Increasing prevalence of ciprofloxacin-resistant food-borne *Salmonella* strains harboring multiple PMQR elements but not target gene mutations

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Fluoroquinolone resistance in *Salmonella* has become increasingly prevalent in recent years. To probe the molecular basis of this phenomenon, the genetic and phenotypic features of fluoroquinolone resistant *Salmonella* strains isolated from food samples were characterized. Among the 82 *Salmonella* strains tested, resistance rate of the three front line antibiotics of ceftriaxone, ciprofloxacin and azithromycin was 10%, 39% and 25% respectively, which is significantly higher than that reported in other countries. Ciprofloxacin resistant strains typically exhibited cross-resistance to multiple antibiotics including ceftriaxone, primarily due to the presence of multiple PMQR genes and the *bla*<sub>CTX-M-65</sub>, *bla*<sub>CTX-M-55</sub>, *bla*<sub>CMY-2</sub> and *bla*<sub>CMY-72</sub> elements. The prevalence rate of the *oqxAB* and *aac(6')-Ib-cr* genes were 91% and 75% respectively, followed by *qnrS* (66%), *qnrB* (16%) and *qnrD* (3%). The most common PMQR combination observable was *aac(6')-Ib-cr-oqxAB-qnrS₂*, which accounted for 50% of the ciprofloxacin resistant strains. Interestingly, such isolates contained either no target mutations or only a single *gyrA* mutation. Conjugation and hybridization experiments suggested that most PMQR genes were located either in the chromosome or a non-transferrable plasmid. To summarize, findings in this work suggested that PMQRs greatly facilitate development of fluoroquinolone resistance in *Salmonella* by abolishing the requirement of target gene mutations.

Foodborne salmonellosis is one of the leading causes of foodborne illnesses worldwide. Although antimicrobial treatment is usually not necessary due to the self-limiting nature of salmonellosis, it can be lifesaving in cases of invasive infections¹, with ceftriaxone and ciprofloxacin being the key drugs of choice². Resistance to ceftriaxone or other extended spectrum beta-lactams is usually due to intracellular production of extended spectrum beta-lactamases (ESBLs) such as the CTX-M group and AmpC beta-lactamase, including the CMY-2 enzyme, which are usually located on transmissible plasmids that tend to disseminate among members of Enterobacteriaceae³⁴. Prevalence of resistance to ceftriaxone in *Salmonella* appears to be slowly increasing, reaching a rate of around 3 ~ 4% at present⁵. However, the rate of resistance to ciprofloxacin has increased dramatically both in clinical and food isolates around the world, in particular China and the adjacent areas⁶. Ciprofloxacin resistance is mainly attributed to double mutations in the *gyrA* gene and single mutation in the *parC* gene in *Salmonella*⁷. Efflux pumps and the presence of plasmid-mediated quinolone resistance (PMQR) determinants have also been regarded as contributive factors of development of low level resistance to nalidixic acid. At least

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### Table 1. Prevalence of antimicrobial resistance in different Salmonella serotypes.

