Quinolone Susceptibility and Detection of qnr and aac(6’)-ib-cr Genes in Community Isolates of Klebsiella pneumoniae

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1. Background

Klebsiella pneumoniae is an opportunistic pathogen responsible for up to 10% of all nosocomial infections (1, 2). These infections are often treated with extended-spectrum cephalosporins, fluoroquinolones and carbapenems. However, resistance mechanisms such as production of β-lactamases, plasmid-mediated quinolone resistance (PMQR) and carbapenemases by the organisms have created serious therapeutic problems (3-5).

PMQR determinants comprise; QnrA, QnrB, QnrS, QnrC and QnrD proteins which protect DNA gyrase and topoisomerase IV from inhibition by quinolones; the aminoglycoside acetyltransferase variant, aac (6’)-ib-cr capable of acetylating and subsequently reducing the activity of norfloxacin and ciprofloxacin; and finally, the recently described fluoroquinolone specific efflux pump protein, qepA (5). Although PMQR determinants confer low level of quinolone resistance on their own, they have been shown to facilitate the acquisition of high level resistance among initially susceptible strains (6, 7).

PMQR determinants have been mostly identified in clinical isolates of Enterobacteriaceae, including K. pneumoniae, and have been shown to play not only an important role in quinolone resistance, but also resistance to other antibiotics, particularly β-lactams and aminoglycosides (8, 9). In fact, a number of studies have shown the presence of qnr genes along with various lactamases determinants on the same plasmids (10-14). Presence of PMQR genes in the community isolates of K. pneumoniae has also been shown, which provides a wider reservoir for the spread of these organisms (15).

2. Objectives

We studied the presence of qnrA, qnrB, qnrS and aac (6’)-ib-cr determinants among the cephalosporin and/or quinolone resistant community isolates of K. pneumoniae.
Table 1. Primers Used For Detection of qnr and aac (6')-Ib-cr Genes

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer Type</th>
<th>Primer Sequence</th>
<th>PCR Product Size</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>qnrA</td>
<td>Forward</td>
<td>TTCTCACGCCAGGATTGAG</td>
<td>571 bp</td>
<td>(17)</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>TGCCAGGGACAGAATCCGTGAC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>qnrB</td>
<td>Forward</td>
<td>TGGCGAAAAAATGACAGAAA</td>
<td>594 bp</td>
<td>(17)</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>GAGCAAGATGCCCTGGTAG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>qnrS</td>
<td>Forward</td>
<td>GACGTGCTAACGTGCTGA</td>
<td>388 bp</td>
<td>(17)</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>AACACCTGACATGGTCAAGA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>aac(6')-Ib-cr</td>
<td>Forward</td>
<td>TTGGCATGTCTCATGTGCTCTCA</td>
<td>482 bp</td>
<td>(12)</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>CTGAAATGCGCGCGTGTTT</td>
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</tbody>
</table>

Figure 1. Antibiotic Resistance Profile of 52 K. pneumoniae Isolates Collected From Outpatients.


3. Materials and Methods

3.1. Bacteria

Fifty-two K. pneumoniae isolates were collected from the Central laboratory in Karaj between July 2010 and January 2011 of which, 80.8% were from urine and 19.2% from stool samples. All isolates were identified by conventional biochemical and microbiological tests and were maintained in brain heart infusion broth (Oxoid, UK) containing 10% dimethyl sulfoxide (v/v) at -20ºC until use.

3.2. Antibacterial Susceptibility

Susceptibility to antibiotics was determined by the disc diffusion method using the CLSI recommendations and the following antibiotics (Himedia, India): amoxiclav (AMC, 20+10 µg), aztreonam (ATM, 30 µg), cefotaxime (CTX, 30 µg), ceftriaxone (CAZ, 30 µg), imipenem (IPM, 10 µg), ciprofloxacin (CP, 30 µg), levofloxacin (LOM, 5 µg), norfloxacin (NOR, 10 µg), ofloxacin (OFX, 5 µg) and nalidixic acid (NA, 30 µg). K. pneumoniae ATCC 10031 was used as the quality control for antimicrobial susceptibility tests.

3.3. DNA Extraction and PCR Amplification

DNA extraction was performed using an improved phenol/chloroform method where the lysis step was eliminated, and the cells were lysed directly by phenol (16). Presence of qnrA, qnrB, qnrS, and aac (6')-ib-cr genes was detected by PCR using the primers shown in Table 1 (12, 17). The reaction mixture (25 µl) contained 1.5 µl DNA template, 1.5 mM MgCl2, 0.25 mM of dNTP mix (Cinnagen, Iran), 1 unit of DFS-Taq DNA polymerase (Bioron, Germany), and 20 pmol of each primer (Faza Biotech, Iran). Amplifications were performed in a thermal cycler (Bioer TC25/H, Bioer Technology, China) using the following program: initial denaturation at 94°C for 5 min followed by 30 cycles of 1 min at 94 ºC, 1 min at annealing temperature (57ºC for qnrA, qnrB and qnrS, 54ºC for aac (6')-ib-cr), 1 min at 72ºC and a final extension period of 10 min at 72 C. The amplified PCR products were resolved by electrophoresis in 1.5% agarose gel and visualized after staining with ethidium bromide (Merck, Germany).

