

Detection of *qnrB* Gene among Quinolone Resistant *Escherichia coli* Isolated from Kermanshah Hospitals

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ABSTRACT

Background: Urinary tract infections are considered as a major health concern. *Escherichia coli* is the most common cause of urinary tract infections. The presence of *qnr* plasmid genes in bacteria is the main cause of resistance to quinolones. The aim of this study was to investigate the antibiotic resistance pattern and prevalence of *qnrB* gene in *E. coli* strains isolated from patients with urinary tract infections.

Materials & Methods: In this cross-sectional study, samples were taken from patients with urinary tract infections, referred to Kermanshah hospitals during the spring of 2017. *E. coli* strains were identified by biochemical tests. Then antibiotic susceptibility testing was performed for the isolates by the disc diffusion method. Following that, *qnrB* resistance gene was detected by PCR; finally, data were analyzed by SPSS software Ver. 23.

Findings: In this study, 105 *E. coli* strains were isolated from urine specimens. The strains resistance rate to nalidixic acid, ciprofloxacin, and ofloxacin antibiotics was 62.85, 38.09, and 33.33%, respectively. PCR results showed that 67 strains (63.8%) had *qnrB* gene, and 38 strains (36.19%) lacked this gene. Logistic regression analysis showed that there was a significant relationship between the presence of *qnrB* gene and nalidixic resistance.

Conclusion: The results of this study show that the frequency of *qnrB* gene among the *E. coli* strains isolated from urinary tract infections is fairly high in Kermanshah. Therefore, it is necessary to do further investigations using molecular techniques and to take serious preventive measures.

Keywords: *Escherichia coli*, Quinolones, *qnrB*, Urinary tract infection.

CITATION LINKS

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Introduction

Escherichia coli is a Gram-negative, facultative anaerobic, and non-spore-forming bacterium. This bacterium is transmitted from person to person through the fecal-oral route. The optimum growth of the bacterium is at 37 ° C, but it could tolerate up to 49 ° C [1].

Urinary tract infection (UTI) is one of the most common bacterial infections and the second reason for referral to the hospital. Each year in the United States, 150 million people suffer from urinary tract infections, and the total cost of treatment is estimated to be \$ 6 billion. Among all bacterial pathogens causing urinary tract infection, *E. coli* is the most common organism isolated from 75 to 90 % of outpatients with UTI [2-3]. Thus, investigation of the antimicrobial resistance pattern of *E. coli*, as the most common cause of UTI, is crucial. This pattern varies in different parts of geographical regions, which could be due to the use of different types and dosages of antibiotics [4]. Therefore, it is important to use specific classes of antibiotics in a region based on local research and studies.

The early stage of antibiotic treatment of UTI is usually done experimentally; therefore, accurate and up-to-date information about antibiotic susceptibility of local strains is necessary. Today, drug resistance has increased globally among UTI patients. The selection of antibiotic classes in experimental treatment is currently under discussion; up to now, between 20 to 50 % of *E. coli* strains have been resistant to first-line antibiotics, even in developed countries [5]. Determining the antibiotic susceptibility pattern and regular monitoring of this pattern could help prevent the overuse of broad-spectrum antibiotics and reduce costs [6].

It could be said that the increase in multi-drug resistance has become a major concern over the past decade, limiting the treatment

of different types of infection. Antibiotic resistance has been shown to be often due to the overuse and misuse of antimicrobial agents [7].

Objectives: The aim of this study was to investigate the antibiotic resistance pattern and prevalence of *qnrB* gene in *E. coli* strains isolated from urine samples in Kermanshah province.

Materials and Methods

Sampling: In this study, 120 urine culture samples were collected from patients with urinary tract infections in several hospitals in Kermanshah from April to June 2017. Informed consent form was obtained from all the participants. Urine samples of these patients were diagnosed to be infected by urinary tract infections in the relevant laboratories, and the cause of these infections was also diagnosed as *E. coli*. The diagnosis of UTI was based on clinical and laboratory criteria set by the Centers for Disease Control and Prevention (CDC), including having suprapubic and flank pains, the presence of leukocytes or blood in the urine, and finally, a positive culture with a colony count of 10⁵ CFU/mL.

Isolation and identification of the strains: All the samples were cultured on EMB agar. All the colonies on EMB agar were considered as Gram-negative isolates. All the colonies apparently similar to *E. coli* isolates were confirmed by biochemical tests, including Gram staining, citrate, urease, indole, mobility, methyl red, Proskauer-Voges, and triple sugar iron agar tests.