| Antimicrobials          | Overall (n = 82) | Chicken isolates (n = 29) | Pork isolates (n = 53) | S. Derby (n = 16) | S. Typhimurium (n = 8) | S. Heidelberg (n = 8) | S. Rosenthal (n = 4) | S. Indiana (n = 4) | S. Enteritidis (n = 4) |
|-------------------------|-----------------|--------------------------|-----------------------|------------------|------------------------|-----------------------|----------------------|---------------------|------------------------|
| Ampicillin              | 68              | 62                      | 72                    | 69               | 100                    | 100                   | 63                   | 100                 | 25                     |
| Cefotaxime              | 10              | 21                      | 4                     | 3                | 0                      | 25                    | 0                    | 100                 | 25                     |
| Ceftriaxone             | 10              | 21                      | 4                     | 3                | 0                      | 25                    | 0                    | 100                 | 25                     |
| Chloramphenicol         | 74              | 69                      | 75                    | 83               | 79                     | 100                   | 50                   | 75                  | 50                     |
| Gentamicin              | 40              | 19                      | 47                    | 52               | 43                     | 25                    | 0                    | 100                 | 0                      |
| Kanamycin               | 48              | 27                      | 55                    | 69               | 64                     | 25                    | 25                   | 100                 | 0                      |
| Streptomycin            | 50              | 42                      | 53                    | 59               | 57                     | 63                    | 25                   | 100                 | 0                      |
| Nalidixic acid          | 63              | 46                      | 68                    | 72               | 57                     | 50                    | 38                   | 100                 | 25                     |
| Ciprofloxacin           | 39              | 17                      | 51                    | 50               | 57                     | 0                     | 0                    | 75                  | 0                      |
| Sulfamethoxazole        | 100             | 100                     | 100                   | 100              | 100                    | 100                   | 100                  | 100                 | 100                    |
| Tetracycline            | 65              | 42                      | 75                    | 76               | 79                     | 63                    | 63                   | 100                 | 0                      |
| Amikacin                | 4               | 4                       | 4                     | 0                | 0                      | 0                     | 75                   | 0                   | 0                      |
| Azithromycin            | 25              | 23                      | 31                    | 38               | 6                      | 13                    | 25                   | 75                  | 0                      |
| Olaquindox              | 51              | 35                      | 58                    | 90               | 71                     | 50                    | 25                   | 75                  | 25                     |

Overall, these strains exhibited a very high rate of resistance to most antibiotics, with the exception of amikacin, chloramphenicol, and tetracycline. The high prevalence of resistance to ciprofloxacin and other fluoroquinolones highlights the need for continuous surveillance of PMQR genes in Salmonella isolates. This is crucial for monitoring the emergence of resistance mechanisms, particularly in food samples.
of the antibiotics tested. The resistance rate of the three most important front line antibiotics (ceftriaxone, ciprofloxacin and azithromycin) were respectively 10%, 39% and 25%, which is significantly higher than that reported in other countries. *Salmonella* strains isolated from pork samples exhibited a higher rate of resistance to most of the antibiotics tested when compared to *Salmonella* chicken isolates, in particular ciprofloxacin (Table 1). Surprisingly, chicken *Salmonella* isolates exhibited a much higher rate of resistance to ceftriaxone (35%) than that of the pork isolates (11%). This phenomenon is probably due to the high rate of resistance to ceftriaxone in *S. Indiana*. Among the different serotypes tested, *S. Indiana* also exhibited the highest rate of resistance to most antibiotics, including the three front line drugs of ceftriaxone, ciprofloxacin and azithromycin. Such phenotype has only been reported in *S. Typhimurium* and *S. Derby* previously. *S. Typhimurium* and *S. Derby* also exhibited a very high resistance rate except that *S. Typhimurium* did not exhibit resistance to ceftriaxone. Two serotypes, namely *S. Heidelberg* and *S. Rosenthal*, exhibited an intermediate rate of resistance to the test antibiotics. On the other hand, resistance was less commonly observed among *S. Enteritidis*. Another important observation is that, among 32 ciprofloxacin-resistant strains, all were resistant to ampicillin, nalidixic acid, kanamycin, streptomycin, chloramphenicol, tetracycline and sulfamethoxazole; furthermore, the MIC of olaquindox was generally 32 mg/L or higher for these strains. We also observed that, among such isolates, up to 84% were resistant to gentamicin, 25% were resistant to azithromycin (MIC $\geq$ 32 mg/L), and 13% were also resistant to ceftriaxone (Table 2).

**Diverse mechanisms of ceftriaxone resistance in *Salmonella* food isolates.** Mechanisms of resistance in selected strains, in particular those mediating resistance to the front line antibiotics such as ceftriaxone and ciprofloxacin, were investigated. Resistance to ceftriaxone was detectable in 8 *Salmonella* isolates including *S. Indiana*, 2 *S. Heidelberg*, 1 *S. Enteritidis* and 1 *S. Derby*. These 8 strains were examined for their ability to produce Extended-spectrum β-lactamases and AmpC β-lactamases, with results showing that diverse resistance mechanisms were observable. Three out of the four *S. Indiana* strains were found to contain bla*CMY-2*, with the fourth one harboring the bla*CMY-72* gene. The bla*CMY-2* gene was detectable in one *S. Enteritidis* and one *S. Derby* strain. For the two *S. Heidelberg* isolates, the bla*CMY-2* and bla*CMY-72* genes were each detectable in one strain (Table 2).

