Journal of Antimicrobial Agents
ISSN: 2472-1212

Wang et al., J Antimicrob Agents 2019, 5:2
DOI: 10.4172/2472-1212.1000185

Research Article Open Access

Molecular Characterization of CTX-M Producing *Salmonella* Isolates with Concurrent Resistance to Ciprofloxacin and Cefotaxime from Slaughtered Chicken Carcasses in Qingdao, China

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Received date: January 29, 2019; **Accepted date:** April 2, 2019; **Published date:** April 9, 2019

Abstract

Objectives: To describe the molecular characterization of CTX-M ESBL (Extended-spectrum Beta-lactamase)-producing *Salmonella* co-resistant ciprofloxacin and cefotaxime from chicken carcasses in 2014 in Qingdao, China.

Methods: A total of 62 *Salmonella* isolates were confirmed as ESBLs by a combination disc diffusion test from 355 *Salmonella* isolates studied. Antimicrobial susceptibility was determined by the broth dilution method. CTX-M subtypes, Quinolone Resistance-determining Regions (QRDRs), Plasmid-mediated Quinolone Resistance (PMQR) determinants and Multi-locus Sequence Typing (MLST) were identified using PCR (Polymerase Chain Reaction) and sequencing. Plasmids were characterized by S1-nuclease PFGE, PCR-based replicon typing and transformation. Broth mating assays were carried out for all isolates to determine whether the CTX-M or PMQR marker could be transferred by conjugation.

Results: Of the 62 ESBL-producing *Salmonella* isolates, showing resistant to a multiple antimicrobials, 59 isolates were identified as *S. indiana* (ST17) followed by two *S. enteritidis* and one *S. typhimurium*. Moreover, all 62 ESBLs were subtyped to six $bla_{\text{CTX-M}}$ types, with $bla_{\text{CTX-M-123}}$ being the most predominant, followed by $bla_{\text{CTX-M-65}}$, $bla_{\text{CTX-M-130}}$, $bla_{\text{CTX-M-14}}$, $bla_{\text{CTX-M-24}}$, and $bla_{\text{CTX-M-79}}$. Amino acid substitutions in *GyrA* (S83F and D87N) and *ParC* (T57S and S80R) and the PMQR-encoding genes *[aac(6')-Ib-cr, oqxA, oqxB, qnrB*, and *qnrS]* were detected in almost all tested isolates. All isolates contained one to three large plasmids and 8 replicon types were detected with IncHI1, IncHI2, IncA/C, IncFIIAS, and IncFrep replicon types being the predominating incompatibility groups. Twenty-six isolates demonstrated the ability to transfer their CTX-M and PMQR marker at different transfer rates.

Conclusion: The emergence of CTX-M, QRDR and PMQR were found in ciprofloxacin and cefotaxime co-resistant *Salmonella*, especially *S. indiana* among chicken carcasses in Qingdao. To the best of our knowledge, this is the first report of $bla_{\text{CTX-M-123}}$ and $bla_{\text{CTX-M-130}}$ in *Salmonella*. The high prevalence and conjugative characteristics suggest that control strategies are necessary to limit the dissemination of these isolates through food chain.

Keywords: *Salmonella*; Chicken carcasses; CTX-M; QRDRs; PMQR; Horizontal gene transfer; Plasmids replicon type

Introduction

Salmonella is a bacterial genus of importance to public health causing morbidity and mortality world widely [1,2]. In Europe, this bacterium caused 91,034 human infections and 61 people death in 2012 in 27 countries [3]. In the United States, *Salmonella* infection is reported as the second major cause of foodborne pathogen diseases [4]. In China, more than 20% of foodborne diseases being attributed to *Salmonella* [5,6]. Fluoroquinolone-based compounds and third generation cephalosporins are the front line agents of treating invasive infections or severe diarrhea in young children, the elderly and those individuals with low immunity [7].

However, the widespread and discretionary usage of antimicrobial agents in medical science and veterinarian clinical medicine have increased the emergence and spread of Multidrug Resistant (MDR) *Salmonella* species including isolates exhibiting fluoroquinolone resistance and ESBL producing phenotypes in human as well as food animals. This development complicates the subsequent treatment of human salmonellosis [8,9]. Previous studies reported on the prevalence of fluoroquinolone and cephalosporins co-resistant *Salmonella* cultured from animals particularly chicken in China and other countries [10-12]. Moreover, these co-resistant *Salmonella* in chicken could not only directly infect humans, but also cause indirect infections by transmitting to humans *via* the food chain [8].

