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Antimicrobial resistance profiles of *Salmonella* isolates from human diarrhea cases in China: an eight-year surveillance study

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Abstract

Salmonella is widely known as one of the most common foodborne pathogens, and antibiotics remain effective in clinical therapies against its infections. To guide better clinical antibiotic treatment, we analyzed the antimicrobial resistance (AMR) profiles of a nation-wide collection of 36,822 *Salmonella* isolates derived from sporadic diarrhea cases in China from 2014 to 2021. A panel of 15 antibiotics, including 10 critically important and 5 highly important antimicrobial agents for human medicine based on the WHO CIA List, was selected for AMR surveillance. *Salmonella enterica* serovar Typhimurium, Enteritidis, I 4,[5],12:i:-, London, and Stanley were turns to be the top five serotypes from human diarrhea cases in China. Antimicrobial susceptibility testing revealed that the majority of the isolates (87.2%) were resistant to at least one antimicrobial agent, and 66.5% were multidrug resistant (MDR). *Salmonella* isolates were found to be highly resistant to ampicillin (73.4%) while sensitive to imipenem (98.73%). Over the eight years, the isolates were demonstrated generally an increase in resistance to ampicillin, chloramphenicol, trimethoprim-sulfamethoxazole, and azithromycin, but displayed varied resistance profiles in terms of serotypes. Stanley (0.66–27.18%) and Agona (0.78–45.30%) had lower resistance rates compared to Typhimurium (1.11–85.6%), Enteritidis (1.55–91.29%), and I 4,[5],12:i:- (1.02–94.28%). In conclusion, our results provide systematic data on the resistance characteristics of *Salmonella* isolates from human diarrhea cases in China. Furthermore, this data identifies priorities for the clinical treatments of antibiotics.

Keywords Foodborne disease, *Salmonella*, Antimicrobial resistance

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Introduction

Foodborne diseases continue to be a significant cause of human morbidity and mortality worldwide. Among the 22 common foodborne diseases, nontyphoidal *Salmonella enterica* causes the highest burden [1]. In China, data from national foodborne disease surveillance revealed that *Salmonella* was among the top three pathogenic causes of bacterial foodborne outbreaks [2]. The isolation rate of *Salmonella* from sporadic diarrheal cases was highest among the four major bacterial pathogens (*Vibrio parahaemolyticus*, *Salmonella*, diarrheagenic *Escherichia coli*, and *Shigella*) in the foodborne disease active surveillance [3].

Outbreaks associated with resistant *Salmonella* are frequently reported worldwide [4]. In China, to monitor trends of antimicrobial susceptibility among foodborne pathogens, AMR surveillance has been included in the National Foodborne Disease Surveillance Plan since 2014. All 32 Chinese provincial CDC laboratories are required to conduct antimicrobial susceptibility tests (ASTs) and submit the minimum inhibitory concentrations (MICs) to the National Molecular Tracing Network for Foodborne Disease Surveillance (TraNet) [5]. The China National Center for Food Safety Risk Assessment (CFSA) manages and analyzes all AMR data and reports it to the National Health Commission annually.

This study, provides the first summary of AMR data for 36,822 *Salmonella* isolates from sporadic diarrhea cases between 2014 and 2021 in China. Additionally, we analyzed the trends and changes of AMR and serotype in these isolates during an eight-year period. The data presented herein would provide better evidence to support clinicians and policy-makers during their clinical therapies and efforts to combat *Salmonella* infections.

Results

Geographic distribution

From 2014 to 2021, a total of 31 Chinese provincial CDCs reported the MICs of 36,822 *Salmonella* isolates derived from human sporadic diarrhea cases to the CFSA. The number of isolates reported by each province varied from 91 in Chongqing to 9,174 in Guangdong (Fig. 1). 7,766 isolates were reported by 16 northern provinces, and 29,056 isolates were reported by 15 southern provinces.

