

The prevalence of quinolone resistance genes of A, B, S in *Escherichia coli* strains isolated from three major hospitals in Tehran, Iran

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Citation: Abbasi H, Ranjbar R. The prevalence of qnr genes of A, B, S in *Escherichia coli* strains isolated from three major hospitals in Tehran, Iran. Cent European J Urol. 2018; 71: 129-133.

Article history

Submitted: Aug. 18, 2017

Accepted: Jan. 14, 2018

Published online: Jan. 18, 2018

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Introduction *Escherichia coli* (*E. coli*) as an opportunistic pathogen is a major cause of the hospital infections. The main goal of this research was to determine the frequency of quinolone resistance genes (qnr) among *E. coli* pathotypes isolated from patients with urinary tract infections (UTIs).

Material and methods Urine samples were obtained from patients with UTIs in three major hospitals of Mofid, Bu Ali, and Vali-Asr during the year of 2015 in Tehran, Iran. The antibiogram was done for isolated bacterial isolates using nalidixic acid, norfloxacin, gentamicin, streptomycin, and chloramphenicol. Then the plasmids of the bacterial samples were extracted. PCR was used to detect qnr genes. Finally, the PCR products were run on a 1% agarose gel electrophoresis and the results were analyzed by the program SPSS version 22.

Results Overall, 100 *E. coli* strains were isolated from patients with UTIs. The highest resistance rate was against Streptomycin. The frequency of the genes of qnrA, qnrB and qnrS were 0%, 25% and 36%, respectively. Moreover, the presence of the both genes of qnrB and qnrS was recognized in 10% of isolated bacterial strains.

Conclusions Our results indicated increasing rates of quinolone resistant *E. coli* strains circulating in hospitals under the study. Dissemination of these strains harboring qnr determinants is of particular concern.

Key Words: *Escherichia coli* ◊ qnr ◊ urinary tract infections ◊ antibiotic resistance

INTRODUCTION

In recent years, the misuse and irregular consumption of antibiotics have led to the high level appearance of antimicrobial resistance genes in different types of microbial causative agents of infectious diseases like *E. coli* [1–5].

Escherichia coli strains and in particular, uropathogenic *E. coli* (UPEC) pathotypes and *Candida albicans* are known as the most common bacterial and fungal agents of urinary tract infections (UTIs). According to previous reports, the UTIs are considered as the second ranking infectious disease worldwide;

that is why the UTIs are known as an important global concern in public health care systems. Among 5 phylogenetic groups of A, B1, B2, D, E, the majority of UPEC pathotypes belong to the B2 category while others are placed in the D group. On the other hand, UPEC strains are equipped with a huge number of virulence factors which may lead to different types of UTIs and in recent years the spread of drug resistant and multi-drug resistant pathotypes of UPEC has significantly increased. Various pathotypes of UPEC cause the majority of bacterial community acquired UTIs and 50% of nosocomial UTIs. UPEC has been found to possess a variety of virulence and

antibiotic resistance genes located on both of chromosome and plasmids [6–15].

The spread of quinolone resistant genes among gram negative bacteria like UPEC is a big concern for the global health care system. At the beginning of the 1980s, the resistance to quinolone was recognized within chromosomal genes. By the 1990s, some reports revealed that the quinolone resistance genes may be also observed within the plasmids. Now we know that the qnr genes family are mediated by plasmids and the dissemination of qnr genes in the population of gram negative bacteria such as UPEC is a big challenge for the health care systems around the world [16].

According to the previous reports, the plasmids of qnrA and qnrB are bigger than qnrS with different origins. They also contain many other important antimicrobial resistance genes against beta-chloramphenicol, aminoglycosides etc. [16, 17].

The aim of the current study was to determine the prevalence of qnr genes of A, B, S in UPEC strains isolated from a number of patients in different major hospitals in Tehran, the capital city of Iran.

MATERIAL AND METHODS

In a cross-sectional study, a total of 4506 urine specimens were collected from patients with a UTI, admitted to three major hospitals of Mofid, Bu Ali and Vali-Asr during the year of 2015 in Tehran, Iran. The urine samples were cultured on EMB and blood agar media and then were incubated at 37°C for 24 hours. Next after from the well-growth colonies, gram staining and the standard biochemical tests including oxidase, TSI, SCA were performed to isolate the *Escherichia coli* strains. In the following step, the antibiotics of nalidixic acid, norfloxacin, gentamicin, streptomycin, and chloramphenicol were used for achieving the antibiogram [18].

