Infection and Drug Resistance

Dovepress
open access to scientific and medical research

Open Access Full Text Article

ORIGINAL RESEARCH

Overview of the development of quinolone resistance in *Salmonella* species in China, 2005–2016

This article was published in the following Dove Press journal:
Infection and Drug Resistance

Qifa Song[1]
Zhaojun Xu[2]
Hong Gao[1]
Danyang Zhang[1]

[1]Department of Microbiology, Ningbo Municipal Centre for Disease Control and Prevention, Ningbo, Zhejiang, China; [2]Intensive Care Unit, Ningbo No. 2 Hospital, Zhejiang, China

Purpose: Several factors contribute to the complexity of quinolone resistance in *Salmonella*, including >2000 different *Salmonella* serotypes, a variety of hosts for *Salmonella*, and wide use of quinolones in human beings and animals. We thus aimed to obtain an overview of the development of quinolone resistance and relevant molecular mechanisms of such a resistance in *Salmonella* species.

Materials and methods: A total of 1,776 *Salmonella* isolates were collected in Ningbo, China, between 2005 and 2016. Antimicrobial susceptibility to quinolone and relevant genetic mechanisms in these isolates were retrospectively analyzed.

Results: The ratio for ciprofloxacin (CIP) resistant:reduced CIP susceptible:CIP susceptible was 26:522:1,228. CIP resistance was found in nine of 51 serotypes: Derby, London, Kentucky, Indiana, Corvallis, Rissen, Hadar, Typhimurium, and Agona. Of 26 CIP-resistant isolates, all were concurrently resistant to ampicillin and 21 were also concurrently resistant to cefotaxime and produced extended-spectrum β-lactamase (ESBL). The minimal inhibitory concentration values were at three levels: 2–4 μg/mL (serotypes except for Kentucky and Indiana), 16 μg/mL (one Kentucky isolate), and >32 μg/mL (Indiana isolates). As with the three most common serotypes, *Salmonella* Typhi showed quickly increased prevalence of reduced CIP susceptibility in recent years, *Salmonella* Enteritidis remained at a high prevalence of reduced CIP susceptibility throughout the study period, and several isolates of *Salmonella* Typhimurium were resistant to CIP. Transferable plasmid-mediated quinolone resistance gene *qnrB* was only found in all CIP-resistant isolates. In contrast, *gyrA* mutations were often found in reduced CIP-susceptible isolates and were not necessarily found in all CIP-resistant isolates.

Conclusion: We conclude that in *Salmonella*, there exists a high prevalence of reduced CIP susceptibility and a low prevalence of CIP resistance, which focuses on several serotypes. Our study also demonstrates that, rather than *gyrA* mutations, *qnrB* is the most common indicator for CIP resistance.

Keywords: quinolone resistance, *Salmonella* species, ciprofloxacin-resistant *Salmonella* isolates, genetic determinant of quinolone resistance

Introduction

Salmonella species (*Salmonella* spp.) are a group of bacteria that can survive in animals, human beings, and the environment. They can cause from mild to life-threatening salmonellosis, such as enteritis and typhoid fever, both in human beings and animals, and have become a major public health issue worldwide.[1] Of >2,000 *Salmonella* serotypes, <100 serotypes account for most of prevalent and important human infections, including

Correspondence: Zhaojun Xu
Ningbo No. 2 Hospital, No. 41, Northwest Street, Ningbo 315010, Zhejiang, China
Tel +86 574 8387 0933
Email Zhaojunxu168@163.com

submit your manuscript | www.dovepress.com
Dovepress
http://dx.doi.org/10.2147/IDR.S157460

Salmonella Typhi (*S.* typhi), *Salmonella* Enteritidis (*S.* Enteritidis), and *Salmonella* Typhimurium (*S.* Typhimurium).[2,3]

Quinolones are a family of synthetic broad-spectrum antimicrobial drugs.[4] The first quinolone is nalidixic acid, which is considered to be the predecessor of all members of the quinolone family. The majority of quinolones in present clinical use are more powerful fluoroquinolones, such as ciprofloxacin (CIP), which remains the first-line drug of choice for treating salmonellosis.[5]

Quinolone resistance in *Salmonella* spp. is an increasing problem.[6] Based on susceptibility testing results of nalidixic acid and CIP, quinolone resistance can be ranked at three levels: resistant to CIP (resistant to both drugs), of reduced CIP susceptibility (resistant to nalidixic acid and intermediate to CIP), and susceptible to both drugs.[7] Recently, particular concern has been raised about highly CIP-resistant strains of several *Salmonella* serotypes circulating in both human beings and the environment.[8,9] To treat salmonellosis cases caused by CIP-resistant *Salmonella* strains, a third-generation cephalosporin, such as cefotaxime or ceftriaxone, is the first choice.[10]

