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Letters to the Editor

Novel Variants of the *qnrB* Gene, *qnrB22* and *qnrB23*, in *Citrobacter werkmanii* and *Citrobacter freundii*▽

Resistance to quinolones in Gram-negative bacteria is usually mediated by the following: (i) chromosomal mutations that alter the target enzymes, DNA gyrase and topoisomerase IV, in their quinolone resistance-determining regions (QRDR), (ii) changes in drug entry (loss of porin channels), and (iii) the presence of plasmid-mediated quinolone resistance (PMQR) determinants [*qnrA*, *qnrB*, *qnrS*, *qnrC*, and *qnrD*, coding for Qnr proteins that protect DNA gyrase from quinolone attack; *aac(6′)-Ib-cr*, coding for a protein that acetylates quinolones; and *qepA*, coding for a quinolone efflux pump] (2, 12). The recent worldwide emergence of PMQR due to the *qnr* and *aac(6′)-Ib-cr* genes is a concerning fact among human and animal Gram-negative pathogens (8).

The aim of this study was to determine the prevalence of *qnr* genes among 93 consecutive nonrepetitive *Enterobacteriaceae* of animal origin and to characterize positive isolates. These isolates were collected from chickens ($n = 37$) and pigs ($n = 56$) at five farms near the city of Seoul (South Korea) in 2007.

The presence of PMQR determinants and QRDR mutations was investigated by PCR-based detection and sequencing (2, 5, 6). The *qnrA*, *qnrS*, *qnrC*, *qnrD*, *aac(6′)-Ib-cr*, and *qepA* genes were not found. Two isolates (2.2%) were found to carry *qnr*-like genes (*Citrobacter werkmanii* PS012 and *Citrobacter freundii* S008). Sequence analysis identified two novel *qnrB* variants, *qnrB22* and *qnrB23*, in *C. werkmanii* PS012 (isolated from a pig at the Daeyoung Farm) and *C. freundii* S008 (isolated from a chicken at the Hanmi Farm), respectively. These new variants were assigned according to the *qnr* numbering scheme shown in the Lahey website (http://www.lahey.org/qnrStudies). The *qnrB22* gene had 99.7% nucleotide identity with *qnrB4*. The *qnrB23* gene had 99.9% nucleotide identity with *qnrB9*. The deduced QnrB22 product had two amino acid substitutions (Ser36Cys and Gly188Val) compared with the amino acid sequence of QnrB4. Compared with the amino acid sequence of QnrB9, QnrB23 showed one amino acid substitution (Asn27Tyr).

C. werkmanii PS012 showed a reduced susceptibility (MIC > 0.125 μg/ml) to fluoroquinolones (ofloxacin, norfloxacin, levofloxacin, and ciprofloxacin) (Table 1). *C. freundii* S008 was nonsusceptible (resistant or intermediate) to the fluoroquinolones (Table 1). The MICs were determined by E-test (AB Biodisk, Solna, Sweden) and interpreted according to Clinical and Laboratory Standards Institute guidelines (4). The QRDR mutations associated with fluoroquinolone resistance were not detected in the two isolates (Table 1).

The transfer of *qnrB22*- and *qnrB23*-harboring plasmids to *Escherichia coli* J53 Azide^R was accomplished through mating experiments described previously (9). Transconjugants were selected on Mueller-Hinton agar plates containing sodium azide (150 μg/ml) and ciprofloxacin (0.125 μg/ml). Fluoroquinolone (or nalidixic acid) MICs of the two transconjugants (TrcPS012 and TrcS008) were similar to those of the donor strains (Table 1). Strain TrcS008, carrying *qnrB23*, had MIC values for nalidixic acid and fluoroquinolones that were higher than those of TrcPS012, harboring *qnrB22* (Table 1).

