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Quinolone Susceptibility and Detection of *qnr* and *aac*(6')-*Ib-cr* Genes in Community Isolates of *Klebsiella pneumoniae*

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Background: Plasmid-mediated quinolone resistance genes (PMQR) have been shown to play not only an important role in quinolone resistance, but also resistance to other antibiotics, particularly β-lactams and aminoglycosides. These genes are mainly associated with clinical isolates of *Enterobacteriaceae*. However, detection of PMQR genes in the community isolates can increase the dissemination rate of resistance determinants among bacteria.

Objectives: This study aimed to investigate quinolone resistance and distribution of *qnr* and *aac* (6')-*Ib-cr* genes among the community isolates of *Klebsiella pneumoniae*.

Materials and Methods: Fifty-two *K. pneumoniae* isolates were collected from the Central Laboratory in Karaj between July 2010 and January 2011. Antibacterial susceptibility was determined by the disc diffusion method. Quinolone and/or cephalosporin-resistant isolates were screened for the presence of *qnr*A, *qnr*B, *qnr*S and *aac* (6')-*Ib-cr* genes by polymerase chain reaction (PCR).

Results: Of the 52 *K. pneumoniae* isolates, 23 were resistant to cephalosporins and/or quinolones. Overall, 7 out of the 23 resistant isolates harbored *qnr* and/or *aac* (6')-*Ib-cr* genes (30.4%). Among these, 5 isolates were resistant to both classes of antibiotics of which; 3 carried the *aac* (6')-*Ib-cr* gene, one had the *qnr*S, and one harbored both *aac* (6')-*Ib-cr* and *qnr*B genes. None of the isolates contained *qnr*A. Two isolates were sensitive to quinolones and resistant to cephalosporins of which; one had *qnr*S and the other carried the *aac* (6')-*Ib-cr* gene.

Conclusions: Our study showed that 30.4% of the quinolone and/or cephalosporin resistant community isolates of *K. pneumoniae* carried PMQR genes. These results confirm that community isolates can be an important source for spreading antibiotic resistance determinants among Gram negative pathogens. This is the first report from Iran on detection of PMQR in the community isolates of *K. pneumoniae*.

Keywords:Klebsiella Pneumonia; Quinolone Resistance; PMQR; qnr; aac (6')-Ib-cr qnr Genes

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 carbapanemases by the organisms have created serious therapeutic problems (3-5).

PMQR determinants comprise; *Qnr*A, *Qnr*B, *Qnr*S, *Qnr*C and *Qnr*D proteins which protect DNA gyrase and topoisomerase IV from inhibition by quinolones; the aminoglycoside acetylteransferase variant, *aac (6')–Ib–cr* capable of acetylating and subsequently reducing the activity of norfloxacin and ciprofloxacin; and finally, the recently described flouroquinolone 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

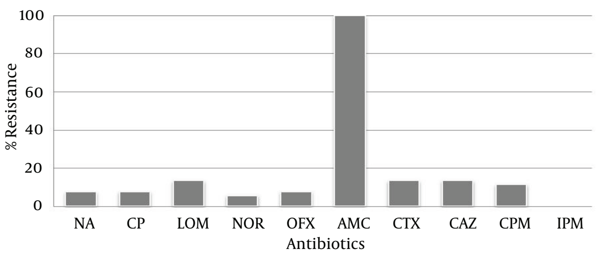
We studied the presence of *qnr*A, *qnr*B, *qnr*S and *aac* (6')–*ib–cr* determinants among the cephalosporin and/or quinolone resistant community isolates of *K. pneumoniae*.

Implication for health policy/practice/research/medical education:
Presence of plasmid-mediated quinolone resistance (PMQR) genes in clinical isolates of *Enterobacteriaceae*, including *K. pneumoniae* have been shown to play an important role in quinolone resistance, as well as resistance to β-lactams and aminoglycosides. PMQR gene carriage in community isolates of *K. pneumoniae* is alarming and can provide a wide reservoir for the spread of these organisms.

