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Comparison of quinolone and β-lactam resistance among *Escherichia coli* strains isolated from urinary tract infections

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SUMMARY

The growing frequency of antibiotic resistances is now a universal problem. Increasing resistance to new generations of β-lactam and quinolone antibiotics in multidrug-resistant Enterobacteriaceae isolates is considered an emergency health issue worldwide. The aim of this study was to evaluate plasmid-mediated quinolone resistance genes in ESBL-producing *Escherichia coli* isolated from urinary tract infections (UTIs). In our study ESBL-producing isolates were assessed by screening methods. After determination of antimicrobial susceptibility, detection of ESBLs and quinolone resistance genes was performed. A total of 97 ESBL-producing *E. coli* were determined. The bla_{TEM}, bla_{SHV} and bla_{CTX-M} genes were detected in 90 isolates. The bla_{TEM} was the most frequently detected gene (46.4%), followed by bla_{SHV} (31.9%) and bla_{CTX-M} (14.4%). The most prevalent quinolone resistance gene among ESBL-producing isolates was *oqxAB* which found in 67 isolates (69.1%). The frequencies of the *aac(6')-Ib-cr*, *qnr* and *qepA* were 65 (67%), 8 (8.2%) and 6 (6.2%), respectively. Our data indicate that the prevalence of plasmid-mediated quinolone resistance genes in ESBL-positive isolates is increasing. The co-dissemination of PMQR and ESBL genes among *E. coli* isolates can be considered a threat to public health. Therefore, prescription of antibiotics against infectious disease should be managed carefully.

Keywords: quinolone, ESBL, *E. coli*, UTI, Iran.

INTRODUCTION

The growing frequency of antibiotic resistances is now a universal problem [1]. Antimicrobial resistance occurs through various mechanisms such as biofilm formation, enzyme modification and efflux pump [2-5]. Increasing resistance to new generations of β-lactam and quinolone antibiotics in multidrug-resistant Enterobacteriaceae isolates has been considered as an emergency health issues worldwide [6, 7]. It is believed that plasmids act as efficient transporters for the spread of antibiotic resistance genes [8, 9].

In the recent decades, due to widespread prescribing of fluoroquinolones, resistance of the Enterobacteriaceae to these agents has become prevalent. The emergence of plasmid-mediated quinolone resistance (PMQR) first was discovered in a multi resistant urinary *Klebsiella pneumoniae* isolate, which demonstrated that quinolone resistance can also be acquired through horizontal

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gene transmission. The responsible quinolone resistance gene was named *qnr* [10, 11]. Two additional mechanisms of PMQR have been described including drug modification by acetyltransferase *AAC(6')-Ib-cr* and active efflux by *QepA* and *OqxAB* [12].

Escherichia coli is a major cause of nosocomial infections and represent a serious public health burden [13]. Treatment of *E. coli* infections has been progressively complicated by the emergence of co-resistance to β-lactams and fluoroquinolones [14]. PMQR have been recognized worldwide with a quite high prevalence among extended spectrum β-lactamases (ESBLs) producing *E. coli* but there is limited comparative information regarding quinolone and β-lactam co-resistance in Iran [15].

In the present study, we aimed to assess the incidence of quinolone resistance genes in ESBL-producing *E. coli* isolated from patients with urinary tract infections (UTIs).

MATERIALS AND METHODS

Bacterial isolates

In this cross-sectional study, from January 2013 to January 2014, 1896 midstream urine specimens were collected from inpatients and outpatients, suspected of having a bacterial urinary-tract infection (UTI), who had not received antibiotic therapy 5-7 days before sampling and referred to hospitals of Sari, Iran. Primary isolation of uropathogens was performed by a streak plate technique on MacConkey agar (Merck Co., Germany) and incubation for 24 hours at 37°C. In order to identify *E. coli*, biochemical tests (oxidase, citrate, fermentation of glucose, lactose, motility, urease, gas and SH2 production) were performed. All identified isolates were stored at -80°C in Luria–Bertani broth (Merck Co., Germany) with 20% glycerol.

Antimicrobial susceptibility testing

Antibiotic susceptibility testing was carried out by disc diffusion method on Mueller Hinton Agar (Merck Co., Germany) according to the Clinical Laboratory Standards Institute (CLSI) guidelines 2015 [16]. The tested antibiotics (Mast Group Ltd., Merseyside, UK.) were gentamicin (10 µg), amikacin (30 µg), tobramycin (10 µg), ceftriaxone (30 µg) piperacillin/tazobactam (100/10 µg), nitrofurantoin (300 µg), cotrimoxazole (1.25/23.75), chloramphenicol (30 µg), doxycycline (30 µg), meropenem (10 µg) and ciprofloxacin (5 µg).

