High frequency of mutations in gyrA gene associated with quinolones resistance in uropathogenic Escherichia coli isolates from the north of Iran

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ABSTRACT

Objective(s): Regarding the global burden of uropathogenic Escherichia coli (UPEC) infections, prevention and treatment of such infections play a significant role in healthcare management. The inappropriate use of fluoroquinolones led to a worldwide spread of quinolone-resistant strains. Therefore, this study aimed to investigate mutations in codons 83 and 106 of gyrA gene in UPEC isolates in the north of Iran.

Materials and Methods: This cross-sectional study performed on a total of 223 UPEC isolates which were recovered within 6 months in 2017. Isolates were identified and confirmed by standard microbiologic tests, and antimicrobial susceptibility testing was carried out by disk diffusion and E-test methods. PCR reaction was performed to amplify gyrA gene, and PCR-RFLP was performed using BsiEI and BstU¬I restriction enzymes to investigate mutations in gyrA gene.

Results: The nalidixic acid, ciprofloxacin, ofloxacin, and norfloxacin resistance rates were 61.9%, 50.2%, 48.25, and 45.3%, respectively. Overall, 55.2% of E. coli isolates had a mutation in gyrA gene in codon 83, and 20.2% in codon 106. Also, 15.2% of isolates had simultaneously mutation. Moreover, a significant association was found between mutations in gyrA gene and quinolone and fluoroquinolones resistance pattern of UPEC isolates.

Conclusion: Our results revealed a high level of quinolone resistance associated with the mutations in gyrA among the clinical isolates of UPEC in our region. To the best of our knowledge, this study is the first investigation on the role of gyrA alteration in quinolone resistance among UPEC isolates from the north of Iran.

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Introduction

Quinolones are one of the synthetic antibiotics which extensively used worldwide (1, 2). Urinary tract infections (UTIs) were treated by first-generation (acidic) quinolones, including nalidixic acid (1). However, the range of effectiveness has improved by alteration of the following generations. One of these changes was the addition of a fluorine atom at position C-6 of antibiotic molecules which leads to extensive powerful activity against different Gram-negative bacteria (3, 4). Fluoroquinolones bind to and impede the activity of topoisomerase II (DNA gyrase) and topoisomerase IV (parC and parE) (4). DNA gyrase comprised of two subunits A and two subunits B, which are encoded by the gyrA and gyrB genes, respectively (5).

Extensive and inordinate consumption of antibiotics over the recent years has been leading to increasing trends of antibiotic resistant bacteria (6, 7). Nowadays, with the advancement of antibiotic resistance mechanisms, the issue of antibiotic resistance becomes an important concern in the health systems (6, 8, 9).

In Escherichia coli, resistance to quinolones frequently occurs through mutation in gyrA and less often by gyrB genes, which catalyzes ATP-dependent DNA supercoiling (5). Some other mechanisms of E. coli resistance to quinolones and fluoroquinolones are through efflux pumps and reduced drug accumulation in the bacteria due to changes in the purine protein (4, 10). Many studies have revealed that mutations in a small part of gyrA N-terminal (Amino acids 67 (Ala-67) to 106 (Gln-106)) leads to quinolones and fluoroquinolones resistance which is named quinolone resistance-determining region (QRDR) (11). Meanwhile, the most of the mutations arose in nucleotide 248 and 620, causes amino acids aspartic acid 83 and serine 87 alterations (12).

Among the point mutations, the most relevant alteration is that on nucleotide 247 (Ser-83) of the gyrA gene (10, 13). In clinical isolates, the second most commonly observed mutation is at codon 87 of gyrA gene (14). Some studies have reported that resistant bacteria to quinolones had no mutation in the codon 83 gyrA gene. Also in some cases despite mutation in codon 83 gyrA gene, the bacteria were susceptible to quinolones. It has been supposed that such resistant strains may have a point mutation in other sites or along with codon 83 in the gyrA gene which may lead to high-level resistance to quinolones (5, 14). A few studies have surveyed the effect of the mutation on codon 106 in conferring resistance to quinolones (15). To our best
knowledge, there is no report about the investigation on mutation in the codon 106 among clinical E. coli in Iran. Mutations in gyrA gene can be identified with several methods such as sequencing, single-strand conformational polymorphism (SSCP) and mismatch amplification mutation assay PCR (MAMA-PCR), but these techniques are quite expensive and time-consuming (16). PCR-RFLP is one of the best methods for identifying the point mutation in a sequence of DNA. Therefore, this study aimed to investigate mutations in codons 83 and 106 of gyrA gene in susceptible and resistant isolates of uropathogenic E. coli (UPEC) in the north of Iran.

