled for 10 minutes and chilled immediately on the ice for 5 minutes. After the ice incubation, the tubes were centrifuged at 10 000 rpm for 5 minutes at 4°C and the supernatant was transferred into a new tube. An aliquot of 3 µL of the supernatant was used in the PCR mixes (18). This method of DNA purification was selected to harvest both plasmid and chromosomal DNA.

Genotypic Detection of ESBL Genes

All the ESBL-producing *E. coli* strains confirmed by the phenotypic assay were screened using the uniplex PCR for the detection of bla_{TEM}, $bla_{SHV,}$ and bla_{CTX-M} genes. The PCRs were carried out in a final volume of 25 µL. The oligonucleotide primers used in the investigation are listed in Table 1.

The PCRs were performed with a 25 µL reaction mixture containing 3 µL of the solution containing DNA, 12.5 µL of super PCR master mix 2X (Yekta Tajhiz Azma), 1 µL of each primer (20 pmol), and 7.5 µL of distilled water. In addition, the PCR was perfor.for 5 minutes. The BLAST analysis revealed that the PCR product sequences all 3 genes show high similarity with the corresponding genes in the GenBank database thus confirming the genes. A clinical isolate containing bla_{TEM}, $bla_{SHV,}$ and bla_{CTX} genes confirmed by the PCR and sequencing techniques were used as positive control and sterile distilled water was utilized as a negative control. The PCR products were identified on 2% agarose gel electrophoresis and the

Table 1. The Primers Used in This Investigation

Gene	Primer Sequence 5′→3′	Amplicon Size (bp)	Annealing Temperature	Reference
TEM -F *TEM*-R	TTGGGTGCACGAGTGGGTTA TAATTGTTGCCGGGAAGCTA	500	52	(19)
SHV-F *SHV*-R	AGGATTGACTGCCTTTTTG ATTTGCTGATTTCGCTCG	392	54	(20)
CTX-M-F *CTX-M*-R	ACCGCCGATAATTCGCAGAT GATATCGTTGGTGGTGCCATAA	585	58	(21)

gels were stained with ethidium bromide and then were visualized by the UV trans-illuminator (19,20, 21).

Data Analysis

The data were analyzed by SPSS software, version 16. The prevalence of resistance genes was calculated using χ^2 and fisher extract tests for each gene. Statistical significance was considered at the $P<0.05$.

Results

The urine samples were collected from hospitalized patients (inpatients) and outpatients. Of the 3192 urine samples cultured on the eosin methylene blue and blood agars, 192 isolates were identified as *E. coli* by IMViC and biochemical tests. Among these 192 *E. coli* strains, the ESBL producers were detected in 45 cases (28/12%) by the combined disc test. Figures 1, 2, and 3 illustrate the PCR product bands of bla_{TEM}, $bla_{SHV,}$ and bla_{CTX-M} genes. The amplified PCR products for bla_{TEM}, $bla_{SHV,}$ and bla_{CTX-M} genes were 500, 392, and 585 base pairs (bp), respectively. All the 45 *E. coli* isolates confirmed by the phenotypic methods were also positive for at least one ESBL gene by the molecular technique ($P<0.001$), the details of which are provided in Table 2.

The uniplex PCR of the 45 ESBL-producing *E. coli* strains indicated that the bla_{TEM} was the most abundant gene (89%), followed by *CTX-M* (27%) and *SHV* (20%). The co-existence of the bla_{CTX-M} and bla_{TEM}, as well as bla_{SHV} *and* bla_{TEM} genes was detected in 9 (20%) and 5 (12%) isolates, respectively. The co-existence of all 3 genes (i.e., bla_{SHV}, bla_{CTX-M}, and bla_{TEM}) were observed in 3 isolates (7%).

Table 2. Prevalence of ESBL Genes Among 54 Isolates

ESBL Genes	Number	Percent
bla_{TEM}	40	89
bla_{CTX-M}	12	27
bla_{SHV}	9	20
bla_{CTX-M} and bla_{TEM}	9	20
bla_{SHV} *and* bla_{TEM}	5	12
bla_{SHV}, bla_{CTX-M} and bla_{TEM}	3	7

Note. ESBL: Extended-spectrum β-lactamases.

Discussion

Cephalexin or amoxicillin/clavulanate is considered as one of the first options in the treatment of acute uncomplicated cystitis and can be prescribed in the treatment of cystitis caused by gram-positive cocci because it is effective against enterococci and staphylococci (22). The ESBL-producing bacteria, particularly *E. coli* and *K. pneumoniae* has emerged as a significant problem in the treatment of bacterial infections worldwide (23). The value of the current study was 0.001 thus the results demonstrated that there is a significant relationship between phenotypic and genotypic methods for the detection of the ESBLs. In our study, the prevalence of ESBL-producing *E. coli* strains was 28.12%. Further, Yılmaz et al. found that the prevalence of ESBL-producing *E. coli* strains was 24% (24). In another study, Jena et al reported that the prevalence of ESBL-producing *E. coli* strains isolated from patients with UTIs was 59.74% (25). Although mutations can cause antibiotic-resistant, the overuse of antibiotics increases the selection and emergence of resistant bacteria strains. Therefore, the prevalence of ESBL-producing UPEC varies in different regions. In the present study, 40 (89%) isolates were positive for the bla_{TEM} gene, which is consistent with the results of several studies. For example, Liu et al reported a prevalence of

