AAC Journals.ASM.org

Emergence of Clinical *Salmonella enterica* Serovar Typhimurium Isolates with Concurrent Resistance to Ciprofloxacin, Ceftriaxone, and Azithromycin

Marcus Ho Yin Wong,[a,d] Meiying Yan,[b] Edward Wai Chi Chan,[c] Kan Biao,[b] Sheng Chen[a,d]

Department of Applied Biology and Chemical Technology, The Hong Kong Polytechnic University, Hung Hom, Kowloon, Hong Kong, SAR[a]; State Key Laboratory for Infectious Disease Prevention and Control, National Institute for Communicable Disease Control and Prevention (ICDC), Chinese Center for Disease Control and Prevention, Beijing, People's Republic of China[b]; Department of Microbiology, The Chinese University of Hong Kong, The Prince of Wales Hospital, Shatin, New Territories, Hong Kong, SAR[c]; Food Safety and Technology Research Center, Hong Kong PolyU Shenzhen Research Institute, Shenzhen, People's Republic of China[d]

***Salmonella* infection is an important public health issue for which the needs of antimicrobial treatment are increasing. A total of 546 human clinical *S. enterica* serovar Typhimurium isolates were recovered from patients in hospitals in China during the period of 2005 to ~2011. Twenty percent of the isolates exhibited resistance to ciprofloxacin, and 4% were resistant to ceftriaxone. Importantly, for the first time, 12 (2%) *S.* Typhimurium isolates resistant to both ciprofloxacin and ceftriaxone were recovered; among these 12 isolates, two were also resistant to azithromycin, and one was resistant to all other drugs tested. The combined effects of various transferrable extended-spectrum β-lactamase determinants and a novel efflux-based ciprofloxacin resistance mechanism encoded by the mobile efflux gene *oqxAB* were responsible for the emergence of these extremely (highly) drug-resistant (XDR) *S.* Typhimurium isolates. The dissemination of resistance genes, such as those encoding ESBLs and the OqxAB pump, among *Salmonella* organisms will speed up the selection of XDR *Salmonella*, posing a huge threat to public health and *Salmonella* infection control.**

Food-borne salmonellosis is an important public health problem worldwide and the leading cause of food-borne illnesses in many countries, including the United States and China. Although antimicrobial treatment is not necessary for most cases of salmonellosis, it can be lifesaving in invasive infections (1). Ceftriaxone and ciprofloxacin are the key drugs of choice for treatment of invasive *Salmonella* infections (2). Recently, azithromycin was also approved by the FDA to be an additional agent for treatment of *Salmonella* infections (3). Resistance to ceftriaxone or other extended-spectrum beta-lactams is usually due to intracellular production of extended-spectrum β-lactamases (ESBLs). In *Salmonella*, the most commonly found ESBL types in Asia are in the CTX-M group (4), which are usually located on transmissible plasmids that tend to disseminate among members of the *Enterobacteriaceae* (5). The most commonly reported CTX-M enzymes in *Salmonella* strains are CTX-M-9, CTX-M-14, and CTX-M-15 (5). In other regions, such as the United States and Canada, the AmpC β-lactamase CMY-2 is the major contributor to ceftriaxone resistance in *Salmonella* (6). On the other hand, ciprofloxacin resistance is mainly attributed to double mutations in *gyrA* and a single mutation in *parC* in *Salmonella* (7). Efflux pumps and the presence of plasmid-mediated quinolone resistance (PMQR) determinants may also contribute to ciprofloxacin resistance. Qnr genes such as *qnrA*, *qnrB*, *qnrD*, and *qnrS* have been reported (8, 9). Recent studies also detected *oqxAB*, a plasmid-borne gene which encoded an RND efflux pump, in *Salmonella* isolated from food, animals, and humans (10–12). Although resistance to fluoroquinolone or extended-spectrum cephalosporins was increasingly reported in *S. enterica* serovar Typhimurium, concurrent resistance to ciprofloxacin and ceftriaxone (i.e., extremely drug resistant [XDR]) has been reported in other serovars, including *S.* Choleraesuis, *S.* Kentucky, *S.* Senftenberg, and *S.* Oranienburg, but not in *S.* Typhimurium (13–16). This work illustrated the underlying molecular mechanisms responsible for the production of extremely drug-resistant phenotypes in *S.* Typhimurium.

