



# Prevalence of Plasmid-Mediated Quinolone Resistance Genes and Molecular Typing of *Klebsiella pneumoniae* Isolates from Blood Cultures in Milad Hospital, Tehran, Iran, Within 2018 - 2019

Maryam Ghane <sup>1,\*</sup>, Laleh Babaeekhou <sup>1</sup> and Morteza Asgharifard <sup>1</sup>

<sup>1</sup>Department of Biology, Islamshahr Branch, Islamic Azad University, Islamshahr, Iran

\*Corresponding author: Department of Biology, Islamshahr Branch, Islamic Azad University, Islamshahr, Iran. Email: ghane@iaau.ac.ir

Received 2022 March 26; Revised 2022 July 13; Accepted 2022 July 13.

## Abstract

**Background:** Plasmid-mediated quinolone resistance (PMQR) determinants are commonly characterized in *Klebsiella pneumoniae* isolates worldwide and complicate the treatment of infections caused by this bacterium.

**Objectives:** This study aimed to investigate the prevalence of PMQR determinants and molecular typing of blood isolates of *K. pneumoniae* in Milad hospital in Tehran, Iran, within 2018 - 2019.

**Methods:** A total of 100 *K. pneumoniae* isolates were tested for susceptibility to quinolones using the disk diffusion method. The minimum inhibitory concentrations (MICs) of ciprofloxacin (CIP) and levofloxacin (LEV) were determined using the microdilution broth method. The PMQR determinants were detected by polymerase chain reaction (PCR) assay, and the genetic relationship between the isolates was assessed using enterobacterial repetitive intergenic consensus (ERIC)-PCR.

**Results:** The resistance rates of the isolates to LEV, CIP, nalidixic acid, and norfloxacin were determined to be 62, 46, 29, and 23%, respectively. Eighty-one isolates were resistant to at least one tested quinolone. A high-level CIP and LEV resistance (MIC > 32 mg/L) was observed in 15 (18.51%) and 36 (44.44%) isolates, respectively. The PMQR genes were detected in 71 (87.65%) isolates. The *oqxAB*, *qnrS*, *qnrD*, *qnrB*, *aac(6)-Ib-cr*, *qnrA*, *qepA*, and *qnrC* genes were detected in 71 (87.65%), 30 (37%), 25 (30.8%), 24 (29.6%), 18 (22.2%), 17 (21%), and 8 (9.9%) isolates, respectively. The ERIC-PCR revealed 64 genotypes among quinolone-resistant isolates.

**Conclusions:** The high prevalence of PMQR genes observed in this study is a significant concern for public health since they can contribute to the spread of fluoroquinolone resistance among clinical isolates. The ERIC-PCR revealed high heterogeneity among the studied isolates, indicating that they emerged from different sources.

**Keywords:** Ciprofloxacin, Drug Resistance, *K. pneumoniae*, Levofloxacin, Molecular Typing

## 1. Background

*Klebsiella pneumoniae*, a member of the *Enterobacteriaceae* family, is a normal microbiota of the intestinal tract of humans and animals. This bacterium is a common opportunistic hospital-related pathogen, causing a broad spectrum of infections, such as pneumonia, wound infection, urinary tract infections, pyogenic liver abscesses, endocarditis, and septicemia (1). Bloodstream infections caused by *K. pneumoniae* occur with remarkable incidence and high mortality worldwide (2). Due to the emergence of drug-resistant strains, infections caused by *K. pneumoniae* are often associated with high mortality, long hospital stay, and high costs (3). Quinolones are a class of antimicrobial agents that are frequently applied for the treatment of *K. pneumoniae* infections due to their broad antibacte-

rial spectrum. However, in recent years, numerous studies have shown that resistance to fluoroquinolones has increased worldwide, especially in *K. pneumoniae* (4).

Three mechanisms of resistance to quinolones have been recognized, namely (1) efflux pump expression, (2) mutational alterations in drug target affinity, and (3) plasmid-mediated quinolone resistance (PMQR) determinants (4). Multidrug efflux pumps are regarded as one of the mechanisms that increase resistance against various types of antimicrobials, such as quinolones. In *K. pneumoniae*, AcrAB and OqxAB are known as the two main multidrug efflux pumps (5). Resistance mutations generally occur in a localized domain of the ParC and GyrA subunits of topoisomerase IV and gyrase, respectively, and reduce the binding of the drug to the enzyme-deoxyribonucleic acid (DNA) complex (6). These mutations have most com-

monly occurred at Ser80 and Glu84 codons of the ParC subunit and Ser83 and Asp87 codons of the GyrA subunit (7). The PMQR is another mechanism of fluoroquinolones' resistance among Gram-negative bacteria, which was first described in 1998 from *K. pneumoniae* clinical isolates. The PMQR confers low-level fluoroquinolones' resistance. However, they can provide background for the development of chromosomally encoded fluoroquinolone resistance mechanisms (8).

To date, three types of PMQR have been recognized. The first is the contribution of plasmid-mediated efflux pumps, such as OqxAB and QepA, which cause reduced susceptibility to quinolones and resistance to multiple agents. Most efflux pumps reside on the chromosomes of bacteria. Nevertheless, plasmid-mediated efflux pumps have been reported in recent years (9). These plasmid-mediated efflux pumps might pose a resistance problem, as they can facilitate the development of multidrug resistance and the spread of antimicrobial resistance through horizontal gene transfer (9). Another PMQR mechanism involves the modification of fluoroquinolones by a particular aminoglycoside acetyltransferase (AAC(6')-Ib-cr). AAC(6')-Ib-cr is able to reduce ciprofloxacin (CIP) activity by the acetylation of its piperazinyl substituent (10, 11). Qnr proteins are another PMQR mechanism among Gram-negative bacteria. To date, seven Qnr proteins, including QnrA, QnrB, QnrC, QnrD, QnrE, QnrS, and QnrVC, have been characterized. These proteins keep bacterial enzymes, such as DNA gyrase and DNA topoisomerase IV, from inhibition by quinolone and have been distributed globally in a variety of bacterial genera (11).

Plasmids carrying quinolone resistance genes have been characterized as an emerging problem since they can be transmitted horizontally between bacterial species. Furthermore, they can coexist on the same plasmid harboring genes encoding extended-spectrum  $\beta$ -lactamases and be cotransferred to other Gram-negative bacteria (12).

