Annals of Clinical Microbiology and Antimicrobials

RESEARCH

Open Access

Prevalence, antimicrobial resistance, and genomic characterization of *Salmonella* strains isolated in Hangzhou, China: a two-year study

Lifei Yu[1,2,3†], Jianzhong Fan[4†], Shanshan Lu[2,5†], Junxin Zhou[2,5], Huangdu Hu[6], Caiping Mao[7], Xiaoting Hua[2,5], Yan Jiang[2,5], Ying Fu[2], Yunsong Yu[6*] and Xinhong Han[7*]

Abstract

This study explored the molecular epidemiology and resistance mechanisms of 271 non-duplicate *Salmonella enterica* (*S. enterica*) strains, isolated mainly from adults (209/271) in a tertiary hospital in Hangzhou between 2020 and 2021. Through whole-genome sequencing and bioinformatics, the bacterial strains were classified into 46 serotypes and 54 sequence types (ST), with *S.* Enteritidis, *S.* 1,4,[5],12:i:-, and *S.* Typhimurium being the most prevalent serotypes and ST11, ST34, and ST19 the most common STs. The strains isolated from adults were primarily *S.* Enteritidis (59/209), while from children were mainly *S.* 1,4,[5],12:i:- (20/62). Worryingly, 12.55% strains were multi-drug resistant (MDR), with resistance rates to cefepime (FEP), ceftazidime (CAZ), ceftriaxone (CRO) and cefotaxime (CTX) of 7.38%, 9.23%, 15.87% and 16.24%, respectively, and resistance rates to levofloxacin (LEV) and ciprofloxacin (CIP) of 8.49% and 19.19%, respectively. It is worth noting that the resistance rates of CRO and CTX in children reached 30.65%. A total of 34 strains carried extended-spectrum β-lactamase (ESBL) genes, dominated by $bla_{\text{CTX-M-65}}$ (13/34) and $bla_{\text{CTX-M-55}}$ (12/34); it is notable that one strain of *S.* Saintpaul carried both $bla_{\text{CTX-M-27}}$ and $bla_{\text{CTX-M-55}}$. The resistance mechanism to cephalosporins was mainly due to ESBL genes (20/43), and other genes included AmpC and β-lactamase genes. The strains resistant to quinolones mainly carried *qnrS1* (27/53), and others included *qnrB6*, *aac(6')-Ib-cr*, and mutations in *gyrA* and *parC*. One strain did not carry common quinolone resistance genes but had a *parC* (p.T57S) mutation to cause CIP resistance. This research provides vital insights into the molecular epidemiology and resistance mechanisms of clinical *S. enterica*, implicating possible infection control strategies.

Keywords *Salmonella enterica*, Prevalence, Molecular epidemiology, Antimicrobial resistance

†Lifei Yu, Jianzhong Fan and ShanshanLu authors are contributed equally to this study.

*Correspondence:
Yunsong Yu
yvys119@zju.edu.cn
Xinhong Han
hanxh@zju.edu.cn
Full list of author information is available at the end of the article

Introduction

S. enterica is a major cause of foodborne infections worldwide, responsible for a significant disease burden, including diarrhea and severe multi-organ invasive diseases [1–4]. An analysis of the global burden of Non-typhoidal *Salmonella* (NTS) gastroenteritis estimates approximately 93.8 million cases annually, resulting in 155,000 deaths [5]. Alarmingly, the incidence of *Salmonella* infections has risen in recent years. For instance, the occurrence of NTS infections has shown an upward trend since 2012 in the Netherlands [6]. Similarly, in Zhejiang, China, the positive detection rate for *Salmonella* increased markedly from 1.69% in 2012 to 6.61% in 2021 [7].

Antibiotic resistance in *S. enterica* has become a critical challenge in clinical treatment. The widespread use of antibiotics has led to increased resistance among clinical isolates, particularly to third-generation cephalosporins and fluoroquinolones [8, 9], complicating treatment and increasing the risk of failure and mortality. According to the standard of Clinical and Laboratory Standards Institute (CLSI), *Salmonella* isolated from intestinal tract in medical institutions does not routinely carry out antimicrobial resistance test, and clinicians need to adopt treatment strategies according to the epidemiological situation in their areas. Therefore, understanding the prevalence, antimicrobial resistance patterns, and genetic characteristics of *Salmonella* strains is crucial for effective surveillance, treatment and control strategies.

In recent years, there has been increasing attention on the molecular epidemiology and mechanisms of drug resistance in clinical isolates of *S. enterica* [10–12]. However, there is a knowledge gap in antimicrobial resistance profile and molecular characteristics of *Salmonella* isolated from human in Zhejiang, China [7].

This study aims to address this gap by assessing the prevalence, distribution, and antimicrobial resistance profiles of *Salmonella* strains isolated in Hangzhou, China, during 2020–2021. Additionally, we analyzed the differences in antimicrobial resistance between adult and pediatric populations and investigated the genomic characteristics and resistance mechanisms, particularly focusing on cephalosporins and quinolones, key antibiotics used in treating *Salmonella* infections.

Materials and methods

Bacteria isolation

Between January 1st 2020 and December 31st 2021, a total of 285 *Salmonella* strains were collected from Affiliated Hangzhou First People's Hospital. The source of patients included outpatient service (intestinal outpatient service, pediatric outpatient service and digestive medicine outpatient service) and ward. The stools of outpatients with diarrhea as the first symptom were cultured and *Salmonella* strains were preserved. Clinical samples of patients suffering from infectious disease in wards were sent for culture, and *Salmonella* strains were preserved. Strains isolated from the same patient within 7 days were defined as replicated strains and were excluded. Finally, 271 *Salmonella* strains were included in our study. A Vitek 2 system (BioMérieux, Marcy-l'Étoile, France) and matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS) (Bruker, Bremen, Germany) were used for bacterial species identification.

Whole genome sequencing and analyses

Genomic DNA of 271 *Salmonella* strains were extracted using a QIAamp DNA minikit (Qiagen) and subjected to the Illumina HiSeq (Illumina, San Diego, USA) platform. Shovill was used for de novo assembly of raw reads. Short reads generated by Illumina sequencing were assembled by Shovill 0.9.0 (https://github.com/tseemann/shovill). Multi-locus sequence typing (MLST) was identified using mlst v2.19.0 (https://github.com/tseemann/mlst). Antibiotic resistance genes were identified using ABRicate v0.8.13 with the refinder database. Samonella Serotyping were conducted using Seqsero2 v1.1.0 (http://www.denglab.info/SeqSero2). Alignment of core genes from all 271 *Salmonella* isolates was performed with panaroo v1.2.7 (https://github.com/gtonkinhill/panaroo). IQ-TREE v2.1.2 was then used for building the maximum-likelihood phylogenetic tree with the best-fit model according to BIC (http://www.iqtree.org/). The phylogenetic tree was visualized using iTOL v6 (https://itol.embl.de/).

Antimicrobial susceptibility testing

Antimicrobial susceptibility tests (ASTs) with cefoxitin (FOX), CAZ, FEP, CRO, CTX, piperacillin/tazabactam (PTZ), cefoperazone/sulbactam (F/S), ceftolozane/tazobactam (C/T), meropenem (MEM), imipenem (IMP), ertapenem (ETP), aztreonam (AZT), amikacin (AMI), LEV, CIP, ceftazidime/avibactam (CZA), fosfomycin (FOS), colistin (COL) and tigecycline (TGC) were performed using the broth microdilution method according to the CLSI guideline M100 [13], and the results were interpreted on the basis of the breakpoints from the CLSI except for tigecycline, which was determined according to the FDA 2019 guideline (https://www.fda.gov/drugs/development-resources/tigecycline-injection-products).

