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Frequency of Plasmid-Located Quinolone Resistance Genes in Clinical Isolates of *Klebsiella pneumoniae* in Northern Iran

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ABSTRACT

Background and objectives: Fluoroquinolones are a class of broad-spectrum antimicrobials typically used for the treatment of lower urinary tract infections. We aimed to determine the frequency of quinolone resistance genes in *Klebsiella pneumoniae* isolates from urinary tract infections in Guilan Province, Iran.

Methods: The resistance of 114 clinical isolates of *K. pneumoniae* to common fluoroquinolones and the minimum inhibitory concentration of ciprofloxacin were determined by disk diffusion and broth microdilution methods, respectively. Frequency of five plasmid-mediated quinolone resistance (PMQR) genes including *qnrA*, *qnrB*, *qnrS*, *qepA*, and *aac (6')-Ib-cr* was determined by PCR.

Results. According to phenotypic assays, 60 isolates (52.6%) were resistant to at least one quinolone compound, 42 isolates (36.8%) were resistant to all tested quinolones, and 28 isolates (24.6%) showed a high level of ciprofloxacin resistance. In addition, *aac(6')-Ib-cr* was the most common PMQR gene (n = 44), followed by *qnrS* (n = 32), and *qnrB* (n = 21).

Conclusion: The possible dissemination of PMQR genes poses a serious threat to the management of infections by resistant *Klebsiella pneumoniae*.

Keywords: Plasmids, quinolone, Klebsiella pneumoniae.

INTRODUCTION

Klebsiella pneumoniae is an opportunistic pathogen that causes various infections such as pneumonia, septicemia, diarrhea, endophthalmitis, meningitis, bacteremia, and urinary tract infections (UTIs), which is known as the most common hospital-acquired infection (1, 2). The infections caused by drug-resistant strains of *K. pneumoniae* are becoming an important health problem worldwide, and in Iran, a relatively high prevalence of drug-resistant isolates of *K. pneumoniae* has further highlighted the importance of controlling the infections caused by this bacterium (3).

Fluoroquinolones are a class of broad-spectrum antimicrobial agents often used for the treatment of lower UTIs but are effective in treating the infections caused by *K. pneumoniae* and other members of *Enterobacteriaceae*. However, a high level of resistance to fluoroquinolones in clinical isolates of these bacteria has been reported due to chromosomal mutations in the subunits of DNA gyrase (*GyrA* and *GyrB*) and DNA topoisomerase IV (*ParC* and *ParE*) (4, 5). These mutations, which are mostly located in a region designated as the quinolone-resistance determining region in *gyr* and *par* genes, change the enzyme structure and reduce their binding to fluoroquinolones (4). As the main mechanism appears to be mutations in the chromosomally-located genes, there is reason to believe that clonal spread may be important for the dissemination of fluoroquinolones resistance (6). Other resistance mechanisms are plasmid-mediated quinolone resistance (PMQR) determinants, overexpression of efflux pumps, and alteration in membrane permeability (7). It has been demonstrated that PMQR can rapidly spread among *Enterobacteriaceae* and has been increasingly reported in most parts of the world (8-12).

Three mechanisms of PMQR including Qnr proteins, AAC(6')-Ib-cr enzyme, and the efflux pumps QepA and OqxAB have been described. The *qnr* genes, including *qnrA*, *qnrB*, *qnrC*, *qnrD*, *qnrS*, and *qnrVC* encode proteins that interact with DNA gyrase and topoisomerase IV enzymes and protect the quinolone targets. The *aac(6')-Ib-cr* gene encodes aminoglycoside acetyltransferase that facilitates the acetylation of aminoglycosides, ciprofloxacin, and norfloxacin. The *qepAB* and *oqxAB* genes encode quinolone efflux pumps, which confer reduced susceptibility to quinolones by drug extrusion from the cell (11, 13).

Despite the importance of plasmid-located resistance genes in the spread of resistant microorganisms, no recent research had been performed on the prevalence of PMQR genes among *K. pneumonia* isolates in the Guilan Province, Iran. The present study aimed to investigate fluoroquinolone resistance and the frequency of plasmid-mediated quinolone resistance genes in clinical isolates of *K. pneumoniae* in the Guilan Province, northern Iran.

MATERIALS AND METHODS

A total of 114 clinical isolates of *K. pneumoniae* were collected from patients with UTI in the Guilan Province, northern Iran. The isolates were identified through conventional microbiological methods and amplification of *K. pneumoniae*-specific *phoE* gene in a PCR reaction.

Antimicrobial susceptibility testing was performed by disc diffusion method on Mueller-Hinton agar (Merck, Germany) using levofloxacin (5 µg), norfloxacin (10 µg), ofloxacin (5 µg), and gatifloxacin (5 µg). The antibiotic disks were purchased from High Media Co., India. In addition, the minimum inhibitory concentration (MIC) of ciprofloxacin and nalidixic acid was determined using the broth microdilution test (14). *K. pneumoniae* ATCC 10031 was used as the control strain.

