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High Prevalence of Plasmid-Mediated Quinolone Resistance Genes *qnr* and *aac(6′)-Ib-cr* in Clinical Isolates of *Enterobacteriaceae* from Nine Teaching Hospitals in China▽

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Quinolone resistance is an emerging problem in China. To investigate the prevalence of the plasmid-mediated quinolone resistance genes *qnr* and *aac(6′)-Ib-cr*, a total of 265 clinical isolates of *Escherichia coli*, *Klebsiella pneumoniae*, *Citrobacter freundii*, and *Enterobacter cloacae* with ciprofloxacin MICs of ≥0.25 μg/ml were screened at nine teaching hospitals in China. The *qnrA*, *qnrB*, *qnrS*, and *aac(6′)-Ib* genes were detected by PCR. The *aac(6′)-Ib-cr* gene was further identified by digestion with BtsCI and/or direct sequencing. The *qnr* gene was present in significantly smaller numbers of isolates with cefotaxime MICs of <2 μg/ml than isolates with higher MICs (≥2.0 μg/ml) (20.6% and 42.1%, respectively; $P < 0.05$). *aac(6′)-Ib-cr* was present in 17.0% of the isolates tested, and 7.9% of the isolates carried both the *qnr* and the *aac(6′)-Ib-cr* genes. Among the isolates with cefotaxime MICs of ≥2.0 μg/ml, *qnr* and *aac(6′)-Ib-cr* were present in 65.7% and 8.6% of *E. cloacae* isolates, respectively; 65.5% and 21.8% of *K. pneumoniae* isolates, respectively; 63.3% and 26.7% of *C. freundii* isolates, respectively; and 6.5% and 16.9% of *E. coli* isolates, respectively. The 20 transconjugants showed 16- to 128-fold increases in ciprofloxacin MICs, 14 showed 16- to 2,000-fold increases in cefotaxime MICs, and 5 showed 8- to 32-fold increases in cefoxitin MICs relative to those of the recipient due to the cotransmission of $bla_{\text{CTX-M-14}}$, $bla_{\text{CTX-M-3}}$, $bla_{\text{DHA-1}}$, $bla_{\text{SHV-2}}$, and $bla_{\text{SHV-12}}$ with the *qnr* and *aac(6′)-Ib-cr* genes. Southern hybridization analysis showed that these genes were located on large plasmids of different sizes (53 to 193 kb). These findings indicate the high prevalence of *qnr* and *aac(6′)-Ib-cr* in members of the family *Enterobacteriaceae* and the widespread dissemination of multidrug resistance in China.

Plasmids carrying *qnr* genes have been found to mediate quinolone resistance (8). These genes encode pentapeptide repeat proteins that block the action of ciprofloxacin on bacterial DNA gyrase and topoisomerase IV (18–20). The plasmid-borne *qnr* genes currently comprise three families, *qnrA*, *qnrB*, and *qnrS*, whose nucleotide sequences differ from each other by 40% or more. The geographical distribution of *qnrA* genes is known to be wide (10), but that of the newer *qnr* types, *qnrB* (6) and *qnrS* (5), have seldom reported within China. Recently, a new mechanism of transferable quinolone resistance was reported: enzymatic inactivation of certain quinolones. The *cr* variant of *aac(6′)-Ib* encodes an aminoglycoside acetyltransferase that confers reduced susceptibility to ciprofloxacin by N-acetylation of its piperazinyl amine (16).

Plasmids harboring *qnrA* may also encode extended-spectrum β-lactamases (ESBLs). Previous studies showed that *qnr*-positive strains frequently expressed ESBLs, such as CTX-M-15 and SHV-12 (6, 15). No previous nationwide survey has evaluated clinical isolates of *Enterobacteriaceae* with reduced susceptibility to ciprofloxacin and extended-spectrum cephalosporins in China for the presence of *qnrA*, *qnrB*, *qnrS*, and *aac(6′)-Ib-cr*. Therefore, we investigated clinical isolates of *Citrobacter freundii*, *Enterobacter cloacae*, *Escherichia coli*, and *Klebsiella pneumoniae* collected from nine teaching hospitals in China for the presence of these genes and whether these genes are linked with ESBLs or plasmid-mediated AmpC genes, in order to more broadly characterize the epidemiology of these resistance elements in a population of clinical isolates.

