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Prevalence and Dissemination of *oqxAB* in *Escherichia coli* Isolates from Animals, Farmworkers, and the Environment[▽]

Jingjing Zhao,[1] Zhangliu Chen,[1] Sheng Chen,[2] Yuting Deng,[1] Yahong Liu,[1] Wei Tian,[1] Xianhui Huang,[1] Congming Wu,[1,3] Yongxu Sun,[1] Yan Sun,[1] Zhenling Zeng,[1] and Jian-Hua Liu[1]*

College of Veterinary Medicine, National Reference Laboratory of Veterinary Drug Residues (SCAU), South China Agricultural University, Guangzhou 510642, China[1]; Department of Applied Biology and Chemical Technology, The Hong Kong Polytechnic University, Hung Hom, Kowloon, Hong Kong SAR[2]; and College of Veterinary Medicine, China Agricultural University, Beijing 100193, China[3]

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OqxAB has recently been identified as one of the mechanisms of plasmid-mediated quinolone resistance (PMQR). Compared to what is observed for other PMQR determinants, there is a paucity of data with regard to the prevalence and epidemiology of OqxAB and its contribution to resistance to different antimicrobials. In this study, the prevalence and dissemination of *oqxAB* and other PMQR genes in *Escherichia coli* isolates from animals, farmworkers, and the environment in 2002 in China were investigated. Of the 172 *E. coli* isolates, 39.0% carried *oqxA*, while only 4.1%, 2.9%, and 0.6% carried *qnr* (1 *qnrB6* isolate, 5 *qnrS1* isolates, and 1 *qnrD* isolate), *qepA*, and *aac(6′)-Ib-cr*, respectively. Among the 33 isolates from farmworkers, 10 (30.3%) were positive for *oqxA*. *oqxAB* was associated with IS*26* and was carried on the 43- to 115-kb IncF transferable plasmid. Transconjugants carrying *oqxAB* showed 4- to 16-fold increases in the MICs of quinolones, 16- to 64-fold increases in the MICs of quinoxalines, 8- to 32-fold increases in the MICs of chloramphenicol and trimethoprim-sulfamethoxazole, and 4- to 8-fold increases in the MICs of florfenicol compared to the levels for the recipient. The pulsed-field gel electrophoresis (PFGE) analysis showed that the high levels of prevalence and dissemination of *oqxAB* in *E. coli* in animal farms were primarily due to the transmission of plasmids carrying *oqxAB*, although clonal transmission between human and swine *E. coli* isolates was observed. It is concluded that *oqxAB* was widespread in animal farms in China, which may be due to the overuse of quinoxalines in animals. This study warrants the prudent use of quinoxalines in food animals.

Fluoroquinolone resistance in animal bacterial isolates became an important public health problem due to the concern regarding the transmission of resistant bacterial pathogens to humans, since fluoroquinolones are the first choice of treatment for some human bacterial infections. The mechanisms of quinolone resistance were initially identified to be mediated by target mutations and overexpression of chromosomally encoded efflux pumps (13). In 1998, a plasmid-mediated quinolone resistance (PMQR) mechanism was firstly described to occur in a *Klebsiella pneumoniae* isolate from the United States (19). To date, three types of plasmid-mediated-quinolone-resistance determinants, including Qnr peptides (QnrA, QnrB, QnrS, QnrD, and QnrC), AAC(6′)-Ib-cr, and QepA, have been identified in clinical isolates (2, 23, 26, 28, 33, 36). Although the PMQR determinants can confer only low-level resistance to quinolones, their significant role may lie in that the low-level resistance ensures that the bacteria survive and subsequently generate target mutations for high-level fluoroquinolone resistance (25). A RND family pump, OqxAB, which confers resistance to olaquindox [*N*-(2-hydroxyethyl)-3-methyl-2-quinoxalinecarboxamide-1,4-di-*N*-oxide], one of the quinoxaline-*N*,*N*-dioxides, was discovered in *Escherichia coli* isolated from swine manure (10, 27). This pump was later identified to be a multidrug efflux pump that confers resistance to multiple agents, including fluoroquinolones (10, 11). OqxAB is encoded by the genes *oqxA* and *oqxB* in the same operon. OqxA and OqxB consist of several conserved blocks of amino acids similar to other verified and putative RND family proteins (10). The putative OqxB protein contains 12 transmembrane α helices, the numbers and positions of which are consistent with the crystal structure of the *E. coli* AcrB and MexF efflux pumps from *Xanthomonas axonopodis* (10). Similar to other members of the RND family of efflux pumps, the OqxAB efflux system also requires TolC to form the transmembrane channel (10). However, OqxAB was not recognized as a PMQR determinant until recently, and the data on the prevalence and epidemiology of OqxAB are limited compared to those observed for other PMQR determinants (28).

