Infection and Drug Resistance

Dovepress
Taylor & Francis Group

Open Access Full Text Article

ORIGINAL RESEARCH

Antimicrobial Resistance and Genomic Characterization of Salmonella Serovars Typhimurium and 4,[5],12:i:- in Huzhou, China

Wei Yan, Lei Ji, Yunfeng Zha, Fenfen Dong, Deshun Xu

Huzhou Center for Disease Control and Prevention, Huzhou, 313000, People's Republic of China

Correspondence: Deshun Xu, Huzhou Center for Disease Control and Prevention, 999 Changxing Road, Huzhou, Zhejiang, 313000, People's Republic of China, Email xds666092@126.com

Objective: *Salmonella* serovar Typhimurium (*S.* Typhimurium) and its monophasic variant, *Salmonella* 4,[5],12:i:-, have become two of the most frequently isolated serovars worldwide, in both humans and animals. This study investigated the antimicrobial resistance and genomic characteristics of these two serovar *Salmonella*.

Methods: Between 2021 and 2023, a total of 90 *S.* Typhimurium and *Salmonella* 4,[5],12:i:-were collected from clinical and food samples in Huzhou. Their antimicrobial resistance phenotype and genes, virulence genes, and phylogenetic relationship were analyzed.

Results: *Salmonella* 4,[5],12:i:-, which all belong to ST34, has become the main serotype of *Salmonella* isolated in Huzhou instead of *S.* Typhimurium. Notably, we observed a higher incidence of infections among the young population (<5 years old). The 90 *Salmonella* isolates were mainly resistant to tetracycline (94.4%), ampicillin (72.2%), and trimethoprim/sulfamethoxazole (70.0%), with multidrug resistance (MDR) rates as high as 93.3%. Genome sequencing indicated that these isolates possessed 39 antimicrobial resistance genes and 184 virulence genes.

Conclusion: This research enhances our understanding of *S.* Typhimurium and *Salmonella* 4,[5],12:i:- infections, which is helpful to guide clinical responses.

Keywords: *Salmonella* typhimurium, monophasic variant, antimicrobial resistance, multidrug resistance, whole genome sequencing

Introduction

Salmonella is a diverse genus comprising over 2600 serovars that can cause a range of infections. It is estimated to cause more than 300,000 deaths annually, mostly in developing countries.[1,2] Based on different pathogenic and disease manifestations, the serotypes of *Salmonella* can be classified into typhoidal and non-typhoidal (NTS). NTS have a broad host range and in humans, most often cause self-limiting gastroenteritis that is typically acquired through the consumption of contaminated food or water.[3,4] The incidence of NTS was estimated at 627 cases per 100,000 persons in China.[5] Of all NTS, *Salmonella* serovar Typhimurium (*S.* Typhimurium, 1,4,[5],12:i:1,2) is reported among the most common serovar linked with human diseases worldwide.[6,7] *S.* Typhimurium infections consistently exhibit the highest incidence rate among all salmonellosis cases in China, maintaining its position as the predominant serotype.[8]

Salmonella 4,[5],12:i:- is considered a monophasic variant of *S.* Typhimurium, lacking the ability to express the second-phase flagellar antigen,[9] and has become a major global cause of NTS diseases in human during the past two decades.[10–12] After identification in the mid-1990s,[13] *Salmonella* 4,[5],12:i:- has been reported in various countries at different times,[9,14] and some reports suggest that it is *the* global pandemic clone.[15,16] According to European studies, Salmonella 4,[5],12:i:- has become one of the top five serovars among human clinical isolates.[17] In 2015, the detection rate of *Salmonella* 4,[5],12:i:- surpassed that of other serotypes, establishing it as the predominant serotype among human *Salmonella* isolates in Guangdong Province, China.[18] This trend was also observed in Huzhou.