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MATERIALS AND METHODS

Bacterial isolates. The test isolates were drawn from the Chinese Meropenem Susceptibility Surveillance study collection, which consists of nonrepeat clinical isolates of specified enterobacterial genera annually collected by specified laboratories in each of the big nine cities in China. These cities are distributed in southern China (including Shanghai, Wuhan, Nanjing, Guangzhou, and Fuzhou) and northern China (including Beijing, Tianjin, Shenyang, and Jinan). A total of 265 isolates of *C. freundii*, *E. cloacae*, *E. coli*, and *K. pneumoniae* with ciprofloxacin MICs of ≥0.25 μg/ml were obtained from a screening of the 421 isolates of the four species mentioned above. The screened isolates were divided into two groups: group 1 had cefotaxime MICs of ≥2.0 μg/ml and ceftriaxone MICs of ≥2.0 μg/ml, and group 2 had cefotaxime or ceftriaxone MICs of <2.0 μg/ml. Group 1 included 30 isolates of *C. freundii*, 35 isolates of *E. cloacae*, 77 isolates of *E. coli*, and 55 isolates of *K. pneumoniae*.

***qnr* and *aac(6′)-Ib-cr* detection.** All 265 isolates selected were screened for the *qnr* (*qnrA*, *qnrB*, and *qnrS*) genes by multiplex PCR (15) and for *aac(6′)-Ib* by PCR (11). All isolates positive for the *aac(6′)-Ib* gene were further analyzed by digestion with BtsCI (New England Biolabs, Beverly, MA) and/or direct sequencing of the purified PCR products to identify *aac(6′)-Ib-cr*, which lacks the BtsCI restriction site present in the wild-type gene.

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TABLE 1. Prevalence of *qnr* and *aac(6′)-Ib-cr* genes in the selected *Enterobacteriaceae* isolates from nine teaching hospitals in China

Group and organism[a]	No. of isolates with locus/total no. of isolates (%)						
	qnrA	*qnrB*	*qnrA* + *qnrB*	*qnrS*	*qnrB* + *qnrS*	*aac(6′)-Ib*	*aac(6′)-Ib-cr*
Group 1 (n = 197)							
E. coli	0/77 (0.0)	3/77 (3.9)	0/77 (0.0)	2/77 (2.6)	0/77 (0.0)	33/77 (42.9)	13/77 (16.9)
K. pneumoniae	2/55 (3.6)	19/55 (34.5)	0/55 (0.0)	14/55 (25.5)	1/55 (1.8)	20/55 (36.4)	12/55 (21.8)
E. cloacae	8/35 (22.9)	8/35 (22.9)	0/35 (0.0)	6/35 (17.1)	1/35 (2.9)	22/35 (62.9)	3/35 (8.6)
C. freundii	5/30 (16.7)	12/30 (40.0)	2/30 (6.7)	0/30 (0.0)	0/30 (0.0)	15/30 (50.0)	8/30 (26.7)
Group 2 (n = 68)							
E. coli	0/28 (0.0)	0/28 (0.0)	0/28 (0.0)	0/28 (0.0)	0/28 (0.0)	2/28 (7.1)	1/28 (3.5)
K. pneumoniae	0/22 (0.0)	1/22 (4.5)	0/22 (0/0)	3/22 (13.6)	0/22 (0.0)	4/22 (18.2)	4/22 (18.2)
E. cloacae	0/8 (0.0)	0/8 (0.0)	0/8 (0.0)	6/8 (75.0)	0/8 (0.0)	1/8 (12.5)	1/8 (12.5)
C. freundii	1/10 (10.0)	1/10 (10.0)	0/10 (0.0)	1/10 (10.0)	1/10 (10.0)	3/10 (30.0)	3/10 (30.0)

[a] Group 1 comprised isolates with ciprofloxacin MICs of ≥0.25 μg/ml, cefotaxime MICs of ≥2.0 μg/ml, and ceftriaxone MICs of ≥2.0 μg/ml; group 2 comprised isolates with ciprofloxacin MICs of ≥0.25 μg/ml and cefotaxime or ceftriaxone MICs of <2.0 μg/ml.