Serotype distribution

During the eight years, 94.0% (34,631/36,822) of the isolates were serotyped. A total of 466 serotypes were detected, and *S. Typhimurium*, Enteritidis, I 4,[5],12:i:-, London, Stanley, Derby, Rissen, Thompson, Agona, and Goldcoast were the top 10 serotypes among sporadic clinical isolates in China (Table 1). The distribution of serotypes exhibited regional differences. Enteritidis (39.7%,

3,082/7,766) was the most dominant serotype in northern China; however, in southern China, Typhimurium and I 4,[5],12:i:- (47.1%, 13,699/29,056) were the most prevalent serotypes.

Antimicrobial resistance

Of the 36,822 isolates, 87.2% (32,102/36,822) were resistant to at least one antimicrobial agent, and 66.5% (24,478/36,822) were MDR strains. A total of 73.4% (26,560/36,822) were resistant to ampicillin, while only 1.3% isolates (432/36,822) showed resistance to imipenem (Fig. 2). Among all MDR isolates, 53.9% (13,194/24,478) exhibited resistance to over five classes of antimicrobial agents.

A total of 1,814 AMR profiles were determined, and 1,701 were MDR profiles. The ACSuT profile (resistance to ampicillin, chloramphenicol, sulfonamide, and tetracycline) and ACSuTAs profile (ACSuT plus ampicillin/sulbactam) were the two most common MDR profiles, accounting for 25.1% (9,247/36,882) and 16.7% (6,170/36,882) of *Salmonella* isolates, respectively. The most common serotypes with the ACSuT profile were Typhimurium ($n=4,372$), I 4,[5],12:i:- ($n=1,135$), and London ($n=690$).

The trend analysis based on the linear regression model showed that the isolates exhibited upward resistance trends to ampicillin, chloramphenicol, trimethoprim-sulfamethoxazole, and azithromycin, and the resistance rates increased by 2.2%, 1.8%, 1.7%, and 0.4% for each additional year, respectively. While decreasing resistance rates were observed for gentamicin, nalidixic acid, and ciprofloxacin, with the resistance rates decreasing by 1.4%, 1.1% and 0.6% for each additional year, respectively. Resistance to colistin E decreased from 27.5% in 2019 to 15.9% in 2021 (Fig. 3); however, three-year data could not fit the linear regression model to conduct statistical tests.

The AMR of *Salmonella* isolates varied among serotypes, with significant differences in resistance rates and MDR rates were observed. For instance, *S. Stanley* and *S. Agona* had lower resistance rates than *S. Typhimurium*, I 4,[5],12:i:-, Enteritidis, and London (Fig. 4).

Salmonella isolates that were categorized as intermediate (0.125–0.5 µg/mL) or resistant (MIC ≥ 1 µg/mL) to ciprofloxacin were previously defined as having decreased susceptibility to ciprofloxacin (DSC) [6]. Although 16.2% of the tested isolates showed resistance to ciprofloxacin, 70.5% had DSC, as 54.3% of isolates were intermediate to ciprofloxacin. *S. Enteritidis* was the most common serotype among the top 10 *Salmonella* serotypes with DSC, and 92.4% (7,278/7,877) of *S. Enteritidis* isolates had DSC. *S. Typhimurium* (5.4%, 661/12,331), *S. London* (35.6%, 435/1,221), and I 4,[5],12:i:- (7.3%, 243/3,341) were the most common serotypes with resistance to azithromycin. Only 6.4% (28/439) of typhoidal *Salmonella* isolates