MATERIALS AND METHODS

Bacterial isolates and serotyping. Human clinical *S.* Typhimurium isolates were collected from the State Key Laboratory for Infectious Disease Prevention and Control, National Institute for Communicable Disease Control and Prevention (ICDC), Chinese Center for Disease Control and Prevention, Beijing, China. These isolates originated from hospitals in eight participating cities and provinces in mainland China, including Guangdong, Guangxi, Henan, Fujian, Sichuan, Beijing, Shanghai, and Chongqing. All isolates were serotyped according to the Kauffmann-White scheme.

Antimicrobial susceptibility testing. 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 (17). Nineteen antimicrobials were tested: ampicillin, amoxicillin-clavulanic acid, ceftazidime, ceftiofur, cefotaxime, ceftriaxone, meropenem, chloramphenicol, gentamicin, kanamycin, streptomycin, nalidixic acid, ciprofloxacin, sulfamethoxazole, tetracycline, trimethoprim, erythromycin, amikacin, and azithromycin. Olaquindox was also tested. *Escherichia coli* strains ATCC 25922 and 35218, *Enterococcus faecalis* strain ATCC 29212, *Staphylococcus aureus* strain ATCC 29213, and *Pseudomonas aeruginosa* ATCC 27853 were used as quality controls.

Received 20 December 2013 Returned for modification 27 January 2014
Accepted 13 April 2014
Published ahead of print 21 April 2014
Address correspondence to Sheng Chen, sheng.chen@polyu.edu.hk.
M.H.Y.W. and M.Y. contributed equally to the work.

doi:10.1128/AAC.02770-13

PMQR genes and target gene mutation screening. The quinolone resistance-determining regions (QRDRs) of *gyrA* and *parC* were amplified by PCR as previously described (18), followed by determination of their nucleotide sequences and comparison to the wild-type *Salmonella* Typhimurium LT2 strain to identify target gene mutations in the test isolates. The presence of the PMQR genes *qnrA*, *qnrB*, *qnrC*, *qnrD*, *qnrS*, *qepA*, *oqxAB*, and *aac(6′)Ib-cr* was determined by PCR using primers described previously and sequencing (18, 19). The prevalence of ESBLs was determined by PCR targeting known β-lactamases as previously described (10). The full lengths of β-lactamase genes were amplified by specific primers for CTX-M group 1 (forward [F], 5′-ATGGTTAAAAAATCACTGCG; reverse [R], 5′ TTACAAACCGTCGGTGAC), CTX-M group 2 (F, 5′-ATGATGACTCAGAGCATTCGC; R, 5′-TCAGAAACCGTGGGTTACGAT), CTX-M group 9 (F, 5′-ATTCAGAGCTCATGGTGACAAAGAGAGTGC; R, 5′-TAGTAGGATCCTTACAGCCCTTCGGCGATG), and CMY (F, 5′-ATGATGAAAAAATCGTTATGCT; R, 5′-ATTGCAGCTTTTCAAGAAT), followed by nucleotide sequencing to determine their type specificity.

Conjugation experiments. A conjugative experiment was carried out as previously described (20) using sodium azide-resistant *E. coli* strain J53 as the recipient. Briefly, overnight cultures of donor and recipient strains were mixed and collected on a filter, which was subjected to overnight incubation on a blood agar plate. The mixture was then spread on double selective blood agar plates containing ceftriaxone (2 μg/ml) and sodium azide (100 μg/ml) to select drug-resistant transconjugants. Plasmid replicon typing was performed as previously described (21).

Molecular typing. The clonal relationship between representative *Salmonella* isolates was examined by pulsed-field gel electrophoresis (PFGE) according to the PulseNet PFGE protocol for *Salmonella* (22). S1-PFGE was conducted to determine the size of large plasmids. Briefly, agarose-embedded DNA was digested with S1 nuclease (New England BioLabs) at 37°C for 1 h. The restriction fragments were separated by electrophoresis in 0.5 mM 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. A phage Lambda PFGE ladder (New England BioLabs) was used as the DNA size marker. The gels were stained with GelRed, and DNA bands were visualized with UV transillumination (Bio-Rad). Multilocus sequence typing was performed using primer sets as suggested at www.mlst.net.

RESULTS AND DISCUSSION

A total of 546 human clinical *S.* Typhimurium isolates, accounting for approximately 40% (1,380) of all *S.* Typhimurium isolates and 9% (5,873) of all *Salmonella* isolates collected from the State Key Laboratory for Infectious Disease Prevention and Control, National Institute for Communicable Disease Control and Prevention (ICDC) of China, during 2005 to ~2011 were included in this study. A substantial proportion of these isolates displayed resistance (MIC, ≥4 μg/ml) (20%) or intermediate resistance (MIC, 2 μg/ml) (16%) to ciprofloxacin. Around 4% of the isolates were resistant to extended-spectrum cephalosporins (Table 1). Profiles of resistance to other antimicrobials are shown in Table 1. All *Salmonella* isolates were susceptible to meropenem.

