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The phenotypic and molecular characteristics of antimicrobial resistance of *Salmonella enterica* subsp. *enterica* serovar Typhimurium in Henan Province, China

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Abstract

Background: *Salmonella enterica* subsp. *enterica* serovar Typhimurium infections continue to be a significant public health threat worldwide. The aim of this study was to investigate antibiotic resistance among 147 *S. Typhimurium* isolates collected from patients in Henan, China from 2006 to 2015.

Methods: 147 *S. Typhimurium* isolates were collected from March 2006 to November 2015 in Henan Province, China. Antimicrobial susceptibility testing was performed, and the resistant genes of ciprofloxacin, cephalosporins (ceftriaxone and ceftiofuran) and azithromycin were detected and sequenced. Clonal relationships were assessed by multilocus sequence typing (MLST) and pulsed-field gel electrophoresis (PFGE).

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Results: Of the 147 isolates, 91.1% were multidrug resistant (MDR), with 4.1% being resistant to all antibiotic classes tested. Of concern, 13 MDR isolates were co-resistant to the first-line treatments cephalosporins and ciprofloxacin, while three were also resistant to azithromycin. Seven PFGE patterns were identified among the 13 isolates. All of the isolates could be assigned to one of four main groups, with a similarity value of 89%. MLST assigned the 147 isolates into five STs, including two dominant STs (ST19 and ST34). Of the 43 ciprofloxacin-resistant isolates, 39 carried double *gyrA* mutations (Ser83Phe, Asp87Asn/Tyr/Gly) and a single *parC* (Ser80Arg) mutation, including 1 isolate with four mutations (*gyrA*: Ser83Phe, Asp87Gly; *parC*: Ser80Arg; *parE*: Ser458Pro). In addition, 12 isolates not only carried mutations in *gyrA* and *parC* but also had at least one plasmid-mediated quinolone resistance (PMQR) gene. Among the 32 cephalosporin-resistant isolates, the most common extended-spectrum β -lactamase (ESBL) gene was *bla*_{OXA-1}, followed by *bla*_{CTX-M}, *bla*_{TEM-1}, and *bla*_{CMY-2}. Moreover, the *mphA* gene was identified in 5 of the 15 azithromycin-resistant isolates. Four MDR isolates contained ESBL and PMQR genes, and one of them also carried *mphA* in addition.

Conclusion: The high level of antibiotic resistance observed in *S. Typhimurium* poses a great danger to public health, so continuous surveillance of changes in antibiotic resistance is necessary.

Keywords: *S. Typhimurium*, Antibiotic resistance, Multidrug-resistant, ESBL, PMQR, Multilocus sequence typing

Background

Salmonella enterica subsp. enterica serovar Typhimurium is an important causative agent of human gastroenteritis and bacteremia in many countries. Worldwide, there are 93.8 million cases of human gastroenteritis due to *Salmonella* infection annually, associated with a death toll as high as 150,000 [1]. Over 2700 serovars have been identified using the White-Kauffmann-Le Minor scheme [1, 2], of which, *S. Typhimurium* is the most commonly associated with human and animal disease globally. *S. Typhimurium* is also the most prevalent serovars causing invasive Nontyphoidal *Salmonella* infections (iNTS), among iNTS cases, 63.7% occurred in children < 5 years of age globally [3]. Moreover, *S. Typhimurium* is the second most prevalent serotype in China [4].

Following the emergence of antimicrobial-resistant *Salmonella*, and in particular, the increasing prevalence of an epidemic multidrug-resistant (MDR) *S. Typhimurium* strain definitive phage type 104 (DT104) first observed during the 1990s [4], the antimicrobial resistance of *Salmonella* has become a matter of concern worldwide. Therefore, the appropriate selection of antimicrobial drugs in treating *Salmonella* infections is necessary. As previously described, cephalosporins (CEP) and ciprofloxacin (CIP) are first-line treatment agents for such infections [5], and azithromycin (AZI) has recently been approved by the US Food and Drug Administration as an additional therapy [6]. *Salmonella* isolates with reduced susceptibility to fluoroquinolones have been frequently reported in several countries, such as Kuwait, the United Arab Emirates, France [7–9]. Moreover, strains resistant to third-generation CEP have been described [10]. Given that these drugs are also first-line treatments for *Salmonella* infections in children, for whom fluoroquinolones are contraindicated, this represents a troubling development [10, 11].

Furthermore, a study of *Salmonella* in Cambodia revealed high rates of decreased susceptibility to CIP and AZI [12]. Worryingly, a recent report also identified a number of strains with concurrent resistance to CEP, CIP, and AZI in China [13].

Within China, significant regional differences in bacterial antibiotic resistance profiles have been demonstrated [14]. Henan is the most populous province of Central China and a region of substantial labor productivity. High levels of migration are expected to accelerate the spread of drug-resistant bacteria, exacerbating the threat to public health. Therefore, the objectives of this study were to determine the antimicrobial resistance profiles and molecular epidemiological characteristics of *S. Typhimurium* present in Henan between 2006 and 2015. This investigation will assist in establishing a scientific basis for the prevention and control of intestinal infectious diseases and guide the selection of appropriate antimicrobials for treatment of *S. Typhimurium* infections.

