

Detection of *TEM*, *SHV* and *CTX-M* Antibiotic Resistance Genes in *Escherichia coli* Isolates from Infected Wounds

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ABSTRACT

Background and Objective: *Escherichia coli* is one of the most common causes of hospital-acquired infections. Extended-spectrum β -lactamase (ESBL)-producing *E. coli* strains are resistant to third-generation cephalosporins. The three main genes involved in ESBL production are *TEM*, *SHV* and *CTX-M*. Detection of ESBL-producing *E. coli* is of importance for infection control, reduction of excessive antibiotic use and epidemiological surveillance. This study aimed to detect ESBL-producing *E. coli* strains isolated from wound infections using phenotypic and molecular methods.

Methods: During 2013- early 2015, 86 strains were collected from three hospitals in Isfahan, Iran. Antibiotic susceptibility testing was done using ceftazidime and ceftazidime + clavulanic acid discs. Polymerase chain reaction was used for the detection of the three resistance genes.

Results: The resistance genes *SHV*, *CTX-M* and *TEM* were detected in 49 isolates (56.9%). In addition, 39 isolates (45%) were ESBL-producing strains. According to the results, 5 (5.8%), 14 (16.2%), 19 (22%) and 11 (12.7%) isolates contained the *SHV*, *CTX-M*, *TEM* and *CTX-M* + *TEM* genes, respectively. The frequency of *CTX* and *TEM* were significantly higher than that of *SHV* gene ($P < 0.05$). Most of the isolated bacteria were resistant to cefazolin and sensitive to nitrofurantoin.

Conclusions: There is a difference between the frequency of ESBL-positive isolates reported in the phenotypic and genotypic methods, which could be due to the lower sensitivity of the phenotypic method and impact of environmental factors on the emergence of antibiotic resistance.

Keywords: Antibiotic resistance genes, ESBL, *TEM*, *SHV*, *CTX-M*, *Escherichia coli*.

INTRODUCTION

Bacterial causes of wound infections vary in different geographical locations (1). Although *Escherichia coli* is a common commensal gastrointestinal tract bacterium that contributes in the maintenance of human health, it is one of the most common causes of both community- and hospital-acquired infections such as bacteremia, meningitis, wound infection and urinary tract infection (1-3).

Indiscriminate use of antibiotics has increased the prevalence of antibiotic resistance among bacteria (4). Factors such as interconnected travel, horizontal gene transfer and bacterial evolution increase the global burden of diseases caused by antibiotic-resistant pathogens (5). B-lactam antibiotics are the most widely used class of antimicrobial agents for treatment of bacterial infections (6). Resistance of pathogenic bacteria and particularly Gram-negative bacteria to β -lactam antibiotics has become a worldwide problem (7). Production of extended-spectrum β -lactamases (ESBLs) is a significant resistance-mechanism that impedes the antimicrobial treatment of infections caused by *Enterobacteriaceae* (6). According to the Ambler scheme, β -lactamases are divided into four classes (A, B, C and D) (8). ESBL-producing *Enterobacteriaceae* causes more severe infections compared to non-ESBL-producing *Enterobacteriaceae* (9). The main mechanism of function in class A ESBLs is hydrolysis penicillins, cephalosporins (except cephamycins) and monobactams (6). *TEM*, *SHV* and *CTX-M* are the main ESBL-encoding genes. Bacterial cells containing the *SHV* gene are resistant to broad-spectrum penicillins such as piperacillin and ampicillin but not to the oxyimino substituted cephalosporins and tigecycline. Bacteria containing the *TEM* gene are resistant to penicillins and first-generation cephalosporins but sensitive to oxyimino cephalosporin. In addition, the *CTX-M* gene is associated with cefotaxime resistance. There is no direct correlation between the *TEM* or *SHV* β -lactamases and *CTX-M*. *CTX-M*-type β -lactamases hydrolyze cephalothin or cephaloridine better than benzyl penicillin, and they preferentially hydrolyze cefotaxime over ceftazidime (8). Detection of ESBL-producing *E. coli* is important for infection control,

reduction of excessive antibiotic use and epidemiological surveillance (10). Different phenotypic or genotypic methods are used for the detection of ESBL-producing *E. coli* (11). The aim of this study was to detect antibiotic resistance genes (*TEM*, *SHV* and *CTX-M*) in *E. coli* strains isolated from wound infections using the phenotypic and genotypic methods.

MATERIAL AND METHODS

Overall, 86 isolates were collected from three hospitals in Isfahan during January 2013-February 2015. The study was approved by the Ethics Committee of University of Isfahan, Iran. Basic clinical data for each patient was collected. Wound specimens were collected with sterile swabs and then transferred to laboratory in sterile containers. Pus samples were cultured in blood and BHI agar media, and then incubated aerobically at 37° C for 24 hours.

Biochemical assays (urease, indole, oxidase, citrate, methyl red, Voges-Proskauer, and lactose fermentation on triple sugar iron agar) and motility test were performed on pure clones of the isolates (12).