The isolates with quinolone MIC profiles corresponding to the presence of a possible plasmid-mediated quinolone resistance gene (i.e. ciprofloxacin MIC ≥0.06 mg/L combined with nalidixic acid MICs 4–32 mg/L) (6) were screened for *qnrA*, *qnrB*, *qnrS*, *qepA* and *aac(6')-Ib-cr* genes by PCR using specific primers for the corresponding genes as described previously (15). The PCR reaction solution (25 µL) contained 12.5 µL of PCR Master Mix (CinnaGen, Iran), 10 pmol of each primer, and 2.5 µL of the template DNA. Table 1 shows the sequences of the primers used in the PCR reactions. The resulting PCR products were detected by electrophoresis on 1% agarose gel.

Table 1- The sequences of the primers used in this study

Primer	Sequences 5' to 3'	Annealing temperature	Amplicon size (bp)	Reference
phoE	CGACCTACCGCAACACCGACT CGACAGCACATAGCCGAGGGAC	65	413	This study
qnrA	AGAGGATTTCTCACGCCAGG TGCCAGGCACAGATCTTGAC	54	580	Al-Agamy et al.,2018
qnrB	GGMATHGAAATTCGCCACTG TTTGCYGYYCGCCAGTCGAA	54	264	Al-Agamy et al.,2018
qnrS	GCAAGTTCATTGAACAGGGT TCTAAACCGTCGAGTTCGGCG	54	428	Al-Agamy et al.,2018
qepA	AACTGCTTGAGCCCGTAGAT GTCTACGCCATGGACCTCAC		596	Al-Agamy et al.,2018
aac(6')-Ib-cr	TTGCGATGCTCTATGAGTGGCTA CTCGAATGCCTGGCGTGTTT	55	482	Al-Agamy et al.,2018

RESULTS

The results of agarose gel electrophoresis of *phoE* gene PCR amplicons are shown in figure 1.

According to phenotypic assays, 60 isolates (52.6%) were resistant to at least two quinolone compounds, 42 isolates (36.8%) were resistant to all tested quinolones, and 28 isolates (24.6%) showed a high level of resistance to ciprofloxacin according to MIC level (>64 µg/mL).

The resistance rates of *K. pneumoniae* isolates against the tested quinolone compounds were as follows: nalidixic acid (52.6%), ciprofloxacin (47.4%), gatifloxacin (46.5%), norfloxacin (43.9%), and levofloxacin (47.4%).

In addition, PMQR was determined in 54 (90%) quinolone-resistant *K. pneumoniae* *aac(6')-Ib-cr* was the most common PMQR isolates. Among the PMQR tested genes, gene (*n*=44), followed by *qnrS* (*n*=32), *qnrB* (*n*=21), and *qepA* (n=3). The *qnrA* gene was not detected in the isolates. Moreover, 45 isolates co-harbored PMQRs. In 26 of them, the *aac(6')-Ib-cr* gene was associated with *qnrS*, and the coexistence of *qnrB with aac(6')-Ib-cr* and *qnrS* was found in 14 and 9 isolates, respectively. All *qepA*-positive isolates co-harbored *aac(6')-Ib-cr*, and the coexistence of three PMQR genes was detected in 8 isolates. On the other hand, 6 of 60 quinolone-resistant isolates in the phenotypic tests did not contain the tested PMQR genes. Agarose gel electrophoresis of *aac (6')-Ib-cr*, *qnr*B, *qnrS*, and *qepA* PCR amplicons are shown in figures 2-4.

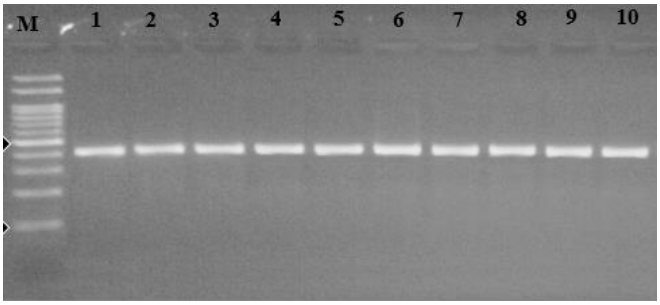

Figure 1- Agarose gel electrophoresis of the *phoE* gene PCR amplicons. Lanes 1-10: 413 bp PCR amplicons; Lane M: 100 bp DNA marker.

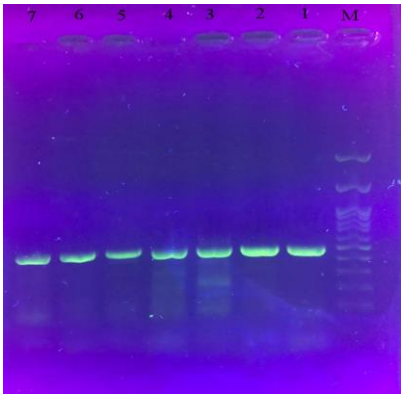

Figure 2- Agarose gel electrophoresis of the *aac (6')-Ib-cr* gene PCR amplicons. Lanes 1-7: 482 bp PCR amplicons; Lane M: 100 bp DNA marker.