Resistance to cephalosporins of *Salmonella* and other Enterobacteriacea is mostly because of acquisition of genes encoding ESBLs locating on mobile genetic elements particularly plasmids [12].

Over the last decade, the most frequently encountered ESBL types in Asia are the CTX-M group that are commonly carried on transmissible plasmids, which disseminate among *Salmonella* and other Enterobacteriacea [13]. Moreover, these CTX-M encoding plasmids may also possess resistant determinants for other antimicrobials like fluoroquinolones, which bring large challenges to clinical treatment [14]. Plasmid-mediated quinolone resistance (PMQR) can be classified into three different resistant mechanisms: *AAC(6')-Ib-cr* acetylating ciprofloxacin and norfloxacin; Qnr proteins mediating target protection and the OqxAB and QepA mediating drug efflux [15].

Although these PMQRs lead only low resistance to fluoroquinolone, their appearance (especially the *qnr* genes) can make bacteria survival once exposed to fluoroquinolone-based compounds and accelerate their subsequent development of high resistance phenotype to fluoroquinolone [16]. Additionally, chromosomal mutations in the Quinolone Resistance-determining Regions (QRDRs) of genes that encode gyrase or topoisomerase IV, are also frequently described in fluoroquinolone resistant *Salmonella* isolates [15]. It becomes especially troublesome when ESBLs and PMQR genes are present in isolates and co-transmitted through transferable plasmids.

Recently, ciprofloxacin and cefotaxime co-resistant *Salmonella* isolated from slaughtered chicken carcasses have been reported in several regions in China, posing a potential public health risk [17]. Here, the current study reports on the characterization of these concurrent resistant *Salmonella* isolates recovered from chicken carcasses in Qingdao, China.

Methods

Bacterial isolates

Sixty-two ciprofloxacin and cefotaxime co-resistant isolates were cultured from 355 *Salmonella* strains isolated from slaughtered chicken carcasses in 2014 in Qingdao, China. Laboratory protocols for isolating *Salmonella* were described previously [17]. All *Salmonella* isolates were confirmed through amplifying of the *invA* gene using PCR [18]. The serology was accomplished by the Luminex 200 system based on an xMAP® *Salmonella* Serotyping Assay Kit (Luminex 200, Austin, USA).

Those *Salmonella* expressing a co-resistance phenotype were selectively enriched for study with Brain Heart Infusion Broth (BHI) (Land Bridge, Beijing, China) supplemented with 2 mg/L cefotaxime and 1 mg/L ciprofloxacin, respectively. All recovered *Salmonella* isolates were further screened for ESBL production by a combination disc diffusion test by cefotaxime and ceftazidime discs, with and without clavulanic acid (Land Bridge, Beijing, China) according to CLSI guidelines [19]. *Escherichia coli* ATCC™25922 and *Klebsiella pneumoniae* ATCC™700603 were applied as reference strain in Antimicrobial Susceptibility Tests (AST). All identified isolates were preserved in Brain Heart Infusion Broth (Land Bridge, Beijing, China) containing 40% [v/v] glycerol in -80 for subsequent study.

Antimicrobial susceptibility testing

All 62 *Salmonella* isolates was detected for their antimicrobial susceptibility using the broth dilution method by the Biofosun® Gram-negative panels (Shanghai Biofosun Biotech, China) according to the CLSI (Clinical & Laboratory Standards Institute) guidelines [19]. The following antimicrobials were assessed: ampicillin (AMP, 1–32 mg/L), ampicillin-sulbactam (SAM, 0.25/0.125-32/16 mg/L), ceftazidime (CAZ, 0.25-32 mg/L), cefotaxime (CTX, 0.25-32 mg/L), imipenem (IPM, 0.125–16 mg/L), meropenem (MEM, 0.125-16 mg/L), trimethoprim–sulfamethoxazole (SXT, 0.125/2.38-16/304 mg/L), gentamicin (GEN, 0.25–32 mg/L), tetracycline (TET, 0.25-32 mg/L), ciprofloxacin (CIP, 0.03-64 mg/L), nalidixic acid (NAL, 0.25-128 mg/L), chloramphenicol (CHL, 0.25–32 mg/L). The Minimum Inhibitor Concentration (MIC) values for imipenem and meropenem were subsequently confirmed by Etest® (bioMérieux, France).