Antimicrobial susceptibility of the isolates was evaluated against various antibiotics (Table 1) using the Kirby-Bauer disk diffusion method according to guidelines from the Clinical and Laboratory Standards Institute (2012) (13). ESBL production was confirmed by inhibition zone diameter of ≥ 5 mm for the combination disc (ceftazidime + clavulanic acid (30 μg /10 μg) compared with the ceftazidime disc alone (13).

DNA was extracted according to a method described previously (12). Resistance genes (*SHV*, *TEM*, and *CTX-M*) and maltose/maltodextrin transporter ATP-binding gene (specific for *E. coli* species) were detected by PCR (14-17). Randomly selected PCR products were sequenced (Macrogen, South Korea). The sequences were registered on GenBank with the following accession numbers: KT321529 (*TEM*), KT321528 (*SHV*), KT321527 (*CTX-M*), and KT321531 (*E. coli*).

GraphPad Prism software (version 6.1, GraphPad Software Inc., USA) and Fisher's exact test were used for statistical analysis.

RESULTS

The *SHV*, *TEM*, *CTX-M* and maltose/maltodextrin transporter ATP-binding genes were detected by PCR (Figure 1).

According to the results of the antibiogram test, most bacteria were resistant to cefazolin while sensitive to nitrofurantoin (Table 1).

The frequency of *CTX-M* and *TEM* were significantly higher than that of *SHV* ($P < 0.05$). However, there was no statistically significant difference in the frequency of *CTX-M* and *TEM*. In addition, no bacteria contained *SHV* and *CTX-M*, *SHV* and *TEM* or all 3 genes at the same time. The highest frequency for the

resistance genes (78.94%) was observed in bacterial strains isolated from surgical wounds (Table 2).

Of 86 *E. coli* isolates, 39 were ESBL-producing strains (45%) isolated from diabetic foot (10), bedsore (13), surgical wound (14) and miscellaneous wound (2) samples. Among the 39 ESBL-producing *E. coli*, 32 (82%) carried one or two ESBL genes. However, seven ESBL-producing isolates contained no resistance genes. Moreover, 17 non-ESBL-producing isolates contained the resistance genes (Table 2).

Table 1- Results of antimicrobial susceptibility testing and screening of *E. coli* isolates for ESBL production.

Antibiotic disc	Pattern of antimicrobial susceptibility						β-Lactamase inhibitor			
	Sensitive		Intermediate		Resistant		ESBL +		ESBL -	
	No.*	%	No.	%	No.	%	No.	%	No.	%
Amikacin (30 µg)	10	11.6	16	18.6	60	69.7	—	—	—	—
Cefepime (30 µg)	44	51.1	9	10.4	33	38.3	—	—	—	—
Cefotaxime (30 µg)	8	9.3	8	9.3	70	81.3	—	—	—	—
Ceftazidime (30 µg)	40	46.5	9	10.4	37	43	—	—	—	—
Levofloxacin (5 µg)	43	50	3	3.4	40	46.5	—	—	—	—
Cefazolin (30 µg)	6	6.9	3	3.4	77	89.5	—	—	—	—
Aztreonam (30 µg)	25	29	8	9.3	53	61.6	—	—	—	—
Tetracycline (30 µg)	27	31.3	5	5.8	54	62.7	—	—	—	—
Nalidixic acid (30 µg)	17	19.7	6	6.9	63	73.2	—	—	—	—
Nitrofurantoin(300 µg)	57	66.2	8	9.3	21	24.4	—	—	—	—
Ceftazidime/Clavulanate	—	—	—	—	—	—	39	45	47	54.6

* = Number of isolates

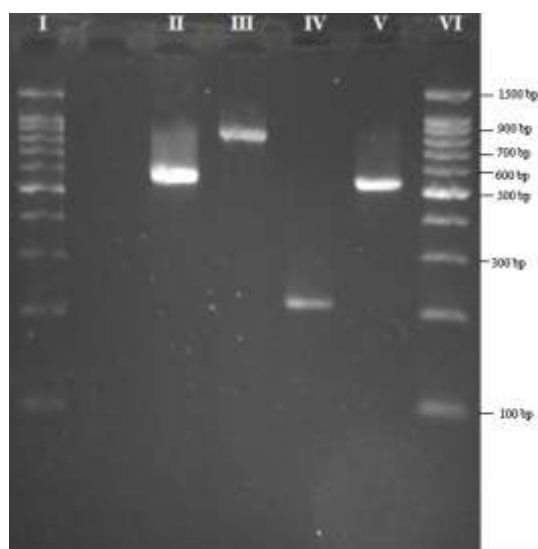
Table 2- Frequency of resistance genes in bacteria isolated from different types of wounds

Wound	Total	Age range	Sex		ESBL genes						
			M	F	<i>SHV</i>	<i>CTX-M</i>	<i>TEM</i>	<i>SHV & CTX-M</i>	<i>SHV & TEM</i>	<i>CTX-M & TEM</i>	<i>SHV & CTX-M & TEM</i>
Diabetic foot infection	25	59-78	1	7	1	3	5	0	0	2	0
Bedsore	28	19-89	1	18	3	5	6	0	0	4	0
Surgical wounds	19	30-75	1	8	0	4	7	0	0	4	0
Miscellaneous	14	18-61	8	6	1	2	1	0	0	1	0
Total	86	—	4	39	5	14	19	0	0	11	0

Table 3- Frequency of *SHV*, *CTX-M* and *TEM* in ESBL-producing *E. coli* in our study and some other studies.

