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Prevalence of *qnr* Genes and Antibiotic Susceptibility Patterns among Clinical Isolates of *Klebsiella Pneumoniae* in West of Iran

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Abstract

Background: This study aimed to define prevalence of qnr genes and antibiotic susceptibility patterns among clinical isolates of *K. pneumoniae* in Lorestan province, west of Iran.

Methods: Totally, 107 *K. pneumoniae* isolates were randomly collected since December until September 2012 from hospitalized patients at general hospitals in Lorestan, Iran. The isolates were from different clinical samples including urine, sputum, etc. Biochemical characterizations were performed for detecting isolates. Antibiotic susceptibility testing by disk diffusion method was performed according to recommendations of Clinical and Laboratory Standards Institute using 12 antibiotic disks. *K. pneumonia* isolates were screened by multiplex PCR amplification of qnrA, qnrB and qnrS using specific primers and sequence analysis of amplified regions of the isolates was also performed.

Results: 43 (40.2%) out of 107 isolates were multidrug-resistant (MDR). Ciprofloxacin (Quinolone) susceptibility testing showed that 34 isolates were resistant, 7 isolates were intermediately resistant and 66 isolates were sensitive. 18 (16.8%) out of 107 *K. pneumoniae* clinical isolates were positive for qnr gene. Among all the qnr-positive isolates, 16 isolates (88.9%) carried qnrB, 1 isolate (5.55%) carried qnrS and the rest (5.55%) carried both qnrB and qnrS genes while no qnrA was detected in these clinical isolates. qnr determinants were detected in 8 (23.5%) of the ciprofloxacin-resistant isolates as well as 1 (14.3%) and 9 (13.6%) intermediate and sensitive isolates, respectively. No significant association was observed between ciprofloxacin resistance and presence of qnr genes (P>0.05).

Conclusion: Findings of the present study indicated that emergence of qnr determinants contributed to development and spread of quinolone resistance in Iranian isolates of *K. pneumonia*.

Keywords: *Klebsiella pneumonia*, qnr gene, Quinolone resistance, Lorestan, Iran

Introduction

Klebsiella pneumonia (family Enterobacteriaceae) is an opportunistic pathogen that usually causes hospital and community acquired bacterial infections in humans [1]. In recent years, emergence of multidrug-resistant *K. pneumoniae* isolates has become a serious antibiotic management problem and led to great concern around the world [1,2]. At present, quinolone resistance is a widespread phenomenon among the Enterobacteriaceae [2,3]. Main mechanisms of resistance to quinolones in this family including mutations in the quinolone resistance determining regions (QRDRs) of DNA gyrase and topoisomerase IV, efflux pumps enhancement or decreased accumulation mediated by reduce in permeability of bacterial cell wall are chromosomally mediated [4-8]. Recently, mechanisms of plasmid-mediated quinolones resistance (PMQR) by qnr genes have been reported [9] These qnr genes (qnrA, qnrB and qnrS) encode proteins of the pentapeptide repeat family that interfere with the action of quinolones on bacterial DNA gyrase and topoisomerase IV [9-11]. Although the qnr gene indicates a low level of resistance to quinolones, its attendance aids the selection of chromosomal mutations, facilitating increased resistance in the host strain [9-11]. Frequency of qnr genes PMQR associated with the qnr genes in different human clinical enterobacterial isolates was determined first in the USA in 1994 using a *K. pneumoniae* isolate (later termed qnrA1) [9], which was then widely reported worldwide [12-19]. However, no study has been done on prevalence of qnr genes among enterobacterial clinical isolates in Iran. For the first time, this study aimed to define prevalence of qnr genes and antibiotic susceptibility patterns among clinical isolates of *K. pneumoniae* from general hospitals in Lorestan province, west of Iran.

Materials and Methods

Studied area

This descriptive study was carried out since December until September 2012 on hospitalized patients at general hospitals of Lorestan province, located between valleys of Zagros Mountain in west of Iran and bordering with provinces of Markazi, Hamedan, Kermanshah, Khuzestan, Ilam and Isfahan. Lorestan covers an area of 28.294 km^2 and its population is approximately 2 million people. Major cities of this province are Khorramabad, Borujerd, Aligoodarz, Dorood, Koohdasht, Azna, Alashtar, Noorabad and Pol-e-Dokhtar (Figure 1) [20].

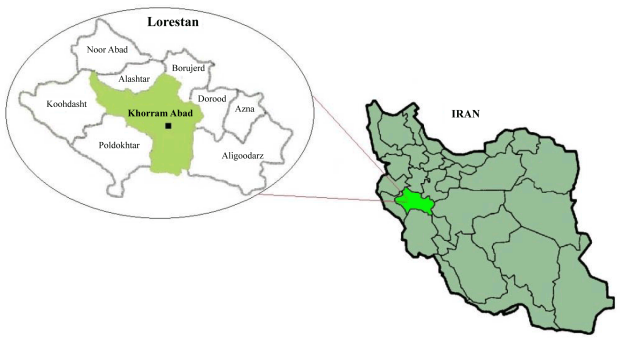

Figure 1: Geographical locations where this study was carried out

Bacterial isolates

Totally, 107 *K. pneumoniae* isolates were randomly collected since December until September 2012 from hospitalized patients at general hospitals of Lorestan, Iran. The isolates were collected from different specimens, including urine, sputum, lesion, blood and other specimens. All the isolates were routinely cultured on Mueller-Hinton (MH) agar plates and typical colonies were picked up and identified by biochemical tests using the API®-20E test kits (bioMérieux, Lyon, France). The bacteria were grown at 37°C for 18–24 h in order to prepare bacterial suspension and DNA extraction.

