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Emergence and Diversity of *Salmonella enterica* Serovar Indiana Isolates with Concurrent Resistance to Ciprofloxacin and Cefotaxime from Patients and Food-Producing Animals in China

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Salmonellosis is a major global foodborne infection, and strains that are resistant to a great variety of antibiotics have become a major public health concern. The aim of this study was to identify genes conferring resistance to fluoroquinolones and extended-spectrum β-lactams in nontyphoidal *Salmonella* (NTS) from patients and food-producing animals in China. In total, 133 and 21 NTS isolates from animals and humans, respectively, exhibiting concurrent resistance to ciprofloxacin and cefotaxime were cultured independently from 2009 to ~2013. All of the isolates were identified, serotyped, and subjected to antimicrobial susceptibility testing. Importantly, the isolates with concurrent resistance to ciprofloxacin and cefotaxime all were confirmed as *S. enterica* serovar Indiana. The presence of fluoroquinolone resistance genes and extended-spectrum β-lactamases (ESBLs) was established by PCR and DNA sequencing. The occurrence and diversity of different genes conferring fluoroquinolone resistance [*qepA*, *oqxAB*, and *aac*(*6′*)*-Ib-cr*] with mutations in topoisomerase-encoding genes (*gyrA* and *parC*) and several ESBLs (including CTX-M-65, CTX-M-27, CTX-M-15, CTX-M-14, and CTX-M-14/CTX-M-15) were noteworthy. Genes located on mobile genetic elements were identified by conjugation and transformation. Pulsed-field gel electrophoresis, used to determine the genetic relationships between these isolates, generated 91 pulsotypes from 133 chicken isolates and 17 pulsotypes from the 21 clinical isolates that showed considerable diversity. Analysis of the pulsotypes obtained with the isolates showed some clones appeared to have existed for several years and had been disseminating between humans and food-producing animals. This study highlights the emergence of ciprofloxacin- and cefotaxime-resistant *S. enterica* serovar Indiana, posing a threat to public health.

Salmonellosis, caused by *Salmonella enterica*, is a global foodborne disease of humans and livestock worldwide (1, 2). A total of 91,034 cases of human laboratory-confirmed *Salmonella* infections and 61 fatal cases were reported in 2012 in 27 European countries (3). In high-income regions of North America, there are an estimated 1.0 million *Salmonella* infections per year (4). In China, approximately 75% (30 million) of foodborne diseases every year are attributed to this bacterium (5). Specifically, children younger than 5 years were the group most at risk of infection by *Salmonella*, as noted in data obtained from several cities in China (2, 6, 7).

Salmonellosis is generally a self-limiting illness; however, antimicrobial agents may be required in severe cases, particularly in immunocompromised individuals, children, and the elderly (8). Fluoroquinolones and extended-spectrum β-lactams (ESBLs) are the front-line drugs of choice for treating salmonellosis (6, 9). However, in recent years, an increase in the occurrence of multidrug-resistant (MDR) *Salmonella* spp. expressing resistance to these compounds has been observed in several countries. Dissemination of antimicrobial resistance among nontyphoidal *Salmonella* isolates in humans is thought to be predominantly due to the use of antimicrobial agents in food animals (6, 10). Furthermore, infections with such drug-resistant *Salmonella* species are associated with increased morbidity and mortality.

Ciprofloxacin resistance is attributed mainly to mutations of quinolone resistance-determining regions (QRDRs) of the genes encoding the target bacterial topoisomerase enzymes (11). The presence of plasmid-mediated quinolone resistance (PMQR)-encoding genes also may contribute to the ciprofloxacin resistance phenotype (12). Although these PMQR genes confer only low-level resistance to fluoroquinolones, the presence of PMQR genes (particularly *qnr* genes) may provide a selective advantage for bacteria exposed to fluoroquinolones and facilitate the development of high-level chromosomal quinolone resistance.

On the other hand, it has been acknowledged that the horizontal transfer of plasmids carrying the ESBL genes is an important contributory factor in the epidemiology of this bacterial ecosystem. The predominant ESBL families of clinical importance in-

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TABLE 1 Phenotypes and topoisomerase and PMQR genotypes of 21 human ciprofloxacin- and cefotaxime-resistant *S. enterica* serovar Indiana isolates

