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Coprevalence of Plasmid-Mediated Quinolone Resistance Determinants QepA, Qnr, and AAC(6′)-Ib-cr among 16S rRNA Methylase RmtB-Producing *Escherichia coli* Isolates from Pigs[▽]

Plasmid-mediated quinolone resistance determinants, including Qnr peptides and AAC(6′)-Ib-cr, are increasingly identified worldwide among various clinical isolates of *Enterobacteriaceae* (7, 9, 10). Very recently, a novel plasmid-mediated fluoroquinolone-resistant determinant, QepA (quinolone efflux pump), which showed a considerable similarity to the major facilitator superfamily-type efflux pumps, was first identified in an *Escherichia coli* clinical isolate from Japan (13) and later found also in an *E. coli* isolate in Belgium (6). Interestingly, both of the two *qepA*-harboring *E. coli* isolates also contained the *rmtB* gene encoding a 16S rRNA methyltransferase, an emerging new molecular mechanism responsible for high-level pan-aminoglycoside resistance among gram-negative pathogens (3, 4, 6, 13, 14).

Our previous study showed that *rmtB* was highly prevalent among *E. coli* isolates from pigs in China (1). The aim of this study was to investigate the prevalence of plasmid-mediated quinolone resistance determinants among *rmtB*-producing *E. coli* isolates from pigs in China and to identify the association of the *qepA* gene with *rmtB*.

One hundred fifty-one *E. coli* isolates were obtained from pig feces sampled at two pig farms. These isolates were collected from 2005 to 2006, and 48 of them were identified as producers of RmtB. (Some of these data were published previously [1].) Screening for *qepA*, *qnrA*, *qnrB*, *qnrS*, and *aac(6′)-Ib-cr* genes was carried out by PCR amplification among the 48 *rmtB*-positive isolates. For *qepA*, the following primers were used to produce a 218-bp amplicon: *qepA*-F (5′-GCAGGTCCAGCAGCGGGTAG-3′) and *qepA*-R (5′-CTTCCTGCCCGAGTATCGTG-3′). Positive results were confirmed by direct sequencing of PCR products. *qnrA*, *qnrB*, *qnrS*, and *aac(6′)-Ib-cr* genes were detected by PCR using specific primers (the used *qnrB* primers were able to detect almost all known *qnrB* alleles except *qnrB8*), as previously described (5, 8, 11), and were finally confirmed by sequencing of each PCR product.

Overall, *qepA*, *qnrB*, *qnrS*, and *aac(6′)-Ib-cr* were detected in 28 (58.3%), 1 (2.1%), 9 (18.8%), and 6 (12.5%) of 48 RmtB-producing *E. coli* isolates, respectively (Table 1). The *qnrB* genes were identified as *qnrB6* alleles by sequencing. The *qnrS* genes were confirmed as *qnrS1* (four isolates) and *qnrS2* (five isolates) alleles by sequencing. Four isolates with uniform pulsed-field gel electrophoresis (PFGE) patterns harbored *qepA*, *qnrS2*, and *aac(6′)-Ib-cr* genes concurrently.

To investigate the association of *rmtB* and *qepA*, *rmtB*-positive *E. coli* transconjugants described previously (1) were subjected to PCR amplification of *qepA*, and all transconjugants

TABLE 1. Characteristics of *E. coli* isolates and transconjugants harboring *rmtB*, as well as *qnr*, *qepA*, and/or *aac(6′)-Ib-cr*