ESBL-producing isolates were phenotypically screened by double-disk synergy test using cefotaxime (30 µg) and ceftazidime (30 µg) (Mast Co., UK) in the presence and absence of clavulanic acid (10 µg). The *E. coli* ATCC 25922 was used as the standard strain.

DNA extraction & gene detection

The colonies of isolates were suspended in TE buffer and their DNA were extracted by boiling method. To detection of ESBL responsible genes (bla_{SHV}, bla_{CTX-M} and bla_{TEM}), PCR was performed as described previously [17].

The ESBL-producing isolates were investigated for the presence of *oqxAB*, *qnr*, *aac(6´)-Ib-cr* and *qepA* genes according to previous studies [18,19]. Briefly, the PCR mixture was prepared containing 10 pmol of each primers, 1 µl DNA template (3 µg/µl), 1.5 mM MgCl2, 0.2 mM each dNTP, and 5 u Taq DNA polymerase (Cinagen, Iran) in a total number of 25 µl of PCR reaction. Amplification of *oqxAB*, *qnr*, *aac(6´)-Ib-cr* and *qepA* genes was performed by this protocol: A predenaturation step at 95°C for 5 min, followed by 35 cycles of 60 s at 95°C, 40 s at 53°C, and 60 s at 72°C. A final extension step was performed at 72°C for 5 min. PCR products electrophoresis was done in 1% agarose gels with 0.5X TBE (Tris/Boric acid/EDTA) buffer. DNA bands were seen by staining with KBC power load dye (Kawsar Biotech Co. Iran) under UV (ultraviolet) illumination.

Statistical analysis

Presence of quinolone resistance genes among ESBL positive isolates was calculated by Fisher's exact test for each gene. A p-value of 0.05 was considered as statistically significant.

RESULTS

A total of 225 *E. coli* strains were isolated from patients with UTI during 13 months.

Antimicrobial resistance pattern

ESBL production was detected by the screening method in 97 of the 225 clinical isolates, representing 43.1% of the *E. coli* isolates. Only 2% of

Table 1 - Frequency of ESBL genes among 97 *E. coli* isolates.

Genes	*TEM only*	*SHV only*	*CTX-M only*	*TEM & SHV*	*TEM & CTX-M*	*CTX-M & SHV*	*TEM & SHV & CTX-M*
No. (%)	34 (35%)	21 (21.6%)	10 (10.03%)	7 (7.2%)	1 (1.03%)	0	3 (3.1%)

Table 2 - Frequency of quinolone resistance genes among ESBL-producing *E. coli*.

Genes	*oqxAB only*	*aac(6')-Ib-cr only*	*qnr only*	*qep-A only*	*oqxAB & aac(6')-Ib-cr*	*oqxAB & aac(6')-Ib-cr & qnr*	*oqxAB & aac(6')-Ib-cr & qnr & qep-A*
No. (%)	19 (19.5%)	18 (18.5%)	0	0	35 (36%)	4 (4.1%)	0

Table 3 - Co-presence of quinolone and ESBL resistance genes among ESBL-producing *E. coli*.

ESBL gene / *PMQR gene*	*TEM positive*	*SHV positive*	*CTX-M positive*
oqxAB positive	32 (33%)	20 (20.6%)	10 (10.3%)
aac(6')-Ib-cr positive	26 (26.8%)	23 (23.7%)	9 (9.3%)
qnr positive	7 (7.2%)	1 (1.03%)	2 (2.06%)
qepA positive	4 (4.1%)	1 (1.03%)	1 (1.03%)

the ESBL-producing *E. coli* isolates showed resistance to meropenem. The antimicrobial resistance rates to other antibiotics were as follows: gentamicin (76%), amikacin (17%), tobramycin (88%), ceftriaxone (96%) piperacillin/tazobactam (4%), nitrofurantoin (9%), cotrimoxazole (80%), chloramphenicol (89.6%) and doxycycline (73%). Resistance rate to ciprofloxacin was determined about 77%.

Distribution of resistance genes

Of 97 ESBL producer strains, bla_{TEM}, bla_{SHV} and bla_{CTX-M} genes were detected in 90 isolates. The frequencies of bla_{TEM}, bla_{SHV} and bla_{CTX-M} were 45(46.4%), 31(31.9%) and 14(14.4%), respectively (Table 1).

The most prevalent PMQR gene among ESBL-producing isolates was *oqxAB* which found in 67 isolates (69.1%). The number of strains harboring *aac(6')-Ib-cr*, *qnr* and *qepA* were 65 (67%), 8 (8.2%) and 6 (6.2%), respectively. Frequency and co-presence of quinolone resistance genes are shown in Table 2.