Methods

Bacterial isolates and serotypes

From 7 March 2006 to 28 November 2015, *S. Typhimurium* isolates were obtained from human patients with diarrhea and clinically suspected *Salmonella* infections, following previously described procedures [15]. The strains were isolated from 15 sentinel hospitals located in 11 different geographic regions in Henan Province, which covers an area of 167,000 km² and is one of the most populous province (more than 95 million people) in China. Five of the hospitals were located in rural areas and the remaining 10 in urban areas. These hospitals consisted of second- and third-grade hospitals, township hospitals and village clinics. Patients generally have fever, diarrhea, abdominal pain, vomiting, watery stool and

other clinical symptoms. Fresh fecal specimens collected from diarrhea patients of all ages were inoculated into Carry-Blair medium and forwarded to the regional CDC laboratories within 4 h. The stool samples were enriched in selenite brilliant green broth (Becton Dickinson and Co., Sparks, MD, USA) for 16–18 h at 37 °C, followed by incubation on *Salmonella/Shigella* agar (Land Bridge, Beijing, China) overnight at 37 °C. The resulting black colonies were streaked onto CHROMagar *Salmonella* agar (CHROMagar, Paris, France) and kept for 16–18 h at 37 °C to confirm their identity. The resultant purple colonies were then subjected to triple sugar iron agar, motility indole urea agar, L-lysine decarboxylase, and β -galactosidase (*o*-nitrophenyl- β -D-galactopyranoside) tests. Subsequently, a presumptive *Salmonella* colony from each sample was stored in semi-solid agar and submitted to our laboratory for further confirmation. Age, gender, geographic origin, date and hospitals were recorded as part of the standard information present on the laboratory request forms. All isolates were identified using API 20E strips (bioMérieux Vitek, Marcy-l'Etoile, France) and tests for O and H antigens by slide agglutination with hyperimmune sera (State Serum Institute, Copenhagen, Denmark). Isolates were assigned to serovars according to the Kauffman-White scheme [7].

Antimicrobial susceptibility testing

The susceptibility of each isolate to the following 13 antimicrobial agents was evaluated: ceftriaxone (AXO), cefoxitin (FOX), ampicillin (AMP), amoxicillin/clavulanic acid, 2:1 ratio (AUG2), CIP, nalidixic acid (NAL), AZI, tetracycline (TET), chloramphenicol (CHL), trimethoprim/sulfamethoxazole (SXT), sulfisoxazole (FIS), gentamicin (GEN), and streptomycin (STR). MICs were determined by broth microdilution using a 96-well microtiter plate (Sensititre CMV3AGNF, Trek Diagnostic Systems; Thermo Fisher Scientific, Inc., West Sussex, UK), with results being interpreted according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI) [16]. Isolates resistant to three or more classes of antimicrobial agents were defined as MDR. The ACSSuT MDR profile, which was the prevalent resistance pattern in *S. Typhimurium*, was defined as resistance to AMP, CHL, STR, FIS, and TET [17]. *Escherichia coli* ATCC 25922 was used in susceptibility tests as a quality control strain, as specified by the Clinical and Laboratory Standards Institute.

Multilocus sequence typing (MLST)

MLST analysis of the *S. Typhimurium* isolates was conducted according to previously described protocols [18]. Seven housekeeping genes (*thrA*, *dnaN*, *aroC*, *purE*, *hisD*, *hemD*, and *sucA*) were amplified by PCR using *Ex Taq* DNA polymerase (TaKaRa, Dalian, China) and primers whose sequences were downloaded from the

abovementioned MLST resource. The PCR cycling conditions were as follows: 95 °C for 5 min; 30 cycles of 95 °C for 30 s, 55 °C for 45 s, and 72 °C for 1 min; and 72 °C for 7 min. The amplicons were sequenced by BGI (BGI, Beijing, China) and the resulting sequence data were checked against the MLST database [19] to establish sequence types (STs). The eBURST analysis was used with multilocus data to define groups of closely related isolates and display the relatedness between very different multilocus genotypes as previously described [20].

Pulsed-field gel electrophoresis (PFGE)

S. Typhimurium isolates co-resistant to CIP and CEP were analyzed by PFGE according to the PulseNet protocol for *Salmonella* [21]. *Salmonella* standard strain Braenderup H9812 was employed as the molecular weight marker [22]. Agarose plug slices were digested with *Xba*I (TaKaRa) at 37 °C for 3 h, and electrophoresis was performed under the following conditions: voltage, 6 V/cm; switch time, 2.16–63.8 s (linear ramping); electric field angle, 120°; electrophoresis time, 19 h; and buffer temperature, 14 °C. PFGE patterns were analyzed using BioNumerics software version 6.0 (Applied Maths, Sint-Martens-Latem, Belgium), employing the Dice correlation coefficient and unweighted pair group method with arithmetic mean, with tolerance set to 1.2%.

PCR amplification and DNA sequencing

Quinolone resistance-determining regions of the DNA topoisomerase genes *gyrA*, *gyrB*, *parC*, and *parE* were amplified by PCR assays as previously described [23]. Presence of the plasmid-mediated quinolone resistance (PMQR) genes *aac(6')-Ib-cr*, *qnrA*, *qnrB*, *qnrD*, and *qnrS* was also established by PCR [24]. Amplification of antibiotic-resistance genes of the *bla*_{TEM}, *bla*_{SHV}, *bla*_{OXA}, and *bla*_{CTX-M} groups and *bla*_{CMY} groups were performed by PCR for all isolates resistant to CEP [8, 24, 25]. In addition, for the AZI-resistant isolates, presence of the macrolide-resistance genes *mphA*, *mphB*, *ermA*, *ermB*, *ereA*, *mefA*, and *msrA* were tested [26]. PCR products were fully sequenced by BGI, and all nucleotide sequences were analyzed by comparisons against corresponding sequences in GenBank.

Statistical analysis

The chi-squared test and Fisher exact probability test were used for data analysis in SPSS version 17.0 (SPSS Inc., Chicago, IL, USA). *P*-values < 0.05 were considered to indicate statistical significance.