Isolate(s)[a]	PFGE type	Resistance gene detected	MIC (μg/ml) of enrofloxacin	Fold increase in quinolone MIC for transconjugant vs recipient[b]				
				NOR	ENR	CIP	NAL	LEV
GZ3	A1	*qepA*	64	16	4	8	8	2
GZ4	A2	*qepA*	32	4	4	8	2	2
GZ5, GZ6	B	*qepA*	16	16,8	8, 2	8, 16	1	2, 4
GZ8	C	*qepA*	64	8	16	4	4	1
GZ9	D	*qepA*	32	16	2	8	4	2
GZ11	E	*qepA*	128	4	4	4	2	2
GZ12, GZ13, GZ14	F	*qepA*	16, 8, 16	16, 8, 16	4, 2, 8	8, 16, 4	1, 4, 1	8, 2, 4
GZ15	G	*qepA*	8	4	8	4	1	2
GZ16	H	*qepA*	>128	8	2	8	2	4
CQ15	I1	*qepA*	2	8	16	16	1	1
CQ18, CQ2, CQ5	I2	*qepA*	2, 2, 4	8, 16, 16	1, 16, 2	1, 3, 2, 8	1	1, 4, 1
CQ4[c]	J1	*qepA*	0.03	2	1	1	2	1
CQ20	J1	*qepA*	0.03	4	4	2	1	2
CQ26	K	*qepA*	0.5	4	2	4	1	1
CQ10	L	*qepA*	0.25	32	2	8	1	2
CQ14	M	*qepA*	16	32	2	4	1	2
GZ7	N	*qepA*, *qnrS1*	32	16	1	2	16	1
GZ1	O	*qnrS1*	4	16	32	16	4	4
GZ2[c]	O	*qnrS1*	2	2	2	4	4	2
CQ22[c]	P	*qnrS1*	4	2	2	1	1	1
CQ13	K	*qnrS2*	0.5	4	8	4	4	4
CQ6, CQ7, CQ12, CQ16	Q	*qepA*, *qnrS2*, *aac(6′)-Ib-cr*	2	16	2, 2, 4, 2	16, 4, 8, 8	16, 1, 1, 2	4, 1, 2, 2
GZ10	R	*qepA*, *aac(6′)-Ib-cr*	16	16	4	16	16	4
CQ19	S	*qepA*, *aac(6′)-Ib-cr*	2	16	4	4	1	1
CQ1[c]	U	*qnrB6*	0.25	2	1	1	2	1

[a] Isolates with the same letters were isolated from the same farm.
[b] The quinolone MICs of the recipient strains were 4 μg/ml for nalidixic acid (NAL); 0.015 μg/ml for ciprofloxacin (CIP); and 0.03 μg/ml for norfloxacin (NOR), enrofloxacin (ENR), and levofloxacin (LEV).
[c] RmtB-positive transconjugants not containing any plasmid-mediated quinolone resistance determinants.

that originated from the 28 *qepA*-positive isolates selected with aminoglycoside resistance were positive for the *qepA* gene except one, suggesting an strong linkage of *qepA* with *rmtB*. Two *rmtB*-positive transconjugants also harbored *qnrS1* or *qnrS2*.

MICs of ciprofloxacin, enrofloxacin, levofloxacin, nalidixic acid, and norfloxacin for the 27 *qepA*-positive and 2 *qnrS*-positive transconjugants were determined by the agar dilution method according to CLSI guidelines (2). The increase (fold) in quinolone MICs for transconjugants compared with those of recipients is shown in Table 1. The MICs for transconjugants strongly indicated that *qepA* as well as *qnrS* conferred quinolone resistance, with a 4- to 32-fold increase in norfloxacin MICs and 1- to 32-fold increase in enrofloxacin and ciprofloxacin MICs. However, variations in the quinolone MICs for different transconjugants suggested that the QepA may be expressed at variable levels. Xu et al. (12) recently reported that different promoter strengths may cause the differences in *qnrA* expression levels and in ciprofloxacin MICs of different transconjugants. Further studies are needed to find out whether the wide range of MICs of quinolones for different *qepA*-harboring transconjugants depends on the diversities in *qepA* expression levels due to different promoter strengths. MICs of enrofloxacin for all isolates were also determined by the agar dilution method according to CLSI guidelines. As indicated in Table 1, most isolates were resistant to enrofloxacin (MIC, ≥2 μg/ml), but six isolates were susceptible to enrofloxacin.

This study shows the high prevalence of plasmid-mediated quinolone resistance determinants among *E. coli* isolates recovered from food-producing animals. A total of 58.3% (28/48) of *rmtB*-positive *E. coli* isolates harbored *qepA* gene, indicating a close relationship between *qepA* and *rmtB*, which has been reported in the previous studies (6, 13). This is also the first time three different plasmid-mediated quinolone resistance determinants (QepA, Qnr, and AAC(6′)-Ib-cr) were identified in an *E. coli* strain. Coproduction of QepA, Qnr, AAC(6′)-Ib-cr, and RmtB may well facilitate the survival of bacteria under selective pressure of antimicrobial agents in both veterinary and human clinical environments, and the resistance determinants in food-producing animals could be transmitted to humans via the food chain. Further spread of these resistance determinants among pathogenic microbes may occur in the near future. Thus, it is necessary to monitor and minimize the spread of such resistance determinants among hazardous bacteria in both humans and animals.

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