**Novel mechanisms of fluoroquinolone resistance in *Salmonella* food isolates.** A total of 32 ciprofloxacin-resistant *Salmonella* strains were subjected to investigation of the mechanisms involved. Contrary to the resistance mechanisms commonly observable in clinical ciprofloxacin resistant strains, in which double and single mutations often occur in the *gyrA* and *parC* genes respectively, most of the 32 ciprofloxacin resistant *Salmonella* strains tested in this work were found to contain either only a single mutation in *gyrA*, with S83T, S83F, and D87N being the most common amino acid changes, or no mutation in both target genes (Table 2). The few exceptions were all *S. Indiana* isolates which harbored the double *gyrA* mutations S83F and D87N, with or without the single *parC* mutation S80R. It should also be noted that a pair of novel double *gyrA* mutations which resulted in the H80N and S83T changes, and single *parC* mutation causing the Q91H substitution, were detectable in a *S. Rissen* isolate; however, the roles of such mutations in development of *Salmonella* fluoroquinolone resistance are not well defined at present. Other less common mutations that were detectable include the C72G change in the *gyrA* protein of a *S. Derby* isolate. The nature of contribution of these novel mutations to the development of ciprofloxacin resistance in *Salmonella* needs further investigation. No mutations were detected in *gyrB* and *parE*.

The presence of PMQR genes in ciprofloxacin resistant *Salmonella* isolates were also screened by PCR and sequencing (Table 2). Surprisingly, all isolates were found to carry PMQRs, with *oqxAB* and *aac(6’)-Ib-cr*, the most prevalent elements, reaching a rate of 91% and 75% respectively. Other PMQR genes detectable included *qnrS* (66%), *qnrB* (16%) and *qnrD* (3%). The most common PMQR combination observable was *aac(6’)-Ib-cr-oqxAB-qnrS2*, which accounted for 50% of all the ciprofloxacin resistant *Salmonella* strains tested. To determine if other resistance mechanisms such as efflux activities contribute to ciprofloxacin resistance in such isolates, the MIC of ciprofloxacin against these isolates was determined in presence and absence of the efflux pump inhibitor, Phenylalanine-arginine-β-naphthylamide (PAβN). The results showed that PAβN caused a mild reduction in the MIC level, suggesting that drug efflux only played a partial role in ciprofloxacin resistance development in these organisms (Table 2). Detailed analysis of the relative roles of PMQRs and target gene mutations in conferring ciprofloxacin resistance phenotypes suggested that several PMQRs including *aac(6’)-Ib-cr*, *oqxAB* and *qnrS*, alone or in combination, could mediate ciprofloxacin resistance development in *Salmonella* isolates which did not contain target gene mutations. In particular, the presence of four different PMQRs, such as the *aac(6’)-Ib-cr-oqxAB-qnrS2-qnrB8* and *aac-oqxAB-qnrS2-qnrD* combinations, was consistently observable in ciprofloxacin-resistant *Salmonella* isolates without any target mutations, suggesting that effects of such elements in conferring antibiotic resistance in *Salmonella* are additive or synergistic in nature (Table 2). It should also be noted that PMQR-mediated ciprofloxacin resistance is commonly associated with a MIC level of 4 to 8 μg/ml, which is comparable to those conferred by target mutations.

Conjugation experiments were performed on these 32 ciprofloxacin-resistant *Salmonella* isolates to confirm if PMQRs were readily transferable to other *Enterobacteriaceae* species, using the *E. coli* J53 strain as recipient. Surprisingly, none of the ciprofloxacin resistance phenotypes tested could be transferred to...
Table 2. Phenotypic and genotypic characteristics of Salmonella strains isolated from retail meat products. All isolates were resistant to the antibiotic profile of Amp-Cip-Nal-Kan-Str-Chl-Tet-Ola (olaquindox); specific strains were also resistant to Gen, gentamicin; Azi, azithromycin; and Cro, ceftriaxone. Pa(3)N, Phenylalanine-arginine β-naphthylamide. C, Chicken; P, Pork; aac, aac(6′)-Ib-cr, *selected for S1-PFGE and Southern hybridization.