The *qnr* gene was higher in bla_{TEM} positive isolates compared to other isolates significantly (P=0.02). Also, co-presence of quinolone and ESBL resistance genes among isolates is mentioned in Table 3.

DISCUSSION

ESBL-producing *E. coli* strains have been emerged worldwide as an important cause of both community and hospital acquired UTI [20]. Since ciprofloxacin is available in oral and intravenous preparations, it is ranked as one of the highest priority critically important antibiotics against UTI caused by *E. coli*, especially ESBL-producing *E. coli* [21]. However, during the last decade, the resistance rate to fluoroquinolones such as ciprofloxacin has increased and the continuation of this trend can have serious clinical consequences [22]. In the present study, a high prevalence of PMQR determinants was found in the 97 ESBL- producing *E. coli* isolates. Over the past ten years, PMQR determinants have emerged as an important concern, because they can easily spread among susceptible bacterial populations [23].

In our study, the prevalence of PMQR genes (*oqxAB*, *aac(6')-Ib-cr*, *qepA*, and *qnr*) among clinical isolates of ESBL-producing *E.coli* from patients with UTI was investigated. Our findings showed that 67 clinical isolates of *E.coli* harbored both of ESBL and PMQR genes simultaneously. Surveys on the coexistence of PMQR (especially *oqxAB*) and ESBL genes among Enterobacteriaceae have been reported in several studies [24-26].

Frequency of ESBL-producing strains of *E. coli* vary in different countries. They have been reported at an incidence rate of 43% in Bangladesh, while less than 1% of *E. coli* isolates produce ESBL in the European countries [27, 28]. In our evaluations, the combined disc method confirmed ESBL production in 97 (43.1%) *E. coli* isolates, which is in agreement with the results of other investigation in some developing counties [29]. In current study, *bla* genes were not detected in some of iso-

lates. It seems that their ESBLs production had been related to other β-lactamase encoding genes. OqxAB is an efflux pump that has a wide substrate specificity including chloramphenicol, trimethoprim, and quinolones such as nalidixic acid, ciprofloxacin and norfloxacin [11]. The *oqxAB* gene was the most frequent PMQR determinant (69.1%) among ESBL-producing *E. coli*. This result contrasts with reports showing that *oqxAB* gene was found in 0.4% of clinical isolates of *E. coli* in Korea, and 6.6% in China [30, 31]. The high levels of prevalence and dissemination of *oqxAB* among UTI isolates may be due to the overuse of ciprofloxacin in patients suspected with UTI in Iran. Previously, high levels of prevalence of *oqxAB* (60.2%) detected among ESBL-producing *Klebsiella pneumoniae* in Iran [32]. These findings suggest that *oqxAB* genes are conjugative genes among ESBL-producing members of Enterobacteriaceae family.

The frequencies of the *aac(6')-Ib-cr*, *qnr* and *qepA* genes in urinary tract *E. coli* isolates in present investigation were a similar magnitude to global epidemic [33]. *aac(6')-Ib-cr* was the second common PMQR gene in this study. AAC(6')-Ib-cr, an aminoglycoside acetyl- transferase enzyme, which is accountable for reduced susceptibility to ciprofloxacin by modifying this antibiotic. In addition, the *aac(6')-Ib-cr* positive clinical isolates can indicate resistance to co-trimoxazole [34]. Our results indicated that in all of ciprofloxacin and co-trimoxazole resistant isolates the *aac(6')-Ib-cr* gene was found.

We detected 8.2% and 6.1% of isolates harboring *qepA* and *qnr*, respectively, in accordance with another study in West of Iran [35]. The results of Sedighi *et al.* indicated that only 1-2% of uropathogenic ESBL-producing *E. coli* carried *qnr genes* [17]. This difference can be due to the number of samples. However, a noticeable increase in strains harboring the resistance genes seems possible.

Presence of *qnr* gene among isolates carrying bla_{TEM} was significant and this may be due to co-existence of the *qnr* and bla_{TEM} genes in a common transmissible plasmid. The co-dissemination of PMQR and ESBL genes among *E. coli* isolates can be considered a threat to public health. Therefore, antimicrobial susceptibility testing is crucial in management of UTI cases and antibiotic drugs must be prescribed judiciously.

In conclusion, our study indicated that 43.1% of isolates were ESBL positive. The bla_{TEM} was the most frequently detected ESBL responsible gene. The most prevalent plasmid mediated quinolone resistance genes among ESBL-producing isolates was *oqxAB* followed by *aac(6')-Ib-cr*, *qnr* and *qepA*. Our data indicated that prevalence of plasmid mediated quinolone resistance genes in ESBL positive isolates is increasing.

Conflicts of interest.

All authors declare no conflicts of interest.

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