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ORIGINAL RESEARCH

# Prevalence, Serotypes and Antimicrobial Resistance of Salmonella Isolated from Children in Guangzhou, China, 2018–2023

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Purpose: Acute gastroenteritis caused by Salmonella spp. among children post a great threat for global public health. The increasing rate of drug-resistant Salmonella spp. has also become a challenging problem worldwide. In this study, the prevalence, serotypes, and antimicrobial characteristics of Salmonella isolated from children in Guangzhou, China, were investigated to provide supporting information for clinical treatment and prevention.

Methods: Clinical data of children featured with gastroenteritis symptoms from 2018 to 2023 in Guangdong Women and Children Hospital were collected. The difference and fluctuation of antimicrobial resistance between serotypes and years were retrospectively analyzed.

Results: A total of 1304 Salmonella isolates were cultural-confirmed. The overall positive rate of Salmonella isolated from stool samples was 22.0% (1304/5924). Salmonella infections occur mainly from June to September and the majority of infected children aged under 4 years. Serogroup B was the most common serogroup among Salmonella isolates (74.6%, 973/1304). The predominant serotypes of Salmonella isolates were Typhimurium (63.1%, 823/1304). Higher drug resistance rate of Salmonella spp. to ceftriaxone was observed in 2023. The drug resistance rates of Salmonella isolates to sulfamethoxazole/trimethoprim and ampicillin are at high level during the past 6 years. Notably, higher multi-drug resistance (MDR) rate was demonstrated in Salmonella Typhimurium compared with other serotypes.

**Conclusion:** Salmonella Typhimurium was the most common serotype isolated from children in Guangzhou, China, and it may mainly account for the high drug resistance rate in Salmonella spp. to most of the antimicrobial profiles. For controlling the high drug resistance rate of Salmonella spp. continuous surveillance of drug resistance and appropriate use of antibiotics based on clinical and laboratory results are of great significance.

Keywords: gastroenteritis, Salmonella, antimicrobial resistance, Guangzhou, China

#### Introduction

Gastroenterology infection caused by enteropathogenic bacteria, viruses and parasites are known to be the major cause of acute diarrhea among children aged under 5 years.<sup>1-3</sup> Frequent isolations of Salmonella spp. derived from a wide variety of polluted water, animal feeds, and food animal environment continuously occur worldwide.<sup>4</sup> It was estimated that approximately 93.8 million cases of gastroenteritis were attributed to Salmonella infection,<sup>5</sup> which even resulted in more than 300,000 deaths per year in developing countries.<sup>5–7</sup> Salmonella is a rod-shaped, gram-negative bacteria with no capsule and spore, commonly transmitted through contaminated food products.<sup>8</sup> Salmonella can be classified into typhoidal Salmonella and non-typhoidal Salmonella (NTS) serovars.<sup>9</sup> NTS that caused bacterial enteritis, especially

serotypes Typhimurium and Enteritidis, has been recognized as a global burden.<sup>2,5</sup> *Salmonella* comprises 392 species based on more than 2600 serotypes,<sup>10–12</sup> while A-F serogroups are the major serotypes prevalent in China.<sup>13</sup>

A large-scale observation showed that the proportion of *Salmonella* Typhimurium in London, Rissen, American and European countries displayed an increasing tendency during 2006–2019.<sup>14</sup> In China, NTS was one of the leading bacterial pathogens that was identified from pediatric patients.<sup>15</sup> Moreover, the positive rate of NTS in China was reported to be the highest in south regions compared with other areas, which was considered to be related with higher temperature.<sup>16</sup> Currently, increasing resistance to broad range of antibiotics in non-typhoidal *Salmonella* strains has become an emerging threat,<sup>17–19</sup> involving enteric and invasive infection. For the susceptibility to antimicrobial agents, different serotypes of *Salmonella* carrying various virulence genes may exhibit differently. However, the information about the antibiotic-resistance and characteristics of *Salmonella* infection among pediatric patients in the south of China is limited yet.

Therefore, this study analyzed enteric *Salmonella* infection among children in Guangzhou, China, and retrospectively investigated the distribution, characteristics and pattern of drug-resistance from 2018 to 2023 to promote the understanding of *Salmonella* infection and improve the clinical treatment.

# **Methods and Materials**

#### Population

This retrospective study was conducted in Guangdong Women and Children Hospital, which is one of the largest women's and children's hospitals in Guangdong Province. Clinical information involving date of tests, sex, age, the results of bacterial culture and antimicrobial resistance were included. Children aged under 14 years old who were defined as patients with gastroenteritis symptoms were enrolled in this study.<sup>20</sup> Stool samples (2–5mL liquid stools or 2–5g solid stools) were collected using sterile and dry container for immediate detection (within 2 h). Rectal swab samples should only be collected from infants or severe diarrheal patients without stool for test. The related clinical information in this study was collected from January 2018 to December 2023.

#### Salmonella Identification and Serotyping

Isolation of *Salmonella* spp. was conducted according to reported methods.<sup>21,22</sup> A 1 g or 1 mL stool samples were plated and incubated onto XLD (Xylose Lysine Desoxycholate) agars at 36°C for 18–48 h. The suspicious colonies (transparent, round, with or without black centers that reflect H<sub>2</sub>S production) were further identified by MALDI-TOF-MS (Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry). Subsequently, O and H antigens of validated *Salmonella* spp. were characterized by commercial anti-serum (Tianrun Bio-Pharmaceutical, China) and the serotypes were classified according to Kauffmann-Le Minor scheme,<sup>23</sup> with PBS (Phosphate Buffered Saline) as negative control and *Salmonella* Typhimurium ATCC 14028 as positive control.