Furthermore, the total bacterial genome was extracted from the isolated *E. coli* strains as done in our pre-

vious study [19]. Obviously, the related primers for recognizing qnrA, qnrB and qnrS genes were taken from different papers which are shown in Table 1 [20]. Then, the PCR was done to detect the qnr genes. In brief, 1 µl of the plasmid extraction, 1 µl of the related forward and reverse primers (Cinnagen Company) regarding each of genes were added in separate microtubes. Afterwards, the total volume of each specimen was increased to 25 µl by adding double distilled water. The PCR cycle program was achieved for the genes of qnrA, qnrB qnrS as below: 95°C for 5 minutes as the primary denaturation temperature, 1 min x 30 cycles in 94°C; 53.4°C, 52.2°C, and 55.6°C, respectively for the genes as the annealing temperatures; 72°C for 1 minute as the elongation temperature and a final temperature 72°C for 7 minutes (1 cycle). The validation of the PCR products were then checked by running 5 µ of each on 1% of agarose gel electrophoresis and the electrophoretic data was interpreted by GelClust [21]. Having collected the data, the results were represented in the form of frequency tables.

RESULTS

Overall, 100 *Escherichia coli* strains were isolated from patients with UTIs and subjected for the antibiogram and molecular analysis. The results of antibiogram revealed a high drug resistant property against streptomycin. In contrast to streptomycin, the lowest drug resistant property was recognized against chloramphenicol. The other sensitive or resistant strains are indicated in Table 2.

Regarding the PCR achievements for the three genes of qnrA, qnrB, and qnrS, the results showed that, 36 cases of the strains bearing qnrS genes, 25 cases carrying qnrB genes and 10 cases possessed both genes of qnrB and qnrS; but no one had the qnrA gene (Table 2).

According to the results, the frequency of the qnrA, qnrB, and qnrS separately and encompassing both

Table 1. Primers used in PCR

Reference	Band size (bp)	Sequence(5'-3')	Length	Primer	Target
(20)	516	ATTCTCACGCCAGGATTG	20 mer	forward	qnrA
		GATCGGCAAAGGTTAGGTCA	20 mer	Reverse	
(21)	526	Sequence (5'-3')	Length	Primer	Target
		GTTGGCGAAAAATTGACAGAA	22 mer	forward	qnrB
		ACTCCGAATTGGTCAGATCG	20 mer	Reverse	
(20)	417	Sequence (5/-3/)	Length	Primer	Target
		ACGACATTGCTCAACTGCAA	20 mer	forward	qnrS
		TAAATTGGCACCCCTGAGGC	20 mer	Reverse	

genes of *qnrB* and *qnrS* in nalidixic acid resistant strains was 0%, 28.1%, 36.6%, and 14%. These values for norfloxacin resistant strains were 0%, 23.9%, 30.4%, and 6.5%, respectively.

According to Figure 2, the highest frequency was recognized for *qnrS* gene. The frequency of the genes of *qnrA*, *qnrB*, and *qnrS* in separate and *qnrB* and *qnrS* together is 0, 25%, 36%, and 10%, respectively.

DISCUSSION

The increased rates of usage for quinolones to treat UTIs caused by UPEC and the other enteric bacteria, has led to the serious failures of treatment [22]. The worst part of the quinolone resistant genes is the bi-dimensional property of the problem. These genes are recognized in both of chromosomes and plasmids with different mechanisms and target molecules [16, 23].

Tarchouna et al. have reported a frequency of 32% for the *qnr* genes: 12.5% for *qnrB*, 5.3% for *qnrA*, 3.5% for *qnrS*, and 2.6% for both *qnrS* and *qnrB*, and 3.5% for *qnrA* and *qnrB*. With exception to the *qnrA*, the frequency of the genes is lesser than our results [24]. In a previous study the frequency of the *qnrA* genes was examined. The frequency of the *qnrA* genes was 87%, 82%, 65%, and 39%, respectively. The high use fluoroquinolones has led to the increase of resistance genes against the related antibiotics. As a result, this increasing trend should be prevented by using appropriate guidelines for prescription [25]. In another study, from 14199 urine specimens, 16.2% of samples were positive for bacterial infections. The isolated samples included 921 *Escherichia coli* isolates, 412 *Klebsiella* spp. isolates, 285 coagulase negative *Staphylococci* isolates, 202 *Enterococcus* spp. isolates, 158 *Pseudomonas* spp. isolates and 83 *Staphylococcus aureus* isolates. The highest resistance genes against carbenicillin, ampicillin, trimethoprim-sulfomethoxazol and kanamycin were recognized with the percentage of 68%, 96%, 70% and 65% respectively, among the *E.coli* strains [3].

In a survey reported by Bouchakor et al., 39 clinical samples of ESBL-producing strains were isolated in which 16 isolates were in association with the *E.coli* quinolone resistant strains with the frequency of 2.56%, 10.25%, and 23.07% relating to *qnrA*, *qnrB*, and *qnrS* genes, respectively. In comparison with our research, a higher level percentage of *qnrA* and lower level percentage relating to *qnrB* and *qnrS* genes were recognized [26].