High prevalences of fluoroquinolone resistance in human and animal *E. coli* isolates have been reported to occur in China, which may be due to the overuse of quinolones as feed additive and therapies in food animals (8, 35, 37). The surveillance of PMQR determinants, in particular OqxAB, in *E. coli* isolates will provide insights into the understanding of the epidemiology and dissemination of OqxAB as well as the mechanism of the high prevalence of fluoroquinolone resistance in food animal bacterial isolates. In this study, we investigated the *E. coli* isolates from animals, farmworkers, and the

* Corresponding author. Mailing address: College of Veterinary Medicine, South China Agricultural University, Guangzhou 510642, People's Republic of China. Phone: (86-20)-85280237. Fax: (86-20)-85284896. E-mail: jhliu@scau.edu.cn.

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farm environment in four pig farms and a chicken farm to understand the prevalence and dissemination of *oqxAB* and other PMQR genes and their contribution to bacterial antimicrobial resistance.

MATERIALS AND METHODS

Sampling and bacterial isolates. Fecal samples or rectal swabs were randomly obtained from sows, piglets, weaners, and boars in four swine farms and chickens in a chicken farm located in different regions of Guangdong Province during 2002. Environmental samples from the farms, including surface soil, sewage, sullage, drinking water, and pond water samples, were randomly collected from different locations in each farm. Rectal swabs were obtained from consenting farmworkers. All samples were cultured on eosin methylene blue (EMB) agar plates and incubated at 37°C for 24 h. One suspicious colony with typical *E. coli* morphology was selected from each sample for identification.

PMQR gene detection. All isolates were screened for *oqxA* and other PMQR genes [*qnrA*, *qnrB*, *qnrC*, *qnrD*, *qnrS*, *aac(6′)-Ib-cr*, and *qepA*] by PCR using specific primers as previously described (2, 12, 15, 18). The whole coding region of *qnrD* was amplified using the primers qnrD-F (5′-TTTTCGCTAACTAACT CGC-3′) and qnrD-R (5′-GAAAGGATAAACAGGCAAAT-3′). All *oqxA*-positive isolates were also screened for the *oqxB* gene (16). As *qepA* was always associated with the 16S rRNA methylase gene *rmtB* (17), *qepA*-positive isolates were also screened for *rmtB* as previously described (4). The association of IS*26* with *oqxA*, as reported previously (16, 22), was investigated by PCR using forward primer IS26-F (5′-GCTGTTACGACGGGAGGAG-3′) located in IS*26* and reverse primer oqxA-R (5′-GGAGACGAGGTTGGTATGGA-3′) located in *oqxA*. All PCR products were sequenced and underwent BLAST searches to confirm the correct amplifications.

After PCR confirmation, the whole coding region of the *oqxAB* gene was amplified using the primers oqxAB-F (5′-CCCTGGACCGCACATAAAG-3′) and oqxAB-R (5′-AAAGAACAAGATTCACCGCAAC-3′). The resultant 5,140-bp PCR product of the *oqxAB* gene was then cloned into the pMD18-T vector (TaKaRa Biotechnology, Dalian, China) to construct pMD18-T::*oqxAB* and sequenced.

***gyrA* and *parC* mutations.** The quinolone resistance-determining regions (QRDRs) of the *gyrA* and *parC* genes in PMQR-positive isolates were sequenced to confirm the mutations as previously described (21).

Conjugation experiments and plasmid analysis. The transferability of *oqxAB* genes was studied by conjugation experiments using streptomycin-resistant *E. coli* C600 as the recipient strain as previously described (4). Briefly, 5 to 10 *oqxAB*-positive *E. coli* isolates from each farm with distinct pulsed-field gel electrophoresis (PFGE) patterns or sources (animals, workers, or the environment) were selected for conjugation experiments. A donor bacterium and recipient were grown in tryptic soy broth (TSB) to logarithmic phase, mixed at a 1:4 ratio (vol/vol), collected in a filter, and incubated at 37°C for 20 h. Transconjugants were selected on MacConkey agar plates containing olaquindox (64 μg/ml) and streptomycin (1,000 μg/ml). Restriction fragment length polymorphism (RFLP) analysis was performed on plasmids from transconjugants. Briefly, plasmids from transconjugants were extracted using a rapid alkaline lysis procedure (29) and digested with the endonuclease EcoRI (TaKaRa Biotechnology, Dalian, China) to analyze the RFLP profile and estimate the sizes of the plasmids.