CIP resistance in *Salmonella* is considered to be mainly attributed to point mutations in *gyrA*, *gyrB*, and *parC* in the quinolone resistance-determining regions (QRDRs).[11] In addition, transferable plasmid-mediated quinolone resistance (PMQR) genes may cause low-level resistance to fluoroquinolones.[11] These genes include pentapeptide repeat protein-encoding *qnrA*, *qnrB*, and *qnrS*; an efflux-pump-encoding *qepA*; and an aminoglycoside acetyltransferase-encoding enzyme variant *aac(6′)-Ib-cr*.[12,13]

A lot of studies have been conducted to explore CIP-resistant *Salmonella* strains of several particular serotypes. Most of these studies are some kind of "case report analysis" with limited information on overall quinolone resistance in *Salmonella* species. There are several factors making quinolone resistance complex in *Salmonella*, including >2,000 different *Salmonella* serotypes, a variety of hosts for *Salmonella*, and wide use of quinolones in human beings and animals. This situation calls for a more general conclusion for explaining CIP resistance development in *Salmonella* species as a whole.

Therefore, through investigating the development of quinolone resistance in all *Salmonella* species, what serotypes account for most CIP-resistant isolates, and the relevant molecular mechanisms, we aimed to obtain an overview of development of quinolone resistance and the relevant molecular mechanisms in *Salmonella*.

Materials and methods

Bacterial isolates

A total of 1,776 *Salmonella* isolates, 1,226 from non-duplicate patient specimens (1,006 feces and 220 blood samples) and 550 from environmental samples (445 food samples and 105 river water samples), were collected in Ningbo, a city in mid-east China that has a population of 9 million and a land area of 9,780 km^2, between 2005 and 2016 (Table 1). These isolates were from an enteric disease surveillance project and clinical patients. Approval of clinical isolates from the ethics committee of Ningbo No. 2 Hospital, Zhejiang, China, and written informed consent of patients were obtained. These isolates were identified by API 20E biochemical identification system (bioMerieux, Paris, France) and were typed into 51 serotypes using *Salmonella*-specific antisera (Denka Seiken, Japan). In Ningbo, the top three most found serotypes were

Table 1 Distribution of *Salmonella* isolates by isolation year and their susceptibility to CIP

Serotype	Resistance level	Number of isolates			Total
		2005–2008	2009–2012	2013–2016	
S. typhi (n=188)	Susceptibility	49	39	8	96
	Reduced CIP susceptibility	5	21	66	92
	CIP resistance	–	–	–	–
S. Enteritidis (n=163)	Susceptibility	16	32	17	65
	Reduced CIP susceptibility	23	41	34	98
	CIP resistance	–	–	–	–
S. Typhimurium (n=156)	Susceptibility	22	28	39	89
	Reduced CIP susceptibility	17	16	27	60
	CIP resistance	–	2	5	7
Other serotypes	Susceptibility	220	286	472	978
(48 serotypes and	Reduced CIP susceptibility	36	71	165	272
untypeable; n=1,269)	CIP resistance	1	4	14	19
Total		389	540	847	1,776

Abbreviations: CIP, ciprofloxacin; *S.* typhi; *Salmonella* Typhi; *S.* Enteritidis, *Salmonella* Enteritidis; *S.* Typhimurium, *Salmonella* Typhimurium.

S. typhi (188 isolates), *S.* Enteritidis (163 isolates), and *S.* Typhimurium (156 isolates). All *S.* typhi isolates came from patients. *S.* Typhimurium and S. Enteritidis isolates came from both patients and environmental sources.

Antimicrobial susceptibility testing (AST)

Since comparative study has proved high categorical agreements between E-test and disk diffusion and broth microdilution for the detection of fluoroquinolone resistance in typhoidal and nontyphoidal *Salmonella* serotypes,[14] to screen for quinolone-resistant isolates, we performed AST on all isolates using the disk diffusion method specified in Clinical and Laboratory Standards Institute (CLSI) document M02-A12.[15] AST results were interpreted according to guidelines in CLSI document M100-S27.[16] The antibiotic disks (Oxoid, Hampshire, UK) included CIP (5 μg) and nalidixic acid (30 μg), as well as ampicillin (10 μg), cefotaxime (30 μg), cefotaxime–clavulanate (30/10 μg), gentamicin (10 μg), chloramphenicol (30 μg), and trimethoprim–sulfamethoxazole (1.25/23.75 μg). *Escherichia coli* ATCC 25922 was used as a quality control strain. Isolates with ≥5 mm increase in the zone diameter for cefotaxime–clavulanate vs. the zone diameter for cefotaxime were defined to be producing extended-spectrum β-lactamase (ESBL). Then, minimal inhibitory concentrations (MICs) of CIP and cefotaxime in CIP-resistant isolates were determined using the E-test (Oxoid) method. MICs of nalidixic acid in CIP-resistant isolates were determined using broth microdilution. Based on AST results, isolates were classified into three groups with different CIP resistance levels: susceptibility, reduced CIP susceptibility, and resistance to CIP.