Conjugation experiments. The transfer of quinolone resistance was studied by performing conjugation experiments, as described previously (21b). Conjugation experiments were performed with 34 isolates in group 1 (including *C. freundii*, *E. cloacae*, *E. coli*, and *K. pneumoniae* isolates) with *qnr* and/or *aac(6′)-Ib-cr* as the donors and with azide-resistant *E. coli* J53 as the recipient. Transconjugants were selected on Trypticase soy agar plates containing sodium azide (150 μg/ml; Sigma Chemical Co., St. Louis, MO) for counterselection and sulfamethoxazole (180 μg/ml) to select for plasmid-mediated resistance. To determine if quinolone resistance was cotransferred, colonies were replica plated onto Trypticase soy agar plates with and without ciprofloxacin (0.05 μg/ml). The *qnrA*, *qnrB*, *qnrS*, and *aac(6′)-Ib-cr* genes were detected in the transconjugants.

Antimicrobial susceptibility testing. The MICs of ciprofloxacin and the other antimicrobial agents tested were determined by Clinical and Laboratory Standards Institute (CLSI) agar dilution method M7-A7 (3) and were interpreted according to CLSI performance standard M100-S17 (4). The antimicrobials were supplied and stored according to the manufacturer's instructions. *E. coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as reference strains for susceptibility testing.

PCR and sequencing of β-lactamase genes for transconjugants and respective donors. Genes coding for Ambler class A serine enzymes were detected by PCR with primers specific for $bla_{\text{CTX-M}}$, bla_{TEM}, and bla_{SHV} (21a). Plasmid-mediated AmpC β-lactamase genes were sought by use of a multiple PCR system, as described previously (12). The PCR products were purified by using a QIAquick PCR purification kit (Qiagen, Hilden, Germany). DNA sequencing of both strands was performed by the direct sequencing method with an ABI Prism 3100 genetic analyzer (Applied Biosystems, Foster City, CA).

PCR amplification and DNA sequencing of *gyrA*, *gyrB*, and *parC*. Mutations in the *gyrA*, *gyrB*, and *parC* genes were identified by DNA sequencing of their PCR products. PCR amplification of the quinolone resistance-determining regions (QRDRs) of *gyrA*, *gyrB*, and *parC* was performed as described previously (16a, 21). Both strands of the purified PCR products were sequenced; and the DNA sequences of the QRDRs of *gyrA*, *parC*, and *gyrB* were compared with the DNA sequences of the QRDRs of *E. cloacae*, *E. coli*, *C. freundii*, and *K. pneumoniae* (GenBank accession numbers AF052256, AE000312, AF052253, and DQ673325, respectively, for *gyrA*; D88981, AE000384, AB003914, and NC009648, respectively, for *parC*; and AF302677, AE000447, AF071877, and NC009648, respectively, for *gyrB*).

Plasmid detection and Southern hybridization. Plasmid DNA was extracted with a Qiagen plasmid Miniprep kit (Qiagen), according to the manufacturer's recommendations. *E. coli* V517 (plasmid sizes, 54, 5.6, 5.1, 3.9, 3.0, 2.7, and 2.1 kb) and *E. coli* J53 containing plasmid R1 (92 kb) or R27 (182 kb) were used as standards. The sizes of the plasmids were calculated by using Quantity One software (Bio-Rad Laboratories, Hercules, CA). The *qnrA*, *qnrB*, *qnrS*, *aac(6′)-Ib-cr*, $bla_{\text{CTX-M}}$, bla_{SHV}, and bla_{DHA} genes were purified by using a DNA and gel band purification kit (GFX PCR; Amersham Pharmacia) and were then labeled by supplementing the master mixture with digoxigenin-dUTP (Roche Applied Science, Mannheim, Germany). Southern hybridization and detection steps were accomplished with the digoxigenin-dUTP detection kit, as recommended by the manufacturer (Roche Applied Science).

RESULTS

Prevalence of *qnr* and *aac(6′)-Ib-cr* genes. The *qnr* gene was present in 83 (42.1%) of 197 isolates in group 1, and of these, 17 isolates carried *qnrA* (8.6%), 46 isolates carried *qnrB* (23.4%), and 24 isolates carried *qnrS* (12.2%) (Table 1). *qnr* was present in 19 (63.3%) of 30 *C. freundii* isolates, 23 (65.7%) of 35 *E. cloacae* isolates, 5 (6.5%) of 77 *E. coli* isolates, and 36 (65.5%) of 55 *K. pneumoniae* isolates (Table 1). *aac(6′)-Ib-cr* was detected in 36 (18.3%) of 197 isolates. It was present in 26.7% of *C. freundii* isolates, 16.9% of *E. coli* isolates, 8.6% of *E. cloacae* isolates, and 21.8% of *K. pneumoniae* isolates. Two *C. freundii* isolates from sputum carried *qnrA*, *qnrB*, and *aac(6′)-Ib-cr*; one *K. pneumoniae* isolate from sputum carried *qnrB*, *qnrS*, and *aac(6′)-Ib-cr*; and one *E. cloacae* isolate from blood carried *qnrB* and *qnrS*.