Results

S. Typhimurium isolates from patients in Henan, China, from 2006 to 2015

A total of 147 *S. Typhimurium* isolates were recovered from 15 hospitals in 11 different geographic regions of

Henan Province from 2006 to 2015, and the strains were distributed every year in each region (Table S1). Of these, 103 (70.1%) were isolated from patients below 6 years of age, and 91 (61.9%) from those less than 2 years old. Nine (6.1%) isolates were retrieved from patients 7 to 18 years of age, 21 (14.3%) from those 19 to 59 years of age, and 12 (8.1%) from those more than 59 years old. Two isolates (1.4%) derived from individuals of unknown age.

Antimicrobial susceptibility testing

The resistance patterns of the 147 *S. Typhimurium* isolates in response to the 13 antimicrobials tested are shown in Tables 1 and 2. There were 89% of the isolates which were resistant to AMP, TET or FIS, followed by NAL (115, 78.2%), SXT (114, 77.6%), CHL (113, 76.9%), GEN (102, 69.3%), STR (101, 68.7%), and AUG2 (97, 66%). Importantly, some isolates demonstrated strong resistance to at least one of four first-line treatment agents, namely, CIP (43, 29.2%), AXO (24, 16.3%), AZI (15, 10.2%), and FOX (9, 6.1%) (Table 1). All of the antimicrobial resistance levels of *S. Typhimurium* isolates in Henan were significantly higher than the level described in the 2014 National Antimicrobial Resistance Monitoring System report from the USA [27] ($P < 0.05$), and the resistance rate of the older generation of antibiotics

(AUG2, NAL, CHL, SXT, GEN, STR) remained at a relatively high level, which showed a decline after 2011 (Fig. S1). Meanwhile, the resistance rate of two first-line treatment agents (FOX and AZI) remained at a relatively low level, which showed a rise after 2011. Particularly, the resistance rate of CIP began to decline significantly after 2008 from high (60%) to low (0%), but the resistance rate of AMP and TET remained at a high level (more than 80%) from 2006 to 2015, and the resistance rate of AXO was decreased from 35.7 to 18.2% (Fig. 1). Furthermore, 13 (8.8%) isolates were co-resistant to CIP and CEP, of which, three were also resistant to AZI.

In total, 134 (91.1%) isolates were MDR (Table 2). Of these, 133 (90.5%), 117 (79.6%), 97 (65.9%), and six (4.1%) were resistant to at least four classes of antimicrobials, five classes of antimicrobials, six classes of antimicrobials, and all classes of antimicrobials tested, respectively. Seventy-five (51.0%) MDR isolates exhibited the ACSSuT resistance pattern, of which, 41.3% were co-resistant to CIP, 26.6% were co-resistant to CEP, and 9.3% were co-resistant to AZI. Notably, two (1.4%) ACSSuT-type isolates were co-resistant to all three of these antimicrobials (Table 2). The MDR (≥ 3 and ≥ 4 antimicrobial classes) rate remained at a high level, while MDR (≥ 5 and ≥ 6 antimicrobial classes) and ACSSuT resistance pattern showed a decline over time

Table 1 Antimicrobial susceptibility of 147 *S. Typhimurium* isolates collected from patients in Henan, China, between 2006 and 2015

Antimicrobial	No. isolates (%)			*NARMS Report n(%)	P-value
	Resistant	Intermediate	Susceptible		
β-Lactams					
Ceftriaxone	24 (16.3)	0 (0.0)	123 (83.7)	14 (5.3)	$P < 0.05$
Cefoxitin	9 (6.1)	0 (0.0)	138 (93.8)	14 (5.3)	$P < 0.05$
Ampicillin	132 (89.8)	0 (0.0)	15 (10.2)	52 (19.8)	$P < 0.05$
Amoxicillin/clavulanic acid, 2:1 ratio	97 (66.0)	0 (0.0)	50 (34.0)	14 (5.3)	$P < 0.05$
Quinolones					
Ciprofloxacin	43 (29.2)	0 (0.0)	104 (70.7)	1 (0.4)	$P < 0.05$
Nalidixic acid	115 (78.2)	0 (0.0)	32 (21.8)	7 (2.7)	$P < 0.05$
Macrolides					
Azithromycin	15 (10.2)	0 (0.0)	132 (89.8)	1 (0.4)	$P < 0.05$
Tetracyclines					
Tetracycline	132 (89.8)	0 (0.0)	15 (10.2)	59 (22.5)	$P < 0.05$
Amphenicols					
Chloramphenicol	113 (76.9)	2 (1.4)	32 (21.7)	42 (16.0)	$P < 0.05$
Sulfonamides and synergistic agents					
Trimethoprim/sulfamethoxazole	114 (77.6)	0 (0.0)	33 (22.4)	6 (2.3)	$P < 0.05$
Sulfisoxazole	132 (89.8)	0 (0.0)	15 (10.2)	66 (25.2)	$P < 0.05$
Aminoglycosides					
Gentamicin	102 (69.3)	1 (0.7)	44 (30.0)	8(3.1)	$P < 0.05$
Streptomycin	101 (68.7)	25 (17.0)	21 (14.3)	65 (24.8)	$P < 0.05$

* The antimicrobial resistant number and rate of 262 *S. Typhimurium* isolates were cited from the National Antimicrobial Resistance Monitoring System report from the USA in 2014 [27]

Table 2 Multidrug resistance patterns of *S. Typhimurium* isolates collected from patients in Henan, China, between 2006 and 2015