Results

Bacteria isolation

During the period from January 1st, 2020, to December 31st, 2021, a total of 271 non-replicated *Salmonella* strains were successfully isolated. Among these isolates,

the majority (263 strains) were derived from fecal samples, while 7 strains were obtained from blood samples and 1 strain from pleural puncture fluid. Of the total isolates, 209 strains were recovered from adults, whereas 62 strains were obtained from children (below 18 years old). The monthly distribution of isolates is graphically represented in Fig. 1, highlighting a concentration of strains between May and October (summer and autumn) annually.

Salmonella serotyping, sequence typing and phylogenetic analyse

The 271 *Salmonella* isolates were classified into 46 different serotypes, as depicted in Fig. 2. The most prevalent serotype was *S.* Enteritidis, accounting for 27.68% of the isolates (75/271), followed by *S.* 1,4,[5],12:i:- at 18.08% (49/271), *S.* Typhimurium at 9.59% (26/271), *S.* London at 7.38% (20/271), *S.* Thompson at 4.43% (12/271), *S.* Goldcoast at 3.69% (10/271), *S.* Agona at 2.58% (7/271), *S.* Give at 2.21% (6/271), and *S.* Rissen at 1.85% (5/271). Additional serotype and bacterial information can be found in Table S1. Among the 209 isolates from adults, 41 serotypes were identified, with *S.* Enteritidis being the most prevalent at 28.23% (59/209), followed by *S.* 1,4,[5],12:i:- at 13.88% (29/209), and *S.* Typhimurium at 10.45% (21/209). Among the 62 isolates from children, 18 serotypes were identified, with *S.* 1,4,[5],12:i:- being the most common at 32.26% (20/62), followed by *S.* Enteritidis at 25.81% (16/62), and *S.* Typhimurium at 8.06% (5/62). *Salmonella* serotypes including *S.* Indiana, *S.* Jangwani, *S.* 1,4,[5],12:i:-, *S.* Ohio, and *S.* Sereban were exclusively isolated from children.

Phylogenetic analysis was conducted on the 271 *Salmonella* isolates, resulting in the identification of 54 sequence types (ST). The majority of the isolates belonged to ST11 at 27.68% (75/271), followed by ST34 at 18.08% (49/271), ST19 at 8.86% (24/271), ST155 at 7.38% (20/271), ST26 at 4.43% (12/271), ST358 at 2.95% (8/271), ST13 at 2.58% (7/271), ST516 at 2.21% (6/271), and ST469 at 1.85% (5/271). Notably, all *S.* Enteritidis strains belonged to ST11, *S.* 1,4,[5],12:i:- belonged to ST34, *S.* London belonged to ST155, *S.* Thompson belonged to ST26, *S.* Agona belonged to ST13, *S.* Give belonged to ST516, and *S.* Rissen belonged to ST469. *S.* 1,4,[5],12:i:- and *S.* Typhimurium exhibited a close genetic relationship, with the majority of *S.* Typhimurium isolates (92.31%, 24/26) belonging to ST19.

Plasmid characteristic of 271 isolates

Our findings reveal the presence of 12 distinct plasmid replicon types, including Col, IncA/C, IncFI, IncFII, IncHI, IncI, IncN, IncQ, IncR, IncX, IncY, and p0111.

S. Enteritidis predominantly harbors the IncX type plasmid, found in 64 out of 75 isolates, followed by IncFII in 60, IncFI in 58, Col in 8, and IncI in 4. Notably, 44 isolates carry three plasmid replicons, and 9 carry four different types. Specifically, *S.* 1,4,[5],12:i:- exhibit a diverse plasmid profile, with 8 types identified, including Col in 19 out of 49, IncFI in 3, IncFII in 3, IncHI in 13, IncI in 5, IncQ in 27, IncR in 1, and p011 in 5. Seven of these isolates carry three plasmid replicon types. In the case of *S.* Typhimurium, the IncFII type is the most prevalent, found in 13 out of 26 isolates, closely followed by IncFI, also in 13. Other types present include Col in 4, IncA/C in 1, IncHI in 1, IncI in 1, and IncQ in 1. *S.* London isolates are characterized by the IncFI type plasmid in 12 out of 20, with Col, IncI, IncQ, IncR, and IncHI types also identified in varying frequencies. A detailed

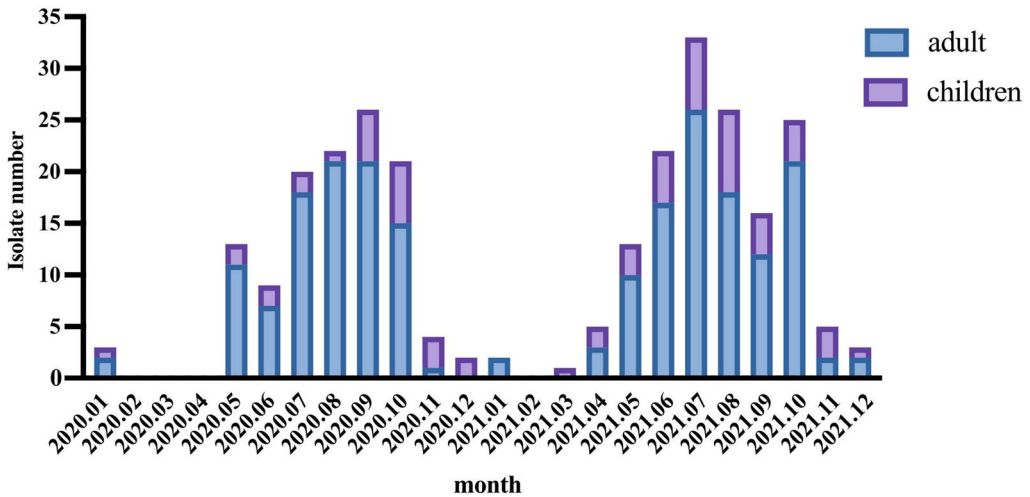

Fig. 1 Temporal distribution characteristics of 271 *Salmonella* strains originating from adults and children

