RESEARCH ARTICLE

Prevalence and Characterization of Plasmid-mediated Quinolone Resistance Genes among *Escherichia coli* Strains Isolated from Different Water Sources in Alborz Province, Iran

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Abstract

BACKGROUND: This study was conducted to investigate the prevalence of quinolone resistance associated (*qnr*) antibiotic resistance among *Escherichia coli* strains isolated from different water sources in Alborz province, Iran.

METHODS: *E. coli* strains were isolated and identified by standard microbiological and biochemical tests from surface water sources in Alborz province, Iran in 2013. Fluoroquinolone-resistant isolates were determined using the antimicrobial susceptibility test determined by the Kirby–Bauer assay. Total genomic and plasmid DNA were extracted by boiling method. The presence of *gnr* genes in all nalidixic-acid and ciprofloxacin-resistant *E. coli* strains was determined by Polymerase Chain Reaction (PCR). The PCR amplicons were visualized after electrophoresis stained with ethidium bromide.

RESULTS: One hundred *E. coli* strains were isolated from the water sources examined in this study. As much as 22.7% and 7.3% of the isolates were resistant to nalidixic acid and ciprofloxacin respectively. While *qnrS*, *qnrB* and *qnrA* genes were detected in 28%, 9% and 1% of fluoroquinolone-resistant isolates respectively. All fluoroquinolone-susceptible isolates however did not contain any of the *qnr* genes.

CONCLUSION: This study reflects an increasing prevalence of fluoroquinolone-resistant *E. coli* strains in surface water sources. Underlining the importance of surface water sources as reservoirs for dissemination of potentially pathogenic *E. coli* and horizontal gene transfer between other waterborne bacterial species. Other possible mechanisms of resistance should also be investigated for better characterization of quinolone-resistant *E. coli* isolates. Therefore, immediate measures are needed to control and treat water sources more effectively.

KEYWORDS: antibiotic resistance, *E. coli*, *qnr* genes, water sources

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Introduction

Escherichia coli (*E. coli*) is important enteric bacteria which exist as a commensal and could become pathogenic to humans and various animal species.(1-5) It is responsible for significant socio-economic and health concerns in various countries worldwide.(6-8) The abuse of antimicrobials during animal husbandry, human and animal chemotherapy has over time encouraged the development and dissemination of antibiotic resistance genes by microorganisms.(9) This increasing antimicrobial resistance by microorganisms,

suggested to be mediated by antimicrobial resistance determinants which are carried by genetic components such as plasmids, transposons and integrons (9,10), conotes fatal consequences for human and animal health worldwide.

Several studies have recently determined that plasmids play a vital role in the propagation of antibiotic resistance genes from pathogenic strains to generic strains of various microbes including *E. coli*.(1-5) Presently, it is more difficult to therapeutically manage infections due to the high incidence and prevalence of antibiotic resistance which is mainly due to the abundance of transmissible multiple drug resistance plasmids among Gram-negative bacteria such as the enterobacteriaceae.(6-8) For example, the treatment of *E. coli* infections has been complicated by the emergence and dissemination of plasmid-mediated resistance to fluoroquinolones.(11-21)

The emergence of plasmid-mediated quinolone resistance (PMQR), first discovered in a multi-drug resistant urinary *Klebsiella pneumoniae* isolate in 1998, has been associated with the Plasmid-Mediated Quinolone-Resistance (*qnr*) gene. Genetic studies have identified five qnr genes namely *qnrA*, *qnrB*, *qnrS*, *qnrC* and *qnrD*.(5, 9-11) Two additional mechanisms of PMQR have been described, including the drug modification by the acetyl transferase AAC(6′)-Ib-cr and active efflux by QepA and OqxAB, which are pumps related to major facilitator superfamily transporters.(14,22)

There is currently insufficient data on the prevalence of *qnr* genes among *E. coli* isolates recovered from water sources in Iran. This study was therefore conducted to investigate the presence of plasmid-mediated *qnrA*, *qnrS* and *qnrB* genes among the quinolone-resistant *E. coli* isolated from different water sources in Alborz province, Iran.