The *qnr* gene was detected in 14 of 68 isolates in group 2, which was significantly less than the number of isolates in group 1 in which it was detected (20.6% and 42.1%, respectively; $\chi^2 = 10.11$; $P < 0.05$). However, there was no significant difference in the prevalence of *aac(6′)-Ib-cr* between the two groups (18.3% in group 1 versus 13.2% in group 2; $\chi^2 = 1.849$; $P > 0.05$) (Table 1). For the *E. cloacae* isolates, the *qnrA* and *qnrB* genes were more common among isolates in group 1 than those in group 2. Unexpectedly, the *qnrS* gene was the most prevalent in the non-ESBL-producing *E. cloacae* isolates (six of eight isolates [75.0%]). For the *K. pneumoniae* and *C. freundii* isolates, the *qnrB* gene was more prevalent among isolates in group 2 than those in group 1. There was no significant difference between the two groups in the prevalence of isolates carrying both the *qnr* and the *aac(6′)-Ib-cr* genes (9.1% and 4.4%, respectively; $\chi^2 = 1.547$; $P > 0.05$).

All *qnr*-positive PCR products from isolates in group 1 were sequenced. The *qnr* gene nomenclature proposed recently was used to define the subtypes of *qnr* (6a). The sequences of the *qnrA*-positive and *qnrS*-positive isolates were all shown to match those of *qnrA1* and *qnrS1*, respectively. The sequences of *qnrB*-positive isolates were shown to match those of *qnrB1*, *qnrB2*, *qnrB4*, *qnrB6*, *qnrB10*, and *qnrB16*.

Conjugation experiments and antimicrobial susceptibility testing. Twenty transconjugants were obtained. Fourteen other

TABLE 2. Plasmid-mediated quinolone resistance genes and MICs of antimicrobial agents for 20 donors and transconjugants

