Journal of Antimicrobial Chemotherapy

J Antimicrob Chemother 2010; **65:** 2041–2053
doi:10.1093/jac/dkq258
Advance publication 8 July 2010

Prevalence and characteristics of *qnr* determinants and *aac(6′)-Ib-cr* among ciprofloxacin-susceptible isolates of *Klebsiella pneumoniae* in Korea

Yeon-Joon Park[1]*, Jin Kyung Yu[1], Soo Young Kim[1], Seungok Lee[1] and Seok Hoon Jeong[2]

[1]*Department of Laboratory Medicine, College of Medicine, Catholic University of Korea, Seoul, Korea;* [2]*Department of Laboratory Medicine, Yonsei University College of Medicine, Seoul, Korea*

*Corresponding author. Tel: +82-2-2258-1640; Fax: +82-2-2258-1719; E-mail: yjpk@catholic.ac.kr

Keywords: plasmids, incompatibility groups, breakpoints

Sir,

qnr genes (*qnrA*, *qnrB* and *qnrS*) have been reported worldwide and *qnrC* and *qnrD* were recently described.[1] Although it has been reported that a substantial fraction of *qnr*-carrying isolates are susceptible to ciprofloxacin according to the CLSI criteria, to the best of our knowledge this is the first study investigating the prevalence and characteristics of *qnr* or *aac(6′)-Ib-cr* in *Klebsiella pneumoniae* susceptible to ciprofloxacin (MIC ≤1 mg/L).

A total of 858 consecutive, non-duplicate isolates were collected from clinical specimens at 20 clinical laboratories in Korea between April and July 2008. The ciprofloxacin MICs were determined by an agar dilution method according to CLSI guidelines.

The presence of *qnrA*, *qnrB*, *qnrS* and *aac(6′)-Ib* was detected by PCR[2,3] and confirmed by sequencing. The nucleotide and deduced protein sequences were analysed with software available from the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov). The presence of extended-spectrum β-lactamases (ESBLs) was investigated in isolates carrying *qnr* determinants and/or *aac(6′)-Ib-cr*, and in isolates with ciprofloxacin MICs of 0.5 or 1 mg/L. For isolates showing a positive reaction for ESBL by a phenotypic test, multiplex PCR for bla_{CTX-M} was performed. Plasmid mediated AmpC β-lactamases (pAmpCs) were detected by using a cefoxitin disc with or without boronic acid (400 μg) and this was confirmed by multiplex PCR. The presence of IS*CR1*, *orf1005* and IS*Ecl2* was investigated in isolates harbouring *qnr* or *aac(6′)-Ib-cr*, isolates harbouring *qnrB1* and isolates harbouring *qnrS1*, respectively, The plasmids were isolated and classified according to their incompatibility group using the PCR replicon typing scheme,[4] and were additionally classified for IncU and IncR.[5] To elucidate the clonal relatedness, PFGE was carried out using a CHEF Mapper (Bio-Rad Laboratories, Hercules, CA, USA). The whole-cell DNA was digested with XbaI and the results were interpreted in accordance with the criteria of Tenover *et al*.[6] Conjugation experiments were performed with 86 *qnr*- or *aac(6′)-Ib-cr*-positive isolates with azide-resistant *Escherichia coli* J53 as a recipient. Transconjugants were selected on Luria (L) agar plates supplemented with sodium azide (150 mg/L) and ciprofloxacin (0.06 mg/L). As positive controls, RA1 (A), R40a (C), R386 (FI), AFp4041JR (A/C) and AFp5031JR (L/M), which were generous gifts from Professor Lee (Kyungpook National University, Daegu, Korea), were used.

The prevalence of *qnr*/*aac(6′)-Ib-cr* according to the ciprofloxacin MIC is shown in Table 1. None harboured both *qnr* and *aac(6′)-Ib-cr*. The pulsotype was available from 82 isolates and clonality was found in 2 out of 15, 4 out of 9, 4 out of 6, 2 out of 6, 2 out of 6 and 2 out of 6 isolates from six clinical laboratories (data not shown).

The ESBL and pAmpC production rates in *K. pneumoniae* harbouring *qnr or aac(6′)-Ib-cr* were 57.0% (49/86) and 23.3% (20/86), respectively. In contrast, of the 15 isolates with ciprofloxacin MICs of 0.5 or 1 mg/L not harbouring *qnr* or *aac(6′)-Ib-cr*, only 4 isolates showed a positive reaction for the ESBL test and none harboured pAmpC. The occurrence of ESBLs, pAmpCs and mobile elements according to *qnr* types or *aac(6′)-Ib-cr* is summarized in Table 2.