Susceptibility testing

Antibiotic susceptibility of the isolates to amikacin (10 µg), ampicillin (10 µg), meropenem (10 µg), nalidixic acid (30 µg), cefotaxime (30 µg), ceftazidime(30 µg), cefteriaxone (30 µg), cephalexine, ciprofloxacin (5 µg), cefexim (30 µg), gentamicin (10 µg) and imipenem(10 µg) (all the antibiotics were purchased from Oxoid, UK) was determined by disk diffusion method as recommended by Clinical and Laboratory Standards Institute (CLSI) [21] on Mueller–Hinton agar plates. In addition, *K. pneumoniae* ATTC BAA-1705 was used as a quality control strain.

Screening for qnr genes

The 107 clinical isolates of *K. pneumoniae* were screened by multiplex PCR amplification of qnrA, qnrB and qnrS using specific primers shown in Table 1, as previously described by Robicsek et al. [3]. Briefly, the colonies were transferred to an Eppendorf tube filled with water and boiled to prepare DNA templates for PCR. Target fragments were amplified under PCR conditions of 94°C for 45 s, 53°C for 45 s and 72°C for 60 s with cycle number of 32. Positive (containing strains with known qnr genes) and negative (without DNA template) controls were included in each run. Amplification products were provisionally identified according to their sizes in ethidium bromide-stained agarose gels.

Amplicon	Primers	Sequence (5'–3')	Size (bp)
QnrA	*qnrA-A* *qnrA-B*	ATTTCTCACGCCAGGATTTG GATCGGCAAAGGTTAGGTCA	516
QnrB	*qnrB-A* *qnrB-B*	GATCGTGAAAGCCAGAAAGG ACGATGCCTGGTAGTTGTCC	469
QnrS	*qnrS-A* *qnrS-B*	ACGACATTCGTCAACTGCAA TAAATTGGCACCCTGTAGGC	417

Table 1: Sequences of primers used for multiplex PCR

DNA sequencing

DNA sequence analysis was performed using direct sequencing of both strands using an auto-sequencer. The obtained DNA sequences were compared and analyzed using BLAST online search engine from GenBank in website of National Center for Biotechnology Information (http://www.ncbi.nlm. nih.gov/blast).

Statistical analysis

Data analysis was carried out using SPSS statistical package (version 17.0) (SPSS Inc., Chicago, IL, USA). P value of <0.05 was considered statistically significant.

Results

Out of 107 isolates, 43 isolates (40.2%) were multidrug-resistant (MDR). The highest rate of resistance was observed in ceftazidime and cefotaxime. Moreover, the lowest rate of resistance was seen in imipenem, meropenem and amikacin, respectively. Ciprofloxacin (Quinolone) susceptibility testing showed that 34 isolates (31.8%) were resistant, 7 isolates (6.5%) were intermediately resistant and 66 isolates (61.7%) were sensitive. 18 (16.8%) out of 107 *K. pneumoniae* clinical isolates screened by multiplex PCR, were positive for the qnr gene. Among all the qnr-positive isolates, 16 isolates (88.9%) carried qnrB, 1 isolate (5.55%) carried qnrS and the rest (5.55%) carried both qnrB and qnrS genes while no qnrA was detected in the present clinical isolates. Table 2 shows clinical characteristics of these isolates and distribution of qnrA, qnrB and qnrS. Qnr determinants were detected in 8 (23.5%) of the ciprofloxacin-resistant isolates as well as 1 (14.3%) and 9 (13.6%) intermediate and sensitive isolates, respectively (Table 3). No significant association was found between ciprofloxacin resistance and presence of qnr genes (P=0.074). Moreover, complete sequences of positive qnrB and qnrS isolates were submitted to the GenBank database and assigned accession numbers AB894351, AB894352, AB894353 and AB894354.

Discussion

Klebsiella pneumoniae colonizes >75% of hospitalized patients and causes the estimated 8% of all nosocomial infections including pneumonia, urinary tract, wound and diarrhoea infections [22]. At

present, emergence of multidrug-resistant *K. pneumoniae* isolates have become a serious antibiotic management problem and led to great concern worldwide [1,2]. This investigation provided the first epidemiological survey on the frequency of *qnrA, qnrB* and *qnrS* genes by multiplex PCR in Lorestan province, Iran, between December and September 2012. In this survey, *qnr* genes were determined in 18 (16.8%) of the isolates. This rate of *qnr* prevalence was consistent with some studies carried out in Taiwan and the USA (15, 16). Prevalence of *qnr* in the present study was also higher than that shown in other areas in Brazil (2.3%), Singapore (5.2%) and the USA (11.1%) [12,13,17]. In contrast, it was much lower than the prevalence of *qnr* genes detected in Malaysia (48.9%) and China (65.5%) [14,19].