Typing of *K. pneumoniae* is crucial to epidemiological surveillance and control of infections caused by multidrug-resistant isolates. One of the most critical measures to prevent the dissemination of health-related infections and enhance infection control is to identify the genetic relationships between pathogenic strains. Among molecular methods, enterobacterial repetitive intergenic consensus-polymerase chain reaction (ERIC-PCR) is a cost-effective, reliable, and quick method to identify the genetic diversity among the *Enterobacteriaceae* family (13). In this method, the repetitive nucleotide sequences in the microbial genome are amplified, and bacterial isolates are clus-

tered and recognized from each other.

Bloodstream infection due to drug-resistant *K. pneumoniae* causes a high rate of morbidity and mortality in hospitals, and the presence of resistance determinants on the transferable plasmids makes this problem more complicated. To the best of our knowledge, there are very rare reports on the presence of different types of PMQR genes (e.g., *oqxAB*, *qepA*, *qnrS*, and *aac(6')-Ib-cr*) from the blood isolates of *K. pneumoniae* in Iran. Knowledge about the genotypes and molecular mechanisms of resistance in each region can be helpful in providing strategies for infectious control and preventing the dissemination of resistant strains.

## 2. Objectives

The main objective of the present study was to assess the presence of PMQR genes in *K. pneumoniae* blood isolates. In the subsequent sections, the molecular diversity between these isolates was characterized using the ERIC-PCR method.

## 3. Methods

### 3.1. Bacterial Isolates

In the present study, 100 plates (each plate belonged to one patient) of *K. pneumoniae* clinical isolates were obtained from Milad hospital in Tehran, Iran. The source of isolates was the blood of the intensive care unit (ICU) patients (n = 100) admitted to the hospital within December 2018 to October 2019. The strains were approved as *K. pneumoniae* using routine laboratory biochemical tests (14).

### 3.2. Antimicrobial Susceptibility Testing

All *K. pneumoniae* isolates were tested for susceptibility to quinolones by the Kirby-Bauer standard disk diffusion method in accordance with the Clinical and Laboratory Standards Institute (CLSI) recommendations (15). The quinolone antibiotics applied in this study were nalidixic acid (30  $\mu$ g), CIP (5  $\mu$ g), norfloxacin (5  $\mu$ g), and levofloxacin (LEV) (5  $\mu$ g) (Mast Diagnostics, UK). The quality control strain was *Escherichia coli* ATCC 25922. The minimum inhibitory concentrations (MICs) for LEV and CIP (Sigma-Aldrich, India) were also assessed by broth microdilution assay in accordance with the CLSI recommendations (15). The serial dilutions of CIP or LEV in Mueller-Hinton broth (MHB) (Merck, Germany) were prepared. Then, 100  $\mu$ L of obtained solutions was distributed into each well of 96-well enzyme-linked immuno-absorbent assay microplates,

and 100  $\mu\text{L}$  of the bacterial suspension (optical density = 0.8 at 650 nm) was added to each well. A negative control (i.e., MHB medium with bacterial suspension) was also used. After the incubation at 37°C for 18 - 24 hours, the lowest concentration of the antibiotics with no evident growth was considered MIC. For the assessment of the minimum bactericidal concentration (MBC), 5  $\mu\text{L}$  of the last three wells with no evident growth was spread plated onto the nutrient agar (HiMedia, India). After incubation, the lowest concentration with no bacterial growth was considered MBC (16).

### 3.3. DNA Extraction and PCR Assay

The DNA extraction steps were performed using the bacteria genomic DNA extraction kit (National Center for Genetic Resources, Iran) in sterile conditions, and purity was quantified using a Nanodrop spectrophotometer 2000 (Thermo Fisher Scientific, USA). For this purpose, 2  $\mu\text{L}$  of extracted DNA was placed on a spectrophotometer, and measures in 260, and 280 nm were performed. The DNA concentration in ng/ $\mu\text{L}$  and absorbance ratio A260/280 were provided automatically by system software. A 260/280 ratio of 1.8-2.0 was regarded as pure. The targeted sequence of PMQR determinants, including *qepA*, *qnrA*, *qnrB*, *qnrC*, *qnrD*, *qnrS*, *aac(6')-Ib-cr*, *oqxA*, and *oqxB*, were amplified as previously described (17, 18). Table 1 shows the primers used for PCR amplification.

The PCR amplification was carried out as predenaturation at 95°C for 15 minutes, denaturation at 95°C for 45 seconds, annealing at 63°C for all genes except for *oqxA* and *oqxB* (both at 59°C) for 60 seconds, elongation at 72°C for 90 seconds (33 cycles), and final elongation at 72°C for 10 minutes (17, 18). Amplified PCR products were electrophoresed through 2% agarose tris-acetate-EDTA gel and visualized using ultraviolet illumination. *K. pneumoniae* and *E. coli* strains positive for *oqxAB*, *aac(6)-Ib*, *qepA*, and *qnr* genes (confirmed by sequencing) were provided from the University of Tehran (Faculty of Veterinary Medicine) and used as positive controls. The PCR mixture without template DNA was used as a negative control.

### 3.4. Genotyping of Quinolone-Resistant Isolates

The clonal relatedness between the quinolone-resistant (QRE) isolates was evaluated by ERIC-PCR using previously proposed primers (Table 1) (19). The PCR mixture contained 1  $\mu\text{L}$  primer (10 pmol), 2  $\mu\text{L}$  of the DNA template, 12.5  $\mu\text{L}$  Master Mix (Ampliqon, Denmark), and 9.5  $\mu\text{L}$  ddH<sub>2</sub>O. The PCR condition included predenaturation at 94°C for 15 minutes, 40 cycles of denaturation at 94°C for

60 seconds, annealing at 37°C for 60 seconds, elongation at 72°C for 60 seconds, and final elongation at 72°C for 8 minutes. The PCR products were then run on 1.5% agarose gels. The obtained fingerprints were examined visually, and the presence or absence of bands was considered 1 or 0, respectively. The data obtained in this step were analyzed by NTSYS pc 2.02 software, and the dendrogram was drawn using Unweighted Pair Group Method with Arithmetic Mean.

### 3.5. Statistical Analysis

A Chi-square test using SPSS software (version 20) was applied to analyze the categorical variables. A P-value  $\leq$  0.05 was set as statistically significant.

