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Short Communication

Co-spread of *oqxAB* and $bla_{CTX\text{-}M\text{-}9G}$ in non-Typhi *Salmonella enterica* isolates mediated by ST2-IncHI2 plasmids

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ABSTRACT

In this study, 130 non-Typhi *Salmonella enterica* isolates from chickens were analysed for the prevalence of plasmid-mediated quinolone resistance (PMQR) determinants as well as the co-existence of *oqxAB* and extended-spectrum β-lactamase (ESBL) genes. The genes *oqxAB*, *aac(6′)-Ib-cr*, $bla_{CTX\text{-}M\text{-}9G}$ and bla_{TEM} were present alone or in combination in 40 (30.8%), 40 (30.8%), 55 (42.3%) and 2 (1.5%) isolates, respectively. Most of the *oqxAB*–$bla_{CTX\text{-}M\text{-}9G}$-positive isolates (17/28) carried transferable ST2-IncHI2 plasmids containing an *oqxAB* cassette and $bla_{CTX\text{-}M\text{-}14}$ flanked by insertion sequences IS*10* or IS*Ecp1* upstream and IS*903* downstream. The *oqxAB*–$bla_{CTX\text{-}M\text{-}9G}$-positive isolates from a local area showed similar pulsed-field gel electrophoresis (PFGE) patterns, whilst the isolates from different areas were genetically divergent, suggesting that both clonal expansion in local areas and horizontal transmission contributed to the spread of ST2-IncHI2 plasmids containing *oqxAB* and $bla_{CTX\text{-}M\text{-}14}$. This is the first report on the prevalence of ST2-IncHI2 plasmids concomitantly carrying *oqxAB* and $bla_{CTX\text{-}M\text{-}14}$ in *Salmonella* and also the first description of the genetic environment of *oqxAB*–$bla_{CTX\text{-}M}$. The genetic linkage of *oqxAB*–$bla_{CTX\text{-}M\text{-}9G}$ in non-Typhi *Salmonella* likely facilitates the spread of antibiotic-resistant *Salmonella* and poses a threat for clinical treatment of salmonellosis.

1. Introduction

To date, three types of plasmid-mediated quinolone resistance (PMQR) mechanisms have been described: the pentapeptide repeat family Qnr proteins (QnrA, QnrB, QnrS, QnrD and QnrC); AAC(6′)-Ib-cr, an aminoglycoside acetyltransferase responsible for reduced susceptibility to ciprofloxacin by modifying antibiotics; and the efflux pumps QepA and OqxAB [1]. PMQR determinants have been shown to be widespread and can confer low-level resistance to quinolones [1,2]. OqxAB is one of the first plasmid-borne efflux pumps of the resistance–nodulation–cell division (RND) family and the first identified mechanism of resistance to olaquindox [3]. OqxAB is encoded by the *oqxA* and *oqxB* genes and confers resistance to multiple agents, including (fluoro)quinolones such as nalidixic acid, ciprofloxacin and norfloxacin as well as chloramphenicol and biocides [4]. Carriage of *oqxAB* on transferable plasmids facilitates the emergence of fluoroquinolone resistance and its transmission via horizontal gene transfer [5]. Recently, our group observed clonal dissemination of IncHI2-type plasmids containing *oqxAB* in *Salmonella enterica* serotype Typhimurium in food-producing animals [6]. Fortunately, these plasmids could not confer resistance to broad-spectrum cephalosporins. However, IncHI2-type plasmids have recently been implicated in the spread of genes encoding extended-spectrum β-lactamases (ESBLs) and are known to play a key role in the acquisition of antibiotic resistance [7].

Reports on the prevalence of coexistence of PMQR, especially *aac(6′)-Ib-cr*, and ESBL genes in the same *Salmonella* and *Escherichia coli* isolates have increased in recent years [7,8]. However, there is little information regarding the distribution of *oqxAB*–ESBL-positive isolates, and detailed information on the plasmid containing *oqxAB* and ESBLs in non-Typhi serotypes of *S. enterica* (hereafter referred to as non-Typhi *Salmonella*) is lacking. The aim of this study was to investigate PMQR determinants and ESBL genes as well as gain a molecular insight into the distribution of non-Typhi *Salmonella* isolates harbouring both *oqxAB* and ESBLs derived from chickens.

