Journal of Coastal Life Medicine

journal homepage: www.jclmm.com

Original artical doi: 10.12980/jclm.4.2016J6-38

Prevalence of ESBL phenotype, *bla*$_{CTX-M-1}$, *bla*$_{SHV}$ and *bla*$_{TEM}$ genes among uropathogenic *Escherichia coli* isolates from 3 military hospitals of Tehran, Iran

Farshad Nojoomi, Abdolmajid Ghasemian*

Microbiology Department, Faculty of Medicine, AJA University of Medical Sciences, Tehran, Iran

ARTICLE INFO

Article history:
Received 15 Feb 2016
Accepted 24 Apr 2016
Available online 8 Jul 2016

Keywords:
Uropathogenic *Escherichia coli*
ESBL
Military hospitals
Hospitalized patients
Combine disk

ABSTRACT

Objective: To determine the extended-spectrum beta-lactamase (ESBL) production and prevalence of *bla*$_{CTX-M-1}$, *bla*$_{SHV}$ and *bla*$_{TEM}$ genes among uropathogenic *Escherichia coli* (UPEC) isolates from 3 military hospitals of Tehran during 2015–2016.

Methods: One-hundred and eleven isolates were adopted. The antibiotic susceptibility testing was conducted according to Clinical and Laboratory Standards Institute guidelines. The combine disk was used for phenotypic ESBL production. The ceftazidime MIC was conducted with the micro-broth dilution test. The PCR assay was used to detect the *bla*$_{CTX-M-1}$, *bla*$_{SHV}$ and *bla*$_{TEM}$ genes.

Results: In the broth microdilution method, 103 (92.7%) isolates showed minimal inhibitory concentration (MIC) ⩾ 1 μg/mL, and also in the combined disk method, 89 (80.1% of all) were ESBL positive. On the other hand, among 91 ceftazidime resistant isolates, 86 (77.4% of all) were ESBL positive. The difference between the two methods for ESBL confirmation was not significant. The result of MIC was similar to the disk diffusion method in the detection of phenotypic ESBL production. Among ESBL producer isolates, 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), respectively. These genes were amplified in a wide range MIC of ceftazidime.

Conclusions: The prevalence of multi-drug resistant UPEC and ESBL positive isolates was high in military hospitals. The majority of UPEC isolates amplified *bla*$_{CTX-M-I}$ and *bla*$_{SHV}$ type β-lactamase genes. One-third of isolates were positive in presence of both these genes. There was no relation between ceftazidime MIC and presence of beta-lactamase genes.

1. Introduction

Uropathogenic *Escherichia coli* (UPEC) isolates can persist in urothelial cells and cause recurrent infections. Furthermore, multi-drug resistant isolates carry plasmids (Inc FII/IncI1, *etc.*) that confer the resistance to multiple classes of antibiotics in addition to cephalosporins. The genetic location of extended-spectrum beta-lactamases (ESBLs) include the mobile elements and chromosome of Enterobacteriaceae[1]. Recent data have shown that *bla*$_{CTX-M-1}$ clones are mostly widespread at an endemic status worldwide similar to results from Iran[2]. The frequency of ESBLs is increasing everywhere[3]. These ESBLs are inhibited by clavulanic acid, sulbactam, and tazobactam. This phenomena can help to detect these β-lactamases in the phenotypic confirmatory test[4]. On the other hand, ESBLs are often associated with resistance to other antibiotics, including fluoroquinolones, aminoglycosides and sulfamethoxazole/trimethoprim[5]. The pandemic *Escherichia coli* ST131 clone encoding CTX-M-15 with a high virulent potential was characterized by a multidrug resistance result and co-production of *OXA-1* or *TEM-1b* as well as *aac(6')-Ib-cr*. This clone produces *bla*$_{CTX-M-15}$ beta lactamase worldwide[6-8]. CTX-M-type ESBLs are a complex and heterogeneous family and may be subdivided into 5 major groups (CTX-M-1, 2, 8, 9 and CTX-M-25)[9,10]. These enzymes have spread worldwide and include most ESBLs detected in Enterobacteriaceae. They are not only found in hospitals, but also in the community settings, thus changing the epidemiology of ESBLs[11]. The *bla*$_{CTX-M}$ and *bla*$_{TEM}$ ESBLs can hydrolyze third and fourth generation cephalosporins. Several studies have demonstrated a relationship between ESBL enzymes and minimal inhibitory concentration (MIC) of 3rd and 4th generation cephalosporins, including ceftazidime, cefepime and cefotaxim[12]. The aim of this study was determination of ESBL positive UPEC strains and prevalence of *bla*$_{CTX-M}$, *bla*$_{SHV}$ and *bla*$_{TEM}$ types among ESBL positive UPEC strains among 3 military hospitals in Tehran.

*Corresponding author: Abdolmajid Ghasemian, Microbiology Department, Faculty of Medicine, AJA University of Medical Sciences, Tehran, Iran.
Tel: +98 9394514860
Fax: +98 82884555
E-mail: majidghasemian86@gmail.com

The study protocol was performed according to the AJA University declaration and approved by AJA University of Medical Sciences ethical Comittee. Informed written consent was obtained from the patients.

