

Expansion of *Salmonella enterica* Serovar Typhimurium ST34 Clone Carrying Multiple Resistance Determinants in China

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Nontyphoidal salmonellae are among the principal bacterial pathogens implicated in cases of food-borne gastroenteritis worldwide (1). Multidrug resistance, including ampicillin, chloramphenicol, streptomycin, sulbactam, and tetracycline (ACSSuT) resistance and ciprofloxacin or ceftriaxone resistance, has occurred in strains of *Salmonella enterica* serovar Typhimurium and has had significant impact on the effectiveness of current strategies to control and manage diseases associated with food-borne infections (2–5). A comprehensive review of data from the literature indicated that *S. Typhimurium* produced up to a 3-fold-higher mortality rate than an average *Salmonella* infection. Worse still, the ACSSuT, nalidixic acid (Nal), and ACSSuT-Nal resistance types were, respectively, associated with 4.8-, 10.3-, and 13.1-fold-higher mortality rates (6).

In this study, 239 human clinical *S. Typhimurium* isolates from hospitals in Hong Kong during 2005 to 2011 and 546 human clinical *S. Typhimurium* isolates 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, in China during 200 to 2011 were characterized for their resistance to 15 antimicrobials, as shown in Table 1, according to CLSI guidelines (7). *S. Typhimurium* from both Hong Kong and China showed high levels of resistance to quinolone, fluoroquinolones, and other antibiotics, such as ACSSuT and trimethoprim. In particular, the rates of resistance to nalidixic acid of *S. Typhimurium*

strains from Hong Kong and China were 73% and 63%, respectively. The corresponding rates of resistance to ciprofloxacin (MIC \geq 2 mg/liter) were 34% and 36%, respectively (Table 1).

The mechanisms mediating the high prevalence of fluoroquinolone resistance in *S. Typhimurium* was investigated. We previously found that *oqxAB*, a plasmid-mediated resistance-nodulation-cell division (RND) efflux pump conferring resistance to multiple antibiotics, existed in *Salmonella* isolates recovered from food samples (8). We then assessed the prevalence of *oqxAB* in human clinical *S. Typhimurium* isolates. It showed that the prevalence of *oqxAB* increased markedly in *S. Typhimurium* from 2005 to 2011. Among the Hong Kong isolates, the overall *oqxAB*-positive rate was about 28% (67 out of 239 isolates), yet this resistance determinant was not detectable in salmonellae in 2005 and an increasing trend was observed only from 2006 onwards, with positivity rates of 12%, 24%, 34%, 34%, 37%, and 43% detectable among the *S. Typhimurium* isolates recovered in 2006 through 2011, respectively. A similar trend was observable among the

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TABLE 1 Rates of resistance to 15 antimicrobial agents of *oqxAB*-positive and -negative *S. Typhimurium* isolates from Hong Kong and China

Antibiotics(s)	Breakpoint(s) (μ g/ml)	% of resistant strains (% of strains exhibiting intermediate resistance)					
		Hong Kong isolates (<i>n</i> = 239)			ICDC isolates (<i>n</i> = 546)		
		Overall	<i>oqxAB</i> positive (<i>n</i> = 67)	<i>oqxAB</i> negative (<i>n</i> = 172)	Overall	<i>oqxAB</i> positive (<i>n</i> = 159)	<i>oqxAB</i> negative (<i>n</i> = 387)
Ampicillin	\geq 32	67	100	55	47 (3)	96 (1)	27
Amoxicillin-clavulanic acid	\geq 32, \geq 16	6 (18)	15 (40)	3 (9)	6 (20)	17 (45)	2 (10)
Cefotaxime	\geq 4	3	3	3	4	3	5
Ceftriaxone	\geq 4	3	3	3	4	3	5
Ceftazidime	\geq 16	3	3	3	2	1	2
Chloramphenicol	\geq 32	50	100	30	43	100	20
Gentamicin	\geq 16	23	42	15	35	70	21
Nalidixic acid	\geq 32	73	100	63	63	100	48
Ciprofloxacin	\geq 4	11 (23)	18 (76)	9 (2)	20 (16)	48 (50)	9 (2)
Streptomycin		52	61	49	28	42	22
Sulfamethoxazole	\geq 512	70	100	59	55	100	36
Tetracycline	\geq 16	68	88	60	49	96	30
Kanamycin	\geq 32	36	63	26	44	85	27
Trimethoprim	\geq 16	38	73	25	42	100	18
Olaquinox	\geq 32	28	100	0	29	100	0

