



Association between ESBLs Genes and Quinolone Resistance in Uropathogenic *Escherichia coli* Isolated from Patients with Urinary Tract Infection

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ABSTRACT

Aims: Urinary tract infection (UTI) is one of the most common infections worldwide. The aim of this study was to investigate the association between the ESBLs genes and quinolone resistance in uropathogenic *Escherichia coli* strains isolated from patients with urinary tract infection.

Materials & Methods: A total of 150 *E. coli* isolates were collected from patients with urinary tract infection, referring to Firouzgar hospital in Tehran, Iran. Antimicrobial susceptibility of isolates was determined by disk diffusion method. Double-disk diffusion test was performed for phenotypic identification of extended-spectrum β -lactamase (ESBL)-producing isolates. PCR was used for the detection of ESBL-encoding and quinolone (qnr) resistance genes.

Findings: There was a high resistance rate to most of the studied antimicrobial agents. Phenotypically, 75% of the isolates produced an ESBL enzyme and were resistant to different antimicrobial classes. Overall, 83% of the isolates carried ESBL genes, especially *bla*_{TEM} and *bla*_{CTX-M}. Also, 75% of the isolates were positive for the presence of quinolone resistance genes, including *qnrA*, *qnrB*, *qnrS*, and *qepA*. The present study results indicated the association between the presence of various ESBLs genes and quinolone resistance in uropathogenic *E. coli* strains.

Conclusion: Resistance patterns showed an increase in the incidence of antibacterial resistance in *E. coli* strains. The current study results indicated the high prevalence rate of ESBL-producing isolates and quinolone resistance genes. Simultaneous presence of genes responsible for antibacterial resistance has made the treatment of UTI more challenging than before.

Keywords: Antimicrobial resistance; ESBL; Uropathogenic *Escherichia coli*; Quinolones

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[1] Acinetobacter baumannii: Evolution of a global pathogen... [2] Dunitz M. Urinary Tract Infection in the Female... [3] Martinez-Martinez L, et al. Prevalence of... [4] Plasmid-mediated quinolone resistance: An update. J Infect Chemother... [5] Plasmid-mediated quinolone... [6] Jiang Y, Zhou Z, Qian Y, Wei Z, Yu Y, Hu S, et al... [7] Cattoir V, Poirel L, Rotimi V, Soussy C-J, Nordmann P. Multiplex PCR for detection of plasmid-mediated... [8] Shams E, Firoozeh F, Moniri R, Zibaei M. Prevalence of... [9] Raei F, Eftekhari F, Feizabadi M. Prevalence of ... [10] Arsalane L, Zerouali K, Katfy K, Zouhair S. Molecular... [11] BMJ Group. Risks of extended-spectrum ... [12] Reller LB, Weinstein M, Jorgensen JH, Ferraro MJ... [13] Klumpp DJ, Rycyk MT, Chen MC, Thumbikat P, Sengupta S, S... [14] Heydari Chaleshtori M, Tajbakhsh E, Arbab Soleymani NJP... [15] Lavilla S, Gonzalez-Lopez J, Sabate M, Garcia-Fernandez... [16] Strahilevitz J, Jacoby GA, Hooper DC, Robicsek A. . [17] Cao X, Cavaco LM, Lv Y, Li Y, Zheng... [18] Colodner R, Kometiani I, Chazan B, Raz R. Risk factors... [19] Molazade A, Shahi A, Najafipour S, Mobasheri F, Norouzi F, Abdollahi Kheirabadi S, et al... [20] Fesharakinia A, Malekaneh M, Hooshyar H, Aval M, Gandomy-Sany... [21] Wintermans B, Reuland E, Wintermans R, Bergmans A... [22] Paterson DL, Bonomo RA... [23] Randegger CC, Hächler H. Amino acid substitutions... [24] Pérez-Llarena FJ, Kerff F, Abián O, Mallo S, Fernández MC, Galleni M, et al. Distant and... [25] Paltansing S, Kraakman M, Ras J, Wessels E, Bernards A. Characterization of fluoroquinolone and ... [26] Hashim RB, Husin S, Rahman MM. Detection... [27] Pereira A, Santos A, Tacão M, Alves A, Henriques I, Correia A. Genetic diversity and... [28] Sedighi I, Arabestani MR, Rahimbakhsh A, Karimitabar Z, Alikhani... [29] Mirzaee M, Owlia P, Mansouri S. Distribution of CTX-M β -lactamase genes ... [30] Yamane K, Wachino J-i, Suzuki S, Kimura K, Shibata N, Kato H, et al... [31] Périchon B, Courvalin P, Galimand MJ... [32] Hassan W, Hashim A, Domany RA... [33] Goudarzi M, Azad M, Seyedjavadi SS. Prevalence... [34] Alheib O, Al Kayali R, Abajy MY. Prevalence of... [35] Bouchakour M, Zerouali K, Claude JD, Amarouch H, El Mdaghri N, Courvalin P, et al. Plasmid-mediated.