Received: 10 February 2025
Accepted: 9 May 2025
Published: 31 May 2025

The prevalence of antibiotic-resistant strains, which pose serious risks to the public's health, is a serious challenge. Because of the indiscriminate use of antibiotics for both clinical purposes and food production, antimicrobial resistance (AMR) has become increasingly common.[19] *S*. Typhimurium and *Salmonella* 4,[5],12:i:- have been identified as exhibiting a high prevalence of multidrug resistance (MDR) among various *Salmonella* serovars, raising considerable public health issues worldwide.[20,21] Multidrug-resistant strains show resistance to a wide range of clinical antimicrobial agents, such as third-generation cephalosporins and colistin,[22] making clinical treatment difficult and necessitating the study of drug resistance in these two *Salmonella* serovars.[23]

Because whole genome sequencing (WGS) has reduced in price, it is replacing prior conventional technologies for investigating disease outbreaks and public health surveillance.[24–26] WGS could generate vast amounts of genetic data rapidly for species identification, determining virulence and resistance characteristics, and phylogenetic analyses.[27,28] It has been instrumental in improving identification of public health pathogens.[29]

To provide improved knowledge regarding *S*. Typhimurium and *Salmonella* 4,[5],12:i:- infections, we carried out this retrospective analysis to assess the AMR and genomic characterization of 90 *S*. Typhimurium and *Salmonella* 4,[5],12:i:- isolates recovered from hospitalized patients and food in Huzhou between 2021 and 2023. This study aimed to explore AMR, distribution characteristics of resistance genes and virulence genes, and the evolutionary relationships of isolates. Our findings could provide valuable insights for the prevention and control of MDR in *S*. Typhimurium and *Salmonella* 4,[5],12:i:-.

Materials and Methods

Isolate Collection, Salmonella Identification and Serotyping

Between 2021 and 2023, a total of 80 *S*. Typhimurium and *S*. 4,[5],12:i:- isolates were collected from six sentinel hospitals of the Chinese Pathogen Identification Net of Huzhou, as well as 10 strains isolated from retail food in wet markets (raw animal meat, raw poultry meat and pre-made dishes). Sample collection from patients were conducted year-round, whereas food sampling was restricted to the period from March to October annually.

Human origin strains were isolated from hospitals, and food strains were isolated by our laboratory according to the National Food Safety Standard (GB 4789.4–2016) rules.[30] After suspicious colonies were isolated by Columbia Blood Agar Plates (Chromagar, France), colonies were identified using matrix-assisted laser desorption ionization time-of-flight (MALDITOF) mass spectrometry (bioMérieux, France). The strains were stored in glycerol broth at –70°C.

Serotypes of *Salmonella* isolates were determined by slide agglutination for somatic antigen O and flagellar antigens Husing commercially available antiserum (Denka Seiken, Japan) according to the Kauffmann–White–LeMinor Scheme,[1] with normal saline used as a negative control.

Antimicrobial Susceptibility Testing

The broth micro-dilution method was used to determine the minimum inhibitory concentrations (MICs) of 17 antimicrobial drugs for 90 isolates and breakpoints were interpreted according to Clinical and Laboratory Standards Institute (CLSI) protocols.[31] The following antimicrobials (Thermo, USA) were tested: ampicillin (AMP), ampicillin/sulbactam (AMS), ceftazidime (CAZ), cefotaxime (CTX), ertapenem (ETP), meropenem (MEM), nalidixic acid (NAL), ciprofloxacin (CIP), azithromycin (AZM), amikacin (AMI), streptomycin (STR), tetracycline (TET), tigecycline (TIG), chloramphenicol (CHL), trimethoprim-sulfamethoxazole (SXT), and colistin (CT). *Escherichia coli* ATCC 25922 was used as the control strain for susceptibility testing.

Whole-Genome Sequencing

Salmonella DNA was extracted from overnight cultures using QIAamp DNA Mini Kits (Qiagen, Germany), following the manufacturer's instructions. DNA concentration was tested by the Qubit 4 (Thermo, USA). Libraries were constructed using a Metagenomic DNA Library Kit (Matridx Biotechnology, China) and sequenced by the Next Seq 550 high Output Reagent Cartridge (Illumina, USA).

