Plasmid-Mediated Quinolone-Resistance (qnr) Genes in Clinical Isolates of Escherichia coli Collected from Several Hospitals of Qazvin and Zanjan Provinces, Iran

Maryam Rezazadeh a, Hamid Baghchesarai a, Amir Peymani b,*

aDepartment of Microbiology, Faculty of Medicine, Zanjan University of Medical Sciences, Zanjan, Iran.
bMedical Microbiology Research Center, Qazvin University of Medical Sciences, Qazvin, Iran.

Abstract
Objectives: Escherichia coli is regarded as the most important etiological agent of urinary tract infections. Fluoroquinolones are routinely used in the treatment of these infections; however, in recent years, a growing rate of resistance to these drugs has been reported globally. The aims of this study were to detect plasmid-mediated qnrA, qnrB, and qnrS genes among the quinolone-nonsusceptible E. coli isolates and to investigate their clonal relatedness in Qazvin and Zanjan Provinces, Iran.

Methods: A total of 200 clinical isolates of E. coli were collected from hospitalized patients. The bacterial isolates were identified through standard laboratory protocols and further confirmed using API 20E test strips. Antimicrobial susceptibility was determined by the standard disk diffusion method. Polymerase chain reaction (PCR) and sequencing were used for detecting qnrA, qnrB, and qnrS genes and the clonal relatedness of qnr-positive isolates was evaluated by enterobacterial repetitive intergenic consensus-PCR (ERIC-PCR) method.

Results: In total, 136 (68%) isolates were nonsusceptible to quinolone compounds, among which 45 (33.1%) and 71 (52.2%) isolates showed high- and low-level quinolone resistance, respectively. Of the 136 isolates, four (2.9%) isolates were positive for the qnrS1 gene. The results from ERIC-PCR revealed that two (50%) cases of qnr-positive isolates were related genetically.

Conclusion: Our study results were indicative of the presence of low frequency of qnr genes among the clinical isolates of E. coli in Qazvin and Zanjan Provinces, which emphasizes the need for establishing tactful policies associated with infection-control measures in our hospital settings.

1. Introduction

Clinically, Escherichia coli is an important Gram-negative bacteria with the potential to cause serious disease including urinary tract infections (UTIs), pyelonephritis, and bacteremia [1]. UTIs, known as the most common hospital-acquired infections, account for up to 35% of infections associated with health-care
system and *E. coli* is reported to be the most frequent cause of UTIs [2]. Fluoroquinolones are synthetic and broad-spectrum antibacterial agents often used for the treatment of lower UTIs [3]. Inappropriate and unnecessary administration of these antibiotics has led to an increase in the appearance of multidrug-resistant *E. coli* isolates, limiting treatment options. Serious health-care-associated infections caused by these resistant organisms have been associated with considerable morbidity and mortality [4].

Fluoroquinolones inhibit two bacterial enzymes, DNA gyrase and topoisomerase IV, both of which play essential roles in bacterial DNA replication [5]. Resistance to quinolone is often linked to amino acid substitutions in the quinolone-resistance-determining regions of DNA gyrase (gyrA and gyrB) and DNA topoisomerase IV (parC and parE) subunits, leading to target modification [6]. Decreased outer membrane permeability through porin changes and overexpression of naturally occurring efflux systems may also contribute to chromosomal quinolone resistance [7]. However, recent reports indicate that quinolone resistance can also be mediated by mobile genetic elements such as plasmids. Plasmid-mediated quinolone resistance is mediated by the genes (*qnr*) encoding proteins that belong to the pentapeptide repeat family and protect DNA gyrase and topoisomerase IV against quinolone compounds [8]. The three major groups of *qnr* determinants are *qnrA*, *qnrB*, and *qnrS* [9,10]. The first plasmid-mediated quinolone-resistance gene (*qnrA*) was identified in a clinical strain of *Klebsiella pneumoniae* isolated in Alabama in 1998 [11]. The other two determinants of *qnr* (*qnrB* and *qnrS*) have subsequently been observed in other enterobacterial species including *E. coli*, *Enterobacter* spp., *Salmonella* spp., and *Klebsiella pneumonia* [12].