Detection of genetic determinants associated with quinolone and β-lactam resistance

CIP-resistant isolates, 100 randomly selected reduced CIP-susceptible isolates, and 100 randomly selected CIP-susceptible isolates were tested for genes related to quinolone resistance using primers previously described (Table 2), including *gyrA*, *gyrB*, and *parC*; PMQR genes *qnrA*, *qnrB*, and *qnrS*; efflux-pump-encoding *qepA*; and aminoglycoside acetyltransferase-encoding enzyme variant *aac(6′)-Ib-cr*. Amplified sequences of *gyr* and *parC* were sequenced (Thermo Fisher Scientific,

Table 2 Primers used for amplifying genes related to quinolone and β-lactam resistance

Gene	Primers (5′→3′)	Amplicon size (bp)	Reference
For mutations in QRDR			17
gyrA	gyrA-1: CGTTGGTGACGTAATCGGTA	251	
	gyrA-2: CCGTACCGTCATAGTTATCC		
gyrB	gyrB-1: GCGCTGTCCGAACTGTACCT		
	gyrB-2: TGATCAGCGTCGCCACTTCC		
parC	parC-1: CTATGCGATGTCAGAGCTGG	270	
	parC-2: TAACAGCAGCTCGGCGTATT		
For PMQR genes			18
aac(6′)-Ib-cr	aac(6′)-Ib-1: TTGCGATGCTCTATGAGTGGCTA	482	
	aac(6′)-Ib-2: CTCGAATGCCTGGCGTGTTT		
qnrA	qnrA-1: TCAGCAAGAGGATTTCTCA	627	
	qnrA-2: GGCAGCACTATTACTCCCA		
qnrB	qnrB-1: GGMATHGAAATTCGCCACTG	264	
	qnrB-2: TTTGCYGYYCGCCAGTCGAA		
qnrS	qnrS-1: ATGGAAACCTACAATCATAC	467	
	qnrS-2: AAAAACACCTCGACTTAAGT		
qepA	qepA-1: GCAGGTCCAGCAGCGGGTAG	199	
	qepA-2: CTTCCTGCCCGAGTATCGTG		
For β-lactam resistance genes			This study
$bla_{CTX\text{-}M14\text{-}like}$	CTX-M14-1: AAAACTTGCCGAATTAGAGC	711	
	CTX-M14-2: TTAGGTTGAGGCTGGGTGAA		
$bla_{CTX\text{-}M15\text{-}like}$	CTX-M15-1: ATGAACGCTTTCCAATGTGC	461	
	CTX-M15-2: GGTCGTATTGCCTTTGAGCC		
bla_{OXA}	OXA-1: AATGGCACCAGATTCAACTT	593	
	OXA-2: TGGCTTTTATGCTTGATGTT		
bla_{TEM}	TEM-1: TGTCGCCCTTATTCCCTTTT	783	
	TEM-2: ATAGTTGCCTGACTCCCCGT		

Abbreviations: QRDR, quinolone resistance-determining region; PMQR, plasmid-mediated quinolone resistance.

Waltham, MA, USA) and their mutations were determined by aligning against the homologous sequence of *S.* Typhimurium LT2. Additionally, CIP- and β-lactam-resistant isolates were tested for reported β-lactamase genes ($bla_{CTX\text{-}M\text{-}like}$, bla_{TEM}, and bla_{OXA}).

Pulsed-field gel electrophoresis (PFGE)

CIP-resistant isolates were subtyped by PFGE using restriction enzyme *XbaI* following PulseNet standardized protocol.[19] *Salmonella* Braenderup (H9812) was used as a reference strain. The PFGE patterns were determined by BioNumerics software (Applied Maths, Sint-Martens-Latum, Belgium). A dendrogram of PFGE patterns was constructed using the unweighted pair-group method with arithmetic mean (UPGMA) with a position tolerance of 1.2%. PFGE patterns were defined by groups of bands that formed at least 90% Dice similarity cutoffs on the dendrogram. In this study, a cluster was defined as more than one isolate with identical PFGE patterns. Sporadic cases were defined when there was a unique PFGE pattern for one isolate.

Interpretation of results for overall quinolone resistance

We assessed overall quinolone resistance in *Salmonella* species as follows. First, levels of quinolone resistance in the three most common serotypes, *S.* typhi, *S.* Typhimurium, and *S.* Enteritidis, were derived for different isolation periods. Second, what serotypes accounted for, and what PFGE features were found in, highly quinolone-resistant isolates that were resistant to CIP and nalidixic acid, were assessed. Third, what genes related to quinolone resistance existed in quinolone-resistant isolates.

We used SPSS 20 software to calculate Pearson's chi-squared test for comparison of resistance rates between different groups of isolates. Statistical significance was accepted at P value of <0.05.