Multidrug resistance pattern	Number of isolates	Percentage	* NARMS Report(%)	P-value
≥ 3 antimicrobial classes	134	91.1	21.8	<i>P</i> < 0.05
≥ 4 antimicrobial classes	133	90.5	18.7	<i>P</i> < 0.05
≥ 5 antimicrobial classes	117	79.6	15.6	<i>P</i> < 0.05
≥ 6 antimicrobial classes	97	65.9	–	–
= 7 antimicrobial classes	6	4.1	–	–
ACSSuT	75	51.0	14.5	<i>P</i> < 0.05
ACSSuT+AZI	7	4.8	–	–
ACSSuT+CIP	31	21.1	–	–
ACSSuT+CEP	20	13.6	–	–
ACSSuT+CEP+CIP	10	6.8	–	–
ACSSuT+CEP+CIP + AZI	2	1.4	–	–
Sensitive to all	4	2.7	–	–

CEP cephalosporins, CIP ciprofloxacin, AZI, azithromycin

* The percentage of multidrug resistance patterns of *S. Typhimurium* isolates were cited from the National Antimicrobial Resistance Monitoring System report from the USA in 2014 [27]

(Fig. 2). But the rates of MDR (≥3, ≥4 and ≥5 antimicrobial classes) and ACSSuT were significantly higher than the rate described in the 2014 NARMS report [27] (*P* < 0.05) (Table 2).

MLST and PFGE analyses

MLST assigned the 147 isolates into five STs as follows: ST19 (72, 49.0%), ST34 (67, 45.6%), ST36 (6, 4.0%), ST3265 (1, 0.7%), and ST13(1, 0.7%) (Fig. 3). The eBURST analysis showed there were four eBURST groups between the five STs, ST19 and ST34 belonged to one group, and ST36, ST3265 and ST13 belonged to another groups, respectively. ST19 and ST34 are single locus variants (SLVs) in which one of the seven MLST loci has been altered, due to different sequence values of *dnaN*. PFGE was performed to determine the genetic relatedness of those isolates co-resistant to CIP and CEP.

Seven PFGE patterns (Profile 1–Profile 7) were identified among the 13 isolates. All of the isolates could be assigned to one of four main groups (A–D), with a similarity value of 89%. PFGE patterns revealed both diversity and the predominance of certain profiles. Eight isolates belonged to group A, in which three profiles were observed and Profile 3 was the main pattern type including six isolates. Group C and group D contained two isolates each, while only one isolate was present in group B (Fig. 4).

PCR amplification and DNA sequencing

Mechanisms of resistance in the 43 CIP-resistant *S. Typhimurium* isolates were investigated. All of these isolates were found to have *gyrA* mutation and 39 (90.7%) of them carrying two mutations (Ser83Phe and Asp87Asn/Tyr/Gly), and four (9.3%) carrying only one

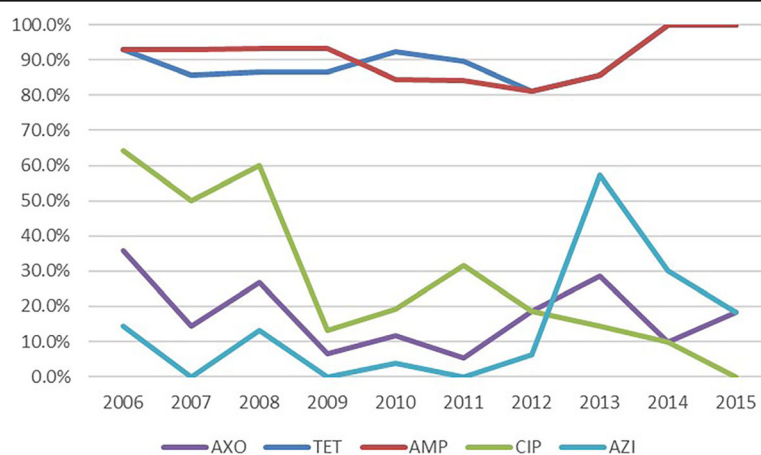


Fig. 1 Trend of resistance rate of 5 common antibiotics in treatment between 2006 and 2015

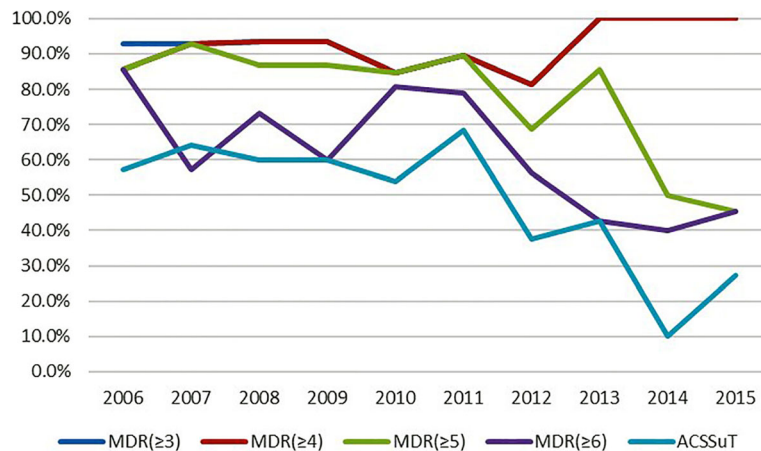


Fig. 2 The change of multidrug resistance pattern of *S. Typhimurium* isolates collected from patients in Henan, China, between 2006 and 2015. MDR (≥3): ≥3 antimicrobial classes, MDR (≥4): ≥4 antimicrobial classes, MDR (≥5): ≥5 antimicrobial classes, MDR (≥6): ≥6 antimicrobial classes

(Asp87Asn or Asp87Tyr). Thirty-nine isolates (90.7%) had the *parC* Ser80Arg mutation. In addition, double *gyrA* mutations together with a single *parC* (Ser80Arg) mutation were detected in 38 (88.4%) of the 43 isolates. None carried a *gyrB* mutation; however, a *parE* mutation (Ser458Pro) was identified in one isolate, which interestingly also carried two *gyrA* mutations (Ser83Phe and Asp87Gly) and a single *parC* (Ser80Arg) mutation. Furthermore, two PMQR determinants were detected: *qnrS1* (11 isolates, 25.6%) and *aac(6′)-Ib-cr* (seven isolates, 16.3%). Among the four isolates with a single *gyrA* mutation (Asp87Tyr or Asp87Asn), two had the *aac(6′)-Ib-cr* gene, one had the *qnrS* gene and one did not have either. None of the isolates carried *qnrA*, *qnrB*, or *qnrD*, and 28

(65.1%) of 43 isolates didn't carry any PMQR determinants tested.