Bacteria preservation: Maintenance of the strains in order to avoid any genetic mutation is important. In the current study, TSB was used to store the strains. Several pure colonies were inoculated, completely dissolved, and kept at 37° C for 24 hours. Then 200 µL of glycerol was added. These samples could be stored for at least 6 months.

Antimicrobial susceptibility test: The susceptibility of *E.coli* isolates against 3 antibiotics ciprofloxacin, nalidixic acid, and ofloxacin was tested by agar disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines 2019. *E. coli* ATCC25922 was used as a control for the disk diffusion method.

DNA extraction by boiling method: Two colonies of overnight grown bacteria were used. The colonies were put in a test tube containing one mL of distilled water, boiled for 10 minutes in a water bath, and centrifuged for 5 minutes at 10000 rpm. Then 5 μ L of supernatant was used for PCR. Agarose gel electrophoresis was used to check DNA samples.

The proliferation of *qnrB* gene by polymerase chain reaction: The primers used to amplify *qnrB* gene were described previously [8]. PCR was performed in a total volume of 25 μ L reaction mixture containing 12.5 μ L of Taq Green PCR master mixture (2X) (Ampliqon, Denmark), 7.5 μ L of double-distilled water, 1 μ L of each primer (10pmol/ μ L), and 3 μ L of DNA template. PCR conditions consisted of an initial denaturation step at 93°C for 5 min; followed by 30 cycles of denaturation at 93°C for 60 sec, 57.4°C for 45 sec, and 72°C for 45 sec; and a final extension step at 72°C for 10 min. To prepare a 3% agarose gel, 9 g of agarose powder was added into 30 mL of TBE 1X buffer and heated for 30 seconds. About 6 μ L of PCR product was used for electrophoresis.

Findings

Sampling, isolation, and identification of strains: In this study, 105 *E. coli* strains were isolated from urine specimens in Kermanshah hospitals from April to June 2017. Information about patients was collected from their hospital records, including gender, age, previous history of UTI, symptoms of lower or upper urinary

tract infection, and history of hospitalization in the past month (possibility of nosocomial infection). Out of 105 patients, 26.3% were outpatients, and 73.7% were inpatients. All *E. coli* strains were isolated from adult women in the age range of 19 to 65 years. Out of 105 samples, 73.5% of the samples were collected from patients with cystitis, and 26.5% were collected from patients with pyelonephritis. The biochemical results are shown in Figure 1.



Figure 1) Differential biochemical tests

Antibiogram Results: In order to determine the resistance profile of 105 *E.coli* isolates against ciprofloxacin, nalidixic acid, and ofloxacin antibiotics, the disk diffusion method was performed. The results indicated that 66 (62.85%), 36 (34.28%), and 3 (2.58%) strains were resistant, intermediate, and susceptible to nalidixic acid, respectively. Also, 40 (38.09%), 59 (56.19%), and 6 (5.71%) strains were resistant, intermediate, and susceptible to ciprofloxacin, respectively. Lastly, 35 (33.33%), 64 (61.9%), and 6 (4.78%) strains were resistant, intermediate, and susceptible to ofloxacin. The highest overall resistance was observed to nalidixic acid (62.85%), while the highest susceptibility was observed to ofloxacin (61.9%).

The results of the identification of *qnrB* gene: In this study, *qnrB* gene sequences were amplified by PCR method using specific primers. Of the 105 strains examined, 67

strains (63.8%) had *qnrB* gene, and 38 strains (36.19%) were negative for the presence of *qnrB* gene (Figure 2).

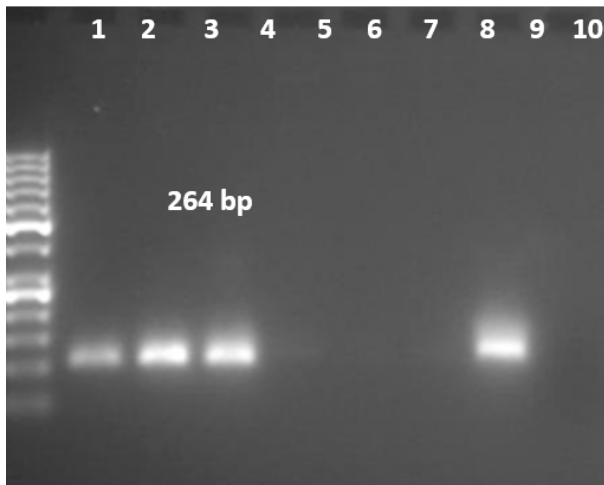


Figure 2) The PCR results for *qnrB* gene. Marker 100 bp, 1: positive control, 2, 3, 4, and 8: positive samples, 5, 6, and 7: negative samples, Column 9: negative control

Statistical analysis: Statistical analysis was performed by SPSS software Version ... using chi-square (X^2) test showing that there was a significant relationship between the presence of *qnrB* gene and resistance to nalidixic acid, ciprofloxacin, and ofloxacin (p -value $<.001$).