Introduction

Urinary tract infection (UTI) is considered as one of the most common infections worldwide. *Escherichia coli* accounts for more than 80% of UTIs and is therefore considered as the most common cause of this infection [1]. Routine antibacterial treatment of UTI could reduce the mortality rate of these infections [2]. The overuse and misuse of antibiotics are considered as a contributing factor to the increase in the prevalence of uropathogenic strains such as multidrug resistant extended-spectrum β -lactamase (ESBL)-producing UPEC strains, limiting the antibacterial treatment of UTI. In recent years, fluoroquinolones have been commonly used as a suitable medication for the treatment of UTIs, but their widespread use has led to antibacterial resistance in bacteria. Quinolone resistance in family *Enterobacteriaceae* is mostly due to the mutations in topoisomerase-encoding chromosomal genes [3]. However, recent studies have suggested the mediation of plasmid genes in quinolone resistance [4]. Plasmid-mediated quinolone resistance (PMQR) was initially detected in *Klebsiella pneumoniae* isolates in the United States [5]. Two groups of PMQR genes were detected, including *qepA* and *qnr*; each group harbored subgroup genes. *qnr* proteins (*qnrA*, *qnrB*, *qnrS*, *qnrD*, and *qnrC*) belong to the pentapeptide repeat protein (PRP) family with the ability to inhibit the effect of quinolones on DNA gyrase and DNA topoisomerase IV enzymes [5]. Three types of *qnr* genes have been detected in family *Enterobacteriaceae*, including *qnrS*, *qnrB*, and *qnrA* [5]. *qepA* is also a proton-dependent transmitter gene with the ability to increase the MIC of hydrophilic fluoroquinolones (ciprofloxacin and norfloxacin) for up to 32 or 64 times [6]. Studies have shown a strong positive correlation between the presence of *qnr* genes and ESBL-producing isolates [7]. In fact, PMQR genes are prevalently found in

ESBL-producing isolates worldwide [8]. It has been also reported that *qnr* plasmids harbor a variety of beta-lactamase genes. Since many antibacterial resistance genes are carried by plasmids, ESBL-producing isolates show simultaneous resistance to various antibiotics [9]. The emergence of fluoroquinolone resistance has hampered the effective treatment of UTIs, subsequently leading to the increased duration of hospitalization and mortality rate of patients with UTIs. Rapid identification of ESBL-producing isolates is of great concern for infection control and antibiotics proper choice [10]. Hence, the identification of beta-lactamase enzymes are necessary for the prevention from the increase in antibacterial resistance rate and for the proper use of antibiotics [11].

Objective: This study aimed to investigate the prevalence of beta-lactamase-encoding (*TEM*, *SHV*, *CTX-M*) and quinolone resistance-encoding (*qepA*, *qnrS*, *qnrB*, *qnrA*) genes in beta-lactamase-producing UPEC strains isolated from patients with UTIs.