Strain	Source	Age	Yr of isolation	MIC (mg/liter)		QRDR amino acid substitutions[a]				Gene(s)	
				CIP	CTX	*gyrA*	*gyrB*	*parC*	*parE*	PMQR	β-Lactamase
P1	Zhoukou	1 mo	2009	128	128	S83F/D87G	WT	T57S/S80R	WT	*aac(6′)-Ib-cr*, *oqxAB*	$bla_{CTX-M-15}$
P2	Jiyuan	7 yr	2010	8	128	S83F/D87G	WT	T57S/S80R	WT	*oqxAB*	$bla_{CTX-M-14}$
P3	Zhoukou	28 yr	2010	128	128	S83F/D87N	WT	T57S/S80R	WT	*aac(6′)-Ib-cr*, *oqxAB*	$bla_{CTX-M-65}$
P4	Zhenzhou	1 mo	2011	32	128	S83F/D87G	WT	T57S/S80R	WT	*aac(6′)-Ib-cr*	$bla_{CTX-M-15}$
P5	Jiyuan	28 yr	2011	128	128	S83F/D87N	WT	T57S/S80R	WT	*aac(6′)-Ib-cr*, *oqxAB*	$bla_{CTX-M-65}$
P6	Jiyuan	1 mo	2011	128	128	S83F/D87N	WT	T57S/S80R	WT	*aac(6′)-Ib-cr*, *oqxAB*	$bla_{CTX-M-65}$
P7	Jiyuan	36 yr	2011	128	128	S83F/D87N	WT	T57S/S80R	WT	*aac(6′)-Ib-cr*, *oqxAB*	$bla_{CTX-M-65}$
P8	Jiyuan	1 yr	2011	128	128	S83F/D87N	WT	T57S/S80R	WT	*aac(6′)-Ib-cr*, *oqxAB*	$bla_{CTX-M-65}$
P9	Shangqiu	22 yr	2011	128	128	S83F/D87G	WT	T57S/S80R	WT	*aac(6′)-Ib-cr*, *oqxAB*	$bla_{CTX-M-15}$
P10	Shangqiu	50 yr	2011	128	128	S83F/D87G	WT	T57S/S80R	WT	*aac(6′)-Ib-cr*, *oqxAB*	$bla_{CTX-M-15}$
P11	Shangqiu	49 yr	2011	128	128	S83F/D87G	WT	T57S/S80R	WT	*aac(6′)-Ib-cr*, *oqxAB*	$bla_{CTX-M-14}$, $bla_{CTX-M-15}$
P13	Shangqiu	1 mo	2011	128	128	S83F/D87N	WT	T57S/S80R	WT	*aac(6′)-Ib-cr*, *oqxAB*	$bla_{CTX-M-27}$
P14	Zhenzhou	4 mo	2012	128	128	S83F/D87N	WT	T57S/S80R	WT	*aac(6′)-Ib-cr*, *oqxAB*	$bla_{CTX-M-65}$
P17	Shangqiu	15 mo	2012	32	128	S83F/D87G	WT	T57S/S80R	WT	*oqxAB*	$bla_{CTX-M-14}$
P18	Zhoukou	17 mo	2012	128	128	S83F/D87G	WT	T57S/S80R	WT	*aac(6′)-Ib-cr*, *oqxAB*	$bla_{CTX-M-15}$
P19	Zhoukou	9 mo	2012	128	128	S83F/D87G	WT	T57S/S80R	WT	*aac(6′)-Ib-cr*, *oqxAB*	$bla_{CTX-M-65}$
P21	Shangqiu	5 mo	2012	128	128	S83F/D87G	WT	T57S/S80R	WT	*aac(6′)-Ib-cr*, *oqxAB*	$bla_{CTX-M-65}$
P22	Shangqiu	74 yr	2012	128	128	S83F/D87G	WT	T57S/S80R	WT	*aac(6′)-Ib-cr*, *oqxAB*	$bla_{CTX-M-65}$
P23	Dengfeng	6 mo	2012	128	128	S83F/D87G	WT	T57S/S80R	WT	*aac(6′)-Ib-cr*, *oqxAB*	$bla_{CTX-M-65}$
P24	Zhenzhou	9 yr	2013	128	128	S83F/D87N	WT	T57S/S80R	WT	*aac(6′)-Ib-cr*, *oqxAB*	$bla_{CTX-M-14}$
P26	Zhoukou	9 mo	2013	32	128	S83F/D87G	WT	T57S/S80R	WT	*oqxAB*	$bla_{CTX-M-14}$

[a] WT, wild type.

clude TEM, SHV, and CTX-M (13). In *Salmonella*, the most commonly found ESBL types in Asia are those of the CTX-M group, which are usually located on transmissible plasmids that have the capacity to disseminate among members of the *Enterobacteriaceae* (13). In other regions of the world, such as in the United States and Canada, the AmpC β-lactamase CMY-2 is the major contributor to ceftriaxone resistance in *Salmonella* (14).

Recently, concurrent resistance to ceftriaxone/cefotaxime and ciprofloxacin in *S. enterica* serovar Indiana has been reported for isolates cultured from food-producing animals (15, 16). *Salmonella* spp. have a variety of animal reservoirs and routes of transmission that can result in human infection (17). However, foods of animal origin, especially poultry and poultry products, often are involved in sporadic cases and outbreaks of human salmonellosis (15), and contaminated raw or undercooked chicken products are primary vehicles of *Salmonella* transmission to human beings (18). Consequently, concurrent resistance to ceftriaxone/cefotaxime and ciprofloxacin for *S. enterica* serovar Indiana poses a risk to humans, and this feature limits the treatment options available when the organisms are encountered. Currently, there is limited information describing the molecular epidemiology of ceftriaxone/cefotaxime- and ciprofloxacin-resistant *S. enterica* serovar Indiana isolates.

Therefore, the objectives of this study are to investigate and characterize those genes conferring resistance to cefotaxime and ciprofloxacin in coresistant *S. enterica* serovar Indiana isolates from both humans and food-producing animals in China.

MATERIALS AND METHODS

Ethics statement. The fecal samples were acquired with written informed consent from the patients. This study was reviewed and approved by the ethics committee of the China National Center for Food Safety Risk Assessment (CFSA) according to the medical research regulations of the Ministry of Health, China. This research was conducted within China.

Bacteria and growth conditions. In total, 133 *S. enterica* serovar Indiana isolates with concurrent resistance to ciprofloxacin and cefotaxime were recovered from poultry slaughterhouses (10.1%; $n = 1{,}320$; isolates from whole chicken carcasses after dehairing and precooling bath) in 3 cities (Hebi [$n = 88$], Kaifeng [$n = 40$], and Luohe [$n = 5$]) in Henan Province from February through November 2012. Twenty-one *S. enterica* serovar Indiana isolates with concurrent resistance to ciprofloxacin and cefotaxime were cultured from patients (2.6%; $n = 802$; isolates from fresh fecal swabs) in five different geographical regions (Dengfeng [$n = 1$], Jiyuan [$n = 5$], Shangqiu [$n = 7$], Zhenzhou [$n = 3$], and Zhoukou[$n = 5$]) in 28 sentinel hospitals and 6 regional Center for Disease Control and Prevention of Henan Province diagnostic laboratories, located in Henan Province, from 2009 to 2013. The ages of the patients varied from 1 month to 70 years, and more than half were less than 2 years old (Table 1).