The vast majority of 109 (20%) isolates which were resistant to ciprofloxacin also exhibited high-level resistance to other classes of antimicrobials, including sulfamethoxazole (100%), chloramphenicol (97%), ampicillin (94%), tetracycline (94%), kanamycin (92%), trimethoprim (92%), amoxicillin-clavulanic acid (87%), gentamicin (82%), streptomycin (67%), and extended-spectrum cephalosporins (9%) (Table 1). The annual distribution of ciprofloxacin-resistant *Salmonella* isolates from 2005 to ~2011 was 11%, 37%, 20%, 12%, 9%, and 33%, respectively. Among the 22 ceftriaxone-resistant *S.* Typhimurium isolates, the rates of resistance to different antimicrobials were the following: kanamycin (92%), nalidixic acid (91%), sulfamethoxazole (86%), tetracycline (86%), gentamicin (82%), trimethoprim (72%), streptomycin (64%), and chloramphenicol (59%). Most importantly, 12 of the 22 ceftriaxone-resistant *S.* Typhimurium isolates also exhibited resistance to ciprofloxacin. Not surprisingly, these isolates exhibited extremely high rates and levels of resistance to all other antimicrobials. Three of them were susceptible to only one antimicrobial (Table 1). Among these 12 extremely drug-resistant (XDR) *S.* Typhimurium isolates, two were also resistant to azithromycin; thus, these two isolates were concurrently resistant to ciprofloxacin, ceftriaxone, and azithromycin (Table 1). Human clinical *Salmonella* isolates that were resistant to all three of these choices of treatment were only recently reported for *S.* Kentucky ST198-X1, a much less prevalent serotype (16). The emergence of *S.* Typhimurium strains resistant to these three antimicrobials warrants continuous monitoring of the trend of development of antimicrobial resistance in China.

Mechanisms of resistance to ciprofloxacin and ceftriaxone in the 12 XDR *S.* Typhimurium isolates were investigated. Double *gyrA* (S83F, D87N) mutations and a single *parC* (S80R) mutation were detectable in 8 out of 12 XDR *S.* Typhimurium isolates. The PMQR genes *aac(6′)-Ib-cr* and *qeqA* were detected in two isolates (Table 2). In contrast to other reports, the remaining 4 isolates harbored only a single *gyrA* (D87N) mutation. However, *oqxAB* was detected in all four of these isolates. One of them also con-

TABLE 1 Rates of *S.* Typhimurium resistance to 18 different antimicrobial agents

Antimicrobial	% Resistance[a]			
	Overall (n = 546)	CIP (MIC, ≥4 μg/ml) (n = 109)	CTZ (MIC, ≥4 μg/ml) (n = 22)	CIP, CTZ (MIC, ≥4 μg/ml) (n = 12)
Ampicillin	47	94	100	100
Amoxicillin-clavulanic acid[b]	6 (20)	87 (6)	100	100
Ceftazidime	2	9	50	50
Ceftiofur	4	9	100	100
Cefotaxime	4	9	100	100
Ceftriaxone	4	9	100	100
Meropenem	0	0	0	0
Chloramphenicol	43	97	59	100
Gentamicin	35	82	82	92
Kanamycin	44	92	92	100
Streptomycin	28	67	64	92
Nalidixic acid	63	100	91	100
Ciprofloxacin[b]	20 (16)	100	55	100
Sulfamethoxazole	55	100	86	100
Tetracycline	49	94	86	92
Trimethoprim	42	92	73	100
Erythromycin	93[c]	97[c]	100[c]	100
Amikacin	ND	ND	ND	17
Azithromycin	ND	ND	ND	17[d]

[a] CIP, ciprofloxacin; CTZ, ceftriaxone; ND, not determined. CIP (MIC, ≥4 μg/ml), *Salmonella* isolates with ciprofloxacin MIC of ≥4 μg/ml; CTZ (MIC, ≥4 μg/ml), *Salmonella* isolates with ceftriaxone MIC of ≥4 μg/ml; CIP, CTZ (MIC, ≥4 μg/ml), *Salmonella* isolates with both ciprofloxacin and ceftriaxone MICs of ≥4 μg/ml.

[b] Values in parentheses indicate percentages of isolates with intermediate resistance.