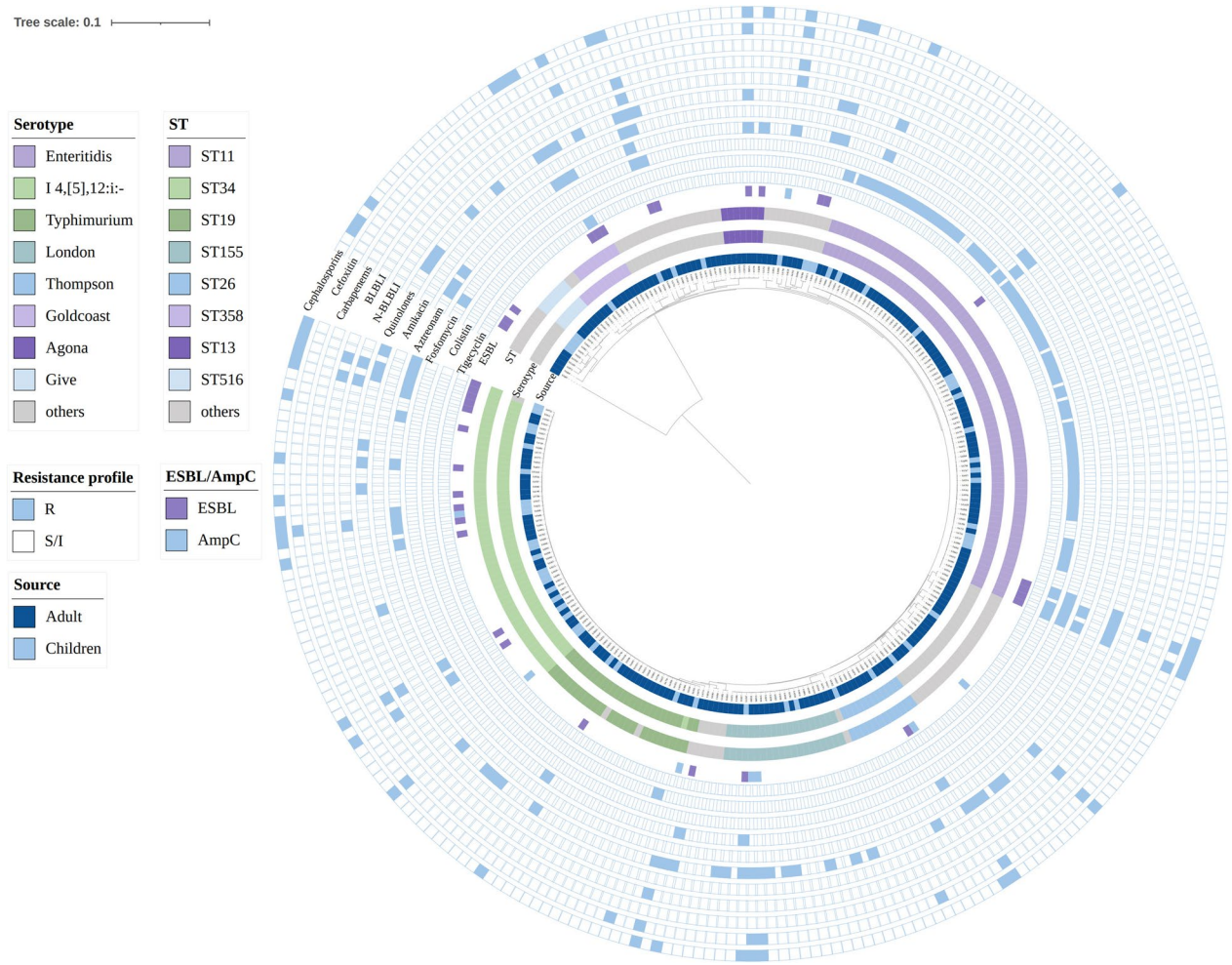

Fig. 2 Phylogenetic analysis and molecular characteristics of 271 *Salmonella* strains. Phylogenetic tree of ST11 CRKP isolates generated by panaroo v 1.2.7 and IQ-TREE 2.1.2, including information about isolate source, serotype, ST type, ESBL/AmpC genes and antibiotic resistance profile. The ESBL includes $bla_{CTX\text{-}M\text{-}55}$, $bla_{CTX\text{-}M\text{-}65}$, $bla_{CTX\text{-}M\text{-}14}$, $bla_{CTX\text{-}M\text{-}27}$, $bla_{CTX\text{-}M\text{-}130}$, and $bla_{CTX\text{-}M\text{-}199}$

account of the plasmid profiles for other serotype isolates is provided in Table S2. It is important to highlight that 74 isolates lacked plasmid replicons, spanning a range of serotypes.

Antibiotic resistance profile

AST was conducted on 19 antibiotics, and 34 of the *Salmonella* strains were classified as MDR. The distribution of Minimum Inhibitory Concentrations (MIC) is presented in Table 1. Overall, the resistance rates of the 271 strains to the 19 antibiotics were below 25%. The resistance rate to FOX was 4.80%. Among cephalosporin antibiotics, the resistance rates to FEP, CAZ, CRO, and CTX were 7.38%, 9.23%, 15.87%, and 16.24%, respectively. Carbapenems exhibited high sensitivity, with sensitivity rates of 100%, 100% and 99.63% for MEM, IMP, and ETP, respectively. β-lactam-β-lactamase inhibitor combinations also demonstrated good sensitivity, with rates of 96.68% for PTZ, 90.77% for F/S, 100% for CZA, and 97.05% for C/T. The resistance rates to quinolones LEV and CIP were 8.49% and 19.19%, respectively, while the resistance rate to AMI was 0.74%. The resistance rates to AZT, FOS, COL, and TGC were 12.55%, 2.95%, 22.14%, and 0.37%, respectively.

Differences in the antibiotic resistance profiles were observed between children and adult source isolates (Fig. 3a, b). Specifically, among the 19 antibiotics, strains isolated from children exhibited higher resistance rates, except for carbapenems, CZA, LEV, FOS, and TGC. The resistance rate of strains isolated from children to AZT was 27.42%. Notably, the resistance rates of strains isolated from children to CRO and CTX were both 30.65%.

The comparative analysis of antibiotic resistance rates among various serotypes was conducted using a chi-square test, as depicted in Fig. 3c. For this comparison,

Table 1 Distribution of MIC50 and MIC90 for 19 antibiotics of 271 *Salmonella* strains

	All			Adult			Children		
	MIC range	MIC50	MIC90	MIC range	MIC50	MIC90	MIC range	MIC50	MIC90
FOX	1–>256	2	8	1–256	2	4	2–>256	2	8
FEP	≤0.06–>128	0.06	4	≤0.06–>128	<0.06	2	≤0.06–128	≤0.06	8
CAZ	0.25–>128	0.25	4	0.25–64	0.25	4	0.25–>128	0.25	4
CRO	≤0.015–>512	0.06	256	≤0.015–>512	0.06	32	≤0.015–>512	0.06	512
CTX	0.03–>512	0.06	128	0.03–>512	0.06	16	0.03–>512	0.06	512
MEM	0.0015–0.25	0.03	0.06	0.0015–0.25	0.03	0.06	0.015–0.125	0.03	0.06
IMP	0.015–0.5	0.125	0.25	0.015–0.5	0.125	0.25	0.06–0.25	0.125	0.25
ETP	0.0015–4	0.015	0.03	0.0015–4	0.015	0.03	0.003–0.5	0.015	0.06
PTZ	1–512	2	8	1–512	2	4	2–512	4	8
F/S	0.25–>128	4	16	0.25–>128	4	8	0.5–128	4	32
CZA	≤0.06–2	0.25	0.5	≤0.06–2	0.25	0.5	0.125–2	0.25	0.5
C/T	0.25–64	0.5	1	0.25–64	0.5	1	0.25–64	0.5	2
AZT	≤0.25–>128	0.25	32	≤0.25–>128	≤0.25	4	≤0.25–128	≤0.25	64
LEV	≤0.03–64	0.25	1	≤0.03–32	0.25	1	0.06–64	0.25	1
CIP	≤0.06–>128	0.25	2	≤0.06–>32	0.25	1	≤0.06–>128	0.25	2
AMI	0.25–>256	2	2	0.25–>256	2	2	1–>256	1	4
FOS	0.25–>128	1	4	0.5–>128	1	4	0.25–>128	1	2
COL	0.5–8	1	4	1–8	1	4	0.5–8	1	4
TGC	0.125–8	0.25	1	0.125–8	0.25	1	0.125–2	0.25	0.5