The protocols for isolating bacteria from animals used a modified method based on the United States Department of Agriculture-Food Safety and Inspection Service *Microbiology Laboratory Guidebook* (19), and protocols applied to patient samples were described previously (20). Finally, isolates from both animals and patients with typical *Salmonella* phenotypes were confirmed by the API 20E test (bioMérieux, Beijing, China) and amplification of the *invA* gene by PCR (21). For selecting concurrent resistance to ciprofloxacin and cefotaxime in *Salmonella* isolates, all confirmed isolates were incubated in LB supplemented with 1 mg/liter ciprofloxacin and 4 mg/liter cefotaxime. For all of the *Salmonella* isolates that grew, serotypes were determined by slide agglutination with commercial *Salmonella* antisera (Statens Serum Institute, Denmark) by following the Kauffmann-White scheme.

Antimicrobial susceptibility testing. The antimicrobial susceptibility of all of the *Salmonella* isolates was determined by the agar dilution method and interpreted according to Clinical and Laboratory Standards Institute guidelines (22) and the European Committee on Antimicrobial

Susceptibility Testing (35). The following antimicrobial compounds were assessed: ampicillin (AMP), cefotaxime (CTX), cefotaxime-clavulanic acid (CTX-CLA), ceftazidime (CAZ), ceftazidime-clavulanic acid (CAZ-CLA), chloramphenicol (CHL), ciprofloxacin (CIP), gentamicin (GEN), imipenem (IPM), tetracycline (TET), tigecycline (TGC), and trimethoprim-sulfamethoxazole (SXT). Multidrug resistance was defined as resistance to three or more different classes of agents. Isolates with MIC values of 1 mg/liter for either cefotaxime or ceftazidime were further screened for ESBL production by determination of synergy between 0.25 and 128 mg/liter ceftazidime or cefotaxime and 4 mg/liter clavulanate. *Escherichia coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 700603 were used as quality-control organisms in antimicrobial susceptibility tests.

PCR amplification and DNA sequence analysis. The quinolone resistance determination regions (QRDRs) of *gyrA*, *gyrB*, *parC*, and *parE* in *Salmonella* isolates were amplified by PCR as described previously (11). All of the *Salmonella* isolates were screened by PCR for transferable PMQR-encoding genes, including *qnrA*, *qnrB*, *qnrC*, *qnrD*, *qnrS*, *oqxAB*, *aac*-(6′)-*Ib*, and *qepA*, as previously described (11, 12). A multiplex PCR was used to screen for $bla_{\text{CTX-M}}$ in all of the ESBL-producing isolates (23). Another multiplex PCR method was applied to screen for plasmid-mediated AmpC-encoding genes in clavulanic acid-resistant isolates (24). All of the PCR products were directly sequenced (TaKaRa Biotechnology Cooperation, Dalian, China) for sequence analysis and aligned using the BLAST program (http://www.ncbi.nlm.nih.gov/BLAST/).

Plasmid replicon typing and conjugation experiment. *Salmonella* isolates were examined for the presence of 18 replicon (Inc) types by PCR-based replicon typing (PBRT) (25). Transfer of resistance was tested by filter-mating assays. Conjugation experiments were performed with *Salmonella* isolates as donors using a modified laboratory-based method and *E. coli* MG1655 as the recipient (26). Transconjugants were selected as pink colonies on MacConkey agar plates containing cefotaxime (4 mg/liter), and PCR and S1-pulsed-field gel electrophoresis (S1-PFGE) subsequently were performed to reconfirm the plasmid profiles in these transconjugants (6).

PFGE. All *Salmonella* isolates were subtyped by PFGE to determine their DNA fingerprint profiles following digestion by the macrorestriction enzyme XbaI (New England BioLabs, Ltd., Beijing) according to the procedures developed by the U.S. Centers for Disease Control and Prevention PulseNet program (27). The interpretation of the PFGE patterns was performed with BioNumerics software (Applied Maths, St-Martens-Latem, Belgium) using the Dice similarity coefficient. Dendrograms were constructed on the basis of the unweighted pair group method using average linkages (UPGMA) with a position tolerance of 1%. Clusters were defined as DNA patterns sharing ≥85% similarity.

RESULTS

Antimicrobial susceptibility testing. All 154 *S. enterica* serovar Indiana isolates were resistant to ampicillin, cefotaxime, ceftazidime, and ciprofloxacin and susceptible to imipenem and tigecycline. Resistance to chloramphenicol was common (98.7%; 152/154), followed by resistance to gentamicin (96.1%; 148/154), trimethoprim-sulfamethoxazole (92.2%; 142/154), and tetracycline (81.2%; 125/154) (Table 2). All *Salmonella* isolates expressed a multidrug-resistant phenotype (being resistant to at least three different classes of antimicrobial compounds). In total, 12 antimicrobial resistance profiles were identified among 154 *S. enterica* serovar Indiana isolates. The dominant resistance profiles identified were determined to be AMP-CAZ-CHL-CIP-CTX-GEN-SXT-TET ($n = 109$) and AMP-CAZ-CHL-CIP-CTX-GEN-SXT ($n = 27$) from both chicken (87.2%; 116/133) and human (95.2% 20/21) isolates. In total, 153 isolates (99.4%; 153/154) were confirmed as ESBL positive.