Table 1. Primers Used For Detection of *qnr* and *aac* (6')-*Ib-cr* Genes

Gene	Primer Type	Primer Sequence	PCR Product Size	Reference
qnrA	Forward	TTCTCACGCCAGGATTTGAG	571 bp	(17)
	Reverse	TGCCAGGCACAGATCTTGAC		
qnrB	Forward	TGGCGAAAAAATTG*AAC*AGAA	594 bp	(17)
	Reverse	GAGC*AAC*GATCGCCTGGTAG		
qnrS	Forward	GACGTGCT*AAC*TTGCGTGAT	388 bp	(17)
	Reverse	*AAC*ACCTCGACTTAAGTCTGA		
aac(6')-Ib-cr	Forward	TTGCGATGCTCTATGAGTGGCTA	482 bp	(12)
	Reverse	CTCGAATGCCTGGCGTGTTT		

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



NA: nalidixic acid, CP: ciprofloxacin, LOM: levofloxacin, NOR: norfloxacin, IPM: Imipenem, OFX: ofloxacin, AMC: amoxiclav, CTX: cefotaxime, CAZ: ceftazidime, CPM: cefepime.

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 -20°C 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), cefepime (CPM, 30 µg), cefotaxime (CTX, 30 µg), ceftazidime (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 *qnr*A, *qnr*B, *qnr*S, 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 Biothec, Iran). Amplifications were performed in a thermal cycler (Bioer TC25/H, Bioer Technolongy, China) using the following program: initial denaturation at 94°C for 5 min followed by 30 cycles of 1 min at 94 °C, 1 min at annealing temperature (57°C for *qnr*A, *qnr*B and *qnr*S, 54°C for *aac* (6')-*Ib-cr*), 1 min at 72°C 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

