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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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 commune 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 previous 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 | ATTCTCTACGCCCGAGATTG | 20 mer | forward | qnrA |
| | | GATCGGCAAGTGTAGTCA | 20 mer | Reverse | qnrA |
| (21) | 526 | GTTGGCCAAAAATGGACAGAA | 22 mer | forward | qnrB |
| | | ACTCCGAAATTGTCAGATCG | 20 mer | Reverse | qnrB |
| (20) | 417 | AGGATCGCGCCTAGGCA | 20 mer | forward | qnrS |
| | | TAAATTGCCAACCTGTAGGC | 20 mer | Reverse | qnrS |
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 1, 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 (Figure 2).

**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 gene 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 distribution 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

**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.
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.

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