Frequency of *E. coli* clinical isolates producing *bla*$_{SHV}$ and *bla*$_{TEM}$ extended-spectrum beta-lactamases

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Original Article

Abstract

Introduction: Production of extended-spectrum beta-lactamase enzymes (ESBLs) in *E. coli* creates many problems for patients. These enzymes are located on transferable elements and can hydrolyze penicillins, broad-spectrum cephalosporins, and aztreonam. This study aimed to determine the clinical isolates of *E. coli* producing ESBLs of *bla*$_{SHV}$ and *bla*$_{TEM}$ in the city of Zanjan.

Methods: This cross-sectional study was performed on 200 *E. coli* isolates from clinical samples, including urine, feces, and secretions. The samples were cultured on EMB agar medium and the isolates were confirmed with various diagnostic tests. Then the sensitivity of strains to antibiotics and the production of ESBLs were determined by disc diffusion and combined disc methods, respectively. Finally, the presence of *bla*$_{SHV}$ and *bla*$_{TEM}$ genes was investigated by PCR using specific primers.

Results: Amoxicillin had the highest resistance by 68.5% (137 isolates) and imipenem the lowest by zero percent. Resistance to the studied antibiotics were as follows; co-trimoxazole 46.5% (93 isolates), cefotaxime 34.5% (69 isolates), ceftazidime 31.5% (63 isolates), cefepime 29.5% (59 isolates), gentamycin 28.5% (57 isolates), aztreonam 45% (90 isolates), ciprofloxacin 25.5% (51 isolates), co-amoxiclave 18.5% (37 isolates), cefoxitin 19% (38 isolates), and amikacin 4.5% (9 isolates). According to the combined disc test, 66 strains (33%) were ESBL-producing enzymes and the frequency of *bla*$_{TEM}$ *and bla*$_{SHV}$ genes was 46.9% (31 isolates) and 56% (37 isolates), respectively.

Conclusion: Given the resistance of ESBL strains to existing antibiotics and the ability to transfer these genes to other clinical isolates, performing antibiotic sensitivity tests and detection of ESBLs in laboratories is necessary for reducing treatment failure.

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Introduction:

Antibiotic resistance is dependent per se on the use of antimicrobial agents or antibiotics; this has attracted the world's attention and is one of the health care problems in different community from long times ago. Production of beta-lactamase enzymes is a way for antibiotic resistance against beta-lactam antibiotics. These enzymes have been widely distributed among bacteria and play a key role in intrinsic and acquired resistance of bacteria. In recent years, some strains of enteric gram-negative bacilli such as *Escherichia coli* have produced certain types of beta-lactamase enzymes called extended-spectrum beta-lactamases (ESBLs). These strains are resistant to penicillins, broad-spectrum cephalosporins, and aztreonam.

Beta-lactamases were reported in the early 1980s from Europe (1) and now they are reported from all over the world (2). Extended-spectrum beta-lactamases (ESBLs) were emerged after production and mass consumption of broad-spectrum cephalosporins (3). ESBLs are capable of hydrolyzing oxyimino-containing beta-lactam antibiotics such as ceftazidime, cefotaxime, ceftriaxone, cefuroxime, and aztreonam (4). TEM-1 is the most common plasmid-related beta-lactamase, and has been reported in 75-80% of resistance to broad-spectrum, plasmid-dependent beta-lactamses. TEM beta-lactamases were isolated from strains of *E. coli* for the first time in 1965 from blood cultures of a patient named Temoniera in Athens, Greece (5). This enzyme is currently known as a source of resistance to beta-lactam antibiotics among gram-negative bacilli. The gene of this enzyme is usually located on a transposon and is capable of transferring to and inducing antibiotic resistance in other strains (6).

SHV beta-lactamases were first isolated from *Klebsiella pneumoniae* strains, and *Klebsiella pneumoniae* is the origin of this class of enzymes. In many strains of *Klebsiella pneumoniae*, the enzyme is encoded by a chromosomal gene. But the gene was inserted into a plasmid over time and thus easily distributed among bacterial strains. SHV-1 leads to resistance in broad-spectrum penicillins such as ampicillin, piperacillin, and ticarcillin, so that this enzyme is responsible for more than 20% of resistance to ampicillin in many isolates (7, 8).

Antibiotic resistance in nosocomial infections is a critical issue; because resistance to antimicrobial agents is commonly seen in a large variety of hospital pathogens. *E. coli* is a common bacteria which is isolated from human infections and leads to urinary and gastrointestinal tract infections and meningitis in newborns.