Materials and methods

**Study design and bacterial isolation**

This cross-sectional study was performed to assess the importance of gyrA gene mutations in quinolone and fluoroquinolone resistance in 1250 urine samples (mid-stream, clean catch) which were collected in a period of months from 6 months from February 2017 to July 2017. Samples obtained from the patients with UTIs who referred to a tertiary hospital (Razi hospital) in the Rasht, the north of Iran. This study was in accordance with the declaration of Helsinki and approved by the regional Ethics Committee. The specimens plated on Blood agar (Quelab, Canada) and Eosin Methylene Blue agar (Pronadisa, Italy) plates. The plates were incubated overnight at 37 ºC. Then, colonies with green metallic agar (Pronadisa, Italy) plates. The plates were incubated for 24 hours at 37 ºC for long preservation.

**DNA extraction and polymerase chain reaction (PCR)**

Genomic DNA of all isolates was extracted using High pure DNA template preparation kit (Roche, Germany) as stated by manufacturer instruction. PCR reaction was performed to amplify gyrA gene in Quinolone resistant determining region (QRDR) using specific primers, gyrA-F: 5′-GCT GCC AGA TGT CCG AGA T-3′; gyrA-R: 5′-TCC GTG CCG TCA TAG TTA CCA-3′. Reaction condition was initiated by pre-denaturation at 95 ºC for 5 min followed by 45 cycles (94 ºC for 1 min; 60 ºC for 30 sec; 72 ºC for 30 sec) and final extension cycle (72 ºC for 5 min). A 360 bp band on agarose gel containing DNA safe stain (Cinnagen, Iran) was visualized under UV Tran illuminator.

**PCR-Restriction fragment length polymorphism (RFLP)**

In order to detection of mutation in 83 and 106 codons of gyrA gene, PCR-RFLP was performed using BsiEI and BstUI restriction enzymes (Thermo fisher scientific Inc., USA). The source of BstUI restriction enzyme is Bacillus stearothermophilus U458 and, BsiEI was getting from an E. coli strain that carries the cloned BsiEI gene from Bacillus sp. The cutting site for BstUI is (5′ CG ↓ CG 3′ or 3′ GC ↑ GC 5′) and for BsiEI is (5′…CGRY ↓ CG…3′ or 3′…GC ↑ YRGC…5′). The 360 bp PCR products were digested using both enzymes simultaneously according to manufacturer guideline. The digested fragments were subjected to electrophoresis on a 2% agarose gel stained with DNA safe stain and was visualized under UV illuminator.

**Statistical analysis**

Resistance rates among the isolates and comparisons of fluoroquinolones resistance and mutation in the gyrA gene were analyzed with Chi-square or Fisher's exact tests. A difference was considered statistically significant if the P-value was less than 0.05.

**Results**

Among 223 isolated UPEC isolates, ciprofloxacin, ofloxacin, and norfloxacin resistance rates were approximately similar (50.2%, 48.25%, and 45.3%, respectively), whereas a higher level of resistance to nalidixic acid was seen (61.9%) (Table1). Moreover, the full results of antibiotic susceptibility testing including MIC$_{50}$ and MIC$_{90}$ (MIC at which 50% and 90% of isolates were inhibited) of the tested isolates are shown in Table 2.

In the present study, 55.2% of E. coli isolates had a
mutation in gyrA gene in codon 83. Mutation in codon 106 occurred in 20.2% of cases. Also, simultaneously mutation (codons 83 and 106) was observed in 15.2% of isolates.

Among wild-type strains there is one restriction site for BstUI at nucleotide 42 which yields 42 and 318 bp fragments after digestion, while in mutants, gyrA gene attains another restriction site at nucleotide 149 (Serine → Alanine). So, three fragments (42, 107 and 211 bp) are observed after digestion and electrophoresis. Also, BsiEI has two restriction sites at nucleotide 168 and 330 in wild-type strains which produces three fragments (390, 168 and 132 bp). If a mutation occurs in gyrA gene, a new restriction site developed at nucleotide 217, which produces 4 fragments (30, 168, 138 and 49 bp).

The association between a mutation in codon 83, 106 or both and ciprofloxacin resistance pattern among E. coli isolates showed that mutation in codon 106 had the most effect on resistance to ciprofloxacin (Table 3). Our results revealed that simultaneously mutation in codon 83 and 106 conferred more resistance rate to norfloxacin among E. coli isolates (Table 4). In the present study, a mutation in codon 106 gyrA gene had the most effect on resistance to ofloxacin in E. coli isolates as such as ciprofloxacin (Table 5). Finally, E. coli isolates which had a mutation in both codons 83 and 106 gyrA gene were more resistant to nalidixic acid which was similar to norfloxacin (Table 6).

