Gene Cell Tissue. 2020 April; 7(2):e102700. doi: 10.5812/gct.102700.

Published online 2020 May 26. **Research Article**

Prevalence of *bla*$_{CTX-M}$, *bla*$_{SHV}$, and *bla*$_{TEM}$ Genes in *Escherichia coli* Strains Isolated From Urinary Tract Infection Samples of Patients in the Intensive Care Unit in Qom, Iran

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Received 2020 March 16; **Revised** 2020 April 22; **Accepted** 2020 April 25.

Abstract

Background: *Escherichia coli* is considered as one of the causes of opportunistic infections. Nowadays, due to the increase in drug resistance, the treatment of these infections has become very difficult and they are recognized as the main causes of death in hospitalized patients.
Objectives: The aim of this study was to determine the prevalence of *blaTEM*, *blaSHV*, and *blaCTX-M* genes in *E. coli* strains isolated from the urinary tract infection in patients in Intensive Care Units of three different hospitals in Qom, Iran.
Methods: This study was conducted in three months from October to December 2014. A total of 200 *E. coli* samples were taken from the patients with urinary tract infections in Intensive Care units of Qom hospital. The disc diffusion method was used to determine the susceptibility pattern of antibiotic and phenotypic confirmatory tests for screening of the expanded spectrum beta-lactamase (ESBL) isolates. The presence of *bla*$_{TEM}$, *bla*$_{SHV}$, and *bla*$_{CTX-M}$ genes was evaluated by the polymerase chain reaction (PCR) assay.
Results: Of 200 samples, ampicillin (96%) and nitrofurantoin (19.5%) showed the highest and lowest drug resistance, respectively. A total of 156 isolates (78%) were identified as ESBLs using the phenotypic method. Moreover, 76 (38%), 90 (45%), and 123 (61.5%) isolates consisted of *bla*$_{CTX-M}$, *bla*$_{SHV}$, and *bla*$_{TEM}$, respectively.
Conclusions: Overall, the findings of this study showed that *bla*$_{TEM}$ was the most common gene with a frequency of 61.5% in ESBL *E. coli*.

Keywords: *Escherichia Coli*, Cephalosporins, Beta-Lactamase, Intensive Care Units (ICU)

1. Background

Escherichia coli (*E. coli*) is recognized as one of the causes of nosocomial infections (1). Nowadays, due to the high prevalence of antibiotic resistance, *E. coli* is recognized as one of the most resistant bacteria to broad-spectrum antibiotics (2). Also, beta-lactam antibiotics are the most important drugs that are commonly used worldwide to treat bacterial infections (3). Resistance to beta-lactam antibiotics is created by different mechanisms, such as beta-lactamase enzymes, such as expanded spectrum beta-lactamase (ESBL), efflux pumps, and porins (4). ESBL-producing bacteria are associated with several crucial health problems in the world (5). Currently, more than 300 ESBLs have been identified, forming after mutation of beta-lactamase enzymes (6). The main encoding genes of the ESBLs are CTX-M, TEM, and SHV groups of Amber molecular class (7).

ESBL-producing bacteria are mainly found in Intensive Care Units (ICUs) of the hospitals (8) Also, they are resistant to other antibiotic groups (3). Therefore, suitable drugs to treat the bacteria will be very limited, which is the main concern regarding the spread of ESBL strains (9). Identification of this bacteria and increasing knowledge about its presence in a geographical location are effective in the proper and effective use of suitable antibiotics in the region (10). Accordingly, it is the responsibility of health and microbiological officials of each region to monitor and track the growth rate of bacteria resistant constantly, especially ESBLs strains, to control and prevent drug-resistant outbreaks (11). Also, the information on the growth and spread rate of these enzymes helps in the treatment and prevention of infections caused by Gram-positive and Gram-negative bacteria (12).