Discussion

In this study, 105 *E. coli* strains were isolated from urine specimens during 2017. The highest overall resistance was observed to nalidixic acid (62.85%), while the lowest resistance was observed to ofloxacin (33.33%). Of the 105 strains, 67 strains (63.8%) had *qnrB* gene, and 38 strains (36.19%) were *qnrB* negative.

Based on the statistical results, the relationship between the presence of *qnrB* gene and resistance to both nalidixic acid and ciprofloxacin was significant (p -value $<.001$). Here, OR (as $x_p(B)$) was 24.167. This means that the strains containing *qnrB* gene were 24.167 times more likely to be resistant to nalidixic acid. But the relationship between

the presence of *qnrB* gene and ofloxacin resistance was not significant (p -value $>.05$). In the present study, the resistance rate to nalidixic acid, ciprofloxacin, and ofloxacin was 62.85, 38.09, and 33.33%, respectively. Since nalidixic acid was produced in 1962 and has been used for more than seven decades to treat urinary tract infections, bacterial resistance to this antibiotic is expected to be higher than other quinolone antibiotics.

In a study conducted by Nakhchivani et al. (2007) on *E. coli* strains isolated from patients with urinary tract infections in Tehran, the resistance rate to nalidixic acid and ciprofloxacin was reported to be 49.3 and 40.2 %, respectively [9]. Comparing the results, it was found that nalidixic acid resistance in the present study was much higher, which could be due to the increasing trend of drug resistance over a period of 11 years. However, in both studies, the level of resistance to nalidixic acid was high.

In another study conducted on *E. coli* strains isolated from urine specimens in Tehran in 2010. the resistance rate to nalidixic acid and ciprofloxacin was reported as 74 and 54.5%, respectively [10].

In a study conducted in Pakistan in 2011, the resistance rate of *E. coli* isolates to ciprofloxacin and nalidixic acid was 36.45 and 84.16%, respectively, indicating a relatively high resistance [11]. This could be due to the overuse without precise monitoring of drugs in developing countries.

In a study conducted in the United States in 2006, quinolones and fluoroquinolones resistance of uropathogenic *E. coli* isolates was 21 and 12% , respectively [12]. The difference in drug resistance rate between these two regions (United States and Between the two groups of antibiotics) could be due to the implementation of more accurate surveillance programs in the United States.

In another study by Talan et al. (2008) in USA, *E. coli* strains causing urinary tract infections

showed the highest rate of resistance to fluoroquinolones. Among the 403 patients with uncomplicated pulmonary disease, the mean prevalence of *E. coli* strains resistant to TMP-SMX was 24 % (range: 13- 45%). The mean resistance to ciprofloxacin and levofloxacin was 1 and 3%, respectively [13]. The difference in drug resistance rates between the present and Talan studies could be related to the difference in geographical areas.

Resistance to ciprofloxacin and imipenem in Fasa was determined as 22.62 and 11.11% using the disk diffusion method, respectively [14]. The difference in resistance rates between the present and Fasa studies could be related to the time difference between the two studies.

In a study by Zhou et al. (2002-2005) in China, the frequency of *qnrS*, *qnrB*, and *qnrA* genes in 514 *E. coli* isolates was determined as 0.4, 1.2, and 2.7 %, respectively [15]. According to the results, the prevalence of *qnrB* gene was much less than that obtained in the current study. This difference could be due to the existence of precise monitoring programs in China and time difference between the two studies.

In 2009, Shane et al. examined the frequency of *aac* (6) -*lb-c* gene in ESBL-producing clinical isolates of *E. coli* and *Klebsiella*. Their findings showed that 61.1% of the isolates carried this gene, and the frequency of *aac* (6) -*lb-c* gene in the isolates carrying *qnr* genes (67.4%) was higher than in those which were *qnr* negative (51.7%) [16]. The results of the current study also indicated that the presence of *qnr* genes had a significant relationship with the resistance to nalidixic acid. Therefore, proper treatment of patients with *qnr*-positive bacterial infection is crucial.