## 4. Results

### 4.1. Antimicrobial Susceptibility Testing

According to the results of the disk diffusion method, the resistance rates of isolates to LEV, CIP, nalidixic acid, and norfloxacin were determined to be 62%, 46%, 29%, and 23%, respectively. Of 100 study isolates, 81 (81%) isolates were resistant to at least one tested quinolone and identified as QRE isolates, among which 7 isolates were resistant to all tested quinolones. Broth microdilution assay on 81 QRE isolates revealed a high-level CIP and LEV resistance (MIC > 32 mg/L) in 15 (18.51%) and 36 (44.44%) isolates, respectively. Moreover, 37 isolates (45.6%) were resistant to both tested fluoroquinolones. Table 2 shows the MIC and MBC ranges of CIP and LEV and MIC<sub>50</sub> and MIC<sub>90</sub> of these isolates.

### 4.2. Detection of PMQR Genes

The PMQR genes were detected in 71 (87.65%) isolates. However, no significant association was observed between higher CIP MIC (> 32  $\mu\text{g}/\text{mL}$ ) and the presence of PMQR genes ( $P = 0.592$ ). In addition, higher LEV MIC was not associated with the presence of PMQR genes ( $P = 0.156$ ). In the present study, the PCR results showed the dominant presence of *oqxAB* (87.65%); accordingly, only 10 isolates lacked this gene. The *qnrS*, *qnrD*, *qnrB*, *aac(6')-Ib-cr*, *qnrA*, *qepA*, and *qnrC* genes were detected in 30 (37%), 25 (30.8%), 24 (29.6%), 18 (22.2%), 17 (21%), 17 (21%), and 8 (9.9%) isolates, respectively. In the present study, higher CIP MIC was not associated with the presence of *qnrA* ( $P = 0.48$ ), *qnrB* ( $P = 0.23$ ), *qnrC* ( $P = 0.52$ ), *qnrD* ( $P = 0.32$ ), *qnrS* ( $P = 0.42$ ), *aac(6')-Ib-cr* ( $P = 0.55$ ), *qepA* ( $P = 0.51$ ), and *oqxAB* ( $P = 0.52$ ). Furthermore, no significant association was observed between higher LEV MIC and the presence of *qnrA* ( $P = 0.28$ ), *qnrB* ( $P = 0.48$ ), *qnrC* ( $P = 0.48$ ), *qnrD* ( $P = 0.42$ ), *qnrS* ( $P = 0.51$ ), *aac(6')-Ib-cr* ( $P = 0.33$ ),

**Table 1.** Primers Applied in This Study

Genes	Sequence	Annealing Temperature (°C)	Amplicon Size (bp)	References
<i>qnrA</i>	F: CAGCAAGAGGATTTCTCACG	60	630	(17)
	R: AATCCGGCAGCACTATTACTC			
<i>qnrB</i>	F: GGCTGTCAGTTCTATGATCG	60	488	
	R: GAGCAACGATGCCTGGTAG			
<i>qnrC</i>	F: GCAGAATTCAGGGGTGTGAT	60	118	
	R: AAC TGCTCCAAAAGCTGCTC			
<i>qnrD</i>	F: CGAGATCAATTTACGGGAATA	60	581	
	R: AAC AAGCTGAAGCGCCTG			
<i>qnrS</i>	F: GCAAGTTCATTGAAC AGGGT	60	428	
	R: TCTAAAC CGTCGAGTTCGGCG			
<i>aac(6')-Ib-cr</i>	F: TTGGAAGCGGGACGGAM	60	260	
	R: ACACGGCTGGACCATA			
<i>qepA</i>	F: GCAGTCCAGCAGCGGGTAG	60	218	
	R: CTCCTGCCCGAGTATCGTG			
<i>oqxA</i>	F: CTCGGCGCATGATGCT	60	392	
	R: CCACTCTTACGGGAGACGA			
<i>oqxB</i>	F: TTCTCCCCGGGGGAAGTAC	58	512	
	R: CTCGGCCATTTGGCGCGTA			
ERIC	AAGTAAGTACTGGGGTGAGCG			(19)

**Table 2.** Antimicrobial Susceptibility of 81 Quinolone-Resistant *Klebsiella pneumoniae* Isolates Against Ciprofloxacin and Levofloxacin by Broth Microdilution Assay

Antibiotics	R, No. (%)	I, No. (%)	S, No. (%)	MIC range ( $\mu\text{g/mL}$ )	MBC range ( $\mu\text{g/mL}$ )	MIC50 ( $\mu\text{g/mL}$ )	MIC90 ( $\mu\text{g/mL}$ )
CIP	47 (58.0)	22 (27.2)	12 (14.8)	0.125 > 32	0.25 > 32	4	> 32
LEV	61 (75.3)	16 (19.7)	4 (4.9)	0.5 > 32	1 > 32	> 32	> 32

Abbreviations: R, resistant; I, intermediate; S, susceptible; CIP, ciprofloxacin; LEV, levofloxacin; MIC, minimum inhibitory concentration; MBC, minimum bactericidal concentration

*qepA* ( $P = 0.23$ ), and *oqxAB* ( $P = 0.38$ ). Figure 1 illustrates the PCR results of PMQR genes.

