

Prevalence of *bla*_{OXA-1} and *bla*_{SHV} Genes in *E. coli* Isolates from Hospitalized Patients in Rasht

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ABSTRACT

Background and Objective: Extended-spectrum beta-lactamases (ESBL) are widely produced by *Escherichia coli* strains. The aim of this study was to determine frequency of *bla*_{OXA-1} and *bla*_{SHV} genes in *E. coli* strains isolated from patients hospitalized in city of Rasht, Iran.

Methods: In this cross-sectional study, 200 samples were collected from patients. The *E. coli* strains were identified using morphological characteristics and biochemical tests. Antimicrobial susceptibility testing was performed. The prevalence of the *bla*_{OXA-1} and *bla*_{SHV} genes in the *E. coli* isolated was assessed by PCR method.

Results: Overall, 160 *E. coli* strains were isolated. Of these, 83 (51.9%) showed ESBL activity while 71 (48.1%) did not. All positive strains were resistant to cephalothin. Moreover, 98.8% of ESBL-producing strains were resistance to amoxicillin, cefotaxime and ceftriaxone. The prevalence of the *bla*_{OXA-1} and *bla*_{SHV} genes in ESBL-producing strains were 45% and 17%, respectively. In addition, 28.9% of the strains had both genes while the genes were absent in 9.6% of the strains.

Conclusion: Prevalence of the *bla*_{OXA-1} gene is higher than that of the *bla*_{SHV} gene. The absence of both genes in some isolates indicates the possible role of other genes in the ESBL activity.

Keywords: Prevalence, ESBLs, *Escherichia coli*, *bla*_{OXA-1} gene, *bla*_{SHV} gene.

INTRODUCTION

Nowadays, nosocomial infection is one of the main health problems worldwide. *Escherichia coli* is regarded as the most common cause of these infections. The main clinical infections caused by this bacterium include kidney, bladder and respiratory infections, neonatal meningitis, pulmonary disease in immunocompromised patients and sepsis (1). Treatment of such infections is usually challenging due to acquisition of plasmids encoding broad-spectrum β -lactamases (2). Beta-lactamases are divided into classes A to D based on their molecular sequence. Class A β -lactamases have been only described in gram-negative bacilli, and are able to hydrolyze narrow-spectrum antibiotics such as penicillin and cephalosporin. Class B β -lactamases include metalloproteases containing zinc that are capable of carbapenem hydrolysis. These β -lactamases are found in *Pseudomonas aeruginosa* and *Serratia marcescens*. Class C β -lactamases are usually chromosomal, and have been reported in *Enterobacter cloacae* with high-resistance frequency. Class D β -lactamases' hydrolytic activity against oxacillin and cloxacillin is weakly prevented by clavulanic acid, which have plasmid origin (3, 4). Most broad-spectrum β -lactamases originate from genes related to TEM and SHV. TEM β -lactamases are common in most gram-negative bacteria, and account for 90% of ampicillin-resistance in *E. coli* and hydrolysis of first-generation cephalosporin such as cephalothin and cephaloridine. In recent years, a new family of plasmid-encoded broad-spectrum β -lactamases (CTX-M) has been identified in *Salmonella typhimurium* and *E. coli*. These types of β -lactamase are genetically related to TEM and SHV β -lactamases. In *Klebsiella pneumoniae*, SHV β -lactamases are responsible for more than 20% of resistance to ampicillin. Unlike TEM β -lactamases, there are not many derivatives from this type of β -lactamases. OXA β -lactamases are another group of broad-spectrum β -lactamases belonging to class D resulting in resistance to ampicillin and cephalothin (2, 5). Due to increased antibiotic resistance in *E. coli* isolates and availability of information on the prevalence of TEM and CTX genes, the study of other genes responsible for resistance is important. *E. coli* isolates resistant to amoxicillin were

susceptible to co-amoxiclav until 1987. Resistance to this antibiotic is through four mechanisms including overproduction of TEM β -lactamases, change in outer-membrane proteins that inhibit the absorption of antibiotics into the cell, production of OXA enzymes, and production of IRT β -lactamases from TEM β -lactamases by replacing a few amino acids. Thus, studying the prevalence of OXA β -lactamases is important (6). Moreover, the increased resistance to ceftazidime depends on the presence and increased production of SHV β -lactamases (7). Hence, this study aimed to investigate the frequency of *bla_{OXA-1}* and *bla_{SHV}* genes in extended spectrum beta-lactamases (ESBLs)-producing *E. coli* strains isolated from patients admitted to hospitals in city of Rasht.