# Antimicrobial Susceptibility Testing (AST)

The antimicrobial susceptibility of *Salmonella* isolates was determined according to the guidelines of Clinical and Laboratory Standards Institute (CLSI).<sup>24,25</sup> Antimicrobial susceptibility testing (AST) was carried out on the VITEK 2-Compact system (bioMerieux, Inc., France), using AST-GN09 commercial card (antimicrobial classes and details were displayed in <u>Supplementary Materials</u> and <u>Methods</u>). The MacFarland 0.5 inoculums were prepared and swabbed on the whole surface of Mueller–Hinton agar for dusk diffusion assays. *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 were used as control strains. Alternative antibiotic susceptibility tests were conducted by Kirby–Bauer disk diffusion method (Oxoid, UK), and classified as resistant (R), intermediate (I) and sensitive (S) according to the CLSI guidelines.

# Definitions

Multidrug resistance (MDR) was defined as resistance to three or more antibacterial classes, such as aminopenicillin (ampicillin, amoxicillin),  $\beta$ -lactam combination agents (ampicillin/sulbactam, piperacillin/tazobactam), cephalosporins (ceftriaxone, ceftazidime, cefepime, cefotaxime), monobactams (aztreonam), carbapenems (imipenem), dihydrofolate

reductase inhibitors (trimethoprim/sulfamethoxazole), and fluoroquinolones (ciprofloxacin, levofloxacin).<sup>26,27</sup> First-line agents include the following antimicrobial agents: ampicillin (AMP), ceftriaxone (CRO), ciprofloxacin (CIP), sulfamethoxazole/trimethoprim (SXT).<sup>28</sup>

### Statistical Analysis

Information of patients and clinical data in this study were exported from Hospital Information System and categorized in Microsoft Excel. SPSS version 26.0 was utilized to statistical analysis involving *Chi-square* test or *Fisher's* exact probability method in this study. Tables in this study were conducted via Microsoft Excel and Figures were made by R Packages. *P*<0.05 indicates statistically significant differences.

# Results

#### Clinical Information and Prevalence of Salmonella Isolates

A total of 5924 cases were included in this study (Table 1). From 2018 to 2023, the positive rates of *Salmonella* isolation were 20.7%, 20.8%, 21.9%, 21.2%, 25.1%, 24.2%, respectively. The fecal isolation of *Salmonella* was statistically higher in 2022 ( $\chi^2$ =5.548, *P*=0.019) than in 2018. *Salmonella* isolation exhibits in season distribution as 9.4% in spring, 26.3% in summer, 30.0% in autumn and 18.8% in winter. Notably, *Salmonella* isolation in summer, autumn and winter were significantly higher than in spring (*P*<0.001). *Salmonella* isolated from in-patients and out-patients were 18.45% and 29.68%, respectively. There were 22.23% male patients and 21.69% female patients were detected as positive in *Salmonella*. Of all the age groups, the positive rates of cultural-confirmed *Salmonella* were 19.7% in <1 age group, 28.2% in 1–3 age group, 15.8% in 4–6 age group and 9.4% in 7–14 age group, respectively. Compared with children under 1 year, children aged 1–3 years old exhibited higher isolation in *Salmonella* ( $\chi^2$ =52.045, *P*<0.001), while children aged 7–14 years old were statistically lower in positive rate ( $\chi^2$ =11.570, *P*=0.001).

Monthly distribution of *Salmonella* isolates was analyzed. As is shown in Figure 1, the fluctuation of the positive rate from January to December in the past 6 years was similar in total. Higher trend was demonstrated from June to September than other months and the positive rate of *salmonellosis* always reached peak in June or July.

Category	Subcategory		S	almonella cultu	OR (95% CI) <sup>#</sup>	χ²	P		
		n	Negative	Proportion (%)	Positive	Proportion (%)			
Years	2018	1400	1110	79.3	290	20.7	-	-	-
	2019	1238	981	79.2	257	20.8	1.00 (0.83–1.21)	0.001	0.977
	2020	636	497	78.1	139	21.9	1.07 (0.85–1.35)	0.342	0.558
	2021	996	785	78.8	211	21.2	1.03 (0.84–1.26)	0.078	0.780
	2022	798	598	74.9	200	25.1	1.28 (1.04–1.57)	5.548	0.019
	2023	856	649	75.8	207	24.2	1.22 (1.00–1.50)	3.719	0.054
Seasons	Spring	1293	1171	90.6	122	9.4	-	-	-
	Summer	1401	1033	73.7	368	26.3	3.42 (2.74–4.27)	128.019	<0.001
	Autumn	1854	1298	70.0	556	30.0	4.11 (3.33–5.08)	190.387	<0.001
	Winter	1376	1118	81.3	258	18.8	2.22 (1.76–2.79)	47.366	<0.001
Sources	In	4027	3284	81.5	743	18.5	-	-	-
	Out	1890	1329	70.3	561	29.7	1.87 (1.64–2.12)	94.451	<0.001
Gender	Male	3562	2770	77.8	792	22.2	-	-	-
	Female	2360	1848	78.3	512	21.7	0.97 (0.85–1.10)	0.241	0.624
Age groups (years)	<1	3496	2808	80.3	688	19.7	-	-	-
	I_3	1964	1410	71.8	554	28.2	1.60 (1.41–1.82)	52.045	<0.001
	4–6	284	239	84.2	45	15.8	0.77 (0.55–1.69)	2.471	0.116
	7–14	180	163	90.6	17	9.4	0.43 (0.26–0.71)	11.570	0.001

Table I Clinical Information of Samples for Suspected Salmonella Infection

Notes: <sup>#</sup>OR, odd ratio. Cl, confidence interval.