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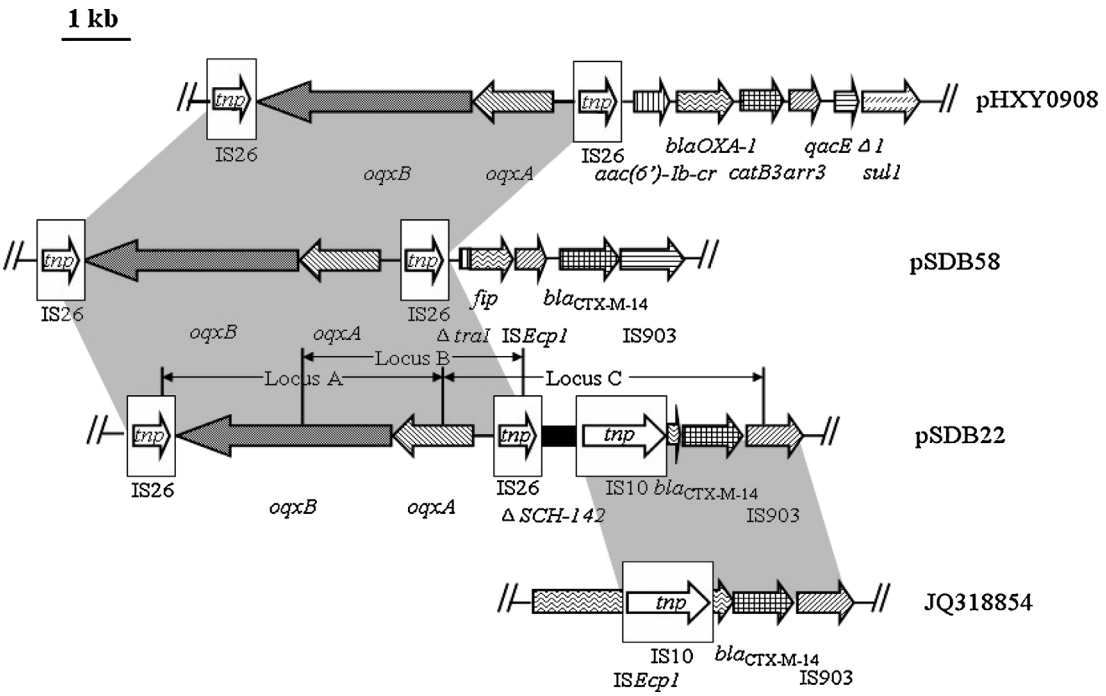

Fig. 1. Flanking regions of *oqxAB*–$bla_{CTX\text{-}M\text{-}14}$ genes in plasmids pSDB22 and pSDB58 of *Salmonella enterica* serotype Indiana strains B22 and B58, respectively, and structural comparison with plasmids pHXY0908 from *S.* Typhimurium and JQ318854 from *Escherichia coli*. Arrows indicate the positions and directions of open reading frames. Different genes are displayed using different shading. The solid black box indicates the truncated hypothetical protein ΔSCH-142. The insertion sequence IS*26* elements are shown as light grey boxes, with the white arrows indicating the *tnp* genes. Regions of >96% homology are marked by grey shading. The marked locus A to locus C regions are the three sets of long-range PCR targets.

2. Materials and methods

2.1. Bacterial strains and susceptibility testing

In total, 130 non-Typhi *Salmonella* isolates were obtained from 1500 faecal swabs of chickens at 12 poultry farms in Shandong Province (China) between March 2012 and May 2013. Minimum inhibitory concentrations (MICs) of quinolones (nalidixic acid), fluoroquinolones (ciprofloxacin, enrofloxacin and levofloxacin), third-generation cephalosporins (ceftiofur, cefotaxime and ceftazidime) and other antimicrobials [olaquindox, ampicillin, trimethoprim/sulfamethoxazole (SXT), tetracycline, gentamicin, amikacin, chloramphenicol and florfenicol] (Sigma Chemical Co., St Louis, MO) were determined by the agar dilution method following the guidelines of the Clinical and Laboratory Standards Institute (CLSI). Breakpoints for each antimicrobial were recommended by the CLSI (M100-S18 and VET01-A4/VET01-S2) [9,10] and DANMAP 98 (olaquindox). *Escherichia coli* ATCC 25922 was used as a quality control strain. ESBL-producing isolates were inferred by the double-disc synergy test using cefotaxime or ceftazidime and ticarcillin/clavulanic acid (Sigma Chemical Co.) as recommended by the CLSI [9,10].

2.2. Detection of resistance genes

The presence of PMQR determinants, including *qnrA*, *qnrB*, *qnrS*, *qnrD*, *qnrC*, *aac(6′)-Ib-cr*, *qepA* and *oqxAB*, was detected among the 130 non-Typhi *Salmonella* isolates by PCR as described previously [6,11,12]. ESBL genes in ESBL-producing isolates were determined by PCR with previously reported oligonucleotide primers, including bla_{TEM}, bla_{SHV} and $bla_{CTX\text{-}M}$ [13]. All PCR products were directly sequenced and the results were compared with those in the GenBank nucleotide database (http://www.ncbi.nlm.nih.gov/blast/).