Plasmid-mediated resistance is of growing clinical concern as they may transfer resistance genes to other species via horizontal gene transfer, conferring resistance against these antibiotics [13]. Moreover, the simultaneous presence of extended-spectrum beta-lactamases (ESBLs), AmpC, and *qnr* genes on the same plasmid has been well documented and this highlights the complexity of determinants involved in plasmid-mediated resistance among the enterobacterial isolates in medical settings [14]. Obviously, the widespread appearance of a growing trend associated with the prevalence of plasmid-mediated resistance among enterobacterial isolates is undeniable; however, only limited numbers of studies have been reported from Iran addressing the prevalence of *qnr* genes among the clinical isolates of *E. coli*. The aim of this study was, therefore, to investigate the prevalence of *qnr* determinants among *E. coli* isolates collected from a number of hospitals in two Iranian provinces—Zanjani and Qazvin.

2. Materials and Methods

2.1. Study design and bacterial isolates

In this cross-sectional study, 200 nonrepetitive *E. coli* isolates were obtained from the clinical sample of UTI patients admitted to hospitals in Zanjani (1 hospital) and Qazvin (3 hospitals) between July 2014 and December 2015. The organisms were identified by standard laboratory methods and later confirmed using the API 20 E test strips (bioMérieux, Marcy l’Etoile, France). The isolates were stored at −70°C in Trypticase soy broth containing 20% glycerol and subcultured two times prior to testing. The mean age of patients was 50.47 ± 18.8 years (range, 13–85 years). There were 153 (76.5%) female and 47 (23.5%) male patients.

2.2. Antimicrobial susceptibility

The Kirby–Bauer disk diffusion method was performed according to the Clinical Laboratory Standards Institute guidelines [15] to detect quinolone-resistance phenotype using nalidixic acid (10 µg), ciprofloxacin (5 µg), gatifloxacin (5 µg), norfloxacin (10 µg), levofloxacin (5 µg), imipenem (10 µg), and meropenem (10 µg) disks. If the results of antibiotic-susceptibility test confirmed the presence of resistance to both ciprofloxacin and nalidixic acid, the isolates were marked as high-level quinolone-resistant bacteria, whereas nalidixic acid-resistant or intermediate isolates and ciprofloxacin-susceptible isolates were marked as low-level quinolone-resistant bacteria [16]. Antibiotic disks were purchased from Mast Company (Mast Diagnostics Group Ltd, Merseyside, UK). *E. coli* American Type Culture Collection (ATCC) 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as quality-control strains in antimicrobial susceptibility testing.

2.3. DNA extraction and detection of *qnr*-encoding genes

The detection of *qnrA*, *qnrB*, and *qnrS* plasmid-mediated quinolone-resistance genes was performed using polymerase chain reaction (PCR) and specific primers (Table 1) [17]. Plasmid DNA was extracted using plasmid mini-extraction kit (Biioneer, Daejeon, South Korea). PCR amplifications were performed in a thermocycler (Applied Biosystems, USA) as follows: 95°C for 5 minutes and 35 cycles of 1 minute at 95°C, 1 minute at specific annealing temperature for each primer, and 1 minute at 72°C. A final extension step of 10 minutes at 72°C was performed. Amplification reactions were prepared in a total volume of 25 µL (24 µL of PCR master mix plus 1 µL of template DNA) including 5 ng of genomic DNA, 2.0 U of *Taq* DNA polymerase (Fermentas, Vilnius, Lithuania), 10 mM deoxyribose nucleotide triphosphate mix at a final concentration of 0.2 mM, 50 mM MgCl2 at a final concentration of 1.5 mM, 1 µM of each primer, and 1× PCR buffer (final
concentration). PCR products were electrophoresed on 1% agarose gel at 100 V and stained with ethidium bromide solution and finally visualized in gel documentation system (UVItc Limited, Cambridge, UK).

2.4. Clonal analysis of qnr-positive isolates

All qnr-positive E. coli isolates were tested for epidemiological relationships using enterobacterial repetitive intergenic consensus-PCR (ERIC-PCR) as previously described by Smith et al [18]. PCR cycling conditions were as follows: denaturation at 94°C for 1 second, annealing at 52°C for 10 seconds, and extension at 72°C for 35 seconds for 30 cycles, followed by a final extension at 72°C for 4 minutes. The final products were electrophoresed on 1.5% agarose gels. Visual comparison was employed to examine the fingerprints, and the patterns varying by two or more bands were classified as different.