Isolate[a]	Organism	Topoisomerase mutation(s)[b]			Plasmid-mediated quinolone resistance gene(s) present		MIC (μg/ml)[c]												
		GyrA	ParC	GyrB	*qnr*	*aac(6′)-Ib-cr*[d]	CIP	LVX	FOX	CTX	CTC	CAZ	CCV	FEP	TZP	GEN	AMK	SMZ	MEM
FJ3	*E. coli*	S83L	S80R	WT	*qnrB*	+	16	4	8	128	0.032	4	0.125	8	2	128	4	>256	0.032
FJ3T		WT	WT	WT	*qnrB*	+	0.5	0.25	2	64	0.032	2	0.125	4	1	0.5	2	>256	0.032
FJ61	*E. cloacae*	S83I, D87A	S80I	WT	*qnrS*	−	>32	>32	256	256	8	16	16	32	8	128	2	16	0.25
FJ61T		ND	ND	ND	*qnrS*	−	0.5	0.5	4	0.032	0.032	0.25	0.125	0.032	1	1	2	16	0.016
FJ63	*E. cloacae*	S83I, D87A	S80I	WT	*qnrS*	+	>32	>32	256	>256	1	128	4	64	>256	>256	>256	>256	1
FJ63T		ND	ND	ND	*qnrS*	+	1	0.5	4	32	0.032	4	0.125	1	2	>256	64	>256	0.064
FJ67	*E. cloacae*	S83I	WT	WT	*qnrS*	−	8	8	>256	256	0.25	4	0.5	32	1	2	32	>256	0.032
FJ67T		ND	ND	ND	*qnrS*	−	1	1	4	64	0.032	4	0.125	16	1	1	16	>256	0.032
GZ40	*K. pneumoniae*	S83I	S80I	WT	*qnrB*	+	>32	>32	32	>256	0.5	256	0.25	64	>256	1	8	>256	0.125
GZ40T		ND	ND	ND	*qnrB*	+	1	0.25	2	0.032	0.032	0.25	0.125	0.016	1	0.5	4	>256	0.016
GZ47	*E. cloacae*	WT	WT	WT	*qnrB*, *qnrS*	−	4	2	256	64	8	128	16	16	4	256	1	>256	0.032
GZ47T		ND	ND	ND	*qnrB*	−	0.125	0.25	32	0.25	0.5	2	0.25	0.016	2	128	1	256	0.016
GZ51	*E. cloacae*	WT	WT	WT	*qnrA*	−	0.5	0.5	>256	64	32	64	16	16	2	>256	>256	>256	0.064
GZ51T		WT	WT	WT	*qnrA*	−	0.25	0.25	4	32	0.032	32	0.125	8	2	>256	>256	>256	0.016
JN1	*C. freundii*	T83I	S80I	WT	*qnrA*	+	>32	16	>256	>256	128	64	256	16	16	1	4	256	0.125
JN1T		ND	ND	ND	−	+	1	0.25	2	8	0.032	1	0.125	1	2	1	2	32	0.016
JN132	*K. pneumoniae*	S83I	S80I	WT	*qnr B*	+	>32	8	64	32	1	32	4	2	4	>256	64	>256	0.032
JN132T		ND	ND	ND	*qnr B*	+	1	0.25	64	32	1	32	4	2	4	>256	64	>256	0.032
JN41	*K. pneumoniae*	S83I	S80I	WT	*qnrB*	+	>32	>32	4	32	0.032	2	0.125	2	4	1	4	>256	0.032
JN41T		ND	ND	ND	*qnrB*	+	0.5	0.25	2	16	0.032	1	0.125	1	2	1	4	>256	0.016
JN64	*C. freundii*	T83I	WT	WT	*qnrA*	−	8	8	>256	256	128	64	256	64	>256	>256	>256	>256	0.25
JN64T		ND	ND	ND	*qnrA*	−	0.25	0.5	8	16	0.064	2	0.25	4	4	>256	>256	>256	0.016
JS33	*C. freundii*	T83I	WT	WT	*qnrA*, *qnrB*	+	4	2	>256	8	64	16	128	0.25	8	64	4	>256	0.125
JS33T		ND	ND	ND	*qnrA*	+	0.5	0.25	2	0.125	0.032	0.25	0.125	0.125	4	16	4	>256	0.032
PU12	*E. coli*	S83L	WT	WT	*qnrS*	−	4	4	4	16	0.032	4	0.125	2	1	2	8	>256	0.016
PU12T		WT	WT	WT	*qnrS*	−	0.5	0.5	4	16	0.032	1	0.125	2	1	1	4	>256	0.016
PU55	*C. freundii*	T83I	S80I	WT	*qnrA*	+	>32	32	>256	128	256	64	128	1	64	1	4	>256	0.25
PU55T		WT	WT	WT	*qnrA*	+	1	0.25	2	0.064	0.032	0.25	0.125	0.125	4	0.5	4	>256	0.016
SH39	*E. cloacae*	S83I	S80I	WT	*qnrA*	−	32	32	>256	4	32	2	64	0.125	2	8	2	>256	0.125
SH39T		ND	ND	ND	*qnrA*	−	0.5	0.5	4	0.032	0.032	0.25	0.25	0.032	1	4	2	256	0.016
SY22	*K. pneumoniae*	S83I	S80I	WT	*qnrS*	−	>32	>32	8	2	0.064	4	0.25	1	4	128	2	64	0.016
SY22T		ND	ND	ND	*qnrS*	−	0.5	0.5	4	0.5	0.032	1	0.064	0.125	1	32	2	16	0.016
SY26	*K. pneumoniae*	S83I	S80I	WT	*qnrB*	+	>32	32	256	8	8	256	128	0.5	16	>256	64	>256	0.125
SY26T		WT	WT	WT	*qnrB*	+	0.5	0.25	16	4	0.032	16	0.125	0.125	2	>256	32	>256	0.016
TJ15	*C. freundii*	T83I	S80I	WT	*qnrB*	+	16	4	256	1	16	4	64	0.125	4	256	4	>256	0.032
TJ15T		WT	WT	WT	−	+	0.25	0.5	64	0.125	0.125	2	4	0.016	1	1	4	>256	0.016
WH42	*E. cloacae*	WT	WT	WT	*qnrA*	−	2	2	>256	256	256	256	64	32	64	16	16	>256	0.125
WH42T		ND	ND	ND	*qnrA*	−	0.25	0.25	2	8	0.032	16	0.125	0.5	1	8	16	>256	0.016
WH99	*K. pneumoniae*	WT	WT	WT	*qnrB*	+	2	0.5	256	32	64	16	128	2	8	128	2	>256	0.032
WH99T		ND	ND	ND	*qnrB*	+	1	0.25	64	16	0.064	4	1	1	2	128	2	>256	0.016
J53	*E. coli*	WT	WT	WT	−		0.008	0.016	2	0.032	0.032	0.25	0.125	0.016	1	0.5	1	16	0.016

