

# Molecular Detection of Plasmid-Mediated Quinolone Resistant Genes in Uropathogenic *E. coli* from Tertiary Referral Hospital in Tehran, Iran

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## Abstract

**Background:** Fluoroquinolone antibiotics are usually used for the treatment of urinary tract infections. The aim of this study was to determine the prevalence and molecular characterization of Plasmid-Mediated Quinolone Resistance (PMQR) genes among ESBL-producing *Escherichia coli* isolates obtained from tertiary referral hospital in Tehran, Iran.

**Methods:** A total of 150 uropathogenic *E. coli* isolates were obtained from Rasool-e-Akram hospital. The bacterial isolates were identified by standard laboratory methods. Then, the susceptibility to quinolone antibiotics was assessed by standard disk diffusion method. The PCR method was used to show presence of *qnrA*, *qnrB*, *qnrS*, *aac(6)-Ib-cr* and *qepA* genes.

**Results:** Overall, 79 of the 150 isolates (52.6%) were non-susceptible to quinolone antibiotics. Out of 79 quinolone non-susceptible isolates, 46 (58.2%) isolates harboured PMQR-encoding genes. Further, 36 (24%) had *aac(6)-Ib-cr* gene and interestingly, amplification assays showed that 33 (41.8%) out of 79 quinolone non-susceptible isolates carried only *qnrB* gene. Also *qnrA*, *qnrB* (30.9%), *qepA* (7.3%) and *qnrS* (25.4%) genes showed.

**Conclusions:** This study showed a high prevalence of *aac(6)-Ib-cr*, *qnrB*, *qnrS* and *qnrA* genes in the uropathogenic *E. coli* isolates from tertiary referral hospital. Therefore, the application of proper infection control and well-established antibiotic prescription guidelines seems to be highly needed in our medical centers.

**Keywords:** Quinolones resistance; Urinary tract infection; *aac(6)-Ib-cr*; Pyelonephritis; Cystitis

## Introduction

Urinary Tract Infection (UTI) due to *Escherichia coli* is the most common bacterial infection. Fluoroquinolones are commonly used for the treatment of UTI because

isolated microorganisms are frequently resistant to aminopenicillins and trimethoprim-sulfamethoxazole and fluoroquinolones which are given orally [1].

Quinolones are among the main groups of antimicrobial

compounds which are used against bacterial infections. However, many recent studies have shed light on the fact that the resistance of various members of Enterobacteriaceae, likely *Salmonella* and *Klebsiella*, to quinolones and fluoroquinolones is increasing, globally [2]. Resistance to the new generations of antibiotics and thereafter the development of resistant strains has become prevalent not only among the nosocomial infections but also among community-acquired infections.

Recent studies have highlighted the increasing rate of the detection of quinolone-resistant *E. coli* strains [3]. Interestingly, the infections by such strains show a multi-drug resistance to beta-lactams and aminoglycosides [4]. Recent finding showed that the plasmid-mediated quinolone resistance (PMQR) might be the main cause of bacterial resistance to quinolones. There are three major groups of gene markers on qnr plasmids; *qnrA*, *qnrB*, and *qnrS* genes [5,6].

There have never been any reports concerning the prevalence of *qnr* genes in *E. coli* isolates from patients with urinary infection in Tehran Hospitals. Thus, the aim of survey was studying the *qnr* markers in *E. coli* isolates which their ESBL capability was already confirmed. These isolates were originally isolated from children with urinary infection.

## Materials and Methods

### Patients

The inclusion criteria were age between 1 day until 5 years and the presence of cystitis symptoms (pain on urination, pollakiuria, supra-pubic pain). Patients with recurrent UTIs (three episodes of UTI during the common year), could be included.

### Sampling urine and microbiological evaluation

Exclusion criteria were antibiotic treatment in the previous two weeks. Every patient gave their informed consent. A fresh midstream urine or catheter sample or supra pubic was collected from all eligible patients, respecting aseptic conditions. The microbiological definition of UTI was leukocyturia  $\geq 10$  leukocytes/mL and bacteriuria  $\geq 10^5$  cfu/ml in urine collected from midstream and in urine collected with catheter  $>10^2$  cfu/ml for supra-pubic.