2.5. Statistical analysis

Statistical data analysis was performed for descriptive statistics including frequencies, cross tabulation of microbiological and clinical features, and demographic characteristics using the computer software program SPSS version 16 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Resistance to quinolone compounds

A total of 200 E. coli isolates were obtained from patients admitted to internal medicine (82; 41%), intensive care unit (52; 26%), infectious diseases (49; 24.5%), surgery (11; 5.5%), and neurosurgery (6; 3%) wards. According to the results of the disk diffusion method, the highest resistance rate of isolates was against nalidixic acid (67.5%) and gatifloxacin (58%), whereas 44.5% and 44% of isolates demonstrated the highest rate of susceptibility to norfloxacin and ciprofloxacin, respectively. Overall, 136 (68%) isolates were nonsusceptible to quinolone compounds used in this study. High-level quinolone resistance was found in 45 (33.1%) isolates, and 71 (52.2%) bacterial samples revealed low-level quinolone resistance. In total, 93% and 91% of isolates were sensitive to meropenem and imipenem, respectively (Table 2).

3.2. Presence of qnr-encoding genes

PCR and sequencing showed that four (2.9%) of the 136 quinolone-nonsusceptible E. coli isolates carried qnrS1. The qnrA and qnrB genes were not found among the clinical isolates of this study. As shown in Table 3, qnrS1-positive isolates were mostly isolated from the internal medicine wards. Three of four (75%) isolates showed high quinolone-resistance level.

3.3. Clonal relatedness of qnr-positive isolates

The results obtained by ERIC-PCR were indicative of the presence of two (50%) qnr-positive E. coli clinical strains isolated from Zanjan hospital with similar ERIC-PCR patterns but, as shown in Figure 1, with a genotypic pattern unrelated to the two isolates collected from Qazvin hospitals.

4. Discussion

UTIs are the commonest type of bacterial infections and E. coli is the most prevalent cause of UTIs [1]. Quinolones are the most widely used antibacterial agents in fighting against serious infections caused by E. coli and other members of Enterobacteriaceae in Iran [19]. However, plasmid-mediated quinolone resistance

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### Table 1. Primers used for detection of qnr genes in urinary Escherichia coli isolates.

| qnr genes | Sequence (5’ → 3’) | Annealing temperatures (°C) | References |
|-----------|--------------------|----------------------------|------------|
| qnrA1–6   | ACG CCAGGATTTGAGTGAC CGAAGGATGAGATTCGAG | 53 | [17] |
| qnrB1–3, 5, 6, 8 | GGCCTCAGCTTTCTTCTGAG | 49 | [17] |
| qnrB4     | AGTTTGATCTTCTCCATGGC CGGATATCTAAATCGCCCAG | 53 | [17] |
| qnrS1–2   | CCTACAATCATACAT ATCCGGC GCCTCGAGAATCAGTTCC | 53 | [17] |

### Table 2. Antimicrobial susceptibility of Escherichia coli isolates against carbapenem and quinolone compounds.

| Antibiotics       | S (%) | I (%) | R (%) |
|-------------------|-------|-------|-------|
| Nalidixic acid    | 65 (32.5) | 2 (1) | 133 (66.5) |
| Gatifloxacin      | 84 (42) | —     | 116 (58) |
| Levofloxacin      | 85 (42.5) | 3 (1.5) | 112 (56) |
| Ciprofloxacin     | 88 (44) | —     | 112 (56) |
| Norfloxacin       | 89 (44.5) | —     | 111 (55.5) |
| Imipenem          | 182 (91) | 16 (8) | 2 (1) |
| Meropenem         | 186 (93) | 12 (6) | 2 (1) |

I = intermediate; R = resistant; S = susceptible.
in the genus belonging to Enterobacteriaceae, especially E. coli, has led to treatment failures and currently is becoming a significant public health concern. Plasmid-mediated resistance to quinolones is being increasingly reported in studies from Asia, Europe, Australia, and the United States [20]. However, the number of reports on prevalence of qnr genes among Iranian enterobacterial isolates is only limited to few studies.

