Frequency of Plasmid-Mediated Quinolone Resistance Genes qnr A, qnr B and qnr S Among Clinical Isolates of Klebsiella pneumoniae

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Abstract
Introduction: Plasmid-mediated quinolone resistance (PMQR) is a growing clinical concern throughout the world. The purpose of this study was to detect qnr-encoding genes and to evaluate the clonal relatedness of qnr-positive Klebsiella pneumoniae isolates.

Materials and Methods: A total of 88 K. pneumoniae isolates assessed to quinolone which were obtained from Tehran hospital in Tehran, Iran. Bacterial identification was administrated using standard laboratory methods. Quinolone resistance was determined using the Kirby-Bauer disk diffusion method according to the Clinical Laboratory Standards Institute (CLSI) guidelines. The PCR was employed to detect qnrA, qnrB and qnrS genes.

Results: The results of disk diffusion showed that 39.3%, 12.1%, 27.4%, 27.1%, 22.6% of strains were fully resistant to nalidixic, norfloxacin, ofloxacin, ciprofloxacin and levofloxacin, respectively. The qnrB (43% isolates) was the most commonly detected gene, followed by qnrS (34% isolates) and qnrA (23 % isolates) either alone or in combination with other genes.

Conclusions: This study describes the high prevalence of the qnrB, qnrS, and qnrA genes among K. pneumoniae isolates in Iran. The detection of qnr genes accentuates the need for organizing tactful policies associated with infection control measures in hospital settings in Iran.

Keywords: Klebsiella pneumoniae, PCR, Plasmid-Mediated Quinolone Resistance


Introduction
Klebsiella pneumoniae is an opportunistic pathogen that frequently causes severe nosocomial infections such as pneumonia, urinary tract infections, soft tissue infections and septicemia.¹² This organism is also known as a community-acquired pathogen.¹ Over the last few years, infections by carbapenem-resistant Klebsiella pneumoniae have caused high rates of mortality and morbidity.¹³

Quinolones were introduced for the treatment of bacterial infections in the 1960s.⁵ In the 1980s, more systemically active drugs became available.⁷ Quinolone agents inhibit DNA gyrase as their target site. In recent decades, fluoroquinolone resistance among Enterobacteriaceae has been increased. The primary mechanism of fluoroquinolone resistance in the family of Enterobacteriaceae is related to the mutation of chromosomal genes encoding topoisomerase IV, DNA gyrase, efflux pumps and porins. The recent reports demonstrate that quinolone resistance may also be mediated by plasmid-mediated quinolone resistance (PMQR) genes including qnrA, qnrB, qnrC, qnrD, qnrS, qnrVC, aac(6')-Ib-cr, qepAB and oqxAB.²³ Quinolone resistance genes code the pentapeptide repeat family proteins interacting with DNA gyrase and topoisomerase IV enzymes to hinder quinolone inhibition.¹⁰

The first PMQR (qnr) gene was detected in a K. pneumoniae isolate from Birmingham, Alabama, in 1994.¹¹ It occurred in an integron-like structure near Orf513 in pMG252 plasmid.¹² The second PMQR mechanism involves the acetylation of the drug by a variant of the gene encoding aminoglycoside acetyltransferase AAC (6')-Ib.¹³ The third mechanism of PMQR were the transporters, known as quinolone efflux pumps (QepAB and OqxAB), which exhibit resistance to the hydrophilic quinolones, especially to ciprofloxacin, enrofloxacin, and norfloxacin.⁷ The relationship between quinolone resistance and resistance to other antimicrobial agents, particularly aminoglycosides and β-lactams, is a serious problem in managing these infections. In-vitro studies have shown that after exposure to antibiotics, high levels of resistance can occur in isolates carrying the PMQR genes.¹⁴,¹⁵ Therefore, monitoring and the surveillance of PMQR genes among Enterobacteriaceae are required. The disk diffusion test, could not detect PMQR positive isolates because of the low-level resistance. Molecular techniques such as PCR assays are required as confirmatory tests for PMQR detection in epidemiological studies. Data on the frequency of qnrA, qnrB,
and qnrS genes is limited in K. pneumoniae isolates collected from clinical samples from humans in Iran. Accordingly, the aim of this study was to determine the prevalence of qnrA, qnrB, and qnrS genes in K. pneumoniae isolated from clinical specimens of patients who had referred to a hospital in Tehran, Iran.