Methods

Bacterial Isolates and Antimicrobial Susceptibility Testing

Between October, 2011 and October, 2012, water samples were collected from surface water sources in Alborz province, Iran. One-hundred *E. coli* strains were identified using standard methods (APHA/AWWA/WEF 2012) and previously described biochemical tests.(23) All isolates were screened for quinolone and fluoroquinolone resistance using the disk diffusion method described by Kirby-Bauer test. The following antibiotics were tested: nalidixic acid (30 µg/disk), norfloxacin (10 µg/disk), ciprofloxacin (5 µg/disk), rifampin (30 µg/disk), tetracycline (30 u/disk), tobramycin (25 µg/disk), nitrofuranton (10 µg/disk), chloramphenicol (30 µg/disk), amikacin (30 u/disk) and gentamicin (30 µg/disk). Results were read as susceptible or resistant according to the criteria recommended via the CLSI and the manufacturer's protocol (Mast Group, Bootle, UK).

DNA Extraction and Primer Design

Total genomic DNA of *E. coli* isolates found to be resistant to at least one antibiotic in the disk diffusion method were extracted using a DNA extraction kit (AccuPrep® Genomic DNA Extraction Kit, Bioneer, Daejeon, South Korea).

The desired gene sequence was extracted from the National Center for Biotechnology Information (NCBI) database and then a pair of specific primers was designed for each gene by using the online application, Primer3 (http://frodo.wi.mit.edu/primer3). The specificities of the primers were evaluated using Primer-blast by Uniform Resource Locator (URL) (http://www.ncbi.nih.gov/tools/primer-blast).

qnr Gene Amplification by Polymerase Chain Reaction (PCR)

The *qnrS*, *qnrA* and *qnrB* genes were amplified in quinolone and fluoroquinolone- resistant *E. coli* strains using the following primers: Forward: 5′-ACG ACA TTC GTC AAC TGC AA-3′ and Reverse: 5′-TTA ATT GGC ACC CTG TAG GC -3′ to amplify the 417 bp fragment of the *qnrS* gene; Forward: 5′-ATTTCTCACGCCAGGATTTG-3′ and Reverse: 5′- GATCGGCAAAGGTTAGGTCA-3′ to amplify the 516 bp fragment of the *qnrA* gene and Forward: 5'-GTT GGC GAA AAA ATT GAC AGA A-3' and Reverse: 5′-ACT CCG AAT TGG TCA GAT CG-3′ to amplify the 469 bp fragment of the *qnrB* gene.(24,25)

The amplification conditions included an initial denaturation step of 95ºC for 5 minutes, 30 cycles consisting of 94°C for 1 minute, 52.2°C, 53.4°C 51.2°C for 1 minute for *qnrS*, *qnrB* and *qnrA* genes, respectively and 72°C for 1 minute, and 72°C for 7 minutes in the final extension. The *E. coli* ATCC 25922 obtained from the Institute of Hygiene, University of Palermo in Italy were used as a positive control and strains which lack the qnr genes in Molecular Biology Research Center, University of Medical Sciences, Tehran were selected as negative control. The electrophoresis of PCR products was performed on 1.5% agarose gel, and then the gels were stained in ethidium bromide for 15 minutes and visualized in a gel document system (BioRad XR+, Hemel Hempsted, UK).

Results

All 100 *E. coli* isolates tested showed resistance to all the antibiotics used with the greatest resistance being recorded to rifampin (93.6%), followed by gentamicin (27.1%) while the least resistance was to nitrofuranton (1.0%) (Figure 1).

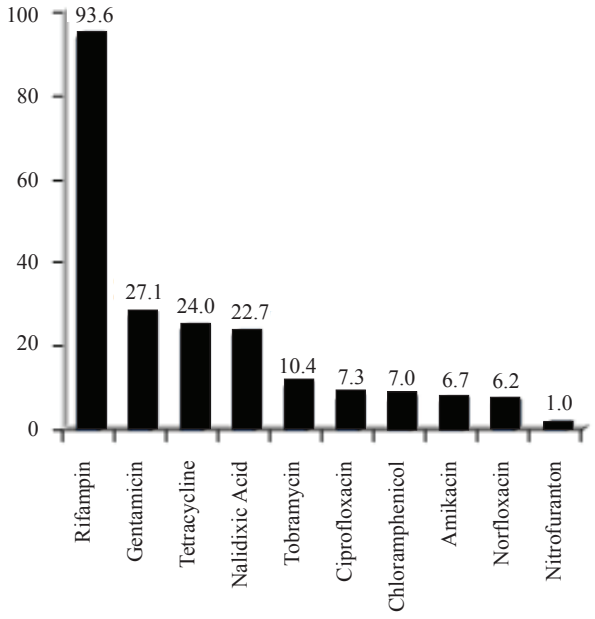

Figure 1. The rate and percent of resistance *E. coli* isolates to antibiotics was shown.