FOX cefoxitin, *FEP* cefepime, *CAZ* ceftazidime, *CRO* ceftriaxone, *CTX* cefotaxime, *MEM* meropenem, *IPM* imipenem, *ETP* ertapenem, *PTZ* piperacillin/tazabactam, *F/S* cefoperazone/sulbactam(2:1), *CZA* ceftazidime/avibactam, *C/T* ceftolozane/tazobactam, *AZT* aztreonam, *LEV* levofloxacin, *CIP* ciprofloxacin, *AMI* amikacin, *FOS* fosfomycin, *COL* colistin, *TGC* tigecycline

we included serotypes with no fewer than 20 isolates: *S.* Enteritidis, *S.* 1, 4, [5], 12:i:-, *S.* Typhimurium, and *S.* London. These four predominant serotypes exhibited significantly distinct resistance profiles towards quinolones, cephalosporins, N-BIBIL, aztreonam, and colistin. Among them, strains of *S.* 1, 4, [5], 12:i:- showed the highest resistance rate to cephalosporins, at 28.57% (4/10), surpassing *S.* London with a 15% resistance rate (3/20), followed by *S.* Enteritidis at 8% (6/75), and *S.* Typhimurium at 7.69% (2/26). When it came to quinolone resistance, strains of *S.* London had the highest rate at 55% (11/20), followed by *S.* Typhimurium at 23.08% (6/26), *S.* 1, 4, [5], 12:i:- at 14.29% (7/49), and *S.* Enteritidis at 6.67% (5/ 75). Serotype *S.* 1, 4, [5], 12:i:- also presented the highest resistance rates towards both N-BIBIL (8.16%, 4/50) and aztreonam (24.49%, 12/50). In contrast, *S.* Enteritidis demonstrated the highest resistance rate towards colistin, with a striking 78.67% (59/75) of the isolates showing resistance. This detailed examination underscores the variability in antibiotic resistance among different serotypes of *Salmonella*, highlighting the importance of serotype-specific surveillance and antibiotic stewardship programs.

Resistance mechanism of cephalosporins and quinolones

A total of 34 isolates were found to carry ESBL genes (Fig. 4), which included $bla_{\text{CTX-M-55}}$, $bla_{\text{CTX-M-65}}$, $bla_{\text{CTX-M-14}}$, $bla_{\text{CTX-M-27}}$, $bla_{\text{CTX-M-130}}$, $bla_{\text{CTX-M-199}}$. Among these, thirteen isolates carried $bla_{\text{CTX-M-65}}$, with the majority of them belonging to *S.* 1,4,[5],12:i:- (8/13). Twelve isolates carried $bla_{\text{CTX-M-55}}$, distributed among *S.* Goldcoast (3/12), *S.* Agona (2/12), *S.* Indiana (2/12), *S.* Enteritidis (1/12), *S.* 1,4,[5],12:i:- (1/12), *S.* Typhimurium (1/12), and *S.* Muenster (1/12). Six isolates carried $bla_{\text{CTX-M-14}}$, specifically found in *S.* Enteritidis (4/6) and *S.* 1,4,[5],12:i:- (2/6). One *S.* 1,4,[5],12:i:- isolate carried $bla_{\text{CTX-M-130}}$, while one *S. Kentucky* isolate carried $bla_{\text{CTX-M-199}}$. Notably, one *S. Saintpaul* isolate carried both $bla_{\text{CTX-M-27}}$ and $bla_{\text{CTX-M-55}}$.

A total of forty-three isolates exhibited resistance to cephalosporins (Fig. 5a). Among them, twenty isolates demonstrated resistance to both third and fourth generation cephalosporins, and all of these isolates carried ESBL genes. The predominant ESBL gene was $bla_{\text{CTX-M-55}}$ (12), followed by $bla_{\text{CTX-M-14}}$ (4), $bla_{\text{CTX-M-65}}$ (2), $bla_{\text{CTX-M-199}}$ (1), $bla_{\text{CTX-M-130}}$ (1), and one isolate carried both $bla_{\text{CTX-M-27}}$ and $bla_{\text{CTX-M-55}}$. Among the remaining twenty-three isolates that were only resistant to third

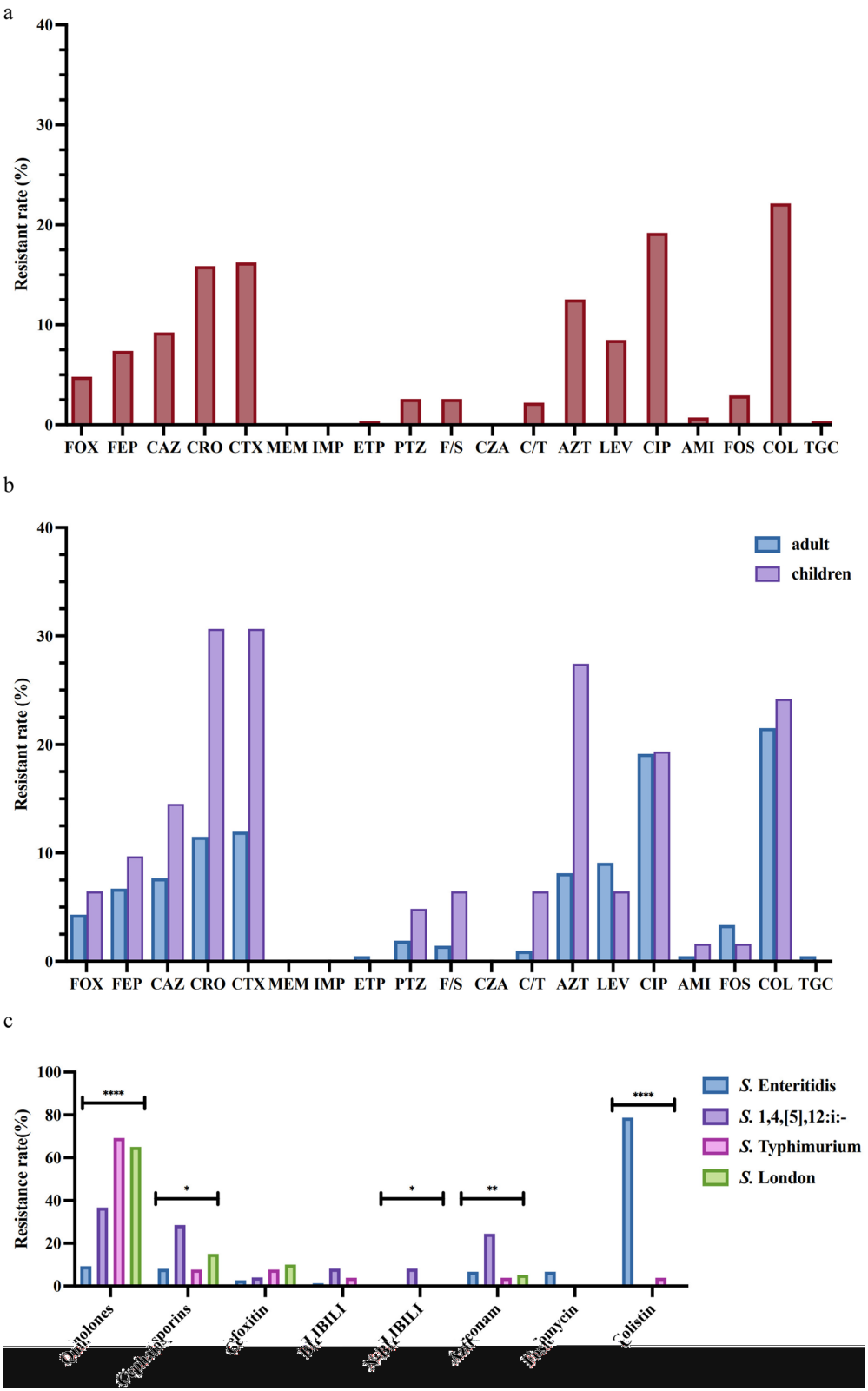

Fig. 3 **a** Resistance rates of 271 *Salmonella* strains to 19 antibiotics; **b** Comparative analysis of antibiotic resistance rates in *Salmonella* strains derived from children and adults; **c** Comparative analysis of antibiotic resistance rates in *Salmonella* strains derived from *S.* Enteritidis, *S.* 1, 4, [5], 12:i:-, *S.* Typhimurium, and *S.* London. Statistical analysis was performed with chi-square tests. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{****}P < 0.0001$