PCR-based replicon typing was performed on conjugative plasmids as described by Carattoli et al. (1). Eighteen primer pairs, targeting the FIA, FIB, FIC, HI1, HI2, I1-Iγ, L/M, N, P, W, T, A/C, K, B/O, X, Y, F, and FII replicons, were used.

Antimicrobial susceptibility testing. Susceptibilities to ampicillin, cefazolin, streptomycin, kanamycin, gentamicin, amikacin, tetracycline, and trimethoprim-sulfamethoxazole (SXT) were determined by the antimicrobial disk diffusion test according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (6). In addition, the MICs for ciprofloxacin, mequindox, olaquindox, chloramphenicol, and enrofloxacin were determined by the agar dilution method. The breakpoints for each antimicrobial were recommended by the CLSI (6, 7). Resistance rates were calculated by dividing the number of intermediate-resistant and resistant strains by the total number of strains.

The MICs of 13 antimicrobial agents for donors, their corresponding *oqxAB*-positive transconjugants, and *E. coli* DH5α carrying pMD18-T::*oqxAB* were determined using the agar dilution method.

Epidemiological typing. PFGE analysis of XbaI-digested genomic DNA of all PMQR-positive isolates was performed using a CHEF Mapper system (Bio-Rad Laboratories, Hercules, CA) as described by Gautom (9). PFGE patterns were interpreted according to the criteria of Tenover et al. (30). The isolates that had PFGE patterns with no more than four band differences were considered clonally related. The phylogenetic group of the PMQR-positive isolates was determined by the multiplex PCR-based method as previously described by Clermont et al. (5).

Multilocus sequence typing (MLST) of some representative strains from different sources and farms was performed according to the previously described protocol (http://www.shigatox.net/mlst). The seven housekeeping genes (*aspC*, *clpX*, *fadD*, *icdA*, *lysP*, *mdh*, and *uidA*) were amplified and sequenced. The allelic profile of the seven gene sequences and the sequence types (STs) were obtained via the electronic database at the *E. coli* MLST website.

Nucleotide sequence accession numbers. The *oqxAB* and *qnrD* sequences were deposited into the GenBank database under the assigned accession numbers GQ497565, GU453932, and GU477622.

RESULTS

Prevalence of *oqxAB* and other PMQR genes. As shown in Table 1, 172 *E. coli* isolates were randomly isolated from 172 samples of animals, farmworkers, and the environment from five farms (18 to 40 isolates per farm). The *oqxA* gene was present in 67 (39.0%) *E. coli* isolates. About 39.8% (39/98) *E. coli* isolates from animals, 43.9% (18/41) from the farm environment, and 30.3% (10/33) from farmworkers were positive for *oqxA*. About 46.3% of *E. coli* isolates from pig farms were positive for *oqxA*, while only ~13% from the chicken farm were positive, significantly lower than the percentage of isolates from pig farms ($P < 0.01$). All except one *oqxA*-positive isolate were also positive for *oqxB*. The *qnr*, *qepA*, and *aac(6′)-Ib-cr* genes were detected in 7 (1 *qnrB6* isolate, 5 *qnrS1* isolates, and 1 *qnrD* isolate) (4.1%), 5 (2.9%), and 1 (0.6%) of the total 172 *E. coli* isolates, respectively. All 7 *qnr*-positive isolates were also positive for *oqxAB*, and all 5 *qepA*-positive isolates were also positive for *rmtB*. No *qnrA* and *qnrC* genes were detected in any of the *E. coli* isolates (Table 1). In addition, 49 of the 67 *oqxA*-positive isolates were positive for IS*26*. The whole coding region of the *oqxAB* genes was entirely sequenced for one isolate and was found to be nearly identical to that previously reported, with only two silent mutations in the *oqxB* gene (9, 16).