MATERIAL AND METHODS

In this cross-sectional descriptive study, 200 samples from urine (160), blood (28), ascites (2), renal (2), wound (4) and peritoneal secretion (4) were collected from patients hospitalized in Golsar, Razi, Alzahra, 17th of Shahrivar, Rasoul Akram, and Poursina hospitals in Rasht during a six-month period. For isolating *E. coli* spp., the samples were cultured on blood agar and eosin methylene blue agar. Incubation was performed at 37 °C for 24 hours. The samples were identified according to morphological characteristics and biochemical tests including triple sugar iron, Simmons citrate, sulfur-indole-motility, urea agar, methyl red-voges proskauer, and lysine iron agar. Media were incubated at 37°C for 24 hours. Antibiotic susceptibility testing was carried out using the Kirby-Bauer and disk diffusion methods according to Clinical and Laboratory Standards Institute guidelines. For this purpose, 0.1 mL of bacterial culture suspension equivalent to half McFarland standard was cultured on Mueller Hinton agar (MHA). After placing antibiotic discs on the media, the growth inhibition zones were measured. The standard antibiotic discs including ciprofloxacin, cefotaxime, imipenem, amoxicillin, cefepime, cephalothin, cefixime, ampicillin, ceftriaxone, ceftazidime,

and ceftazidime/clavulanic acid were obtained from MAST Group Ltd, UK. The double disk method was used to confirm the production of broad-spectrum β -lactamase enzymes. The isolates were cultured on 10-cm plate containing MHA. Two ceftriaxone and co-amoxiclav discs with ceftazidime and ceftazidime clavulanic acid were placed at a distance of 30 mm but center to center on plates. Incubation was performed at 37 °C for 18-24 hours. Identification of a clear zone from edge of inhibition zone of disc lacking clavulanic acid towards the disc containing clavulanic acid indicates synergy. Moreover, ≥ 5 -mm increase in the diameter of the inhibition zone of cephalosporin/clavulanic acid against cephalosporin represents production of broad-spectrum β -lactamase enzyme. The frequency of the *bla_{SHV}* and *bla_{OXA-1}* genes was investigated using PCR. For amplification of the *bla_{SHV}* gene, the following forward and reverse primers were used:

Forward: ATTTGCTGATTTTCGCTCG

Reverse: AGGATTGACTGCCTTTTTG

Primers used for amplification of the *bla_{OXA-1}* gene were as follows (8):

Forward: ATATCTCTACTGTTGCATCTCC

Reverse: AAACCCTTCAAACCATCC

Amplification of the *bla_{SHV}* gene was performed under the following conditions: initial denaturation for 3m at 94°C for the first cycle, denaturation of other cycles for 30s at 94 °C, annealing for 30s at 45 °C, elongation for 1min at 72 °C, and final elongation for 7 min at 72 °C. Amplification of the *bla_{OXA-1}* gene was performed under the following conditions: initial denaturation for 3min at 94 °C in the first cycle, denaturation of other cycles for 30s at 94 °C, annealing for 30s at 51 °C, elongation for 45s at 72 °C, and final elongation for 7min at 72 °C. PCR products were electrophoresed on 1.5% agarose gel. *E. coli* strain C600 (R1010) was used as positive control for the *bla_{SHV}* and *bla_{OXA-1}* genes. The

results obtained were analyzed by the SPSS software and presented as frequency percentage of resistance to β -lactam antibiotics. The presence of genes was evaluated by Chi-square test. The P-value of less than 0.05 was considered as statistically significant.

RESULTS

Among the 200 samples tested, 160 *E. coli* isolates were identified that were from urine (140 ;87.5%), blood (14 ;8.8%), peritoneal secretion (2 ;1.3%), wounds (2 ;1.3%), ascites (1 ;0.6%), and renal secretion (1 ;0.6%) samples. In addition, 54 isolates were from males and 106 from females. The highest and lowest level of resistance was observed against amoxicillin (79.3%) and nitrofurantoin (1.4%), respectively. Maximum and minimum level of resistance was against amoxicillin (90%), cefoxitin and co-amoxiclav (20%), respectively. The lowest level of resistance was observed against imipenem.

According to the results obtained from the double disk method, among the 160 *E. coli* isolates, 83 (51.9%) produced broad-spectrum β -lactamases while 77 (48.1%) did not. All positive strains were resistant to cephalothin. Moreover, 98.8% of the isolates were resistant to amoxicillin, cefotaxime, and ceftriaxone. Overall, the isolates from 64.8% of males and 45.3% of females produced broad-spectrum β -lactamases.