2.3. Molecular typing

Genomic DNA of *oqxAB*–ESBL-positive isolates was analysed by pulsed-field gel electrophoresis (PFGE) following digestion with *Xba*I [14]. Comparison of PFGE patterns was performed with BioNumerics software (Applied Maths, Sint-Martens-Latem, Belgium). Dendrograms were generated with the Dice similarity coefficient (1.5% optimisation and 1.5% tolerance) using the unweighted pair-group method with arithmetic mean (UPGMA), and PFGE types were defined with >90% similarity between clusters [8].

2.4. Conjugation and transformation analysis

Isolates harbouring both *oqxAB* and ESBLs were selected for conjugation and transformation experiments. Conjugation experiments were carried out by the liquid mating-out assay as described previously [15]. Transconjugants were selected by plating onto MacConkey agar (Huankai Co. Ltd., Guangzhou, China) containing 50 mg/L sodium azide and 0.05 mg/L ciprofloxacin. Plasmids not transferable by conjugation were studied by transformation assay. Plasmid DNA from donors was extracted using a QIAGEN Prep Plasmid Midi Kit (QIAGEN, Hilden, Germany). Purified plasmids were used to transform *E. coli* DH5α (TaKaRa Biotechnology, Dalian, China) by electroporation following the manufacturer's instructions. Transformants were incubated at 37 °C for 1 h and were then selected on Luria–Bertani agar (Huankai Co. Ltd.) containing 0.05 mg/L ciprofloxacin.

2.5. Plasmid analyses

Incompatibility (Inc) groups were assigned by PCR-based replicon typing of transconjugants [16]. Plasmids in transconjugants/transformants were analysed by S1 nuclease-PFGE and

Southern blot hybridisation with probes specific for *oqxA*, *oqxB*, *aac(6′)-Ib-cr* and ESBL genes as well as with probes for each gene used to identify the plasmid replicon type (Roche Diagnostics GmbH, Mannheim, Germany). Each probe was made by PCR amplification with the primers in Supplementary Table S1. Mobilisable plasmid relaxases (MOB) typing and plasmid double-locus sequence typing (pDLST) for IncHI2 plasmids were performed as previously described [7,17]. The *oqxAB*–ESBL-positive plasmids were further analysed by restriction fragment length polymorphism (RFLP) using *Eco*RI as the endonuclease (TaKaRa Biotechnology).

Supplementary Table S1 related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ijantimicag.2014.05.014.

The flanking regions of *oqxAB*–ESBLs were determined by the primer walking method on both DNA strands, and the partial sequence was used to design primers for PCR-RFLP analysis of the remaining *oqxAB*–ESBL-positive plasmids (Supplementary Table S1). The detailed locations of primers are presented in Fig. 1 (designated A–C). In addition, the genetic environment of *oqxAB*–*aac(6′)-Ib-cr*-positive plasmids was analysed as described previously [6].

2.6. Nucleotide sequence accession numbers

The partial nucleotide sequences in *oqxAB*–$bla_{CTX\text{-}M\text{-}14}$-positive plasmids pSDB22 and pSDB58 have been submitted to GenBank and assigned accession nos. KF746160 and KF840373, respectively.

3. Results

3.1. Antimicrobial susceptibility

Amongst the 130 non-Typhi *Salmonella* isolates examined in this study, 81 (62.3%) presented resistance (MIC ≥ 4 mg/L) to ciprofloxacin. Approximately one-half of the isolates showed resistance to olaquindox, ceftiofur, gentamicin and tetracycline. The antimicrobial resistance rates to other antibiotics tested were as follows: ampicillin, 72.3%; SXT, 70%; florfenicol, 66.9%; and amikacin, 11.5%. Of the 130 strains, 59 (45.4%) and 27 (20.8%) showed intermediate susceptibility to cefotaxime and ceftazidime (MIC ≥ 16 mg/L), respectively. The MICs of ceftiofur were ≥8 mg/L in all *oqxAB*–ESBL-positive non-Typhi *Salmonella* isolates ($n = 28$), and these isolates also exhibited a multiresistant phenotype, including resistance to olaquindox (Table 1).