### Isolation and bacterial identification of bacteria and assessment of their antibiotic susceptibility

*E. coli* isolates of urine samples were incubated in LB medium for 18h, 37°C. The plasmid DNA was extracted using a Bioneer kit (Kit number: K-3030-2). The used primers for the amplification of the given *qnr* genes are listed in (Table 1). The PCR was directed in a thermal cycler (SENSOQUEST, Germany) and the program was as below: a cycle of 94°C, 5 min followed by 35 cycles of 95°C,

Target genes	PCR product	Primer sequences (5'→3')	Annealing temperatures(°C)
<i>qnrA-F</i>	516 (bp)	ATTTCTCACGCCAGGATTTG	56
<i>qnrA R</i>		GATCGGCAAAGGTTAGGTCA	
<i>qnrB-F</i>	469 (bp)	GATCGTGAAAGCCAGAAAGG	55
<i>qnrB R</i>		ACGATGCCTGGTAGTTGTCC	
<i>qnrS-F</i>	417 (bp)	ACGACATTCGTCAACTGCA	57
<i>qnrS R</i>		TAAATTGGCCTGTAGGC	

**Table 1:** Primers used in this study to screen the isolates for plasmid-mediated Ciprofloxacin-resistance genes.

1 min (denaturation), 54°C, 1 min (annealing), and 72°C, 1 min (extension). The PCR products were electrophoresed on a 2% agarose gel to visualize the amplified genomic fragments. Finally, the collected data was analyzed statistically using SPSS22 package. Results are for analyses of virulence genes as detected by PCR only.

## Results

In general, 33.3% (50 people) of the studied people were outpatients and the remaining 66.6% (100 people) were

in-patients. After studying these patients for 12 months, 74 (49.3%) patients showed at least a relapse of urinary infection, and in these cases, 68% of the isolates were resistant to ampicillin, (64%) tetracyclin, (60.6%) co-trimoxazole, (57.3%) piperacillin, respectively. Besides, the highest antibiotic susceptibilities were detected for imipeneme, meropeneme (100%), amikacin (98%), and (96.7%) piperacillin/tazobactam, respectively (Figure 1).

Further, 82 isolates (54.6%) showed resistance to three or more antibiotic classes and were categorized as multidrug resistance (MDR) strains. The prevalence of antibiotic