In a study conducted by Soleimani and colleagues (2012) in Khorramabad on 140 isolates of *E. coli* to investigate the prevalence of *qnrA* gene by PCR method, it was deter-

mined that from a total of 140 isolates, 116 (82.8%) and 63 (43%) isolates were resistant to nalidixic acid and ciprofloxacin, respectively. Of which 14 (12.1%) nalidixic acid-resistant isolates and 9 (14.3%) ciprofloxacin-resistant isolates contained *qnrA* gene [17]. This comparison shows that resistance to nalidixic acid and ciprofloxacin in Khorramabad is more than in Kermanshah, which may be due to the appropriate monitoring of standards in Kermanshah, especially in Kermanshah hospitals.

In a study carried out by Naderi Nasab (2011) in Imam Reza hospital in Mashhad on 200 clinical isolates of *E. coli* to investigate the prevalence of *qnr* and ESBL genes, it was found that out of 200 isolates, 63 (31%) isolates contained *qnrA* gene, 34 (17%) isolates contained *qnrB* gene, 14 (7%) isolates contained *qnrS* gene, and 85 (42.5%) isolates were ESBLs producer [18]. The comparison of these studies results suggests the difference in the prevalence of *qnrB* gene between the provinces of Iran. It could be concluded that the prevalence of resistance genes varies in the provinces of a country. The difference in the prevalence rates of resistant genes between the present and Naderi's studies could be due to the differences in demographic features, type of samples, antibiotics used, geographical conditions, and the prevalence of *E. coli* infections.

In the present study, the prevalence of *qnrB* gene was 63.8%, while in another study by Park et al. (2011), it was reported to be 11.52% [19]. Preventing uncontrolled use of antibiotics by individuals, observing personal and public health, controlling health standards in hospitals and health centers, and observing the standards of infectious waste disposal in health centers may be the reasons for the lower prevalence of this gene in Korea than in Kermanshah-Iran.

In a study conducted by Shyamala (2011-2012) in India on a total of 464 intensive

care unit patients, it was found that 44 cases were positive for *E. coli*, of which 31 (70.45%) isolates were resistant to ciprofloxacin [20]. Resistance to ciprofloxacin in the present study was 38.09 %, which is much lower than that reported by Shyamala in India. This comparison shows that India has not done well in terms of observing health standards and monitoring antimicrobial resistance pattern.

Cai and his colleagues (2011) in China examined a total of 179 Gram-negative bacteria and investigated the prevalence of *qnrA*, *qnrB*, and *qnrS* genes. They reported that *qnrB* gene was present in 6.2 and 7.6% of *E. coli* and *E. cloacae* isolates, respectively [21]. Comparing the present and their studies results indicates a very high prevalence of *qnrB* resistance gene in Iran (Kermanshah) in comparison to the developed country of China.

In a study conducted by Mansori et al. (2011) in Milad hospital in Tehran on 150 *E. coli* isolates to investigate resistance to quinolones and frequency of ESBLs, it was found that 69 samples (46%) were ciprofloxacin resistant, and 106 samples (70.6%) were resistant to at least one quinolone. The frequency of *qnrA*, *qnrB*, and *qnrS* genes was 22 (56.5%), 39 (31.8%), and 20 (28.9%), respectively. The highest frequency was related to *qnrB* 39 (56.5%) [22]. In both studies conducted in Tehran and Kermanshah, the prevalence of *qnrB* gene was high, but it was slightly higher in Kermanshah (63.8%), although this difference was not significant.

In the present study, there was a significant correlation between the presence of *qnrB* gene and the rate of nalidixic acid resistance. Akya et al. (2017) also found that there was a significant correlation between the presence of *qnrB* gene and resistance to ciprofloxacin, levofloxacin, and nalidixic acid in *E. coli* isolates [23].

The association between the presence of *qnrB* gene and nalidixic acid resistance is

more important than before, which should be considered as an important reason to prevent further spread of *E. coli* strains containing *qnrB* gene.

Conclusion

In the present study, the resistance rate to quinolones was high among *E. coli* strains isolated from urine specimens in Kermanshah. *qnrB* gene was also highly prevalent in these strains, which could be due to the overuse of antibiotics without prescribing.

Finally, the relationship between the presence of *qnrB* gene and nalidixic acid resistance is important in terms of controlling and adhering to personal, public, and hospital health-promotion standards related to antibiotics use. Therefore, the results of this study could be used by physicians to prescribe appropriate antibiotics and prevent the wild spread of resistant isolates in the healthcare system.

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