| Strain # | Isolation date | Sources | Serotypes | PFGE | Resistance Profiles* | CIP | CIP/PABN | PMQRs | Mutations in gyrA | Mutations in parC |
|----------|----------------|---------|-----------|------|----------------------|-----|----------|-------|-----------------|-----------------|
| S34      | 12/12/12       | P        | Derby     | DER1 | Gen                  | 16  | 2        | aac-oqxAB-qnrS2 | —               | —               |
| S24      | 01/12/13       | P        | Derby     | DER3 | Gen                  | 4   | 2        | oqxAB           | S83I            | —               |
| S35      | 01/26/13       | P        | Derby     | DER5 | Gen                  | 4   | 4        | aac-oqxAB-qnrS2 | S83T            | —               |
| S36      | 01/26/13       | P        | Derby     | DER5 | Gen-Azi              | 8   | 2        | aac-oqxAB-qnrS2 | —               | —               |
| S37      | 01/26/13       | P        | Derby     | DER5 | Gen                  | 4   | 2        | aac-oqxAB-qnrS2 | —               | —               |
| S38*     | 01/26/13       | P        | Derby     | DER5 | Gen                  | 2   | 2        | aac-oqxAB-qnrS2 | N78H            | —               |
| S39      | 01/26/13       | P        | Derby     | DER5 | Gen                  | 8   | 4        | aac-oqxAB-qnrS2 | —               | —               |
| S40      | 01/26/13       | P        | Derby     | DER5 | Gen-Azi              | 8   | 4        | aac-oqxAB-qnrS2 | —               | —               |
| S41      | 01/26/13       | P        | Derby     | DER5 | Gen                  | 4   | 4        | aac-oqxAB-qnrS2 | —               | —               |
| S42      | 01/26/13       | P        | Derby     | DER5 | Gen                  | 4   | 2        | aac-oqxAB-qnrS2 | —               | —               |
| S43      | 01/26/13       | P        | Derby     | DER5 | Gen                  | 4   | 4        | aac-oqxAB-qnrS2 | —               | —               |
| S44      | 01/26/13       | P        | Derby     | DER5 | Gen                  | 4   | 4        | aac-oqxAB-qnrS2 | —               | —               |
| S45      | 01/26/13       | P        | Derby     | DER6 | Gen                  | 2   | 2        | aac-oqxAB-qnrS2 | —               | —               |
| S46      | 01/26/13       | P        | Derby     | DER6 | Gen                  | 4   | 0.5      | aac-oqxAB-qnrS2 | S83T            | —               |
| S47      | 01/26/13       | C        | Derby     | DER8 | Gen                  | 2   | 0.12     | oqxAB-qnrS8-qnrB | S83T            | —               |
| S48      | 03/13/13       | C        | Derby     | DER1 | Gen                  | 32  | >8       | oqxAB-qnrS1-qnrB | S83T            | —               |
| S49      | 03/13/13       | P        | Derby     | DER10| Gen-Azi              | 32  | >8       | oqxAB-qnrS1-qnrB | S83T            | —               |
| S50      | 03/13/13       | C        | Derby     | DER11| Gen                  | 2   | 0.5      | qnrS1           | S83T            | —               |
| S70      | 12/12/12       | P        | Typhimurium | TR1  | Gen                  | 4   | 0.25     | aac-oqxAB       | D87N            | —               |
| S8       | 12/12/12       | P        | Typhimurium | TR1  | Gen                  | 4   | 0.25     | oqxAB           | D87N            | —               |
| S11      | 12/12/12       | P        | Typhimurium | TR1  | Gen                  | 2   | 0.25     | aac-oqxAB       | D87N            | —               |
| S79      | 05/17/13       | P        | Typhimurium | TR2  | Gen                  | 4   | 0.12     | oqxAB           | S83F            | —               |
| S65      | 03/21/13       | P        | Typhimurium RH2 | TRH1 | Gen                  | 2   | 0.5      | aac-oqxAB-qnrS1 | D87N            | —               |
| S66      | 03/21/13       | P        | Typhimurium RH2 | TRH2 | Gen                  | 4   | 0.5      | aac-oqxAB-qnrS1 | —               | —               |
| S71*     | 05/01/13       | P        | Typhimurium RH2 | TRH2 | Gen                  | >32 | >8       | aac-oqxAB-qnrS1 | D87N            | —               |
| S6       | 12/12/12       | P        | Typhimurium RH2 | TRH2 | Gen                  | 4   | 0.25     | aac-oqxAB-qnrS1 | —               | —               |
| S20      | 01/12/13       | C        | Typhimurium RH2 | TRH3 | Gen                  | 2   | 0.5      | aac-qnrB        | D87N            | —               |
| S13      | 12/25/12       | P        | Indiana   | I1   | Gen-Azi-Cro          | >32 | >8       | aac-oqxAB       | S83F,D87N       | —               |
| S14      | 12/25/12       | C        | Indiana   | I1   | Gen-Azi-Cro          | >32 | >8       | aac-oqxAB-qnrB  | S83F,D87N       | S80 R           |
| S16      | 12/25/12       | P        | Indiana   | I1   | Gen-Azi-Cro          | >32 | >8       | aac-oqxAB       | S83F,D87N       | C72G,S80 R       |
| S27      | 01/19/13       | P        | Riesen    | R1   | Gen                  | 8   | 0.12     | oqxAB           | H80N,S83T       | Q94H            |
| S59      | 03/16/13       | C        | London    | L1   | Gen                  | 2   | 0.12     |                 | D87N            | —               |
| S2       | 12/12/12       | P        | Sanierberg | S1   | Gen                  | 4   | 4        | aac-oqxAB-qnrS2 | —               | —               |
| S45      | 02/22/13       | P        | Virchow   | V2   | Gen                  | 4   | 4        | aac-oqxAB-qnrS8-qnrD | — | — |

