Prevalence of Plasmid-Mediated Quinolones Resistance among *Klebsiella pneumoniae* Strains Isolated from Hospitals in Borujerd, Iran

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ABSTRACT

Background and Objective: *Klebsiella pneumoniae* is one of the most common causes of bacterial infections. Presence of plasmid-mediated quinolone resistance genes causes low level of resistance in *K. pneumoniae*. This study investigated the prevalence of resistance to quinolones and fluoroquinolones, and the frequency of *qnrA*, *qnrB* and *qnrS* genes among *K. pneumoniae* strains.

Methods: The study was performed on 100 *K. pneumoniae* strains isolated from hospitals in city of Borujerd (Iran) during April to September 2014. Susceptibility of the isolates to nalidixic acid, ciprofloxacin, norfloxacin and ofloxacin was evaluated. Minimum inhibitory concentration (MIC) of ciprofloxacin was determined using ciprofloxacin Etest strips. Polymerase chain reaction was performed to detect *qnrA*, *qnrB and qnrS* genes in quinolone-resistant isolates using specific primers.

Results: The results showed that 38% of the isolates were resistance to both nalidixic acid and ciprofloxacin. The prevalence of ofloxacin- and norfloxacin-resistant isolates was determined to be 18% and 15%, respectively. The MIC values for ciprofloxacin were ranging from 0.064 to ≥256 μg/ml. In addition, four ciprofloxacin-resistant isolates (10%) had MIC of ≥256 μg/ml. The *qnrA* gene was not detected in any of the quinolone-resistant isolates. Moreover, 23.6% (n=9) and 5.2% (n=2) of the quinolones-resistant isolates contained the *qnrB* and *qnrS* genes, respectively.

Conclusion: Although 38 isolates were ciprofloxacin-resistant, the *qnrB*, qnrS genes were detected in a small number of isolates. This indicates the involvement of factors other than the *qnr* genes in resistance of these isolates to quinolones.

Keywords: Klebsiella Pneumoniae, Qnr protein, Borujerd.

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INTRODUCTION

Klebsiella pneumonia is one of the most common causes of bacterial infections such as pneumonia, sepsis, urinary tract infection and nosocomial infections. *K. pneumoniae* infections are more acute in immunocompromised patients (1). Several cases of antibiotic-resistant *K. pneumonia* have been reported in recent years. Beta-lactams and cephalosporins are usually used to treat infections caused by *K. pneumonia*, but due to increasing rate of resistance to these antibiotics, broad-spectrum fluoroquinolones could be used as alternative (2, 3). Nalidixic acid and ciprofloxacin have been known as quinolone and fluoroquinolone agents, respectively. Fluoroquinolones are mainly used to treat genital and urinary tract infections (4). Quinolones kill bacteria by inhibiting bacterial DNA gyrase. Resistance to quinolones occurs via changes in target enzymes (such as DNA gyrase and topoisomerase IV), changes in input and efflux of antibiotics, and plasmid-quinolone resistance gene (*qnr*) (5, 6). In plasmid-mediated quinolone-resistance (PMQR), genes *aac* (6)-I*b-cr*, *qnr* and efflux pumps are involved in the low-level resistance to quinolones (7-9). About six *qnr* gene families have been identified so far, including *qnr A, B, C, D, S,* and *VC*. Presence of *qnrA, qnrB* and *qnrS* genes in *K. pneumoniae* strains have been reported from various countries (10-12). The *qnr*A gene was primarily found in *Escherichia coli*, preventing DNA gyrase inhibition by ciprofloxacin. Low prevalence of *qnrC* and *qnrD* has been reported in *K. pneumoniae* strains isolated in China. Moreover, *qnrS1* of *Shigella flexneri* and *qnrB1* of *K. pneumoniae* show 41% and 59% homology with *qnrA*, respectively (13-15). Due to lack of enough data regarding the frequency of these genes in Iran, this study aimed to evaluate resistance to quinolones and fluoroquinolones, and the frequency of *qnrA*, *qnrB* and *qnrS* genes among clinical *K. pneumoniae* isolates from hospitals in Borujerd, Iran.