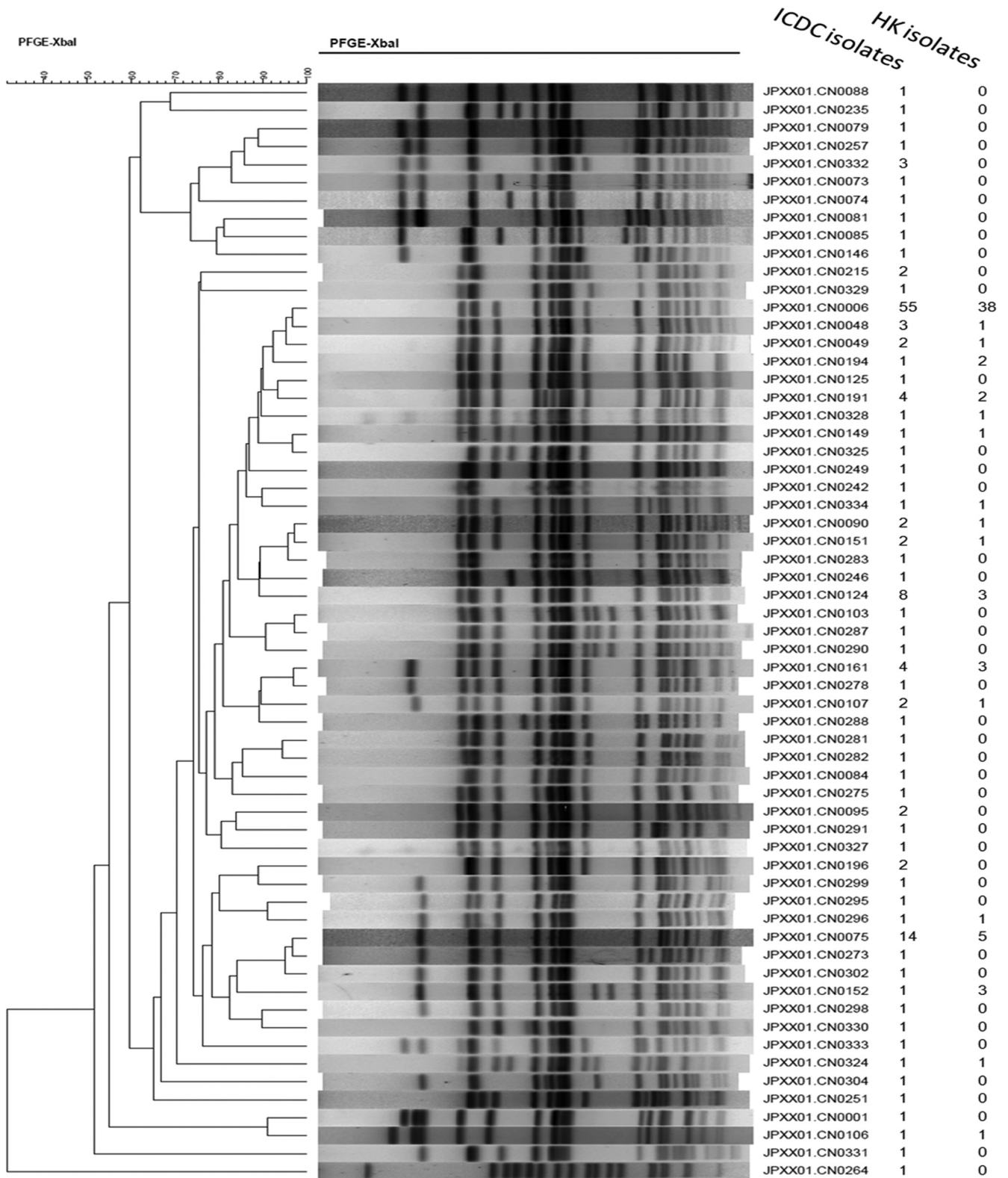


FIG 1 PFGE patterns of *oqxAB*-positive *S. Typhimurium* strains recovered in Hong Kong and China during the period of 2006 to 2011. All *oqxAB*-positive *S. Typhimurium* strains in this study from Hong Kong (HK) and China (ICDC) were subjected to XbaI digestion and PFGE analysis.

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ICDC isolates. The overall *oqxAB*-positive rate of *S. Typhimurium* strains was 29% (159 out of 546 isolates), and the yearly rates were 0%, 13%, 26%, 32%, 36%, 39%, and 42% during the years 2005 to 2011, respectively.

Screening of other plasmid-mediated quinolone resistance (PMQR) genes [*qnrA*, *qnrB*, *qnrC*, *qnrD*, *qnrS*, *qepA*, *oqxAB*, and *aac(6')Ib-cr*] in these *S. Typhimurium* isolates detected the high prevalence of *aac(6')Ib-cr* in 85% and 83% of the *oqxAB*-positive *S. Typhimurium* isolates from Hong Kong and China, respectively, while none of the *oqxAB*-negative *S. Typhimurium* isolates were positive for *aac(6')Ib-cr*. No other PMQR genes were detected in these *S. Typhimurium* isolates. *oqxAB* was also found to be associated with multidrug resistance in *S. Typhimurium*. Among the *S. Typhimurium* isolates of the *oqxAB*-positive group, 94% and 98% from Hong Kong and the ICDC, respectively, were resistant to ciprofloxacin (MIC \geq 2 mg/liter), while the corresponding resistance rate in *oqxAB*-negative *S. Typhimurium* Hong Kong and ICDC isolates was only 11% (Table 1). In addition, the ACSSuT resistance type was detected in 58% and 53% of the *oqxAB*-positive *S. Typhimurium* isolates from Hong Kong and China, respectively, while the corresponding rate in *oqxAB*-negative *S. Typhimurium* was only around 10%.