DNA purification

Frozen isolated were incubated for 18-24 h at 37 Luria-Bertani broth. A commercial bacterial DNA extraction kit (Bacterial DNA Kit D3350, Guangzhou, China) was employed to extract pure genomic DNA from the bacterial culture. A Qubit® 3.0 fluorometer (Thermo Fisher Scientific, NH, USA) was used to detect the quality of DNA. DNA samples were diluted into a concentration of 50 mg/L with sterile deionized water for subsequent PCR assay.

PCR and DNA sequencing

Extracted Genomic DNA of the *Salmonella* isolates were used as template for the detection of ESBL bla_{CTX-M} gene cluster by PCR [20]. PCR amplification of QRDRs (*gyrA, gyrB, parC, and parE*) and PMQR determinants [*qepA, aac(6')-Ib-cr, oqxA/B*, and *qnrA/B/C/D/S*] were performed on the ciprofloxacin-resistant strains [21-23]. All PCR products were commercially sequenced (Thermo Fisher Scientific China, Shanghai, China) and subsequently analyzed by DNAstar (DNAstar Inc., Madison, WI, USA) and then, the resulted sequences were blasted with reference sequences from NCBI.

Plasmid profiling

The plasmid profiles of all 62 strains were determined by S1-nuclease (Promega, Madison, WI, USA) digestion and PFGE. Briefly, the bacterial cells were embedded in agarose plugs and digested with 8 U of S1 nuclease at 37 for 45 min. Each plasmid sample was analyzed by PFGE in a Chef-Mapper® XA System (Bio-Rad, USA) at 14, with a switch time between 1 s and 12 s, at 6 V/cm on a 120 angle in 0.5 × TBE buffer for 18 h. A unit length of linear plasmid was identified by each visible DNA band. *Salmonella* Braenderup H9812 was used to determine the approximate sizes of plasmids.

PCR-based plasmid replicon typing (PBRT)

The incompatibility groups of the studied plasmids of all *Salmonella* strains were identified by the PBRT according the protocols described previously [24].

Conjugation-based mating experiments and verification

All 62 isolates were studied for their capability to transfer the plasmids mediated CTX-M enzymes and PMQR markers by broth mating to a plasmid-free *E. coli* J53 strain [25,26]. PCR of CTX-M and PMRQs markers, AST, PBRT, and S1-PFGE were again performed for all the transconjugations to analyze the presence of CTX-M and PMQR genes, antimicrobial susceptibility and plasmid features, respectively.

MLST

MLST was performed following the protocols described at the MLST website (http://mlst.ucc.ie/mlst/dbs/Senterica/documents/primersEnterica_html).

Seven conserved housekeeping genes (*aroC, dnaN, hemD, hisD, purE, sucA*, and *thrA*) of *Salmonella* enterica were amplified and sequenced at Thermo Fisher Scientific (China) Co. Ltd (Shanghai, China). Sequences were submitted to the *Salmonella* MLST database website (http://mlst.warwick.ac.uk/mlst/dbs/Senterica) to assign the Sequence Types (STs).

Results

Antimicrobial susceptibility testing

In this study, all 62 *Salmonella* isolates (17.5%, 62/355) were confirmed to be ESBL-positive and co-resistant to ampicillin, ampicillin-sulbactam, cefotaxime, ciprofloxacin, nalidixic acid, gentamicin, and chloramphenicol. Moreover, resistance to trimethoprim/sulfamethoxazole (61/62, 98.4%) was common, followed by ceftazidime (48/62, 77.4%), tetracycline (18/62, 29.0%). Notably, one isolate (1/62, 1.6%) was found to be resistant to carbapenems (imipenem and meropenem).

All *Salmonella* isolates expressed a MDR phenotype. The 62 *Salmonella* isolates were subtyped into three serotypes including 59 (95.2%, 59/62) *S. indiana*, 2 (3.2%, 2/62) *S. enteritidis* and 1 (1.6%, 1/62) *S. typhimurium* isolates. The antimicrobial resistance profiles of the collected *Salmonella* isolates were showed in Table 1, Table S1 and Figure 1.