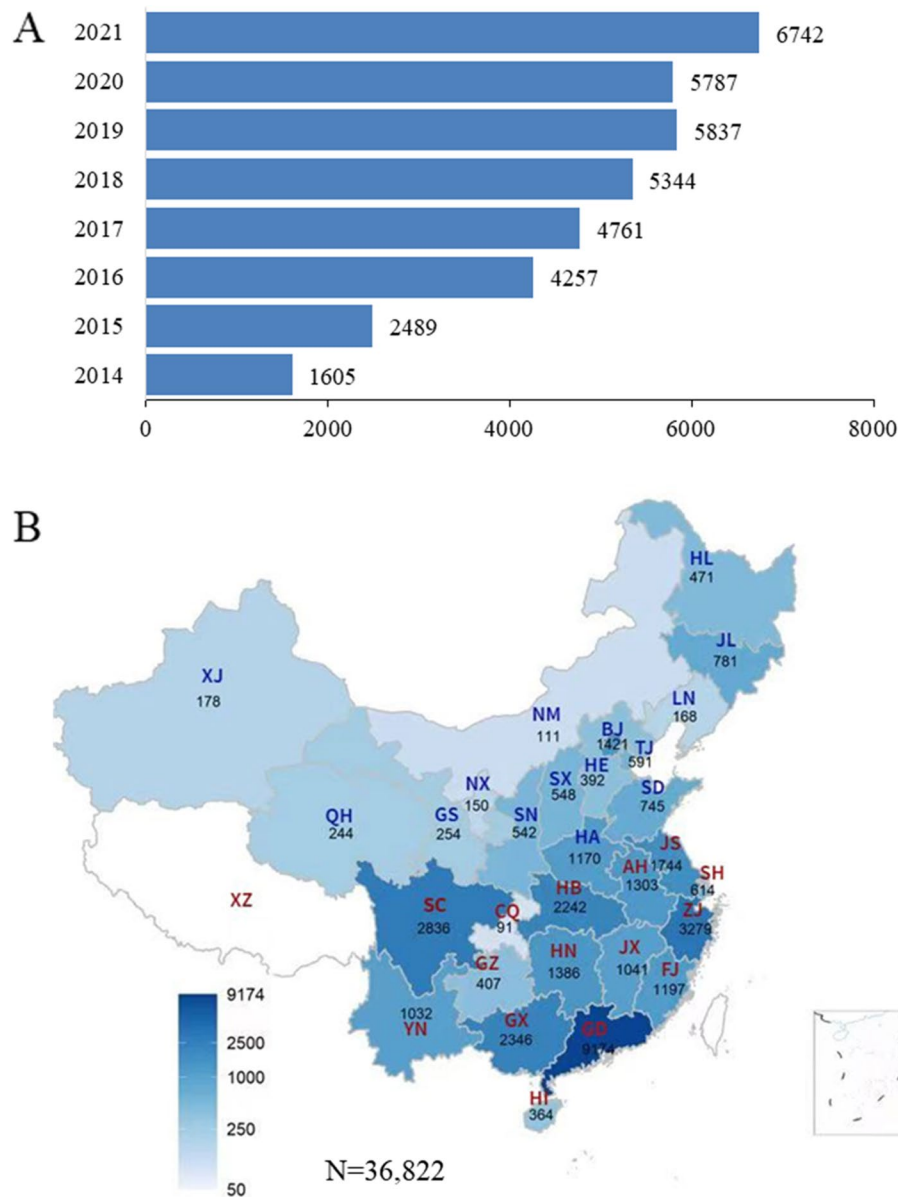


Fig. 1 Year (A) and geographical (B) distribution of 36,822 *Salmonella* isolates from sporadic diarrhea cases in China, 2014–2021. * Provinces in blue font belong to northern China, and provinces in red font belong to southern China

(*S. typhi*, *S. paratyphi* A, *S. paratyphi* B, and *S. paratyphi* C) were resistant to azithromycin. The resistance rate of *Salmonella* isolates to third-generation cephalosporins was 19.3%. The percentages of resistance to ceftazidime and cefotaxime were 10.4% and 19.1%, respectively, indicating the production of extended-spectrum β -lactamase (ESBL) among these resistant isolates. The most common serotypes of these cephalosporin-resistant isolates were Typhimurium ($n=2,830$), I 4,[5],12:i:-($n=997$) and Enteritidis ($n=871$). 1.27% of isolates showed resistance to imipenem, Typhimurium, and I 4,[5],12:i:-being the most

common *Salmonella* serotypes, accounting for 30.1% and 26.2% of imipenem-resistant isolates, respectively.