MATERIAL AND METHODS

Overall, 100 *K. pneumoniae* isolates were randomly collected from urine (78%), trachea (15%), wounds (4%) and blood (3%) samples of patients in three hospitals of Borujerd during April to September 2014. All *K. pneumoniae* isolates were identified via conventional microbiological testing. Antimicrobial susceptibility testing of *K. pneumoniae* isolates was performed using nalidixic acid, ciprofloxacin, norfloxacin and ofloxacin disks (Rosco, Denmark) using disk diffusion method. Minimum inhibitory concentration (MIC) of ciprofloxacin was determined using ciprofloxacin Etest strips (Hi media, India). According to the CLSI criteria, MIC values of ≥1 μg/ml, ≥2 μg/ml and ≥4 μg/ml for ciprofloxacin were considered as sensitive, intermediate and resistant, respectively (16). DNA of all isolates was extracted using commercial mini column DNA extraction Kit (Cinnagen, Iran). Polymerase chain reaction (PCR) assay was performed to detect *qnrA, qnrB* and *qnrS* genes using specific primers (Metabion, Germany) (Table 1) (17). The amplification program consisted of initial denaturation for 3 min at 94 °C, 35 cycles of denaturation for 30s at 94 °C, annealing for 30s at 55 °C, extension for 30s at 72 °C, and final extension for 5 min at 72 °C in a thermocycler (Peq star, Germany).

RESULTS

Results of antimicrobial susceptibility testing showed that 38% of all isolates were resistant against both nalidixic acid and ciprofloxacin. In addition, 18% and 15% of the isolates were resistant to ofloxacin and norfloxacin, respectively. Quinolone-resistance was detected in 33%, 4%, and 1% of urine, trachea, and blood samples, respectively. In addition, 15% of the isolates were resistant to nalidixic acid, ciprofloxacin, ofloxacin and norfloxacin, while 21% of the isolates were resistant to nalidixic acid and ciprofloxacin. Based on the results of the Etest, MIC values ranged from 0.064 to ≥256 μg/ml. Moreover, four ciprofloxacin-resistant isolates (10%) had MIC of ≥256 μg/ml. The minimum concentration of ciprofloxacin that inhibited growth in 50% (MIC_{50}) of *K. pneumoniae* isolates was determined as ≥16 μg/ml. The *qnrA* gene was not detected in any of the quinolone-resistant *K. pneumoniae* isolates. The Genes *qnrB* and *qnrS* were detected in 23.6% (n=9) and 5.2% (n=2) of the quinolones-resistant isolates, respectively. Simultaneous presence of *qnrB* and *qnrS* genes was not detected in any of the isolates (Figure1). The MIC of ciprofloxacin in strains containing both *qnrB* and *qnrS* genes are shown in in table 2.

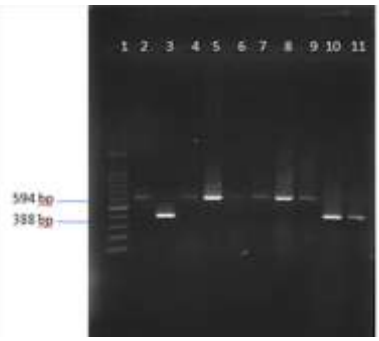
Table 1- Primers used for detection of *qnrA*, *qnrB* and *qnrS* in quinolone-resistant *K. pneumonia* strains

Gene	Primer sequence	Length of fragment
*qnr*A	Forward: 5′- TTC TCA CGC CAG GAT TTG AG-3′	571 bp
	Reverse: 5′- TGC CAG GCA CAG ATC TTG AC-3′	
*qnr*B	Forward : 5′- TGG CGA AAA AAT TGA ACA GAA-3′	594 bp
	Reverse : 5′- GAG CAA CGA TCG CCT GGT AG-3	
*qnr*S	Forward: 5′- GAC GTG CTA ACT TGC GTG AT-3′	388 bp
	Reverse: 5′- AAC ACC TCG ACT TAA GTC TGA-3′	