[a] J53, recipient; T, transconjugant.
[b] WT, wild-type; ND, not determined
[c] CIP, ciprofloxacin; LVX, levofloxacin; FOX, cefoxitin; CTX, cefotaxime; CTC, cefotaxime-clavulanic acid; CAZ, ceftazidime; CCV, ceftazidime-clavulanic acid; FEP, cefepime; TZP, piperacillin-tazobactam; GEN, gentamicin; AMK, amikacin; MEM, meropenem; SMZ, sulfamethoxazole.
[d] +, present; −, absent.

TABLE 3. Properties of the transconjugants

Transconjugant	*qnr* gene present[a]	Presence of *aac(6′)-Ib-cr*[a]	β-Lactamase(s) present	Size (kb) of plasmid(s)
FJ3T	*qnrB6*	+	CTX-M-3	76
FJ61T	*qnrS1*	−	−	120, 86, 4.8
FJ63T	*qnrS1*	+	TEM-1	169, 6.0
FJ67T	*qnrS1*	−	CTX-M-3	136, 69, 54, 6
GZ40T	*qnrB6*	+	−	53
GZ47T	*qnrB4*	−	DHA-1, TEM-1, SHV-12	119
GZ51T	*qnrA1*	−	SHV-12, CTX-M-14	81
JN132T	*qnrB6*	+	CTX-M-3, DHA-1	191, 104
JN1T	−	+	CTX-M-3	200, 172, 107
JN41T	*qnrB2*	+	CTX-M-14	164
JN64T	*qnrA1*	−	CTX-M-14	146, 81
JS33T	*qnrA1*	+	−	193
PU12T	*qnrS1*	−	CTX-M-14	129, 65, 9.8
PU55T	*qnrA1*	+	−	63
SH39T	*qnrA1*	−	−	182, 81
SY22T	*qnrS1*	−	SHV-2	182, 91
SY26T	*qnrB4*	+	DHA-1, SHV-12	162, 4.3, 3.5
TJ15T	−	+	DHA-1	161
WH42T	*qnrA1*	−	SHV-12	195
WH99T	*qnrB4*	+	CTX-M-14, DHA-1, TEM-1	177

[a] +, present; −, absent.

qnr- and/or *aac(6′)-Ib-cr*-bearing isolates failed to produce transconjugants, although multiple agents were used for selection. The *qnr* and *aac(6′)-Ib-cr* genes can be cotransferred from different donors. Two isolates (isolates GZ40 and JS33) harboring different *qnr* genes transferred only a single *qnr* gene to the recipient, which indicated that different *qnr* genes were located on different plasmids (Table 2). The 20 transconjugants showed 16- to 128-fold increases in the MICs of ciprofloxacin and 16- to 64-fold increases in the MICs of levofloxacin relative to those of the recipient (Table 2). Fourteen of 20 transconjugants showed 16- to 2,000-fold increases in the MICs of cefotaxime and 4- to 128-fold increases in the MICs of ceftazidime. Clavulanic acid decreased greater than eightfold the MICs of extended-spectrum cephalosporins for these transconjugants. Five transconjugants showed 8- to 32-fold increases in the MICs of cefoxitin and seven showed more than a 256-fold increase in the MICs of gentamicin relative to those of the recipient. It is obvious that ciprofloxacin resistance was cotransferred with resistance to other antimicrobial agents, such as extended-spectrum cephalosporins, cephamycins, aminoglycosides, and sulfamethoxazole.