2. Objectives

The purpose of this study was to determine the prevalence of antibiotic resistance and ESBL in *E. coli* isolates of the urine samples taken from the patients in Qom hospitals using the phenotypic and genotypic methods.

3. Methods

3.1. Separation of the Isolates

As stated earlier, during three months (October to December 2014), 200 samples suspected of *E. coli* were taken from the urinary tract infection (UTI) of the hospitalized patients in ICUs of three different hospitals in Qom, Iran, and then evaluated. Microbiological standard tests, such as gram stain, IMViC, oxidase, catalase, and fermentation/oxidation tests were used to identify the isolates (13) (all strains were Gram-negative bacilli, indol positive, citrate negative, Voges-Proskauer (VP) negative, and methyl red (MR) positive).

3.2. Antibiotic Susceptibility Pattern

To identify the antibiotic susceptibility pattern, the disk diffusion agar test (Kirby Bauer method) (14) was performed using 17 different antibiotic types (Mast, UK). These antibiotic disks included ampicillin (30 μg), Piperacillin (30 μg), cefuroxime (30 μg), imipenem (30 μg), nitrofurantoin (30 μg), amikacin (30μg), aztreonam (30 μg), carboncillin (30 μg), Cefepime (30 μg), Ceftriaxone (30 μg), Ciprofloxacin (30 μg), Trimethoprim-sulfamethoxazole (30 μg), Gentamicin (30 μg), Ofloxacin (30 μg), Nalidixic acid (30 μg), Cefotaxime (30 μg), and Ceftazidime (30 μg) in terms of the Clinical and Laboratory Standards Institute (CLSI) procedures (14).

3.3. ESBLs-Producing Isolates by Combination Disc Method

Combined carbohydrate-deficient transferrin (CDT) testing was applied to screen the ESBL-producing *E. coli*. The CDT phenotypic test was performed by the disks of ceftazidime (30 μg) and cefotaxime (30 μg) alone and in combination with clavulanic acid (10 μg) in Muller Hinton Agar. After 24 h of incubation at 37°C, an increase in zone size of $\geq$ 5 mm was considered as a positive ESBL isolate. In the present study, K. pneumoniae ATCC 700603 (prepared by the Pasteur Institute, Tehran, Iran) was used as positive control (15).

3.4. DNA Extraction and PCR Amplification for TEM, SHV, and CTX-M Genes

DNA extraction was performed using the boiling method. Then, the quality and quantity of all the extracted DNAs were evaluated by the spectrophotometer and gel electrophoresis. The presence of TEM, CTX-M, and SHV were studied using the polymerase chain reaction (PCR) assay (16). The ingredients of the PCR mixture were as follows for 25 mL PCR reaction: 50 mm of Tris-HCL, 50 mM of (pH = 8) KCL, 15 mm of $MgCl_2$, 0.2 mm dNTP mix, 20 pM of each primer (Table 1), and 5 mg of the extracted DNA. PCR was performed at the temperature conditions shown in Table 2. The PCRs products were exposed to electrophoresis in 1.5% agarose gel.

3.5. Statistical Analysis

The results of antibiotic susceptibility pattern, a confirmatory test for ESBL production, and the existence of bla_{TEM}, bla_{SHV}, and bla_{CTX-M} genes were analyzed using SPSS software (version 16) and Fisher's exact test.

4. Results

The experiment was performed on 200 *E. coli* isolates taken from the urine samples of cases in the ICU in Qom hospitals, of whom 152 (76%), 25 (12.5%), and 23 (11.5%) patients were women, men, and infants, respectively. The result of the antibiotic sensitivity test is shown in Table 3. According to the results, most of the isolates (192, 96%) were resistant to ampicillin and the least resistance was found to Nitrofurantoin (39, 19.5%). Based on the results of the combined disk tests, 156 samples (78%) were producers of ESBLs, and 44 strains (22%) were non-ESBLs producers.

