

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

Majid Komijani (PhD)

Department of Biology, Faculty of Sciences, University of Isfahan, Isfahan, Iran

Majid Bouzari (PhD)

Department of Biology, Faculty of Sciences, University of Isfahan, Isfahan, Iran

Fateh Rahimi (PhD)

Department of Biology, Faculty of Sciences, University of Isfahan, Isfahan, Iran

Corresponding author: Majid Bouzari

Address: Department of Biology, Faculty of Sciences, University of Isfahan, Hezarjerib St., Isfahan 81746-73441, Iran

Tel: 031-37932459

E-mail: bouzari@sci.ui.ac.ir

Address: Faculty of Science, University of Isfahan, Isfahan, Iran

Received : 24 Sep 2015

Revised: 01 Nov 2015

Accepted: 10 Nov 2017

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). β -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 μ g/10 μ g) 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 ceftazidime 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

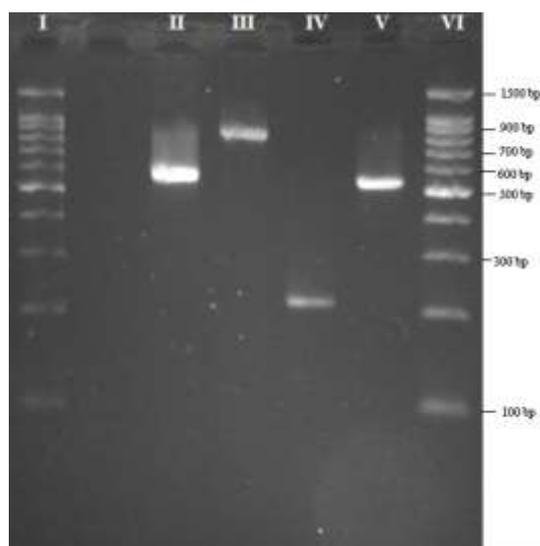
Table 2- Frequency of resistance genes in bacteria isolated from different types of wounds

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).

REFERENCES

1. Bowler P, Duerden B, Armstrong D. *Wound microbiology and associated approaches to wound management*. *Clinical Microbiology Reviews*. 2001; 14(2): 244-69. doi: 10.1128/CMR.14.2.244-269.2001.
2. Johora FT, Ahmed Z. *Bacteriological study of post-operative abdominal wound infection-a Case Study*. *Bangladesh Journal of Medical Science*. 2013; 12(1): 86-90. DOI: <http://dx.doi.org/10.3329/bjms.v12i1.10982>.
3. Jacobsen S, Stickler D, Mobley H, Shirtliff M. *Complicated catheter-associated urinary tract infections due to Escherichia coli and Proteus mirabilis*. *Clinical Microbiology Reviews*. 2008; 21(1): 26-59. doi: 10.1128/CMR.00019-07.
4. Roca I, Akova M, Baquero F, Carlet J, Cavaleri M, Coenen S, et al. *The global threat of antimicrobial resistance: science for intervention*. *New Microbes New Infect*. 2015; 6: 22-9. doi: 10.1016/j.nmni.2015.02.007.
5. Lu TK, Koeris MS. *The next generation of bacteriophage therapy*. *Current Opinion in Microbiology*. 2011; 14(5): 524-31. doi: 10.1016/j.mib.2011.07.028.
6. Alfaresi MS, Elkoush AA, Alshehhi HM, Abdulsalam AI. *Molecular characterization and epidemiology of extended-spectrum beta-lactamase-producing Escherichia coli and Klebsiella pneumoniae isolates in the United Arab Emirates*. *Med Princ Pract*. 2011; 20(2): 177-80. doi: 10.1159/000319912.
7. Kaftandzиеva A, Trajkovska-Dokic E, Kotevska V, Cekovska Z, Jankoska G. *Genotypes of ESBL producing Escherichia coli and Klebsiella pneumoniae in relation to resistance to antimicrobial drugs*. *Pril (Makedon Akad Nauk Umet Odd Med Nauki)*. 2014; 35(2): 31-8.
8. Shaikh S, Fatima J, Shakil S, Rizvi SMD, Kamal MA. *Antibiotic resistance and extended spectrum beta-lactamases: Types, epidemiology and treatment*. *Saudi Journal of Biological Sciences*. 2015; 22(1): 90-101.

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.

ACKNOWLEDGMENTS

This study was funded by the Department of Research and Graduate Studies of the University of Isfahan, Iran.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