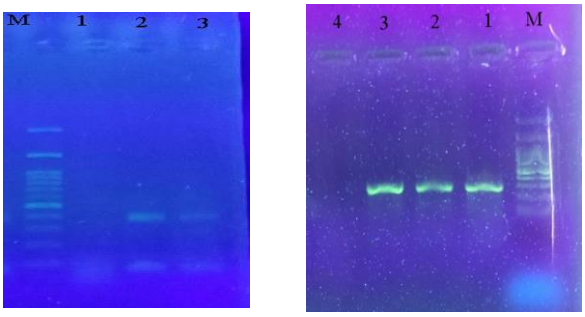

Figure 3- Agarose gel electrophoresis of the *qnrB* and *qnrS* genes PCR amplicons. (Left) Lanes 1-3: 264 bp PCR amplicons of *qnrB*; Lane M: 100bp DNA marker. (Right) Lanes 2 and 3: 428 bp PCR amplicons of *qnrS*; Lane M: 100 bp DNA marker

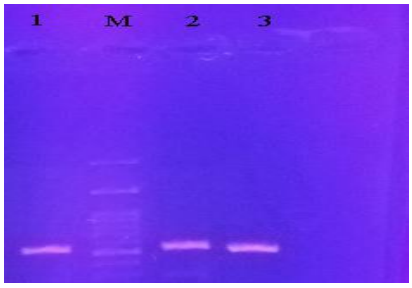

Figure 4-Agarose gel electrophoresis of the *qepA* gene PCR amplicons. Lanes 1-3: 596 bp PCR amplicons of *qepA*; Lane M: 100 bp DNA marker

DISCUSSION

Due to the widespread use of fluoroquinolones in the treatment of UTIs, resistance to this group of antibiotics has increased worldwide (16, 17). Mutations in the *gyr* and *par* genes have been reported as major mechanisms of fluoroquinolone/quinolone resistance associated with DNA gyrase and topoisomerase IV alterations in *Enterobacteriaceae*, as the PMQR genes and extrusion by intrinsic efflux pumps commonly mediate low-level fluoroquinolone/quinolone resistance (18).

The present study revealed quinolone resistance in more than half of clinical *K. pneumonia* isolates from patients with UTI. Moreover, 90% of resistant isolates harbored at least one PMQR gene. This high frequency of PMQR determinants in the present study is comparable with the results of a study carried out by Azargun et al., (2019) in Tabriz (Iran) and higher than the rate reported by studies in other parts of Iran (7, 11, 13).

In our study, *aac(6')-Ib-cr* was the predominant (73.3%) PMQR gene among quinolone-resistant *K. pneumonia* isolates. This finding is in agreement with the results of previous studies (11, 19, 20). Moreover, the *qnrS* and *qnrB* genes were detected in 53.3% and 35% of resistant isolates. However, *qnrA* was not detected in the isolates. These findings

are in line with the findings of a study in Hamedan, Iran (11). In a similar research by Yugderan et al. in India, the prevalence of the *aac(6')-Ib-cr* gene was highest (64%) in clinical isolates of *Enterobacteriaceae*, and *qnrA* was not detected in resistant isolates (21). In a study by Badamchi et al., 58.2% of quinolone nonsusceptible uropathogenic *Escherichia coli* isolates harbored the PMQR-encoding genes with *aac(6)-Ib-cr* as the predominant gene (22).

In the present study, *qepA* was detected in 5% of quinolone-resistant isolates, which is similar to the rate reported by previous studies (19).

Most *qnrS*/*qnrB*-positive *K. pneumoniae* isolates had both *aac (6')-1b-cr* and *qnr* genes. The co-transmission of *qnr* with *aac(6')-Ib-cr* genes that speeds up the formation of multidrug resistance in *Enterobacteriaceae* has been previously reported (22, 23). In the present study, 9 isolates carried two types of *qnr* genes. Similarly, in a study by Wang et al., *qnrA*, *qnrB*, and *qnrS* were detected either alone or in combination. However, some studies did not report the co-presence of different types of *qnr* genes (11, 24). In this study, six fluoroquinolones-resistant isolates did not harbor any of the tested genes. This indicates the possible involvement of other resistance mechanisms.

In general, the prevalence of PMQR determinants in *Enterobacteriaceae* may vary depending on the geographical location, study period, the pattern of antibiotic use, and the origin of bacteria.

CONCLUSION

In the present study, a high frequency and diversity of PMQR genes were detected among clinical isolates of *K. pneumoniae* isolates. This along with the increasing use of fluoroquinolones can lead to the emergence of highly resistant strains, which may pose a serious threat to the management of infections caused by these bacteria.

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DECLARATIONS

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Ethics approval and consent to participate

Not applicable since the study did not involve human or animal subjects.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article.

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