Country	Number of isolates tested	<i>SHV</i>	<i>CTX-M</i>	<i>TEM</i>	Reference
Iran (Our study)	80	12.8	38.4	51.3	—
Iran (North)	100	44	28	49	(19)
Iran (Tehran)	42	40.5	—	19	(24)
India	138	66	71	75	(25)
Spain	112	32	69	6	(26)
Sweden	81	6	92	63	(27)

Figure 1- Columns I and VI= 100 bp DNA Ladder RTU (CinnaGen, Iran), Column II= *CTX-M* gene (590 bp), Column III= *TEM* gene (867 bp), Column IV= *SHV* gene (214 bp) and Column V= maltose/maltodextrin transporter ATP-binding gene (585 bp)



DISCUSSION

Investigation of antibiotic resistance patterns as well as molecular identification of *TEM* and *SHV* genes in ESBL-producing bacteria can be useful in epidemiological studies (18). High prevalence of ESBLs has been reported in different countries (8, 11, 19). Considering the high risk of treatment failure in patients infected with ESBL-producing bacteria, determining the frequency of these bacterial strains in different regions could be of great importance (8). In the present study, the frequency of ESBL-positive isolates in the genotypic and phenotypic methods was 56.9% and 45%, respectively. This difference could be related to the lower sensitivity of the phenotypic method, and the influence of environmental factors on emergence of resistance and expression of some resistance genes. On the other hand, seven ESBL-producing *E. coli* isolates contained none of the three resistance genes studied. This could be due to the presence of other β -lactamase genes such as *OXA*, *PER*, *GES*, *BES* and *CME*, and the structural changes in penicillin-binding proteins that result in resistance to β -lactam antibiotics.

In this study, the frequency of *SHV*, *CTX-M* and *TEM* was 8.4%, 41.6% and 50%, respectively. Study of Fatemi et al. on 41 *E. coli* strains isolated from bile samples reported the frequency of *SHV*, *CTX-M* and *TEM* as 92%, 80% and 70%, respectively (20), which are higher compared to our findings.

This could be due to the difference in the source of samples tested in the two studies. In study of Etok et al. in Nigeria, all *E. coli* isolates from wound infections were ESBL-producing strains (21). In study of Islam et al. the frequency of ESBL-producing isolates was 55% (22). Based on our findings, the frequency of ESBL-producing *E. coli* strains isolated from wound infections was 63.7%. The high frequency of these bacterial strains could complicate the treatment of wound infections.

The frequency of the *SHV*, *CTX-M* and *TEM* genes in the ESBL-producing *E. coli* was 12.8%, 38.4% and 51.3%, respectively. Frequency of *CTX-M* and *TEM* was nearly the same, while the frequency of *SHV* was lower compared to frequency rates reported in study of Rezai et al. on 100 ESBL-producing *E. coli* in north of Iran (23). This indicates that the frequency of the genes differs in areas of Iran. Differences in frequencies of these genes in other countries (Table 3), highlights the importance of the study of varied genes in different geographical locations. In addition, international travel may play an important role in worldwide spread of multidrug-resistant *E. coli*. In the present study, we determined the resistance rate of isolates to amikacin (69.7%), cefepime (38.3%), cefotaxime (81.3%),

ceftazidime (43%), levofloxacin (46.5%), cefazolin (89.5%), aztreonam (61.6%), tetracycline (62.7%), nalidixic acid (73.2%) and nitrofurantoin (24.4%). A similar study in north of Iran reported that 38.1%, 21.4%, 14% and 19% of the *E. coli* strains isolated from children with diarrhea were resistant to tetracycline, cefotaxime, ceftazidime and aztreonam, respectively (23).

The lower resistance rates reported by the mentioned study in comparison with our study could be due to the difference in source of sampling. In study of Mendonca et al. in Portugal, 91%, 93% and 89% of isolates were resistant to ceftazidime, quinolones and aminoglycoside, respectively, which are much higher than the resistance rates found in our study. This could be related to different antibiotics use policies (19).

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CONCLUSION

Compared to previous studies, our findings demonstrate that resistance to the third generation cephalosporins may have been increased, especially during last decade. The deference between results of the phenotypic and genotypic methods indicates the higher sensitivity of the genotypic method. It is recommended continuously monitoring antibiotic resistance patterns, and utilizing molecular method for the detection of ESBL-positive strains.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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