According to the result of PCR assay, 123 (61.5%), 76 (38%), and 90 (45%) *Escherichia coli* strains posed bla_{TEM}, bla_{CTX-M}, and bla_{SHV} genes, respectively (Figure 1).

5. Discussion

Over one million people hospitalized for different medical conditions and during a hospital stay have been infected with nosocomial infections. Nosocomial infection is the most common cause of complications and problems for medical personnel, patients, and all hospitals in Iran (17).

The risk of nosocomial infections has been reported to be from at least 0.27 (0%) to more than 6/27 (22.2%) between 2010 and 2015, respectively (18). These infections can easily be transmitted among patients and their visitors, hospital personnel, and those with direct contact with the hospital

Table 1. Primers Used for Amplification of bla_{TEM}, bla_{SHV}, and bla_{CTX-M} Genes in the Present Study

Genes	Primer Sequences (5' → 3')	Temperature, °C	Length, bp	References
blaSHV	Forward: -GCTTTCCCATGATGAGCACC	60	292	Primers were designed by the authors
	Reverse: -AGGCGGGTGACGTTGTCGC	61		
blaTEM	Forward: -GGTGAAAGTAAAAGATGCTGAAG	59	559	
	Reverse: -AACTTTATCCGCCTCCATC	60		
blaCTX-M	Forward: -GCGGAAAAGCACGTCAAT GGG	62	427	
	Reverse: -GCCAGATCACCGCGATATC	61		

Table 2. Polymerase Chain Reaction Conditions for Amplification of bla_{TEM}, bla_{SHV}, and bla_{CTX-M} Genes in the Present Study

Steps	Temperature, °C			Time, min		
	SHV	CTX-M	TEM	SHV	CTX-M	TEM
Initial denaturation	95	95	95	3	3	3
Denaturation	95	95	95	0.5	0.5	0.5
Annealing	57	60	55	0.5	0.5	0.5
Extension	72	72	72	1	1	1
Final extension	72	72	72	5	5	5
Cycle number	35 Cycle					

Table 3. Antibiotics Sensitivity Pattern of the *Escherichia coli* Strains[a]

Antibiotic	Resistant	Intermediate	Sensitive
Carbenicillin	90 (45)	11 (5.5)	99 (49.5)
Piperacillin	175 (87.5)	21 (10.5)	4 (2)
Ofloxacin	69 (34.5)	19 (9. 5)	112 (56)
Nitrofurantoin	39 (19.5)	110 (55)	51 (25.5)
Amikacin	57 (28.5)	71 (35.5)	72 (36)
Ceftriaxone	90 (45)	62 (31)	48 (24)
Ampicillin	192 (96)	8 (4)	-
Imipenem	77 (38.5)	43 (21.5)	80 (40)
Ciprofloxacin	72 (36)	27 (13.5)	101 (50.5)
Aztreonam	182 (91)	16 (8)	2 (1)
Cefepime	83 (41.5)	52 (26)	65 (32.5)
Gentamicin	55 (27.5)	41 (20.5)	104 (52)
Nalidixic acid	133 (66.5)	23 (11.5)	44 (22)
Co-trimoxazole	137 (38.5)	4 (2)	59 (29.5)
Cefuroxime	180 (90.5)	19 (9. 5)	59 (29.5)
Cefotaxime	160 (80)	31 (15.5)	9 (4.5)
Ceftazidime	94 (47)	60 (30)	46 (23)

[a]Values are expressed as No. (%).

environment (1). The highest number of nosocomial infections occurs in the ICU, therefore, it is known as one of the most important responsibilities of lab technicians to identify and control these infections (9). Additionally, *E. coli* is the most evident and frequent organism, which is also a critical pathogen for UTI (19).