Materials and Methods
Bacterial Isolation From Clinical Samples
In the present cross-sectional study, 88 non-repetitive K. pneumoniae isolates were collected from the clinical samples of patients admitted to the Tehran hospital from 21 September 2018 to 24 September 2019. Various clinical specimens including; sputum, blood, and urine were assayed. Identification of bacteria was performed by gram stain, culture characteristic and biochemical methods. For the preservation of bacteria at -70°C, trypticase soy broth containing 20% glycerol was used.

Antimicrobial Susceptibility Testing
Antibiotic susceptibility to detection of quinolone resistance against nalidixic acid (30 μg), norfloxacin (10 μg), ofloxacin (5 μg), levofloxacin (5 μg) and ciprofloxacin (5 μg) was used by Kirby-Bauer disk diffusion testing according to the Clinical Laboratory Standards Institute (CLSI) guidelines. The antibiotic disks were purchased from the Rosco. If isolates resistance to two of the quinolone antibiotics were classified as high-level quinolone resistance and resistance to nalidixic acid were classified as intermediate isolates and ciprofloxacin-susceptible isolates were classified as low-level quinolone resistance. For quality control in antibiotic susceptibility, the Escherichia coli American Type Culture Collection (ATCC) 25922 and Pseudomonas aeruginosa ATCC 27853 were used.

DNA Extraction and PCR Assay
The DNA of every isolate was extracted using fresh overnight culture in TSB by Favorgen; Taiwan Kit. Extracted DNA was subjected to PCR assay targeting three genes of qnrK. pneumoniae. Primers used for the detection of qnrK. pneumoniae Table 1. Genes in Isolates

<table>
<thead>
<tr>
<th>Target Genes</th>
<th>Primer Sequence (5’-3’)</th>
<th>Annealing Temperature (°C)</th>
<th>Size (bp)</th>
<th>Reference</th>
</tr>
</thead>
</table>
| qnrA         | Forward: ATTTCTACCCGCGAGATTG  
Reverse: GATCGGCAAGGTTAGGTCG  | 54                         | 516        | 17        |
| qnrB         | Forward: GATCGTGAAAGCCCGAGG  
Reverse: ACATGTCTAGATGTGTCG  | 53                         | 469        | 17        |
| qnrS         | Forward: ACGACATCCGTCAAACCTGCA  
Reverse: TAAAATGGACCCTGTAGGG  | 53                         | 417        | 17        |

qnr: quinolone resistance.

Discussion
The prevalence of qnrA, qnrB and qnrS genes in quinolone – non-susceptible K. pneumoniae isolated was investigated from different samples in Iran. Quinolones are among the most commonly prescribed antimicrobial agents for the treatment of major infections caused by the Enterobacteriaceae family. Also, resistance development to these antibiotics in bacteria complicates treatments and may sometimes lead to treatment defeat. Recently, PMQRs have been reported in various studies, especially among enterobacteria. However, there are few data on the prevalence of qnr genes among Enterobacteriaceae isolates in Iran. The high resistance rate observed among urine isolates (93.5%) in this study emphasizes the need of a local and national antimicrobial
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resistance surveillance system for monitoring the operation of antimicrobials and outward of antibiotic resistance in bacterial isolates present in hospital settings.

The present study demonstrated a high (50%) prevalence of PMQR determinants between quinolone resistant K. pneumoniae isolates collected from a hospital in Iran. The PMQR genes reported in our hospital were similar to those reported from many countries, particularly in China. However, the frequency of the genes found in our study was higher than that reported by Rodriguez-Martinez et al from Spain (1.8%).