[c] Percentage of isolates with a MIC of ≥64 μg/ml.

[d] Since there is no breakpoint for azithromycin, it is commonly accepted that azithromycin MIC of ≥64 μg/ml in *Salmonella* is considered resistant to azithromycin.

TABLE 2 Summary of genotypes and phenotypes of *S.* Typhimurium isolates and their corresponding transconjugants

Isolate	MLST	MIC[a] (μg/ml)			Non-β-lactam resistance profile[b]	β-Lactamase	QRDR mutation(s)[c]		PMQR gene(s)	Plasmid replicon(s)	Plasmid size(s) (ca. kb)
		CIP	CTX	CEF			GyrA	ParC			
ST414	19	≥8	≥128	≥64	NAL-CHL-GEN-KAN-SUL-TRI-TET	CTX-M-28	S83F, D87N	S80R		FIB, FIIAs	ND
ST429	19	≥8	≥128	8	NAL-CHL-AMK-GEN-KAN-SUL-TRI-TET-AZR	CTX-M-14	S83F, D87N	S80R	*qepA*	FIB, FIIAs	70, 120, 170
TC-ST429		<0.025	≥128	4	AMK-GEN	CTX-M-14	ND	ND		FIB	70
ST494	34	≥8	≥128	2	NAL-CHL-GEN-SUL-KAN-TRI-TET	CTX-M-104	S83F, D87N	S80R	*aac(′)-Ib-cr*	HI2	ND
ST249	19	≥8	≥128	8	NAL-CHL-GEN-SUL-KAN-TRI-AZR	CTX-M-14	S83F, D87N	S80R		FIB, FIIAs	50, 97
ST512	19	≥8	≥128	4	NAL-CHL-GEN-SUL-KAN-TRI-TET	CTX-M-14	S83F, D87N	S80R		FIIAs	100
ST588	34	4	≥128	4	NAL-CHL-GEN-SUL-KAN-TRI-TET	CTX-M-14	D87N	WT	*oqxAB*	I1-Iγ	120, 200
TC-ST588		<0.025	≥128	4	GEN	CTX-M-14	ND	ND		I1-Iγ	120
ST297	19	4	≥128	4	NAL-GEN-TET-SUL-KAN-TRI	CTX-M-14	S83F, D87N	S80R		I1-Iγ, FIIAs	47, 90, 110, 200
TC-ST297		<0.025	64	4	GEN	CTX-M-14	ND	ND		I1-Iγ	110
ST277	19	≥8	≥128	64	NAL-CHL-KAN-SUL-TRI-TET	CTX-M-79	S83F, D87N	S80R		I1-Iγ, HI1	80, 220
TC-ST277		<0.025	64	32		CTX-M-79	ND	ND		I1-Iγ	80
ST241	34	≥8	≥128	4	NAL-CHL-GEN-SUL-KAN-TRI-TET	CTX-M-14	S83F, D87N	S80R	*aac(6′)-Ib-cr*, *oqxAB*	FIB, FIIAs	90, 97
ST426	34	4	≥128	4	NAL-CHL-GEN-SUL-KAN-TRI-TET	CTX-M-14	D87Y	WT	*oqxAB*	B/O, HI2	48.5, 242
ST566	19	4	64	64	NAL-CHL-GEN-SUL-KAN-TRI-TET	CMY-2	S83F	WT	*oqxAB*	Y, FIB	ND
SH111302	34	4	≥128	32	NAL-CHL-GEN-SUL-KAN-TRI-TET	CTX-M-27	D87Y	WT	*oqxAB*	HI2	ND

[a] CIP, ciprofloxacin; CTX, cefotaxime; CEF, ceftazidime.
[b] NAL, nalidixic acid; CHL, chloramphenicol; AMK, amikacin; GEN, gentamicin; KAN, kanamycin; SUL, sulfamethoxazole; TRI, trimethoprim; TET, tetracycline; AZR, azithromycin.
[c] WT, wild type; ND, not determined.

tained *aac(6′)-Ib-cr* (Table 2). A single *gyrA* mutation, together with *oqxAB*-mediated efflux, was shown to be a novel mechanism of ciprofloxacin resistance in *S.* Typhimurium. Further works will be needed to evaluate the relative roles of OqxAB and single-target-gene mutation in the development of fluoroquinolone resistance in *S.* Typhimurium. Recently, our laboratory confirmed that clonal expansion of an *S.* Typhimurium ST34 clone carrying multiple resistance determinants, in particular *oqxAB* and *aac(6′)-Ib-cr*, occurred in both China and Hong Kong (12). Therefore, findings of this work further highlight the role of the mobile OqxAB pump in the emergence of XDR phenotypes in *Salmonella* and the subsequent clonal expansion of such XDR isolates.