E. coli, suggesting these PMQR genes may be present on a non-conjugative plasmid or the chromosomal DNA of Salmonella. To test these possibilities, five representative Salmonella strains including S. Derby strains S3, S4 and S38, and S. Typhimurium strains S7 and S71, all exhibiting different PFGE types and harboring different PMQRs, were selected for S1-PFGE and southern hybridization analysis (Fig. 1). Among these isolates, the aac(6′)-Ib-cr gene was shown to be located in both chromosomal DNA and a ~200 kb size plasmid of the S. Derby strain S38, in the chromosome of the S. Derby strain S3 and S. Typhimurium strain S71, and in a ~200 kb plasmid in the S. Derby strain S4 and S. Typhimurium strain S7 (Fig. 1). The oqxAB element was shown to be located in the chromosome of S. Typhimurium strain S71. However, the

Mutations in

 gyrA

parC

aac(6′)-Ib-cr, *selected for S1-PFGE and Southern hybridization.

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hybridization experiment was not successful even though the presence of \( \text{qnrB} \) in this strain has been confirmed by both PCR and sequencing.

*Salmonella* isolates that exhibited resistance to both ceftriaxone and ciprofloxacin included three *S. Indiana* strains, S13, S14 and S16. Again, conjugation experiments failed to transfer either the ciprofloxacin or ceftriaxone resistance phenotype to *E. coli J53*. Southern hybridization was performed on strains S13 and S14 to determine the genetic location of the \( \text{bla}_{\text{CTX-M-65}} \) element and the PMQR genes. Our data demonstrated that the \( \text{bla}_{\text{CTX-M-65}}, \text{oqxAB} \) and \( \text{aac(6′)-Ib-cr} \) elements were all located on the same ~200 kb plasmid (Fig. 2). However, hybridization experiment performed to confirm the genetic location of the \( \text{qnrB} \) gene in strain S14 was not successful even though it was proven to be present in the isolate by PCR.