4. Results

4.1. Antibacterial Susceptibility

The antibiotic susceptibility results of the 52 K. pneumoniae isolates are shown in Figure 1. All isolates were resistant to amoxiclav and susceptible to imipenem. Resistance rates to the other antibiotics were 13.5% to ceftazidime, cefotaxime and levofloxacin; 11.5% to cefepime; 7.7% to ciprofloxacin, nalidixic acid, and ofloxacin; and 5.8% to norfloxacin (Figure 1). Twenty-three isolates were chosen for PCR studies based on their resistance to quinolones and/or cephalosporins.

4.2. Detection of qnr and aac (6')-ib-cr Determinants

Figure 2 shows the PCR amplification products of qnr and aac (6')-ib-cr genes among the 23 selected isolates. Overall, 7 out of the 23 selected isolates harbored qnr and/or aac (6')-ib-cr genes (30.4%), 6 of which were urinary isolates and 1, a stool isolate. None of the isolates harbored qnrA. Five isolates were resistant to all test quinolones and cephalosporins, of which, 3 carried aac (6')-ib-cr, one had qnrS, and one carried both aac (6')-ib-cr and qnrB genes. QnrB was detected in 2 isolates both of which were quinolone and cephalosporin resistant. Two isolates
Figure 2. PCR Amplification Products of qnr and aac (6’)-Ib-cr Genes

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<table>
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<tr>
<th></th>
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<tbody>
<tr>
<td>A</td>
<td>B</td>
<td>C</td>
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</table>

A, qnrB; B, qnrS and C, aac(6’)-Ib-cr genes in 23 community isolates of K. pneumoniae. L, 130bp ladder; C-, negative control.

A, harbored the qnrS gene; one of which was resistant to quinolones and cephalosporins and the other was quinolone susceptible, cephalexin resistant. Finally, one quinolone susceptible, cephalosporin resistant isolate carried the aac (6’)-Ib-cr gene.

5. Discussion

Presence of qnr and aac (6’)-Ib-cr genes in clinical isolates of E. coli and K. pneumoniae has been reported worldwide (4-9). A large number of studies have also shown the presence of qnr genes along with resistance to various β-lactamases, including the AmpC and extended-spectrum β-lactamases (10, 11, 14, 18, 19). However, studies on the presence of PMQR genes in the community isolates are far fewer. In the present study, majority of the community isolates of K. pneumoniae were susceptible to all test antibiotics except for amoxicillin. However, despite the low rate of antibiotic resistance, 13.5% of all test isolates and 30.4% of the quinolone and/or cephalosporin resistant isolates carried qnr and/or aac (6’)-ib-cr genes.

In a study conducted on Escherichia coli in Italy, the rate of qnr gene carriage was 27.8% of which; aac (6’)-ib-cr was detected in 11% of the community isolates (20). In another study conducted in northern Italy between 2004 and 2006, the aac (6’)-ib-cr gene was found in 3.9% of the community isolates of the uropathogenic E. coli (21). PMQR genes were also reported in commensal isolates of Enterobacteriaceae from Vietnam, including 45 K. pneumoniae isolates of which 35.5% carried the qnrS and aac (6’)-ib-cr genes (22). More recently, a study from Morocco showed that among 34 community isolates of K. pneumoniae, 41% harbored plasmid-mediated qnr genes, including qnrA, qnrB and qnrS, and 76.4% carried the aac (6’)-ib-cr gene (15). Our results were closer to the report from Vietnam but much lower than the Moroccan study. We did not detect the qnrA gene among our isolates. Although qnrA was the first PMQR gene discovered, several studies have indicated that qnrS, qnrB and aac (6’)-ib-cr are more commonly found among Enterobacteriaceae (5, 6, 23).

Consistent with the previous studies, we also showed the presence of aac (6’)-ib-cr, qnrB, and qnrS genes but not qnrA among our community isolates. We believe that this is the first report on the presence of PMQR genes in K. pneumoniae isolates collected from outpatients in Iran. There is one other study from Iran where the prevalence of PMQR genes (qnrA and qnrB but not qnrS) was detected in E. coli (13). Since quinolone resistant genes are plasmid-mediated, dissemination of these antibiotic resistance determinants could easily occur between opportunistic Gram-negative pathogens, which can be problematic and further limit treatment of these infections.

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Authors’ Contributions

Collection of the bacteria and implementation of the research: Seyed Mohsen Seyedpour; Research design,
interpretation of results and preparing the manuscript: Fereshteh Eftekhar.

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References