Genomic Analysis

Quality control analysis of raw reads was performed with Fast QC (0.11.9) and fastp (0.23.2) was used to remove low-quality data. Reads were assembled using SPAdes (3.15.4). The serotype of isolates from the Kauffmann–White scheme was confirmed by SeqSero2 (1.1.1). Based on the results of WGS analysis, the CARD (https://card.mcmaster.ca/) and VFDB (https://www.mgc.ac.cn/VFs/) databases were used to predict the resistance genes and virulence genes by the parameter identity of >99.0% and coverage of >99.0%. The core single-nucleotide polymorphism (SNP) for 90 isolates was determined by Snippy (4.4.5) using the reference sequence S. Typhimurium LT2 (GenBank BioSample ID SAMN02604315). FastTree (2.1.11) was used for sequence alignment and homology analysis, and Gubbins was used for reassembly. The phylogenetic tree and the heatmap of genes were visualized using tvBOT.[32] The genomes of the 90 isolates have been deposited in the National Center for Biotechnology Information (NCBI) database under the Bioproject number PRJNA1195539.

Statistical Analysis

The data were analyzed using SPSS (Statistical Package for the Social Sciences) 23.0 (SPSS, Chicago, USA). Differences in *Salmonella* infection rates by sex and age were analyzed using the chi-square test. *P*-values below 0.05 were considered significant.

Results

Description of S. Typhimurium and Salmonella 4,[5],12:i:-

A total of 30 *S.* Typhimurium and 60 monophasic variant *Salmonella* 4,[5],12:i:-isolates were recovered between 2021 and 2023, including 80 from clinical samples of patients with diarrhea and 10 from food samples (Table S1). Of the 80 patients infected with *Salmonella*, 32 (40.0%) were aged between 0 and 5 years (Figure 1a), and the detection rate in the older age group was >60 was 15.0%. No significant association in the incidence of salmonellosis was noted between sex and age group (p >0.05).

The distribution of serotypes in different age groups was also different, and the main serotypes in patients under 60 years of age were *S.*1,4,[5],12:i:-, while *S.* Typhimurium were predominant in those over 60 years of age (Figure 1b).

Multilocus sequence typing (MLST) analysis generated three STs from 90 isolates. ST34 was the most frequent sequence type (67.8%, 61/90), followed by ST19 (30.0%, 27/90) and ST1544 (2.2%, 2/90). The proportion of the ST types also differed between the two serotypes, the isolates of *Salmonella* 4,[5],12:i:- were all ST34. The distribution of ST types varied across different age groups: ST34 was the predominant ST type in patients under 60 years of age, while ST19 was the primary ST type in those over 60 (Figure 1c).

The frequency and severity of *S.* Typhimurium infections varied between males and females. In this study, we observed a higher incidence of *S.* Typhimurium infections among male patients below 30 and over 60 years of age. Females aged 30–60 years exhibited higher susceptibility (58.3%, 14/24) to *S.* Typhimurium infection (Figure 1d).

Phenotypic AMR Among S. Typhimurium and Salmonella 4,[5],12:i:-

Antibiotic susceptibility tests were performed for the 90 isolates by 17 antimicrobials (Table 1 and Table S2). The highest recorded resistance rates were observed for tetracycline (85/90, 94.4%), with ampicillin (65/90, 72.2%) and trimethoprim/sulfamethoxazole (63/90, 70.0%). Conversely, the resistance rates for ceftazidime (0/90, 0.0%), amikacin (1/90, 1.1%) and meropenem (2/90, 2.2%) were relatively low. Only two isolates (2/90, 2.2%) demonstrated susceptibility to all 17 antibiotics tested. When analyzing antimicrobial resistance patterns by serovar, *Salmonella* 4,[5],12:i:- demonstrated significantly elevated resistance rates to tetracycline (83.3%), ampicillin (80.0%), streptomycin (63.3%), chloramphenicol (63.3%), and trimethoprim-sulfamethoxazole (63.3%). For *S.* Typhimurium, there were some differences: the highest resistance rates were to tetracycline (60/60, 100.0%), trimethoprim/sulfamethoxazole (44/60, 73.3%), and streptomycin (40/60, 70.0%). Source of isolate resulted in different resistance rates: however, the highest resistance rate for both was to tetracycline (patients: 93.8%; food: 100.0%).