Results

Totally, of 1,776 isolates, 26 (1.5%) were resistant to CIP and 522 (29%) were of reduced CIP susceptibility (Table 3). The three most common *Salmonella* serotypes were *S.* typhi, *S.* Enteritidis, and *S.* Typhimurium; each had distinct features of quinolone resistance development during three periods from 2005 to 2016 (Table 1). During the three periods of 2005–2008, 2009–2012, and 2013–2016, S. typhi was characteristic of apparent increase in reduced CIP susceptibility: from 9% (5/54) to a moderate level of 35% (21/60) and a high rate of 89% (66/74); *S.* Enteritidis remained at steady, high rates of reduced CIP susceptibility: 59% (23/39), 56% (41/73), and 67% (34/51); in contrast, *S.* Typhimurium showed both a high level of reduced CIP susceptibility, i.e., 44% (17/39), 35% (16/46), and 38% (27/71), and a prevalence of 5% of CIP resistance. Overall, CIP resistance was found in nine of 51 serotypes: Derby, London, Kentucky, Indiana, Corvallis, Rissen, Hadar, Typhimurium, and Agona. Especially, six *Salmonella* Indiana (*S.* Indiana) isolates were resistant to CIP. The MIC values were at three levels in CIP-resistant isolates: 2–4 μg/mL (seven serotypes except for Kentucky and Indiana), 16 μg/mL (one Kentucky isolate), and >32 μg/mL (all Indiana isolates; Table 4).

As to other antimicrobial agents, prevalence of resistance (33.8%) to ampicillin was the highest (Table 3). Average prevalence of resistance to other antimicrobials was significantly higher in the group of CIP-resistant isolates than in the other two groups (P<0.01). Of 26 CIP-resistant isolates, all were concurrently resistant to ampicillin and 21 were also concurrently resistant to cefotaxime and produced ESBL. In addition, 19 CIP-resistant isolates were from patients and seven were from environmental sources. All *S.* Indiana CIP-resistant isolates and a number of *S.* Typhimurium, *S.* Agona, and S. Rissen CIP-resistant isolates were from environmental sources (Figure 1).

There were two types of *gyrA* mutations at loci 83, three types of *gyrA* mutations at loci 87, and four PMQR genes

Table 3 Results of resistance to antimicrobials tested in isolates with different CIP susceptibilities

Antimicrobial	Reduced CIP susceptible (n=522)	CIP resistant (n=26)	Susceptible (n=1,228)	Total (n=1,776)
Ampicillin	210 (40.2[a])*	26 (100)*	365 (29.7)	601 (33.8)
Cefotaxime	13 (2.5)*	15 (57.7)*	5 (0.4)	33 (1.9)
ESBL producing	8 (1.5)*	14 (53.8)*	4 (0.3)	26 (1.5)
Gentamicin	50 (9.6)*	8 (30.8)*	84 (6.8)	142 (8.0)
Chloramphenicol	38 (7.3)	20 (76.9)*	67 (5.5)	125 (7.0)
Trimethoprim–sulfamethoxazole	55 (10.5)*	21 (80.8)*	75 (6.1)	151 (8.5)
Average resistance rate (%)	11.9*	66.7*	8.1	10.1

Notes: [a]Percentage of number of resistant isolates to number of all isolates. *Statistically significant compared with group of susceptible isolates.
Abbreviations: CIP, ciprofloxacin; ESBL, extended-spectrum β-lactamase.

Table 4 Serotype distribution, CIP MICs, topoisomerases and PMQR genes, and β-lactamase genes of 26 CIP-resistant isolates

Serotypes	No. of isolates	Source (n)	MIC (μg/mL)		ESBL	*gyrA*	*parC*	PMQR	β-lactamase
			CIP	Cefotaxime					
Indiana	6	Water (4), feces (2)	>32	>128	Yes	S83F, D87N	S80R	*qnrB*, *aac(6′)-Ib-cr*	OXA, CTX-M
Typhimurium	4	Food (2), feces (2)	2–4	128	Yes	–	S80R	*qnrB*, *qnrS*	CTX-M
Typhimurium	2	Feces (2)	2–4	64	Yes	D87N	WT	*qnrB*, *aac(6′)-Ib-cr*	OXA
Agona	2	Feces (1), food (1)	2–4	64	Yes	S(TCC)83S(TCT)	WT	*qnrB*	TEM
Derby	5	Feces (5)	2	64	Yes	S(TCC)83S(TCT)	WT	*qnrB*, *qnrS*, *aac(6′)-Ib-cr*	OXA, TEM
London	3	Feces (3)	2	<1	No	–	WT	*qnrB*, *aac(6′)-Ib-cr*	–
Rissen	1	Blood (1)	2	4	No	–	WT	*qnrB*	TEM
Corvallis	1	Feces (1)	2	<1	No	–	WT	*qnrB* + *qnrS*	–
Kentucky	1	Food (1)	16	64	Yes	S83F, D87G	S80R	*qnrB*	CTX-M
Hadar	1	Feces (1)	2	<1	No	–	WT	*qnrB*	–

Abbreviations: CIP, ciprofloxacin; MIC, minimal inhibitory concentration; PMQR, plasmid-mediated quinolone resistance; ESBL, extended-spectrum β-lactamase; WT, wild type.