3.2. Detection of plasmid-mediated quinolone resistance determinants and extended-spectrum β-lactamase-encoding genes

Among the 130 non-Typhi *Salmonella* isolates, PMQR determinants were present in 73 (56.2%), with *oqxAB* and *aac(6′)-Ib-cr* being detected in 40 (30.8%) and 40 (30.8%) strains, respectively. $bla_{CTX\text{-}M\text{-}9G}$ genes (55/130; 42.3%) were found to be dominant in the isolates with ESBL production, and two $bla_{TEM\text{-}1}$-positive isolates were negative for *oqxAB* (2/130; 1.5%). The *qnrA*, *qnrB*, *qnrC*, *qnrD* and *qepA* genes as well as the ESBL-encoding gene bla_{SHV} were not detected in any of the isolates tested. Of the 40 *oqxAB*-positive isolates, 28 were detected to harbour $bla_{CTX\text{-}M\text{-}9G}$ ($bla_{CTX\text{-}M\text{-}14}$ and $bla_{CTX\text{-}M\text{-}65}$), whereas it was detected in 27 of the 90 *oqxAB*-negative isolates ($P < 0.001$). In addition, four *oqxAB*–$bla_{CTX\text{-}M\text{-}9G}$-positive isolates were found to harbour *aac(6′)-Ib-cr* (Table 1).

3.3. Pulsed-field gel electrophoresis

Of the 28 *oqxAB*–$bla_{CTX\text{-}M\text{-}9G}$-positive non-Typhi *Salmonella* isolates, 26 were successfully typed by PFGE and were grouped into 13 PFGE clusters designated A–M (Fig. 2). Three major PFGE clusters A, E and K accounted for 26.9% (7/26), 15.4% (4/26) and 15.4% (4/26) of the isolates, respectively. In addition, most of these isolates except one (B51) were identified as serotype Indiana, whereas the remaining PFGE clusters comprised only one or two isolates each, and three isolates belonging to clusters D, H and L were identified as serotype Enteritidis.

3.4. Co-transfer of *oqxAB* and $bla_{CTX\text{-}M\text{-}9G}$ genes

In this study, 5 transconjugants and 23 transformants were successfully obtained from 28 *oqxAB*–$bla_{CTX\text{-}M\text{-}9G}$-positive isolates by conjugation/transformation experiments. Five transconjugants and 14 transformants carried both *oqxAB* and $bla_{CTX\text{-}M\text{-}9G}$ genes. The ciprofloxacin MICs of the transconjugants/transformants increased 4–16-fold (0.03125–0.125 mg/L) compared with the recipient *E. coli* J53 (0.008 mg/L) and *E. coli* DH5α (0.016 mg/L), respectively. For third-generation cephalosporins, all transconjugants/transformants showed extremely high-level resistance to cefotaxime and ceftiofur (32–128 mg/L) at the same level as the donor strains. In addition, co-transfer of resistance to olaquindox, nalidixic acid, ampicillin, chloramphenicol, florfenicol, tetracycline, gentamicin and SXT was also observed in some transconjugants/transformants.

3.5. Plasmid analysis

The results of S1-PFGE and Southern blot hybridisation revealed that *oqxAB* and $bla_{CTX\text{-}M\text{-}9G}$ co-located on the same plasmid in 19 (67.9%) of the 28 *oqxAB*–$bla_{CTX\text{-}M\text{-}9G}$-positive isolates, and all of them belonged to the IncHI2 type with size varying between 145.5 kb and 388 kb. Furthermore, results obtained by MOB typing and pDLST indicated that all IncHI2 plasmids detected belonged to the MOB_{H11} subgroup, and 89.5% (17/19) of them belonged to group ST2 (Table 1). Meanwhile, all five $bla_{CTX\text{-}M\text{-}65}$-positive plasmids were negative for *oqxAB*. In addition, *aac(6′)-Ib-cr* and *oqxAB* were co-localised on the plasmid extracted from isolate D25, and it presented the same genetic environment of *oqxAB* as described previously (Table 1) [6]. Most of the *oqxAB*–$bla_{CTX\text{-}M\text{-}14}$-positive plasmids shared very different RFLP patterns. However, the plasmids isolated from A44, B6, D20, B32 and A100 showed identical RFLP patterns (Supplementary Fig. S1). The flanking regions of *oqxAB*–$bla_{CTX\text{-}M\text{-}14}$ were determined in plasmid pSDB22 and pSDB58 isolated from B22 and B58, respectively. The genetic environment of *oqxAB*–$bla_{CTX\text{-}M\text{-}14}$ consisted of an *oqxAB* cassette flanked by IS*26* and a $bla_{CTX\text{-}M\text{-}14}$ region flanked by IS*10* and IS*903* in pSDB22 (Fig. 1). In addition, the aforementioned two regions were linked by a hypothetical protein ΔSCH-142, which was truncated by IS*26* and IS*10*. However, $bla_{CTX\text{-}M\text{-}14}$ was flanked by IS*Ecp1* and IS*903* in pSDB58, with *fip* and Δ*traI* located upstream (Fig. 1). Digestion of the amplicons indicated that 89.5% (17/19) of the *oqxAB*–$bla_{CTX\text{-}M\text{-}14}$-positive plasmids presented the same genetic environment of *oqxAB* as pSDB22, whilst the others (B58 and B21) showed a genetic environment identical to that of pSDB58 (Supplementary Fig. S2).