Antibiotic class	Antimicrobial agent	Total, n (rate)	*S. indiana*, n (rate)	*S. enteritidis*, n	*S. typhimurium*, n
Penicillin	Ampicillin	62 (100)	59 (100)	2	1
Cephalosporin	Cefotaxime	62 (100)	59 (100)	2	1
	Ceftazidime	48 (77.4)	46 (78.0)	1	1
Fluoroquinolone	Ciprofloxacin	62 (100)	59 (100)	2	1
	Nalidixic Acid	62 (100)	59 (100)	2	1
β-lactam	Ampicillin-Sulbactam	62 (100)	59 (100)	2	1
Aminoglicosides	Gentamicin	62 (100)	59 (100)	2	1
Phenicol	Chloramphenicol	62 (100)	59 (100)	2	1
Folate pathway inhibitors	Trimethoprim/Sulfamethoxazole	61 (98.4)	59 (100)	1	1
Tetracycline	Tetracycline	18 (29.0)	15 (25.4)	2	1
Carbapenems	Imipenem	1 (1.6)	1 (1.7)	0	0
	Meropenem	1 (1.6)	1 (1.7)	0	0

Table 1: Antimicrobial resistance of 62 *Salmonella* isolates within serogroups.

Detection of bla_{CTX-M}, QRDRs, and PMQR-encoding genes

As shown in Figure 1, bla_{CTX-M} genes were found in all 62 ESBL-producing *Salmonella* isolates by PCR. Sequence analysis revealed six bla_{CTX-M} subtypes including $bla_{CTX-M-123}$ (n=43), $bla_{CTX-M-65}$ (n=38), $bla_{CTX-M-130}$ (n=10), $bla_{CTX-M-14}$ (n=7), $bla_{CTX-M-24}$ (n=5), and $bla_{CTX-M-79}$ (n=1).

Moreover, four QRDRs point mutations, two in *gyrA* (giving rise to S83F and D87N amino acid substitutions) and two in *parC* (giving rise to T57S and S80R amino acid substitutions), respectively, were found in almost all 62 *Salmonella* isolates with two exceptions wherein the latter *ParC* mutations (T57S and S80R) were absent in isolates CH60, while *GyrA* mutations (S83F) and *ParC* mutations (T57S and S80R) were absent in isolates CH62.

Additionally, all 62 *Salmonella* isolates were detected for the presence and frequency distribution of each PMQR-encoding gene. No *qepA, qnrA, qnrC*, and *qnrD* genes were detected in all the isolates. While the *aac(6')-Ib-cr, oqxA, oqxB, qnrB*, and *qnrS* were found in almost all tested isolates except that the qnrB gene was not identified in two isolates (denominated as isolate CH36 and CH50, respectively).

Plasmids profiles and PCR-based plasmid replicon types

All 62 *Salmonella* isolates contained detectable large plasmids by S1 nuclease-based plasmid analysis (Figure 1). In brief, 40 isolates (64.5%, 40/62) possessed one plasmid; 19 isolates (30.6%, 19/62) possessed two plasmids and 3 isolates (4.8%, 3/62) possessed three plasmids, respectively.

Plasmid replicon types of all 62 isolates were detected by PCR to evaluate the heterogeneity among the profiles (Figure 1). These results showed that 8 of 18 replicons were detected, while the IncX, IncL/M, IncN, IncW, IncY, IncP, IncFIC, IncT, IncK/B, and IncB/O types were not detected in this study.

IncHI1 (n=34), IncHI2 (n=23), IncA/C (n=18), IncFIIAS (n=16), and IncFrep (n=12) replicon types were the frequently present types. Moreover, 43 of 62 isolates were found to have for more than 1 replicon type.