**Discussion**

Regarding the global burden of UPEC infections, prevention and treatment of such infections play a significant role in healthcare management (18). Fluoroquinolones are an important class of antibiotics for the treatment of UPEC; however, the inordinate use of these agents led to a worldwide spread of quinolone-resistant strains, particularly in developing countries (19). In the present study, a remarkable rate
of quinolones resistance (ranging from 45.3% to 61.9%) in 223 tested UPEC isolates were found. Despite the great discrepancy, the prevalence of quinolone-resistant UPEC isolates in our findings was consistent with the median values (range 14% to 71%) reported in different regions of the country (20-26). Based on the literature, the level of resistance to quinolones is increasing in other parts of the world, as well. Several reposts from Asian, European, African, and South-American countries indicate to the high prevalence of fluoroquinolones resistance even more than 50% and raised serious concerns (27-33). Meanwhile, our findings regarding the resistance even more than 50% and raised serious concerns. (27-33). The MICs of fluoroquinolones showed that the MIC ranges in Asian, European, African, and South-American countries is mostly associated with quinolones resistance in other countries showed alteration in the GyrA protein with 20.2%. Previously, several studies from Iran and other countries showed alteration in the GyrA protein is mostly associated with quinolones resistance in Enterobacteriaceae and nonfermenting Gram-negative bacilli (4, 33-39).

Moreover, a double concomitant mutation in gyrA (codons 83 and 106) was observed in 15.2% of isolates. Previously, it has been documented that low-level fluoroquinolone resistance in E. coli is associated with a single mutation, while high-level resistance required double mutations (36). In accordance with this finding, we found the majority of double mutation (more than 80%) is associated with quinolone-resistant isolates. Finally, despite the significant role of mutations in the QRDRs of gyrA, we found quinolone-resistant isolates without any mutations in this region. Therefore it is possible that other resistance mechanisms such as mutations in parC or the presence of horizontally acquired genes (qnr genes) may cause of quinolone resistance in our isolates (25, 36-38, 40).

As the main limitations of the present work, the lack of sequencing for confirming the RFLP results to reveals the exact status of base replacements among the mutant strains must be mentioned.

**Conclusion**

Our results revealed a high level of quinolone resistance is associated with the mutations in gyrA among the clinical isolates of UPEC in our region. To the best of our knowledge, this study is the first investigation on the role of gyrA alteration in quinolone resistance among UPEC isolates from north of Iran.

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**Table 5. Association between mutation in codons 83 and 106 gyrA gene and resistance to ofloxacin among Escherichia coli isolates**

| Resistance pattern | Resistant | Intermediate-resistant | Susceptible | P-value |
|-------------------|-----------|------------------------|-------------|---------|
| Mutation          | No %      | No %                   | No %        |         |
| gyrA 83           | No 72 58.5| 1 0.8                  | 50 40.7     | 0.006   |
| gyrA 106          | No 37 37  | 1 1                    | 62 62       | 0.006   |
| gyrA 83 & 106     | No 42 93.3| 0 0                    | 3 6.7       | <0.001  |
|                   | No 67 37.6| 2 1.1                  | 109 61.2    | <0.001  |
|                   | Yes 31 91.2| 0 0                    | 3 8.0       | <0.001  |
|                   | No 78 41.3| 2 1.1                  | 109 57.7    | <0.001  |

**Table 6. Association between mutation in codons 83 and 106 gyrA gene and resistance to Nalidixic acid among Escherichia coli isolates**

| Resistance pattern | Resistant | Intermediate-resistant | Susceptible | P-value |
|-------------------|-----------|------------------------|-------------|---------|
| Mutation          | No %      | No %                   | No %        |         |
| gyrA 83           | No 93 75.6| 3 2.4                  | 27 22       | <0.001  |
| gyrA 106          | No 45 45  | 4 4                    | 51 51       | <0.001  |
| gyrA 83 & 106     | No 39 86.7| 1 2.2                  | 5 11.1      | 0.001   |
|                   | No 99 56.6| 6 3.4                  | 73 41       | 0.001   |
|                   | Yes 31 91.2| 0 0                    | 3 8.8       | <0.001  |
|                   | No 107 56.6| 7 3.7                  | 75 39.7     | <0.001  |
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Conflict of Interest
None declared.

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