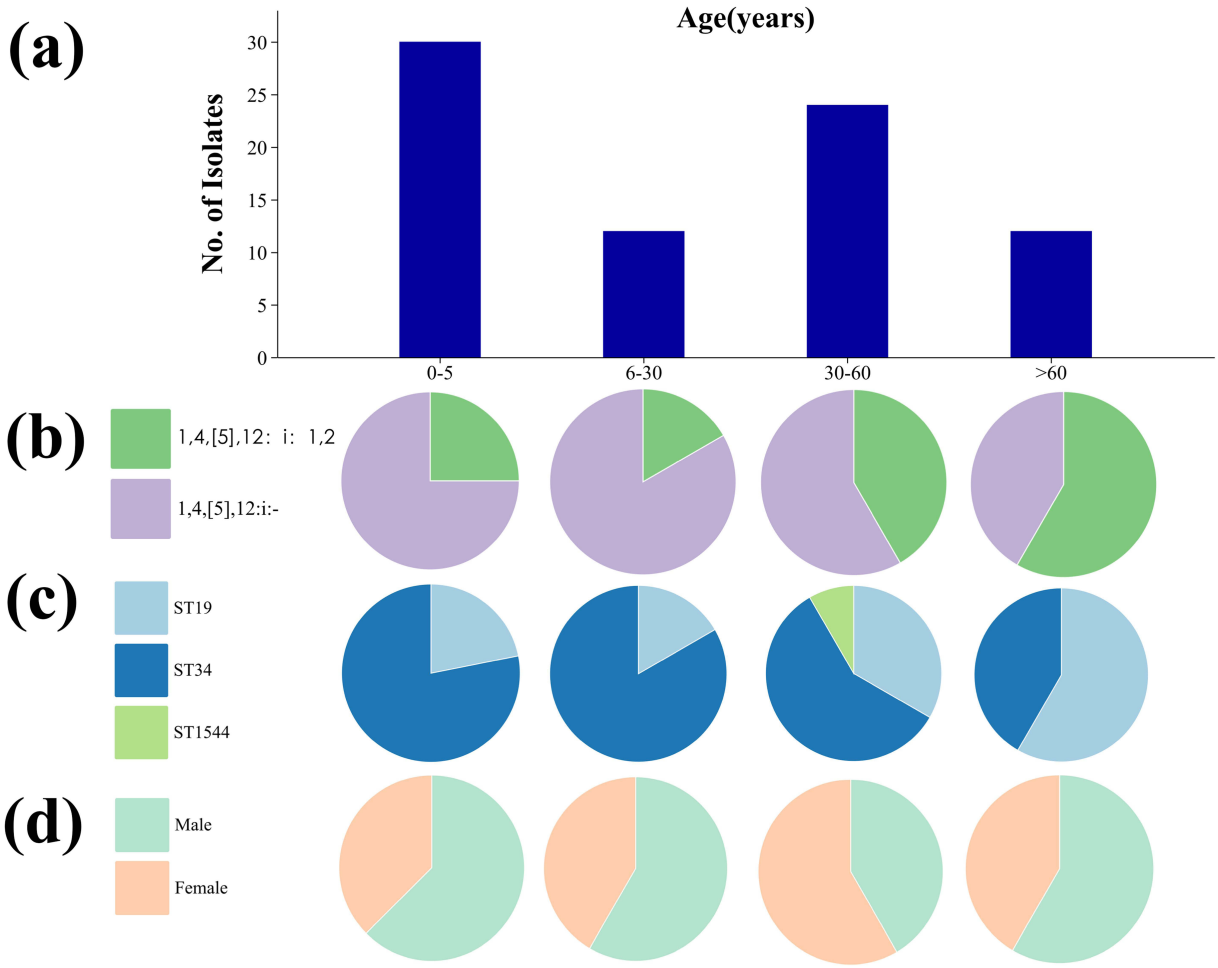

Figure 1 Epidemiological information regarding the 90 isolates in this study: (**a**) number of isolates in each age group; (**b**) composition ratios of serotypes in each age group. (**c**) composition ratios of ST types in each age group; and (**d**) composition ratios of sex in each age group.