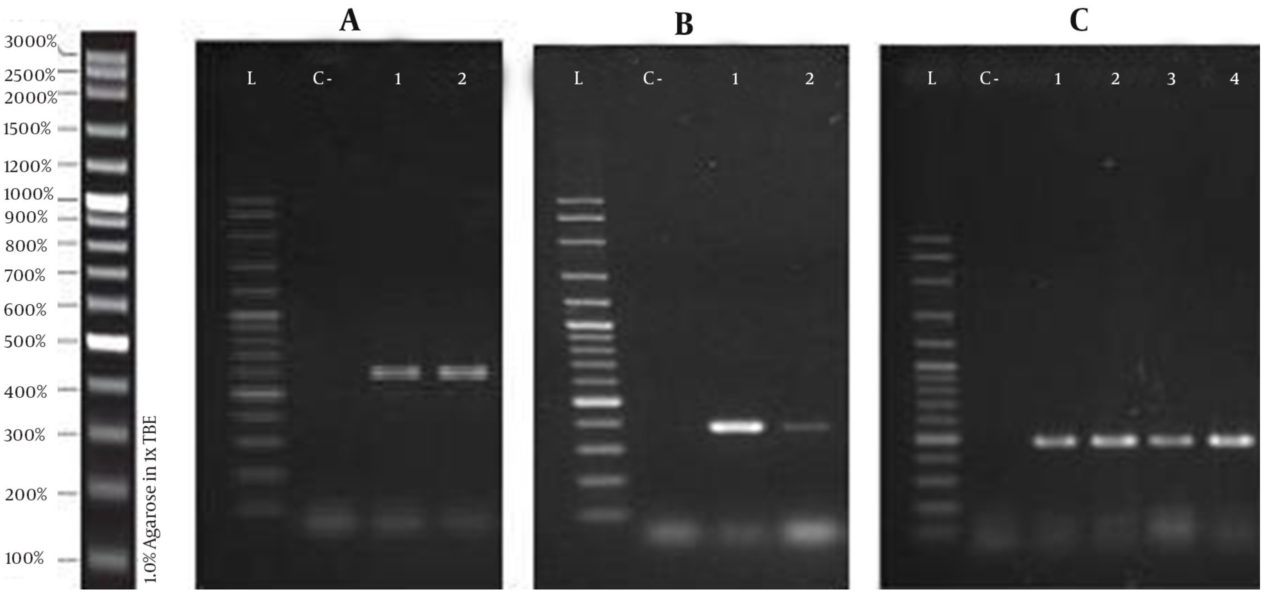
4.1. Antibacterial Susceptibility

The antibiotic susceptibility results of the 52 *K. pneumoniae* isolates are shown in Figure 1. All isolates were resistant to amoxilcav 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 *qnr*A. Five isolates were resistant to all test quinolones and cephalosporins, of which, 3 carried *aac* (6')-*Ib-cr*, one had *qnr*S, and one carried both *aac* (6')-*Ib-cr* and *qnr*B genes. *Qnr*B 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



A, *qnrB*; B, *qnrS* and C, *aac(6')-Ib-cr* genes in 23 community isolates of *K. pneumoniae*. L, 1 Kbp ladder; C-, negative control.

harbored the *qnr*S gene; one of which was resistant to quinolones and cephalosporins and the other was quinolone susceptible, cephalosporin 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 amoxiclav. 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 *qnr*S 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 *qnr*A, *qnr*B and *qnr*S, 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 *qnr*A gene among our isolates. Although *qnr*A1 was the first PMQR gene discovered, several studies have indicated that *qnr*S, *qnr*B 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*, *qnr*B, and *qnr*S genes but not *qnr*A 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 (*qnr*A and *qnr*B but not *qnr*S) 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