The present study aimed to evaluate the frequency of beta-lactamase-producing *E. coli* isolates through phenotypic methods and to determine the prevalence of bla_{SHV} and bla_{TEM} genes in different clinical samples through PCR.

Methods:

In this cross-sectional study, performed during spring and summer of 2012, a total of 200 *E. coli* isolates were collected randomly from clinical samples including blood, secretions, urine, and stool from 4 hospitals in Zanjan. To identify and confirm the clinical isolates, they were cultured in eosin methylene blue agar medium and incubated for 24 hours. The disc diffusion method (Kirby-Baur) was used to determine the antimicrobial sensitivity of *E. coli* isolates. The antibiotic discs were made by MAST Company (England) and were as follows: cefepime (30 μg), gentamicin (10 μg), imipenem (10μg), amikacin (30μg), aztreonam (30μg), ciprofloxacin (5μg), amoxicillin (25μg), cefotaxime (30μg), ceftazidime (30μg), tetracycline (30μg), co-amoxiclave (30μg), co-trimoxazole (25μg). After performing disc diffusion, the diameter of inhibition zone around each disc was measured and the results were reported as sensitive, resistant, and intermediate, according to the standards of CLSI. The combined disc method was used to study the phenotype of ESBL-producing strains. Strains resistant to cefotaxime and ceftazidime were analyzed using cefotaxim (30μg), cefotaxime+clavulanic acid (10-30μg), ceftazidime (30μg), and ceftazidime+clavulanic acid (10-30 μg). After incubation for 24 hours at 37°C, the production of ESBLs was determined based on increased diameter by 5 mm or more around ceftazidime+clavulanic acid or the cefotaxime+clavulanic acid discs compared to ceftazidime of cefotaxime discs. The standard strain of *E. coli* ATCC 25922 (obtained from the Microbiology Department of Zanjan Medical Sciences University) was used for controlling the antibiogram and compound disc method, and the boiling method was used for polymerase chain reaction (PCR). Strains carrying the genes bla_{TEM} and bla_{SHV} were used as positive controls (obtained from the Microbiology Department of Zanjan Medical Sciences University).

Sequences of the primers were as follows: Subject

TEM/F: 5’-TCCGCTCATGAGACAATAACC-3’ (931 bp)

TEM/R: 5’-TTGGTCTGACAGTTACCAATGC-3’

SHV/F: 5’-AAGATCCACTATCGCCAGCAG-3’ (231 bp)

SHV/R: 5’-ATTCAGTTCCGTTTCCCAGCGG-3’

PCR reaction was performed in a final volume of 25μL, containing 1μL dNTP (1mM), 2.5μL 10X buffer, 1 μL of each primer (10 pmol), 5 μL template DNA (50 pmol/μL), 1.5μL Taq polymerase (0.5 U), and 13μL distilled water, in 30 cycles with the following thermocycler program; initial denaturation of DNA at 94°C for 4 min, denaturation at 94 °C for one minute, annealing of primers of TEM at 50°C and of SHV at 57°C for 1 minute, elongation at 72°C for one minute, and final elongation at 72°C for 8 minutes.

The products of PCR were evaluated in the next step with electrophoresis on 1% agarose gel. The amplicons were compared with a 100 bp ladder (Fermentas) and gene segments of TEM and SHV had 931 bp and 231 bp length, respectively.

The frequency of the studied genes was calculated with SPSS 17.

Results:

A total of 200 clinical isolates of *E. coli* were collected from different samples including blood, secretions, urine, and stool from four hospitals of Mousavi, Imam Hussein, Shahid Beheshti, and Vali-e-Asr in Zanjan. Most of isolates were collected from Imam Hussein Hospital with 82 isolates (41%) and the lowest from Vali-e-Asr Hospital with 9 isolates (4.5%). Seventy three percent (146 samples) of the samples were collected from females and 27% (54 samples) from males, and the majority of isolates were from urine samples (80%) followed by stool samples (15%), and secretions (5%).

Table 1 lists the information on the percentage of drug resistance of *E. coli* isolates.

Amoxicillin had the highest resistance by 68.5% (137 isolates) and imipenem the lowest by zero percent. Resistance to the studied antibiotics were as follows; co-trimoxazole 46.5% (93 isolates), cefepime 29.5% (59 isolates), gentamycin 28.5% (57 isolates), aztreonam 45% (90 isolates), ciprofloxacin 25.5% (51 isolates), co-amoxiclave 18.5% (37 isolates), cefoxitin 19% (38 isolates), and amikacin 4.5% (9 isolates). The resistance rate of ceftazidime and cefotaxime was 31.5% (63 isolates) and 34.5% (69 isolates), respectively, and they were used for the combined disc test, which showed that a total of 66 isolates (33%) were ESBL-producing *E. coli*.