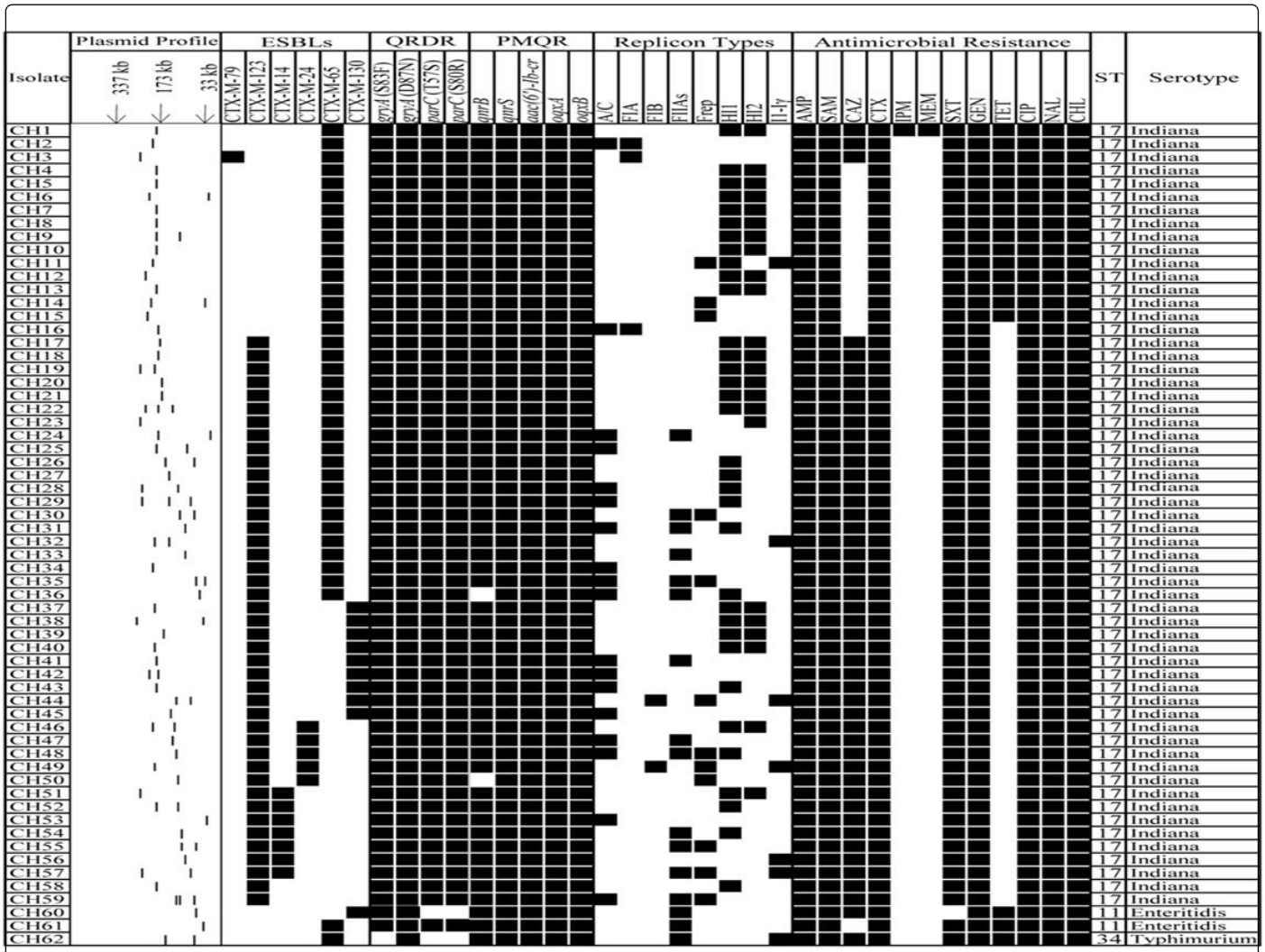

Figure 1. A heat-map summary of the schematic showing the S1 nuclease plasmid profile; ESBL profiles, QRDR mutations and PMQR genes identified by PCR and DNA sequencing, corresponding Inc plasmid type(s), antimicrobial resistance profile, MLST and serotypes for all 62 *Salmonella* isolated from chicken carcasses in Qingdao, China. Black squares shown indicate the features present in the ESBL marker, the QRDR mutations and PMQR genes, the Inc type and the corresponding antimicrobial resistance profile. White squares denote features that are lacking in the corresponding bacterial isolate. Antimicrobial compounds used are abbreviated as follows: AMP: Ampicillin; SAM: Ampicillin-sulbactam; CAZ: Ceftazidime; CTX: Cefotaxime; IPM: Imipenem; MEM: Meropenem; SXT: Trimethoprim/sulfamethoxazole; GEN: Gentamicin; TET: Tetracycline; CIP: Ciprofloxacin; NAL: Nalidixic acid; CHL: Chloramphenicol.

Conjugational transfer of plasmid encoding CTX-M and PMQRs genes carried by the *Salmonella* isolates

Of 62 *Salmonella* isolates tested for conjugation assay, 26 isolates were found that could transfer the cefotaxime and quinolone (ciprofloxacin/nalidixic acid) resistance determinants to *E. coli* J53 with the conjugation frequencies ranging from 4.4×10^{-8} to 7.5×10^{-2} (Figure 2).

Subsequently, the AST results of all transconjugants showed that resistance to antimicrobial agents including ampicillin, trimethoprim-sulfamethoxazole, gentamicin, tetracycline, and chloramphenicol in addition to cephalosporins and fluoroquinolone, were also transferred to the *E. coli* J53 strain.

The predominant $bla_{\text{CTX-M}}$-encoding ESBLs genes detected among the transconjugants were $bla_{\text{CTX-M-65}}$ (18/26) and $bla_{\text{CTX-M-123}}$ (16/26). Following the CTX-M genes, the PMQR genes were also detected among the transconjugants, showing in Figure 2.