Identification of mutations in DNA *gyrA*, *gyrB*, and *parC*. DNA sequencing of the PCR product covering the entire QRDR of *gyrA* demonstrated the presence of mutations at codon 83 in 16 of 20 clinical isolates tested (Table 2). Point mutations in *gyrA* were found in *E. cloacae*, *E. coli*, *C. freundii*, and *K. pneumoniae*; and the resulting amino acid substitutions were Ser83Ile, Ser83Leu, Thr83Ile, and Ser83Ile, respectively. Two *E. cloacae* isolates had an additional mutation, Asp87Ala. In *parC*, the Ser80R or Ser80Ile substitutions were found in 12 of 20 clinical isolates. No mutations were found in the *gyrB* gene of any of 20 clinical isolates tested (Table 2). There was no mutation in three target genes among six transconjugants and the recipient.

TABLE 4. Southern hybridization of plasmid DNAs from eight transconjugants with probes

Transconjugant	Size (kb) of plasmid(s)	Southern hybridization result
GZ40T	53	*qnrB*, *aac(6′)-Ib-cr*
JN132T	191	*qnrB*, *aac(6′)-Ib-cr*, $bla_{CTX\text{-}M}$
JN1T	172, 107	*aac(6′)-Ib-cr*, $bla_{CTX\text{-}M}$
JS33T	193	*qnrA*, *aac(6′)-Ib-cr*
PU12T	129	*qnrS*, $bla_{CTX\text{-}M}$
SY22T	182	*qnrS*, bla_{SHV}
TJ15T	161	*aac(6′)-Ib-cr*, bla_{DHA}
WH99T	177	*qnrB*, $bla_{CTX\text{-}M}$, *aac(6′)-Ib-cr*, bla_{DHA}

Sequencing of β-lactamase genes for transconjugants. The properties of the 20 transconjugants are shown in Table 3. The *qnr* gene could be cotransmitted with *aac(6′)-Ib-cr*, ESBLs, and plasmid-mediated AmpC in one conjugation experiment. CTX-M-14 and CTX-M-3 were the most prevalent ESBL types and DHA-1 was the most common plasmid-mediated AmpC type among the transconjugants. In addition to the *qnr* and *aac(6′)-Ib-cr* genes, some transconjugants carried several β-lactamase genes. Strain SY26T, which had the *qnrB4* and *aac(6′)-Ib-cr* genes, produced DHA-1 and SHV-12; strain GZ51T, which had the *qnrA1* gene, produced SHV-12 and CTX-M-14 (Table 3).

Plasmid profiles and Southern blot analysis. Plasmid DNA was extracted from the 20 transconjugants and some of the respective donors. All the transconjugants and donors had different plasmid profiles. Twelve transconjugants carried one large plasmid of 53 to 195 kb (Table 3); eight carried two to three large plasmids of 65 to 200 kb. Southern hybridization analysis was performed with eight selected transcoujugants and their respective donors. Table 4 shows that the resistance genes detected were located on large plasmids of different sizes. We found that the genes for *qnr*, *aac(6′)-Ib-cr*, $bla_{CTX\text{-}M}$, bla_{SHV}, and bla_{DHA} were located on a single large plasmid for each transconjugant. For example, the *qnrB*, *aac(6′)-Ib-cr*, $bla_{CTX\text{-}M}$, and bla_{DHA} genes were on a plasmid of 177 kb in strain WH99T (Table 4). *aac(6′)-Ib-cr* and $bla_{CTX\text{-}M}$ were detected on two large plasmids of 172 and 107 kb, respectively, in strain JN1T. The results of Southern hybridization analysis with the donors were identical to those for their respective transconjugants.

DISCUSSION

This study showed that the prevalence of plasmid-mediated quinolone resistance due to the *qnr* and *aac(6′)-Ib-cr* genes among clinical isolates of *Enterobacteriaceae* in China is high and that it is much higher than that reported in other areas, such as the United States (15) and Taiwan (22). In our study, the prevalence of *qnr* appeared to be much lower in *E. coli* isolates (4.8%) than in other species of clinical isolates of the *Enterobacteriaceae*. However, the prevalence of *aac(6′)-Ib-cr* appeared to be lower in *E. cloacae* isolates (9.3%) than in isolates of the other species tested. *qnrA* was not found in relatively large numbers of nonfermenting bacilli, including

128 *P. aeruginosa* and 77 *A. baumannii* isolates (23). This implies that the mechanism for the incidence of *qnr* and/or *aac(6′)-Ib-cr* is related to the particular species. Interestingly, we found that the *qnrS* gene was the most prevalent in non-ESBL-producing *E. cloacae* (75.0%), and *qnrB* and *qnrA* were more frequently found in the suspected ESBL producers. A study in Taiwan also found that the *qnrS* gene was more common than other *qnr* genes in non-ESBL-producing isolates of *E. cloacae* (22). The distributions of the different *qnr* genes in non-ESBL-producing *Enterobacteriaceae* need further study.