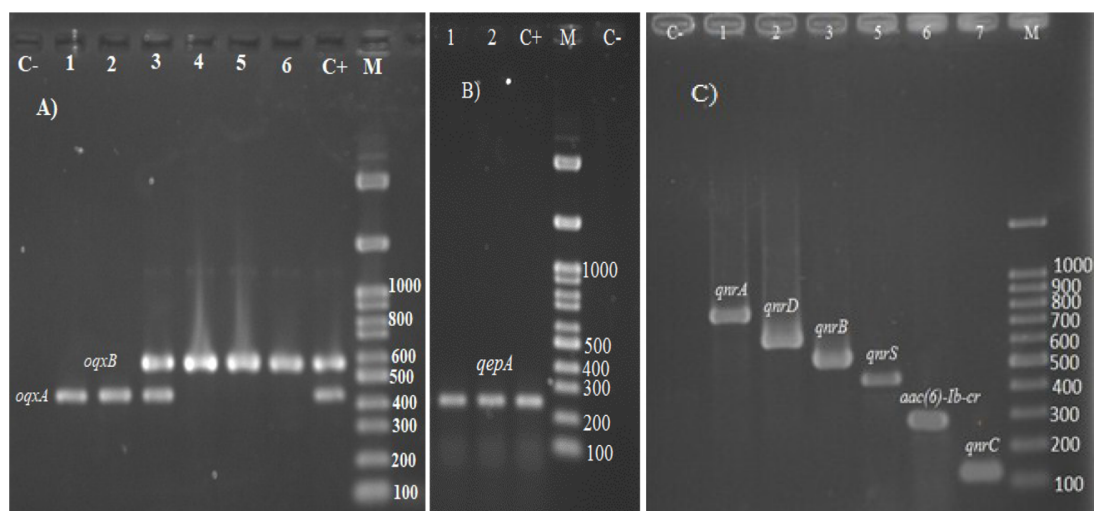
#### 4.3. ERIC-PCR Typing

The ERIC typing was performed on all 81 QRE isolates, and the results showed high diversity among them. Figure 2 depicts the representative DNA fingerprint pattern of *K. pneumoniae* isolates, and Figure 3 illustrates the constructed dendrogram based on DNA fingerprint. Of the total 81 QRE isolates, 64 ERIC types were observed (Figure 2). One genotype contained five isolates; three genotypes included three isolates; seven genotypes contained two QRE isolates. The discriminatory index was 0.97. In the present study, no significant association was observed between the ERIC types and resistant patterns ( $P = 0.42$ ) or the presence of PMQR genes ( $P = 0.62$ ).

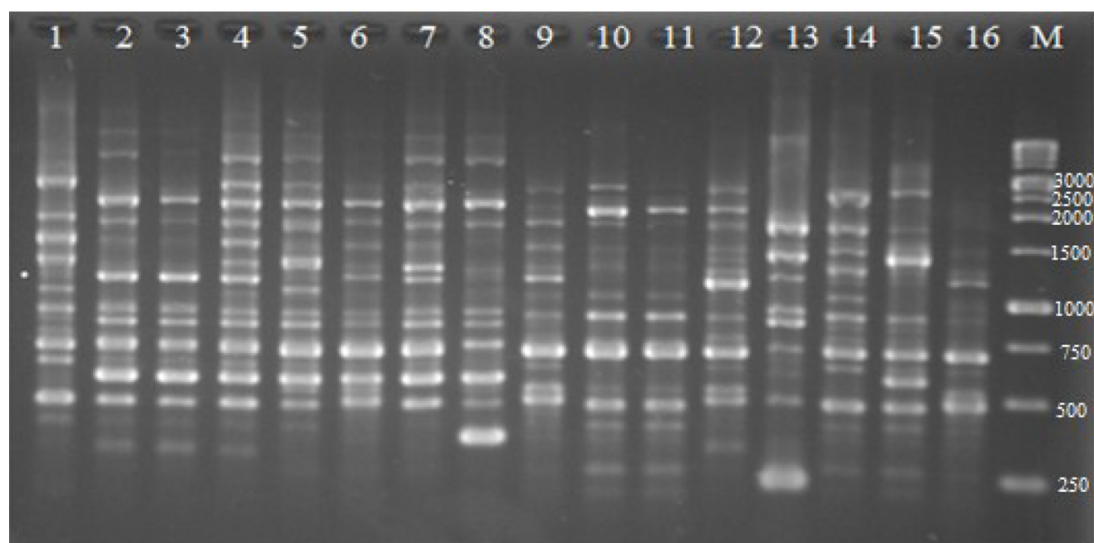
## 5. Discussion

*Klebsiella pneumoniae* is one of the main causes of bloodstream infections that is responsible for high mortality worldwide (2). The emergence and spread of PMQR determinants among the blood isolates of *K. pneumoniae* have become a significant threat to public health. The data on the prevalence of PMQR genes among the blood isolates of *K. pneumoniae* and their epidemiology in Iran is limited. In this study, a high frequency of QRE and a high prevalence of PMQR determinants were observed among the studied isolates. Additionally, high heterogeneity was observed among the isolates indicating that the blood isolates from ICU patients emerged from different sources.

Broth microdilution assay revealed CIP and LEV resistance in 58% and 75% of the studied QRE isolates, and a



**Figure 1.** Agarose gel electrophoretic of *oqxA*, *oqxB*, *qepA*, *aac(6)-Ib-cr*, and *qnr* genes; (A): C-, negative control; lanes 1-6, positive strains for *oqxA* and/or *oqxB*; C+, positive control for both *oqxA* and *oqxB* genes; (B): lanes 1 and 2, positive strains for *qepA* gene; C+, positive control for *qepA*; C-, negative control; (C): C-, negative control; lanes 1-7, positive strains for *qnrA*, *qnrD*, *qnrB*, *qnrS*, *aac(6)-Ib-cr*, and *qnrC*, respectively; M, 100 bp marker



**Figure 2.** Representative deoxyribonucleic acid fingerprints of quinolone-resistant *Klebsiella pneumoniae* isolates obtained by enterobacterial repetitive intergenic consensus-polymerase chain reaction; M, marker

high percentage of them had MICs  $> 32 \mu\text{g/mL}$  against CIP (18.51%) and LEV (44.44%). Compared to the results of the present study, a lower prevalence of CIP and LEV resistance was observed by Ghane et al. (47% and 67%, respectively) (20). In a meta-analysis study conducted in Iran, the mean rate of CIP resistance among *K. pneumoniae* isolates was observed to be 34.8 (21). In another meta-analysis study performed in Iran, it was within the range of 12.7 (in

Shahrekord) to 53.2 (in Isfahan), and the CIP resistance rate in Tehran was observed to be 36.9 (22). The findings of the present study indicated an increase in resistance to CIP and LEV, which might be due to the increased use of these antibiotics in recent years.