Materials and Methods

Collection and identification of isolates:

In this cross-sectional study, 150 urine samples were collected from patients with asymptomatic UTI, referring to Firouzgar hospital, Tehran, Iran from February 2018 to May 2018. Mid-stream urine samples were collected from patients who did not take any antibiotics 30 days prior to sampling. Urine samples were analyzed in lab, and patients were diagnosed with UTI after the detection of *E. coli* strains. Biochemical tests were performed for the isolates to confirm the identity of UPEC isolates.

Determination of antibiotic resistance:

Susceptibility testing was performed for ceftazidime (30 μ g), cefotaxime (30 μ g), cefixime (5 μ g), ciprofloxacin (5 μ g), ceftriaxone (30 μ g), norfloxacin (10 μ g), and nalidixic acid (30 μ g) (Mast Co., UK) using

disk diffusion method. For each test, *E. coli* ATCC 25922 was used as a control.

Determination of ESBL-producing isolates: In order to identify ESBL-producing isolates, screening tests were initially performed using cefotaxime and ceftazidime disks according to the CLSI (2018) guideline [12]. Then confirmation tests were performed using double-disk diffusion method. *E. coli* ATCC 35218 was used as a quality control for ESBL-producing isolates.

Primer design, DNA extraction, and PCR: Genomic DNA was extracted using DNA extraction kit (Roche, Germany). The presence of *TEM*, *SHV*, *CTX-M*, *qnrA*, *qnrB*, *qnrS*, and *qepA* genes was determined by PCR test using specific primers designed in this study. PCR reaction was performed on a final volume of 25 µl consisting of 12.5 µl mastermix (Amplicon, USA), 2 µl of each primer (Forward and Reverse), 2 µl bacterial DNA, and 6.5 µl sterile distilled water. The program consisted of an initial denaturation step at 95°C for 5 min, followed by 35 cycles of 94°C for 1 min, annealing

step (annealing T_m for each primer is shown in Table1) for 30 sec, extension at 72°C for 1 min, and a final extension at 72°C for 5 min. PCR products were then analyzed on 1% agarose gel after electrophoresis.

Statistical analysis: Data were analyzed by Chi-square tests using SPSS software ver.20. P value<0.05 was considered as significant.

Findings

Susceptibility testing: UPEC isolates showed the highest resistance rate to cefotaxime (94%) and the maximum sensitivity to norfloxacin (63.5%). The prevalence of resistance to ceftazidime, nalidixic acid, cefexime, ceftriaxone, and ciprofloxacin was 88%, 86.5%, 84%, 83%, and 68.75%, respectively.

ESBL production: The results of Double Disk Synergy Test (DDST) showed that 75% (105 of 150) of the isolates were ESBL producers. The results of disk diffusion method showed that quinolone resistance rate was higher in ESBL-producing isolates (Table 2). The statistically significant

Table1) Characteristics of primers used in this study for the detection of resistance genes in UPEC isolates.

Target	Primer sequence (5' to 3')	Size	Annealing temperature (°C)
<i>CTX-M</i>	F: AAGCACGTCAATGGGACGAT R: CCTTAGGTTGAGGCTGGGTG	478 bp	55
<i>SHV</i>	F: ATCTCCCTGTTAGCCACCCT R: CAGCTGGCTTTCGCTTTGTT	84 bp	56
<i>TEM</i>	F: GGGAACCGGAGCTGAATGAA R: CAGTGCTGCAATGATACCGC	254 bp	55
<i>qnrA</i>	F: GAGCAATGCCAATCTCAGCG R: CAGATCGCAAAGGTCAGGT	121 bp	57
<i>qnrB</i>	F: TTTTCGCGGCGCAAGTTTAA R: TACCCATCCAGCGGTTTCC	146 bp	55
<i>qnrS</i>	F: GGTGCCAACTTTTCCCGAAC R: CGGGGATCTAAACCGTTCGAG	284 bp	56
<i>qepA</i>	F: CGTTATTCGCATTTCGCCTCG R: GATCACGATACTCGGGCAGG	327 bp	55

F: Forward; R: Reverse

difference between the ESBL production and antibiotic resistance is illustrated in Table 2.