The ESBL screening and characterization for these *S.* Typhimurium isolates showed that different variants of the CTX-M family of β-lactamases were detectable in XDR *S.* Typhimurium and that they most likely contributed to the ceftriaxone resistance phenotypes of the host isolates. $bla_{CTX\text{-}M\text{-}14}$ was detectable in seven isolates, and $bla_{CTX\text{-}M\text{-}27}$, $bla_{CTX\text{-}M\text{-}28}$, $bla_{CTX\text{-}M\text{-}79}$, $bla_{CTX\text{-}M\text{-}104}$, and $bla_{CMY\text{-}2}$ could be detected in each of the other five *S.* Typhimurium isolates (Table 2). Detection of $bla_{CTX\text{-}M\text{-}27}$ and $bla_{CTX\text{-}M\text{-}28}$ was reported for *S.* Livingstone and *S.* Isangi, respectively, in 2005 (23, 24). This work constitutes the first report of $bla_{CTX\text{-}M\text{-}79}$ and $bla_{CTX\text{-}M\text{-}104}$ in *Salmonella*, and $bla_{CTX\text{-}M\text{-}27}$ and $bla_{CTX\text{-}M\text{-}28}$ were also found in *S.* Typhimurium for the first time. Both CTX-M-104 and CTX-M-79 have been identified in *E. coli* (25, 26). CTX-M-104 differs from CTX-M-14 at position 275, with N^{275} in CTX-M-104 and S^{275} in CTX-M-14. CTX-M-79 differs from CTX-M-55 at position 289, with N^{289} in CTX-M-79 and D^{289} in CTX-M-55. Conjugation experiments were conducted for all XDR *Salmonella* isolates listed in Table 2 and showed that four *S.* Typhimurium clones, ST429, ST588, ST297, and ST277, were able to transfer their ESBL determinants to *E. coli* J53. The gentamicin resistance determinants of ST588 and ST297 and gentamicin and amikacin resistance determinants of ST429 were also transferrable (Table 2). The resistance determinant for azithromycin was not transferable.

S1-PFGE showed that most of the isolates possessed several large-sized plasmids (Fig. 1 and Table 2). Transconjugants of *S.* Typhimurium harbored plasmids with sizes ranging from 70 kb to 120 kb. IncF- and I1-Iγ-type plasmids were the most commonly found replicon types, followed by FIIAs, F1B, HI2, Y, and B/O (Table 2). Among the transconjugants, 3 out of 4 harbored an I1-Iγ-type plasmid, and the remaining one carried the F1B type. The I1-Iγ-type plasmids were found to be dominant among *Salmonella* strains carrying $bla_{CMY\text{-}2}$ (6). This study reports the detection of different $bla_{CTX\text{-}M}$-containing I1-Iγ-type plasmids for the first time and provides evidence that plasmids of this type are disseminating among *S.* Typhimurium strains in China.

The clonal relationships among the 12 XDR *S.* Typhimurium isolates were determined. These 12 isolates were found to display unrelated PFGE profiles which were not the dominant PFGE types in China (Fig. 2). XDR *Salmonella* isolates were commonly seen in Henan Province in 2007 and 2008 and became common in Shanghai in 2010 and 2011 (Fig. 2). Nevertheless, multilocus sequence typing showed that 7 of the *S.* Typhimurium isolates belonged to ST19, a common clone causing human infections worldwide (27–29). The remaining 5 isolates belonged to ST34, which is associ-

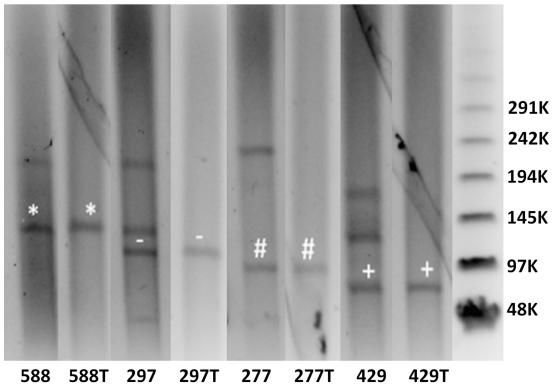
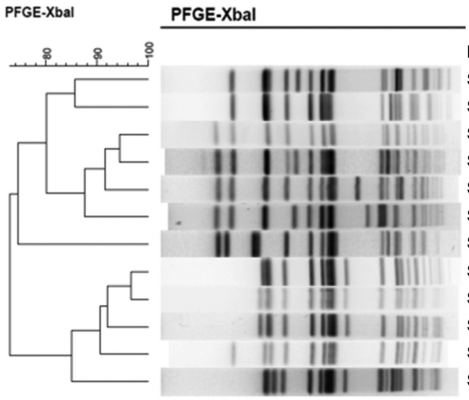