Foundation Project: Supported by AJA University of Medical Sciences of Iran (Grant No. 1649546/6743, 2015).

The journal implements double-blind peer review practiced by specially invited international editorial board members.

2. Materials and methods

2.1. Clinical isolates

One-hundred and eleven UPEC isolates were collected during 2015–2016, from three hospitals in Tehran. These isolates were obtained from different urine cultures of patients with ages ranging from 5 to 73 (mean = 46.0 ± 1.3) years old. Furthermore, seventy patients were female (mean age of 46.63) and 41 (mean age of 34.21) were male. The isolates were identified by both conventional biochemical and molecular tests advised for UPEC strains.

2.2. Susceptibility tests and phenotypic ESBL detection

Susceptibility testing was performed by the disk diffusion method following the guidelines of Clinical and Laboratory Standards Institute (CLSI). Seventeen antimicrobial disks were used including aztreonam (30 μg), piperacillin (100 μg), augmentin (30 μg), cefotaxime (30 μg), cefpodoxime (10 μg), ceftriaxone (30 μg), meropenem (10 μg), piperacillin-tazobactam (110 μg), imipenem (10 μg), ceftazidime (30 μg) and cefepime (30 μg), ofloxacin (5 μg), ciprofloxacin (5 μg), levofloxacin (5 μg), amikacin (30 μg) and tobramycin (10 μg), gentamicin (120 μg).

Escherichia coli ATCC 25922 was used for the quality control of susceptibility testing. The ESBL positive phenotype was detected by combined disk method using ceftazidime and cefotaxime disks with and without clavulanic acid. The MICs of 3rd generation cephalosporin resistant isolates were determined by broth micro dilution method using ceftazidime with range of 0.25–128.00 μg/mL (CLSI 2014). Each isolate with MIC ⩾ 1 μg/mL was further tested for ESBL production in addition to the results of disk diffusion.

2.3. PCR amplification of ESBL genes

The CTX-M, SHV and TEM type ESBLs were amplified with specific primers shown in Table 1. The PCR amplification of the genes was performed with 2 μL of template DNA that was added to a final 50 μL master mix containing: 50 mmol/L KCl, 0.1% Triton X-100, 10 mmol/L Tris-HCl (pH 9), 2 mmol/L $MgCl_2$, 200 μmol/L dNTPs, 1 μL of each primer, 1.25 IU of *Taq* DNA polymerase and 32.25 μL distilled water. For $bla_{CTX\text{-}M\text{-}I}$, 25 cycles with annealing temperature of 55 ℃ (1 min) was used. For bla_{SHV} and bla_{TEM} types, 25 cycles with annealing temperatures of 54 ℃ (30 s) and 56 ℃ (30 s) were used respectively. The primers of TEM included: F 5'-TCG GGG AAA TGT GCG CG-3' and R 5'-TGC TTA ATC AGT GAG GCA CC-3', amplifying a 971 bp sequence.

Table 1
The specific primers used in the present study.

Primer	Sequence (5' to 3')	Target(s)	
CTXM1-F3	GAC GAT GTC ACT GGC TGA GC	CTX-M group I	CTX-M-1, -3, -10 to -12, -15 (UOE-1), -22, -23, -28 to -30
CTXM1-R2	AGC CGC CGA CGC TAA TAC A		
TOHO1-2F	GCG ACC TGG TTA ACT ACA ATCC	CTX-M group II	CTX-M-2, -4 to -7, and -20 and Toho-1
TOHO1-1R	CGG TAG TAT TGC CCT TAA GCC		
CTXM825F	CGC TTT GCC ATG TGC AGC ACC	CTX-M group III	CTX-M-8 and -25
CTXM825R	GCT CAG TAC GAT CGA GCC		
CTXM914F	GCT GGA GAA AAG CAG CGG AG	CTX-M group IV	CTX-M-9, -13, -14, -16 to -19, -21, and -27 and Toho-2
CTXM914F	GTA AGC TGA CGC AAC GTC TG		

2.4. Statistical analysis

Comparisons of variants were conducted using the student unpaired *t*-test. A value of $P < 0.05$ was considered to be significant.

3. Results

3.1. The susceptibility testing and ESBL production

The antibiotic susceptibility testing of ESBL positive and negative UPEC isolates have been depicted in Table 2.

Table 2
The results of disk diffusion test compared between ESBL negative and positive isolates. %.

Antimicrobial	ESBL negative	ESBL positive
Aztreonam	31.2	97.3
Piperacillin	4.4	6.3
Augmentin	87.3	23.5
Cefotaxime	18.9	89.6
Cefpodoxime	36.3	91.4
Ceftriaxone	67.7	89.3
Meropenem	12.1	13.4
Piperacillin-tazobactam	4.6	5.4
Imipenem	11.3	13.5
Ceftazidime	23.1	82.0
Cefepime	27.2	87.6
Ofloxacin	31.1	78.2
Ciprofloxacin	78.2	77.7
Levofloxacin	32.4	73.3
Amikacin	5.3	54.8
Ttobramycin	9.9	57.0
Gentamicin	10.3	60.1

In the broth microdilution method, 103 (92.7%) isolates showed MIC ⩾ 1 μg/mL, and in the combined disk method, 89 (80.1% of all) were ESBL producer strains. On the other hand, among 91 ceftazidime resistant isolates, 86 (77.4% of all) were ESBL positive. The results of these two methods in the ESBL confirmation were similar. The result of MIC was approximately similar to the disk diffusion for isolates in phenotypic ESBL production test.