Of the 86 isolates harbouring *qnr* or *aac(6′)-Ib-cr*, the IncR replicon was the most frequent (51.2%), followed by IncFII (18.6%), IncL/M (7.0%) and IncA/C (3.5%).

Conjugal transfer was successful in 31 out of 86 isolates. The transferability was highest for *qnrB2* (3/4, 75.0%), and followed by *qnrS1* (15/26, 57.7%), *qnrB4* (8/21, 38.1%), *aac(6′)-Ib-cr* (3/9, 33.3%) and *qnrA1* (2/19, 10.5%), and the order was correlated with that of association with IS*CR1* (except *qnrS1*, which is associated with IS*Ecl2*). Transconjugation failed in *qnrB1*- or *qnrB10*-harbouring isolates. Of the 31 isolates for which conjugal transfer was successful, ESBLs and/or pAmpCs were present in 15 isolates, and all of them were transferred to the transconjugants.

In contrast to the previous studies, where *qnrA1* was highly associated with IS*CR1*, only 36.8% of *qnrA1* were associated with IS*CR1* in this study. This difference might be due to the fact that the isolates selected in the previous studies were ESBL- or integron-positive isolates. From our study, IS*CR1* was present in all of the six isolates co-harbouring *qnrA1* and ESBL, suggesting that the association of *qnrA1* with IS*CR1* was derived from that of ESBL with IS*CR1*. Further study of the possible novel environment carrying this gene would be interesting.

In conclusion, the prevalence of *qnr* determinants or *aac(6′)-Ib-cr* was remarkably high (97.4%) in isolates with ciprofloxacin MICs of 1 mg/L and they were present on the plasmids of various backbones. Although *qnr* and *aac(6′)-Ib-cr* confer

Table 1. Prevalence of *qnr* or *aac(6′)-Ib-cr* according to ciprofloxacin MIC

Ciprofloxacin MIC (mg/L)	Number	*qnrA* (%)	*qnrB* (%)	*qnrS* (%)	*qnr* total (%)	*aac(6′)-Ib* (%)	*aac(6′)-Ib-cr* (%)
≤0.25	478	13 (2.7)	8 (1.7)	5 (1.0)	26 (5.4)	11 (2.3)	6 (1.3)
0.5	32	1 (3.1)	12 (37.5)	5 (15.6)	18 (56.3)	9 (28.1)	1 (3.1)
1	38	5 (13.2)	13 (34.2)	17 (44.7)	35 (92.1)	12 (31.6)	2 (5.3)
Total	548	19 (3.5)	33 (6.0)	27 (4.9)	79 (14.4)	32 (5.8)	9 (1.6)

Table 2. Occurrence of ESBLs or pAmpCs according to *qnr* types or *aac(6′)-Ib-cr* in *K. pneumoniae*

	qnr/*aac(6′)-Ib-cr* group (number of isolates)									
	total	*qnrA1* (19)	*qnrB1* (1)	*qnrB2* (4)	*qnrB4* (21)	*qnrB6* (4)	*qnrB10* (1)	*qnrB6* and *qnrS1* (1)	*qnrS1* (26)	*aac(6′)-Ib-cr* (9)
ESBL(s) DDST(+)	49	6	1	3	9	1		1	20	8
CTX-M group 1	9		1		1					7
CTX-M group 9	16	3			3				10	
CTX-M groups 1 and 9	2	1								1
non-CTX-M type	22	2		3	5	1		1	10	
pAmpC(s)										
DHA type	20				19			1		
IS*CR1*	31	7		3	15	1		1		4
IS*Ecl2*	26							1	25	

DDST, double disc synergy test.

low-level resistance to quinolones and/or fluoroquinolones, considering the fact that they may provide a favourable background in which the selection of additional chromosomally encoded quinolone resistance mechanisms[7,8] and the appearance of *qnr* in both *Enterobacter* spp. and *K. pneumoniae* coincided with the rapid increase in ciprofloxacin resistance,[9] ciprofloxacin should be used cautiously in isolates with ciprofloxacin MICs of 1 mg/L. Lowering of the current CLSI breakpoints for ciprofloxacin appears to be warranted.