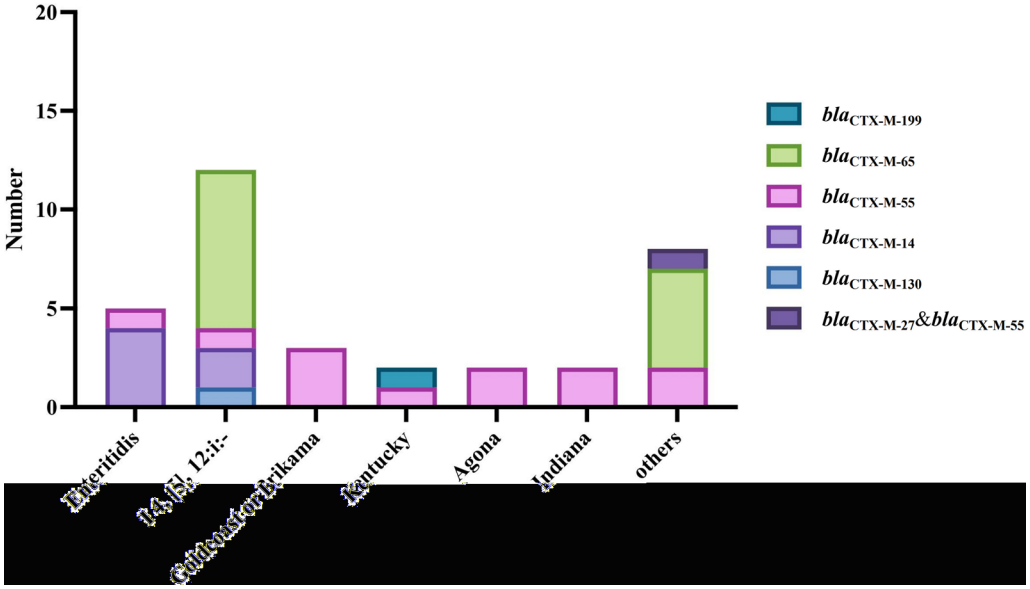

Fig. 4 Distribution characteristics of ESBL genes in 34 *Salmonella* strains

generation cephalosporins, thirteen carried ESBL genes, with $bla_{CTX-M-65}$ (11) being the most prevalent, followed by $bla_{CTX-M-14}$ (2). Eight isolates carried AmpC genes, specifically bla_{CMY-2} (6) and bla_{DHA-1}(2). One isolate sa824 carried bla_{TEM-1} and the gene encoding penicillin-binding protein 3 (PBP3) sal, presenting resistant profile towards third generation cephalosporins, CRO and CTX.

Fifty-three isolates exhibited resistance to quinolones (Fig. 5b). Among the thirty isolates that were solely resistant to CIP, eleven isolates carried *qnrS1* as the underlying mechanism, ten isolates carried *qnrB6* and *aac(6')-Ib-cr* and one isolate showed *parC* (p.T57S) as potential mechanism. One isolate that carried *qnrS1* was solely resistant to LEV. Twenty-two isolates displayed resistance to both CIP and LEV, with the majority (10/22) attributed to *qnrS1* and two isolates presented mutations in *gyrA* and *parC*.

Discussion

In this study, we found that *S. enterica* isolated from clinical settings in the Hangzhou area were predominantly *S.* Enteritidis, with the emergence of MDR strains. The resistance rates to cephalosporins, commonly used clinical antibiotics, were 7.38% to 16.24%, and the resistance rates to quinolones were 8.49% to 19.19%. Notably, the resistance rate to cephalosporins was significantly higher in strains isolated from children compared to adults. The resistance of *S. enterica* to cephalosporins was mainly due to the carriage of ESBL genes, and the resistance to quinolones was mainly due to the carriage of the *qnrS1* gene, which has important value for guiding usage of clinical antimicrobials and public health management in the local area.

We observed that the isolation rate of *S. enterica* strains in the Hangzhou area from 2020 to 2021 increased significantly between May and October each year. Similarly, a 10-year epidemiological investigation by the Zhejiang Provincial Center for Disease Prevention and Control (CDC) from 2012 to 2021 found that the isolation of *Salmonella* has a higher incidence in summer and the isolation rate increased year by year [7]. The possible reason for this analysis may be related to the opening of gastroenterology clinics during this period each year.

S. Enteritidis and *S.* Typhimurium serotypes are the main serotypes of *Salmonella* infections worldwide [14–16] and are prone to invasive infections [17]. *S.*

(See figure on next page.)

Fig. 5 **a** Resistance mechanisms to cephalosporins in 43 *Salmonella* strains. The strains were categorized into five groups based on their resistance profiles: I, Resistant to both CRO and CTX (sa748-sa792). sa824 carried genes encoding PBP3sal. II, Resistant to both CTX and CAZ (sa784). III, Resistant to CRO, CTX, and CAZ (sa848-sa945). IV, Resistant to CRO, CTX, and FEP (sa949-sa1034). V, Resistant to CRO, CTX, FEP, and CAZ (sa759-sa1030); **b** Resistance mechanisms to quinolones in 53 *Salmonella* strains. The strains were classified into three groups based on their resistance profiles: I, Resistant to CIP (sa821-sa971), with sa971 carrying mutations in *parC*(p.T57S). II, Resistant to LEV (sa1036). III, Resistant to both CIP and LEV (sa758-sa908)