The clonal relationships of *oqxAB*-positive salmonella isolates were examined by pulsed-field gel electrophoresis (PFGE) according to the PulseNet PFGE protocol for salmonella (9). For the 159 *oqxAB*-positive ICDC isolates, 61 PFGE patterns were observed among the *oqxAB*-positive strains. Interestingly, the majority (37%) of *oqxAB*-positive isolates belonged to one PFGE type, designated CN0006 (Fig. 1). For 67 *oqxAB*-positive Hong Kong isolates, 18 PFGE patterns were detectable among the *oqxAB*-positive isolates; again 57% of the *oqxAB*-positive strains belonged to the CN0006 type (Fig. 1). Using an 80%-cutoff Dice coefficient index for the PFGE profiles, about 83% and 66% of *S. Typhimurium* isolates from Hong Kong and China, respectively, were related to the CN0006 clone. This finding suggested that CN0006 and related clones are responsible for the expansion of the ACSSuT-ciprofloxacin-*oqxAB*-*aac(6')Ib-cr* type of *S. Typhimurium* in clinical settings in China. Multilocus sequence typing (MLST) was performed using primer sets as suggested at www.mlst.net. Results of MLST showed that all of the 20 randomly selected *oqxAB*-positive strains from Hong Kong and 16 out of the 20 randomly selected *S. Typhimurium* strains from the ICDC belonged to a specific sequence type, ST34, a sequence type that was frequently

associated with the ACSSuT resistance type of *S. Typhimurium*, which is also frequently reported in the European Union (10). The quick expansion of the multidrug-resistant *S. Typhimurium* ST34 clone will pose a notable threat to clinical *Salmonella* infection control. Urgent actions are required to halt its further transmission in both environmental and clinical settings.

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We declare that we have no conflicts of interest.

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