Eighty-four (93.3%) isolates showed resistance to a minimum of three antimicrobial classes and were chosen as demonstrating MDR. *S.* Typhimurium and *Salmonella* 4,[5],12:i:- demonstrated 86.7% and 96.7% MDR, respectively, revealing 47 multidrug-resistant spectra, among which NAL-TET-SXT, seen in 16 isolates, was predominant. The most resistant isolate was detected from a nine-month-old boy in 2023, which was resistant to 16 drugs examined (Table 2).

AMR Genes

As shown in Figure 2, we identified 39 different AMR genes of 9 classes in the 90 *Salmonella* isolates, which included aminoglycoside, ampenicol, beta-lactam, fosfomycin, macrolide, quinolone, rifamycin, sulfonamides, and tetracycline. The *aac(6')-Iaa* point mutation, linked to aminoglycoside resistance, was the most common (100.0% of isolates), followed by *blaTEM-1* (30/90, 33.3%), *tet(B)* (24/90, 26.7%), and *tetR* (24/90, 26.7%). Some AMR genes (such as *aac*[*3*]-*IId*, *aadA5*, *floR*, *blaCTX-M-14*, *fosA3, fosB6*, *mphA*, *qnrD1*, *qnrS2*, *oqxA*, *oqxB*, *dfrA17*, and *tet*[*K*]) were identified in just one isolate. Each strain of *S.* Typhimurium carries an average of 4.5 AMR genes, while *Salmonella* 4,[5],12:i:- carries 3.5 AMR genes. Apart from *aac (6')-Iaa*, the most prevalent AMR genes in *Salmonella* 4,[5],12:i:-were *blaTEM-1* (19/60), followed by *qnrS1* (15/60), *tet(B)* (15/60), and *tetR* (15/60). In contrast, the top three AMR genes carried by S. Typhimurium were *blaTEM-1* (11/30), *tet(B)* (9/30) and*tetR* (9/30).

Table 1 Antimicrobial Resistant Rate of *S.* Typhimurium and *Salmonella* 4,[5],12:i:-Isolates Against 17 Antimicrobials

Antibiotic (Breakpoints, μg/mL)	Resistant Rate (%) of Isolates (n=90)	Resistant Rate (%) of Isolates by Serovar		Resistant Rate (%) of Isolates by Species	
		Typhimurium (n=30)	1,4,[5],12:i:- (n=60)	Patient (n=80)	Food (n=10)
AMP(≥32)	72.2	80.0	68.3	73.8	60.0
AMS(≥32/16)	42.2	36.7	45.0	42.5	40.0
CAZ(≥16)	20.0	20.0	20.0	18.8	30.0
CZA(≥16/4)	0.0	0.0	0.0	0.0	0.0
CTX(≥4)	35.6	30.0	38.3	36.3	30.0
ETP(≥2)	3.3	3.3	3.3	2.5	10.0
MEM(≥4)	2.2	3.3	1.7	2.5	0.0
NAL(≥32)	56.7	60.0	55.0	52.5	90.0
CIP(≥1)	12.2	6.7	15.0	13.8	0.0
AZM(≥32)	20.0	23.3	18.3	20.0	20.0
AMI(≥64)	1.1	0.0	1.7	1.3	0.0
STR(≥32)	67.8	63.3	70.0	68.8	60.0
TET(≥16)	94.4	83.3	100.0	93.8	100.0
TIG(>0.5)	10.0	13.3	8.3	7.5	30.0
CHL(≥32)	56.7	63.3	53.3	57.5	50.0
SXT(≥4/76)	70.0	63.3	73.3	68.8	80.0
CT(≥4)	18.9	20.0	16.7	17.5	20.0

Table 2 ====Number and Percentage of Multidrug-resistant Profiles in S. Typhimurium and Salmonella 4,[5],12:i:-Isolates