Table 1. Antimicrobial Resistance Pattern of Clinical Samples

Antibiotic	Resistant	Intermediate	Sensitive
Co-trimoxazol	93 (46.5%)	102 (51%)	5 (2.5%)
Cefexitin	38 (19%)	162 (81%)	0 (0%)
Cefepime	59 (29.5%)	129 (64.5%)	12 (6%)
Gentamycin	57 (28.5%)	119 (59.5%)	24 (12%)
Imipenem	0 (0%0	196 (98%)	4 (2%)
Amikacin	9 (4.5%)	176 (88%)	15 (7.5%)
Aztreonam	90 (45%)	87 (43.5%)	23 (11.5%)
Ciprofloxacin	51 (25.2%)	144 (72%)	5 (2.5%)
Amoxicilin	137 (68.5%)	63 (31.5%)	0 (0%)
Ceftaxime	69 (34.5%)	128 (64%)	3 (1.5%)
Ceftazdime	63 (31.5%)	119 (59.5%)	18 (%)
Amoxiclave	37 (18.5%)	163 (81%)	0 (0%)

Figure 1 depicts a view of the combination disc test.

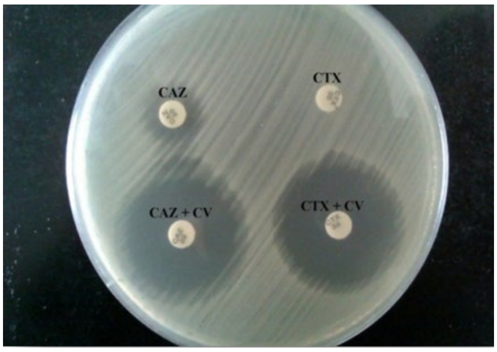

Figure 1. Phenotypic detection of ESBLs producers. CTX (cefotaxime), CAZ (ceftazidime), CV (clavulanic acid)

According to the results of PCR performed to detect the *bla*TEM and *bla*SHV genes, 66 isolates of *E. coli* were ESBL-producing strains, and 31

(46.96%) and 37 (56%) isolates were carrying *blaTEM* and *blaSHV* genes, respectively (Fig. 2 and 3). Out of 66 ESBL-producing isolates, 6 (9%) had any of the *blaTEM* and *blaSHV* genes, while 19 (28.7%) had both simultaneously.

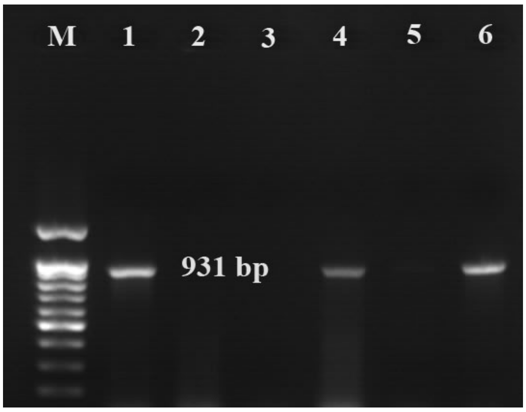

Figure 2. Detection of *blaTEM* gene in agarose gel. Lanes 1, 4, and 6: clinical samples with TEM gene; Lanes 2, 3, and 5: clinical samples without TEM gene; Lane M: DNA size marker

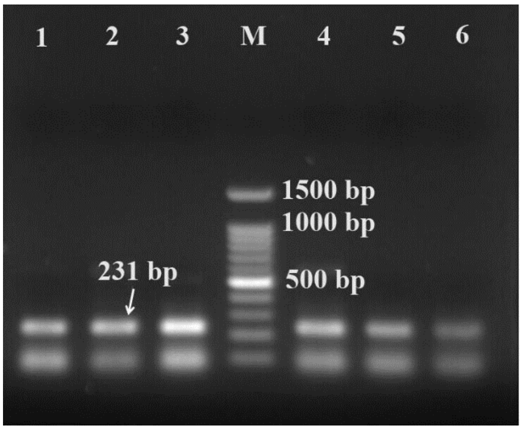

Figure 3. Detection of *blaSHV* gene in agarose gel. Lane 3: positive control; Lane M: DNA size marker; Lanes 1, 2, 4, 5, and 6: clinical samples with SHV

Conclusion:

The present study was performed on 200 isolates of *E. coli* isolated from four hospitals in Zanjan. According to the results, resistance to amoxicillin was over 50%. In a study by Mohajeri in Kermanshah, the highest and the lowest percentages of resistance were seen in ampicillin (77%) and imipenem (zero percent), respectively, which was similar to our results (9). The study of Heike von Baum from 1986 to 2001 showed a significant increase in the rate of resistance to ampicillin and ciprofloxacin (10).