Discussion

The global burden of AMR has led to triggered the development of coordinated national and global action plans [7, 8]. Increasingly, governments around the world have launched and strengthened AMR surveillance programs in both animals and humans [9–13]. In China, the AMR surveillance program for animals was launched in 2008 and for humans in 2005 [14]. In 2014, AMR surveillance

Table 1 Serotype distribution of 34,631 *Salmonella* isolates from sporadic cases, 2014–2021, China

Serotypes	Number of isolates	Rate (%)	95% CI
Typhimurium	12,331	35.6	(35.1, 36.1)
Enteritidis	7,877	22.7	(22.3, 23.2)
I 4,[5],12:i:-	3,341	9.6	(9.3, 10)
London	1,221	3.5	(3.3, 3.7)
Stanley	1,167	3.4	(3.2, 3.6)
Derby	770	2.2	(2.1, 2.4)
Rissen	758	2.2	(2.0, 2.3)
Thompson	569	1.6	(1.5, 1.8)
Agona	479	1.4	(1.3, 1.5)
Goldcoast	447	1.3	(1.2, 1.4)
Kentucky	283	0.8	(0.7, 0.9)
Infantis	277	0.8	(0.7, 0.9)
Weltevreden	244	0.7	(0.6, 0.8)
Newport	226	0.7	(0.6, 0.8)
Paratyphi B	210	0.6	(0.5, 0.7)
Saintpaul	200	0.6	(0.5, 0.7)
Corvallis	186	0.5	(0.5, 0.6)
Bovismorbificans	168	0.5	(0.4, 0.6)
Typhi	157	0.5	(0.4, 0.5)
Litchfield	152	0.4	(0.4, 0.5)
Dublin	137	0.4	(0.3, 0.5)
Senftenberg	128	0.4	(0.3, 0.4)
Blegdam	114	0.3	(0.3, 0.4)
Meleagridis	108	0.3	(0.3, 0.4)
Virchow	108	0.3	(0.3, 0.4)
All other serotypes	2,973	8.6	(8.3, 8.9)
Total	34,631	100.0	

for foodborne pathogens from human diarrhea cases was launched. The main goal of the AMR surveillance program in TraNet was to track the trend in antibiotic resistance of selected foodborne pathogens, identify important resistance patterns, and assess the effect of interventions. This is the first time that the Chinese human diarrhea surveillance data has been officially released.

Fluoroquinolones, third-generation cephalosporins, and macrolides are commonly used to treat human *Salmonella* infections. Over 70% of human *Salmonella* isolates had decreased susceptibility to ciprofloxacin, and the proportion was far higher than the 5.8% shown by the NARMS in the US [15]. Enteritidis was the most common serotype in both China and the US, a high proportion (>90%) of human Enteritidis isolates with DSC collected in this study indicated that ciprofloxacin may be unsuitable for the treatment of Enteritidis infection in China. A similar resistance rate to ciprofloxacin were

observed in *Salmonella* isolates from China (16.2%) and EU (14.1%) [16]. Moreover, 39.8% of the tested *Salmonella* isolates with DSC were susceptible to nalidixic acid, implying a high presence of plasmid-mediated quinolone resistance (PMQR) in human *Salmonella* infections [15]. The use of fluoroquinolones may further facilitate the transmission of PMQR determinants in patients during treatment. Nearly 20% of human *Salmonella* isolates from diarrhea cases were resistant to third-generation cephalosporins in China, and the resistant proportion was much higher than that in the US (2.7%) and EU (0.8%). However, no upward trend in resistance to third-generation cephalosporins was observed in this study, indicating that these antimicrobials are still effective for the treatment of most *Salmonella* infections. Resistance to third-generation cephalosporins is mainly mediated by ESBLs and AmpC-type β -lactamases. *bla*_{CTX-M} was the most prevalent gene among partial ESBL-producing *Salmonella* isolates in China, according to the whole-genome sequencing (WGS) analysis (unpublished data). Although it is still unclear to what extent isolates with ESBL from animals are directly responsible for human infections, some countries have placed voluntary restrictions on the use of these critically important antimicrobials for disease prophylaxis in food-producing animal husbandry under the One Health perspective [17, 18]. It should be noted that the rate of human azithromycin-resistant *Salmonella* isolates (7.9%) in China was not only higher than that reported in the US and EU (<1%), but also showed a gradual upward trend, suggesting that a prudent use of azithromycin is urgently needed in the treatment of *Salmonella* infections in Chinese clinical settings.