FIG 1 Plasmid profiles of representative *S.* Typhimurium isolates and transconjugants determined by S1-PFGE. Transmissible plasmids from *S.* Typhimurium and transconjugants (T) are highlighted. The same symbol in the parental isolate and transconjugant indicates the position of the conjugative plasmid. The marker is a phage lambda concatemer with different bands labeled.



ID	Year	Origin	Location	ESBL	PMQR	MLST
ST566	2010	Stool	Shanghai	CMY-2	*oqxAB*	ST19
ST277	2010	Stool	Shanghai	CTX-M-79		ST19
ST512	2010	Stool	Sichuan	CTX-M-14		ST19
ST241	2007	Stool	Henan	CTX-M-14	*oqxAB, aac(6')-Ib-cr*	ST34
ST429	2008	Stool	Henan	CTX-M-14	qepA	ST19
ST249	2007	Stool	Henan	CTX-M-14		ST19
ST297	2007	Stool	Sichuan	CTX-M-14		ST19
ST494	2010	Stool	Shanghai	CTX-M-104	*aac(6')-Ib-cr*	ST34
ST588	2010	Stool	Guangxi	CTX-M-14	*oqxAB*	ST34
ST414	2008	Stool	Henan	CTX-M-28		ST19
SH111302	2011	Stool	Shanghai	CTX-M-27	*oqxAB*	ST34
ST426	2008	Stool	Henan	CTX-M-14	*oqxAB*	ST34

FIG 2 PFGE patterns of clinical *S.* Typhimurium isolates that are resistant to both ciprofloxacin and ceftriaxone. The isolate identity (ID), year of isolation, origin, and location of isolation, as well as the ESBL, PMQR gene(s), and multilocus sequence types, are also indicated.

ated with a monophasic variant of *S.* Typhimurium in Europe with the ASSuTe R-type (27) (Table 2 and Fig. 2).

In conclusion, this study reported the emergence of *S.* Typhimurium human clinical isolates that were resistant to all classes of antimicrobials tested, including ceftriaxone, ciprofloxacin, and azithromycin, which are common choices of treatment. The selection of these XDR *S.* Typhimurium isolates may be due to the increasing transmission of different ESBL-encoding elements among ciprofloxacin-resistant *S.* Typhimurium organisms whose resistance phenotypes are in turn mediated by conventional and novel mechanisms, including a single *gyrA* mutation and a PMQR gene, *oqxAB*. Transmission of resistance genes such as those encoding ESBLs and the OqxAB pump in *Salmonella* will speed up the selection of these XDR *Salmonella* organisms, which poses a huge threat to human *Salmonella* infection control. Further works are required to trace the transmission routes of XDR *Salmonella* strains in clinical settings and develop appropriate intervention strategies.

ACKNOWLEDGMENTS

This work was supported by the Chinese National Key Basic Research and Development (973) Program (2013CB127200) and the Research Fund for the Control of Infectious Diseases of the Food and Health Bureau, the Government of the Hong Kong SAR (grants 11101192 and 12111542 to S.C.).

We have no conflicts of interests to declare.