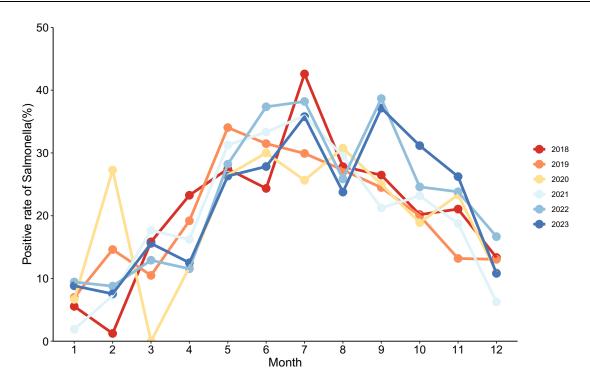


Figure I Monthly distribution of Salmonella isolates.

#### Serotype Distribution of Salmonella Isolates

Serogroup B (74.6%) was the predominant *Salmonella* serogroup (Table 2), followed by serogroup C (10.20), D (7.0%), E (4.4%) and ungrouped (2.6%). Notably, *Salmonella* Typhimurium accounted for the major composition of *Salmonella* isolates (63.1%, 823/1304). The classified *Salmonella* Enteritidis and *Salmonella* Stanley was both 2.1% (28/1304),

Serogroups and Serotypes	Salmonella isolates				
	Number	Proportion (%)			
Typhoidal	14	1.1			
Salmonella Typhi	1	0.1			
Salmonella Paratyphi A	2	0.2			
Salmonella Paratyphi B	11	0.8			
Non-typhoidal (NTS)	1290	98.9			
NTS Serogroup B	973	74.6			
Salmonella Typhimurium	823	63.1			
Salmonella Agona	5	0.4			
Salmonella Stanley	28	2.1			
Salmonella Derby	11	0.8			
Salmonella Saintpaul	2	0.2			
Untyped B	104	8.0			
NTS Serogroup C	133	10.2			
Untyped CI	90	6.9			
Untyped C2	41	3.1			
Untyped C	2	0.2			

Table	2	Distribution	of	1304	Salmonella	Isolates	in
Serogro	oup	s and Serotyp	es				

(Continued)

Serogroups and Serotypes	Salmonella isolates				
	Number	Proportion (%)			
NTS Serogroup D	91	7.0			
Salmonella Enteritidis	28	2.1			
Salmonella Dublin	5	0.4			
Salmonella Blegdam	16	1.2			
Untyped D	42	3.2			
NTS Serogroup E	57	4.4			
Salmonella London	15	1.2			
Salmonella anatum	I	0.1			
Salmonella Weltevreden	6	0.5			
Untyped EI	30	2.3			
Untyped E2	5	0.4			
NTS Serogroup F	2	0.2			
Untyped F	2	0.2			
Ungrouped	34	2.6			

Table 2 (Continued).

respectively. Other serotypes of classified isolates included *Salmonella* Paratyphi B (0.8%, 11/1304), *Salmonella* Derby (0.8%, 11/1304), *Salmonella* London (1.2%, 15/1304) and *Salmonella* Blegdam (1.2%, 16/1304). During the past 6 years, *Salmonella* Typhimurium was always the predominant serotype, and the proportion of *Salmonella* Enteritidis in 2021 was significantly higher than others. The lowest frequency of positive cases was observed in 2020 (Figure 2).

#### Antibiotic Susceptibility Tests

The antibiotic resistance profiles of *Salmonella* isolates were analyzed followed by dividing *Salmonella* isolates into serotypes of Typhimurium, Enteritidis, Stanley and Other (Table 3, <u>Supplementary Table 1</u>). Statistical difference was

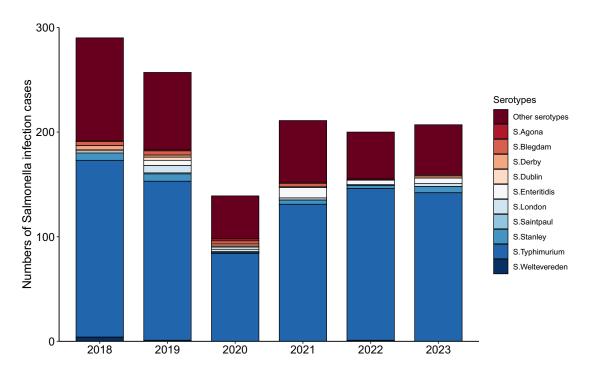


Figure 2 Distribution of Salmonella serotypes by years.