In our investigation, the highest and lowest resistances were found to ampicillin and nitrofurantoin, respectively. However, various resistant rates have been reported from different parts of the world, mostly due to the different patterns of antibiotic use. Rajabnia et al. (19) in Iran indicated that cefotaxime and meropenem had the highest and lowest resistance rates, whereas Jena in India (2017) reported ceftazidime and colistin with the highest and lowest resistance rates (19, 20).

Previous studies conducted in India, Poland, Africa, Iraq, Iran, and other countries showed different rates of antibiotic resistance in *E. coli* strains (20-24).

We also found a high rate of bla_{TEM} (61.5%), bla_{CTX-M} (45%), and bla_{SHV} (38%) genes in clinical strains isolated from the patients with UTI admitted to the ICU. However, bla_{SHV} was less than the other two types of genes. Moreover, another study conducted in India reported that 93.47%, 82.60%, and 4.34% of bla_{TEM}, bla_{CTX-M}, and bla_{SHV} genes in *E. coli* were isolated from adult patients with UTI, respectively (20). Polse et al. (23) in a study performed in Iraq indicated that *E. coli* strains isolated from UTI included 87.2%, 54.5%, and 21.8% of $_{bla}$CTX-M, bla_{TEM}, and bla_{SHV} genes, respectively. In contrast, a recent study by Nojoomi and Ghasemian (25) in 2016 in Iran the prevalence of $bla_{CTX-M-1}$, bla_{SHV} and bla_{TEM} was 77.4% (n = 86), 47.4% (n = 53) and 2.4% (n = 2), respec-