1. Dworkin M, Falkow S, Rosenberg E, Schleifer KH, Stackebrandt E. *The Prokaryotes: a handbook on the biology of bacteria.* Brisse S, Grimont F editors. Berlin: Springer-Verlag; 2006.
2. Podschun R, Ullmann U. Klebsiella spp. as nosocomial pathogens: epidemiology, taxonomy, typing methods, and pathogenicity factors. *Clin Microbiol Rev.* 1998;**11**(4):589–603.
3. Paterson DL, Bonomo RA. Extended-spectrum beta-lactamases: a clinical update. *Clin Microbiol Rev.* 2005;**18**(4):657–86.
4. Martinez-Martinez L, Pascual A, Jacoby GA. Quinolone resistance from a transferable plasmid. *Lancet.* 1998;**351**(9105):797–9.
5. Karah N, Poirel L, Bengtsson S, Sundqvist M, Kahlmeter G, Nordmann P, et al. Plasmid-mediated quinolone resistance determinants qnr and aac(6')-Ib-cr in Escherichia coli and Klebsiella spp. from Norway and Sweden. *Diagn Microbiol Infect Dis.* 2010;**66**(4):425–31.
6. Martinez-Martinez L, Eliecer Cano M, Manuel Rodriguez-Martinez J, Calvo J, Pascual A. Plasmid-mediated quinolone resistance. *Expert Rev Anti Infect Ther.* 2008;**6**(5):685–711.
7. Poirel L, Cattoir V, Nordmann P. Is plasmid-mediated quinolone resistance a clinically significant problem? *Clin Microbiol Infect.* 2008;**14**(4):295–7.
8. Robicsek A, Jacoby GA, Hooper DC. The worldwide emergence of plasmid-mediated quinolone resistance. *Lancet Infect Dis.* 2006;**6**(10):629–40.
9. Nordmann P, Poirel L. Emergence of plasmid-mediated resistance to quinolones in Enterobacteriaceae. *J Antimicrob Chemother.* 2005;**56**(3):463–9.
10. Karisik E, Ellington MJ, Pike R, Warren RE, Livermore DM, Woodford N. Molecular characterization of plasmids encoding CTX-M-15 beta-lactamases from Escherichia coli strains in the United Kingdom. *J Antimicrob Chemother.* 2006;**58**(3):665–8.
11. Rodriguez-Martinez JM, Pascual A, Garcia I, Martinez-Martinez L. Detection of the plasmid-mediated quinolone resistance determinant qnr among clinical isolates of Klebsiella pneumoniae producing AmpC-type beta-lactamase. *J Antimicrob Chemother.* 2003;**52**(4):703–6.
12. Park CH, Robicsek A, Jacoby GA, Sahm D, Hooper DC. Prevalence in the United States of aac(6')-Ib-cr encoding a ciprofloxacin-modifying enzyme. *Antimicrob Agents Chemother.* 2006;**50**(11):3953–5.
13. Pakzad I, Ghafourian S, Taherikalani M, Sadeghifard N, Abtahi H, Rahbar M, et al. Qnr prevalence in extended spectrum beta-lactamases (ESBLs) and non-ESBL producing Escherichia coli isolated from urinary tract infections in Central Iran. *Iran J Basic Med Sci.* 2011;**14**(5):458–64.
14. Cattoir V, Poirel L, Rotimi V, Soussy CJ, Nordmann P. Multiplex PCR for detection of plasmid-mediated quinolone resistance qnr genes in ESBL-producing enterobacterial isolates. *J Antimicrob Chemother.* 2007;**60**(2):394–7.
15. Barguigua A, El Otmani F, Talmi M, Reguig A, Jamali L, Zerouali K, et al. Prevalence and genotypic analysis of plasmid-mediated beta-lactamases among urinary Klebsiella pneumoniae isolates in Moroccan community. *J Antibiot (Tokyo).* 2013;**66**(1):11–6.
16. Cheng HR, Jiang N. Extremely rapid extraction of DNA from bacteria and yeasts. *Biotechnol Lett.* 2006;**28**(1):55–9.
17. Bouchakour M, Zerouali K, Gros Claude JD, Amarouch H, El Mdaghri N, Courvalin P, et al. Plasmid-mediated quinolone resistance in expanded spectrum beta-lactamase producing Enterobacteriaceae in Morocco. *J Infect Dev Ctries.* 2010;**4**(12):779–803.
18. Robicsek A, Strahilevitz J, Sahm DF, Jacoby GA, Hooper DC. qnr prevalence in ceftazidime-resistant Enterobacteriaceae isolates from the United States. *Antimicrob Agents Chemother.* 2006;**50**(8):2872–4.
19. Wang A, Yang Y, Lu Q, Wang Y, Chen Y, Deng L, et al. Occurrence of qnr-positive clinical isolates in Klebsiella pneumoniae producing ESBL or AmpC-type beta-lactamase from five pediatric hospitals in China. *FEMS Microbiol Lett.* 2008;**283**(1):112–6.
20. Longhi C, Conte MP, Marazzato M, Iebba V, Totino V, Santangelo F, et al. Plasmid-mediated fluoroquinolone resistance determinants in Escherichia coli from community uncomplicated urinary tract infection in an area of high prevalence of quinolone resistance. *Eur J Clin Microbiol Infect Dis.* 2012;**31**(8):1917–21.
21. Musumeci R, Rausa M, Giovannoni R, Cialdella A, Bramati S, Sibra B, et al. Prevalence of plasmid-mediated quinolone resistance genes in uropathogenic Escherichia coli isolated in a teaching hospital of northern Italy. *Microb Drug Resist.* 2012;**18**(1):33–41.
22. Le TM, Baker S, Le TP, Cao TT, Tran TT, Nguyen VM, et al. High prevalence of plasmid-mediated quinolone resistance determinants in commensal members of the Enterobacteriaceae in Ho Chi Minh City, Vietnam. *J Med Microbiol.* 2009;**58**(Pt 12):1585–92.
23. Cano ME, Rodriguez-Martinez JM, Aguero J, Pascual A, Calvo J, Garcia-Lobo JM, et al. Detection of plasmid-mediated quinolone resistance genes in clinical isolates of Enterobacter spp. in Spain. *J Clin Microbiol.* 2009;**47**(7):2033–9.