In this recent study, nine PMQR genes were investigated among the studied QRE *K. pneumoniae* isolates. The results showed a high prevalence (87.65%) of PMQR genes



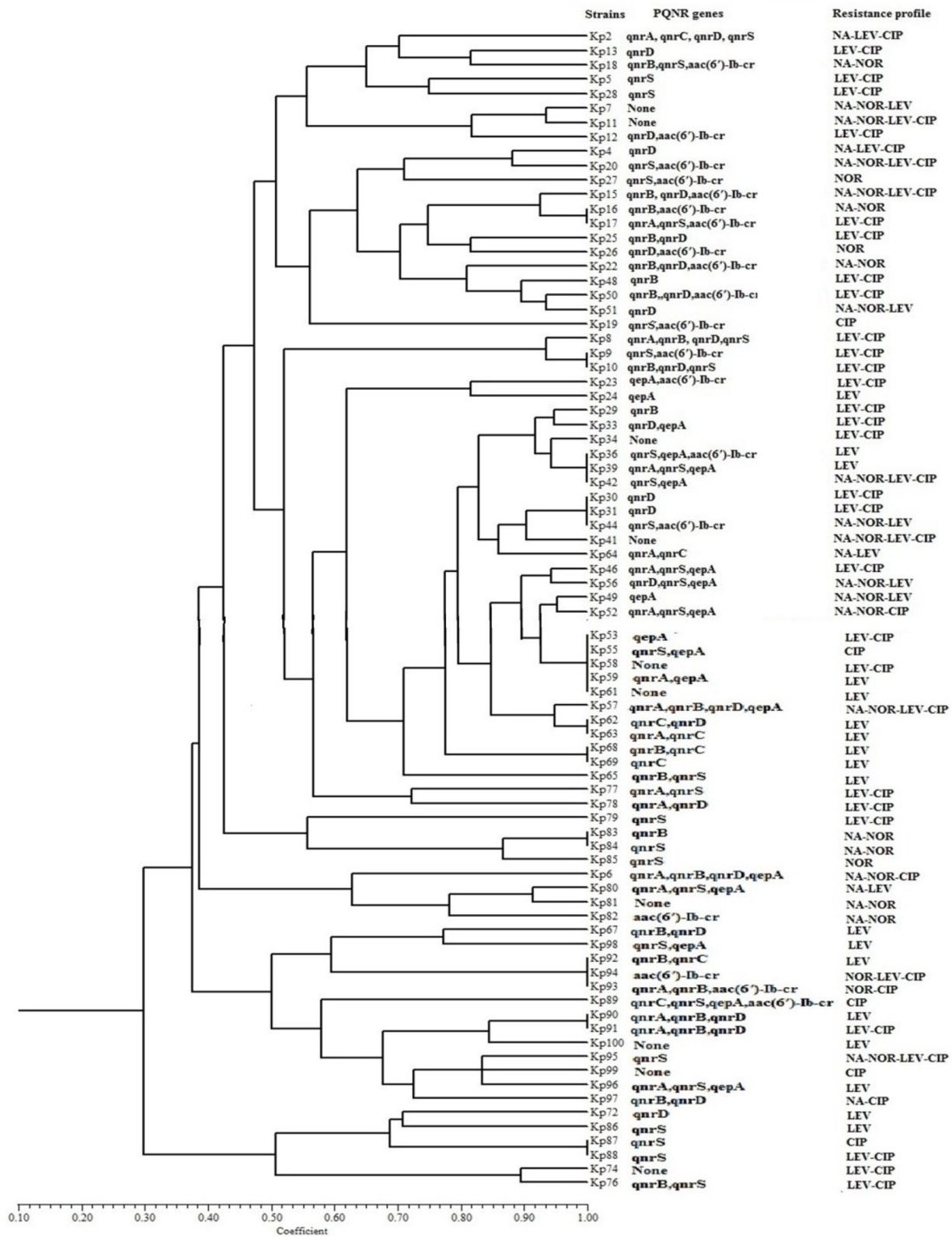


Figure 3. Dendrogram Generated by unweighted pair group method with arithmetic mean showing the genetic relationship between 81 quinolone-resistant *Klebsiella pneumoniae* isolates; NA, nalidixic acid; CIP, ciprofloxacin; LEV, levofloxacin; NOR, norfloxacin.

which is comparable to the results obtained by Alheib et al. in Syria (86.95%) (23). A higher prevalence (100%) of PMQR genes among *K. pneumoniae* isolates was reported in a study by Yang et al. in Korea (24), and a lower prevalence (70.4%) was reported in a study by Hamed et al. in Egypt (11). Although PMQR determinants confer a low level of quinolone resistance, they might provide background for the development of chromosomally encoded quinolone resistance (8). Furthermore, PMQR can disseminate horizontally between Gram-negative bacteria. Therefore, the high frequency of PMQR obtained in the current study and other studies performed worldwide can be a threat to public health and cause a higher level of quinolone resistance in the future.

In the present study, *oqxAB* efflux genes were detected as the most frequent (87.65%) PMQR genes among the studied isolates. Similar to the obtained results in two separate studies in Iran, *oqxAB* was detected to be the most frequent PMQR gene among *K. pneumoniae* isolates (25, 26). The high prevalence of *oqxAB* was also reported in Egypt (96.4%) (11). In the current study, CIP MIC<sub>50</sub> values of the isolates with or without the *oqxAB* genes were the same (4 µg/mL), suggesting that this gene plays a minor role in CIP resistance among the studied isolates. This finding might be due to the low expression level of this gene on account of its chromosomal location. In *K. pneumoniae* isolates, the *oqxAB* operon has mostly resided on the chromosome where the expression level is 80-fold lower than that of plasmid-borne *oqxAB* (27).

The gene encoding QepA, another efflux-mediated PMQR mechanism, was detected in 21% of the studied isolates. A lower prevalence (12%) of *qepA* was reported in a study conducted in Egypt (18). In a study by Azargun et al. in Iran, *qepA* was not detected among *K. pneumoniae* isolates (28). However, in two separate Iranian studies, a very low prevalence (2% and 5.2%) of *qepA* genes was observed among the human isolate of *K. pneumoniae* (25, 29). The *qepA* gene has been reported to be associated with an increase in resistance and an 8-32-fold increase in MIC values of hydrophilic quinolones (30). However, in the present study, higher CIP MIC (> 32 µg/mL) was not associated with the presence of the *qepA* gene (P = 0.51), and most isolates lacking *qepA* genes had higher MIC values. This finding might be due to the presence of other mechanisms of quinolone resistance in these isolates.

Among *qnr* genes, *qnrS* (37%) was the most prevalent. Similarly, in a study by Hamed et al., *qnrS* was detected as the most prevalent *qnr* gene (11). A similar prevalence of *qnrS* (37%) was observed in a study by Kareem et al. in Iraq

(31). However, a higher prevalence of *qnrS* was reported in Iran (46.25%) (32) and Egypt (49%) (18). The lower prevalence of *qnrS* was observed among *K. pneumoniae* isolates in India (4.5%) (4) and Iran (21.7%) (28). In the present study, *qnrD* (30.8%) was detected as the second foremost *qnr* gene. However, its prevalence was lower than in the previous report in Egypt (40%) (18). Additionally, the prevalence of *qnrB* (29.6%) obtained in this study was lower than those reported in Egypt (75%) (18) and Iraq (40.7%) (31).