Drug-Resistant Spectrum	Number of Isolate (n)	T%
NAL-TET-SXT	16	18.6
AMP-STR-TET	3	3.5
AMP-NAL-STR-TET	3	3.5
AMP-TET-CHL-SXT	2	2.3
AMP-AMS-STR-TET	1	1.5
AMP-NAL-CHL-SXT	1	1.5
AMP-STR-TET-SXT	1	1.5
NAL-TET-TIG-SXT	1	1.5
STR-TET-CHL-SXT	1	1.5
AMP-STR-TET-CHL-SXT	6	6.9
AMP-AMS-TET-CHL-SXT	2	2.3
AMP-AMS-CTX-STR-TET	2	2.3
AMP-AZM-STR-TET-CT	1	1.5
AMP-CTX-STR-TET-CHL	1	1.5
AMP-NAL-STR-TET-CT	1	1.5
NAL-STR-TET-CHL-SXT	1	1.5
AMP-AMS-STR-TET-CHL-SXT	4	4.7
AMP-AMS-CTX-NAL-TET-CHL	1	1.5
AMP-CAZ-CTX-NAL-STR-TET	1	1.5
AMP-CAZ-CTX-TET-CHL-SXT	1	1.5
AMP-NAL-AZM-STR-TET-CT	1	1.5
AMP-CTX-NAL-STR-TET-CT	1	1.5

(*Continued*)

Table 2 (Continued).

Drug-Resistant Spectrum	Number of Isolate (n)	T%
AMP-AMS-CTX-STR-TET-CHL-SXT	3	3.5
AMP-AMS-NAL-STR-TET-CHL-SXT	2	2.3
AMP-AMS-AZM-TET-TIG-CHL-CT	1	1.5
AMP-AMS-CAZ-CTX-STR-TET-CHL	1	1.5
AMP-NAL-STR-TET-TIG-CHL-SXT	1	1.5
AMP-AMS-STR-TET-TIG-CHL-SXT	1	1.5
AMP-AMS-CTX-ETP-MEM-NAL-STR-TET	1	1.5
AMP-AMS-CTX-NAL-STR-TET-CHL-SXT	1	1.5
AMP-CIP-ZAM-STR-TET-TIG-CHL-SXT	1	1.5
AMP-AMS-NAL-AZM-STR-TET-TIG-CHL-SXT	1	1.5
AMP-CTX-NAL-CIP-AZM-STR-TET-CHL-SXT	1	1.5
AMP-AMS-CTX-NAL-AZM-STR-TET-CHL-SXT	1	1.5
AMP-AMS-CAZ-CTX-STR-TET-CHL-SXT-CT	1	1.5
AMP-AMS-CAZ-CTX-CIP-STR-TET-CHL-SXT	1	1.5
AMP-AMS-CAZ-CTX-NAL-STR-TET-TIG-CHL	1	1.5
AMP-AMS-CAZ-CTX-NAL-CIP-STR-TET-CHL-SXT	2	2.3
AMP-AMS-CAZ-CTX-AZM-STR-TET-CHL-SXT-CT	1	1.5
AMP-CAZ-CTX-NAL-AZM-STR-TET-CHL-SXT-CT	1	1.5
AMP-AMS-CAZ-CTX-NAL-AZM-STR-TET-CHL-SXT-CT	2	2.3
AMP-AMS-CTX-NAL-CIP-AZM-STR-TET-CHL-SXT-CT	2	2.3
AMP-AMS-CAZ-CTX-NAL-CIP-AZM-STR-TET-CHL-SXT	1	1.5
AMP-AMS-CAZ-CTX-NAL-STR-TET-TIG-CHL-SXT-CT	1	1.5
AMP-AMS-CAZ-CTX-NAL-CIP-AZM-STR-TET-CHL-SXT-CT	2	2.3
AMP-AMS-CAZ-CTX-ETP-NAL-AZM-STR-TET-CHL-SXT-CT	1	1.5
AMP-AMS-CAZ-CTX-ETP-MEM-NAL-CIP-AZM-AMI-STR-TET-TIG-CHL-SXT-CT	1	1.5

Virulence Factors

Virulence analysis using the Virulence Factor Database (VFDB) identified 184 virulence genes across 90 *S*. Typhimurium and *Salmonella* 4,[5],12:i:- isolates. These genes were classified into eight categories based on their roles in pathogenesis. The eight categories included adherence (*csgC, bcfA, lpfA*, etc.), effector delivery system (*tae4, hilC, orgC*, etc.), exotoxin (*spvB*), immune modulation (*rck*), exotoxin (*spvB*), nutritional/metabolic factor (*mgtB, mgtC, iroB*, etc.), stress survival (*sodCI*), antimicrobial activity/competitive advantage (*mig-14* and *mig-5*), and regulation (*fur, phoP, rpoS*, etc.). All detected virulence genes categorized by VF class are outlined in Figure 3.