Supplementary Figs. S1 and S2 related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ijantimicag.2014.05.014.

4. Discussion

Plasmid-mediated genes, such as *oqxAB* and $bla_{CTX\text{-}M}$, may facilitate the spread and increase the prevalence of quinolone-resistant

Table 1
Characteristics of 28 non-Typhi *Salmonella* isolates carrying both *oqxAB* and extended-spectrum β-lactamase (ESBL) genes.

Strain	Serotype	PMQR determinant	ESBL gene	Resistance profile	MIC (mg/L)					*oqxAB*–ESBL-positive plasmid		
					OLA	CIP	CTX	CIF	CAZ	Size (kb)	Replicon/pDLST	RFLP pattern[a]
B51	Enteritidis	*oqxAB*, *aac(6′)-Ib-cr*	$bla_{CTX-M-14}$	FFC, CIF, ENR, AMP, OLA, CHL, LVX, NAL, GEN, AMK, CTX, CIP, TET, SXT	128	256	128	256	4	145.5	HI2/ST2	B3
D20	Indiana	*oqxAB*, *aac(6′)-Ib-cr*	$bla_{CTX-M-14}$	FFC, CIF, ENR, AMP, OLA, CHL, LVX, NAL, GEN, CTX, CIP, TET, SXT	128	64	256	128	8	145.5	HI2/ST1	A2
B37	Indiana	*oqxAB*, *aac(6′)-Ib-cr*	$bla_{CTX-M-14}$	FFC, CIF, ENR, AMP, OLA, CHL, LVX, NAL, GEN, CTX, CIP, TET, SXT	128	32	256	64	2	388	HI2/ST2	G
D25	Indiana	*oqxAB*, *aac(6′)-Ib-cr*	$bla_{CTX-M-65}$	CIF, AMP, CHL, NAL, SXT	64	1	0.25	32	4	N/D	HI2, A/C, N	N/D
A44	Enteritidis	*oqxAB*	$bla_{CTX-M-14}$	FFC, CIF, ENR, AMP, OLA, CHL, LVX, NAL, CIP, SXT	128	64	16	16	1	200	HI2/ST2	A1
A32	Enteritidis	*oqxAB*	$bla_{CTX-M-14}$	FFC, CIF, ENR, AMP, OLA, CHL, NAL, GEN, CIP, TET, SXT	128	4	8	16	0.5	N/D	F, N, B/O	N/D
A100	Enteritidis	*oqxAB*	$bla_{CTX-M-14}$	FFC, CIF, ENR, AMP, OLA, CHL, LVX, NAL, CIP, SXT	128	64	16	32	1	200	HI2/ST2	A1
B14	Indiana	*oqxAB*	$bla_{CTX-M-14}$	FFC, CIF, ENR, AMP, OLA, CHL, LVX, NAL, GEN, CTX, CIP, TET, SXT	128	64	512	256	4	388	HI2/ST2	C2
B54	Indiana	*oqxAB*	$bla_{CTX-M-14}$	FFC, CIF, ENR, AMP, OLA, CHL, LVX, NAL, GEN, CTX, CIP, TET, SXT	128	64	512	512	4	N/D	F, N	N/D
B57	Indiana	*oqxAB*	$bla_{CTX-M-14}$	FFC, CIF, ENR, AMP, OLA, CHL, LVX, NAL, GEN, CTX, CIP, TET, SXT	128	32	128	256	4	N/D	HI2, N, F	N/D
B41	Indiana	*oqxAB*	$bla_{CTX-M-14}$	FFC, CIF, ENR, AMP, OLA, CHL, LVX, NAL, GEN, CTX, CIP, TET, SXT	128	64	256	128	8	N/D	HI2, N	N/D
B58	Indiana	*oqxAB*	$bla_{CTX-M-14}$	FFC, CIF, ENR, AMP, OLA, CHL, LVX, NAL, GEN, AMK, CTX, CIP, TET, SXT	128	64	256	256	4	194	HI2/ST2	F
B8	Indiana	*oqxAB*	$bla_{CTX-M-14}$	FFC, CIF, ENR, AMP, OLA, CHL, LVX, NAL, GEN, CTX, TET, SXT	128	64	256	128	4	N/D	F, P-1α, B/O	N/D