Furthermore, 22.7%, 7.3% and 6.2% of the isolates were resistant to Nalidixic acid, Ciprofloxacin and Norfloxacin respectively. The highest intermediate phenotypes were recorded against nalidixic acid (8.24%) while the lowest intermediate phenotypes were recorded against ciprofloxacin (4.16%). 17% of *E. coli* isolates were resistant to at least one of the tested quinolone antibiotics, and 6% were susceptible to nalidixic acid, ciprofloxacin and norfloxacin, simultaneously (Table 1).

Table 1. Quinolone and fluoroquinolone-resistant isolates by disk diffusion method

Antibiotic	Isolates, n (%)		
	Resistant	Intermediate	Sensitive
Nalidixic acid	22.7	8.1	69.2
Ciprofloxacin	7.3	4.5	88.2
Norfloxacin	6.2	6.3	87.5

Of the 22 quinolone-resistant isolates, 95.4%, 40.9% and 4.5% were positive for *qnrS*, *qnrB* and *qnrA* genes respectively (Figures 2, 3 and 4). The prevalence of qnr genes among eight quinolone-intermediate isolates were 87.5% for *qnrS*, 0% for *qnrB* and *qnrA*. Of the 30 quinolone-resistant and intermediate isolates, 26.6% had the *qnrS* and *qnrB* genes, 3.3% had the *qnrS* and *qnrA* genes while 0 % had the *qnrA* and *qnrB* genes simultaneously.

Discussion

Food and water-borne infections constitute public health concerns especially in under-developed and developing countries, and *E. coli* plays an important pathogen involved gastrointestinal and urinary tract diseases.(26,27) Multi-drug resistance of *E. coli* strains recorded in this study is in tandem with the reports of various studies.(26-30) This may be suggestive of high rate of contamination of various water sources with human and animal wastes with antibiotic resistant *E. coli* strains in the Alborz province, Iran. Furthermore, these antibiotic resistant *E. coli* strains in the aquatic environment may predispose to the spread of the antibiotic resistance among other closely related organisms usually by the exchange of genetic information.(26,27) The relatively high level of resistance to quinolones recorded in the present study could be attributed to misuse or abuse of these antibiotics.

The 22.7%, 7.3% and 6.2% resistance rates to nalidixic acid, ciprofloxacin and norfloxacin respectively recorded among water-borne *E. coli* strains in this study corroborates various reports indicating the increase in fluoroquinolone resistance in *E. coli* isolates of different origins.(24,28-

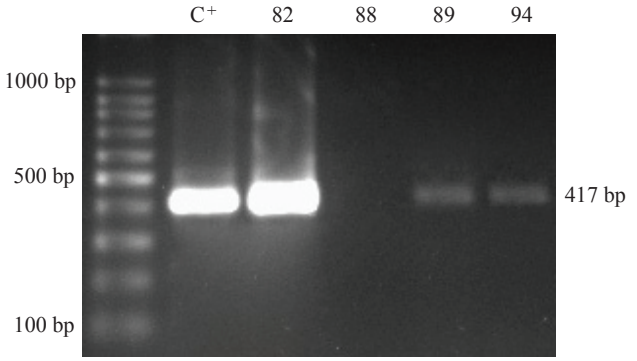

Figure 2. PCR amplification of *qnrS* gene in resistant *E. coli* isolates. Electrophoresis of PCR product on 1.5% agarose gel. First lanes, Marker 100 bp; lane 2 (C+), *E. coli qnrS*-positive control; lane 4 (No.88), *E. coli qnrS*-negative isolate; lanes 3 (No.82), 5 (No.89) and 6 (No.94), *E. coli qnrS*- positive isolates.