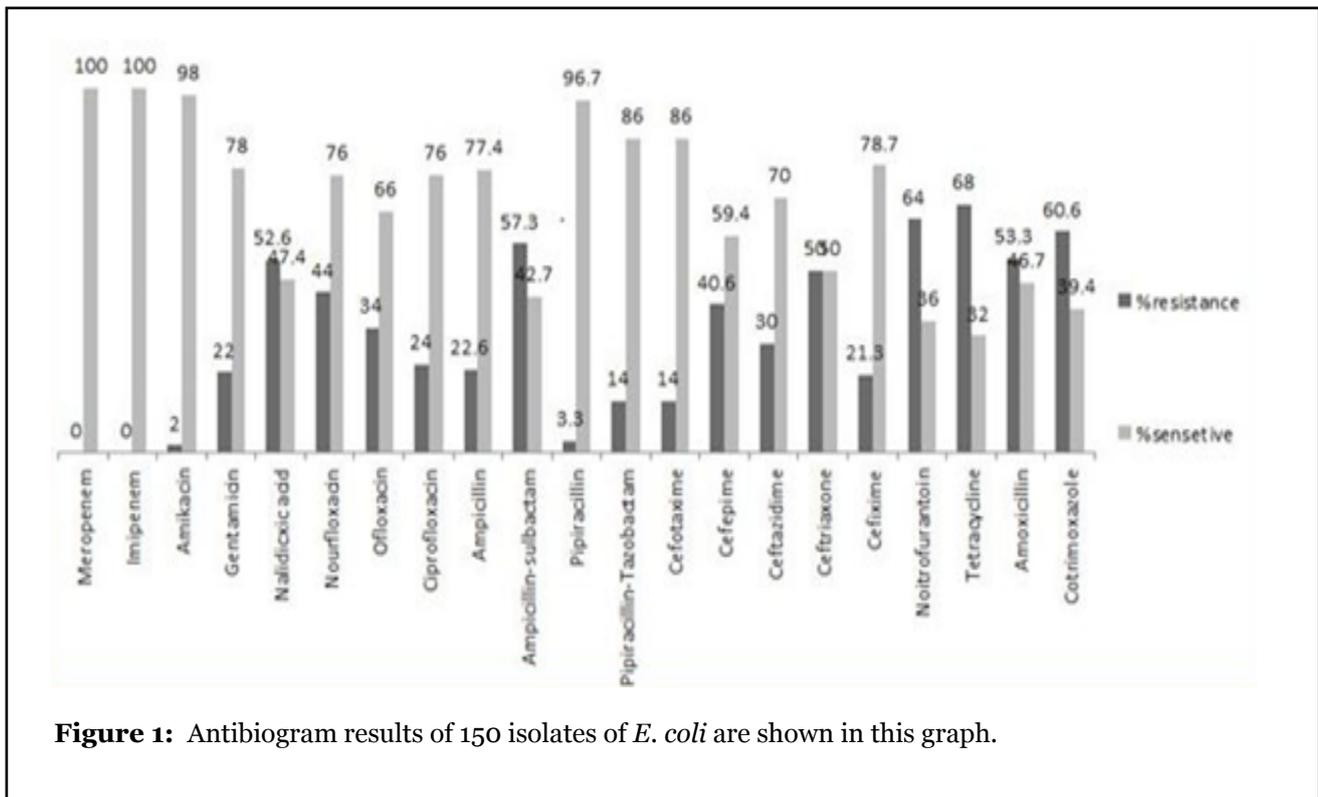


Figure 1: Antibiogram results of 150 isolates of *E. coli* are shown in this graph.

No (%). of positive isolates in UTI patients				Total No (%). of positive isolates (n=150)
Resistance genes	In patients (n=100)	Out patients (n=50)	P value	
<i>qepA</i>	9 (9%)	2 (4%)	0.26	11 (7.3%)
<i>qnrA</i>	4 (4%)	1 (2%)	0.52	5 (3.3%)
<i>qnrB</i>	22 (22%)	11 (22%)	0.05	33 (22%)
<i>qnrS</i>	18 (18%)	9 (18%)	0.5	27 (18%)
<i>aac(6')-Ib-cr</i>	20 (20%)	16 (32%)	0.105	36 (24%)

Table 2: The prevalence of antibiotic resistance genes in UPEC isolates in outpatients and in-patients.

resistance genes and their distribution is demonstrated in (Tables 2).

## Discussion

In this study, 52.6% and 24% resistance was detected to nalidixic acid and ciprofloxacin; these statistics seem to be similar to those of Peimani et al., [7]. But, our results show higher resistance compared to those of Raei et al., [8] [18.57% (NA) and 36.2% (Cp)] and Zamani et al., [9]. [23.8% (NA) and 34.1% (Cp)]. Considering the increasing rise of resistance to extended-spectrum antibiotics in hospitals and also communities, an outbreak of quinolone resistant isolates in hospitals and clinical settings seem inevitable. Also, taking non-prescribed antibiotics can worsen the situation [7-9]. These studies highlight the particularity of fluoroquinolone resistance in *E. coli* for Iran's tertiary hospitals; however, a shortfall exists in information about the molecular epidemiology in those of county status. Also 52.6% of *E. coli* isolates were non-susceptible to quinolone antibiotics. Resistance to fluoroquinolones in *E. coli* is quite high in many European countries ranging from 25% to 50 [10].

In this study, *qnrA* (18%), *qnrB* (30.9%) and *qnrS* (25.4%) were detected in quinolone resistant *E. coli*. This report is the first which concerned the prevalence of quinolone resistance in Rasool-e-Akram Hospital, Iran. Pakzad et al., showed that 37.5% and 20.8% of the detected ESBL *E. coli* were *qnrA*+ and *qnrB*+, respectively [11]. Saiadpour et al. showed that 30.4% of the community which they studied had *qnr* and/or *aac(6)-Ib-cr* genes [12]. Wang et al. reported 2.4%, 6.1% and 15.1% prevalence of *qnrA*, *qnrB* and *qnrS* genes in the *Klebsiella* isolates, respectively [13]. Also, the coincidence of the *qnr* and *bla* (beta-lactamases), which is of a considerable significance, has been reported. Also, *qnrA* gene was detected in only five *E. coli* isolates. Peimani et al., [7], in their study has not detected *aac(6)-Ib-cr* gene in *Klebsiella* isolates of 250 urinary infection samples. Peimani et al. [7] also reported *qnrB* gene as the most prevalent gene in those *Klebsiella* isolates.

In this study, 46% of the *qnr*+ isolates showed the highest resistance to quinolones. Interestingly, a survey in the ICU and CCU hospital units showed that *qnr* genes were detected in the isolates of the patients who experienced multiple relapses of urinary infection, annually. Also in this study, *qnrS*, *qnrB*, *aac(6)-Ib-cr* genes were found as the most prevalent antibiotic genes in UPEC isolates. The critical role of these genes in antibiotic resistance of such bacteria has been already highlighted. Comparing the results to those of similar investigations in Iran and other countries, *aac(6)-Ib-cr* gene was found as the most prevalent antibiotic resistance gene in UPEC isolates. Ma et al. [14] showed a 18.8% prevalence of *aac(6)-Ib-cr* gene. In comparison, Arabi et al. [15] and Momtaz et al. [16]

indicated 34% and 46.34% prevalence of this gene in UPEC isolates. Interestingly, it has been already shown that this gene, which is located on a plasmid, is not transmitted through the well-known plasmid transmission ways. In addition, there is a report concerning the plasmids which may have a role in the distribution of this gene. However, this gene is an integron cassette insertion (ICI) indeed, and so, it might be transmitted among various plasmids. Large-scale administration of quinolones and/or cephalosporines to food-production animals might select for cephalosporin-resistant (*bla<sub>CTX-M</sub>*) and plasmid-mediated quinolone resistant *E. coli* strains in animals.