9. Rottier WC, Ammerlaan HS, Bonten MJ. *Effects of confounders and intermediates on the association of bacteraemia caused by extended-spectrum β -lactamase-producing Enterobacteriaceae and patient outcome: a meta-analysis*. *J Antimicrob Chemother*. 2012; 67(6): 1311-20. doi: 10.1093/jac/dks065.
10. Garrec H, Drieux-Rouzet L, Golmard J-L, Jarlier V, Robert J. *Comparison of nine phenotypic methods for detection of extended-spectrum β -lactamase production by Enterobacteriaceae*. *J Clin Microbiol*. 2011; 49(3): 1048-57. doi: 10.1128/JCM.02130-10.
11. Krishnamurthy V, Vijaykumar G, Kumar S, Prashanth H, Prakash R, Nagaraj E. *Phenotypic and genotypic methods for detection of extended spectrum β lactamase producing Escherichia coli and Klebsiella pneumoniae isolated from ventilator associated pneumonia*. *Journal of Clinical and Diagnostic Research: JCDR*. 2013; 7(9): 1975. doi: 10.7860/JCDR/2013/6544.3376.
12. Kazemnia A, Ahmadi M, Dilmaghani M. *Antibiotic resistance pattern of different Escherichia coli phylogenetic groups isolated from human urinary tract infection and avian colibacillosis*. *Iranian Biomedical Journal*. 2014; 18(4): 219-24.
13. Cockerill FR, Clinical, Institute LS. *Performance standards for antimicrobial susceptibility testing: twenty-second informational supplement*. National committee for clinical laboratory standards; 2012.
14. Kim J, Jeon S, Rhie H, Lee B, Park M, Lee H, et al. *Rapid detection of extended spectrum β -lactamase (ESBL) for Enterobacteriaceae by use of a multiplex PCR-based method*. *Infection and Chemotherapy*. 2009; 41(3):181-4. DOI: 10.3947/ic.2009.41.3.181.

15. Sidjabat HE, Paterson DL, Adams-Haduch JM, Ewan L, Pasculle AW, Muto CA, et al. *Molecular epidemiology of CTX-M-producing Escherichia coli isolates at a tertiary medical center in western Pennsylvania*. Antimicrob Agents Chemother. 2009; 53(11): 4733-9. doi: 10.1128/AAC.00533-09.
16. Bhattacharjee A, Sen MR, Prakash P, Anupurba S. *Role of β -lactamase inhibitors in enterobacterial isolates producing extended-spectrum β -lactamases*. J Antimicrob Chemother. 2008; 61(2): 309-14. doi: 10.1093/jac/dkm494.
17. Rekha R, Alam Rizvi M, Jaishree P. *Designing and validation of genus-specific primers for human gut flora study*. Electronic Journal of Biotechnology. 2006;9(5):0-.
18. Bokaeian M, Zahedani SS, Bajgiran MS, Moghaddam AA. *Frequency of PER, VEB, SHV, TEM and CTX-M genes in resistant strains of Pseudomonas aeruginosa producing extended spectrum β -lactamases*. Jundishapur Journal of Microbiology. 2015; 8(1): e13783. doi: 10.5812/jjm.13783.
19. Mendonça N, Leitão J, Manageiro V, Ferreira E, Caniça M. *Spread of extended-spectrum β -lactamase CTX-M-producing Escherichia coli clinical isolates in community and nosocomial environments in Portugal*. Antimicrobial Agents and Chemotherapy. 2007; 51(6): 1946-55. doi: 10.1128/AAC.01412-06.
20. Fatemi S-M, Doosti A, Tavakoli H, Moayednia R, Ghasemi-Dehkordi P, Kelidari B, et al. *Antibiotic susceptibility patterns of isolated bacteria from bile fluids of patients with gallstone disease in Isfahan city (Iran)*. Archives of Biological Sciences. 2015; 67(2): 611-7. DOI: 10.2298/ABS140506021F.
21. Etok CA, Edem EN, Ochang E. *Aetiology and antimicrobial studies of surgical wound infections in university of Uyo teaching hospital (UUTH), Uyo, Akwa Ibom state, Nigeria*. Open Access Scientific Reports. 2012; 1: 341. doi:10.4172/scientificreports.341.
22. Islam MS, Yusuf MA, Islam MB, Jahan WA. *Frequency of ESBL in Surgical Site Infection at a Tertiary Care Hospital*. Journal of current and advance medical research. 2014; 1(2): 25-9.
23. Rezaei MS, Salehifar E, Rafiei A, Langaee T, Rafati M, Shafahi K, et al. *Characterization of multidrug resistant extended-spectrum beta-lactamase-producing Escherichia coli among uropathogens of pediatrics in north of Iran*. Biomed Res Int. 2015; 2015: 309478. doi: 10.1155/2015/309478.
24. Memariani M, Peerayeh SN, Salehi TZ, Mostafavi SKS. *Occurrence of SHV, TEM and CTX-M β -Lactamase genes among enteropathogenic Escherichia coli strains isolated from children with diarrhea*. Jundishapur J Microbiol. 2015; 8(4): e15620. doi: 10.5812/jjm.8(4)2015.15620.
25. Varkey DR, Balaji V, Abraham J. *Molecular characterisation of extended spectrum beta lactamase producing strains from blood sample*. International Journal of Pharmacy and Pharmaceutical Sciences. 2014; 6(3): 276-8. doi: 10.1186/s12941-015-0098-9.
26. Rodríguez-Baño J, Alcalá J, Cisneros JM, Grill F, Oliver A, Horcajada JP, et al. *Escherichia coli producing SHV-type extended-spectrum β -lactamase is a significant cause of community-acquired infection*. J Antimicrob Chemother. 2009; 63(4): 781-4. doi: 10.1093/jac/dkp028.
27. Fang H, Ataker F, Hedin G, Dornbusch K. *Molecular epidemiology of extended-spectrum β -lactamases among Escherichia coli isolates collected in a Swedish hospital and its associated health care facilities from 2001 to 2006*. Journal of Clinical Microbiology. 2008; 46(2): 707-12. doi: 10.1128/JCM.01943-07.