In this study, 9.9% of the isolates harbored *qnrC*, which is higher than that reported by Abossedgh et al. in Iran (5.9%) (33). However, this gene was not detected in most Iranian studies (25, 28). The difference in the distribution of PMQR genes can be ascribed to the differences in fluoroquinolone usage in each region, geographical area, and study period. In the present study, *aac(6′)-Ib-cr* was detected in 22.2% of the studied isolates. This frequency is lower than those observed in India (89%) (4), Iraq (92.5%) (31), Egypt (61%) (18), and Iran (50%) (26). Lower prevalence was observed in Tunisia (19%) (34) and China (16.1%) (27). In the present study, higher LEV MIC was not associated with the presence of *aac(6′)-Ib-cr* (P = 0.33). Furthermore, no significant association was observed between higher CIP MIC and the presence of *aac(6′)-Ib-cr* (P = 0.52). The aforementioned results contradict the results of previous studies reporting a significant association between a higher level of CIP MIC and the presence of *aac(6′)-Ib-cr* (25). However, the presence of these genes in the transferable multiresistant plasmids could be a major public health threat due to their capability to induce multidrug resistance to other recipient strains.

In the present study, to obtain the epidemiological features and control bacterial resistance, all QRE isolates were typed using ERIC-PCR, and the results showed a high genetic variation among the studied isolates. Previous studies also revealed a high heterogeneity among *K. pneumoniae* isolates (33). The heterogeneity obtained in this study indicated that quinolone resistance in the studied isolates has emerged from various sources and has not been distributed from a specific clone.

The current study had some limitations. First, this study was conducted in only one hospital; therefore, the prevalence of PMQR obtained in this study might not reflect the prevalence of PMQR in Iran. The absence of data on Multilocus sequence typing for characterizing *K. pneumoniae* blood isolates is another limitation of this study.

### 5.1. Conclusions

In this study, the high prevalence of CIP and LEV resistance was reported among *K. pneumoniae* blood isolates. The obtained findings revealed that the majority of the isolates harbored PMQR genes. The high frequency of PMQR genes obtained in this study is alarming since they can extensively spread through plasmids and increase the dissemination of fluoroquinolone resistance among clinical isolates. The ERIC-typing revealed a high heterogeneity among the QRE *K. pneumoniae* blood isolates of the present study, indicating that the isolates emerged from different sources.

### Footnotes

**Authors' Contribution:** M. G.: Conceptualization, investigation, methodology, formal analysis, writing-original draft preparation, writing-review, and editing; L. B.: Methodology, investigation, experimental work, and formal analysis; M. A.: Experimental work

**Conflict of Interests:** The authors declare that there is no conflict of interest.

**Data Reproducibility:** The dataset presented in the study is available on request from the corresponding author during submission or after publication. The data are not publicly available due to privacy.

**Ethical Approval:** This study was approved by the Ethics Committee of Islamic Azad Tehran Medical Sciences University- Pharmacy and Pharmaceutical Branches Faculty (IR-IAU.PS.REC.1399.102) (webpage of ethical approval code: ethics.research.ac.ir/IR-IAU.PS.REC.1399.102).