The resistance to β -lactam and non- β -lactam antibiotics based on presence and absence of *bla_{SHV}* and *bla_{OXA-1}* genes (Tables 1). The Chi-square test showed a significant relationship between β -lactamase activity and presence of *bla_{SHV}* and *bla_{OXA-1}* genes (P=0.0001). In most cases, the presence of these genes increased resistance to β -lactamase and non- β -lactamase antibiotics.

Table 1- Frequency distribution of resistance to β -lactam and non β -lactam antibiotics in *E. coli* isolates based on presence and absence of the *bla*_{SHV} gene (no broad-spectrum β -lactamase activity was observed in the isolates lacking *bla*_{SHV} gene).

Resistance	β -lactamase positive				β -lactamase negative	
	<i>bla</i> _{SHV} positive Number: 56		<i>bla</i> _{SHV} positive Number: 27		<i>bla</i> _{SHV} negative Number: 77	
	Number	Percentage	Number	Percentage	Number	Percentage
Aztreonam	51	91/1	25	92/6	5	6/5
Cefotaxime	56	100	27	100	28	36/4
Imipenem	4	7/1	2	7/4	0	0
Amoxicillin	56	100	26	96/3	47	61
Cefepime	40	71/4	19	55/6	2	2/6
Cephalothin	56	100	26	96/3	13	16/9
Cefixime	14	100	22	95/7	7	10
Cefoxitin	17	30/4	6	22/2	4	5/2
Ampicillin	55	98/2	26	96/3	46	59/7
Ceftriaxone	55	98/2	27	100	10	13
Co-amoxiclav	17	30/4	10	37	8	10/4
Ceftazidime	44	78/6	23	85/2	2	2/6
Gentamicin	34	48/1	13	48/1	5	6/5
Co-trimoxazole	48	85/7	19	70/4	42	54/5
Ciprofloxacin	51	91/1	16	59/3	20	26
Ofloxacin	49	87/5	16	59/3	18	4/23
Tetracycline	44	78/6	19	70/4	37	48/1
Nitrofurantoin	1	5/2	1	4/3	0	0
Nalidixic acid	47	100	19	82/6	36	51/42
Gentamicin	34	48/1	13	48/1	5	6/5

Table 2- Frequency distribution of resistance to β -lactam antibiotics in *E. coli* isolates based on presence and absence of the *bla*_{OXA} gene (no broad-spectrum β -lactamase activity was observed in isolates lacking the *bla*_{OXA-1} gene)

Resistance	β -lactamase positive				β -lactamase negative	
	<i>bla</i> _{OXA-1} negative Number: 11		<i>bla</i> _{OXA-1} positive Number: 72		<i>bla</i> _{OXA-1} negative Number: 77	
	Number	Percentage	Number	Percentage	Number	Percentage
Aztreonam	10	90/1	66	91/7	5	6/5
Cefotaxime	10	90/1	72	100	13	16/9
Imipenem	2	18/2	4	5/6	6	7/8
Amoxicillin	7	63/6	68	66/7	3	2/6
Cefepime	10	90/9	72	100	47	1/6
Cephalothin	11	100	72	100	28	36/4
Cefixime	10	100	59	98/3	7	10
Cefoxitin	5	45/5	18	25	4	5/2
Ampicillin	9	81/8	72	100	46	59/7
Ceftriaxone	11	100	71	98/6	10	13
Co-amoxiclav	6	54/4	21	29/2	8	10/4
Ceftazidime	10	90/9	57	79/2	2	2/6

DISCUSSION

E. coli is regarded as one of the most common causes of nosocomial infections. The widespread use of antibiotics has increased resistance mechanisms in *E. coli* strains (9). One of the main mechanisms of resistance is the production of broad-spectrum β -lactamases. Broad-spectrum β -lactamases-producing gram-negative bacteria have been isolated in many countries since 1980s (10). The main broad-spectrum β -lactamases (TEM and SHV family)-producing strains have been reported in Asia and Western countries. Since 1995, the incidence of CTX-M β -lactamases has increased (11). Study of Sung Hi Li et al. (2001) reported increase in non-TEM and SHV β -lactamases using restriction fragment length dimorphism-PCR technique for identification of β -lactamases such as CMY-3, FOX-3, and OXA-2 (12). Most β -lactamases found in *E. coli* and *K. pneumoniae* belong to the TEM and SHV family (13). Since different genes such as *SHV* and *OXA* affect antibiotic