The *aac(6)-Ib* gene encodes a common aminoglycoside acetyltransferase responsible for resistance to aminoglycoside antibiotics such as kanamycin, amikacin and tobramycin [9]. The co transmission of *qnr* with *aac(6)-Ib-cr* genes which speeds up the formation of multidrug resistance in Enterobacteriaceae has been previously reported in China [14]. However, in the present study we showed that currently there is no significant relationship between *aac(6)-Ib-cr* prevalence and the presence of the *qepA*, *qnrB*, *qnrA*, *qnrS* gene in Iran. According to the fact that gentamycin is being prescribed much more than that of quinolones for urinary infection patients, so it may lead to a natural selection.

In 70.1% of all isolates two or more genes responsible for  $\beta$ -lactam or quinolone resistance were identified. Combinations of several  $\beta$ -lactamase genes are commonly observed regularly within genomes of Gram-negative bacterial pathogens and co-selection of  $\beta$ -lactamase genes and determinants for resistance against other antibiotics e.g. fluoroquinolones has been postulated as a possible mechanism responsible for the widespread distribution of those combined genes [17]. The frequently observed combination of *bla<sub>CTX-M-15</sub>* and *aac(6)-Ib-cr* could provide genetic support for this theory, which has been noted before [18]. However, a strong association between resistance to TMS and the presence of integrons was observed [19]. Trimethoprim-sulfamethoxazole and fluoroquinolones tend to be more effective than many Beta-lactams, in eradicating initial bacteriuria. As a result, use of beta-lactams class for only may yield increased incidence of recurrence and side effects.

Interestingly, in this work, a strong association between fluoroquinolone-resistant *E. coli* and *qnrB* was observed (p value<0.05). Other studies have shown that acquisition of resistance determinants and the expression of a multidrug resistance phenotype is associated with an increase in virulence of *E. coli* isolates [17]. These results suggest that fluoroquinolone-susceptible *E. coli* strains would have more virulence determinants since they belong to group's commensal. In contrast, there was a strong

association between fluoroquinolone-resistance strains and commensal groups, suggesting that the presence of these resistance mechanisms would favor *E. coli* clones to become successful commensals. Interestingly, *qnrB* has been detected on the *CTX-M-15* and *SHV-12* ESBL bla coding plasmids in India and USA, respectively. Considering the fact that plasmid-born *qnrB* attach to the upstream of *LexA* [20], it can cause an increase of *qnrB* expression in ESBL+ *E. coli* when expose to quinolones. Therefore, in this study 30.9% of the isolates were *qnrB*+ and Peimani reported a 35.5% prevalence of this gene [7].

In this study a high prevalence of PMQR was detected in ESBL+ *E. coli*. Shams et al. [21] showed that their *E. coli* isolates were all *qnrA*+ and also 80% of their *E. coli* isolates showed a MDR characteristic. However, Peimani [7] (Iran), and Bouchakour [22] (Morocco) detected 39.5%, and 50% *qnrA*+ *E. coli*. The difference of the results of this study with the above mentioned results may be related to the increase of PMQR in Enterobacteriaceae, especially *E. coli* [22, 7].

MDR is one of the major worries of medical authorities and communities. In this study, a high prevalence of MDR in UPEC isolates was detected and 82 isolates were detected as MDR. There are various reports on the prevalence of MDR isolates. For example; 42% is reported in Slovenia and 65.6% in Iran [23]. Our results showed that there might be an increasing rate of antibiotic resistance and so an increasing rate of MDR in UPEC isolates of Hospitals and clinical settings of Tehran seems to be inevitable.

## Conclusion

This study showed a high prevalence of MDQR which is related to the presence of *qnr* and also *aac(6')-Ib-cr* genes in the *E. coli* isolates. The presence of such an antibiotic resistance in clinical settings of the country seems to be alarming, not only for human health, but also financially. It is particularly important to understand the prevalence and characteristics of fluoroquinolone resistance in county hospitals, so that guidance can be provided on the clinical application of these antibiotics. Our results show that more studies is needed to control the infections. Also, prevention of taking non-prescribed antibiotics is highly needed to pave the way to control such infections.

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## Ethics approval

This study was approved by the institutional review board

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