Table 2- MIC of ciprofloxacin in *qnrB*-positive, *qnrS*-positive, and some *qnr*-negative *K. pneumonia* strains

Number of isolates	MIC	*qnrB*	*qnrS*
13	12	+	-
8	64	+	-
45	8	+	-
33	12	+	-
74	8	+	-
18	16	+	-
66	4	+	-
9	4	+	-
64	32	+	-
72	256	-	+
40	8	-	+
15	4	-	-
11	64	-	-
32	256	-	-

Figure 1- Detection of *qnrB* and *qnrS* genes in quinolone-resistant *K. pneumonia* strains by gel electrophoresis of PCR products. Column 1: 1 kb DNA ladder, column 2: positive control for *qnrB*, column 3: positive control for *qnrS*, columns 4-9: *qnrB*-positive strains, columns 10 and 11: *qnrS*-positive strains



DISCUSSION

Considering the importance of quinolone resistance among immunocompromised and hospitalized patients, the relatively high level (38%) of resistance to nalidixic acid and ciprofloxacin among *K. pneumoniae* is a significant and important finding. Similar to most previous study, we could not find the *qnrA* gene in any of the *K. pneumoniae* isolates (18-20). In a recent study, *qnrB* was identified as the dominant *qnr* gene with frequency of 23.6%. Consistent with our findings, studies in China, Singapore and Malaysia have reported *qnrB* as the dominant *qnr* gene (18-20). In the present study, the gene *qnrS* was detected only in two strains. However, a study in Thailand reported *qnrS* as the most prevalent *qnr* gene in *K. pneumoniae* strains (21). Moreover, a study in Malaysia showed that only 1.1% of *K. pneumoniae* isolates contain the *qnrS* gene (20). Limited number of studies in Iran has been conducted on the frequency of *qnr* genes among other hospital pathogens. Soleimani et al. reported that about 83% of *E. coli* isolates from Khorramabad hospitals (Iran) were resistant to nalidixic acid and ciprofloxacin. They also reported presence of the *qnrA* gene in 1.12% of nalidixic acid-resistant isolates and 3.14%

of ciprofloxacin-resistant isolates (22). Study of Oktem et al. in Turkey on 34 *E. coli* and 44 *K. pneumoniae* isolates showed that 6.78% of the isolates were resistant to both ciprofloxacin and nalidixic acid. They also detected the *qnrA* gene in 3.6% of quinolone-resistant isolates (23). In the present study, only one of the isolates containing the *qnrS* gene had MIC of ≥256 µg/ml. The isolates containing the *qnrB* gene had MIC values ranging from 12 to 64 µg/ml. However, the *qnr*-negative strains also had high MIC values (Table 2), suggesting that resistance mechanisms other than the *qnr* genes may be involved in these isolates. These findings confirm that although the *qnr* genes are not solely involved in resistance to quinolones, they reduce the susceptibility to nalidixic acid and fluoroquinolones. The qnr agents protect quinolones targets in bacteria, and the genes encoding the agents are widely distributed in the *Enterobacteriaceae* family. It is thought that the *qnr* genes induce low to medium quinolone-resistance, whereas strains with mutations in the *gyrA* and *parC* or plasmid mediated *aac* (6') *-Ib-cr* and *qnr* genes exhibit a high level of quinolone resistance. The findings of this study suggest that mechanisms beside *qnr* genes may be involved in quinolone resistance among hospital-adapted pathogens such as *K. pneumoniae*.

CONCLUSION

The results of this study show the involvement of PMQR via *qnrB and qnrS* genes among *K. pneumoniae* strains isolated from hospitals in Broujerd. Thus, more attention should be given to plasmid-mediated mechanism of resistance that could increase the risk of transmission and rapid spread of quinolone-resistance among hospital-adapted bacteria.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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