The 32 *S. Typhimurium* isolates found to be resistant to CEP antibiotics all carried β-lactamase gene and 16 (50%) had at least two ESBL genes. PCR amplification and sequencing showed that 28 (87.5%) isolates were positive for *bla_{OXA-1}*, 13 (40.6%) for *bla_{CTX-M}*, nine (28.1%) for *bla_{TEM-1}*, and two (6.3%) for *bla_{CMY-2}*. All 32 isolates were negative for *bla_{SHV}*. Furthermore, sequencing revealed the presence of *bla_{CTX-M-14}*, *bla_{CTX-M-15}*, and *bla_{CTX-M-55}* in six, two, and five isolates, respectively.

PCR showed that only five (33.3%) of the 15 azithromycin-resistant isolates were positive for *mphA*,

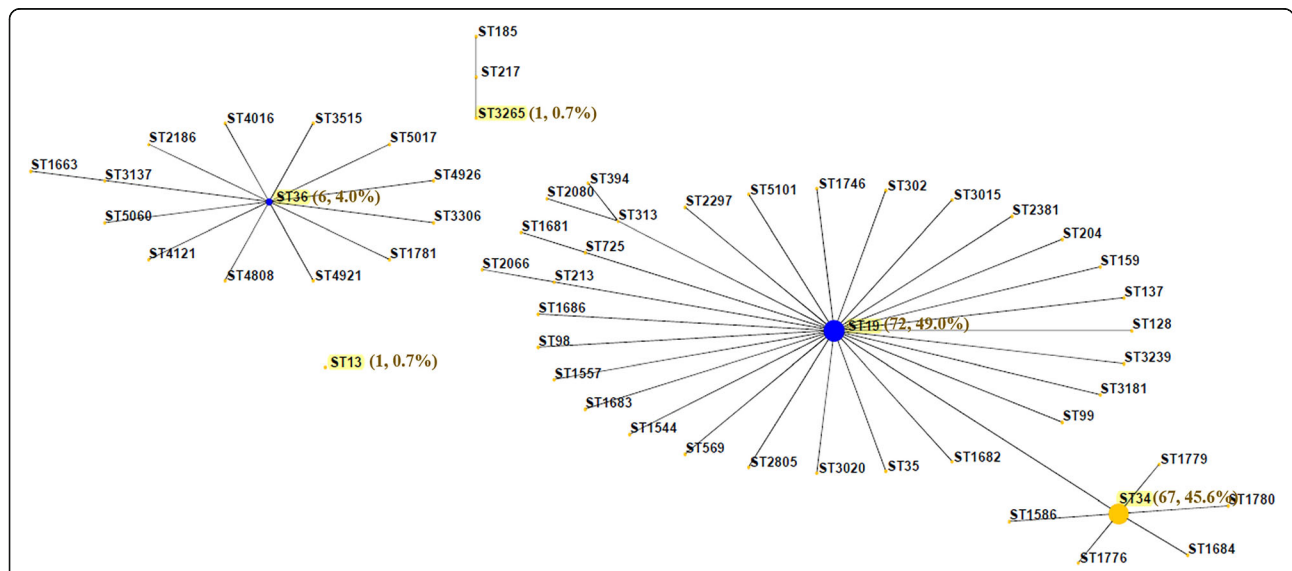


Fig. 3 The eBURST diagram for sequence types (STs) among *S. Typhimurium* isolates. The eBURST diagram was generated with STs of 147 *S. Typhimurium* detected in this study and other STs in the MLST database