Prevalence of resistance genes by PCR method: In UPEC isolates, the frequency of *TEM*, *SHV*, and *CTX* genes was 83.3%, 12.5%, and 79.12%, respectively. Moreover, 83% of the isolates harbored at least one of the above mentioned resistance genes. In addition, 75% (113 of 150) of the isolates carried *qnr* gene; among which 44.66%, 20.3%, and 16.25% of the isolates carried *qnrA*, *qnrB*, and *qnrS* genes, respectively. Interestingly, only 2.6% of the isolates carried *qepA* gene.

Prevalence of beta-lactamase and quinolone-resistance genes in ESBL- and non ESBL-producing isolates: A statistically significant correlation was found between the ESBL production and the presence of *qnrA* and *CTX-M* resistance genes in ESBL- and non ESBL-producing isolates (p value<0.05) (Table3). The prevalence of quinolone resistance genes in ESBL- and non ESBL-producing isolates is presented in Table 4.

Association between the presence of antibiotic resistance genes and antibiotic resistance profile in UPEC isolates: After evaluating the distribution of antibiotic resistance genes among UPEC isolates, a significant association was found between the ceftriaxone resistance and the presence of *SHV* gene. A correlation was also found between the resistance to cefixime, ceftazidime, ceftriaxone, and cefotaxime and the presence of *CTX-M* gene. There was also a significant association between the resistance to cefotaxime, nalidixic acid, and ciprofloxacin and the presence of *qnrB* gene. Finally, resistance to norfloxacin, ciprofloxacin, cefixime, ceftazidime, and cefotaxime was found to be significantly associated with the presence of *qnrS* gene.

Association between the *qnr* genes and ESBL production: There was a significant association between the presence of *TEM*, *qnrB*, and *qnrS* genes. There was also a positive correlation between the presence of *SHV* and *qnrB* genes. Also, a significant

Table2) Antibacterial resistance in ESBL- and non ESBL-producing isolates.

Antimicrobial agents	Resistant rate, % (No. of resistant strains)			p-value
	ESBL+, n=105	ESBL-, n=15	Total, n=150	
Nalidixic acid	74.28 (78)	46.67 (7)	56.6 (85)	<0.001
Ciprofloxacin	62.85 (66)	26.66 (4)	46.6 (70)	0.006
Norfloxacin	30.47 (32)	20 (3)	23.3 (35)	NS
Cefixime	83.81(88)	6.67 (1)	59.3(89)	<0.001
Ceftriaxone	85.71(90)	0(0)	60(90)	<0.001
Ceftazidime	90.48(95)	0(0)	63.3(95)	<0.001
Cefotaxime	96.19(101)	0(0)	67.3(101)	<0.001

ESBL, extended spectrum β -lactamase; NS, not significant

association was found between the presence of *CTM-M*, *qnrA*, and *qnrB* genes.

Discussion

Urinary tract infection (UTI) is a common hospital- and community-acquired infection with high mortality rate and high medical expenses [13]. Epidemiological studies have shown a high prevalence of UTI caused by UPEC, isolated from 80% of all infections [14]. Although quinolones are commonly prescribed for the treatment of UTIs caused by *E. coli* and other *Enterobacteriaceae*, antibacterial resistance has become a challenge in the treatment of these infections [15,16]. In the current study, the prevalence of isolates resistant to nalidixic acid, ciprofloxacin, and norfloxacin was 56.6%, 46.6%, and 23.3%, respectively. In a study by Cao *et al.* (2011) in China, ciprofloxacin resistance rate was reported as 75% in *E.coli* isolates [17]. In another study by Colonder *et al.* (2008), ciprofloxacin resistance rate was reported as 50% [18]. These results are in accordance with the present study results.