Antimicrobial susceptibility analysis. The MICs of quinoxalines, chloramphenicol, and fluoroquinolones were determined for all *E. coli* isolates. The MIC_{50}s of mequindox, olaquindox, chloramphenicol, enrofloxacin, and ciprofloxacin were 8- to 32-fold higher in *oqxAB*-positive isolates than in *oqxAB*-negative isolates. The MICs of mequindox and olaquindox were higher than 32 μg/ml in all *oqxA*-positive isolates except for one isolate (16 μg/ml) that was negative for *oqxB*, while the MICs of mequindox and olaquindox were much lower (≤32 μg/ml) in *oqxAB*-negative isolates. The MICs of chloramphenicol, enrofloxacin, and ciprofloxacin differed among *oqxAB*-positive isolates. However, the rates of resistance to chloramphenicol and ciprofloxacin were higher in *oqxAB*-positive isolates (85.5% and 47.8%, respectively) than in *oqxAB*-negative isolates (38.8% and 21.4%, respectively) ($P < 0.01$). More than 75% of the *oqxAB*-positive isolates were resistant to ampicillin, kanamycin, tetracycline, and trimethoprim-sulfamethoxazole. No significant differences in resistance to gentamicin and amikacin were found between *oqxAB*-positive and *oqxAB*-negative *E. coli* isolates. All isolates were susceptible to cefazolin.

***gyrA* and *parC* mutations.** Of the 60 *E. coli* isolates carrying only *oqxAB*, 25 (41.7%) isolates had wild-type (WT) *gyrA* and *parC* genes, with ciprofloxacin MICs ranging from 0.008 to 0.5

TABLE 1. Prevalence and diversity of PMQR determinants in *E. coli*

Sample source	Total no. of isolates	No. (%) of isolates positive for:						No. of isolates with any PMQR gene (%)	No. of PFGE subtypes of PMQR-positive isolates[a]
		oqxA	*qepA*	*qnrB6*	*qnrS1*	*qnrD*	*aac(6′)-Ib-cr*		
Farms 1–4									
Pigs	73	36	4		4	1		40	
Workers	27	8	1					11	
Environment	34	16		1			1	16	
Total	134	62 (46.3)	5	1	4	1	1	67 (50.0)	53 (6)
Farm 5									
Chickens	25	3			1			3	
Workers	6								
Environment	7	2						2	
Total	38	5 (13.2)			1			5 (13.2)	3
Total farms									
Animals	98	39 (39.8)	4		5	1		43 (43.9)	
Workers	33	10 (30.3)	1					11 (33.3)	
Environment	41	18 (43.9)		1			1	18 (43.9)	
Total	172	67 (39.0)	5	1	5	1	1	72 (41.9)	56 (6)

[a] The number of nontypeable isolates is indicated in parentheses.

μg/ml; 4 had a single mutation in *gyrA* at codon 83 to the L codon, with ciprofloxacin MICs ranging from 0.25 to 1 μg/ml; and 5 had one point mutation in both *gyrA* and *parC*, with ciprofloxacin MICs ranging from 1 to 4 μg/ml (Table 2). Mutations at both codon 83 to the L codon and codon 87 to the N codon in *gyrA* were found in 26 (43.3%) isolates with ciprofloxacin MICs of ≥16 μg/ml. Among these isolates, 23 had a single point mutation at codon 80 or 84 in *parC*, and 2 had both mutations in *parC*. These target mutations explained the difference in MICs of fluoroquinolones for *oqxAB*-positive isolates.

TABLE 2. Distribution of QRDR mutations of *gyrA* and *parC* in the PMQR-positive *E. coli* isolates

PMQR gene(s)	QRDR mutation(s)[a]		No. of isolates	MIC range for ciprofloxacin (μg/ml)
	gyrA	*parC*		
oqxAB (n = 60)	None	None	25	0.008–0.5
	L83	None	4	0.25–1
	L83	I80	3	4
	L83	G84	1	4
	L83	R84	1	1
	L83, N87	None	1	>32
	L83, N87	R80	1	32
	L83, N87	I80	20	16–32
	L83, N87	K84	2	32–>32
	L83, N87	I80, A84	2	>32
qepA	L83, N87	I80	4	32–>32
	L83, N87	K84	1	>32
oqxAB, *qnrD*	L83	R80	1	2
oqxAB, *qnrS1*	None	None	5	1–2
oqxAB, *qnrB6*, *aac(6′)-Ib-cr*	None	None	1	2

[a] "L83" represents a mutation at codon 83 to the L codon, etc. "None" indicates the wild type.