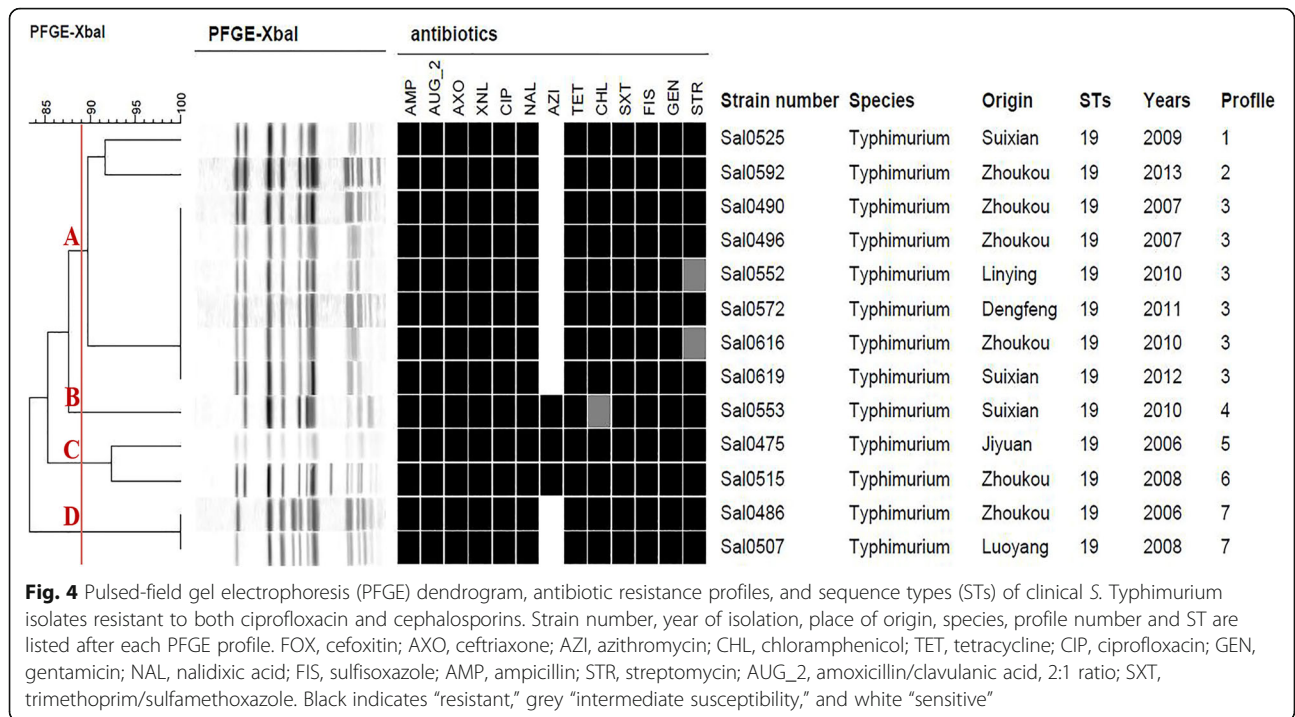


Fig. 4 Pulsed-field gel electrophoresis (PFGE) dendrogram, antibiotic resistance profiles, and sequence types (STs) of clinical *S. Typhimurium* isolates resistant to both ciprofloxacin and cephalosporins. Strain number, year of isolation, place of origin, species, profile number and ST are listed after each PFGE profile. FOX, cefoxitin; AXO, ceftriaxone; AZI, azithromycin; CHL, chloramphenicol; TET, tetracycline; CIP, ciprofloxacin; GEN, gentamicin; NAL, nalidixic acid; FIS, sulfisoxazole; AMP, ampicillin; STR, streptomycin; AUG_2, amoxicillin/clavulanic acid, 2:1 ratio; SXT, trimethoprim/sulfamethoxazole. Black indicates “resistant,” grey “intermediate susceptibility,” and white “sensitive”

and none of them carried *mphB*, *ermA*, *ermB*, *ereA*, *mefA*, or *msrA*.

Among the 13 isolates resistant to both CIP and CEP, 8 harbored ESBL and carried two mutations in *gyrA* and one in *parC*, one harbored ESBL and carried one mutation in *parC*, 4 harbored ESBL and PMQR genes (Table 3). Moreover, each of these four carried two mutations in *gyrA* and one in *parC*. Two isolates each harbored four types of antimicrobial-resistance gene: *qnrS/aac(6′)-Ib-cr/bla_{OXA}/bla_{CTX}* (*n* = 1) and *aac(6′)-Ib-cr/bla_{OXA}/bla_{CTX}*

bla_{TEM} (*n* = 1). One isolate exhibited three antimicrobial-resistance gene types (*qnrS/bla_{CTX}/bla_{OXA}*), and one other was found to have two types (*qnrS/bla_{OXA}*). In addition, 2 of the 13 isolates harbored the *mphA* gene.

Discussion

S. Typhimurium is one of the most prevalent *Salmonella* serotypes in the world, which was frequently resistant to ampicillin, amoxicillin/clavulanic acid, ceftriaxone, chloramphenicol, kanamycin, nalidixic acid, streptomycin,

Table 3 Antimicrobial resistance determinants of clinical *S. Typhimurium* isolates resistant to both ciprofloxacin and cephalosporins

Strain	Years	STs	QRDR mutations		PMQR genes		β-Lactamase genes and types				Macrolide resistance gene
			<i>gyrA</i>	<i>parC</i>	<i>qnrS</i>	<i>aac(6′)-Ib-cr</i>	<i>bla_{CTX-M}</i>	<i>bla_{OXA}</i>	<i>bla_{CMY-2}</i>	<i>bla_{TEM-1}</i>	<i>mphA</i>
Sal0475	2006	19	Ser83Phe, Asp87Asn	Ser80Arg	-	-	<i>bla_{CTX-55}</i>	<i>bla_{OXA-1}</i>	<i>bla_{CMY-2}</i>	-	-
Sal0485	2006	19	Ser83Phe, Asp87Asn	Ser80Arg	-	-	<i>bla_{CTX-15}</i>	<i>bla_{OXA-1}</i>	-	-	-
Sal0490	2007	19	Ser83Phe, Asp87Asn	Ser80Arg	-	-	<i>bla_{CTX-55}</i>	<i>bla_{OXA-1}</i>	-	-	-
Sal0496	2007	19	Ser83Phe, Asp87Asn	Ser80Arg	-	-	<i>bla_{CTX-55}</i>	<i>bla_{OXA-1}</i>	-	-	-
Sal0507	2008	19	Ser83Phe, Asp87Asn	Ser80Arg	-	-	<i>bla_{CTX-15}</i>	<i>bla_{OXA-1}</i>	-	-	-
Sal0515	2008	19	Ser83Phe, Asp87Asn	Ser80Arg	-	<i>aac(6′)-Ib-cr</i>	<i>bla_{CTX-55}</i>	<i>bla_{OXA-1}</i>	-	<i>bla_{TEM-1}</i>	<i>mphA</i>
Sal0525	2009	19	Ser83Phe, Asp87Asn	Ser80Arg	-	-	-	<i>bla_{OXA-1}</i>	-	-	-
Sal0616	2010	19	Ser83Phe, Asp87Asn	Ser80Arg	-	-	<i>bla_{CTX-14}</i>	<i>bla_{OXA-1}</i>	-	-	-
Sal0552	2010	19	Ser83Phe, Asp87Asn	Ser80Arg	-	-	<i>bla_{CTX-14}</i>	<i>bla_{OXA-1}</i>	-	-	-
Sal0553	2010	19	Asp87Tyr	WT	-	-	-	<i>bla_{OXA-1}</i>	-	-	<i>mphA</i>
Sal0572	2011	19	Ser83Phe, Asp87Asn	Ser80Arg	<i>qnrS1</i>	-	<i>bla_{CTX-55}</i>	<i>bla_{OXA-1}</i>	-	-	-
Sal0619	2012	19	Ser83Phe, Asp87Asn	Ser80Arg	<i>qnrS1</i>	<i>aac(6′)-Ib-cr</i>	<i>bla_{CTX-55}</i>	<i>bla_{OXA-1}</i>	-	-	-
Sal0592	2013	19	Ser83Phe, Asp87Asn	Ser80Arg	<i>qnrS1</i>	-	-	<i>bla_{OXA-1}</i>	-	-	-