REFERENCES

1. **White DG, Zhao S, Sudler R, Ayers S, Friedman S, Chen S, McDermott PF, McDermott S, Wagner DD, Meng J.** 2001. The isolation of antibiotic-resistant salmonella from retail ground meats. N. Engl. J. Med. **345:** 1147–1154. http://dx.doi.org/10.1056/NEJMoa010315.
2. **Glynn MK, Bopp C, Dewitt W, Dabney P, Mokhtar M, Angulo FJ.** 1998. Emergence of multidrug-resistant Salmonella enterica serotype typhimurium DT104 infections in the United States. N. Engl. J. Med. **338:**1333–1338. http://dx.doi.org/10.1056/NEJM199805073381901.
3. **Sjolund-Karlsson M, Joyce K, Blickenstaff K, Ball T, Haro J, Medalla FM, Fedorka-Cray P, Zhao S, Crump JA, Whichard JM.** 2011. Antimicrobial susceptibility to azithromycin among Salmonella enterica isolates from the United States. Antimicrob. Agents Chemother. **55:**3985–3989. http://dx.doi.org/10.1128/AAC.00590-11.
4. **Antunes P, Machado J, Sousa JC, Peixe L.** 2004. Dissemination amongst humans and food products of animal origin of a Salmonella typhimurium clone expressing an integron-borne OXA-30 beta-lactamase. J. Antimicrob. Chemother. **54:**429–434. http://dx.doi.org/10.1093/jac/dkh333.
5. **Hopkins KL, Liebana E, Villa L, Batchelor M, Threlfall EJ, Carattoli A.** 2006. Replicon typing of plasmids carrying CTX-M or CMY beta-lactamases circulating among Salmonella and Escherichia coli isolates. Antimicrob. Agents Chemother. **50:**3203–3206. http://dx.doi.org/10.1128/AAC.00149-06.
6. **Mataseje LF, Xiao J, Kost S, Ng LK, Dore K, Mulvey MR.** 2009. Characterization of Canadian cefoxitin-resistant non-typhoidal Salmonella isolates, 2005–06. J. Antimicrob. Chemother. **64:**723–730. http://dx.doi.org/10.1093/jac/dkp249.
7. **Hooper DC.** 2001. Emerging mechanisms of fluoroquinolone resistance. Emerg. Infect. Dis. **7:**337–341. http://dx.doi.org/10.3201/eid0702.010239.
8. **Kim JH, Cho JK, Kim KS.** 2013. Prevalence and characterization of plasmid-mediated quinolone resistance genes in Salmonella isolated from poultry in Korea. Avian Pathol. **42:**221–229. http://dx.doi.org/10.1080/03079457.2013.779636.
9. **Ferrari R, Galiana A, Cremades R, Rodriguez JC, Magnani M, Tognim MC, Oliveira TC, Royo G.** 2011. Plasmid-mediated quinolone resistance by genes qnrA1 and qnrB19 in Salmonella strains isolated in Brazil. J. Infect. Dev. Ctries. **5:**496–498. http://dx.doi.org/10.3855/jidc.1735.
10. **Wong MH, Chen S.** 2013. First detection of oqxAB in Salmonella spp. isolated from food. Antimicrob. Agents Chemother. **57:**658–660. http://dx.doi.org/10.1128/AAC.01144-12.
11. **Li L, Liao X, Yang Y, Sun J, Liu B, Yang S, Ma J, Li X, Zhang Q, Liu Y.** 2013. Spread of oqxAB in Salmonella enterica serotype Typhimurium predominantly by IncHI2 plasmids. J. Antimicrob. Chemother. **68:**2263–2268. http://dx.doi.org/10.1093/jac/dkt209.
12. **Wong MHY, Yan M, Chan EWC, Liu LZ, Kan B, Chen S.** 2013. Expansion of *Salmonella enterica* serovar Typhimurium ST34 clone carrying multiple resistance determinants in China. Antimicrob. Agents Chemother **57:**4599–4601. http://dx.doi.org/10.1128/AAC.01174-13.
13. **Su LH, Teng WS, Chen CL, Lee HY, Li HC, Wu TL, Chiu CH.** 2011. Increasing ceftriaxone resistance in Salmonellae, Taiwan. Emerg. Infect. Dis. **17:**1086–1090. http://dx.doi.org/10.3201/eid/1706.101949.
14. **Wong MH, Zeng L, Liu JH, Chen S.** 2013. Characterization of Salmonella food isolates with concurrent resistance to ceftriaxone and ciprofloxacin. Foodborne Pathog. Dis. **10:**42–46. http://dx.doi.org/10.1089/fpd.2012.1266.
15. **Hendriksen RS, Joensen KG, Lukwesa-Musyani C, Kalondaa A, Leekitcharoenphon P, Nakazwe R, Aarestrup FM, Hasman H, Mwansa JC.** 2013. Extremely drug-resistant Salmonella enterica serovar Senftenberg infections in patients in Zambia. J. Clin. Microbiol. **51:**284–286. http://dx.doi.org/10.1128/JCM.02227-12.
16. **Le Hello S, Harrois D, Bouchrif B, Sontag L, Elhani D, Guibert V, Zerouali K, Weill FX.** 2013. Highly drug-resistant Salmonella enterica serotype Kentucky ST198-X1: a microbiological study. Lancet Infect. Dis. **13:**672–679. http://dx.doi.org/10.1016/S1473-3099(13)70124-5.
17. **CLSI.** 2013. Performance standards for antimicrobial susceptibility testing. CLSI document M100-S23. Clinical and Laboratory Standards Institute, Wayne, PA.
18. **Chen S, Cui S, McDermott PF, Zhao S, White DG, Paulsen I, Meng J.** 2007. Contribution of target gene mutations and efflux to decreased susceptibility of Salmonella enterica serovar typhimurium to fluoroquinolo-

nes and other antimicrobials. Antimicrob. Agents Chemother. **51:**535–542. http://dx.doi.org/10.1128/AAC.00600-06.