Moreover, the prevalence rate of resistance to other antibiotics including cefotaxime, ceftazidime, ceftriaxone, and cefixime was 67.3%, 63.3%, 60%, and 59.3%, respectively. Babaei *et al.* (2012) reported that the prevalence of resistance to ceftriaxone, cefixime, and ceftazidime was 100%, 93.33%, and 88.33%, respectively. They showed an increase in the incidence of resistance to third-generation cephalosporins, including cefixime, ceftriaxone, and ceftazidime [19,20]. The current study also indicated a high resistance rate to cephalosporins. This finding may be due to the increase in the prevalence of ESBL production.

Currently, ESBL-producing UPEC isolates are a challenge worldwide. In the current study, 70% (105 of 150) of the isolates were ESBL-producers. PCR results indicated that among the all 150 isolates, the prevalence

of *CTX-M*, *TEM*, and *SHV* genes was 79.12%, 83.3%, and 12.5%, respectively. In addition, among the 105 ESBL-producing isolates, 17(16%) isolates carried *CTX-M*, *TEM*, and *SHV* genes, simultaneously. Furthermore, among the ESBL-producers, 49 (52.1%) isolates carried *CTX-M* and *TEM* genes, 8 (8.5%) isolates carried *TEM* and *SHV* genes, and 11(11.7%) isolates carried *SHV* and *CTX-M* genes. Furthermore, 4 (2.6%) isolates carried *qepA* gene. It was found that there was a significant association between the ESBL production and the presence of *CTX-M* gene ($p < 0.05$).

The results of molecular and phenotypical tests performed for ESBL identification showed that among the 105 ESBL-producing isolates detected by phenotypical tests, 15 isolates lacked *CTX-M*, *TEM*, and *SHV* genes. This finding could be explained by other resistance mechanisms such as the presence of other beta-lactamase genes whose activity can be inhibited by clavulanic acid [21]. In addition, 45 isolates were non-ESBL producers; among which 41 cases carried at least one of the *CTX-M*, *TEM*, and *SHV* genes. One explanation for this phenomenon could be the presence of *ampC* beta-lactamase gene causing tolerance to the inhibitory effect of clavulanic acid. The other reason may be the occurrence of mutations in the aforementioned genes [22, 23, 24].

Paltancing *et al.* (2012) studied 49 *E. coli* isolates; among which 29 (59.2%) cases were ESBL-producers [25]. Yazdi *et al.* (2012) showed that among the 109 ESBL-producers, *SHV*, *CTX-M*, and *TEM* genes were present in 70.6%, 68.8%, and 87.1% of the isolates, respectively [26]. In the current study, *TEM* and *CTX* genes were the most prevalent genes in UPEC isolates. The results of various studies indicated the important role of these genes in antibacterial resistance [27]. In Sedighi *et al.*'s (2015) study, the prevalence rate of ESBL-producing isolates was

Table 3) Prevalence of beta-lactamase genes and quinolone resistance genes in ESBL- and non ESBL-producing isolates.

Gene	TEM	SHV	CTX-M	qnrA	qnrB	qnrS	qepA
ESBL +	99 (66%)	17(11.33%)	113 (75.33%)	55 (37%)	21 (14%)	25 (17%)	1(0.66%)
ESBL -	26 (17.33%)	2 (1.33%)	6 (4%)	12(8%)	3(2%)	5 (3.33%)	1(0.66%)

Table 4) Prevalence of quinolone resistance in ESBL- and non ESBL-producing isolates