TABLE 2 Resistance phenotypes of *S. enterica* serovar Indiana isolates for each antimicrobial from chicken and patients

Antibiotic	Breakpoint(s)[a] (mg/liter)	No. (%) of resistant isolates from different sources		
		Chicken ($n = 133$)	Patients ($n = 21$)	Total
AMP	≥32	133 (100)	21 (100)	154 (100)
CAZ	≥16	133 (100)	21 (100)	154 (100)
CTX	≥4	133 (100)	21 (100)	154 (100)
CHL	≥32	131 (98.5)	21 (100)	152 (98.7)
CIP	≥1	133 (100)	21 (100)	154 (100)
GEN	≥16	128 (96.2)	20 (95.2)	148 (96.1)
IMP	≥4	0 (0)	0 (0)	0 (0)
TET	≥16	104 (78.2)	21 (100)	125 (81.2)
TGC	≥2	0 (0)	0 (0)	0 (0)
SXT	≥4/76	121 (91.0)	21 (100)	142 (92.2)

[a] All antimicrobial breakpoints were recommended by CLSI M100-S22 (22), except tigecycline, whose breakpoint was recommended by EUCAST-2012 (35).

Identification of quinolone resistance-encoding genes. Of the 154 *S. enterica* serovar Indiana isolates we studied, four point mutations in QRDRs were found in 152 isolates, of which 80 possessed amino acid substitutions in GyrA (S83F and D87G) and ParC (T57S and S80R) as the dominant types (51.9%; 80/154), along with 72 isolates that contained GyrA (S83F and D87N) and ParC (T57S and S80R) alleles. Three point mutations were identified in 2 isolates from poultry slaughterhouses with amino acid substitutions in GyrA (S83F) and ParC (T57S and S80R).

PMQR-encoding genes were identified in 143 isolates (92.9%; 143/154) from both chicken ($n = 122$) and human ($n = 21$) isolates, including *aac*(6′)-*Ib-cr* ($n = 117$), *oqxAB* ($n = 112$), and *qepA* ($n = 2$). The PMQR genes *qnrA*, *qnrB*, *qnrC*, *qnrD*, and *qnrS* were not detected. Ninety-eight isolates (63.6%; 98/154) possessed more than one PMQR gene. Compared to the chicken isolates (60.9%; 81/133), human isolates (81.0%; 17/21) showed a higher prevalence with two PMQR genes. The ciprofloxacin MIC was measured and determined to be between 8 and 128 mg/liter in all of these isolates.

Among 132 isolates that were highly resistant to ciprofloxacin (MIC of ≥32 mg/liter), including 112 poultry and 20 human isolates, four topoisomerase point mutations were detected in each of 130 isolates, and a further three were detected in two isolates. PMQR genes were identified in 127 isolates (two PMQR genes in 96 isolates and one PMQR gene in 31 isolates), and only five isolates were negative for any of the PMQR genes. The 37 isolates that demonstrated a ciprofloxacin MIC of ≥128 mg/liter possessed four point mutations in QRDRs with two PMQR genes [*aac*(6′)-*Ib-cr* and *oqxAB*], of which 27 contained amino acid substitutions in GyrA (S83F and D87N) and ParC (T57S and S80R) and 10 had amino acid substitutions in GyrA (S83F and D87G) and ParC (T57S and S80R).

Identification of β-lactamase resistance-encoding genes. Five $bla_{\text{CTX-M}}$ subtypes were identified among the 153 ESBL-positive *Salmonella* isolates, including $bla_{\text{CTX-M-65}}$ ($n = 131$), $bla_{\text{CTX-M-14}}$ ($n = 15$), $bla_{\text{CTX-M-15}}$ ($n = 5$), $bla_{\text{CTX-M-27}}$ ($n = 1$), and $bla_{\text{CTX-M-14}}$/$bla_{\text{CTX-M-15}}$ ($n = 1$). *Salmonella* isolates containing $bla_{\text{CTX-M-65}}$ were recovered both from poultry slaughterhouses ($n = 121$) in all sampling cities and from humans ($n = 10$). *Salmonella* isolates containing $bla_{\text{CTX-M-14}}$ were also recovered from

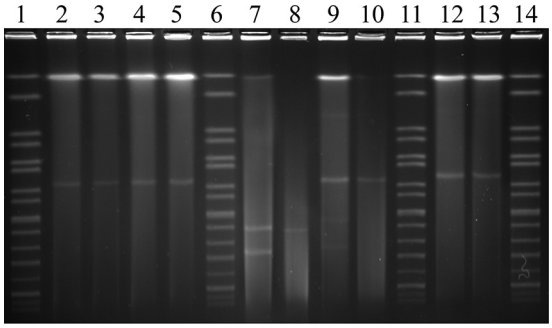


FIG 1 Plasmid profiles of representative *S. enterica* serovar Indiana isolates and transconjugants determined by S1-PFGE. Transmissible plasmids from *S. enterica* serovar Indiana and transconjugants (T) are highlighted. Lanes 1, 6, 11, and 14, marker H9812 with different bands labeled (used as a molecular size marker); lane 2, isolate P9; lane 3, isolate P9-(T); lane 4, isolate P10; lane 5, isolate P10-(T); lane 7, isolate D25; lane 8, isolate D25-(T); lane 9, isolate D26; lane 10, isolate D26-(T); lane 12, isolate D169; lane 13, isolate D169-(T).

poultry slaughterhouses ($n = 11$) in Hebi ($n = 3$) and Kaifeng ($n = 8$) and from humans ($n = 4$). The remaining three $bla_{\text{CTX-M}}$ subtypes were detected only in human isolates. Only three strains were AmpC-producing isolates, and these were cultured from poultry slaughterhouses. The $bla_{\text{CMY-2}}$ gene was identified in two ESBL isolates with $bla_{\text{CTX-M-14}}$ from Kaifeng and one clavulanic acid-resistant isolate from Luohe.