Number of Strains	Specimen	Sex	Age	Diagnoses	*Qnr*
Kp6	Sputum	Male	12 Months	Pneumonia	*qnrB*
Kp 8	Urine	Male	6 years	UTIa[a]	*qnrB*
Kp13	Sputum	Female	23 years	Pneumonia	*qnrB*
Kp17	Urine	Female	9 years	UTIa	*qnrS*
Kp18	Sputum	Male	3 years	Pneumonia	*qnrB*
Kp19	Tracheal	Male	51 years	Pneumonia	*qnrB*
Kp26	Sputum	Male	13 years	Pneumonia	*qnrB*
Kp33	Sputum	Female	6 Months	Pneumonia	*qnrB*
Kp42	Urine	Female	33 years	UTIa	*qnrB*
Kp44	Sputum	Male	14 Months	Pneumonia	*qnrB*
Kp57	Urine	Female	52 years	UTIa	*qnrB*
Kp68	Sputum	Female	12 Months	Pneumonia	*qnrB*
Kp73	Urine	Male	4 years	UTIa	*qnrB*
Kp84	Sputum	Female	8 Months	Pneumonia	*qnrB, qnrS*
Kp93	Sputum	Male	2 years	Pneumonia	*qnrB*
Kp97	Urine	Male	19 Months	UTIa	*qnrB*
Kp101	Sputum	Female	11 years	Pneumonia	*qnrB*
Kp104	Sputum	Male	47 years	Pneumonia	*qnrB*

Table 2: Clinical characteristics and *qnr* genotype of the *qnr*-positive isolates. a :Urinary tract infection

Ciprofloxacin susceptibility	No. (%) isolate with *qnr* determinants				
	qnrA	*qnrB*	*qnrS*	*qnrB* + *qnrS*	Total
Sensitive (n=66)	0 (0)	8 (12.1)	0 (0)	1 (1.5)	9 (13.6)
Intermediate (n=7)	0 (0)	1 (16.6)	0 (0)	0 (0)	1 (14.3)
Resistant (n=34)	0 (0)	7 (20.6)	1 (2.9)	0 (0)	8 (23.5)
Total (n=107)	0 (0)	16 (14.9)	1 (0.93)	1 (0.93)	18(16.8)

Table 3: Frequency of *qnr* genes based on ciprofloxacin (Quinolone) susceptibility in *K. pneumoniae* isolates from Lorestan, Iran

The present findings indicated that *qnrB* was the most prevalent one (88.9%), followed by *qnrS* (5.55%), whereas no isolates carried *qnrA* among all the clinical isolates. In the studies conducted by Minarini et al. [13] and Saiful et al. [19] on clinical isolates in Brazil (2.3%) and Malaysia (31.9%), respectively, *qnrB* has been proven to be more prevalent than other *qnr* genes among the tested clinical isolates. In contrast, in other investigations carried out in China and the USA, it has been shown that *qnrS* (14.9%) and *qnrA* (14%) are the most prevalent of all *K. pneumoniae* clinical isolates screened by multiplex PCR, respectively [16,18]. The present investigation shows no *qnrA* in its clinical isolates; similarly, Minarini et al. [13] and Saiful et al. [19] in Brazil and Malaysia have indicated no *qnrA* or *qnrS*-positive isolates among their clinical isolates of *K. pneumonia*, respectively.

In the present study, *qnr* determinants (*qnrA, qnrB* and *qnrS*) were identified from 8 (23.5%) ciprofloxacin-resistant, 1 (14.3%) intermediate and 9 (13.6%) sensitive isolates. Similar to these results, Saiful et al. [19] reported that the highest percentage of *qnr* determinants (47.8%) was found in ciprofloxacin-resistant isolates. It has been previously demonstrated that clinical isolates with *qnr* determinants are known to harbor multiple ciprofloxacin resistance mechanisms such as variations in *gyrA* or reduce the drug permeability and therefore facilitate high resistance to ciprofloxacin [9,10]. In this survey, it was also proven due to lack of significant association between ciprofloxacin resistance and presence of *qnr* determinants. Moreover, the results reflected considerable frequency of *qnr*-positive *K. pneumonia* among clinical isolates in Iran. This frequency of *qnr* genes indicated that *qnr* genes were disseminating and subsequently prevalence of MDR *K. pneumonia* was increasing because of probable increasing resistance to quinolone.

Conclusion

The findings of our study showed considerable frequency of *qnr*-positive *K. pneumonia* among clinical isolates in Iran. Therefore, it is necessary to properly use antibiotics, especially quinolone antibiotics, and continuously monitor resistance patterns in *K. pneumoniae* in hospital settings.

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