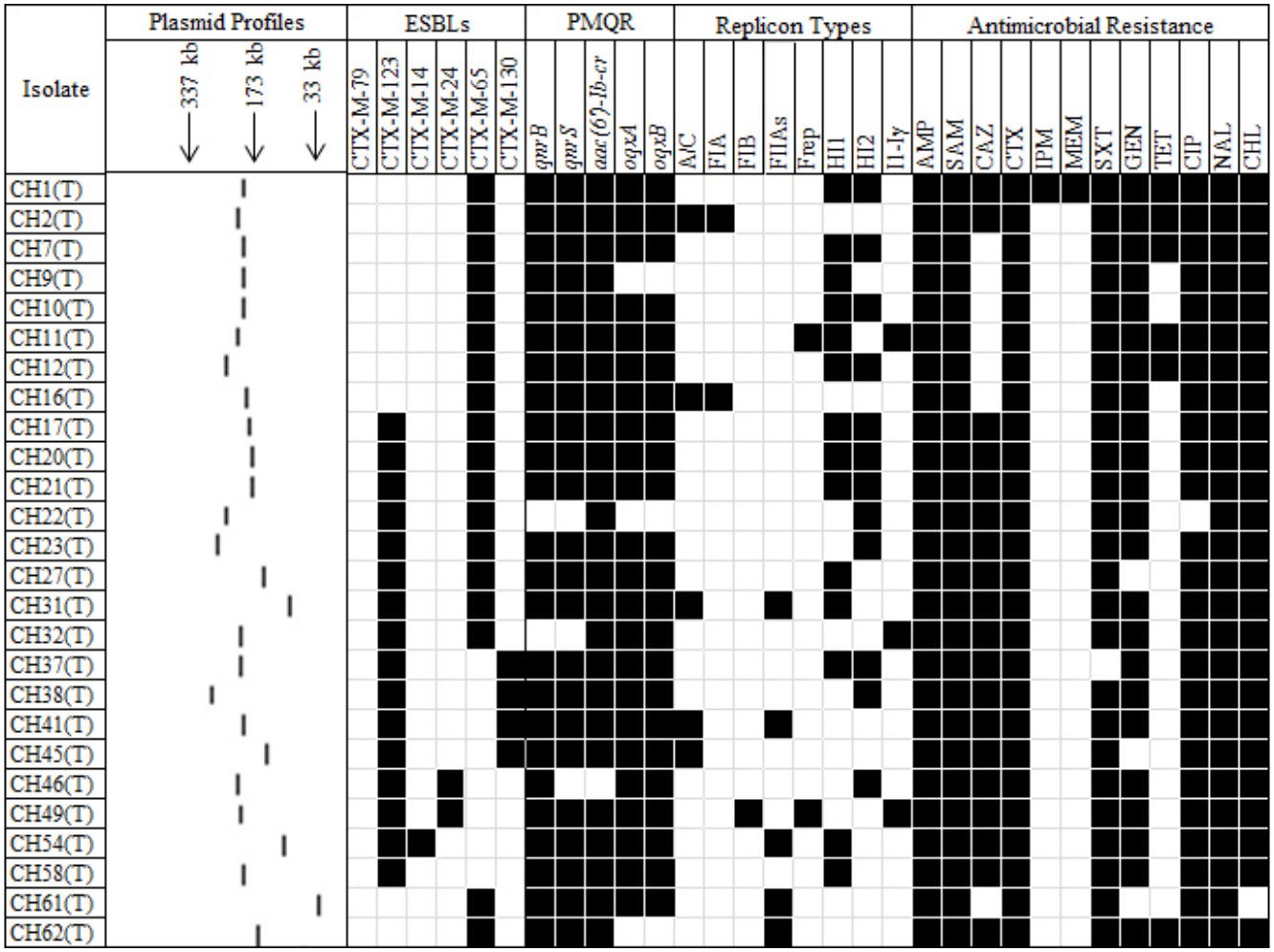

Figure 2: A heat-map showing the resultant transconjugants, characterized on the basis of their plasmid profiles; ESBL-markers and PMQR genes identified by PCR and DNA sequencing; antimicrobial resistance profile; and plasmid replicon type(s). Antimicrobial compounds are abbreviated as described in the legend to Figure 1.

MLST

All 62 *Salmonella* isolates were subtyped by MLST and seven STs were identified (Figure 1). The genotyping results showed that all 59 *S. indiana* isolates were identified as ST17; both 2 *S. enteritidis* were identified as ST11; and 1 *S. typhimurium* isolate was identified as ST34, respectively.