In this study, two *C. freundii* isolates carried *qnrA*, *qnrB*, and *aac(6′)-Ib-cr*; one *K. pneumoniae* isolate carried *qnrB*, *qnrS*, and *aac(6′)-Ib-cr*; and one *E. cloacae* isolate carried *qnrB* and *qnrS*, which indicated that these species could carry *qnr* genes of different subtypes. Conjugation experiments proved that the plasmid-mediated quinolone resistance was transferable. The transferable plasmid-mediated, low-level quinolone resistance associated with different *qnr* genes and *aac(6′)-Ib-cr* was widespread among the isolates of the *Enterobacteriaceae*, and this perhaps contributed to the rapid increase in resistance to quinolones among bacteria in China. This study also indicated that chromosomal QRDR mutations in GyrA and ParC played an important role in mediating high-level quinolone resistance.

ESBLs are one of the most significant mechanisms of resistance to oxyimino-cephalosporins in the *Enterobacteriaceae*. In the 1980s, the ESBLs were predominantly TEM and SHV derivatives (2). However, since 2000, the CTX-M enzymes, originally described in South America, Asia, and Eastern Europe, have spread worldwide (1). In parallel, nosocomial outbreaks because of the expression of plasmid-mediated class C enzymes have increasingly been reported (9, 13). A statistical link between CTX-M production and nalidixic acid or fluoroquinolone resistance has been established, and this association can be explained at least in part by the high incidence of *qnr* genes in this ESBL type (7, 14). Another study showed a significant difference in the numbers of *qnr*-positive strains between the two time periods, 0 of 391 strains from 1991 to 1995 and 10 (3.5%) of 288 strains in 1996 to 2005 ($P < 0.01$), and suggested that ceftazidime resistance in *qnr*-positive *Enterobacter* strains was associated with a true ESBL-mediated mechanism (17). The information in Table 3 also suggests that the *qnr* and *aac(6′)-Ib-cr* genes and certain ESBLs or AmpCs are frequently cotransmitted and coselected, and this study found that there is a genetic linkage between these resistance elements on plasmids. One horizontal transmission event can result in the acquisition of multidrug resistance genes by wild-type strains, so this has presumably contributed to the rapid increase in the prevalence of multidrug resistance among clinical bacteria. Despite these recent findings, the contributions of the *qnr* and *aac(6′)-Ib-cr* genes to the increasing rates of quinolone resistance worldwide and the genetic association between quinolone resistance and ESBL- or AmpC-producing strains remain largely unknown, and further work is needed to examine the genetic environment and array of these resistance genes on plasmids.

In conclusion, transferable, plasmid-mediated quinolone resistance associated with *qnr* and *aac(6′)-Ib-cr* is widely distributed in China. These genes are implicated in low-level fluoroquinolone resistance and may play a significant role in the generation of resistant mutants and therapeutic failure. *qnr*-mediated quinolone resistance associated with multidrug resistance has the additional effect of genetically linking low-level quinolone resistance with resistance to other antibiotics and thus promoting the coselection of resistance upon exposure to other antimicrobials to which resistance is also encoded on plasmids. The emergence of plasmid-mediated quinolone resistance may thus contribute to the rapid increase in bacterial resistance to quinolones in several ways. The cotransmission of *qnr* with *aac(6′)-Ib-cr*, ESBLs, and plasmid-mediated AmpC genes speeds the formation of multidrug resistance in *Enterobacteriaceae* in China.

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ERRATUM

High Prevalence of Plasmid-Mediated Quinolone Resistance Genes *qnr* and *aac(6′)-Ib-cr* in Clinical Isolates of *Enterobacteriaceae* from Nine Teaching Hospitals in China

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Volume 52, no. 12, p. 4268–4273, 2008. Page 4268, column 2, line 11: The first sentence of the Materials and Methods section should read as follows. "The test isolates, which were collected from September to December 2006, were drawn from the Chinese Meropenem Susceptibility Surveillance study collection, which consists of nonrepeat clinical isolates of specified enterobacterial genera annually collected by specified laboratories in each of the big nine cities in China."