Another important aim of TraNet is to assess the effectiveness of interventions. Previous studies have shown that restricting the use of antibiotics in food-producing animals is associated with a reduction in antibiotic-resistant bacteria in both animals and humans [19, 20]. Our surveillance data also indicated a downward resistant trend in colistin resistance in *Salmonella* isolates from diarrhea cases. This decrease further demonstrates that the ban on colistin as an animal growth promoter in 2017 had a significant effect on reducing colistin resistance in bacteria of human origin, which is consistent with the decreasing colistin resistance of *E. coli* from animals and humans in China after the colistin withdrawal policy was implemented [20, 21]. Therefore, continuous surveillance is necessary for early warning of emerging AMR in human foodborne pathogens in hospital settings.

AMR varies by serotypes in different countries/regions. The serotype distribution of sporadic human isolates in China was similar to that in the EU (top 3: Enteritidis, Typhimurium, and I 4,[5],12:i:-), but different from that

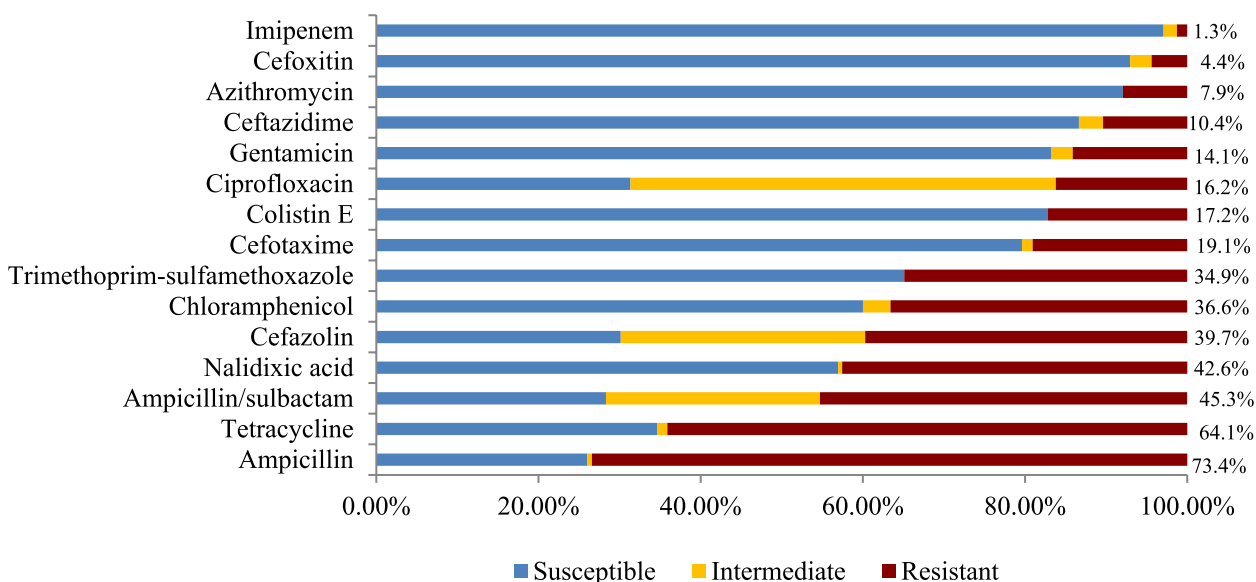


Fig. 2 Resistance profile of *Salmonella* isolates from sporadic cases, 2014–2021, China