Discussion

The emergence of CTX-M-producing *Salmonella* has been increasingly reported among bacteria cultured from poultry throughout the world including China [10,15,16,27]. Of note, poultry especially chicken and chicken products are often involved in human salmonellosis, thereby presenting an important challenge for the clinical and veterinary communities [28-30]. Consequently, the clonal dissemination of CTX-M producing and fluoroquinolone-resistant *Salmonella via* chicken can give rise to serious infections whilst reducing the treatment options available for clinicians. Moreover, ciprofloxacin and cefotaxime co-resistant *Salmonella* isolates could be transferred to non-contaminated chicken carcasses *via* cross-contamination during the slaughtering process, thus increasing the spread of these isolates in the environment and further along the food chain [31]. Previous reports have described the nature of these co-resistant resistant *Salmonella* in farm or retail chicken [11,15,32,33]. However, there is limited reports describing the nature of CTX-M producing-*Salmonella* isolates co-resistant to fluoroquinolones from slaughtered chicken carcasses in China [10,16]. In this study, we described the antimicrobial susceptibility and associated molecular mechanisms for a collection of *Salmonella* isolates that were resistant to both ciprofloxacin and cefotaxime from chicken carcasses in a slaughterhouse in Qingdao, China.

In this study, an extensive resistant phenotype to a multi antimicrobial compounds observed and which was likely linked to the usage of these agents in the poultry production chain in China [4,15,34]. Meanwhile, a total of 17.5% (62/355) ciprofloxacin and cefotaxime co-resistant *Salmonella* isolates were found to be ESBL-positive, a figure that was similar to previous reports in China [16,17,35]. The high frequency of fluoroquinolone resistance in

Salmonella has been considered as an important feature in China and several other developing countries that do not strictly control the application of antimicrobial agents in veterinary settings and hospitals [36,37]. In contrast, more than 3% of *Salmonella* isolates were identified as intermediate or resistant to ciprofloxacin in the United States, where the usage of this class of antimicrobial is well controlled [38]. Therefore, it is necessary to enforce controls on the use of antimicrobial compounds of critical importance the poultry industry. Additionally, in this study, more than 95% of the *Salmonella* isolates were identified as serovar Indiana, which were subtyped as ST17 subsequently. This ST17 clone has been widely reported in food-producing animals and human cases in China [17,29,34]. Moreover, the contribution of chicken-originated *S. indiana* strains in the community infections has been established [16]. Interestingly, one *S. indiana* isolate in our study collection was found to have the resistant phenotype to all tested antimicrobial agents including imipenem and meropenem. The latter phenotype was identified as a New Delhi Metallo-beta-lactamase (NDM) producing isolate expressing an Extensively-drug Resistance (XDR) phenotype [39]. Therefore, the spread of MDR *S. indiana* strains becomes a potential threat to chicken health and would consequently represent an important public health hazard *via* the food chain.

CTX-M has replaced the TEM (Temoneira) and SHV (Sulfhydryl Variable) as the predominant ESBLs since they were discovered in the 1980s [40,41]. ESBL screening and characterization of isolates in the current study showed that different CTX-M subtypes were identified. Previous studies reported that the $bla_{\text{CTX-M-65}}$ and $bla_{\text{CTX-M-14}}$ were the most frequently detected genotypes among *Salmonella* in chicken in China [16,29,42]. However, in the current study, of the 62 CTX-M producing isolates, $bla_{\text{CTX-M-123}}$ (43/62) was the predominant genotype, followed by $bla_{\text{CTX-M-65}}$, and $bla_{\text{CTX-M-130}}$, suggesting that the CTX-M subtypes may have particular epidemic characteristics in different geographical regions. Moreover, both of the $bla_{\text{CTX-M-123}}$ and $bla_{\text{CTX-M-130}}$ genes were originally recovered from *E. coli* in China, but rarely detected in *Salmonella* [43-45]. As far as we know, this is the first study to identify $bla_{\text{CTX-M-123}}$ and $bla_{\text{CTX-M-130}}$ in *Salmonella*. Additionally, other CTX-M subtypes such as $bla_{\text{CTX-M-14}}$, $bla_{\text{CTX-M-24}}$ and $bla_{\text{CTX-M-79}}$ were also found in this study and these have been previously detected in *Salmonella* or *E. coli* from both food-producing animal and human in China [16,42]. *Salmonella* isolates in this study harboring $bla_{\text{CTX-M}}$ genes have the potential to disseminate these markers amongst different Gram-negative bacteria, a feature that confounds our ability to control the spread of these resistance genotypes.