QRDR quinolone resistance-determining region, PMQR plasmid-mediated quinolone resistance, WT wild type

trimethoprim-sulfamethoxazole, and tetracyclines [28–30]. In this study, the *S. Typhimurium* isolates examined exhibited high rates of resistance to older-generation antimicrobials, and MDR (91.1%) showed the predominant resistance profile over the potential 10 year in Henan province, of which, 51.0% exhibited the ACSSuT resistance pattern. CEP, CIP, and AZI are recommended as first-line treatments for *Salmonella* infections [5, 6], and surprisingly, resistance rates to these antibiotics among the isolates tested were far higher than the values given in the 2014 National Antimicrobial Resistance Monitoring System (NARMS) report [27]. In addition, we found that the resistance rate of CEP and AZI showed a rise over time, while the resistance rate of CIP began to decline. Of concern, 91.1% of the isolates in the present investigation were MDR, an estimate higher than those in previous studies in Guangdong and Shanghai and in the abovementioned report [27, 31, 32]. More importantly, it is noteworthy that 13 (8.84%) isolates were co-resistant to CIP and CEP, and that all of these were also resistant to at least five additional antimicrobial classes, indicating that CIP and CEP resistant *S. Typhimurium* isolates have been disseminated among communities in Henan. Notably, three of the 13 isolates also exhibited resistance to AZI. Only some clinical *S. Typhimurium* strains have previously been documented with this resistance profile in China, 12 (2%) of 546 *S. Typhimurium* isolates resistant to both CIP and AXO were recovered from patients in hospitals during the period of 2005 to 2011; among these 12 isolates, two were also resistant to AZI [13]. And ACSuT *Salmonella* co-resistant to quinolones and cephalosporins will make treatment even more difficult, and the spread of these isolates will pose a real threat to global public health. The above analyses therefore reveal that *S. Typhimurium* resistance patterns have obviously changed in recent years (Table 2 and Table S1), and empirical therapy should keep pace with these changes. Of concern is 6 (4.1%) strains are resistant to all antibiotic classes tested in our study, it meant we were almost impossible to find an effective treatment for infections. This situation demands regular surveillance of antimicrobial resistance and implements an efficient infection control program. We should control the injudicious use of antibiotics, and manage the antibiotics which are readily available in pharmacies without a prescription. Because antibiotic resistance could be driven by antibiotic consumption, the changing composition of consumption may also reflect alterations in patterns of resistance [33]. Otherwise, antimicrobial resistance will become more and more serious with such a sustained selective pressure. Henan Province is located in Central China, representing approximately 7% of the Chinese population, and is a major transport hub with substantial population mobility. If these MDR strains identified here become as prevalent as definitive phage type 104 worldwide, a more serious threat to national and international

public health would be posed. It is therefore essential that antimicrobial resistance be monitored and appropriate drugs be chosen for *S. Typhimurium* infections.

In order to facilitate understanding of the fundamental factors underlying bacterial antimicrobial resistance and establish measures for its prevention, examination of the molecular mechanisms responsible is urgently needed. *Salmonella* CIP resistance has principally been attributed to point mutations in the quinolone resistance-determining regions of genes encoding the target gyrase (*gyrA* and *gyrB*) and topoisomerase IV (*parC* and *parE*) enzymes [7, 34]. Two *gyrA* mutations affecting amino acid residues 83 and 87 have been identified in *S. Typhimurium* with high-level resistance to CIP, and variations in other target enzyme-encoding genes such as *parC* and *parE* increase such resistance [35, 36]. In the present study, 43 CIP-resistant isolates were identified, all of which had at least one *gyrA* mutation. Thirty-nine (91%) of these carried two such mutations and showed high-level resistance. One isolate exhibiting a high degree of CIP resistance carried two *gyrA* mutations (Ser83Phe and Asp87Gly), one *parC* mutation (Ser80-Arg), and a single *parE* mutation (Ser458Pro). This *parE* sequence variation has been reported previously in Taiwan, Hong Kong, and Wuhan [37–39], suggesting that it has the potential to become prevalent in China. As previously described, PMQR genes such as *qnr* and *aac(6′)-Ib-cr* have also been established as conferring CIP resistance [40]. The first PMQR gene was identified in a clinical isolate of *Klebsiella pneumoniae* in 1998 [41], and to date, various PMQR gene types have been detected in clinical *Salmonella* isolates from humans worldwide [42–44]. In this study, the genes *qnrS1* and *aac(6′)-Ib-cr* were found to be present in 25.6% (11 isolates) and 16.3% (seven isolates) of the 43 CIP-resistant isolates, respectively. Interestingly, 12 isolates simultaneously carried two *gyrA* mutations, a single *parC* mutation, and at least one PMQR gene, and thus demonstrated a high level of CIP resistance. However, three isolates highly resistant to CIP harbored only single *gyrA* mutations affecting amino acid position 87. The fact that they also carried *qnrS1* or *aac(6′)-Ib-cr* indicates that the presence of PMQR genes can increase CIP MICs [37, 40].