Association between ESBL and plasmid-mediated quinolone resistance genes. Among all 154 *S. enterica* serovar Indiana isolates, the most frequent combination of *aac(6′)-Ib-cr* with *oqxAB* was in CTX-M-65-producing strains ($n = 89$). The combination of *oqxAB* with *qepA* was also found in only two CTX-M-65-producing isolates with amino acid substitutions in GyrA (S83F) and ParC (T57S and S80R). Isolates containing $bla_{\text{CTX-M-14}}$ also possessed two PMQR genes, one PMQR gene, or no PMQR gene. Isolates with $bla_{\text{CTX-M-15}}$ contained two PMQR genes or one PMQR gene.

Plasmid replicon typing and conjugation experiment. Among 154 isolates, 106 isolates carried at least one replicon type and six different plasmid Inc types were detected, ranging from one to three replicon types in these isolates. The three most prevalent replicon types identified by PBRT included IncN (91.5%; 97/106), IncA/C (9.4%; 10/106), and IncI1-Ir (6.6%; 7/106). IncN was detected in all bacterial isolates that were positive for the $bla_{\text{CTX-M-65}}$ gene. IncA/C, IncFIA, IncFIB, IncF, and IncI1-Ir were detected only in isolates positive for $bla_{\text{CTX-M-14}}$ and $bla_{\text{CMY-2}}$ genes. Forty randomly selected poultry isolates containing different plasmid Inc types, along with 21 clinical isolates, were selected as donors in the conjugation experiments. Five transconjugants were obtained. Conjugative transfer frequencies recorded ranged from 10^{-7} to 10^{-8} transconjugants per recipient. Analysis of the plasmids in these transconjugants identified IncA/C, IncI1-Ir, and IncN types along with three other isolates whose plasmid replicon types could not be confirmed with the primer sets used. Transconjugants obtained using *S. enterica* serovar Indiana isolates as donors harbored plasmids with sizes ranging from 130 to 285 kbp (Fig. 1 and Table 3). Genes present in the transconjugants included *oqxA*, *oqxB*, *aac(6′)-Ib-cr*, $bla_{\text{CTX-M-14}}$, $bla_{\text{CTX-M-15}}$, and $bla_{\text{CTX-M-65}}$ (Fig. 1 and Table 3).

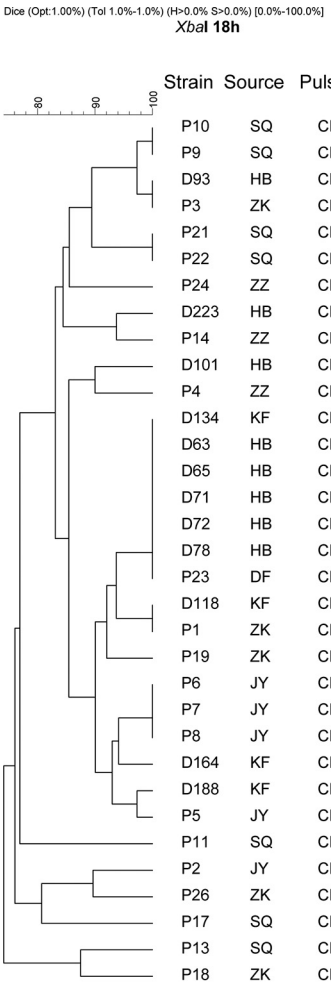
PFGE analysis of *Salmonella* isolates. Ninety-one pulsotypes were identified among the 133 *S. enterica* serovar Indiana isolates from chicken by PFGE, and 14 different clusters were denoted as clusters CC1 to CC14 (see Fig. S1 in the supplemental material). There were 18 separate PFGE patterns containing more than one isolate. Isolates with the same PFGE patterns mostly were recovered from the same regions, but 4 indistinguishable pulsotypes contained isolates from different regions. Some isolates within the same pulsotype possessed the same type of $bla_{\text{CTX-M}}$ but not the same QRDR or PMQR profile. The $bla_{\text{CTX-M-65}}$ gene was identified in isolates in 81 pulsotypes. Cluster CC1-1 had the greatest number of isolates with high-level resistance to ciprofloxacin (MIC, ≥128 mg/liter). In contrast, clusters CC13 and CC14 contained isolates with CTX-M-14 (see Fig. S1). The 21 clinical isolates were divided into 17 pulsotypes (Fig. 2). The predominant pulsotype was denoted INDX11.CN009, which included three isolates from the same city. Two pulsotypes (INDX11.CN004 and INDX11.CN007) were found to contain two each, and the remaining 14 pulsotypes contained a single isolate (Fig. 2).

DNA fingerprint comparisons between isolates cultured from patients and food-producing animals by PFGE exhibited diversity among the strains. Three pulsotypes (INDX11.CN005, INDX11.CN010, and INDX11.CN012) included isolates from both chicken and humans (Fig. 2). Some of the remaining pulsotypes from patients showed profiles highly similar to those from food-producing animals (Fig. 2). The results further confirmed that some predominant clones may have existed in Henan for several years, and chicken could be a

TABLE 3 Characteristics of $bla_{\text{CTX-M}}$-positive *S. enterica* serovar Indiana isolates coharboring PMQR genes and their transconjugants