B21	Indiana	*oqxAB*	$bla_{CTX-M-14}$	FFC, CIF, ENR, AMP, OLA, CHL, LVX, NAL, GEN, CTX, CIP, TET, SXT	128	64	512	256	8	194	HI2/ST2	D1
B24	Indiana	*oqxAB*	$bla_{CTX-M-14}$	FFC, CIF, ENR, AMP, OLA, CHL, LVX, NAL, GEN, CTX, CIP, TET, SXT	128	64	>512	128	4	194	HI2/ST2	D1
B38	Indiana	*oqxAB*	$bla_{CTX-M-14}$	FFC, CIF, ENR, AMP, OLA, CHL, LVX, NAL, GEN, AMK, CIP, TET, SXT	64	64	16	64	2	194	HI2/ST2	D2
B6	Indiana	*oqxAB*	$bla_{CTX-M-14}$	FFC, CIF, ENR, AMP, OLA, CHL, LVX, NAL, CIP, TET, SXT	128	64	32	64	0.25	145.5	HI2/ST2	A1
B59	Indiana	*oqxAB*	$bla_{CTX-M-14}$	FFC, CIF, ENR, AMP, OLA, CHL, LVX, NAL, GEN, AMK, CTX, CIP, TET, CAZ, SXT	128	64	>512	256	64	N/D	HI2, N	N/D
B22	Indiana	*oqxAB*	$bla_{CTX-M-14}$	FFC, CIF, ENR, AMP, CHL, LVX, NAL, CIP, TET, SXT	64	64	16	128	4	194	HI2/ST2	E
B9	Indiana	*oqxAB*	$bla_{CTX-M-14}$	FFC, CIF, ENR, AMP, OLA, CHL, LVX, NAL, GEN, CTX, CIP, TET, CAZ, SXT	128	512	512	256	128	388	HI2/ST2	C1
A52	Indiana	*oqxAB*	$bla_{CTX-M-14}$	FFC, CIF, ENR, AMP, OLA, CHL, LVX, NAL, CIP, SXT	256	64	16	32	1	145.5	HI2/ST2	B1
B32	Indiana	*oqxAB*	$bla_{CTX-M-14}$	FFC, CIF, ENR, AMP, OLA, CHL, LVX, NAL, CTX, CIP, TET, SXT	128	64	64	64	0.25	145.5	HI2/ST2	A1
B56	Indiana	*oqxAB*	$bla_{CTX-M-14}$	FFC, CIF, ENR, AMP, OLA, CHL, LVX, NAL, GEN, CTX, CIP, TET, CAZ, SXT	128	512	>512	256	64	145.5	HI2/ST2	B2
B26	Indiana	*oqxAB*	$bla_{CTX-M-14}$	FFC, CIF, ENR, AMP, OLA, CHL, LVX, NAL, SXT	128	0.25	32	64	0.25	200	HI2/ST2	H
B10	Indiana	*oqxAB*	$bla_{CTX-M-14}$	CIF, ENR, AMP, NAL, SXT	64	0.5	16	8	0.125	145.5	HI2/ST1	A3
B20	Indiana	*oqxAB*	$bla_{CTX-M-14}$	FFC, CIF, ENR, AMP, OLA, CHL, LVX, NAL, GEN, CIP, TET, SXT	128	0.5	32	16	1	145.5	HI2/ST2	A1
B53	Indiana	*oqxAB*	$bla_{CTX-M-65}$	FFC, CIF, ENR, AMP, OLA, CHL, LVX, NAL, GEN, CTX, CIP, TET, SXT	128	32	128	128	1	N/D	F, N	N/D
A50	Indiana	*oqxAB*	$bla_{CTX-M-65}$	CIF, AMP, OLA, CHL, NAL, SXT	64	0.25	16	8	0.25	N/D	F, N	N/D

PMQR, plasmid-mediated quinolone resistance; MIC, minimum inhibitory concentration; pDLST, plasmid double-locus sequence typing; RFLP, restriction fragment length polymorphism; OLA, olaquindox; CIP, ciprofloxacin; CTX, cefotaxime; CIF, ceftiofur; CAZ, ceftazidime; FFC, florfenicol; ENR, enrofloxacin; AMP, ampicillin; CHL, chloramphenicol; LVX, levofloxacin; NAL, nalidixic acid; GEN, gentamicin; AMK, amikacin; TET, tetracycline; SXT, trimethoprim/sulfamethoxazole; N/D, not determined.