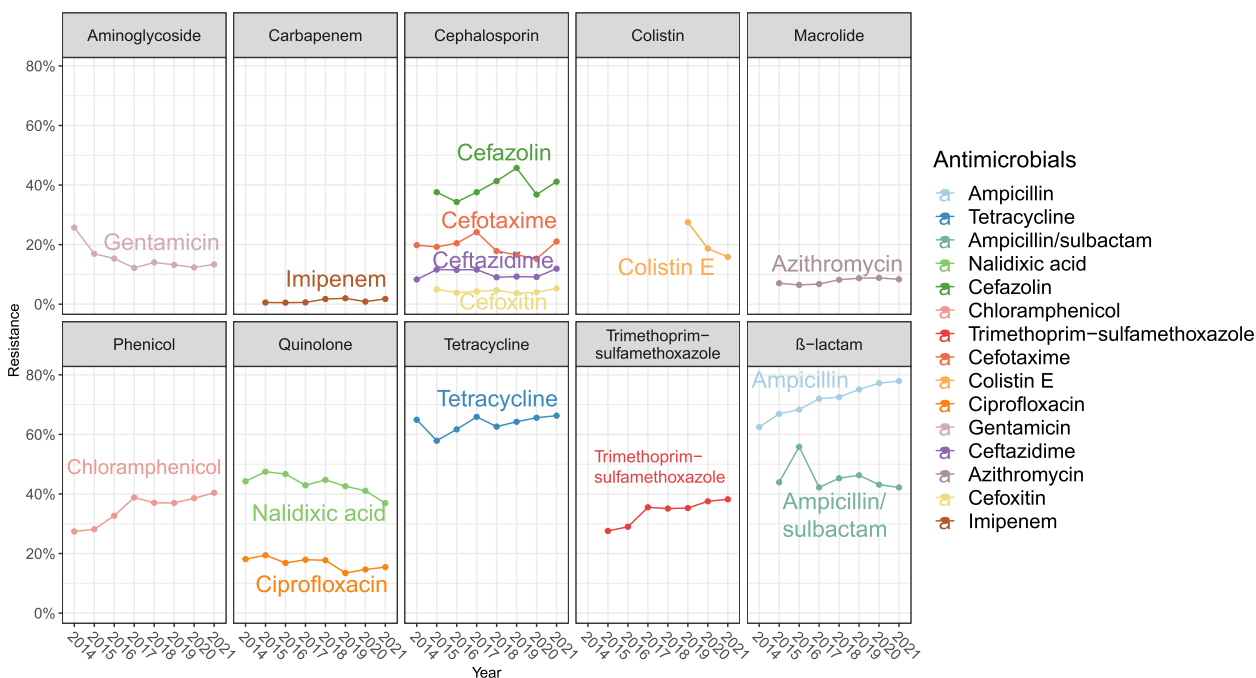


Fig. 3 Resistance rates of 36,822 *Salmonella* isolates to 15 antimicrobial agents by year

in the US (top 3: Enteritidis, Newport, and Typhimurium). Even within China, Enteritidis was the most prevalent serotype in the north of China, whereas Typhimurium and I 4,[5],12:i:- were the dominant serotypes in the south of China. This difference in serotype distribution might be partly explained by dietary and consumption habits or climatic factors. Recent research in China has revealed the dominant mobile genetic elements accounting for the

transmission of MDR I 4,[5],12:i:-, providing evidence for addressing the spread of AMR pathogen [22]. Given the interconnections of AMR amongst the environment, animals, and humans, a national integrated surveillance system across different sectors is needed to better understand the ecology of resistant bacteria, implement evidence-based control measures, and promote effective stewardship.