Antimicrobials	Typhimurium		Enteritidis		Stanley		Other Serotypes		χ <sup>2</sup>	Р
	R (%)	R/n	R (%)	R/n	R (%)	R/n	R (%)	R/n		
AMP	90.73	(587/647)	86.96	(20/23)	8.33	(2/24)	69.62	(236/339)	131.563#	<0.001
AMO	89.47	(221/247)	100.00	(6/6)	8.33	(1/12)	68.10	(111/163)	29.125	<0.001ª
SAM	79.28	(199/251)	100.00	(6/6)	8.33	(1/12)	60.74	(99/163)	16.855	<0.001ª
TZP	2.21	(6/271)	0.00	(0/6)	0.00	(0/13)	2.98	(5/168)	-	-
IPM	0.62	(4/641)	0.00	(0/22)	0.00	(0/24)	0.00	(0/327)	-	-
ATM	21.20	(53/250)	0.00	(0/6)	0.00	(0/12)	11.52	(19/165)	6.502	0.011ª
FEP	10.70	(29/271)	0.00	(0/6)	0.00	(0/13)	4.79	(8/167)	4.668	0.031ª
CAZ	17.46	(110/630)	4.35	(1/23)	0.00	(0/23)	11.64	(39/335)	5.671	0.017 <sup>a</sup>
СТХ	34.72	(25/72)	0.00	(0/1)	0.00	(0/2)	22.22	(6/27)	1.427	0.232 <sup>a</sup>
CRO	27.11	(177/653)	4.55	(1/22)	0.00	(0/23)	15.32	(51/333)	17.246	<0.001ª
LEV	0.74	(2/271)	0.00	(0/6)	0.00	(0/12)	0.60	(1/166)	-	-
CIP	6.58	(42/638)	0.00	(0/22)	0.00	(0/22)	8.92	(29/325)	1.726	0.189 <sup>a</sup>
SXT	79.28	(199/251)	100.00	(6/6)	8.33	(1/12)	60.74	(99/163)	16.855	<0.001ª
First-line agents										
0 agent (no resistant)	8.79	(59/671)	13.04	(3/23)	87.50	(21/24)	28.70	(99/345)	123.698#	<0.001
l agent	28.76	(193/671)	69.57	(16/23)	12.50	(3/24)	22.03	(76/345)	29.018	<0.001
2 agents	45.75	(307/671)	17.39	(4/23)	0.00	(0/24)	34.49	(119/345)	17.491	<0.001 <sup>b</sup>
3 agents	14.90	(100/671)	0.00	(0/23)	0.00	(0/24)	12.75	(44/345)	0.865	0.393 <sup>a</sup>
4 agents	1.79	(12/671)	0.00	(0/23)	0.00	(0/24)	2.03	(7/345)	0.072	0.789 <sup>a</sup>
Class Level										
0 class (no resistant)	8.79	(59/671)	13.04	(3/23)	87.50	(21/24)	28.70	(99/345)	I 23.698 <sup>#</sup>	<0.001
l class	19.82	(133/671)	47.83	(11/23)	8.33	(2/24)	12.46	(43/345)	21.544#	<0.001
2 class	36.66	(246/671)	34.78	(8/23)	4.17	(1/24)	27.25	(94/345)	18.326	<0.001
3 class	23.99	(161/671)	4.35	(1/23)	0.00	(0/24)	23.19	(80/345)	0.082	0.815 <sup>a</sup>
≥4 class	10.73	(72/671)	0.00	(0/23)	0.00	(0/24)	8.41	(29/345)	1.375	0.269 <sup>a</sup>
MDR	34.72	(233/671)	4.35	(1/23)	0.00	(0/24)	31.59	(109/345)	9.749	0.007 <sup>b</sup>

Table 3 Resistance of Salmonella Isolates to Antimicrobials

**Notes**: *<sup>#</sup>Fisher's* exact test. <sup>a</sup>Comparison of Typhimurium, Stanley and Other serotypes. <sup>b</sup>Comparison of Typhimurium versus Other serotypes. **Abbreviations**: AMP, ampicillin; AMO, amoxicillin; SAM, ampicillin/sulbactam; TZP, piperacillin/tazobactam; IPM, imipenem; ATM, aztreonam; FEP, cefepime; CAZ, ceftazidime; CTX, cefotaxime; CRO, ceftriaxone; LEV, levofloxacin; CIP, ciprofloxacin; SXT, sulfamethoxazole/trimethoprim. R, resistance to antimicrobial agents.

First-line agents: ampicillin (AMP), ceftriaxone (CRO), ciprofloxacin (CIP), sulfamethoxazole/trimethoprim (SXT).

observed in ampicillin (AMP,  $\chi^2$ =131.563, *P*<0.001) resistance among the four groups. Moreover, the following antimicrobial resistance between Typhimurium and Other serotypes were also displayed statistical differences: amoxicillin (AMO,  $\chi^2$ =29.125, *P*<0.001), ampicillin/sulbactam (SAM,  $\chi^2$ =16.855, *P*<0.001), aztreonam (ATM,  $\chi^2$ =6.502, *P*=0.011), cefepime (FEP,  $\chi^2$ =4.668, *P*=0.031), ceftazidime (CAZ,  $\chi^2$ =5.671, *P*=0.017), ceftriaxone (CRO,  $\chi^2$ =17.246, *P*<0.001), sulfamethoxazole/trimethoprim (SXT,  $\chi^2$ =16.855, *P*<0.001). These antimicrobial resistances in Typhimurium were significantly higher than the group of other serotypes. Intermediate resistance to ciprofloxacin in *Salmonella* isolates was remarkable among all the antimicrobials, specifically 26.33% in Typhimurium, 45.45% in Enteritidis, 13.64% in Stanley and 21.23% in other serotypes (Supplementary Table 1).

In comparison of first-line agent resistance among the four groups (Table 3), the proportion of no resistance was demonstrated statistically different ( $\chi^2$ =123.698, P<0.001). As was estimated, the proportions of no resistance were Typhimurium 8.79% (59/671), Enteritidis 13.04% (3/23), Stanley 87.50% (21/24), and other serotypes 28.70% (99/345). For 1 agent resistance, the proportions of the four groups were Typhimurium 28.76% (193/671), Enteritidis 69.57% (16/23), Stanley 12.50% (3/24) and other serotypes 22.03% (76/345), and the difference was statistically significant ( $\chi^2$ =29.018, P<0.001). The resistance of the four groups in different class levels displayed similar tendency to the resistance in first-line agents. Notably, the rate of MDR were Typhimurium 34.72% (233/671), Enteritidis 4.35% (1/23) and other serotypes 31.59% (109/345), with statistical difference ( $\chi^2$ =9.749, P=0.007) among them.