Of the 5 *qepA*-positive isolates, all had two mutations in *gyrA* and one mutation in *parC* and had ciprofloxacin MICs of ≥32 μg/ml. In contrast, of the 7 isolates carrying both *qnr* and *oqxAB*, 6 had wild-type *gyrA* and *parC* genes, with ciprofloxacin MICs of 1 to 2 μg/ml (Table 2).

Transferability of the *oqxAB* gene. Thirteen transconjugants were successfully obtained from 41 OqxAB-producing isolates by conjugation experiments. The conjugative transfer frequencies ranged from 10^{-9} to 10^{-5} transconjugants per recipient. The *qnrB6* and *aac(6′)-Ib-cr* genes were also cotransferred with *oqxAB* from an ST2 donor that was isolated from a soil sample from farm 4 (Table 3).

The MICs of mequindox and olaquindox for all *oqxAB*-positive transconjugants were similar to those observed for the donor isolates but were about 16- to 64-fold higher than those observed for the recipient (Table 3). The 12 transconjugants carrying only *oqxAB* showed about 4- to 8-fold increases in the MICs of ciprofloxacin, 8- to 16-fold increases in the MICs of nalidixic acid, and 4- to 16-fold increases in the MICs of enrofloxacin and norfloxacin in comparison to the levels for the recipient, suggesting that *oqxAB* contributed to the decreased susceptibility to quinolones in *E. coli*. However, the MICs of nalidixic acid, norfloxacin, enrofloxacin, and ciprofloxacin in the transconjugants carrying both *oqxAB* and *qnrB6*/*aac(6′)-Ib-cr* were 32- to 128-fold higher than those observed for the recipient, approaching levels similar to those observed for the donor isolates, suggesting that the combination of different PMQR determinants can also confer intermediate resistance to quinolone. The MICs of chloramphenicol and SXT for the *oqxAB* transconjugants ranged from 32 to 128 μg/ml and 4 to 16 μg/ml, respectively, about 8- to 32-fold higher than those observed for the recipient. All *oqxAB*-positive transconju-

TABLE 3. MICs for transconjugants and characterization of plasmids carrying *oqxAB*[a]

Strain	Donor		Plasmid			MIC (μg/ml)[b]												
	Origin	MLST	Size (kb)	RFLP pattern	Replicon	OLA	MEQ	NAL	NOR	CIP	ENR	CHL	FFC	SXT	TET	AMP	GEN	STR
E. coli C600						8	4	2	0.03	0.008	0.016	4	2	0.5	1	4	0.5	>512
W281-T	F1 sow	ST921	ND	ND	ND	128	64	16	0.125	0.06	0.06	128	16	16	2	>128	0.5	>512
W191-T	F1 sullage	ST172	115	I	FII	256	128	16	0.125	0.06	0.125	128	16	16	64	>128	1	>512
W322-T	F1 worker	ND	43	II	UT	512	256	64	0.25	0.06	0.125	64	16	4	1	>128	0.125	>512
W245-T	F1 boar	ST920	58	III	UT	512	128	16	0.125	0.06	0.06	64	16	8	1	>128	0.25	>512
G262-T	F2 soil	ST922	100	IV	FII	256	128	16	0.25	0.06	0.125	128	16	16	32	8	0.5	>512
G062-T	F2 piglet	ND	57	V	FII	256	128	32	0.125	0.06	0.125	32	16	8	4	>128	0.5	>512
G375-T	F2 worker	ND	53	VI	UT	256	256	64	0.25	0.06	0.125	32	16	8	4	16	0.25	>512
X1B1-T	F3 weaner	ST134	ND	ND	ND	256	64	16	0.125	0.06	0.125	128	16	16	32	16	0.5	>512
XT11-T	F3 soil	ST928	115	VII	FIVC	256	128	16	0.25	0.06	0.06	128	16	16	64	>128	0.5	>512
SW8-T	F4 pond water	ST926	115	VII	FII	512	256	64	0.5	0.06	0.125	128	16	16	1	>128	32	>512
ST2-T[c]	F4 soil	ST925	81	VIII	UT	512	128	256	4	2	2	32	4	16	2	4	0.25	>512
SP8-T	F4 pig	ND	91	IX	UT	256	256	32	0.125	0.06	0.06	64	16	8	2	>128	0.25	>512
D83-T	F5 chicken	ND	ND	ND	ND	256	128	8	0.125	0.03	0.06	32	8	8	4	16	0.25	>512
E. coli DH5α/ pMD18-T::*oqxAB*						128	64	32	0.25	0.125	0.125	64	16	8	4	>128	0.125	2
E. coli DH5α						8	4	2	0.03	0.008	0.016	4	4	0.5	2	>128	0.5	4