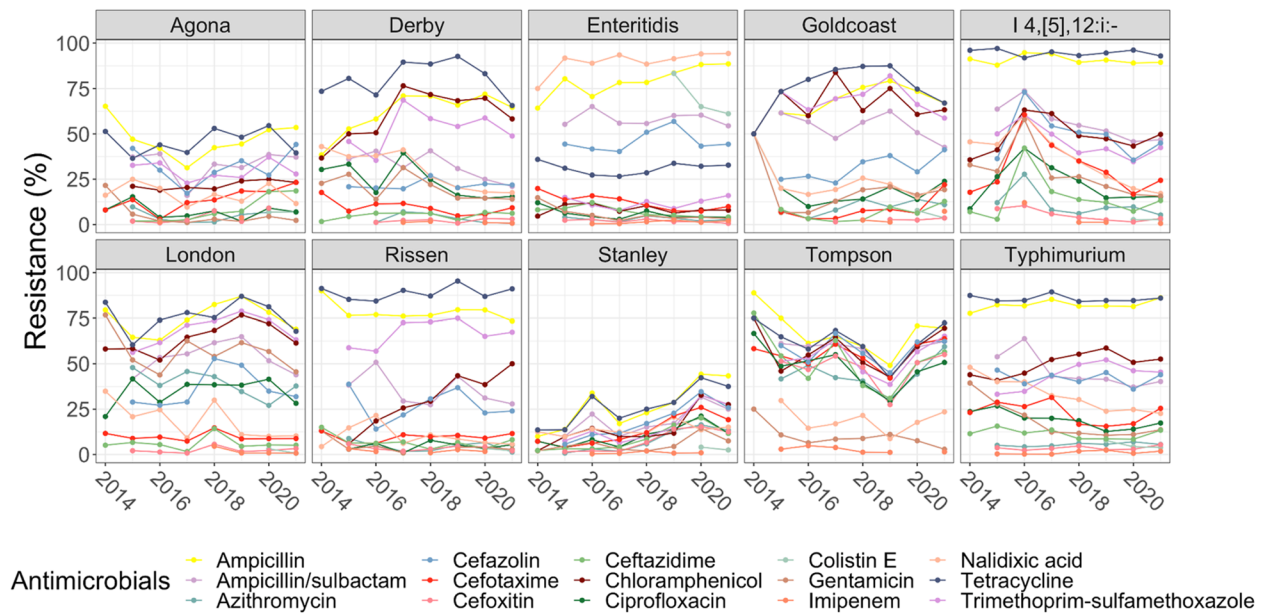


Fig. 4 Antimicrobial resistance of top 10 *Salmonella* serotypes

Limitations

We acknowledge four limitations of the study. First, all *Salmonella* isolates were cultured from selected sentinel hospitals in China, and only a fraction of diarrheal cases were sampled in these hospitals, so the conclusions might not fully represent the *Salmonella* resistance profiles in China. Second, not all isolates were clearly identified due to insufficient data. I 4,[5],12:i:- was underreported in previous years because the second-phase flagellar antigen was not detected. Resistance profiles by different serotypes might have been influenced by this, especially the serovar Typhimurium and I 4,[5],12:i:-. Third, although standard procedures were applied in participating laboratories, the selected antimicrobials and concentration ranges in the panels changed, and not all the isolates were tested against the same antimicrobials. For instance, it was inappropriate to assess the trend of colistin E because of the lack of AST data before 2019. Fourth, WGS analysis, including confirmation of *Salmonella* serotypes, core genome multilocus sequence typing, and determination of virulent and AMR genes (ARGs), was not used in TraNet to facilitate surveillance until 2019. Isolates collected before 2019 were not subjected to the prediction of ARGs; therefore, this study only focused on phenotypes. There is a need to combine AMR testing and WGS techniques to link the resistance of food and human isolates.

Conclusion

We described an eight-year dataset of the AMR profile of *Salmonella* isolates from active foodborne disease in China, and found that *Salmonella* AMR varied

by serotype. Although the isolates displayed downward resistance trends to nalidixic acid, gentamicin, ciprofloxacin, and colistin, the resistance to ampicillin, chloramphenicol, trimethoprim-sulfamethoxazole, and azithromycin presented upward trends. Compared with the surveillance data in the US and EU, the resistance profiles in human *Salmonella* isolates in China suggest that prudent use of antibiotics for treating *Salmonella*-caused foodborne diseases is needed.

Materials and methods

Human *Salmonella* Isolates

Based on the national foodborne disease surveillance plan in China, sentinel hospitals were chosen by each provincial health administration to conduct laboratory-based active foodborne disease surveillance. At least 10 stool specimens per hospital every month were collected from sporadic diarrheal cases to identify the five most common foodborne pathogens, including *Salmonella* species, *Shigella* species, *Vibrio parahaemolyticus*, DEC, and norovirus. According to the guidelines and techniques of the national foodborne disease surveillance manual, all isolates were ultimately sent to provincial CDCs for final identification, molecular subtyping, and AMR testing. Then, provincial CDC laboratories were instructed to submit the epidemiological information and experimental data of isolates to the CFSA via TraNet. In this study, all *Salmonella* isolates were derived from sporadic diarrhea cases under the framework of active foodborne disease surveillance, and isolates from foodborne outbreaks were not included.