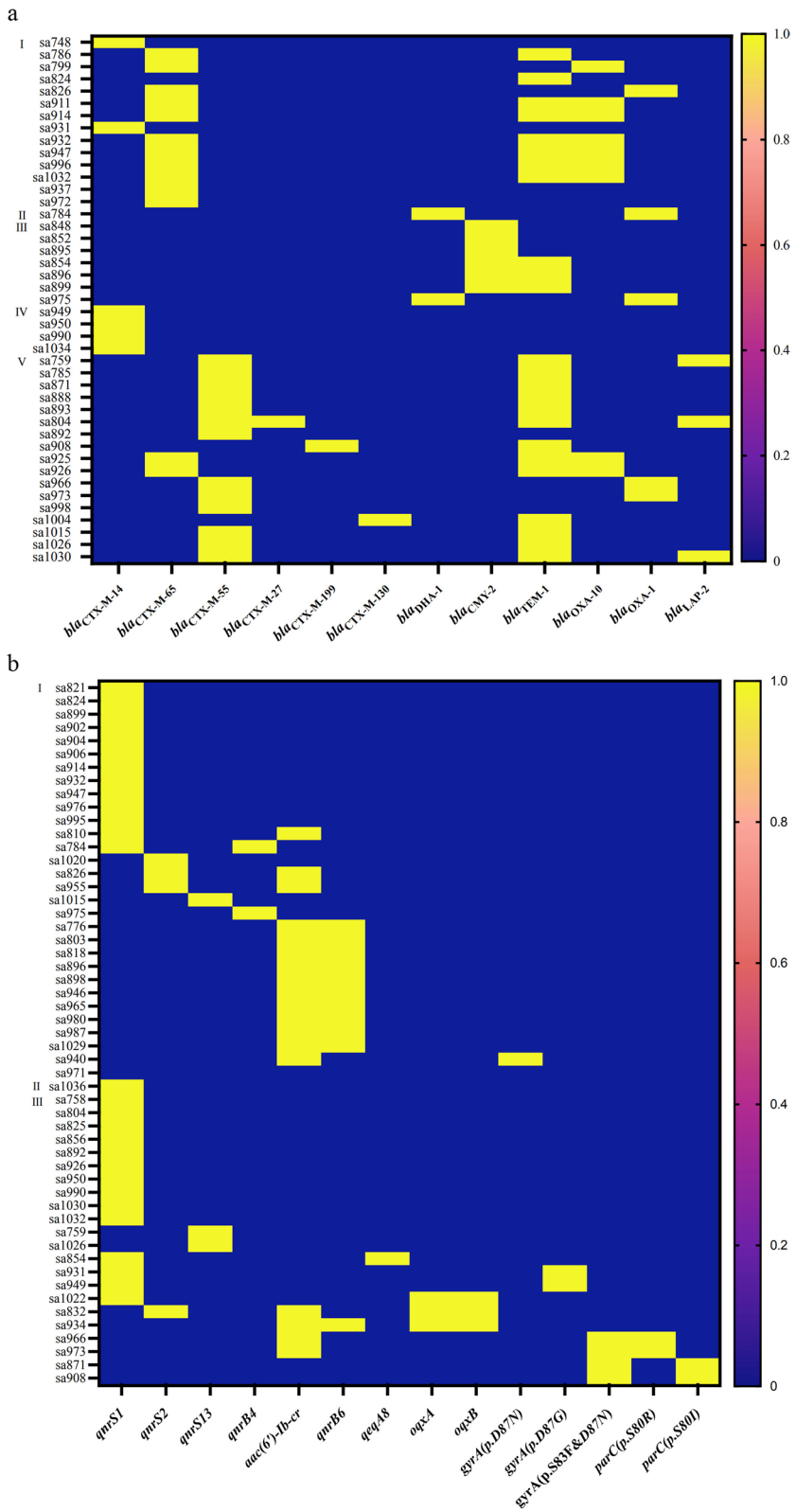

Fig. 5 (See legend on previous page.)

1,4,[5],12:i:- is a monophasic variant of *S.* Typhimurium [18], and was observed in our study, presenting a close genetic relationship with *S.* Typhimurium through phylogenetic analysis. In our study, the most prevalent serotype was *S.* Enteritidis, accounting for 27.68% of the isolates, followed by *S.* 1,4,[5],12:i:- at 18.08% and *S.* Typhimurium at 9.59%. Similarly, data from the China National Foodborne Disease Surveillance Network from 2010 to 2019 showed that *S.* Enteritidis is the most common serotype, followed by *S.* Typhimurium, and is prone to outbreaks [19]. Interestingly, we observed a difference in the prevalence of *Salmonella* serotypes between adults and children. In adults, *S.* Enteritidis was most common, followed by *S.* 1,4,[5],12:i:- and *S.* Typhimurium. Conversely, in children, *S.* 1,4,[5],12:i:- was the most prevalent, followed by *S.* Enteritidis and *S.* Typhimurium. *S.* Typhimurium was observed to be the predominant serotype in children [20, 21]. Serotyping of clinical strains is conducive to a better understanding of the local *Salmonella* epidemiology and is crucial for the prevention and control of infections. At the same time, for clinicians, understanding the *Salmonella* serotype can help them make informed decisions about disease diagnosis, progression, and prognosis.

With the widespread use of antibiotics, the resistance of *Salmonella* has increased year by year. Epidemiological investigations around the world have shown that the prevalence of MDR strains and even extensively drug-resistant (XDR) strains has emerged in some regions [22–24]. Salmonella is a leading cause of foodborne bacterial illnesses, primarily transmitted to humans through animal-derived foods [25]. In China, Salmonella is a widespread foodborne pathogen, posing a significant risk to food safety and public health [26]. Studies have revealed a high prevalence of multidrug-resistant (MDR) Salmonella strains in various food samples, along with numerous virulence factors [27]. Since 2016, there has been a growing concern about the emergence of Salmonella strains carrying the mcr-1 gene, which confers resistance to colistin, a last-resort antibiotic [28–30]. In this study, the resistance rates of 271 strains of *S. enterica* to third-generation cephalosporins (CRO and CTX) were 15.87% and 16.24%, respectively, and the resistance rates to quinolones (LEV and CIP) were 8.49% and 19.19%, respectively. Among the 271 *S. enterica*, 34 strains were MDR. Third-generation cephalosporins and quinolones are the first-line antibiotics for the clinical treatment of *Salmonella* infections [31]. Once invasive infections related to antimicrobial resistance occur, the use of effective antibiotics will be restricted. Fortunately, *S. enterica* in this region showed high susceptibility to β-lactam-β-lactamase inhibitor combinations and carbapenems.

Studies have shown that the antimicrobial resistance of *Salmonella* isolated from humans is related to the use of antibiotics in animal husbandry and food contamination [32]. The MDR patterns of *Salmonella* isolated from food are resistance to ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole [33, 34]. According to the results of a global survey of foodborne diseases [35], $bla_{\text{CTX-M-14}}$, *fosA3*, *tet(A)*, and $bla_{\text{TEM-1}}$ are common resistance genes in China, while *qnrS1* is uncommon. Most of the *Salmonella* isolated from a slaughterhouse in China possess $bla_{\text{TEM-1}}$ and/or $bla_{\text{CTX-M}}$ genes that are resistant to β-lactams, and the vast majority of quinolone-resistant strains have at least one mutation in *gyrA* or *parC* [36]. Similarly, we observed that the strains resistant to cephalosporins mainly carried ESBL genes predominantly $bla_{\text{CTX-M-65}}$ and $bla_{\text{CTX-M-14}}$. It is worth noting that one strain of *S. Saintpaul* carried $bla_{\text{CTX-M-27}}$ and $bla_{\text{CTX-M-55}}$ at the same time. Moreover, one of the isolates demonstrated resistance to CRO and CTX but did not possess the ESBL or AmpC genes. Upon analysis, it was noted that this isolate carried the PBP3sal gene, which is reportedly characterized by low affinity towards β-lactams [37, 38]. However, strains resistant to quinolones mainly carried the *qnrS1* gene (27/53 strains). Most (10/22) of the strains that were resistant to both CIP and LEV were attributed to the carriage of *qnrS1*, and two strains that did not carry common resistance genes had *gyrA* and *parC* mutations. Among the 30 strains that were only resistant to CIP, it was shown that the carriage of *qnrS1* (11/30), *qnrB6* and *aac(6')-Ib-cr* (10/30) were the potential mechanisms, and one strain did not carry common quinolone resistance genes and had a *par*C (p.T57S) mutation to cause resistance (Fig. 5b). A recent report has shown that *S.* Worthington ST592, isolated from raw milk in Brazil, carries a conserved Col(pHAD28) plasmid, which contains the antimicrobial resistance determinant *parC* (p.T57S) that can confer resistance to CIP [39]. Our strain is *S.* London ST155, which also has a *parC* (p.T57S) mutation associated with CIP resistance. However, whether the *parC* (p.T57S) substitution contributes to quinolone resistance remains controversial [40]. We will conduct further analysis in future studies to identify the root cause. Studies have shown that the IncHI2 plasmid of *Salmonella* contains two class I integrons, which can simultaneously carry $bla_{\text{CTX-M-15}}$ and $bla_{\text{OXA-1}}$ resistance genes [41]. The presence of such a set of resistance genes on a mobile genetic element may increase the rapid spread of resistant pathogens in the environment. Other studies have shown that *S. enterica*