**Discussion**

PMQRs play an important role in the development of fluoroquinolone resistance in *Enterobacteriaceae*\(^{18,19}\). These elements have mainly been reported in *E. coli* strains isolated from various sources and their prevalence has been shown to increase dramatically in recent years. In contrast, PMRQs have only been recoverable from *Salmonella* since 2005; nevertheless, their prevalence remains extremely low in *Salmonella* until the emergence of a new PMQR determinant, namely \( \text{oqxAB} \), which encoded an efflux pump mediating resistance to olaquindox, chloramphenicol, nalidixic acid and elevated MICs of other antimicrobial reagents including ampicillin and gentamicin\(^{14}\). The \( \text{oqxAB} \) operon was first found to be present in an IncX1 type plasmid designated as pOLA52, which was recoverable from swine *Escherichia coli* isolates\(^{15,22}\). More recently, \( \text{oqxAB} \) was reported to be prevalent in organisms isolated from pork as well as pig farms in China\(^{23-25}\). In fact, various lines of evidence suggest that this mobile resistance element already existed in poultry *E. coli* isolates as early as 1994\(^{23}\). On the other hand, \( \text{oqxAB} \) has not been found in clinical isolates until recently, when it became detectable in clinical strains of *E. coli* and *Klebsiella pneumoniae*\(^{26-29}\).
In *Salmonella*, oqxAB was first found to be present in the chromosomal DNA of two S. Derby strains of food origin in 2013\(^\text{37}\). Retrospective study of clinical isolates of *Salmonella* in China revealed that oqxAB could be detected in *Salmonella* as early as 2006\(^\text{6}\), and that it was often genetically associated with the aac(6\(^\text{′}\))-Ic-\(br\) element, contributing to transmission of drug resistant organisms in clinical setting in both clonal and non-clonal manner. Further studies showed that oqxAB and aac(6\(^\text{′}\))-Ic-\(br\) could greatly facilitate development of fluoroquinolone resistance by abolishing the requirement of target gene mutations, thereby potentially causing a dramatic increase of fluoroquinolone resistance in *Salmonella*\(^\text{37}\). Our data confirmed that this is indeed the case, and suggested that by further acquiring other PMQRs in *Salmonella* strains which already harbored the oqxAB and aac(6\(^\text{′}\))-Ib-cr elements, fluoroquinolone resistance at a level comparable to that conferred by target mutations is consistently achievable in organisms that do not even harbor a single *gyrA* mutation. The finding that multiple PMQR elements can simultaneously or synergistically produce a fluoroquinolone resistance phenotype via the mechanisms of enzymatic inactivation, drug efflux, and competitive inhibition of drug binding is intriguing. Since oqxAB or other PMQR determinants that confer reduced susceptibility of the host organism to fluoroquinolones may also enhance the rate of mutational changes in the drug target gene (12), the phenomenon of rapid transmission of PMQR elements among members of *Enterobacteriaceae* is alarming.

Although our data showed that the PMQRs detectable in *Salmonella* were often found to be located in chromosome or plasmids that could not be transferred to other bacterial through conjugation, these elements must have been, at some stages, harbored by mobile elements that are capable of transferring its contents to the chromosome of the host strain via transposition events. This was evidenced by the observation that the oqxAB element can be recoverable in both chromosome and mobile elements containing the IS26 element\(^\text{7,18}\). With the fast progress of oqxAB-associated PMQR evolution in *Salmonella*, transmission of plasmids mediating fluoroquinolone resistance among *Salmonella*, or between *Salmonella* and other bacterial species, may become even more efficient, posing a huge threat to *Salmonella* infection control in clinical settings. Plasmids carrying the *blaCTX-M-65* gene and multiple PMQR cassettes are of particular concern, despite the fact that they are currently restricted to specific strains such as S. Indiana.