According to Momtaz et al. report, the genes of *aadA1* (52.84%), and *qnr* (46.34%) were the most frequent antibiotic resistant genes; while the distri-

Table 2. Results of antibiogram test

Antibiotics	Resistant	Sensitive	Semi-sensitive	Total
Nalidixic acid	71	28	1	100
Norfloxacin	46	52	2	100
Streptomycin	90	3	7	100
Gentamicin	21	72	7	100
Chloramphenicol	12	88	–	100

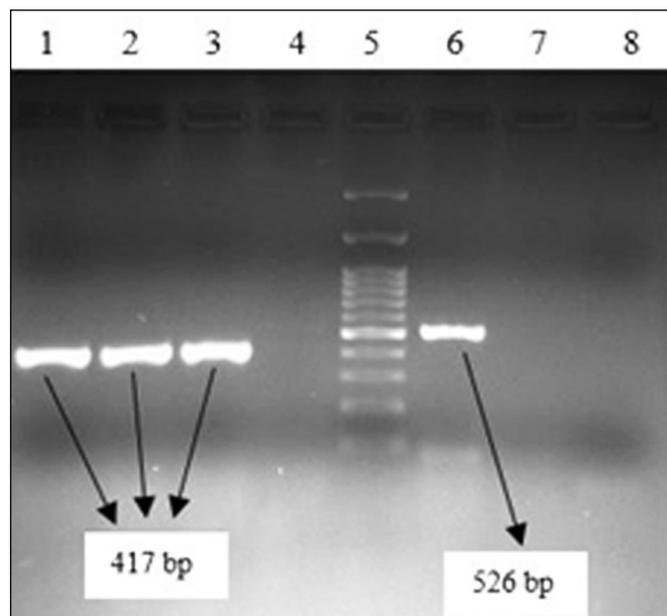


Figure 1. The PCR results to confirm presence of the *qnrA*, *qnrB* and *qnrS* genes. lanes 1, 2, and 3: positive *qnrS*. lane 4: negative *qnrS*. lane 5: 1 kb ladder. lane 6: positive *qnrB*. lane 7: negative *qnrB*. lane 8: negative *qnrA*.

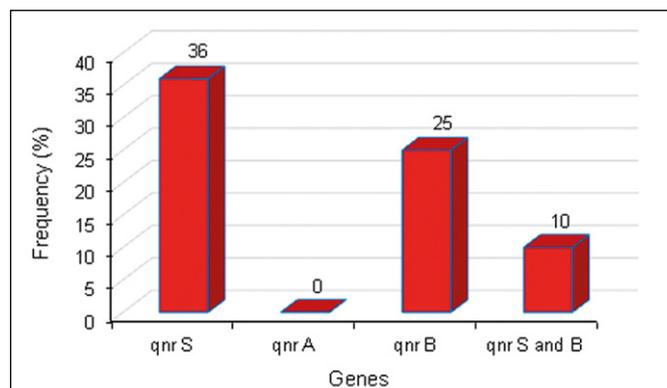


Figure 2. Frequency of the *qnr* genes in the population studied.

bution of the genes of *cat1* (15.44%), *cmlA* (15.44%) and *dfrA1* (21.95%) were the least. Besides, the resistance genes against penicillin (100%) and tetracycline (73.98%) were detected as the highest, while

the resistance genes against nitrofurantoin (5.69%) and trimethoprim (16.26%) were recognized as the lowest [13].

Anvarinejad et al. in their studies found 30 different genetic integrons and genotype patterns for quinolone-resistant strains of UPEC. The gene cassettes observed in class 1 and 2 integrons had no complete similarity with the quinolone resistance strains isolated in this study [14].

In other words, the frequency of the *qnrB* and *qnrS* genes in our isolates which were resistant against the nalidixic acid (28.1% and 36.6%, respectively), is higher than the percentage reported by Bouchakor et al. Prabhat Kumar Talukdar et al. in their investigation showed that among the isolated strains of *E. coli*, 16 different antibiotic resistance genes including the *qnr* genes were detected [27].

Finally, in a recent study carried out on 80 *E. coli* strains isolated from the hospital wastewater sources in Tehran, Iran, we reported the highest rates of antibiotic resistance for nalidixic acid (60%), followed by norfloxacin (30%), and ciprofloxacin (25%). More than 24 isolates harbored *qnr* genes. The *qnrA* gene was not observed in any strain, while 11 and 7 isolates contained the *qnrB* and *qnrS*, respectively [19]. The PCR technique used in this study was able to detect various *qnr* genes successfully. The use of molecular techniques like PCR is a good choice

when the number of samples is low. But in the case of a large number of specimens, an advanced pangenomic diagnostic tool like the DNA microarray is fully recommended [28–32].

CONCLUSIONS

Compared with previous studies, our results indicated an increasing rate of quinolone resistant *E. coli* strains circulating in hospitals under the study. Our findings emphasized the usage limitation of these antibiotics for treatment of urinary tract infections caused by uropathogenic *Escherichia coli* strains.

The *qnrS* gene had the greatest degree of frequency among the other quinolone resistance genes (*qnr*) between isolated strains. Dissemination of these *qnr* harboring strains is of particular concern. Rapid detection and identification of antibiotic resistance genes is an urgent need in epidemiological researches.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

ACKNOWLEDGEMENTS

We would like to thank from the “Clinical Research Development Center of Baqiyatallah hospital” for their kindly cooperation. This study was financially supported in part by “Clinical Research Development Center of Baqiyatallah hospital”.

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