Table 2 Antimicrobial agents used for susceptibility testing of *Salmonella* isolates, 2014–2021

WHO category level	Importance	Antimicrobial Class	Antimicrobial Agent	Years Tested	Antimicrobial Agent Concentration Range (µg/mL)	MIC Interpretive Standard (µg/mL)		
						Susceptible (S)	Intermediate (I)	Resistant (R)
I	Critically important ^a	Cephalosporins (3rd, 4th and 5th generation)	Cefotaxime (CTX)	2014–2021	0.25~8	1	2	4
			Ceftazidime (CAZ)	2014–2021	1~32	4	8	16
		Macrolides	Azithromycin (AZM)	2015–2021	4~64	16	-	32
		Polymyxins	Colistin E (CT)	2019–2021	0.12~4	2	-	4
		Quinolones	Nalidixic acid (NAL)	2014–2021	2~64	16	-	32
			Ciprofloxacin (CIP)	2014–2021	0.03~32	0.06	0.125~0.5	1
		Aminoglycosides	Gentamicin (GEN)	2014–2021	1~32	4	8	16
		Carbapenems	Imipenem (IPM)	2015–2021	0.25~8	1	2	4
		Penicillins	Ampicillin (AMP)	2014–2021	2~64	8	16	32
		Penicillins (with β-lactamase inhibitors)	Ampicillin/sulbactam (AMS)	2015–2021	2/1~64/32	8/4	16/8	32/16
		II	Highly important	Cephalosporins (1st and 2nd generation) and Cephamycins	Cefazolin (CFZ)	2015–2021	0.5~16	2
Cefoxitin (CFX)	2015–2021				2~64	8	16	32
Phenicol	Chloramphenicol (CHL)			2014–2021	2~64	8	16	32
Tetracyclines	Tetracycline (TET)			2014–2021	1~32	4	8	16
Folate pathway inhibitors	Trimethoprim-sulfamethoxazole (SXT)			2015–2021	0.25/4.75~8/152	2/38	-	4/76

^a The antimicrobials in bold letters were Highest Priority Critically Important Antimicrobials, and others are High Priority Critically Important Antimicrobials

Antimicrobial susceptibility testing (AST)

The broth microdilution method was applied to test the antimicrobial susceptibility in participating TraNet laboratories. The Clinical and Laboratory Standards Institute (CLSI) recommended antibiotics and commonly used antibiotics in clinical therapy. A panel of 15 antibiotic agents from 11 classes was selected for surveillance, including ampicillin, ampicillin/sulbactam, cefazolin, cefotaxime, ceftazidime, cefoxitin, tetracycline, chloramphenicol, nalidixic acid, ciprofloxacin, azithromycin, gentamicin, trimethoprim-sulfamethoxazole, imipenem, and colistin. All antibiotics were grouped into critically important and highly important antimicrobials based on the World Health Organization (WHO) List of Critically Important Antimicrobials for Human Medicine (WHO CIA List) [23] (Table 2). The MIC for each antimicrobial was used to categorize isolates as susceptible, intermediate (if applicable) or resistant based on CLSI M100-S32 clinical breakpoints. *Escherichia coli* ATCC 25,922 was included to ensure the reliability and reproducibility of AST.

Data analysis

All reported data were audited and checked and then exported and managed using Microsoft Excel 2016. All variable values were reported as counts or percentages (%). Statistical analyses, such as resistance rate, MDR profile, and resistance trend analysis based on a linear regression model, were conducted with R version 4.1.2. Isolates that were resistant to at least one of the tested antimicrobials were considered resistant isolates, and MDR was defined as “resistance to three or more classes of antimicrobials”.

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Author contributions

W.L. analyzed the data and wrote the manuscript; H.H. reviewed the data; J.L. designed the experiments; B.K., L.Z., X.Y., D.T., B.Y., X.H., X.M., S.C., Y.S., W.C., Y.L., C.L., Z.Y., performed the experiments and submitted the data; T.W. conducted statistical analysis; Y.G. conceived and managed the study. All authors read and approved the final manuscript.

Declarations

Ethics approval and consent to participate

Not applicable.

Competing interests

The authors have declared that no conflicts of interest exist.

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