Strain[a]	Source	Resistance gene(s)	Antimicrobial resistance profile	Replicon typing[b]	Plasmid size(s) (kb)
D25	Chicken	$bla_{\text{CTX-M-14}}$, bla_{CMY2}	AMP-CAZ-CHL-CIP-CTX-SXT-TET	I1-Iγ,A/C	130, 83
D25-(T)		$bla_{\text{CTX-M-14}}$	AMP-CAZ-CHL-CTX-TET	A/C	130
D26	Chicken	*oqxAB*, $bla_{\text{CTX-M-14}}$	AMP-CAZ-CHL-CIP-CTX-GEN-SXT-TET	I1-Iγ, N, A/C	260, 155, 94
D26-(T)		*oqxAB*, $bla_{\text{CTX-M-14}}$	AMP-CAZ-CHL-CTX-SXT-TET	I1-Iγ, N, A/C	260
D169	Chicken	*oqxAB*, $bla_{\text{CTX-M-65}}$	AMP-CAZ-CHL-CIP-CTX-GEN-TET	NT	285
D169-(T)		$bla_{\text{CTX-M-65}}$	AMP-CAZ-CHL-CTX	NT	285
P9	Human	*aac(6′)-Ib-cr*, *oqxAB*, $bla_{\text{CTX-M-15}}$	AMP-CAZ-CHL-CIP-CTX-GEN-SXT-TET	NT	256
P9-(T)		*aac(6′)-Ib-cr*, *oqxAB*, $bla_{\text{CTX-M-15}}$	AMP-CAZ-CHL-CTX-GEN-SXT-TET	NT	256
P10	Human	*aac(6′)-Ib-cr*, *oqxAB*, $bla_{\text{CTX-M-15}}$	AMP-CAZ-CHL-CIP-CTX-GEN-SXT-TET	NT	256
P10-(T)		*aac(6′)-Ib-cr*, *oqxAB*, $bla_{\text{CTX-M-15}}$	AMP-CAZ-CHL-CTX-GEN-SXT-TET	NT	256

[a] (T), transconjugant.
[b] NT, an isolate whose plasmid replicon was nontypeable.



Strain	Source	Pulsotype	Mutations in QRDRs		PMQR determinants	β-Lactamase genes	Antimicrobial resistance profile	Year	Replicon typing
			gyrA	*parC*					
P10	SQ	CN004	S83F/D87G	T57S/S80R	*aac(6')-Ib-cr, oqxAB*	CTX-M-15	AMP-CAZ-CHL-CIP-CTX-GEN-SXT-TET	2011	NT
P9	SQ	CN004	S83F/D87G	T57S/S80R	*aac(6')-Ib-cr, oqxAB*	CTX-M-15	AMP-CAZ-CHL-CIP-CTX-GEN-SXT-TET	2011	NT
D93	HB	CN005	S83F/D87N	T57S/S80R	*aac(6')-Ib-cr, oqxAB*	CTX-M-65	AMP-CAZ-CHL-CIP-CTX-GEN-SXT-TET	2012	N
P3	ZK	CN005	S83F/D87N	T57S/S80R	*aac(6')-Ib-cr, oqxAB*	CTX-M-65	AMP-CAZ-CHL-CIP-CTX-GEN-SXT-TET	2010	N
P21	SQ	CN007	S83F/D87G	T57S/S80R	*aac(6')-Ib-cr, oqxAB*	CTX-M-65	AMP-CAZ-CHL-CIP-CTX-GEN-SXT-TET	2012	I1-Ir
P22	SQ	CN007	S83F/D87G	T57S/S80R	*aac(6')-Ib-cr, oqxAB*	CTX-M-65	AMP-CAZ-CHL-CIP-CTX-GEN-SXT-TET	2012	I1-Ir
P24	ZZ	CN008	S83F/D87N	T57S/S80R	*aac(6')-Ib-cr, oqxAB*	CTX-M-14	AMP-CAZ-CHL-CIP-CTX-GEN-SXT-TET	2013	NT
D223	HB	CN021	S83F/D87N	T57S/S80R	*aac(6')-Ib-cr, oqxAB*	CTX-M-65	AMP-CAZ-CHL-CIP-CTX-GEN-SXT	2012	NT
P14	ZZ	CN006	S83F/D87N	T57S/S80R	*aac(6')-Ib-cr, oqxAB*	CTX-M-65	AMP-CAZ-CHL-CIP-CTX-GEN-SXT-TET	2012	I1-Ir,N
D101	HB	CN018	S83F/D87N	T57S/S80R	*aac(6')-Ib-cr, oqxAB*	CTX-M-65	AMP-CAZ-CHL-CIP-CTX-GEN-SXT-TET	2012	N
P4	ZZ	CN014	S83F/D87G	T57S/S80R	*aac(6')-Ib-cr*	CTX-M-15	AMP-CAZ-CHL-CIP-CTX-GEN-SXT-TET	2011	NT
D134	KF	CN010	S83F/D87G	T57S/S80R	*aac(6')-Ib-c, roqxAB*	CTX-M-65	AMP-CAZ-CHL-CIP-CTX-GEN-SXT-TET	2012	NT
D63	HB	CN010	S83F/D87G	T57S/S80R	*aac(6')-Ib-cr, oqxAB*	CTX-M-65	AMP-CAZ-CHL-CIP-CTX-GEN-SXT-TET	2012	N
D65	HB	CN010	S83F/D87G	T57S/S80R	*aac(6')-Ib-cr, oqxAB*	CTX-M-65	AMP-CAZ-CHL-CIP-CTX-GEN-SXT-TET	2012	N
D71	HB	CN010	S83F/D87G	T57S/S80R	*aac(6')-Ib-cr, oqxAB*	CTX-M-65	AMP-CAZ-CHL-CIP-CTX-GEN-SXT-TET	2012	N
D72	HB	CN010	S83F/D87G	T57S/S80R	*aac(6')-Ib-cr, oqxAB*	CTX-M-65	AMP-CAZ-CHL-CIP-CTX-GEN-SXT-TET	2012	N
D78	HB	CN010	S83F/D87G	T57S/S80R	*aac(6')-Ib-cr, oqxAB*	CTX-M-65	AMP-CAZ-CHL-CIP-CTX-GEN-SXT-TET	2012	N
P23	DF	CN010	S83F/D87G	T57S/S80R	*aac(6')-Ib-cr, oqxAB*	CTX-M-65	AMP-CAZ-CHL-CIP-CTX-GEN-SXT-TET	2012	NT
D118	KF	CN012	S83F/D87G	T57S/S80R	*aac(6')-Ib-cr, oqxAB*	CTX-M-65	AMP-CAZ-CHL-CIP-CTX-GEN-SXT-TET	2012	NT
P1	ZK	CN012	S83F/D87G	T57S/S80R	*aac(6')-Ib-cr, oqxAB*	CTX-M-15	AMP-CAZ-CHL-CIP-CTX-GEN-SXT-TET	2009	NT
P19	ZK	CN013	S83F/D87G	T57S/S80R	*aac(6')-Ib-cr, oqxAB*	CTX-M-65	AMP-CAZ-CHL-CIP-CTX-GEN-SXT-TET	2012	N
P6	JY	CN009	S83F/D87N	T57S/S80R	*aac(6')-Ib-cr, oqxAB*	CTX-M-65	AMP-CAZ-CHL-CIP-CTX-GEN-SXT-TET	2011	N
P7	JY	CN009	S83F/D87N	T57S/S80R	*aac(6')-Ib-cr, oqxAB*	CTX-M-65	AMP-CAZ-CHL-CIP-CTX-GEN-SXT-TET	2011	N
P8	JY	CN009	S83F/D87N	T57S/S80R	*aac(6')-Ib-cr, oqxAB*	CTX-M-65	AMP-CAZ-CHL-CIP-CTX-GEN-SXT-TET	2011	N
D164	KF	CN019	S83F/D87N	T57S/S80R	*aac(6')-Ib-cr, oqxAB*	CTX-M-65	AMP-CAZ-CHL-CIP-CTX-GEN-SXT-TET	2012	N
D188	KF	CN020	S83F/D87N	T57S/S80R	*aac(6')-Ib-cr, oqxAB*	CTX-M-65	AMP-CAZ-CHL-CIP-CTX-GEN-SXT-TET	2012	N
P5	JY	CN011	S83F/D87N	T57S/S80R	*aac(6')-Ib-cr, oqxAB*	CTX-M-65	AMP-CAZ-CHL-CIP-CTX-GEN-SXT-TET	2011	N
P11	SQ	CN017	S83F/D87G	T57S/S80R	*aac(6')-Ib-cr, oqxAB*	CTX-M-14 CTX-M-15	AMP-CAZ-CHL-CIP-CTX-GEN-SXT-TET	2011	NT
P2	JY	CN001	S83F/D87G	T57S/S80R	*oqxAB*	CTX-M-14	AMP-CAZ-CHL-CIP-CTX-GEN-SXT-TET	2010	N
P26	ZK	CN002	S83F/D87G	T57S/S80R	*oqxAB*	CTX-M-14	AMP-CAZ-CHL-CIP-CTX-GEN-SXT-TET	2013	N
P17	SQ	CN003	S83F/D87G	T57S/S80R	*oqxAB*	CTX-M-14	AMP-CAZ-CHL-CIP-CTX-GEN-SXT-TET	2012	N,A/C
P13	SQ	CN015	S83F/D87N	T57S/S80R	*aac(6')-Ib-cr, oqxAB*	CTX-M-27	AMP-CAZ-CHL-CIP-CTX-GEN-SXT-TET	2011	NT
P18	ZK	CN016	S83F/D87G	T57S/S80R	*aac(6')-Ib-cr, oqxAB*	CTX-M-15	AMP-CAZ-CHL-CIP-CTX-GEN-SXT-TET	2012	NT