The fluoroquinolone resistant determinants were also analyzed among 62 ESBLs in this study. Amino acid substitutions in *GyrA* (S83F and D87N) and *ParC* (T57S and S80R) were found in almost all tested isolates, and which were detected previously [16,26,46]. In this study, the PMQR-encoding genes were detected in all 62 isolates including *aac(6')-Ib-cr, oqxA/B, qnrB/S*. The *oqxA/B* and *aac(6')-Ib-cr* genes were commonly detected in previous studies [16,35,47]. Accordingly, *OqxAB* encoded by the oqxA and oqxB genes is the first identified mechanism of resistance to olaquindox [48,49]. Olaquindox is used in animal to promote their growth for decades in China, suggesting that a constant selective pressure may contribute the acquisition and dissemination of *oqxA/B* [29,50]. The *oqxA/B* and *aac(6')-Ib-cr* genes are often located on plasmids, which may contain other resistance markers such as ESBL-encoding genes, as well as chloramphenicol and biocides mechanisms [29,49,51]. Moreover, the *qnrB/S* genes were found in almost all isolates in this study. These two qnr genes together with *aac(6')-Ib-cr* and *oqxA/B*, co-existing in a single isolate was seldom reported in *Salmonella* from the USA, Europe or Japan [52,53]. Additionally, several studies indicated that PMQR-encoding genes could only confer only low-level prevalence of fluoroquinolone resistance [16,54]. Nonetheless, they could facilitate the acceleration of the emergence of high fluoroquinolone resistance by subsequent mutations in the topoisomerase genes [52]. Further studies showed that mutations only in *GyrA* confer low fluoroquinolone resistance while additional *GyrA* mutation(s) together with *ParC* could increase their resistance to a high level [53-56]. Interestingly, we found two isolates with mutations only in *GyrA* in this study, and which expressed a lower-level resistance to ciprofloxacin (MIC=2 mg/L) than those with multiple mutations in both *GyrA* and *ParC* (MIC>64 mg/L).

Characterization of high molecular-weight plasmids by S1-PFGE and PBRT showed that PMQR co-located with $bla_{\text{CTX-M}}$ genes on plasmids of IncHI1, IncHI2, IncA/C, IncFIIAS, IncFrep replicon types of different sizes. Furthermore, several of the PMQR and $bla_{\text{CTX-M}}$ genes (26/62) could be horizontally transferred. Accordingly, the IncHI1, IncHI2 and IncA/C plasmids have been intensively studied due to their temperature-dependent conjugation, which are also associated with ESBLs and MDR determinants emerging in animals and humans and which were reported to have originated in *Salmonella* in China and elsewhere [57-59]. Besides, IncFIIAS and IncFrep plasmids were rarely reported in *Salmonella* but commonly present in *E. coli* in China, suggesting that these plasmids might have been acquired by *Salmonella* from *E. coli* more recently [60]. Hence, these conjugative plasmids of different replicons types have the potential to drive such increased transfer of PMQR and $bla_{\text{CTX-M}}$ genes within and/or between bacterial species colonizing chicken, posing a risk of the transfer of these resistant isolates into the food chain and leading to human infections.

Conclusions

The CTX-M producing MDR *S. indiana* isolates were predominant among chicken carcasses in this study. As far as we know, this study firstly reported $bla_{\text{CTX-M-123}}$ and $bla_{\text{CTX-M-130}}$ in *Salmonella*. A high prevalence of PMQR genes was found among the studied isolates, while the data confirms the observation that mutations present in both of *GyrA* and *ParC* are essential for high resistance level of ciprofloxacin. The conjugative resistant plasmids harboring $bla_{\text{CTX–M}}$ and PMQR genes were responsible for the dissemination of *Salmonella* isolates, posing a potential threat for public health. Our data suggest that there should be a reassessment and reduction usage of cephalosporins, fluoroquinolones, and other antimicrobial compounds, critical to human health, in the poultry industry in China, as an important step to improve food safety controls.

Funding

This study was funded by the National Key R&D Program of China (2016YFD0401102), and China Food Safety Talent Competency Development Initiative: CFSA 523 Program.

Author Contributions

WW, XJ, L-FQ, and SF designed the experiments and wrote the manuscript. ZL, H-YJ, BY, GX, and D-YP carried out the experiments. WW, P-ZX, Y-SF and L-MH analyzed the experimental results.

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