Isolate	Quinolones					
	Nalidixic acid		Norfloxacin		Ciprofloxacin	
	R	S	R	S	R	S
ESBL +	110 (73.5%)	15 (10%)	40 (27%)	78 (52%)	95 (64%)	30 (20%)
ESBL -	20 (13%)	5 (3.33%)	15 (10%)	17 (11.33%)	8 (5%)	17 (11%)

R: Resistant; S: Sensitive

reported as 27.3%. In their study, *CTX-M* was the most common gene with the prevalence of 66.7%. In addition, the prevalence rate of *TEM* and *SHV* genes was 40.8% and 20.8%, respectively [28]. In another study in 2009 conducted on outpatients and inpatients in Tehran, Iran, 37.8% of ESBL-producing isolates carried *CTX-M* gene [29]. The present and previous studies results showed an increasing trend in the prevalence of ESBL-producing isolates and *CTX-M* beta-lactamase gene. Paterson *et al.* also found a significant correlation between the fluoroquinolone resistance and ESBL production [29]. In the current study, among the 120 UPEC isolates, 29 isolates were simultaneously resistant to nalidixic acid, norfloxacin, and ciprofloxacin; among which 27 isolates were ESBL-producers. Statistical analysis showed a significant association between the ESBL production and resistance to nalidixic acid and ciprofloxacin. In addition, there was a significant association between the ESBL production and the presence of *qnrA* gene.

Overall, antibacterial resistance pattern among

the UPEC isolates indicated a statistically significant association between the ESBL production and resistance to ceftazidime, ceftriaxone, cefixime, nalidixic acid, cefepime, and ciprofloxacin.

The current study showed a high prevalence rate of quinolone resistance (60%, 72 of 120) in UPEC isolates; among which 45.8%, 10.8%, and 29.1% carried *qnrA*, *qnrB*, and *qnrS* genes, respectively. Sedighi *et al.* (2014) indicated that the prevalence of *qnrB* and *qnrS* genes in 150 *E. coli* isolates was 6.6% and 5%, respectively. In their study, *qepA* was present in 2.6% of the isolates. In 2007, *qepA* efflux pump was initially found in two *E. coli* isolates in Japan and Belgium [30, 31]. A new variant called QepA2 was also found in France [31]. Therefore, this gene has not been widely distributed, and most studies have reported the low prevalence of this gene [32, 33, 34].

In a study by Bouchakour *et al.* (2010), the prevalence rate of *qnr* gene was reported to be 37.8% among the 83 ESBL-producing *E. coli* strains [35]. This prevalence rate was lower than that reported in the current study.

The present study results showed a high prevalence of PMQR genes in ESBL-producing *E. coli* isolates in Tehran, Iran. Although the presence of *qepA* gene has been rarely reported in Iran, this gene was detected in the isolates under study. There was a positive correlation between the resistance to beta-lactams and quinolones, suggesting the transfer of quinolone resistance genes along with plasmids carrying ESBL genes. Identification of UPEC isolates with ESBL genes indicated the emergence of high resistance to quinolones.

Conclusion

Resistance patterns showed an increase in the prevalence of antibacterial resistance in *E. coli* strains. The current study results indicated a high prevalence of ESBL-producing isolates and quinolone resistance genes. Moreover, a high prevalence of quinolone resistance genes and a low prevalence of *qepA* gene were indicated in this study. Simultaneous presence of genes responsible for antibacterial resistance has made UTI treatment more challenging than before.

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Conflict of Interests: The authors declare that they have no conflict of interest.

Ethical Permissions: No animal or human model was used in this study. All isolates were collected from the Microbiology bank of the Department of Microbiology, Iran University of Medical Sciences.

Authors' Contribution: Shivae A. (First author), Statistical analyst / Original researcher / Discussion author (20%); Mirshekar M. (Second author), Original researcher / Methodologist / Discussion author (20%); Mohammadzadeh R. (Third author), Introduction author / Methodologist / Original researcher / Discussion author (20%); Shahbazi Sh. (Fourth author), Statistical analyst / Discussion author (40%).

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