**Funding/Support:** No funding/support was received for this study.

### References

- Navon-Venezia S, Kondratyeva K, Carattoli A. Klebsiella pneumoniae: a major worldwide source and shuttle for antibiotic resistance. *FEMS Microbiol Rev.* 2017;**41**(3):252-75. doi: [10.1093/femsre/fux013](https://doi.org/10.1093/femsre/fux013). [PubMed: 28521338].
- Xu M, Fu Y, Kong H, Chen X, Chen Y, Li L, et al. Bloodstream infections caused by Klebsiella pneumoniae: prevalence of blaKPC, virulence factors and their impacts on clinical outcome. *BMC Infect Dis.* 2018;**18**(1):1-9. doi: [10.1186/s12879-018-3263-x](https://doi.org/10.1186/s12879-018-3263-x). [PubMed: 30064360]. [PubMed Central: PMC6069789].
- Effah CY, Sun T, Liu S, Wu Y. Klebsiella pneumoniae: an increasing threat to public health. *Ann Clin Microbiol Antimicrob.* 2020;**19**(1):1-9. doi: [10.1186/s12941-019-0343-8](https://doi.org/10.1186/s12941-019-0343-8). [PubMed: 31918737]. [PubMed Central: PMC7050612].
- Geetha PV, Aishwarya KVL, Mariappan S, Sekar U. Fluoroquinolone resistance in clinical isolates of Klebsiella pneumoniae. *J Lab Physicians.* 2020;**12**(2):121-5. doi: [10.1055/s-0040-1716478](https://doi.org/10.1055/s-0040-1716478). [PubMed: 32905353]. [PubMed Central: PMC7467831].
- Ni RT, Onishi M, Mizusawa M, Kitagawa R, Kishino T, Matsubara F, et al. The role of RND-type efflux pumps in multidrug-resistant mutants of Klebsiella pneumoniae. *Sci Rep.* 2020;**10**(1):1-10. doi: [10.1038/s41598-020-67820-x](https://doi.org/10.1038/s41598-020-67820-x). [PubMed: 32616840]. [PubMed Central: PMC7331594].
- Hooper DC, Jacoby GA. Mechanisms of drug resistance: quinolone resistance. *Ann N Y Acad Sci.* 2015;**1354**:12-31. doi: [10.1111/nyas.12830](https://doi.org/10.1111/nyas.12830). [PubMed: 26190223]. [PubMed Central: PMC4626314].
- Aldred KJ, Kerns RJ, Osheroff N. Mechanism of quinolone action and resistance. *Biochemistry.* 2014;**53**(10):1565-74. doi: [10.1021/bi5000564](https://doi.org/10.1021/bi5000564). [PubMed: 24576155]. [PubMed Central: PMC3985860].
- Araujo BF, Campos PA, Royer S, Ferreira ML, Goncalves IR, Batistao D, et al. High frequency of the combined presence of QRDR mutations and PMQR determinants in multidrug-resistant Klebsiella pneumoniae and Escherichia coli isolates from nosocomial and community-acquired infections. *J Med Microbiol.* 2017;**66**(8):1144-50. doi: [10.1099/jmm.0.000551](https://doi.org/10.1099/jmm.0.000551). [PubMed: 28771139].
- Li J, Zhang H, Ning J, Sajid A, Cheng G, Yuan Z, et al. The nature and epidemiology of OqxAB, a multidrug efflux pump. *Antimicrob Resist Infect Control.* 2019;**8**:44. doi: [10.1186/s13756-019-0489-3](https://doi.org/10.1186/s13756-019-0489-3). [PubMed: 30834112]. [PubMed Central: PMC6387526].
- Xue G, Li J, Feng Y, Xu W, Li S, Yan C, et al. High prevalence of plasmid-mediated quinolone resistance determinants in Escherichia coli and Klebsiella pneumoniae isolates from pediatric patients in China. *Microb Drug Resist.* 2017;**23**(1):107-14. doi: [10.1089/mdr.2016.0004](https://doi.org/10.1089/mdr.2016.0004). [PubMed: 27167505].
- Hamed SM, Elkhatib WF, El-Mahallawy HA, Helmy MM, Ashour MS, Aboshanab KMA. Multiple mechanisms contributing to ciprofloxacin resistance among Gram negative bacteria causing infections to cancer patients. *Sci Rep.* 2018;**8**(1):1-10. doi: [10.1038/s41598-018-30756-4](https://doi.org/10.1038/s41598-018-30756-4). [PubMed: 30115947]. [PubMed Central: PMC6095922].
- Briales A, Rodriguez-Martinez JM, Velasco C, de Alba PD, Rodriguez-Bano J, Martinez-Martinez L, et al. Prevalence of plasmid-mediated quinolone resistance determinants qnr and aac(6)-Ib-cr in Escherichia coli and Klebsiella pneumoniae producing extended-spectrum beta-lactamases in Spain. *Int J Antimicrob Agents.* 2012;**39**(5):431-4. doi: [10.1016/j.ijantimicag.2011.12.009](https://doi.org/10.1016/j.ijantimicag.2011.12.009). [PubMed: 22365240].
- Sedighi P, Zarei O, Karimi K, Taheri M, Karami P, Shokoohzadeh L, et al. Molecular typing of Klebsiella pneumoniae clinical isolates by enterobacterial repetitive intergenic consensus polymerase chain reaction. *Int J Microbiol.* 2020;**2020**:1-5. doi: [10.1155/2020/8894727](https://doi.org/10.1155/2020/8894727).
- Mahon CR, Lehman DC, Manuselis G. *Textbook of diagnostic microbiology-e-book.* Elsevier Health Sciences; 2018. p. 412-47.
- Wayne P. *Performance Standards for Antimicrobial Susceptibility Testing M100-S22.* Pennsylvania, USA: CLSI; 2018.
- Galehdar M, Ghane M, Babaeekhou L. Co-occurrence of carbapenemase-encoding genes among klebsiella pneumoniae clinical isolates: positive relationship of bla NDM and bla SIM with imipenem resistance. *Jundishapur J Microbiol.* 2021;**14**(2). doi: [10.5812/jjm.112486](https://doi.org/10.5812/jjm.112486).
- Ciesielczuk H, Hornsey M, Choi V, Woodford N, Wareham DW. Development and evaluation of a multiplex PCR for eight plasmid-mediated quinolone-resistance determinants. *J Med Microbiol.* 2013;**62**(Pt 12):1823-7. doi: [10.1099/jmm.0.064428-0](https://doi.org/10.1099/jmm.0.064428-0). [PubMed: 24000223].
- El-Badawy MF, Tawakol WM, El-Far SW, Maghrabi IA, Al-Ghamdi SA, Mansy MS, et al. Molecular identification of aminoglycoside-modifying enzymes and plasmid-mediated quinolone resistance genes among Klebsiella pneumoniae clinical isolates recovered from Egyptian patients. *Int J Microbiol.* 2017;**2017**:8050432. doi: [10.1155/2017/8050432](https://doi.org/10.1155/2017/8050432). [PubMed: 28638412]. [PubMed Central: PMC5468591].