The PCR amplicons of the *qnrB22* and *qnrB23* genes were cloned into the vector pCR-BluntII-TOPO and transformed into the *E. coli* DH5α host strain (Invitrogen, Karlsruhe, Germany). Primers used were as follows: for cloning of *qnrB22*, 5′-ATGACTCTGGCGTTAGTTGG-3′ and 5′-TTA ACCCATGACAGCGATACCAA-3′; and for cloning of *qnrB23*, 5′-ATGACGCCATTACTGTATAAAAAAACA-3′ and 5′-CTAGCCAATAATCGCGATGCC-3′. A decrease in quinolone susceptibility was observed with both transformants, even though the *qnrB23*-carrying transformant showed higher MICs than that carrying *qnrB22* (Table 1). Fluoroquinolone (or nalidixic acid) MICs of two transformants (TrfPS012 and TrfS008) were lower than those of two transconjugants (TrcPS012 and TrcS008), which was compatible with a recent finding (11). The differences observed between transconjugants and transformants might be related to recipient susceptibility (*E. coli* DH5α was more susceptible than *E. coli* J53 Azide^R), plasmid copy number, and/or the presence of additional PMQR determinants in the two plasmids.

The conjugative plasmids of *C. werkmanii* PS012 and *C. freundii* S008 showed identical patterns (showing 13 distinct bands) and similar molecular sizes (about 23 kb) in restriction fragment length polymorphism analysis after digestion with BglII, as described previously (1). *qnrB22*- and *qnrB23*-harboring plasmids belonged to an incompatibility group, IncL/M, according to a PCR-based replicon-typing scheme (3). These

TABLE 1. Characteristics of the two *Citrobacter* isolates, their transconjugants, *Escherichia coli* DH5α transformants, and reference (recipient or host) strains

Strain	QRDR mutation[a]	PMQR[b] determinant	MIC (μg/ml) of drug[c]				
			NAL	OFX	NOR	LVX	CIP
Citrobacter field strains[d]							
C. werkmanii PS012	ND	*qnrB22*	32	1	0.5	0.5	0.25
C. freundii S008	ND	*qnrB23*	>256	8	8	3	2
Transconjugants[e]							
TrcPS012		*qnrB22*	16	1	0.25	0.5	0.25
TrcS008		*qnrB23*	>256	8	8	3	2
E. coli J53 Azide^R			3	0.064	0.064	0.023	0.016
Transformants[f]							
TrfPS012		*qnrB22*	4	0.064	0.064	0.004	0.016
TrfS008		*qnrB23*	16	0.25	0.25	0.094	0.064
E. coli DH5α			2	0.023	0.032	<0.002	0.003

[a] QRDR mutation, quinolone resistance-determining region mutation associated with fluoroquinolone resistance; ND, not detected.
[b] PMQR, plasmid-mediated quinolone resistance.
[c] NAL, nalidixic acid; OFX, ofloxacin; NOR, norfloxacin; LVX, levofloxacin; CIP, ciprofloxacin.
[d] The two *Citrobacter* strains were isolated at two farms near the city of Seoul, the capital of South Korea, in 2007. Species assignment was confirmed biochemically and by 16S rRNA gene sequence analysis.
[e] TrcPS012 and TrcS008, *E. coli* J53 Azide^R (recipient strain) transconjugants of *C. werkmanii* PS012 and *C. freundii* S008, respectively.
[f] TrfPS012 and TrfS008, *E. coli* DH5α (host strain) transformants harboring pCR-BluntII-TOPO::*qnrB22* and pCR-BluntII-TOPO::*qnrB23* (expressing QnrB22 and QnrB23 determinants, respectively), respectively.

results suggest that conjugative IncL/M plasmids might play a role in the dissemination and evolution of *qnrB* genes. The association of various antibiotic resistance genes, including PMQR determinants with conjugative IncL/M plasmids from human isolates of the *Enterobacteriaceae*, has been described in several reports (7, 10, 13, 14). Despite the currently low prevalence (2.2%) of *qnrB22* and *qnrB23*, surveillance for bacterial isolates carrying these resistance determinants in animals is warranted.

Nucleotide sequence accession numbers. The nucleotide sequences of *qnrB22* and *qnrB23* have been submitted to the GenBank database and assigned the accession numbers FJ981621 and FJ981622, respectively.

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