resistance in *E. coli*, this study investigated the prevalence of *bla*_{SHV} and *bla*_{OXA-1} genes. According to the results, the presence of the investigated genes increased resistance to β -lactam antibiotics. It has to be noted that the presence of other β -lactamases in the isolates lacking this gene affects the resistance. A low level of resistance was observed in the absence of other β -lactamases and the *bla*_{SHV} gene. Resistance to non- β -lactam antibiotics was significantly lower in isolates lacking the *bla*_{SHV} gene. The resistance to β -lactam antibiotics was higher in the presence of *bla*_{OXA-1} gene and lower in its absence. In this study, 51.9% of isolates resistant to β -lactam antibiotics (83 strains) produced broad-spectrum β -lactamase enzymes in the double disk method using ceftriaxone and co-amoxiclav, ceftazidime and ceftazidime/clavulanic acid discs. This is in agreement with studies of Fazeli (53.9%) and Masjedian (51%) (3, 14). In the present study, 32.5% of ESBL-producing *E. coli* isolates were resistant to co-amoxiclav. High resistance to co-amoxiclav indicates that

some strains may have the ability to acquire β -lactamase inhibitors. The results showed that 98.8% of ESBL-producing isolates were resistant to cefotaxime and ceftriaxone while 80.7% were resistant to ceftazidime. Shahcheraghy et al. (2007) showed resistance of ESBL-producing *E. coli* isolates against cefotaxime (44.77 %), ceftriaxon (48.57%) and ceftazidim (47.6 %) (9). The higher level of resistance to these antibiotics was observed in this study, indicating an increasing pattern of resistance to the antibiotics in Iran. The resistance pattern of ESBL-producing isolates (n=83) and non-ESBL-producing isolates (n=77) to non β -lactam antibiotics was evaluated. β -lactamase producing bacteria were resistance to ciprofloxacin, gentamicin, cotrimoxazol, aflatoxin and nalidixic acid, which is in line with the study of Fazeli (3). In this study, the prevalence of the *bla_{SHV}* and *bla_{OXA-1}* genes was 32.5% and 86.7 %, respectively. Moreover, 28.9% of strains had both *bla_{SHV}* and *bla_{OXA-1}* genes, while 9.6% of isolates lacked both genes. There was a significant relationship between ESBL activity and the presence of the *bla_{SHV}* and *bla_{OXA-1}* genes. In this study, the lowest and the highest frequency of ESBL bacteria was observed in one-year-old children (2.31%) and the participants aged 51-70 years (63.35%), respectively. In addition, the highest frequency of the *bla_{SHV}* (22.7%) and *bla_{OXA-1}* (59.1%) genes was observed in the participants aged 51-70 years. The frequency of the *bla_{SHV}* and *bla_{OXA-1}* genes was 20.4 and 55.6% in males and 15.1 and 39.6 % in females, respectively. Among the 27 *bla_{SHV}* genes, 16.4% and 20% of samples were isolated from urine and non-urine samples, respectively. Among the 72 *bla_{OXA-1}* genes, 42.9% and 60% were related to urine and non-urine samples, respectively. A simultaneous presence of both genes was observed in 14.3% of urine samples and 20% of non-urine samples. The isolates with the *bla_{SHV}* gene showed a maximum resistance to cefotaxim and ceftriaxon while

showing minimum resistance to ceftazidime. Isolates with the *bla_{OXA-1}* gene demonstrated maximum resistance to ampicillin, cefepem, cefalotio, cefotaxime while showing minimum resistance to ceftazidime (25 %). ESBL-producing isolates that had both genes showed the maximum resistance against ampicillin, cefotaxim, cefalotio and ceftazidim (100%) while showing a minimum resistance against ceftazidime (16.7%).

Isolates with ESBL activity showed an increased resistance to non- β -lactamase antibiotics, which could be due to transformation of the resistance genes. This is also consistent with the study of Fazeli et al. in Isfahan that reported β -lactamase gene-carrying plasmids might transfer the resistance genes to other bacteria (3). The frequency of the *bla_{OXA-1}* and *bla_{SHV}* genes in *E. coli* isolates with ESBL activity was 45% and 17%, respectively. The high prevalence of the *bla_{OXA-1}* gene may be due to use of different antibiotics in different regions. A Study by Speldoorn et al. showed that the frequency of *bla_{OXA-1}* gene is higher in London compared to Paris (15).

CONCLUSION

Among the 160 *E. coli* isolates, 51.9% showed ESBL activity. Frequency of the *bla_{OXA-1}* gene is higher than that of *bla_{SHV-1}*. Both genes are absent in 9.4% of strains. This indicates that the genes other than the *bla_{SHV}* and *bla_{OXA-1}* may play significant role in the β -lactamase activity.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interests.

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