3.2 Genotypic detection of ESBLs

The prevalence of $bla_{CTX\text{-}M}$, bla_{SHV} and bla_{TEM} genes among ESBL producer strains was 77.4% (n = 86), 47.4% (n = 53) and 2.4% (n = 2) respectively. The $bla_{CTX\text{-}M}$ was related to higher MIC to ceftazidime. The relation between the MIC of isolates and presence of $bla_{CTX\text{-}M}$, bla_{SHV} and bla_{TEM} genes for 28 community isolates has been depicted in Table 3.

Table 3
The MIC and demographic data regarding 28 $bla_{CTX\text{-}M}$, bla_{SHV} and bla_{TEM} positive isolates.

Isolate	MIC (μg/mL)	ESBL DDST	Gender	Age	$bla_{CTX\text{-}M\text{-}1}$	bla_{TEM}	bla_{SHV}	Number of non-susceptible antibiotics
1	4	+	F	23	+		+	6
2	4	+	F	12	+		+	7
3	4	+	M	14	+		+	5
4	4	+	F	36	+		+	6
5	4	+	F	62	+		+	4
6	8	+	M	13	+			6
7	8	+	M	45	+		+	7
8	8	+	F	52	+	+		7
9	8	+	M	33	+		+	6
10	8	+	M	37	+			5
11	2	+	F	45	+		+	4
12	2	+	F	61	+		+	6
13	32	+	F	16	+		+	5
14	32	+	M	15	+		+	6
15	2	+	M	11	+			4

(*continued on next page*)

Table 3 (*continued*)

Isolate	MIC (μg/mL)	ESBL DDST	Gender	Age	$bla_{CTX-M-1}$	bla_{TEM}	bla_{SHV}	Number of non-susceptible antibiotics
16	16	+	F	17	+			5
17	16	+	F	72	+			5
18	16	+	F	61	+	+		7
19	16	+	M	22	+			5
20	32	+	M	21	+			6
21	64	+	M	46	+		+	8
22	64	+	F	39	+		+	8
23	64	+	M	28	+		+	9
24	128	+	F	18	+		+	10
25	128	+	M	28	+		+	11
26	128	+	M	57	+		+	11
27	128	+	M	47	+		+	12
28	128	+	M	59	+		+	12

DDST: Double disk susceptibility test/combine disk; M: Male; F: Female; +: Indicating a positive result.

4. Discussion

In the present study, the prevalence of ESBL positive strains and multi-drug resistant UPEC isolates was high among military hospitals of Tehran. Results of UPEC susceptibility to antibiotics demonstrated that imipenem (88.86%) and piperacillin (94.87%) were the most effective antibiotics among β-lactam groups. Furthermore, among non-β-lactam antibiotics, amikacin showed the highest activity against UPEC isolates (91.82%). Several previous studies, similar to the results of this study, have demonstrated high rate of ESBLs in Tehran and other cities. Recent data have uncovered the predominance of $bla_{CTX-M-1}$ among ESBL positive phenotype all over the world. In the current study, CTX-M-1 group accounted for 77.4% of ESBL producer strains. This was the first study on ESBL production and molecular detection of beta-lactamases among military hospitals of Tehran. The results of previous molecular studies similarly have shown that $bla_{CTX-M-1}$ was endemic and was present among ST131 clone in hospitals and community settings[13-16]. The $bla_{CTX-M-I}$ was detected in the range of 2-128 μg/mL ceftazidime MIC, mostly because of universal primer that was used in the current study, detecting $bla_{CTX-M1-30}$. It has been revealed that $bla_{CTX-M-1/14}$ and $bla_{CTX-M-15}$ were responsible for high level of resistance to cefepime/ceftriaxone and ceftazidime respectively[12]. However, despite most of these studies we could detect a lower frequency of ESBL encoding genes, suggesting that other beta-lactamase classes are participating in 3rd generation cephalosporins resistance. Our data complemented the study by Mohajeri from west of Iran, in which 93.3%, 68.3% and 43.2% of UPEC were $bla_{CTX-M-1}$, bla_{SHV} and bla_{TEM} positive[17].

The prevalence of multi-drug resistant UPEC and ESBL positive isolates were high. The majority of UPEC isolates amplified $bla_{CTX-M-1}$ and bla_{SHV} type β-lactamases. One-third of isolates were positive for both of the two genes. There was no relation between MIC of ceftazidime and presence of β-lactamase genes.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

The authors acknowledge the AJA University of Medical Sciences of Iran for providing the funds (Grant No. 1649546/6743, 2015) of this study.

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