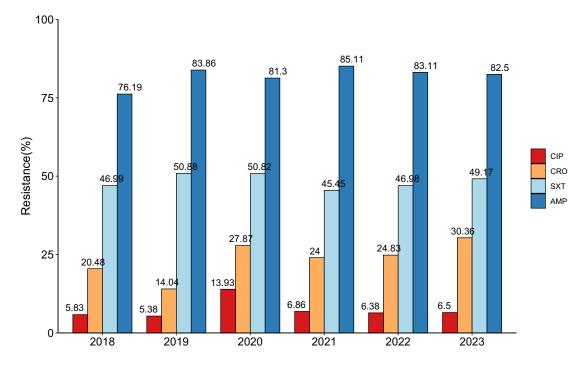


Figure 3 Antimicrobial resistance trends of Salmonella isolates to first-line agents from 2018 to 2023.

Among the first-line antimicrobial profiles (Figure 3), the highest resistance rate was AMP. The overall AMP resistance rate from 2018 to 2023 was 76.19%, 83.86%, 81.30%, 85.11%, 83.11% and 82.50%, respectively, with no statistical difference ( $\chi^2$ =7.127, *P*=0.211). Also, there was no statistical difference ( $\chi^2$ =1.832, *P*=0.872) in SXT resistance among 6 years. However, statistical differences were observed in year distribution of antimicrobial resistance to ceftriaxone (CRO,  $\chi^2$ =17.026, *P*=0.004) and ciprofloxacin (CIP,  $\chi^2$ =182.659, *P*<0.001). The highest rate of CRO resistance was observed in 2023 (30.36%), while the highest rate of CIP resistance was observed in 2020 (13.93%).

#### Discussion

*Salmonella* is one of the leading causes of acute gastroenteritis, with an overall rate of 22.01% from cultural-confirmed tests in this study. From this study, the majority of the cases occurred in children aged under 4 years old, which is consistent with the retrospective studies carried out in other hospitals in Guangzhou, China.<sup>13,29</sup> In a 6-year (2013–2018) surveillance study in Shenzhen,<sup>30</sup> the prevalence of *Salmonella* in children younger than 5 years was 10.1% (137/1361) and in children aged 5–9 years old was 15.1% (11/73), which was lower than the prevalence in this study. Given that Guangzhou and Shenzhen are the two central cities of Guangdong Province that shared with similar economic levels and medical resources, these differences between the studies may be attributed to variation of the number of samples. It was demonstrated in the study of Fujian, China that the proportion of S. Typhimurium and drug-resistance rate was similar to this study.<sup>28</sup> Similar results were also observed in season distributions,<sup>13</sup> and the cases of *Salmonella* (especially NTS) infection were concentrated from June to September.<sup>12,28</sup> Higher environmental temperature was associated with increase of *Salmonella* infections.<sup>31</sup>

The serotype distribution of isolated *Salmonella* varied from regions or countries. In the south of China, S. Typhimurium was the major and dominant serotypes detected from patients,<sup>28,29,32</sup> with the proportion of over 50%, which was also demonstrated in this study. In the southeast of Asia, Vietnam, a relevant study revealed that S. Typhimurium was the most predominant serotype (41.8%) in the NTS isolates.<sup>33</sup> However, in Denmark, the leading non-typhoid *Salmonella* serotypes were S. Enteritidis, monophasic S. Typhimurium and S. Typhimurium.<sup>34</sup> In south America, S. Typhimurium accounted for less than 15% of NTS, although most of the isolates were unclassified.<sup>35</sup> Recently, there were opinions claiming that climate-related foodborne and waterborne diseases have increased and provided possible mitigations against Typhoidal *Salmonella* dissemination.<sup>16</sup> In a nationwide population-based study in Netherlands, the leading serotypes of invasive NTS were

Enteritidis and Typhimurium.<sup>36</sup> An extensive multi-country outbreak of multidrug-resistant monophasic *Salmonella* Typhimurium infection in 10 countries was investigated in 2022, which was linked to chocolate products.<sup>37</sup> The foodborne incidents caused by *Salmonella* are continuous challengeable problems worldwide.

The frequency of multi-drug resistance was common in enteric *Salmonella* isolates. In this study, *Salmonella* Typhimurium attributed to high rate of MDR in 34.72%. The mechanism of MDR occurrence was demonstrated to be related with coexistence of related genes.<sup>38</sup> The transmission of resistance genes may result in great challenge in clinical treatments. Plasmidmediated quinolone resistance (PMQR) determinants and mutations in quinolone resistance determination regions (QRDRs) were commonly used in assessments of quinolone-resistant genes. High frequency of mutations in *gyrA*, *gyrB*, *parC*, and *parE* genes were found in quinolone-resistant strains,<sup>30</sup> which may be associated with the observed high intermediary resistance to ciprofloxacin in this study. Moreover, high prevalence of intermediate resistance to ciprofloxacin in *Salmonella* isolated from poultry production chain should be paid more attention.<sup>39</sup> Among PMQR determinants, aac(6')-Ibcr and oqxA/oqxB were commonly conferred resistance.<sup>40</sup> From this study, the rate of resistance to ceftriaxone in *Salmonella* spp. in 2023 was 30.36%, which was the highest among the past 6 years. The major resistance mechanism of ceftriaxone is the production of AmpC β-lactamases, such as CMY and DHA, and extended-spectrum β-lactamases (ESBL), including TEM, SHV, CTX-M, VEB, and GES.<sup>41</sup> The dissemination of ESBL-producing *Salmonella* isolates calls for more attention. The prevalence and emergence of MDR isolates suggested that ongoing surveillance of antibiotic resistance are needed.