Acknowledgements

We wish to thank all the contributing laboratories that provided isolates for this study and are also indebted to Jung Jun Park and Kang Gyun Park for valuable technical assistance. We are especially grateful to Professor Nordmann (Université Paris-Sud, Kremlin-Bicêtre, France) and Professor Jacoby (Lahey Clinic, USA) for providing us with the *qnr*-positive strain, and to Professor Lee (Kyungpook National University, Daegu, Korea) for the gifts of positive controls for plasmid typing.

Funding

This work was supported by a research grant from the Korea Food and Drug Administration (08072Hangsaengjae 134).

Transparency declarations

None to declare.

References

1 Strahilevitz J, Jacoby GA, Hooper DC *et al*. Plasmid-mediated quinolone resistance: a multifaceted threat. *Clin Microbiol Rev* 2009; **22**: 664–89.

2 Robicsek A, Strahilevitz J, Jacoby GA *et al*. *qnr* prevalence in ceftazidime-resistant Enterobacteriaceae isolates from the United States. *Antimicrob Agents Chemother* 2006; **50**: 2872–4.

3 Park CH, Robicsek A, Jacoby GA *et al*. Prevalence in the United States of *aac(6′)-Ib-cr* encoding a ciprofloxacin-modifying enzyme. *Antimicrob Agents Chemother* 2006; **50**: 3953–5.

4 Carattoli A, Bertini A, Villa L *et al*. Identification of plasmids by PCR-based replicon typing. *J Microbiol Methods* 2005; **63**: 219–28.

5 García-Fernández A, Fortini D, Veldman K *et al*. Characterization of plasmids harbouring *qnrS1*, *qnrB2* and *qnrB19* genes in *Salmonella*. *J Antimicrob Chemother* 2009; **63**: 274–81.

6 Tenover FC, Arbeit RD, Goering RV *et al*. Interpreting chromosomal DNA restriction patterns produced by pulsed-filed gel electrophoresis: criteria for bacterial strain typing. *J Clin Microbiol* 1995; **33**: 2233–9.

7 Rodríguez-Martínez JM, Velasco C, García I *et al*. Mutant prevention concentrations of fluoroquinolones for Enterobacteriaceae expressing the plasmid-carried quinolone resistance determinant *qmrA1*. *Antimicrob Agents Chemother* 2007; **51**: 2236–9.

8 Poirel L, Pitout J, Calvo L *et al*. In vivo selection of fluoroquinolone-resistant *Escherichia coli* isolates expressing plasmid-mediated quinolone resistance and expanded-spectrum β-lactamase. *Antimicrob Agents Chemother* 2006; **50**: 1525–7.
9 Strahilevitz J, Engelstein D, Adler A *et al*. Changes in *qnr* prevalence and fluoroquinolone resistance in clinical isolates of *Klebsiella pneumoniae* and *Enterobacter* spp. collected from 1990 to 2005. *Antimicrob Agents Chemother* 2007; **51**: 3001–3.

J Antimicrob Chemother 2010
doi:10.1093/jac/dkq242
Advance publication 1 July 2010

Plasmid-mediated quinolone resistance determinants in *Salmonella* spp. isolates from reptiles in Germany

Beatriz Guerra*, Reiner Helmuth, Katharina Thomas, Janine Beutlich, Silke Jahn and Andreas Schroeter

Federal Institute for Risk Assessment, BfR, Department for Biological Safety, Diedersdorfer Weg 1, D-12277 Berlin, Germany

*Corresponding author. Tel: +49-30-8412-2082; Fax: +49-30-8412-2953; E-mail: beatriz.guerra@bfr.bund.de