Table 2. Distribution of the qnrA, qnrB and qnrS Genes Among 80 qnr-Positive Klebsiella pneumoniae Isolates

<table>
<thead>
<tr>
<th>qnr-Encoding Genes</th>
<th>Pattern Antibiotic Resistance</th>
<th>N (Isolate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>qnrB</td>
<td>NA</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>OFLX-NA</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>OFLX-NA-NOR-CP</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>OFLX-NA-NOR-CP-LVX</td>
<td>2</td>
</tr>
<tr>
<td>qnrA-qnrB</td>
<td>NA-CP</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>CP</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>NA-NOR</td>
<td>2</td>
</tr>
<tr>
<td>qnrB-qnrS</td>
<td>NA-CP</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>OFLX-NA-NOR</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>OFLX-NA-NOR-CP</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>OFLX-NA-LVX</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>OFLX-NA-NOR-CP-LVX</td>
<td>6</td>
</tr>
<tr>
<td>qnrA-qnrB-qnrS</td>
<td>NA-CP</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>OFLX-NA-NOR-CP-LVX</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>26</td>
</tr>
</tbody>
</table>

NA= Nalidixic acid, OFLX= Ofloxacin, NOR= Norfloxacin, CP=Ciprofloxacin, LVX= Levofloxacin.

Bacterial isolates vary in the range of 1% to 50% in various studies, depending on the selection criteria and study period for bacterial isolates. Moreover, this may indicate that the rate of PMQR is increasing among enterobacteria.

Briales et al reported that the prevalence of qnr genes has increased to 3.7%. Despite the fact that the prevalence of qnr genes in Spain continues to be low, it can be concluded that the wide geographical distribution of PMQR genes and their association with ESBL-producing plasmids should be taken into account as an extra factor that could compromise the therapeutic usefulness of quinolones.

Yang et al also reported the presence of different PMQR genes in clinical isolates of K. pneumoniae, indicating the wide spread of qnr genes among clinical isolates of enterobacteriaceae. Wang et al reported that qnrA, qnrB and qnrS were detected either alone or in combination in 3.8%, 4.7% and 3.8% of these isolates, respectively which had a much lower resistance rate compared to the present study.

In another study in Korea, Shin et al reported that 55.9% of K. pneumoniae ciprofloxacin-resistant isolates only contained qnrB (qnrB2, qnrB4 and/or qnrB6). These data suggest that inappropriate and extensive use of broad-spectrum antibiotics has resulted in the emergence of resistant isolates in hospital settings.

Purified qnrB, like qnrA, protected DNA gyrase from quinolone action. It seems to be even more potent than QnrA in blocking the action of ciprofloxacin. In the present study, qnrB had the highest frequency of resistance.
United States suggest that this resistance mechanism has been present long enough to disseminate widely. Preliminary surveys indicate that in ceftazidime-resistant Enterobacter and Klebsiella isolates from the United States qnrB is as common as qnrA. 26

It has been stated that qnrB2 has been found on plasmids collected in 1996, and the association of qnrB with SHV-12 on plasmids found in both India and the United States suggests that this resistance mechanism has been present long enough to disseminate widely.

Previous studies indicate that in ceftazidime-resistant Enterobacter and Klebsiella isolates from the United States, qnrB is as common as qnrA. 26 It would not be surprising if even more members of the qnr family were discovered.

The frequency of the qnrB and qnrS genes in antibiotic resistance against nalidixic acid and norfloxacin has been observed. This may be due to the change in the presence of plasmid. Resistance to quinolones is associated with porin loss, defined efflux pump or DNA gyrase alterations and plasmid. Resistance to quinolones is associated with porin observed. This may be due to the change in the presence of plasmid.

Conclusions
It has been found in this study that the prevalence of qnr genes has increased to 50%. Despite the fact that the prevalence of qnr genes is warning us that transferable resistance determinants may be transferred to other Enterobacteriaceae and non-fermenting gram-negative bacilli is a serious consideration in the care of hospitalized patients.

Authors’ Contributions
MN and SM participated in the research design and contributed to different parts of the research.

Conflicts of Interest Disclosure
There are no conflicts of interest in the present study.

Acknowledgments
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References