carries bla_{CTX-M} and *qnrS* genes through specific IncY plasmids to form XDR strains [42], which are resistant to both CRO and quinolones and lead to global dissemination, posing a major challenge to public health. We identified specific resistance genes and mutations associated with resistance to these antibiotics, revealing the genetic basis of antimicrobial resistance in *S. enterica* strains isolated clinically in the Hangzhou region. Understanding these resistance mechanisms is essential for selecting appropriate antibiotics and developing effective treatment strategies.

We have investigated the molecular basis of high colistin resistance among the 271 isolates, with a particular focus on the 60 isolates exhibiting resistance. Our findings indicate that none of the isolates carried the acquired colistin resistance mcr genes, nor were there any colistin-associated chromosomal point mutations detected. Among the colistin-resistant isolates, 59 were identified as *S.* Enteritidis, and one as *S.* Typhimurium. It is noteworthy that Group D *Salmonella*, including *S.* Enteritidis and *S.* Typhimurium, are known to exhibit a higher natural tolerance to colistin, as supported by the literatures [43, 44]. This observation is further corroborated by a recent report from the European Food Safety Authority (EFSA) and the European Centre for Disease Prevention and Control (ECDC), which highlighted a significant association between colistin resistance and the prevalence of *S.* Enteritidis [45]. In *S.* Enteritidis and *S.* Typhimurium, the O antigens are structurally similar. Ricci et al. have reported that the O-antigen epitope in these Group D *Salmonella* species is a likely factor attributing to their colistin resistance [44]. This insight provides a plausible explanation for the high natural tolerance observed in our study.

This study also has some limitations. First, the study was conducted in a specific geographical area and may not fully represent the diversity and resistance patterns of *Salmonella* strains in other regions. Second, larger-scale studies are needed to more fully understand the epidemiology and resistance status of *Salmonella* nationwide or globally.

In general, our study investigated the epidemiology of *S. enterica* isolated clinically in a tertiary hospital in Hangzhou for two consecutive years and analyzed the characteristics of antimicrobial resistance. The results of the study revealed the molecular epidemiological characteristics of clinical *S. enterica* infections in this region and provided a theoretical basis for the resistance mechanisms of cephalosporins and quinolones. This is of great significance for clinical doctors to better use antibiotics empirically and for public health interventions and policy formulation. Further research is needed to delve into the underlying molecular mechanisms of antibiotic resistance in *Salmonella* to provide a more comprehensive theoretical basis for the prevention and treatment of *Salmonella* infections.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12941-024-00748-6.

Additional file 1.

Additional file 2.

Author contributions

All authors contributed to the study design and data collection. Lifei Yu performed the analysis, and Xinhong Han verified it. Lifei Yu wrote the first draft of the manuscript. Yunsong Yu and Xinhong Han critically revised the work and gave final approval of the manuscript. All authors contributed to the interpretation of the data, reviewed and revised the manuscript, and approved the final manuscript for submission.

Funding

This work was supported by National Natural Science Foundation of China (32141001), the Program of Health Commission of Zhejiang Province (2023KY171).

Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

This study was approved by ethics committee of Hangzhou First People's Hospital (approval number 2020103-1). This study was not considered a human research study. Therefore, no informed consent to participate was required.

Competing interests

The authors declare no competing interests.

Author details

[1]Department of Infectious Diseases, Hangzhou First People's Hospital, Hangzhou 310006, China. [2]Key Laboratory of Microbial Technology and Bioinformatics of Zhejiang Province, Hangzhou 310016, China. [3]Zhejiang University School of Medicine, Hangzhou 310058, China. [4]Department of Clinical Laboratory, Hangzhou First People's Hospital, Hangzhou 310006, China. [5]Department of Infectious Diseases, Sir Run Run Shaw Hospital, Zhejiang University School of Medicine, Hangzhou 310016, China. [6]Centre for General Practice Medicine, Department of Infectious Diseases, Zhejiang Provincial People's Hospital, People's Hospital of Hangzhou Medical College, Hangzhou, Zhejiang, China. [7]Department of Clinical Laboratory, Zhejiang Cancer Hospital, Hangzhou Institute of Medicine (HIM), Chinese Academy of Sciences, Hangzhou 310022, Zhejiang, China.

Received: 7 July 2024 Accepted: 20 September 2024
Published online: 28 September 2024

References

1. GBD 2019 Antimicrobial Resistance Collaborators. Global mortality associated with 33 bacterial pathogens in 2019 a systematic analysis for the global burden of disease study 2019. Lancet. 2022;400:2221–48.
2. Hu Y, Wang J, Chen S, Li Y, Zhang R, Chen S. Non-typhoidal salmonella invasive infections in China. Lancet Infect Dis. 2022;22:939.
3. Cheng RA, Eade CR, Wiedmann M. Embracing diversity: differences in virulence mechanisms, disease severity, and host adaptations contribute to the success of nontyphoidal salmonella as a foodborne pathogen. Front Microbiol. 2019;10:1–20.