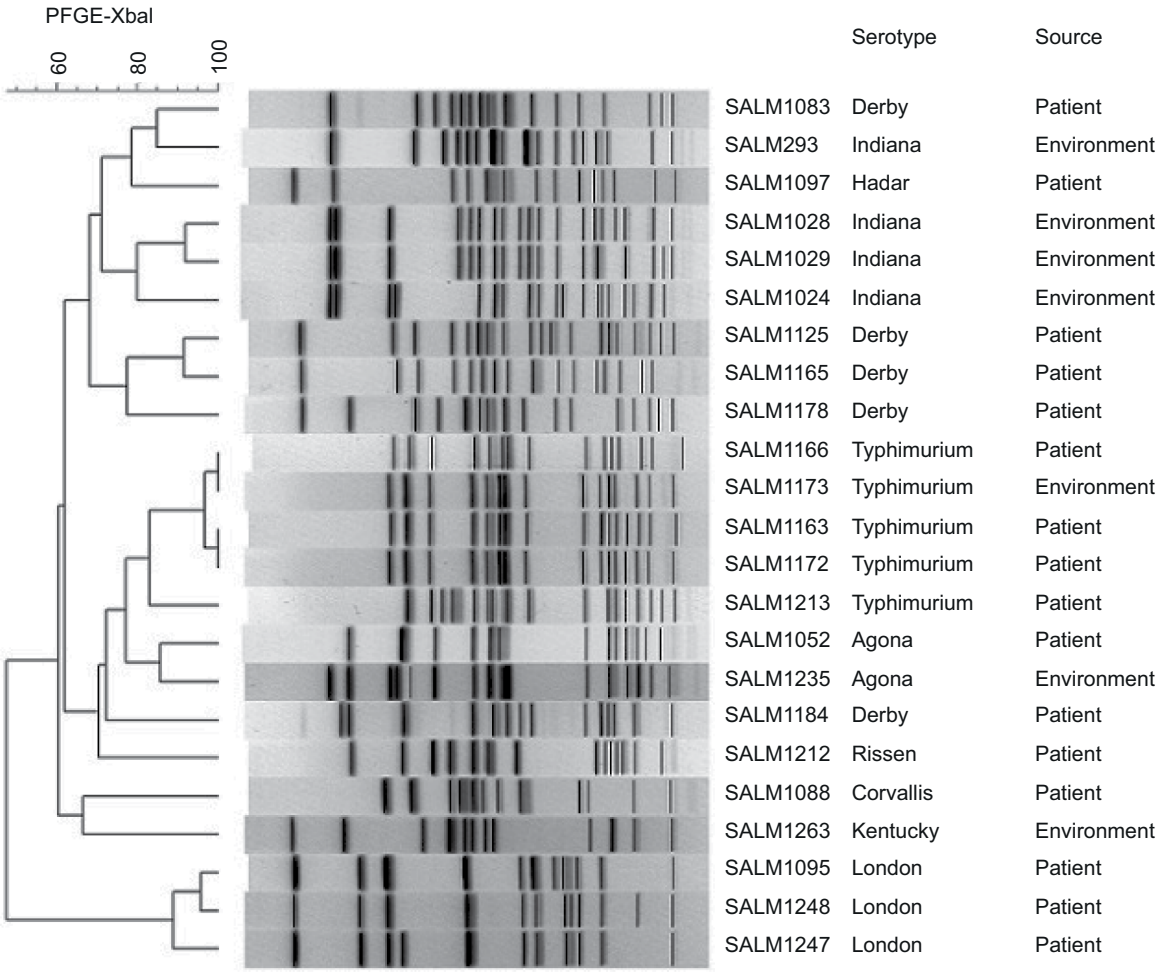

Figure 1 Dendrogram of PFGE patterns among 23 CIP-resistant *Salmonella* isolates, showing no overall clustering and yet genetic homogeneity in Typhimurium, Indiana, and London serotypes.
Abbreviations: PFGE, pulsed-field gel electrophoresis; CIP, ciprofloxacin.