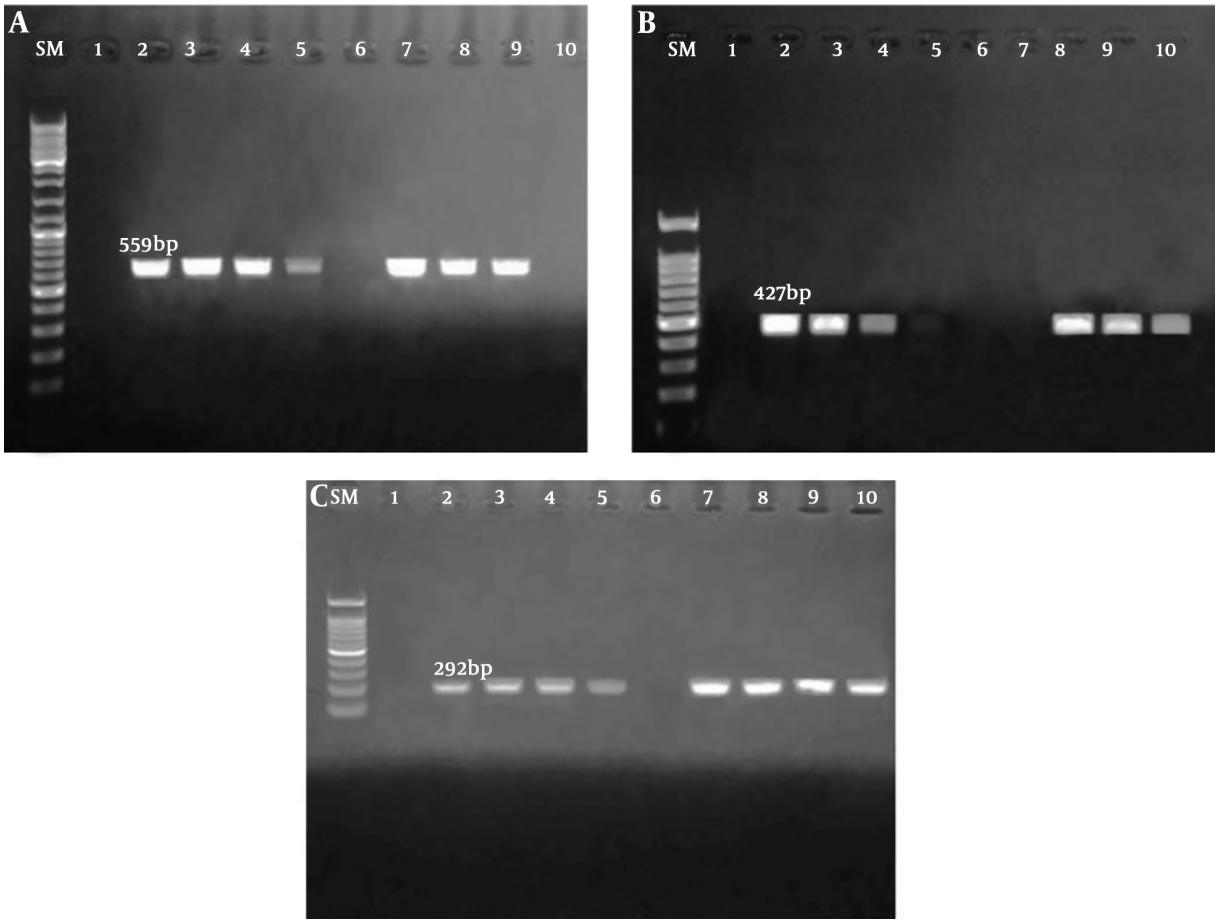

Figure 1. A, SM: Marker size (100 bp), lane 1: negative control, lane 2: positive control, lanes 3, 4, and 7 - 9: *Escherichia coli* strains bla_{TEM} positive, and lane 6 and 10: negative *E. coli* strains for bla_{TEM}; B, SM: marker size (100 bp), lane 1: negative control, lane 2: positive control, lanes 3, 4, 5, and 7 - 10: positive *E. coli* strains for bla_{SHV}, and lane 6: negative *E. coli* strain for bla_{SHV}; C, SM: marker size (100 bp), lane 1: negative control, lane 2: positive control, lanes 3, 4, and 7 - 9: positive *E. coli* strains for *bla*CTX-M, and lane 6: negative *E. coli* strains for bla_{CTX-M}.

tively.

Interestingly, these findings are consistent with a previous study reflected a high rate of ESBL genes in clinical isolates, which clearly indicates the current challenges for the centers for infection control at hospitals and health centers in ICU Qom, Iran. Financial problems and the lack of facilities for molecular typing methods for epidemiology studies, such as pulsed-field gel electrophoresis (PFGE), multilocus sequence typing (MLST) that are effective in finding the relationship between strains, and ultimately finding their origin are some of the limitations of our study. The patients admitted to the ICU in our hospitals were not evaluated for the extent of beta-lactamase genes in the urine samples, which can be considered as the strength of the present study.

It is also hoped that in future studies, we will be able to take some effective steps to help in controlling nosocomial infections through molecular typing methods.

5.1. Conclusions

In conclusion, our results showed that among the examined genes, the most common gene was bla_{TEM}, with a frequency of 61.5% in ESBL-producing *E. coli* taken from the patients with UTI admitted to the ICU in Qom, Iran.

Due to the high level of drug resistance of the studied isolates, it was very difficult to treat the infections. Accordingly, considering the high rate of drug resistance in our study, further studies are needed to find effective drugs, including nanoparticles, for eliminating these bacteria that are resistant to antibiotics.

Acknowledgments

The authors would like to thank the Islamic Azad University, Qom Branch, Qom, Iran.

Footnotes

Authors' Contribution: Study concept and design: Mohammad Reza Zolfaghari. Acquisition of data: Shima Sadat Lesani. Analysis and interpretation of data: Mohammad Soleimani. Drafting of the manuscript: Pegah Shakib. Critical revision of the manuscript for important intellectual content: Mohammad Reza Zolfaghari. Statistical analysis: Mohammad Soleimani. Study supervision: Mohammad Reza Zolfaghari.

Conflict of Interests: None declared.

Ethical Approval: The study was approved by the Islamic Azad University, Qom Branch, Qom, Iran.

Funding/Support: None.

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