19. **Deng Y, Zeng Z, Chen S, He L, Liu Y, Wu C, Chen Z, Yao Q, Hou J, Yang T, Liu JH.** 2011. Dissemination of IncFII plasmids carrying rmtB and qepA in Escherichia coli from pigs, farm workers and the environment. Clin. Microbiol. Infect. **17:**1740–1745. http://dx.doi.org/10.1111/j.1469-0691.2011.03472.x.
20. **Jacoby GA, Chow N, Waites KB.** 2003. Prevalence of plasmid-mediated quinolone resistance. Antimicrob. Agents Chemother. **47:**559–562.
21. **Carattoli A, Bertini A, Villa L, Falbo V, Hopkins KL, Threlfall EJ.** 2005. Identification of plasmids by PCR-based replicon typing. J. Microbiol. Methods **63:**219–228. http://dx.doi.org/10.1016/j.mimet.2005.03.018.
22. **Ribot EM, Fair MA, Gautom R, Cameron DN, Hunter SB, Swaminathan B, Barrett TJ.** 2006. Standardization of pulsed-field gel electrophoresis protocols for the subtyping of Escherichia coli O157:H7, Salmonella, and Shigella for PulseNet. Foodborne Pathog. Dis. **3:**59–67. http://dx.doi.org/10.1089/fpd.2006.3.59.
23. **Bouallegue-Godet O, Ben Salem Y, Fabre L, Demartin M, Grimont PA, Mzoughi R, Weill FX.** 2005. Nosocomial outbreak caused by Salmonella enterica serotype Livingstone producing CTX-M-27 extended-spectrum beta-lactamase in a neonatal unit in Sousse, Tunisia. J. Clin. Microbiol. **43:**1037–1044. http://dx.doi.org/10.1128/JCM.43.3.1037-1044.2005.
24. **Hasman H, Mevius D, Veldman K, Olesen I, Aarestrup FM.** 2005. Beta-lactamases among extended-spectrum beta-lactamase (ESBL)-resistant Salmonella from poultry, poultry products and human patients in The Netherlands. J. Antimicrob. Chemother. **56:**115–121. http://dx.doi.org/10.1093/jac/dki190.
25. **Zheng H, Zeng Z, Chen S, Liu Y, Yao Q, Deng Y, Chen X, Lv L, Zhuo C, Chen Z, Liu JH.** 2012. Prevalence and characterisation of CTX-M beta-lactamases amongst Escherichia coli isolates from healthy food animals in China. Int. J. Antimicrob. Agents **39:**305–310. http://dx.doi.org/10.1016/j.ijantimicag.2011.12.001.
26. **Jiang HX, Tang D, Liu YH, Zhang XH, Zeng ZL, Xu L, Hawkey PM.** 2012. Prevalence and characteristics of beta-lactamase and plasmid-mediated quinolone resistance genes in Escherichia coli isolated from farmed fish in China. J. Antimicrob. Chemother. **67:**2350–2353. http://dx.doi.org/10.1093/jac/dks250.
27. **Antunes P, Mourao J, Pestana N, Peixe L.** 2011. Leakage of emerging clinically relevant multidrug-resistant Salmonella clones from pig farms. J. Antimicrob. Chemother. **66:**2028–2032. http://dx.doi.org/10.1093/jac/dkr228.
28. **Antunes P, Coque TM, Peixe L.** 2010. Emergence of an IncIgamma plasmid encoding CMY-2 ss-lactamase associated with the international ST19 OXA-30-producing ss-lactamase Salmonella Typhimurium multidrug-resistant clone. J. Antimicrob. Chemother. **65:**2097–2100. http://dx.doi.org/10.1093/jac/dkq293.
29. **Cooke FJ, Brown DJ, Fookes M, Pickard D, Ivens A, Wain J, Roberts M, Kingsley RA, Thomson NR, Dougan G.** 2008. Characterization of the genomes of a diverse collection of Salmonella enterica serovar Typhimurium definitive phage type 104. J. Bacteriol. **190:**8155–8162. http://dx.doi.org/10.1128/JB.00636-08.