detected in CIP-resistant and reduced CIP-susceptible isolates (Tables 4 and 5). Most found *gyrA* mutations were S83F and D87N that could be detected in both groups of isolates. A silent mutation S(TCC)83S(TCT) was found in a number of CIP-resistant Agona and Derby isolates. It is important to note that, most reduced CIP-susceptible *S.* typhi had S83F or D87Y mutation. Especially, this D87Y mutation was detected only in reduced CIP-susceptible *S.* typhi. No *gyrB*

mutation was found in such reduced CIP-susceptible *S.* typhi isolates. Regarding PMQR genes, 100% CIP-resistant isolates harbored *qnrB* that, in contrast, could not be detected in reduced CIP-susceptible isolates. Gene *aac(6′)-Ib-cr* in CIP-resistant isolates was also detected at a much higher prevalence in CIP-resistant isolates. *qnrS* existed in both groups. Especially, in Kentucky and Indiana isolates with CIP MIC of >16 μg/mL, two *gyrA* mutations, *qnrB* and usually *aac(6′)-Ib-cr*, concurrently existed. In isolates of other serotypes with smaller CIP MIC, one or no *gyrA* mutation and at least *qnrB* and sometimes plus *aac(6′)-Ib-cr* existed. No genetic determinants of quinolone resistance were detected in 100 susceptible isolates.

As to β-lactam resistance genes in 26 β-lactam- and CIP-resistant isolates, 21 were detected positive for at least one of TEM, OXA, and CTX-M genes (Table 4).

PFGE results

Although there was no overall clustering in 23 (three failed PFGE) CIP-resistant isolates, PFGE results showed that highly identical PFGE patterns were observed in some common serotypes, such as Typhimurium and Indiana, indicating that CIP-resistant isolates of some serotypes had very limited diversity (Figure 1).

Discussion

We found distinctive features about the overall development of quinolone resistance in *Salmonella* species: high prevalence of reduced CIP susceptibility and low prevalence of CIP resistance, which focuses on several serotypes, and PMQR gene *qnrB* being the most common indicator for CIP resistance.

Totally, the ratio for three levels of quinolone susceptibility (CIP resistant:reduced CIP susceptible:CIP susceptible) was 26:522:1,228, indicating a small proportion of CIP-resistant isolates and large proportion of reduced CIP-susceptible isolates. In the literature, a few studies have explored overall CIP resistance development in *Salmonella* and the limited results are varied. In Hong Kong, a study showed that 42% of 963 non-typhoidal *Salmonella* isolates are resistant to nalidixic acid (corresponding to reduced CIP susceptibility in our study) and 13.7% are resistant to CIP,[6] whereas the National Antimicrobial Resistance Monitoring System for Enteric Bacteria (NARMS) found that, of 2,236 non-typhoidal *Salmonella* isolates, only 56 (2%) are resistant to nalidixic acid and eight (0.4%) are resistant to CIP.[20] Our results are distinct from the Hong Kong study that shows a high prevalence of both CIP resistance and reduced CIP susceptibility, and the NARMS report that shows a low prevalence of both. A recent study has reported that 16.9% of isolates of animal sources are resistant to CIP, a prevalence much higher than that (1.5%) in our study. This difference suggests a gap in CIP resistance level between isolates from animals and human beings.[21]

Our results show distinctive phenotypes of quinolone resistance in different serotypes. As to the three most common serotypes, *S.* typhi showed quickly increased prevalence of reduced CIP susceptibility in recent years, a finding that we have reported in a previous study.[22] *S.* Enteritidis remained at a high prevalence of reduced CIP susceptibility throughout the study period. Nevertheless, in addition to a high prevalence of reduced CIP susceptibility, 5% of S. Typhimurium isolates were resistant to CIP. As early as 2005, up to 70% of 44 *S.* Typhimurium isolates from outpatients were reported to be resistant to CIP.[23] However, our findings illustrate that CIP resistance in *S.* Typhimurium has emerged only during recent years. In our study, the most serious situation of CIP resistance was observed in *S.* Indiana, a result consistent with a recent report.[24]

CIP resistance was found centrally in limited serotypes. PFGE results of CIP-resistant isolates show no overall clustering and a predominant clone in several serotypes, as indicated by limited diversity among multiple isolates of one serotype (Figure 1).

In our study, CIP resistance usually exists along with β-lactam resistance and ESBL production in the nine serotypes. Although there were several studies demonstrating concurrent resistance to CIP and cefotaxime in a particular serotype,[25–27] due to the fact that these studies involved only one serotype, it is difficult to answer whether the concurrent resistance is mainly in a particular serotype or a strain or is a general case. This phenomenon proves that concurrent resistance to CIP and β-lactam is more a general phenomenon.