[a] RFLP patterns with no more than three band differences were assigned to the same RFLP profile.

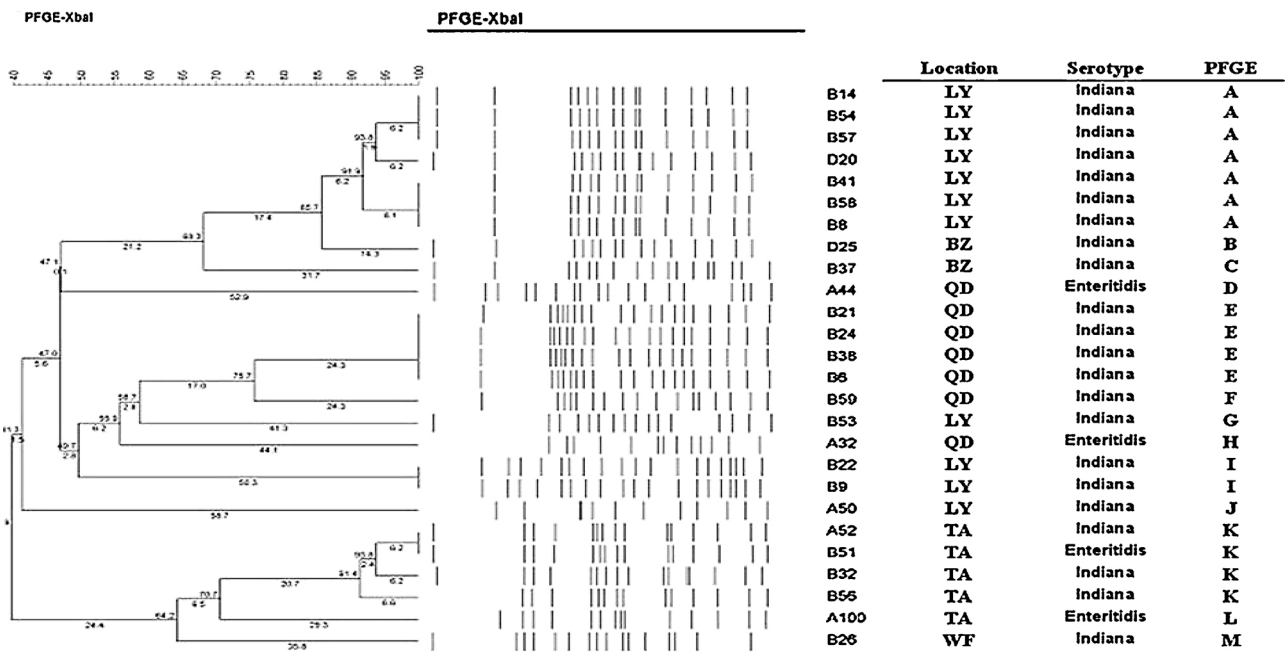

Fig. 2. Pulsed-field gel electrophoresis (PFGE) analysis, serotype and location of 26 *oqxAB*–$bla_{CTX\text{-}M\text{-}9G}$-positive non-Typhi *Salmonella enterica* isolates. LY, BZ, QD, TA and WF represent poultry farms located in Linyi, Binzhou, Qingdao, Tai'an and Weifang, respectively.

and broad-spectrum cephalosporin-resistant strains, respectively [18]. In the present study, a high prevalence of PMQR determinants [*oqxAB* (30.8%) and *aac(6′)-Ib-cr* (30.8%)] was found in the non-Typhi *Salmonella* isolates, similar to our previous work [6]. This was significantly higher than that reported for non-Typhi *Salmonella* isolates in the USA (0.3%) [19]. The ESBL gene $bla_{CTX\text{-}M\text{-}9G}$ (42.3%) was most prevalent in the non-Typhi *Salmonella* isolates, which is higher than that reported for isolates from humans and food animals in the USA [20]. Moreover, a high prevalence (21.5%) of isolates carrying both *oqxAB* and $bla_{CTX\text{-}M\text{-}9G}$ was detected. Fluoroquinolones and third-generation cephalosporins are commonly used to treat Gram-negative bacterial infections [21]. Dissemination of $bla_{CTX\text{-}M\text{-}9G}$ may facilitate the spread and increase the prevalence of cephalosporin-resistant strains. Although the PMQR determinants contribute to small increases in the MICs of quinolones, they are sufficient to facilitate the selection of mutants (e.g. *gyrA*) with higher levels of resistance [1]. Therefore, we should devote enough attention to the high prevalence of isolates carrying both *oqxAB* and $bla_{CTX\text{-}M\text{-}9G}$.