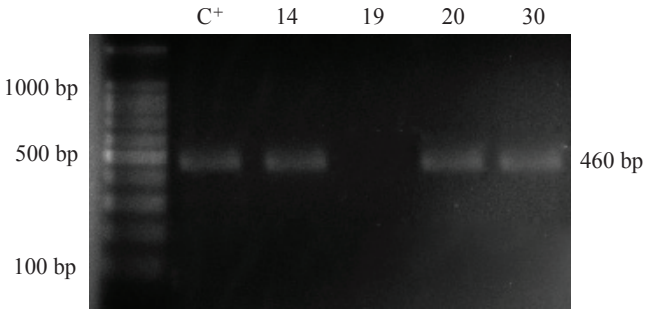

Figure 3. PCR amplification of *qnrB* gene in resistant *E. coli* isolates. Electrophoresis of PCR product on 1.5% agarose gel. First lanes, Marker 100 bp; lanes 3 (No.14), 5 (No.20) and 6 (No.30), *E. coli qnrB*-positive isolates; lanes 4 (No.19), *E. coli qnrB*-negative isolate; lane 2 (C+), *E. coli qnrB*-positive control.

31) Similarly, high resistance rates to fluoroquinolones were reported by various studies on water worldwide.(32-34) The most culpable reason for the resistance patterns of *E. coli* observed in this study is the indiscriminate use of antibiotics for medical and veterinary management of infections which has been reported to predispose to the development of resistance.(30)

However, the discrepancies in the patterns seen in different reports may be associated to the differences in animal husbandry practices and environmental conditions. Several study from India, Canada and Bangladesh, reported the presence of antibiotic resistant *E. coli* in water.(35-37) A higher frequency of resistance against β-lactam, quinolone and floroquinolone antibiotics was observed among the isolates from household water in study by Talukdar, *et al.*(37)

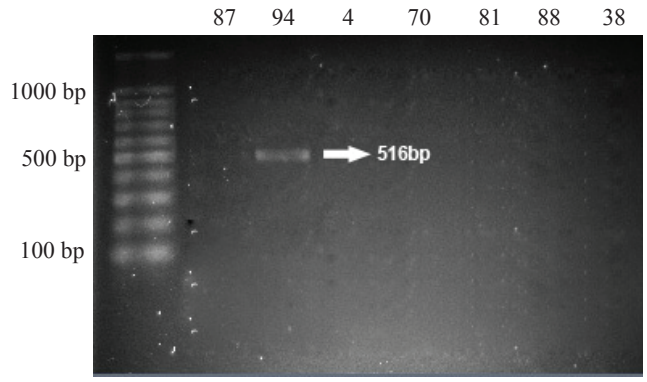

Figure 4. PCR amplification of *qnrA* gene in resistant *E. coli* isolates. Electrophoresis of PCR product on 1.5% agarose gel. First lanes, Marker 100 bp; lane 2 (No.87) and lane 4-8 (No.4, 70, 81, 88, 38), *E. coli qnrA*-negative isolates; lanes 3 (No.94), *E. coli qnrA*- positive isolate.

A *qnrS* gene was identified in a water-borne bacterial species, isolated from the River Seine in Paris and Swiss lake.(37) The prevalence of 28%, 9% and 1% of *qnrS*, *qnrB* and *qnrA* genes respectively in the quinolone-resistant *E. coli* isolates in the present study was higher than the report in India, where the rate of qnr genes among resistant isolates of *E. coli* was 0.85%.39 Quinolone resistance was also recorded in some *qnrS*, *qnrA* and *qnrB*-negative isolates in this study which signifies that other qnr genes or resistance mechanisms, such as mutations in the target enzyme (*e.g.*, DNA gyrase and topoisomerase IV) and/or activation of efflux pumps, may be involved.42 Point mutations related to fluoroquinolone resistance were observed at S83 to L and D87 to N or Y in the GyrA subunit and S80 to R or I and E84 to G in the ParC subunit.(38-42)

Conclusion

In conclusion, to the best of our knowledge, this is the first report of the presence of fluoroquinolone-resistance genes in *E. coli* isolated from surface waters in Iran. The results of this study revealed a higher resistance to nalidixic acid than other fluoroquinolones with a predominance of qnrS gene among the quinolone-resistant *E. coli* isolates while the occurrence of the *qnrA* gene was lowest. The presence of these genes in these *E. coli* strains portends the risk of transfer of genetic information via plasmids to other bacteria there by increasing the antibiotic resistance reported in these bacteria.

These findings emphasize the importance of prescribed use of these antibiotics which will help to limit the potential spread of resistant genes. Proper regulation is needed to ensure that the correct dosages of these drugs are administered when they are indicated as the drug of choice in an infection. To gain more insight into the molecular characterization of quinolone-resistant *E. coli* isolates, other possible mechanisms of resistance, changes in expression of efflux pumps, or even novel mechanisms should also be investigated. Therefore, immediate measures are needed to control and treat water sources more effectively.

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