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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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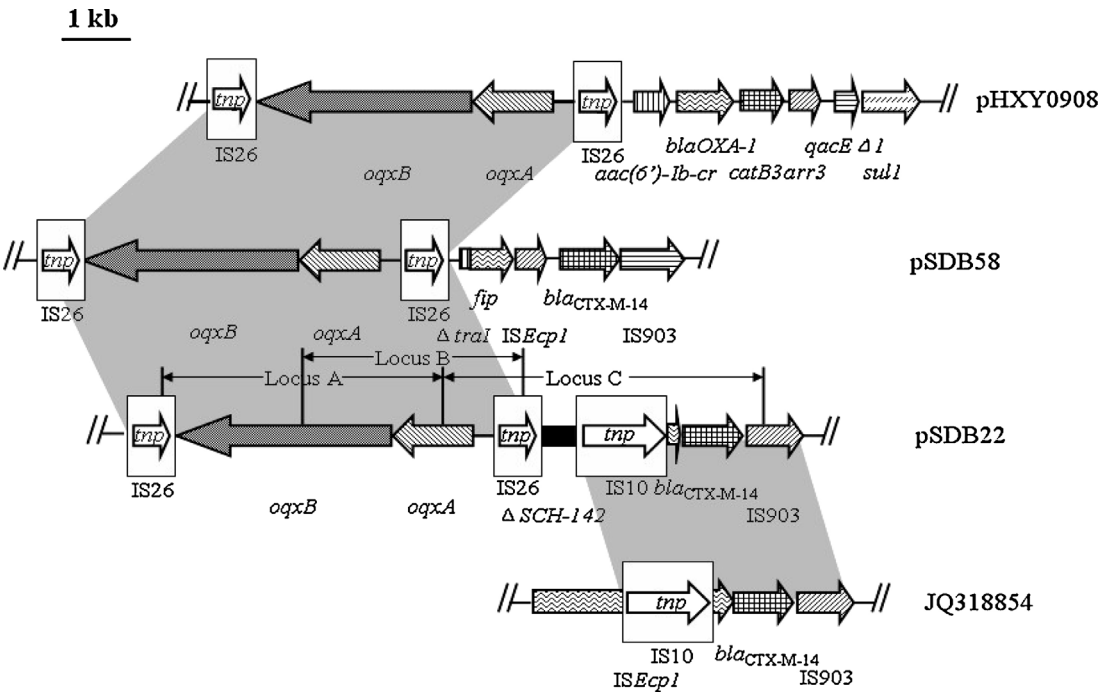

Fig. 1. Flanking regions of *oqxAB*–$bla_{CTX\text{-}M\text{-}14}$ genes in plasmids pSDB22 and pSDB58 of *Salmonella enterica* serotype Indiana strains B22 and B58, respectively, and structural comparison with plasmids pHXY0908 from *S.* Typhimurium and JQ318854 from *Escherichia coli*. Arrows indicate 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