Keywords: antimicrobial resistance, *qnr*, ColE, PMQR, plasmids

Sir,

In 2008, the European Community Reference Laboratory for Antimicrobial Resistance (EURL-AR; at the National Food Institute, Denmark) asked the European National Reference Laboratories within their network (NRLs-AR) to collect retrospective information on the prevalence of plasmid-mediated quinolone resistance (PMQR)[1] determinants in *Salmonella* and *Escherichia coli* isolates.[2] In Germany, we screened the data of >40000 *Salmonella* strains (NRL-*Salm* collection) isolated in all Federal Länder of Germany, originating from routine surveys of different investigation centres involved in public health, or from national or EU monitoring programmes obtained during 2000–08. These isolates originated from food-producing animals (39%), foods (28%), non-food-producing animals (14%) [including 1900 isolates (5%) from reptiles], animal feed (8%), environment (7%), humans (3%) and other sources (2%). Among these isolates, 194 showed database records of MICs (determined by the CLSI broth microdilution method) suggesting the presence of PMQR-encoding genes (nalidixic acid MICs of 8–32 mg/L and ciprofloxacin MICs of 0.06–1 mg/L). However, in secondary tests (Etest and CLSI agar dilution) performed in June 2009 for all these isolates, only for 113 could the initial MIC values be confirmed. Interestingly, 14 of them (12.4%) originated from reptiles. Avoiding duplicated isolates (same isolation place/date), 10 of these reptile isolates were selected for this study.

The 10 isolates showed nalidixic acid MICs of 8–32 mg/L and ciprofloxacin MICs of 0.25–1 mg/L. They were tested for the presence of the PMQR-encoding genes *qnrA*, *qnrB*, *qnrC*, *qnrD*, *qnrS*, *qepA* and *aac(6′)-Ib-cr* by PCR amplifications/sequencing using the primers and conditions described in Table S1 [available as Supplementary data at *JAC* Online (http://jac.oxfordjournals.org/)]. To determine epidemiological relationships, the isolates were typed by PFGE with XbaI (Roche Diagnostics, Germany) using the PulseNet protocol (www.pulsenetinternational.org) [Figure S1, available as Supplementary data at *JAC* Online (http://jac.oxfordjournals.org/)]. Plasmid profile analysis was done by DNA extraction followed by separation on 0.8% vertical agarose gels (Figure S1). Plasmid DNA was transferred via Southern blotting and hybridized with probes for the different PMQR genes detected (Figure S1). The incompatibility group of the plasmids was determined by a PCR replicon-typing scheme.[3]

Nine of the 10 isolates were positive for some of the PMQR genes cited above. In one isolate, none of these genes was present. The *qnrB19* gene was present in seven strains belonging to different *Salmonella* subspecies and serotypes, isolated from different reptiles in several German regions (Table 1). The two *Salmonella* Urbana and one of the rough isolates were isolated from different animals in the same zoo during different periods, and showed very similar XbaI-PFGE and plasmid patterns (Figure S1), suggesting the clonal spread of a *qnrB19*-positive strain. The *qnrS1* gene, and *qnrB6* together with *aac(6′)-Ib-cr*, were found in isolates from turtles. No *qnrA*, *qnrC*, *qnrD* or *qepA* genes were detected.

Plasmid location of the *qnr* genes was confirmed (Figure S1). The *qnrB19* gene was carried on plasmids of ∼4.5 (four isolates) and <3 kb (three isolates). All these plasmids belonged to the incompatibility group ColE1, representing the ColE-Tp variant described for small plasmids (∼10 kb) isolated from different *Salmonella* serotypes.[3] Very small *qnrB19* plasmids (2.7 kb) belonging to this incompatibility group have recently been described.[4] The present study shows that these plasmids seem to be more frequent than previously observed.

The *qnrB6* gene was located on an ∼45 kb plasmid belonging to IncN and *qnrS1* was located on an ∼35 kb IncX plasmid (Figure S1). *qnr* genes have been found on plasmids belonging to several incompatibility groups, being frequent in Inc F, N, L/M and ColE plasmids,[5] but as far as we know they have not been described on IncX plasmids, a family commonly found in *Salmonella*.[6]

Our results show that various determinants conferring PMQR are present in *Salmonella* isolates originating from different reptiles. This has also been found in different bacteria (i.e. *Pseudomonas*, *Klebsiella* and *E. coli*) isolated from zoo reptiles (four isolates from turtles being positive for *qnrB* and *qnrS1*) in Japan,[7] but we could not find in the published literature any other description of the presence of PMQR in *Salmonella* isolated from reptiles.

We do not know if the presence of PMQR could be related to the treatment of zoo or pet animals, or if they are colonized by resistant isolates via feed. Although the *Salmonella* serovars described rarely appear in human disease in Germany (i.e. since 2001, there have been 2–6 cases per year of *Salmonella* Urbana in humans; Robert Koch Institute, www3.rki.de/SurvStat), some of them, such as *Salmonella* Urbana, are highly invasive[8] and infected patients might require treatment with fluoroquinolones. Furthermore, these animals could serve as a reservoir for the further spread of these determinants. For