Indeed, there are also some limits in this study. A larger scale of stool samples, more types of enteric pathogens and clinical information such as syndromes, vaccination and district should be collectively considered. As the gastrointestinal is home to numerous types of microorganisms, the co-infection with multiple pathogens or their communications need to be considered. Accumulating evidence has reported the enteric pathogens co-infection may result in severe outcomes.<sup>42–44</sup> Multiplex polymerase chain reaction assay has been previously used for validation and identification of invasive *Salmonella*.<sup>45</sup> FilmArray Gastrointestinal panel (FAGIP) is a novel multiplex PCR kit used for enteric pathogens detection in infections diarrhea with high sensitivity and efficiency compared to conventional methods.<sup>46,47</sup> Thus, from the evidence above, there would be significant improvement and comprehensive understanding for pediatric enteric infection via updated technology.

#### Conclusion

In general, prevalence and antimicrobial characteristic enteric *Salmonella* derived from pediatric patients in Guangzhou, China, were retrospectively analyzed. This study showed an overall positive rate of *Salmonella* from 2018 to 2023 in Guangzhou, China, and the infection was most prevalent in summer and autumn. Children under 4 years old were the major infected objects with enteric *Salmonella*. Moreover, this study displayed fluctuation of antimicrobial resistance of overall serotypes of *Salmonella*, specifically *Salmonella* Typhimurium involving ampicillin, ceftriaxone, sulfamethoxazole/trimethoprim and ciprofloxacin during the six years. Differences were observed in antimicrobial resistance between *S*. Typhimurium and other serotypes. This study also implicated potential genotype variants in enteric *Salmonella*, which required larger scale of samples and genotype sequencing for further investigation of mechanism underlying.

There were some limitations in this study: 1) Molecular epidemiological investigation for mechanisms underlying antimicrobial resistance was not conducted in this study. 2) Some of *Salmonella* isolates failed to be typed or grouped and not all isolates were carried out in antimicrobial susceptibility testing. 3) The clinical information and source of samples were limited. To fill these gaps, exploring the molecular mechanisms based on the trends and prevalence of antimicrobial resistance, as well as the distinct features of *Salmonella* isolates in different serotypes would be our future directions. Furthermore, it is crucial to develop large-scale and multi-source investigations for comprehensive understanding in epidemiology and evolution of *Salmonella*.

# **Data Sharing Statement**

The data that support the findings are available from the corresponding author upon non-commercial request.

# **Ethics Approval**

This study was approved by the Medical Ethics Committee of Guangdong Women and Children Hospital, China, and it was conducted according to the ethical guidelines of the Declaration of Helsinki.

#### **Consent to Participants**

The informed consent was waived by the Medical Ethics Committee of Guangdong Women and Children Hospital because of the retrospective nature of the study and all data used in this study were strictly anonymous.

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#### Disclosure

The authors declare that they have no competing interests in publishing this paper.