[a] ND, not determined; UT, untypeable; F1 to F5, farm 1 to farm 5, respectively.
[b] OLA, olaquindox; MEQ, mequindox; NAL, nalidixic acid; NOR, norfloxacin; CIP, ciprofloxacin; ENR, enrofloxacin; CHL, chloramphenicol; FFC, florfenicol; SXT, trimethoprim-sulfamethoxazole; TET, tetracycline; AMP, ampicillin; GEN, gentamicin; STR, streptomycin.
[c] ST2-T contained *oqxAB*, *qnrB6*, and *aac(6′)-Ib-cr*.

gants also showed 4- to 8-fold increases in the MICs of florfenicol. In addition, the cotransfer of resistance to ampicillin, tetracycline, and gentamicin was also observed in 8, 4, and 1 of the 13 transconjugants, respectively (Table 3). The *E. coli* DH5α strain carrying pMD18-T::*oqxAB* also showed increased MICs of quinoxalines, quinolones, chloramphenicol, florfenicol, and SXT compared to those observed for WT *E. coli* DH5α (Table 3).

Plasmid analysis. Plasmid DNA was extracted from 12 transconjugants. Two transconjugants carried two plasmids, and the other 10 carried only one plasmid. The RFLP patterns were determined for the 10 transconjugants carrying only one plasmid. The sizes of the plasmids ranged from ~43 to 115 kb. Only two plasmids, one from strain X1B1 isolated from a soil sample from farm 3 and one from strain SW8 recovered from a pond water sample from farm 4, showed identical RFLP patterns. PCR-based *inc* replicon typing showed that plasmids from 4 of the 10 transconjugants carrying only one plasmid belonged to the FII type, 1 belonged to the FIVC type, and the other 5 were untypeable (Table 3).

PFGE and phylogenetic analysis. Sixty-six out of the 72 PMQR-positive isolates underwent PFGE analysis, and 56 different XbaI–pulsed-field gel electrophoresis patterns were observed (Table 1). It is suggested that the dissemination of *oqxAB* was not due to the clonal dissemination of *oqxAB*-positive *E. coli*. However, *E. coli* isolates with indistinguishable PFGE patterns were found in sows and farmworkers from farm 2 as well as sows and farmworkers from farm 3 (Fig. 1).

Phylogenetic analysis showed that the 72 PMQR-positive isolates mainly belonged to phylogenetic group A (52.8%), followed by groups B1 (36.1%), D (8.3%) and B2 (2.8%). MLST analysis of 17 OqxAB-producing *E. coli* isolates of different sources identified 15 different sequence types (8 STs are listed in Table 3). Nine novel sequence types (ST920 to ST928) were detected in these *E. coli* isolates. The *E. coli* isolates from farms 2 and 3 that showed identical PFGE patterns also showed identical sequence types (Fig. 1).

DISCUSSION

The prevalence and dissemination of *oqxAB* in *E. coli* isolated from animals, farmworkers, and the environment were investigated in this study. A surprisingly high prevalence

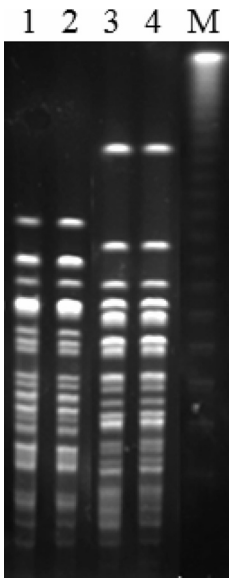

FIG. 1. PFGE fingerprinting patterns of XbaI-digested total DNA preparations from *E. coli* isolates. Lanes: M, lambda ladder PFGE marker used as a molecular size marker; 1, *E. coli* strain from one worker from farm 3, ST923; 2, *E. coli* strain from one sow from farm 3, ST923; 3, *E. coli* strain from one worker from farm 2, ST924; 4, *E. coli* strain from one sow from farm 2, ST924.