Salmonella Indiana *oqxAB*–$bla_{CTX\text{-}M\text{-}9G}$-positive strains isolated from the same location (Linyi, Qingdao and Tai'an) belonged to identical PFGE clusters (A, E and K), indicating that clonal spread especially of *Salmonella* Indiana strains was responsible for the dissemination of *oqxAB*–$bla_{CTX\text{-}M\text{-}9G}$-positive strains in local areas. This result supported the assumption that trade of animals may be an important source of colonisation [22]. In addition, the sporadic *oqxAB*–$bla_{CTX\text{-}M\text{-}9G}$-positive *Salmonella* Enteritidis should be of concern. However, *Salmonella* strains that showed diverse PFGE patterns presented a similar multidrug resistance phenotype. This is probably due to the spread of resistance plasmids.

Characterisation by pDLST, S1-PFGE and Southern blot hybridisation showed that *oqxAB* co-resided with $bla_{CTX\text{-}M\text{-}14}$ on transferable ST2-IncHI2 plasmids in most of the *oqxAB*–$bla_{CTX\text{-}M\text{-}14}$-positive strains. Although RFLP analysis presented a different backbone between pSDB22 and pHXY0908 (data not shown), the analysis provided supporting evidence for our previous hypothesis that *oqxAB*-positive IncHI2-type plasmids are correlated with ESBLs [6]. Different from previously reported findings that PMQR and ESBL genes are frequently co-located on ST1-IncHI2 plasmids, this study found that the PMQR–ESBL genetic linkage was mostly associated with ST2-IncHI2 plasmids, which are also known for epidemic spread in *Salmonella* strains from human and animals in Europe and the USA.

The genetic environment of *oqxAB*–$bla_{CTX\text{-}M\text{-}14}$ in pSDB22 consisted of a backbone that is similar to the *S.* Typhimurium plasmid pHXY0908 and a plasmid from *E. coli* DDC10-15 [6,23]. This organisation was common (17/28) in *oqxAB*–$bla_{CTX\text{-}M\text{-}14}$-positive strains found by PCR-RFLP analysis, indicating that the spread of *oqxAB* in combination with $bla_{CTX\text{-}M\text{-}14}$ will become increasingly prevalent in China [24]. It is noteworthy that both *oqxAB* and $bla_{CTX\text{-}M\text{-}14}$ genes were flanked by insertion sequences, which are widely spread among plasmids and contribute to the dissemination of resistance genes.

Interestingly, some unique features associated with the *oqxAB*–$bla_{CTX\text{-}M\text{-}14}$ genetic environment are identified in this study. (i) The IS*Ecp1* element was frequently found to be associated with several $bla_{CTX\text{-}M}$ genes and could mobilise such genes, and this organisation was also detected in pSDB58 [25]. However, this insertion sequence was disrupted by another insertion sequence, IS*10*, located upstream of $bla_{CTX\text{-}M\text{-}14}$, and IS*10* may play a role in the mobilisation of this ESBL gene [26]. (ii) The hypothetical protein SCH-142 truncated by IS*26* and IS*10* was first identified in plasmid pSC138, which was isolated from a highly invasive and multiresistant non-Typhi *Salmonella* isolate [27]. The residue of IS*Ecp1* located upstream of IS*10* has been replaced by ΔSCH-142. Meanwhile, the MICs of third-generation cephalosporins for strain B58 were higher than in B22. The potential effect of ΔSCH-142 on expression of $bla_{CTX\text{-}M\text{-}14}$ needs further research.

In summary, we report the high prevalence of $bla_{CTX\text{-}M\text{-}9G}$ among *oqxAB*-positive non-Typhi *Salmonella* from chickens in China. The spread of $bla_{CTX\text{-}M\text{-}9G}$ and *oqxAB* in these isolates was mediated by clonal expansion in local areas and horizontal transmission of ST2-IncHI2 plasmids containing the *oqxAB*–$bla_{CTX\text{-}M\text{-}14}$ genes. To our knowledge, this is the first report of the prevalence of ST2-IncHI2 plasmids concomitantly carrying *oqxAB* and $bla_{CTX\text{-}M\text{-}14}$ in non-Typhi *Salmonella*, and also the first description of the genetic environment of *oqxAB*–$bla_{CTX\text{-}M}$. The presence of these genes in non-Typhi *Salmonella* strains isolated from food-producing animals

poses a potential threat to public health, and continued surveillance of the dissemination of these genes in *Salmonella* is urgently needed.

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