#### References

- 1. Cohen R, Minodier P, Hau I, et al. Anti-infective treatment of gastro-intestinal tract infections in children. *Infect Dis Now.* 2023;53(8s):104784. doi:10.1016/j.idnow.2023.104784
- 2. Farthing M, Salam MA, Lindberg G, et al. Acute diarrhea in adults and children: a global perspective. *J Clin Gastroenterol*. 2013;47(1):12–20. doi:10.1097/MCG.0b013e31826df662
- 3. Troeger C, Blacker BF, Khalil IA. Estimates of the global, regional, and national morbidity, mortality, and aetiologies of diarrhoea in 195 countries: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet Infect Dis.* 2018;18(11):1211–1228. doi:10.1016/s1473-3099(18)30362-1
- Magwedere K, Rauff D, De Klerk G, Keddy KH, Dziva F. Incidence of nontyphoidal salmonella in food-producing animals, animal feed, and the associated environment in South Africa, 2012-2014. Clin Infect Dis. 2015;61(Suppl 4):S283–289. doi:10.1093/cid/civ663
- 5. Majowicz SE, Musto J, Scallan E, et al. The global burden of nontyphoidal Salmonella gastroenteritis. *Clin Infect Dis.* 2010;50(6):882-889. doi:10.1086/650733
- Seif Y, Kavvas E, Lachance JC, et al. Genome-scale metabolic reconstructions of multiple Salmonella strains reveal serovar-specific metabolic traits. Nat Commun. 2018;9(1):3771. doi:10.1038/s41467-018-06112-5
- Antillon M, Warren JL, Crawford FW, et al. The burden of typhoid fever in low- and middle-income countries: a meta-regression approach. PLoS Negl Trop Dis. 2017;11(2):e0005376. doi:10.1371/journal.pntd.0005376
- Yang Q, Chen J, Dai J, et al. Total coliforms, microbial diversity and multiple characteristics of Salmonella in soil-irrigation water-fresh vegetable system in Shaanxi, China. Sci Total Environ. 2024;924:171657. doi:10.1016/j.scitotenv.2024.171657
- 9. Chang YJ, Chen YC, Chen NW, et al. Changing antimicrobial resistance and epidemiology of non-typhoidal Salmonella infection in Taiwanese children. *Front Microbiol.* 2021;12:648008. doi:10.3389/fmicb.2021.648008
- Judd MC, Hoekstra RM, Mahon BE, Fields PI, Wong KK. Epidemiologic patterns of human Salmonella serotype diversity in the USA, 1996-2016. Epidemiol Infect. 2019;147:e187. doi:10.1017/s0950268819000724
- Kipper D, Hellfeldt RM, De Carli S, et al. Salmonella serotype assignment by sequencing analysis of intergenic regions of ribosomal RNA operons. *Poult Sci.* 2019;98(11):5989–5998. doi:10.3382/ps/pez285
- 12. Wu LJ, Luo Y, Shi GL, Prevalence LZY. Clinical characteristics and changes of antibiotic resistance in children with nontyphoidal salmonella infections from 2009-2018 in Chongqing, China. *Infect Drug Resist.* 2021;14:1403–1413. doi:10.2147/IDR.S301318
- Gao F, Huang Z, Xiong Z, et al. Prevalence, serotype, and antimicrobial resistance profiles of children infected with Salmonella in Guangzhou, southern China, 2016-2021. Front Pediatr. 2023;11:1077158. doi:10.3389/fped.2023.1077158
- 14. Wang Y, Liu Y, Lyu N, et al. The temporal dynamics of antimicrobial-resistant Salmonella enterica and predominant serovars in China. *Natl Sci Rev.* 2023;10(3):nwac269. doi:10.1093/nsr/nwac269
- 15. Huang X, Huang Q, Dun Z, et al. Nontyphoidal Salmonella Infection, Guangdong Province, China, 2012. *Emerg Infect Dis.* 2016;22(4):726–729. doi:10.3201/eid2204.151372
- 16. Jia C, Cao Q, Wang Z, van den Dool A, Yue M. Climate change affects the spread of typhoid pathogens. *Microb Biotechnol*. 2024;17(2):e14417. doi:10.1111/1751-7915.14417
- 17. Nair S, Ashton P, Doumith M, et al. WGS for surveillance of antimicrobial resistance: a pilot study to detect the prevalence and mechanism of resistance to azithromycin in a UK population of non-typhoidal Salmonella. J Antimicrob Chemother. 2016;71(12):3400–3408. doi:10.1093/jac/dkw318
- 18. Ed-Dra A, Karraouan B, Allaoui AE, et al. Antimicrobial resistance and genetic diversity of Salmonella Infantis isolated from foods and human samples in Morocco. J Glob Antimicrob Resist. 2018;14:297–301. doi:10.1016/j.jgar.2018.05.019
- 19. Ford L, Shah HJ, Eikmeier D, et al. Antimicrobial-resistant nontyphoidal Salmonella infection following international travel-United States, 2018-2019. J Infect Dis. 2023;228(5):533-541. doi:10.1093/infdis/jiad128
- 20. Mughini-Gras L, Pijnacker R, Heusinkveld M, et al. Societal burden and correlates of acute gastroenteritis in families with preschool children. *Sci Rep.* 2016;6(1):22144. doi:10.1038/srep22144
- Nambiar RB, Elbediwi M, Ed-Dra A, Wu B, Yue M. Epidemiology and antimicrobial resistance of Salmonella serovars Typhimurium and 4,[5],12: i- recovered from hospitalized patients in China. *Microbiol Res.* 2024;282:127631. doi:10.1016/j.micres.2024.127631
- 22. Ke Y, Lu W, Liu W, Zhu P, Chen Q, Zhu Z. Non-typhoidal Salmonella infections among children in a tertiary hospital in Ningbo, Zhejiang, China, 2012-2019. PLoS Negl Trop Dis. 2020;14(10):e0008732. doi:10.1371/journal.pntd.0008732
- 23. G PAD, W FX. Antigenic Formulae of the Salmonella Serovars. Paris, France: WHO Collaborating Centre for Reference and Research on Salmonella, Institut Pasteur; 2007.