(39.0%) of *oqxAB* was detected in *E. coli* isolates, significantly higher than previously reported for Denmark, Sweden (1.8%) (12), and South Korea (0.4%) (16). Olaquindox was commonly used as a therapeutic and preventive antibiotic in swine in China. However, it has been forbidden in poultry since 2000 due to its toxic side effects, which may explain the relatively low prevalence of *oqxAB* in the chicken farm. A new synthetic quinoxaline 1,4-dioxide (QdNO) derivative, mequindox (3-methyl-2-acetyl-*N*-1,4-dioxyquinoxaline; $C_{11}H_{10}N_2O_3$), which was developed in China, has also been widely used as an antibacterial and animal feed additive in China since the 1990s (14). Antimicrobial usage in food animals is considered the most important factor in the selection of resistant bacteria (34). Therefore, the high levels of prevalence and dissemination of *oqxAB* in *E. coli* isolates in animals in China may be due to the overuse of olaquindox and mequindox in food animals.

In addition to animal *E. coli* isolates, *oqxAB* was also detected in 30.3% of human commensal *E. coli* isolates from farmworkers without previous antimicrobial treatment or hospital admission, suggesting the transmission of *oqxAB* to human isolates. The diverse PFGE patterns within *oqxAB*-positive *E. coli* isolates suggest the possible horizontal transmission of the *oqxAB* determinant instead of the direct clonal dissemination between animals, farmworkers, and the environment. However, the same PFGE pattern was occasionally observed in *E. coli* isolates from animals and farmworkers, suggesting the transmission of *oqxAB*-positive *E. coli* between humans and animals as described in other studies (20, 32). Further studies are needed to investigate the prevalence of *oqxAB* in human clinical isolates in China and the possible transmission of plasmids carrying *oqxAB* through the food chain.

In contrast to the previous report of Kim et al. (16), the *oqxAB* gene was proven by conjugation experiments to be located in transferable plasmid. Five of the 13 transconjugants carried a broad-host-range IncF-type plasmid, which is different from the previous report of *oqxAB* located in the IncX incompatible group of plasmids (pOLA52) (22). However, *oqxAB* may be located in a different (IncF) group of plasmids, which is evidenced by various RFLP patterns within the plasmids carrying *oqxAB*. Consistent with previous report that the *oqxAB* cassette flanked by IS*26* was identical to the chromosome segment (composite transposon Tn*6010*) of *K. pneumoniae* MGH 78578 (16, 22), the *oqxA* gene in this study was also flanked by IS*26*, which suggests that the dissemination of *oqxAB* among different *E. coli* strains may be mediated by the mobile element.

The *oqxAB* genotype is very consistent with the olaquindox and mequindox resistance phenotype in *E. coli* isolates, suggesting the role of *oqxAB* in olaquindox resistance, which was also supported by the conjugation experiments and other studies (10, 11). In contrast, *oqxAB* contributes only to low-level decreases in susceptibility to quinolones, which was evidenced by the increase of quinolone MICs by 4- to 16-fold in transconjugants, a lower extent of contribution than was observed for the first reported pOLA52-mediated *oqxAB* gene (11, 28). The various levels of MICs of quinolones in *oqxAB*-positive *E. coli* isolates were due to the presence of the target mutations. The *oqxAB*-positive *E. coli* isolates without target mutations showed only low-level decreases in susceptibility to ciprofloxacin and other quinolones.

Though *oqxAB* conferred only low-level quinolone resistance, the significant role of this PMQR may lie in its ability to enable *E. coli* to survive at a low concentration of fluoroquinolones, which is the prerequisite for subsequent generation of target mutations for resistance to higher-level fluoroquinolone (24). Cesaro et al. found that topoisomerase mutations were rarely selected by ciprofloxacin from strains containing *qnr* (3). It is not clear whether the first step of quinolone resistance is the acquisition of the QRDR gene(s) or the occurrence of topoisomerase mutation. However, the relatively high frequencies of topoisomerase mutations in *oqxAB*- and *qepA*-positive isolates compared to those observed in Qnr-producing isolates suggested that the quinolone efflux pump (OqxAB and QepA) might favor the selection of high-level quinolone resistance compared to Qnr proteins which protect QRDR domains from quinolone attacks (31).

In conclusion, the transferable plasmid-mediated multidrug efflux pump gene *oqxAB* was widespread in animal farms. The overuse of quinoxaline as a feed additive in food animals might contribute to the development and dissemination of *oqxAB*, which subsequently promotes the development of high-level fluoroquinolone resistance in bacteria. The data in this study warrant the prudent use of mequindox and olaquindox in farm animals in China.

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