FIG 2 Dendrogram of 21 clinical and 12 poultry *S. enterica* serovar Indiana isolates that were resistant to both ciprofloxacin and cefotaxime, constructed based on PFGE with XbaI on patient samples. The isolate identity, source, pulsotype, year of isolation, antimicrobial resistance profile, ESBL genes, mutations in QRDRs, and PMQR determinants were identified. D in strain names indicates isolates from poultry, and P indicates isolates from patients. DF, Dengfeng; JY, Jiyuan; HB, Hebi; KF, Kaifeng; LH, Luohe; SQ, Shangqiu; ZK, Zhoukou; ZZ, Zhengzhou; NT, an isolate whose plasmid replicon typing was nontypeable.

source of concurrent resistance to ciprofloxacin and cefotaxime *S. enterica* serovar Indiana in humans.

DISCUSSION

In this study, we collected ciprofloxacin- and cefotaxime-resistant *S. enterica* serovar Indiana isolates from both processed chicken in poultry slaughterhouses and patients in Henan province, which is one of the largest production centers in China for broiler meat processing. Genes conferring resistance to fluoroquinolones and extended-spectrum β-lactams in these isolates were characterized, and the collection of bacteria was subtyped by PFGE.

In the present study, our aim was to characterize *Salmonella* isolates that were resistant to both fluoroquinolone and third-generation cephalosporins. All were identified as serovar Indiana, and interestingly this serovar has also been reported in livestock in Beijing and Guangdong (15, 16), suggesting that it has a broad geographical distribution in China. Our data showed that 10.1% of the isolates cultured from chicken were coresistant to cefotaxime and ciprofloxacin, a feature previously reported by Wang et al. (16), suggesting this strain has become established in some poultry flocks in China. Human clinical *Salmonella* isolates that were resistant to these two antimicrobial compounds recently were reported in *S. enterica* serovar Kentucky ST198-X1, a much less prevalent serotype from France (28), and in *S. enterica* serovar Typhimurium from China (29). In this study, only 2.6% of the isolates from humans were identified as cefotaxime- and ciprofloxacin-resistant *S. enterica* serovar Indiana, and this coresistance was first reported by our study. With the large-scale use of antimicrobial compounds over time, *Salmonella* isolates expressing resistance to critically important antimicrobial agents such as fluoroquinolones and/or extended-spectrum cephalosporins have been detected in cultures from animals and patients in numerous locations with various levels of prevalence in China (15, 16, 29).