Production of β -lactamases is considered the predominant mechanism of bacterial resistance to CEP [45]. Among the 32 CEP-resistant isolates in the current work, *bla*_{OXA} was the most frequently observed β -lactamase gene ($n = 28$), with only *bla*_{OXA-1} being detected, suggesting that it is prevalent among *S. Typhimurium* in Henan. OXA-type β -lactamases are characterized by high levels of hydrolytic activity against oxacillin and cloxacillin, and confer resistance to AMP and CEP [46], compounding the difficulty faced in

choosing antimicrobials for treatment of *S. Typhimurium* infections. In recent years, the *bla*_{OXA} gene has also been recorded at high frequencies among other *Enterobacteriaceae* [47]. Previous studies have shown that CMY-2-type β -lactamases encoded by the plasmid-borne *bla*_{CMY-2} gene are the most prevalent and problematic damaging β -lactamase of such enzymes [48]. Although the *bla*_{CMY-2} resistance gene has been documented in *Salmonella* isolates from many countries [49], its presence in China has only been reported in Shandong, Shanxi, and Sichuan Provinces [50–52]. In the current study, one isolate was positive for *bla*_{CMY-2}, *bla*_{CTX-M} and *bla*_{OXA} and resistant to all antimicrobial classes tested. To the best of our knowledge, this is the first report of *bla*_{CMY-2}-positive *Salmonella* in Henan. Of the 32 CEP-resistant isolates in our investigation, 40% harbored *bla*_{CTX-M} genes, while 28.1% carried the *bla*_{TEM-1} resistance gene. We also found that some of these isolates harbored at least two CEP-resistance genes at the same time. As ESBL genes are usually located on antimicrobial-resistance plasmids, they can be easily transferred between different species of bacteria [53]. We should therefore pay consideration to such phenomena and make every effort to take preventive measures.

ST19 and ST34 have been shown to be common *S. Typhimurium* STs responsible for infections worldwide [54–56]. Here, these two STs were also the most commonly encountered, indicating their predominance in Henan. Of the 43 CIP-resistant isolates, 39 were categorized as ST19, indicating a relationship between this ST and resistance to CIP ($P < 0.05$). Moreover, 13 MDR isolates co-resistant to CIP and CEP were identified as ST19, suggesting that the ST19 is prevalent among MDR *S. Typhimurium* in Henan. According to our data, we note that only by combining etiological and epidemiological information can the characteristics of the spread of resistant clonal strains be better understood.

Conclusions

We analyzed the antimicrobial resistance and basic molecular mechanisms underlying CIP and CEP resistance of *S. Typhimurium* isolates in Henan, China, and explored their genetic relatedness. These isolates not only exhibited high rates of resistance to traditional antimicrobials but also show high resistance rates to the first-line treatments for *Salmonella* infection. More importantly, we identified certain MDR isolates co-resistant to CEP, CIP, and AZI, suggesting that the choice of treatment for *Salmonella* infection has become increasingly difficult. Among the isolates, we detected various plasmid-encoded antimicrobial-resistance genes, including PMQR, ESBL, and *mphA* genes, with some isolates even carrying two or more types, posing a serious threat to global public health. Therefore, more comprehensive surveillance is essential to prevent further spread of resistant clonal strains.

Supplementary information

Supplementary information accompanies this paper at <https://doi.org/10.1186/s12879-020-05203-3>.

Additional file 1: Figure S1. Trend of resistance rate of 8 uncommon antibiotics in treatment between 2006 and 2015. It was shown in the supplemental material.

Additional file 2. Table S1

Additional file 3. The original Pulsed-field gel electrophoresis (PFGE) images of clinical *S. Typhimurium* isolates resistant to both ciprofloxacin and cephalosporins. The PFGE profiles of the isolates were distributed in different images, and they were labelled in red below.

Abbreviations

ESBLs: Extended-spectrum β -lactamases; PMQR: Plasmid-mediated quinolone resistance; QRDR: Quinolone resistance determining region; MICs: Minimal inhibition concentration; MLST: Multilocus sequence typing; *gyrA*: DNA gyrase; *parC*: Topoisomerase IV

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Authors' contributions

YS, LJ, SQ, HS and YRL conceived the study, ND and YRL wrote the manuscript, JZ and SX and HM participated in the collection of samples, FW, BL, XY, and CY were responsible for sample preservation, JW, ND and YRL were responsible for the experiments, YRL and HBL analyzed the data, XD, SQ, HS, YS, LJ and YL provided academic revision for the manuscript. All authors have read and approved the final draft of the manuscript.

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Availability of data and materials

The data sets generated and analyzed are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The stool samples from human patients with diarrhea were collected and screened in sentinel hospitals based on a national pathogen monitoring system, during our routine surveillance of *S. Typhimurium*. This study was approved by the ethics committees of the Academy of Military Medical Sciences (Beijing, China). Permission and written informed consent was obtained from patients for sample collection and subsequent usage for research purposes. Written informed consent for participants under 16 was obtained from the parents/guardians of the participants.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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