Finally, while analyzing the molecular mechanisms of resistance to CIP, the most interesting finding was that, rather than the commonly considered *gyrA* mutations, a type of PMQR structure *qnrB* is the most common indicator for CIP resistance because it was only found in all CIP-resistant isolates (Tables 4 and 5). The second most common indicator is *aac(6′)-Ib-cr*. In contrast, *gyrA* mutations are not necessarily the cause of CIP resistance since these mutations were often found in reduced CIP-susceptible isolates and were not found in a number of CIP-resistant isolates. Especially, in reduced CIP-susceptible *S.* typhi, S83F and D87Y usually existed. Moreover, coexisting *gyrA* mutation and one or two PMQR genes were found in a number of reduced CIP-susceptible isolates, inconsistent

Table 5 Prevalence of genetic determinants related to quinolone resistance in CIP-resistant isolates and in 100 reduced CIP-susceptible isolates

Gene		Reduced CIP-susceptible isolates (n=100), %	CIP-resistant isolates (n=26), %	*P*[a]
PMQR gene	*qnrA*	5 (5)	–	–
	qnrB	–	26 (100)	<0.001*
	qnrS	20 (20)	10 (38)	0.14
	aac(6′)-Ib-cr	15 (15)	16 (62)	<0.001*
gyrA mutation	S83F	8 (8)	7 (27)	–
	S(TCC)83S(TCT)	–	7 (27)	–
	D87N	6 (6)	8 (31)	–
	D87Y	4 (4)	–	–
	D87G	–	1 (4)	–
	Event/isolate	0.18	0.88	<0.001*

Notes: [a]*P* value between reduced CIP-susceptible and CIP-resistant isolates. *Statistically significant.
Abbreviations: CIP, ciprofloxacin; PMQR, plasmid-mediated quinolone resistance.

with the previous statement that this coexistence is rare.[25] To conclude, prevalence of *qnrB* and *aac(6′)-Ib-cr* is significantly higher in CIP-resistant isolates, whereas *gyr* mutations in several CIP-resistant isolates (Table 4). Although it is usually believed that CIP resistance is mainly due to *gyrA* mutations[28] and PMQR genes cause low-level resistance to CIP in enteric bacteria,[29] our results demonstrate that it is not always the case in *Salmonella*. Given that it is difficult to obtain a large number of CIP-resistant isolates, we hope there will be more future studies to increase knowledge in this field. In addition, in highly CIP-resistant isolates (Kentucky and Indiana with MIC >16 μg/mL), presence of two *gyrA* mutations plus *qnrB* and/or *aac(6′)-Ib-cr* is common.

Conclusion

Our study shows that in *Salmonella*, there exists a high prevalence of reduced CIP susceptibility and a low prevalence of CIP resistance in limited serotypes. The CIP-resistant isolates generally show concurrent resistance to β-lactams. Our study also demonstrates that, rather than *gyrA* mutations, PMQR gene *qnrB* is the most common indicator for CIP resistance.

Acknowledgment

This study was supported by the Science and Technology Projects of Medicine and Health of Zhejiang (no. 2016KYB274 and 2018KY688).

Disclosure

The authors report no conflicts of interest in this work.

References

1. Majowicz SE, Musto J, Scallan E, et al; International Collaboration on Enteric Disease 'Burden of Illness' Studies. The global burden of nontyphoidal *Salmonella* gastroenteritis. *Clin Infect Dis*. 2010;50(6):882–889.
2. Centres for Disease Control and Prevention. *Foodborne Diseases Active Surveillance Network (FoodNet): FoodNet Surveillance Report for 2012 (Final Report)*. Atlanta, Georgia: U.S. Department of Health and Human Services, CDC; 2014.
3. Centres for Disease Control and Prevention. *An Atlas of Salmonella* in the United States, 1968-2011: Laboratory-based Enteric Disease Surveillance. Atlanta, Georgia: US Department of Health and Human Services, CDC; 2013.
4. Andersson MI, MacGowan AP. Development of the quinolones. *J Antimicrob Chemother*. 2003;51(suppl 1):1–11.
5. Effa EE, Lassi ZS, Critchley JA, et al. Fluoroquinolones for treating typhoid and paratyphoid fever (enteric fever). *Cochrane Database Syst Rev*. 2011;(10):CD004530.
6. Lo NW, Chu MT, Ling JM. Increasing quinolone resistance and multidrug resistant isolates among *Salmonella* enterica in Hong Kong. *J Infect*. 2012;65(6):528–540.
7. Hassing RJ, Goessens WH, Mevius DJ, et al. Decreased ciprofloxacin susceptibility in *Salmonella* Typhi and Paratyphi infections in ill-returned travellers: the impact on clinical outcome and future treatment options. *Eur J Clin Microbiol Infect Dis*. 2013;32(10):1295–1301.
8. Gong J, Wang C, Shi S, et al. Highly drug-resistant *Salmonella* enterica Serovar Indiana clinical isolates recovered from broilers and poultry workers with diarrhea in China. *Antimicrob Agents Chemother*. 2016;60(3):1943–1947.
9. Westrell T, Monnet DL, Gossner C, Heuer O, Takkinen J. Drug-resistant *Salmonella* enterica serotype Kentucky in Europe. *Lancet Infect Dis*. 2014;14(4):270–271.
10. Dutta P, Mitra U, Dutta S, De A, Chatterjee MK, Bhattacharya SK. Ceftriaxone therapy in ciprofloxacin treatment failure typhoid fever in children. *Indian J Med Res*. 2001;113:210–213.
11. Fabrega A, Madurga S, Giralt E, Vila J. Mechanism of action of and resistance to quinolones. *Microb Biotechnol*. 2009;2(1):40–61.
12. Giraud E, Baucheron S, Cloeckaert A. Resistance to fluoroquinolones in *Salmonella*: emerging mechanisms and resistance prevention strategies. *Microbes Infect*. 2006;8(7):1937–1944.
13. Hopkins KL, Davies RH, Threlfall EJ. Mechanisms of quinolone resistance in *Escherichia coli* and *Salmonella*: recent developments. *Int J Antimicrob Agents*. 2005;25(5):358–373.
14. Deak E, Hindler JA, Skov R, Sjolund-Karlsson M, Sokovic A, Humphries RM. Performance of Etest and disk diffusion for detection of ciprofloxacin and levofloxacin resistance in *Salmonella enterica*. *J Clin Microbiol*. 2015;53(1):298–301.
15. CLSI. Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard-Eleventh Edition. Wayne, PA: Clinical and Laboratory Standards Institute; 2014. CLSI document M02-A12.