Olaquindox has been a widely used growth enhancer in the pig-raising industry since the 1970s\(^\text{30,31}\). Its antibacterial activity can be attributed to its ability to inhibit DNA synthesis. This agent was previously considered safe since they were not structurally related to any human drug. Findings in this work constitute part of the evidence that the use of olaquindox as growth promoter in the swine industry has resulted in some unexpected consequences, the impact of which only became evident decades later. First, our recent study confirmed that oqxAB actually originated as a chromosomal efflux pump gene of *Klebsiella pneumoniae*, which was picked up and incorporated into a mobile element by IS26-mediated transposition, presumably under the selection pressure of olaquindox. These events resulted in constitutive expression of the plasmid-borne oqxAB operon. Second, the process of inter-species transmission from *E. coli* to *Salmonella* occurred over a period of at least a decade, during which oqxAB was not detectable in clinical *Salmonella* strains until 2006. Third, amplification of an oqxA-oxidative S. Typhimurium strain resulted in a sharp increase in the prevalence of oqxAB-borne clinical *Salmonella* isolates in subsequent years. Finally, our data demonstrated that co-existence of the oqxAB genes with other PMQR elements has become commonplace, leading to emergence of a new category of fluoroquinolone-resistant organisms that exhibit selective advantages in both the environment and clinical settings where antibiotic selection pressure is high. At present, *Salmonella* strains harboring multiple PMQR/oqxAB elements appear to be confined to zoonotic organisms but the risk of these strains causing human infections is apparently increasing rapidly. To conclude, findings in this work highlight a need to devise specific infection control measures to halt further transmission of the oqxAB/PMQR-borne resistant *Salmonella* strains, and investigate the impact of other animal growth promoters in selection of both bacterial resistance and virulence determinants in a wide range of foodborne and zoonotic pathogens.

Materials and Methods

**Salmonella** isolation from retail meat products. *Salmonella* were isolated from retail meat samples including chicken and pork from supermarkets and wet markets in Shenzhen, China from October 2012 to June 2013\(^\text{32}\). Food samples were collected aseptically in plastic bags and transported on ice to the laboratory for isolation of *Salmonella* within 6h. Twenty-five grams of meat samples were placed in a stomacher bag with 100-ml Buffered Peptone Water (BPW) (Difco, Detroit, MI) which was subjected to homogenization for 5 min. The homogenate was incubated at 35°C for 24 h. One ml aliquot of pre-enriched homogenate was transferred to 10 mL of Tetrathionate broth (Difco) and incubated at 42°C for 24-h. A loopful of the enriched content was streaked on XLT4 agar and incubated for 24 h to 48 h at 37°C. One typical *Salmonella* strain recovered from each sample was purified and subjected to species identification by detection of the *invA* gene and 16S RNA sequencing. All isolates were serotyped according to the Kauffmann-White scheme, using commercial antiserum (Difco, Detroit).

**Antimicrobial susceptibility tests.** Confirmed S. Typhimurium isolates were subjected to antimicrobial susceptibility testing using the agar-dilution method, and the results were interpreted according to the CLSI guidelines\(^\text{33}\). Fourteen antimicrobial agents were tested: ampicillin, cefotaxime, ceftriaxone, amoxicillin/clavulanic acid, sulfamethoxazole, kanamycin, amikacin, gentamicin, tetracycline, chloramphenicol, ciprofloxacin, nalidixic acid, streptomycin, and olaquindox. *E.coli* strains ATCC 25922.
Salmonella by pulsed-field gel electrophoresis (PFGE) according to the PulseNet PFGE protocol for the
and qnrA, qnrB, qnrC, qnrD, qnrS, qepA, oqxAB, strain for identification of target gene mutations in the test strains. The presence of the PMQR genes

Molecular typing. Clonal relationship between representative Salmonella isolates was examined by pulsed-field gel electrophoresis (PFGE) according to the PulseNet PFGE protocol for Salmonella\(^5\). S1-PFGE was conducted to determine the size of large plasmids. Briefly, agarose-embedded DNA was digested with S1 nuclease (New England Bio-Lab) at 37 °C for 1 hr. The restriction fragments were separated by electrophoresis in 0.5 Tris-borate-EDTA buffer at 14 °C for 18 h using a Chef Mapper electrophoresis system (Bio-Rad, Hercules, CA) with pulse times of 2.16 to 63.8 S. Phage Lambda PFGE ladder (New England Biolab) was used as DNA size marker. The gels were stained with GelRed, and DNA bands were visualized with UV transillumination (Bio-Rad). Southern blot hybridization was carried out by following the manufacturer's instructions of the DIG-High Prime DNA Labeling and Detection Starter Kit II (Roche Diagnostics), using the different PMQR gene and bluCTX-M-64 digoxigenin-labeled probes.

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