- 24. Humphries RM, Abbott AN, Hindler JA. Understanding and addressing CLSI breakpoint revisions: a primer for clinical laboratories. J Clin Microbiol. 2019;57(6). doi:10.1128/JCM.00203-19
- 25. Humphries R, Bobenchik AM, Hindler JA, Schuetz AN. Overview of changes to the clinical and laboratory standards institute performance standards for antimicrobial susceptibility testing, M100, 31st edition. J Clin Microbiol. 2021;59(12):e0021321. doi:10.1128/jcm.00213-21
- 26. He J, Sun F, Sun D, et al. Multidrug resistance and prevalence of quinolone resistance genes of Salmonella enterica serotypes 4,[5],12:i:- in China. Int J Food Microbiol. 2020;330:108692. doi:10.1016/j.ijfoodmicro.2020.108692
- 27. Yue M, Liu D, Li X, et al. Epidemiology, serotype and resistance of Salmonella isolates from a children's hospital in Hangzhou, Zhejiang, China, 2006-2021. *Infect Drug Resist.* 2022;15:4735–4748. doi:10.2147/idr.S374658
- Chen H, Qiu H, Zhong H, Cheng F, Wu Z, Shi T. Non-typhoidal Salmonella infections among children in Fuzhou, Fujian, China: a 10-year retrospective review from 2012 to 2021. Infect Drug Resist. 2023;16:2737–2749. doi:10.2147/IDR.S408152
- 29. Liang B, Xie Y, He S, et al. Prevalence, serotypes, and drug resistance of nontyphoidal Salmonella among paediatric patients in a tertiary hospital in Guangzhou, China, 2014-2016. J Infect Public Health. 2019;12(2):252–257. doi:10.1016/j.jiph.2018.10.012
- 30. Shen H, Chen H, Ou Y, et al. Prevalence, serotypes, and antimicrobial resistance of Salmonella isolates from patients with diarrhea in Shenzhen, China. *BMC Microbiol*. 2020;20(1):197. doi:10.1186/s12866-020-01886-5
- 31. Kovats RS, Edwards SJ, Hajat S, Armstrong BG, Ebi KL, Menne B. The effect of temperature on food poisoning: a time-series analysis of salmonellosis in ten European countries. *Epidemiol Infect*. 2004;132(3):443–453. doi:10.1017/s0950268804001992
- 32. Hung YT, Lay CJ, Wang CL, Koo M. Characteristics of nontyphoidal Salmonella gastroenteritis in Taiwanese children: a 9-year period retrospective medical record review. J Infect Public Health. 2017;10(5):518-521. doi:10.1016/j.jiph.2016.09.018
- 33. Duong VT, The HC, Nhu TDH, et al. Genomic serotyping, clinical manifestations, and antimicrobial resistance of nontyphoidal Salmonella gastroenteritis in hospitalized children in Ho Chi Minh city, Vietnam. J Clin Microbiol. 2020;58(12). doi:10.1128/jcm.01465-20
- 34. Aarø NS, Torpdahl M, Rasmussen T, Jensen M, Nielsen HL. Salmonella infections in Denmark from 2013-2022 with focus on serotype distribution, invasiveness, age, sex, and travel exposition. Eur J Clin Microbiol Infect Dis. 2024;43(5):947–957. doi:10.1007/s10096-024-04808-9
- 35. Rosso F, Rebellón-Sánchez DE, Llanos-Torres J, et al. Clinical and microbiological characterization of Salmonella spp. isolates from patients treated in a university hospital in South America between 2012-2021: a cohort study. *BMC Infect Dis.* 2023;23(1):625. doi:10.1186/s12879-023-08589-y
- 36. Mughini-Gras L, Pijnacker R, Duijster J, et al. Changing epidemiology of invasive non-typhoid Salmonella infection: a nationwide population-based registry study. *Clin Microbiol Infect*. 2020;26(7):941e949–941e914. doi:10.1016/j.cmi.2019.11.015
- 37. Larkin L, Pardos de la Gandara M, Hoban A, et al. Investigation of an international outbreak of multidrug-resistant monophasic Salmonella Typhimurium associated with chocolate products, EU/EEA and United Kingdom, February to April 2022. Euro Surveill. 2022;27(15). doi:10.2807/ 1560-7917.Es.2022.27.15.2200314
- Wang J, Li Y, Xu X, et al. Antimicrobial resistance of Salmonella enterica serovar typhimurium in Shanghai, China. Front Microbiol. 2017;8:510. doi:10.3389/fmicb.2017.00510
- 39. Grossi JL, Yamatogi RS, Call DR, Nero LA. High prevalence of intermediate resistance to ciprofloxacin in Salmonella enterica isolated from a Brazilian poultry production chain, located in Minas Gerais state. Int J Food Microbiol. 2023;394:110180. doi:10.1016/j.ijfoodmicro.2023.110180
- 40. Kuang D, Zhang J, Xu X, et al. Emerging high-level ciprofloxacin resistance and molecular basis of resistance in Salmonella enterica from humans, food and animals. *Int J Food Microbiol.* 2018;280:1–9. doi:10.1016/j.ijfoodmicro.2018.05.001
- 41. Shi Q, Ye Y, Lan P, et al. Prevalence and characteristics of ceftriaxone-resistant Salmonella in children's hospital in Hangzhou, China. Front Microbiol. 2021;12:764787. doi:10.3389/fmicb.2021.764787
- 42. Zhang SX, Zhou YM, Xu W, et al. Impact of co-infections with enteric pathogens on children suffering from acute diarrhea in southwest China. Infect Dis Poverty. 2016;5(1):64. doi:10.1186/s40249-016-0157-2
- 43. Potgieter N, Heine L, Ngandu JPK, et al. High burden of co-infection with multiple enteric pathogens in children suffering with diarrhoea from rural and peri-urban communities in South Africa. *Pathogens*. 2023;12(2):315. doi:10.3390/pathogens12020315
- 44. Vergadi E, Maraki S, Dardamani E, Ladomenou F, Galanakis E. Polymicrobial gastroenteritis in children. Acta Paediatr. 2021;110(7):2240–2245. doi:10.1111/apa.15854
- 45. Al-Emran HM, Krumkamp R, Dekker DM, et al. Validation and identification of invasive salmonella serotypes in sub-saharan Africa by multiplex polymerase chain reaction. *Clin Infect Dis.* 2016;62(Suppl 1):S80–82. doi:10.1093/cid/civ782
- 46. Chen CH, Low YY, Liu YH, Lin HH, Ho MW, Hsueh PR. Rapid detection of gastrointestinal pathogens using a multiplex polymerase chain reaction gastrointestinal panel and its role in antimicrobial stewardship. *J Microbiol Immunol Infect*. 2023;56(6):1273–1283. doi:10.1016/j.jmii.2023.10.004
- 47. Xie J, Kim K, Berenger BM, et al. Comparison of a rapid multiplex gastrointestinal panel with standard laboratory testing in the management of children with hematochezia in a pediatric emergency department: randomized controlled trial. *Microbiol Spectr.* 2023;11(3):e0026823. doi:10.1128/spectrum.00268-23

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