This study showed a high level of antimicrobial resistance against quinolone compounds among urinary E. coli isolates. Overall, 67.5% and 56% of isolates were either fully resistant or had intermediate resistance to nalidixic acid and ciprofloxacin, respectively. Our results were partly similar to the resistance levels reported in two previously conducted studies in Iran. Firoozeh et al [19] reported that 82.5% and 45% of urinary E. coli isolates were resistant to nalidixic acid and ciprofloxacin, respectively. In another study from Iran, Khorvash et al [21] found that 76% and 52% E. coli isolates associated with nosocomial infection were resistant to nalidixic acid and ciprofloxacin, respectively. In our neighboring country, Pakistan, Muhammad et al [22] showed that 84.2% and 36.5% of E. coli isolated from UTIs were resistant to nalidixic acid and ciprofloxacin, respectively. In China, the frequency of ciprofloxacin resistance among the urinary E. coli isolates was 59.4% [23]. It seems that unnecessary and widespread administration of these antibacterial agents is the most important predisposing factor that could eventually lead to appearance of resistant bacteria in our hospital settings. Moreover, the resistance rate found in this study emphasizes the need for a local and national antimicrobial resistance surveillance system in bacterial isolates present in our hospital settings.

In this study, meropenem and imipenem showed high-level susceptibility against E. coli infections. Currently, the treatment of infections caused by multidrug-resistant Gram-negative bacteria is achieved by administration of carbapenems as the drugs of choice; however, in this study, 9% and 7% of isolates were either fully resistant or had intermediate susceptibility to imipenem and meropenem, respectively, a finding that would have more clinical impact if these strains become more prevalent in the future.

This study demonstrated a low prevalence rate (2.9%) of plasmid-mediated quinolone resistance (qnrS1) among quinolone-nonsusceptible E. coli isolates in educational hospitals of Qazvin and Zanjan Provinces. We did not find the qnrA and qnrB genes in our clinical isolates. The frequency of qnr genes in our study was lower than those found in the two studies previously conducted in Iran. In a study from Khorraramabad (Iran), Firoozeh et al reported that 14 (12.1%) and nine (7.8%) nalidixic acid-resistant E. coli isolates were positive for qnrA and qnrB genes, respectively [19]. In another study...
from Tehran, Pakzad et al [24] showed that qnrA and qnrB genes were present in 37.5% and 20.8% of ESBL-producing E. coli isolates, respectively. Like our study, a low frequency for qnr gene isolation was also described by other reports. In Brazil, Pereira et al [25] reported that only a single E. coli isolate among 144 ciprofloxacin-resistant isolates was positive for qnr genes. In Singapore, Deepak et al [26] also showed that 1.8% of urinary isolates of E. coli were found to possess the qnrS gene.

In Denmark, Cavaco et al [27] showed only 1.6% of nalidixic acid-resistant E. coli isolates as qnr positive. In France, qnr genes were present in 1.6% of ESBL-producing E. coli and Klebsiella spp. isolates [28]. In Canada, only about 1% of ciprofloxacin- and/or tobramycin-resistant E. coli and Klebsiella spp. isolates were qnr positive [29]. Nevertheless, the high prevalence rate of qnr genes has also been detected in Egypt where 26.6% of ESBL-producing E. coli isolates were positive for qnr genes, among which qnrA1-, qnrB1-, and qnrS1-type genes were detected alone or in combination in 16.6%, 23.3%, and 16.6% isolates, respectively [30]. We previously showed the high appearance of qnrB1, qnrS1, and qnrB4 genes among the clinical isolates of K. pneumoniae in Iran [31].

In this study, most qnr-positive isolates showed high-level resistance. Because qnr genes are responsible for low-level resistance to quinolones, it can be hypothesized that high-level resistance pattern could be linked to the presence of other mechanisms such as secondary changes in DNA gyrase or topoisomerase IV, and porin or efflux systems, which was not evaluated in our study.

In this work, the ERIC-PCR analysis of two qnr-positive isolates from Qazvin confirmed that these two isolates were epidemiologically unrelated; the explanation for this finding may be attributed to the fact that the clinical isolates from Qazvin were collected from two different hospitals, whereas those obtained from Zanjan were collected from the same hospital, resulting in identical genetic profile.

In conclusion, results of this study revealed the low prevalence rate of plasmid-mediated quinolone resistance associated with the presence of qnr genes among the clinical isolates of E. coli in Qazvin and Zanjan Provinces, Iran. The appearance of quinolone resistance through this type of mechanism within the Iranian health-care system could produce serious therapeutic and epidemiological concerns, which can be overcome through establishing appropriate infection control measures as well as comprehensive guidelines on proper administration of antibacterial in our medical centers.

Conflicts of interest

The authors declare no conflicts of interest.

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