The resistance to cotrimoxazole was 46.5% in the present study, while Daza *et al*. (2008) reported a resistance percentage of 35.2 to this antibiotic in a research performed in Spain on *E. coli* isolated from urine samples. In the Daza's study, resistance to ciprofloxacin was 35.2% which was higher than our result (25.5%) (11). In this study, the resistance of *E. coli* strains to cefotaxime and ceftazidime was 34.5% and 31.5%, respectively, while in Brazil, Kiffer *et al*. reported the resistance rate of *E. coli* strains isolated from different wards of hospitals as 14.1% and 14.4% for cefotaxime and ceftazidime, respectively (12). In a study conducted between 2000 and 2009, Yong Hong et al. showed that resistance to these antibiotics in *E. coli* isolates increased from 16.7% and 6.3% in 2000 to 52.3% and 16.6% in 2009, indicating the currently increased resistance to third generation cephalosporins (13). In a study by Mobasher Karjedi et al. on clinical samples isolated from 4 educational and treatment centers of Tabriz, 80.49% and 78.05% of isolates were reported to be resistant to ceftazidime and cefotaxime, respectively, which are higher than our results (14).

Detection of extended-spectrum beta-lactamases (ESBLs) producing bacteria, which have had a significant increase in the last two decades throughout the world, was another important result of the present study. In this research, 33% of *E. coli* strains (66 isolates) were ESBL-producing. In a study conducted in Brooklyn, the frequency of ESBL-producing *E. coli* isolates was 4.7% showing a higher prevalence of these enzymes in the area (15).

In a recent study by Mohajeri *et al*. on samples of *E. coli*, 27% of the strains were producers of ESBL (9). In a research by Masjedian, 51% of *E. Coli* isolates and 49% of Klebsiella isolates were ESBL-producing (16). In the study of Mobasher Karjedi, 97.87% of *E. coli* isolates were ESBL positive (14). In a research by Tassli *et al*. in Turkey, the production of ESBLs in *E. coli* strains was reported 17% (17). In another study conducted by Wu *et al*. in Taiwan's hospitals, ESBL-producing *E. coli* was one of the most common strains which produced these enzymes (18.18%) (18).

According to the results, 46.9% of the studied samples carried *bla_{TEM}* gene, while similar studies reported this figure as 56.4% in Italy and 22.1% in Spain (19). In the study of Tassli in Turkey (2005), 21 isolates contained *bla_{TEM}* gene, out of 24 beta-lactamase positive isolates (17). In the study of Hung Fung *et al*. between 2001 and 2006 in Sweden, from 87 strains of ESBL-producing *E. coli* phenotype, 55 isolates (63%) had *bla_{TEM}* genotype (20).

In research conducted in Iran, the frequency of *bla_{TEM}* gene varied in different geographical areas; so that the prevalence of *bla_{TEM}* carrying *E. coli* strains was reported 24%, 58.3%, and 84.6% in the studies by Shahcheraghi (21), Zaman Zad (22), Masjedian (16), respectively. The results showed that the prevalence of this gene is high in most parts of the country.

The prevalence of *bla_{SHV}* gene in this study was 56%; this was 15.9% in a study by Romiro *et al*. in Spain from 2001 and 2004 (23). In the study of Hung Fung in Sweden, this rate was 6% from 2001 to 2006 (20). Hosoglu *et al*. (2007) reported that 28.6% of isolates had *bla_{SHV}* gene (24).

Bla_{SHV} gene existed in 71.7% of *E. coli* samples and 25.52% of *Klebsiella pneumoniae* samples in a study by Mobasher Karjedi (12). The prevalence of this enzyme was reported 6% by Shahcheraghi et al. (21). The prevalence of *bla_{SHV}* gene in our study was higher compared to studies in other regions which could be a warning regarding an increased resistance.

The prevalence of ESBLs of *bla_{TEM}* and *bla_{SHV}* were evaluated in the present study, and comparison of these results with other studies reveals a relatively high percentage of beta-lactamase resistance of *E. coli* isolates in Zanjan.

Production of ESBLs is a major threat in the use of penicillins and broad-spectrum cephalosporins. Therefore, appropriate antibiotic should be chosen carefully for treatment of ESBL-producing organisms. In order to prevent the spread of these strains and to select effective antibiotic treatment, it is recommended to routinely identify such resistance in microbiology laboratories. Detection of resistant and common strains of infectious agents can be effective in providing treatment strategies.

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