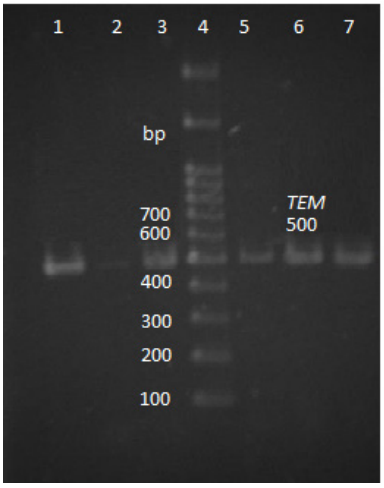

Figure 1. Agarose Gel Electrophoresis of the PCR Products of bla_{TEM} Gene (500 bp).
Note. Lane 4: DNA ladder; Lane 1: Positive control; Lane 2: Negative control; Lanes 3, 5, 6, 7, and 8: positive samples; PCR: Polymerase chain reaction.

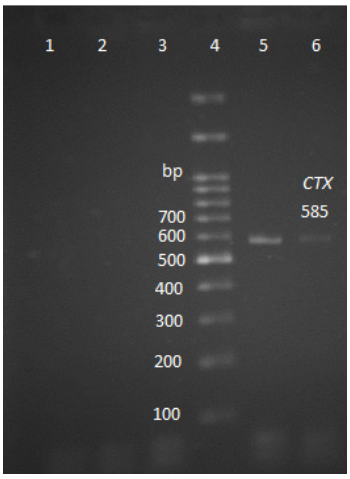

Figure 2. Agarose Gel Electrophoresis of the PCR Products of bla_{SHV} Gene (392 bp).
Note. Lanes 1, 2, 3, and 7: Positive samples; Lane 4: DNA ladder; Lane 6: Negative control; Lane 5: Positive control; PCR: Polymerase chain reaction.

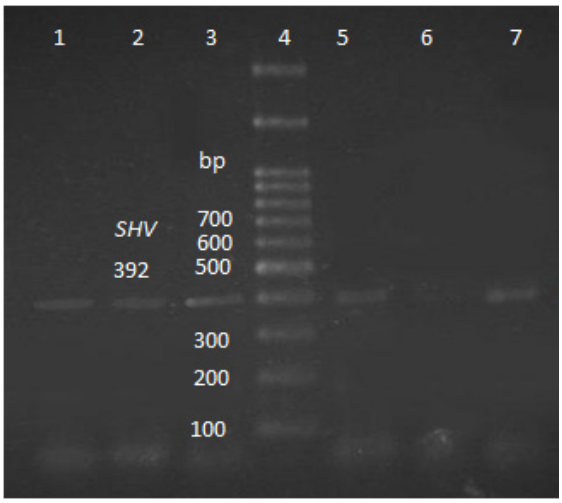

Figure 3. Agarose Gel Electrophoresis of the PCR Products of bla_{CTX-M} Gene (585 bp).
Note. Lanes 1 and 2: Negative samples; Lane 3: Negative control; Lane 4: DNA ladder; Lane 5: Positive control; Lane 6: Positive sample; PCR: Polymerase chain reaction.

72.1% for the bla_{TEM} genotype (26). Furthermore, Jena et al (25) showed that among ESBL-producing *E. coli* isolated from patients with UTI, the prevalence of bla_{TEM} gene was the predominant (93.47%). In the current study, the detection rate for the bla_{CTX-M} gene was 12 (27%). In studies conducted by Ruppé et al and Majeed et al, the detection rate of the bla_{CTX-M} gene in patients with UTI was 26 (76.4 %) and 26%, respectively (27,28). The result of our research is nearly similar to the report of Majeed et al, but it differs from that of Ruppé et al. These contradictory results indicate that the prevalence of the bla_{CTX-M} gene varies in different regions. Moreover, the results of our study revealed that the prevalence of bla_{SHV} was 9 (20%). According to Seyedjavadi et al and Reid et al, the rate of isolation of the bla_{SHV} gene in UPEC was 45% and 2.4%, respectively (29,30). The reason for the different rates of the bla_{SHV} is that the prevalence varies in various areas. Additionally, our finding showed that 39% of the UPEC isolates contained 2 or more ESBL genes. Similarly, Seyedjavadi et al (29) and Manoharan et al (31) reported the co-existence of different ESBL genes within the same isolate. Based on previous evidence, the co-existence of ESBL genes (i.e., bla_{SHV}, $bla_{CTX-M,}$ and bla_{TEM}) is because they are frequently located on the plasmid and can be transferred to other bacteria (32).

Conclusions

Due to the high prevalence of the ESBL uropathogenic *E. coli*, especially bla_{TEM} (89%), it is thought that ESBLs should be detected by phenotypic or genotypic methods for selecting the appropriate antibiotics regarding treating the patients with UTIs. Thus, future studies are suggested to determine the prevalence of the metallo-β-lactamases of the Verona Integron-encoded metallo-β-lactamase, imipenemases, and New Delhi metallo-β-lactamase (33,34).

Ethical Approval

There are no ethical issues for this article.

Conflict of Interests

Authors declare no conflict of interests associated with this study.

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