The emergence of cefotaxime- and ciprofloxacin-resistant *S. enterica* serovar Indiana warrants continuous monitoring of the trend for development of antimicrobial resistance in China.

Double amino acid substitutions in GyrA (S83F and D87N)/GyrA (S83F and D87G) and ParC (T57S and S80R) were noted in most of the study isolates. No mutations were detected in either *gyrB* or *parE*. These data are consistent with a previous report (29)

that showed only those mutations in *gyrA* and *parC* mediate quinolone and fluoroquinolone resistance.

PMQR-encoding genes were identified in 143 isolates, a number that was higher than the previous study reported (30) but similar to the report by Jiang et al. (15). Furthermore, two PMQR-encoding genes coexisting in a single isolate were frequently detected, a feature that was rarely observed in isolates from other countries (30). The frequent use of olaquindox as a growth promoter in animals has been suggested to exert a high selective pressure driving the acquisition of *oqxAB* and contributing to its subsequent dissemination (29). Uniquely, one of the isolates possessed three amino acid substitutions (denoted W102R, D179Y, and A160G) in *aac*(6′)*-Ib-cr* (31). Two others were detected with a *qepA* gene which was identified previously in *E. coli*. To our knowledge, this is the first description of *qepA* in *S. enterica* serovar Indiana in China, and this gene has been reported previously only in *S. enterica* serovar Typhimurium from Spain (32) and China (29). The *qepA* gene was found in CTX-M-65-producing isolates alone, along with substitutions in GyrA (S83F) and ParC (T57S and S80R). These data suggested that the acquisition of this transferable quinolone resistance gene is a rare event. Although PMQR confers only low-level quinolone resistance, it can facilitate the emergence of high-level resistance via mutation(s) in one or more of the topoisomerase genes (33). However, in most isolates, two PMQR-encoding genes combined with multiple mutations in topoisomerase genes resulted in high-level resistance to ciprofloxacin (MIC, ≥128 mg/liter).

ESBL screening and characterization for these *S. enterica* serovar Indiana isolates showed that different variants of the CTX-M family of β-lactamases were detectable in *S. enterica* serovar Indiana and that they most likely contributed to the cefotaxime resistance phenotypes of the host isolates. Subtypes of $bla_{\text{CTX-M}}$ genes identified in humans isolates were more variable than those identified in food-producing animals, which suggests that there were other sources in addition to chickens. $bla_{\text{CTX-M-65}}$ was the predominant type existing both in food-producing animals and in humans, and these differed from the types reported earlier in *S. enterica* serovar Kentucky ST198-X1 ($bla_{\text{CTX-M-1}}$ and $bla_{\text{CTX-M-15}}$) (28) and in *S. enterica* serovar Typhimurium ($bla_{\text{CTX-M-14}}$) (29). Genotypes $bla_{\text{CTX-M-14}}$ and $bla_{\text{CTX-M-27}}$ also have been found in *S. enterica* serovar Indiana isolates from chickens and pigs in Guangdong (15), and $bla_{\text{CTX-M-24}}$ was identified in *S. enterica* serovar Indiana from chickens in Shandong (26). Why the predominant $bla_{\text{CTX-M}}$ in ciprofloxacin- and cefotaxime-resistant *S. enterica* serovar Indiana isolates was $bla_{\text{CTX-M-65}}$ in Henan was not clear, but it suggests that the selection, dissemination, and maintenance of transmissible elements carrying these genes can occur during exposure to any of the agents to which resistance is conferred. Interestingly, $bla_{\text{CTX-M}}$ is often located on conjugative plasmids. It has been reported that ciprofloxacin-resistant *S. enterica* serovar Kentucky ST198-X1 acquired the genes conferring resistance to extended-spectrum cephalosporins only recently (28), and certain genotypes, such as $bla_{\text{CTX-M-55}}$, are borne on plasmids that may have originated from *E. coli* possessing the ability to disseminate to *Salmonella* and other bacterial species (34). Conjugative systems in Gram-negative bacteria support transfer of mobile genetic elements between different genera, and in this study five transconjugants (8.25%; 5/61) were obtained. According to S1-PFGE, most of these harbored large plasmids of greater than 200 kbp (data not shown). Previously, several carbapenemase resistance mechanisms (such as KPC, OXA-48, NDM, and VIM) have been identified in *S. enterica* (28). None of the isolates in this study were found to be resistant to imipenem, although surveillance for the emergence of these types of *S. enterica* serovar Indiana isolates should be undertaken as a routine assay.

The PFGE profile variation of the 154 isolates from poultry slaughterhouses and humans demonstrated extensive genetic heterogeneity. Several bacterial isolates from the same sampling place exhibited similar or indistinguishable PFGE pulsotypes, suggesting the potential for cross-contamination. Similar PFGE patterns of isolates from different regions highlighted the possibility for the clonal spreading of some coresistant strains. A comparison of PFGE patterns between bacteria cultured from food-producing animals and from humans further confirmed the existence of clones in Henan for several years. Most notably, the pulsotypes (INDX11.CN005, INDX11.CN010, and INDX11.CN012) from food-producing animals, such as chicken, suggested that chicken is a source of these coresistant *S. enterica* serovar Indiana isolates in humans.

Conclusions. Characterization of the genetic basis for resistance to both third-generation cephalosporins and fluoroquinolones identified plasmids as a major contributing factor. Transmission of resistance genes, such as those encoding ESBLs, OqxAB, and AAC(6′)-Ib-c in *Salmonella*, will facilitate the selection of these MDR *Salmonella* isolates that pose a threat to public health. Ongoing surveillance by regulatory agencies is required to identify routes of transmission.

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We have no conflicts of interest to declare.

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