4. Knodler LA, Elfenbein JR. Salmonella enterica. Trends Microbiol. 2019;27:964–5.
5. Majowicz SE, Musto J, Scallan E, et al. The global burden of nontyphoidal Salmonella gastroenteritis. Clin Infect Dis. 2010;50:882–9.
6. 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(941):e9-941.e14.
7. He Y, Wang J, Zhang R, et al. Epidemiology of foodborne diseases caused by Salmonella in Zhejiang Province, China, between 2010 and 2021. Front Pub Health. 2023;11:1127925.
8. Alvarez DM, Barrón-Montenegro R, Conejeros J, Rivera D, Undurraga EA, Moreno-Switt AI. A review of the global emergence of multidrug-resistant Salmonella enterica subsp. enterica Serovar Infantis. Int J Food Microbiol. 2023;403:110297.
9. Talukder H, Roky SA, Debnath K, Sharma B, Ahmed J, Roy S. Prevalence and antimicrobial resistance profile of Salmonella isolated from human, animal and environment samples in South Asia: a 10-year meta-analysis. J Epidemiol Glob Health. 2023;13:637–52.
10. Dyson ZA, Ashton PM, Khanam F, et al. Pathogen diversity and antimicrobial resistance transmission of Salmonella enterica serovars Typhi and Paratyphi A in Bangladesh, Nepal, and Malawi: a genomic epidemiological study. Lancet Microbe. 2024;5:100841.
11. Sun R-Y, Fang L-X, Dai J-J, et al. Antimicrobial resistance and population genomics of emerging multidrug-resistant Salmonella 4,[5],12:i:- in Guangdong China. mSystems. 2024;9: e0116423.
12. Cuco A, Mkupasi E, Mzula A, Mdegela R. Non-typhoidal Salmonella contributes to gastrointestinal infections in Morogoro: evidence from patients attending Morogoro regional referral hospital in Tanzania. PLoS Negl Trop Dis. 2024;18: e0012249.
13. Weinstein MP, Lewis JS. The Clinical and laboratory standards institute subcommittee on antimicrobial susceptibility testing: background, organization, functions, and processes. J Clin Microbiol. 2020. https://doi.org/10.1128/JCM.01864-19.
14. Chousalkar K, Gast R, Martelli F, Pande V. Review of egg-related salmonellosis and reduction strategies in United States, Australia, United Kingdom and New Zealand. Crit Rev Microbiol. 2018;44:290–303.
15. Boore AL, Hoekstra RM, Iwamoto M, Fields PI, Bishop RD, Swerdlow DL. Salmonella enterica infections in the United States and assessment of coefficients of variation: a novel approach to identify epidemiologic characteristics of individual serotypes, 1996–2011. PLoS ONE. 2015;10: e0145416.
16. Diaz D, Hernandez-Carreño PE, Velazquez DZ, et al. Prevalence, main serovars and anti-microbial resistance profiles of non-typhoidal Salmonella in poultry samples from the Americas: a systematic review and meta-analysis. Transbound Emerg Dis. 2022;69:2544–58.
17. Fierer J. Invasive non-typhoidal Salmonella (iNTS) infections. Clin Infect Dis. 2022;75:732–8.
18. D'Incau M, Salogni C, Giovannini S, et al. Occurrence of Salmonella Typhimurium and its monophasic variant (4, [5],12:i:-) in healthy and clinically ill pigs in northern Italy. Porc Health Manag. 2021;7:34.
19. Sun L, Zhang H, Chen J, Chen L, Qi X, Zhang R. Epidemiology of foodborne disease outbreaks caused by nontyphoidal Salmonella in Zhejiang Province, China, 2010–2019. Foodborne Pathog Dis. 2021;18:880–6.
20. 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: e0008732.
21. Obaro SK, Hassan-Hanga F, Olateju EK, et al. Salmonella Bacteremia among children in central and Northwest Nigeria, 2008–2015. Clin Infect Dis. 2015;61(Suppl 4):S325–31.
22. Dyson ZA, Klemm EJ, Palmer S, Dougan G. Antibiotic resistance and typhoid. Clin Infect Dis. 2019;68:S165–70.
23. da Silva KE, Tanmoy AM, Pragasam AK, et al. The international and intercontinental spread and expansion of antimicrobial-resistant Salmonella Typhi: a genomic epidemiology study. Lancet Microbe. 2022;3:e567–77.
24. Woh PY, Yeung MPS, Goggins WB, et al. Genomic epidemiology of multidrug-resistant nontyphoidal Salmonella in young children hospitalized for gastroenteritis. Microbiol spectr. 2021;9: e0024821.
25. Paudyal N, Pan H, Liao X, et al. A meta-analysis of major foodborne pathogens in chinese food commodities between 2006 and 2016. Foodborne Pathog Dis. 2018;15:187–97.
26. Tang B, Siddique A, Jia C, et al. Genome-based risk assessment for foodborne Salmonella enterica from food animals in China: a one health perspective. Int J Food Microbiol. 2023;390:110120.
27. Tang B, Elbediwi M, Nambiar RB, Yang H, Lin J, Yue M. Genomic characterization of antimicrobial-resistant Salmonella enterica in duck, chicken, and pig farms and retail markets in eastern China. Microbiol Spectr. 2022;10: e0125722.
28. Tang B, Chang J, Zhang L, et al. Carriage of distinct mcr-1-harboring plasmids by unusual serotypes of Salmonella. Adv Biosyst. 2020;4: e1900219.
29. Yi L, Wang J, Gao Y, et al. mcr-1-harboring Salmonella enterica Serovar typhimurium sequence type 34 in pigs China. Emerg Infect Dis. 2017;23:291–5.
30. Hu Y, Fanning S, Gan X, et al. Salmonella harbouring the mcr-1 gene isolated from food in China between 2012 and 2016. J Antimicrob Chemother. 2019;74:826–8.
31. Crump JA, Sjölund-Karlsson M, Gordon MA, Parry CM. Epidemiology, clinical presentation, laboratory diagnosis, antimicrobial resistance, and antimicrobial management of invasive Salmonella infections. Clin Microbiol Rev. 2015;28:901–37.
32. Threlfall EJ. Antimicrobial drug resistance in Salmonella: problems and perspectives in food- and water-borne infections. FEMS Microbiol Rev. 2002;26:141–8.
33. McDermott PF, Zhao S, Tate H. Antimicrobial resistance in nontyphoidal Salmonella. Microbiol Spectr. 2018;6:780–90.
34. Maltha J, Guiraud I, Kaboré B, et al. Frequency of severe malaria and invasive bacterial infections among children admitted to a rural hospital in Burkina Faso. PLoS ONE. 2014;9: e89103.
35. Yang K, Wang A, Fu M, et al. Investigation of incidents and trends of antimicrobial resistance in foodborne pathogens in eight countries from historical sample data. Int J Environ Res Pub Health. 2020;17:6–8.
36. Zhu Y, Lai H, Zou L, et al. Antimicrobial resistance and resistance genes in Salmonella strains isolated from broiler chickens along the slaughtering process in China. Int J Food Microbiol. 2017;259:43–51.
37. Cestero JJ, Castanheira S, González H, Zaragoza Ó, García-Del PF. Affinity of cefotiam for the alternative penicillin binding protein PBP3SAL used by Salmonella inside host eukaryotic cells. J Antimicrob Chemother. 2023;78:512–20.
38. Castanheira S, Cestero JJ, Rico-Pérez G, et al. A specialized peptidoglycan synthase promotes Salmonella cell division inside host cells. MBio. 2017;8:1–13.
39. Leite EL, Saraiva MMS, Vasconcelos PC, et al. Whole genome sequence datasets of Salmonella enterica serovar Saintpaul ST50 and serovar Worthington ST592 strains isolated from raw milk in Brazil. Data Brief. 2024;53:109965.
40. Chang M-X, Zhang J-F, Sun Y-H, et al. Contribution of different mechanisms to ciprofloxacin resistance in Salmonella spp. Front Microbiol. 2021;12:663731.
41. Kariuki S, Gordon MA, Feasey N, Parry CM. Antimicrobial resistance and management of invasive Salmonella disease. Vaccine. 2015;33(Suppl 3):C21–9.
42. Klemm EJ, Shakoor S, Page AJ, et al. Emergence of an extensively drug-resistant Salmonella enterica serovar typhi clone harboring a promiscuous plasmid encoding resistance to fluoroquinolones and third-generation cephalosporins. MBio. 2018;9:1–10.
43. Agersø Y, Torpdahl M, Zachariasen C, Seyfarth A, Hammerum AM, Nielsen EM. Tentative colistin epidemiological cut-off value for Salmonella spp. Foodborne Pathog Dis. 2012;9:367–9.
44. Ricci V, Zhang D, Teale C, Piddock LJV. The O-antigen epitope governs susceptibility to colistin in Salmonella enterica. MBio. 2020;11:1–10.
45. European Food Safety Authority European Centre for Disease Prevention and Control. The European Union Summary Report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2019–2020. EFSA J Eur Food Saf Auth. 2022;20: e07209.

Publisher's Note