16. CLSI. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fifth Informational Supplement. Wayne, PA: Clinical and Laboratory Standards Institute; 2015. CLSI document M100-S27.
17. Eaves DJ, Randall L, Gray DT, et al. Prevalence of mutations within the quinolone resistance-determining region of gyrA, gyrB, parC, and parE and association with antibiotic resistance in quinolone-resistant *Salmonella* enterica. *Antimicrob Agents Chemother*. 2004;48(10):4012–4015.
18. Le TM, Baker S, Le TP, et al. High prevalence of plasmid-mediated quinolone resistance determinants in commensal members of the Enterobacteriaceae in Ho Chi Minh City, Vietnam. *J Med Microbiol*. 2009;58(pt 12):1585–1592.
19. Centers for Disease Control and Prevention. Standard Operating Procedure for PulseNet PFGE of *Escherichia coli* O157:H7, *Escherichia coli* non-O157 (STEC), *Salmonella* serotypes, *Shigella* sonnei and *Shigella* flexneri. Available from: https://www.cdc.gov/pulsenet/pdf/ecoli-shigella-salmonella-pfge-protocol-508c.pdf. Accessed May 4, 2016.
20. Plant J, Glynn AA. Natural resistance to *Salmonella* infection, delayed hypersensitivity and Ir genes in different strains of mice. *Nature*. 1974;248(446):345–347.
21. Cao TT, Deng GH, Fang LX, et al. Characterization of quinolone resistance in *Salmonella enterica* from farm animals in China. *J Food Prot*. 2017;80(10):1742–1748.
22. Song Q, Yang Y, Lin W, Yi B, Xu G. Epidemiological characteristics and clinical treatment outcome of typhoid fever in Ningbo China, 2005-2014, PFGE results revealing great proportion of common transmission sources. *Jpn J Infect Dis*. 2017;70(5):513–516.
23. Cui S, Li J, Sun Z, et al. Ciprofloxacin-resistant *Salmonella enterica* serotype Typhimurium, China. *Emerg Infect Dis*. 2008;14(3):493–495.
24. Lu Y, Zhao H, Liu Y, et al. Characterization of quinolone resistance in *Salmonella enterica* serovar Indiana from chickens in China. *Poult Sci*. 2015;94(3):454–460.
25. Bai L, Zhao J, Gan X, et al. Emergence and diversity of *Salmonella enterica* Serovar Indiana isolates with concurrent resistance to ciprofloxacin and cefotaxime from patients and food-producing animals in China. *Antimicrob Agents Chemother*. 2016;60(6):3365–3371.
26. Wong MH, Yan M, Chan EW, Biao K, Chen S. Emergence of clinical *Salmonella enterica* serovar Typhimurium isolates with concurrent resistance to ciprofloxacin, ceftriaxone, and azithromycin. *Antimicrob Agents Chemother*. 2014;58(7):3752–3756.
27. Wong MH, Zeng L, Liu JH, Chen S. Characterization of *Salmonella* food isolates with concurrent resistance to ceftriaxone and ciprofloxacin. *Foodborne Pathog Dis*. 2013;10(1):42–46.
28. Chen S, Cui S, McDermott PF, et al. Contribution of target gene mutations and efflux to decreased susceptibility of *Salmonella enterica* serovar typhimurium to fluoroquinolones and other antimicrobials. *Antimicrob Agents Chemother*. 2007;51(2):535–542.
29. Liu JH, Deng YT, Zeng ZL, et al. Coprevalence of plasmid-mediated quinolone resistance determinants QepA, Qnr, and AAC(6')-Ib-cr among 16S rRNA methylase RmtB-producing *Escherichia coli* isolates from pigs. *Antimicrob Agents Chemother*. 2008;52(8):2992–2993.