19. Wasfi R, Elkhatib WF, Ashour HM. Molecular typing and virulence analysis of multidrug resistant *Klebsiella pneumoniae* clinical isolates recovered from Egyptian hospitals. *Sci Rep*. 2016;**6**:1-11. doi: [10.1038/srep38929](https://doi.org/10.1038/srep38929). [PubMed: [28004732](https://pubmed.ncbi.nlm.nih.gov/28004732/)]. [PubMed Central: [PMC5177892](https://pubmed.ncbi.nlm.nih.gov/PMC5177892/)].
20. Ghane M, Babaeekhou L, Jafar Shanjani M. AmpC  $\beta$  lactamases in Urinary *Klebsiella pneumoniae* Isolates: First Report of ACC Type AmpC  $\beta$ -lactamase Resistance in Iran. *J Adv Med Biomed Res*. 2019;**27**(123):23-30. doi: [10.30699/jambs.27.123.23](https://doi.org/10.30699/jambs.27.123.23).
21. Heidary M, Nasiri MJ, Dabiri H, Tarashi S. Prevalence of drug-resistant *Klebsiella pneumoniae* in Iran: A review article. *Iran J Public Health*. 2018;**47**(3):317-26. [PubMed: [29845018](https://pubmed.ncbi.nlm.nih.gov/29845018/)]. [PubMed Central: [PMC5971167](https://pubmed.ncbi.nlm.nih.gov/PMC5971167/)].
22. Vaez H, Sahebkar A, Khademi F. Carbapenem-resistant *Klebsiella pneumoniae* in Iran: A systematic review and meta-analysis. *J Chemother*. 2019;**31**(1):1-8. doi: [10.1080/1120009X.2018.1533266](https://doi.org/10.1080/1120009X.2018.1533266). [PubMed: [30595129](https://pubmed.ncbi.nlm.nih.gov/30595129/)].
23. Alheib O, Al Kayali R, Abajy M. Prevalence of plasmid-mediated quinolone resistance (PMQR) determinants among extended spectrum beta-lactamases (ESBL)-producing isolates of *Escherichia coli* and *Klebsiella pneumoniae* in Aleppo, Syria. *Arch Clin Infect Dis*. 2015;**10**(3). doi: [10.5812/archcid.20631](https://doi.org/10.5812/archcid.20631).
24. Yang HY, Nam YS, Lee HJ. Prevalence of plasmid-mediated quinolone resistance genes among ciprofloxacin-nonsusceptible *Escherichia coli* and *Klebsiella pneumoniae* isolated from blood cultures in Korea. *Can J Infect Dis Med Microbiol*. 2014;**25**(3):163-9. doi: [10.1155/2014/329541](https://doi.org/10.1155/2014/329541). [PubMed: [25285114](https://pubmed.ncbi.nlm.nih.gov/25285114/)]. [PubMed Central: [PMC4173980](https://pubmed.ncbi.nlm.nih.gov/PMC4173980/)].
25. Haeili M, Salehzeinali H, Mirzaei S, Pishnian Z, Ahmadi A. Molecular characterization of quinolone resistance and antimicrobial resistance profiles of *Klebsiella pneumoniae* and *Escherichia coli* isolated from human and broiler chickens. *Int J Environ Health Res*. 2022;**32**(6):1-11. doi: [10.1080/09603123.2021.1885632](https://doi.org/10.1080/09603123.2021.1885632). [PubMed: [33615919](https://pubmed.ncbi.nlm.nih.gov/33615919/)].
26. Malek Jamshidi MR, Zandi H, Eftekhari F. Correlation of quinolone-resistance, qnr genes and integron carriage in multidrug-resistant community isolates of *Klebsiella* spp. *Iran J Basic Med Sci*. 2019;**22**(12):1387-91. doi: [10.22038/IJBMS.2019.13985](https://doi.org/10.22038/IJBMS.2019.13985). [PubMed: [32133055](https://pubmed.ncbi.nlm.nih.gov/32133055/)]. [PubMed Central: [PMC7043881](https://pubmed.ncbi.nlm.nih.gov/PMC7043881/)].
27. Wong MH, Chan EW, Chen S. Evolution and dissemination of OqxAB-like efflux pumps, an emerging quinolone resistance determinant among members of Enterobacteriaceae. *Antimicrob Agents Chemother*. 2015;**59**(6):3290-7. doi: [10.1128/AAC.00310-15](https://doi.org/10.1128/AAC.00310-15). [PubMed: [25801572](https://pubmed.ncbi.nlm.nih.gov/25801572/)]. [PubMed Central: [PMC4432112](https://pubmed.ncbi.nlm.nih.gov/PMC4432112/)].
28. Azargun R, Soroush Barhaghi MH, Samadi Kafil H, Ahangar Oskouei M, Sadeghi V, Memar MY, et al. Frequency of DNA gyrase and topoisomerase IV mutations and plasmid-mediated quinolone resistance genes among *Escherichia coli* and *Klebsiella pneumoniae* isolated from urinary tract infections in Azerbaijan, Iran. *J Glob Antimicrob Resist*. 2019;**17**:39-43. doi: [10.1016/j.jgar.2018.11.003](https://doi.org/10.1016/j.jgar.2018.11.003). [PubMed: [30445211](https://pubmed.ncbi.nlm.nih.gov/30445211/)].
29. Goudarzi M, Azad M, Seyedjavadi SS. Prevalence of plasmid-mediated quinolone resistance determinants and OqxAB efflux pumps among extended-spectrum-lactamase producing *Klebsiella pneumoniae* isolated from patients with nosocomial urinary tract infection in Tehran, Iran. *Scientifica (Cairo)*. 2015;**2015**:518167. doi: [10.1155/2015/518167](https://doi.org/10.1155/2015/518167). [PubMed: [26301114](https://pubmed.ncbi.nlm.nih.gov/26301114/)]. [PubMed Central: [PMC4537771](https://pubmed.ncbi.nlm.nih.gov/PMC4537771/)].
30. Rahman Z, Islam A, Rashid MU, Johura FT, Monira S, Watanabe H, et al. Existence of a novel qepA variant in quinolone resistant *Escherichia coli* from aquatic habitats of Bangladesh. *Gut Pathog*. 2017;**9**:1-4. doi: [10.1186/s13099-017-0207-8](https://doi.org/10.1186/s13099-017-0207-8). [PubMed: [29075330](https://pubmed.ncbi.nlm.nih.gov/29075330/)]. [PubMed Central: [PMC5654019](https://pubmed.ncbi.nlm.nih.gov/PMC5654019/)].
31. Kareem SM, Al-Kadmy IMS, Kazaal SS, Mohammed Ali AN, Aziz SN, Makharrata RR, et al. Detection of gyrA and parC mutations and prevalence of plasmid-mediated quinolone resistance genes in *Klebsiella pneumoniae*. *Infect Drug Resist*. 2021;**14**:555-63. doi: [10.2147/IDR.S275852](https://doi.org/10.2147/IDR.S275852). [PubMed: [33603418](https://pubmed.ncbi.nlm.nih.gov/33603418/)]. [PubMed Central: [PMC7886241](https://pubmed.ncbi.nlm.nih.gov/PMC7886241/)].
32. Roshani M, Goodarzi A, Hashemi A, Afrasiabi F, Goudarzi H, Arabestani M. Detection of qnrA, qnrB, and qnrS genes in *Klebsiella pneumoniae* and *Escherichia coli* isolates from leukemia patients. *Rev Med Microbiol*. 2022;**33**(1):14-9. doi: [10.1097/mrm.0000000000000250](https://doi.org/10.1097/mrm.0000000000000250).
33. Abossedgh R, Ghane M, Babaeekhou L. High Frequency of qnr Genes in Urinary Isolates of Extended-Spectrum  $\beta$ -Lactamase (ESBL)-producing *Klebsiella pneumoniae* in Tehran, Iran. *Shiraz E-Med J*. 2019;**21**(3). doi: [10.5812/semj.92032](https://doi.org/10.5812/semj.92032).
34. Jlili NE, Rejiba S, Smaoui H, Guillard T, Chau F, Kechrid A, et al. Trend of plasmid-mediated quinolone resistance genes at the Children's Hospital in Tunisia. *J Med Microbiol*. 2014;**63**(Pt 2):195-202. doi: [10.1099/jmm.0.062216-0](https://doi.org/10.1099/jmm.0.062216-0). [PubMed: [24194556](https://pubmed.ncbi.nlm.nih.gov/24194556/)].