Phylogenetic Analysis

To assess genetic variation among these isolates, we conducted whole genome single nucleotide polymorphism (cgSNP) phylogenetic analysis of these 90 isolates. As shown in Figure 4, 90 isolates were classified into six major clades, designated A–F. There was no association between lineages and years, district, origin of isolates; however, the lineages were closely related to serotypes and epidemiology. *Salmonella* 4,[5],12:i:- was distributed primarily in clade E, which contains 16 strains from one outbreak, while *S*. Typhimurium isolates were found in the other five clades. The serotype of isolates from clades A to D, and clade F were all 1,4,[5],12:i:1,2. In contrast, 59 isolates from clade E were 1,4,[5],12:i:-, while one isolate was 1,4,[5],12:i:1,2. The ST types of clade A (2 strains), clade C (1 strain), clade D (14 strains) and clade F (2 strains) were ST19. There were two ST types in clade B, with two strains of ST1544 and eight strains of ST19. For clade E, all 60 strains were ST34.

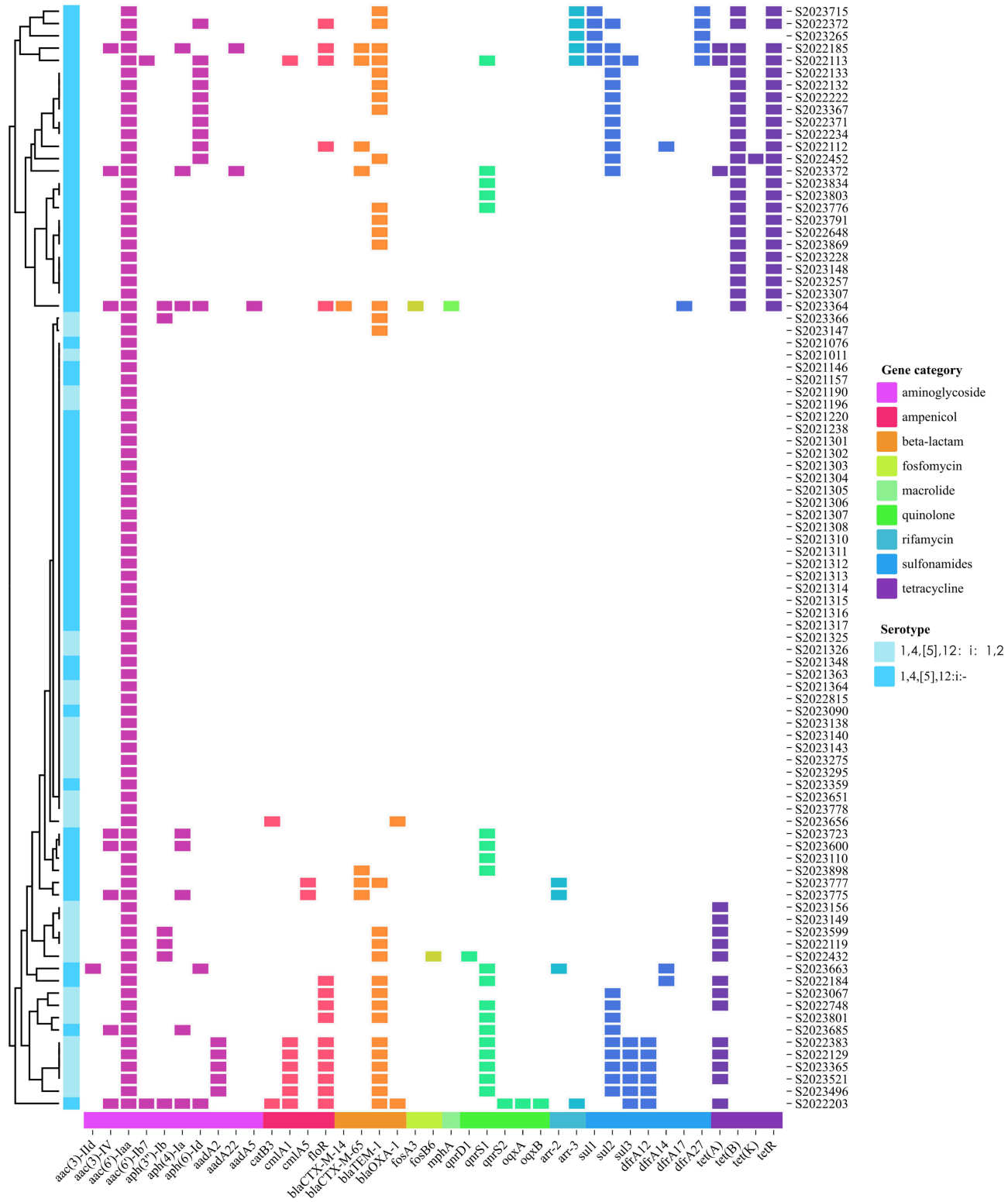

Figure 2 Annotation heatmap of AMR genes among 90 isolates.

https://doi.org/10.2147/IDR.S521802

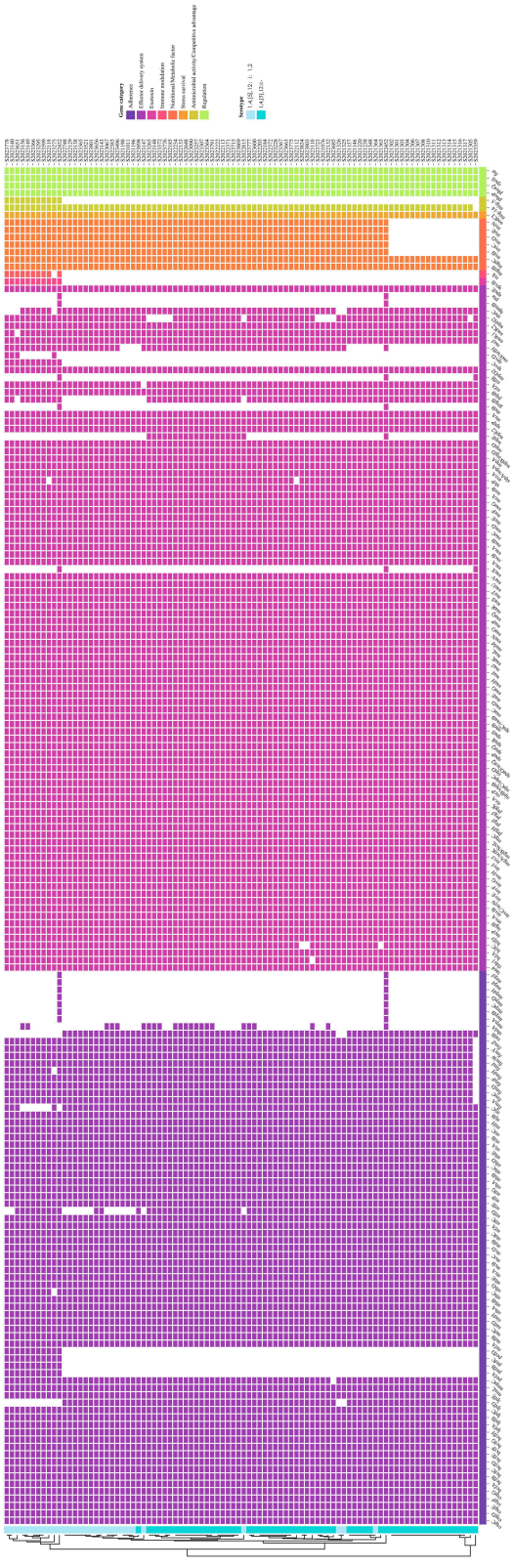

Figure 3 Annotation heatmap of virulence factors among 90 isolates.

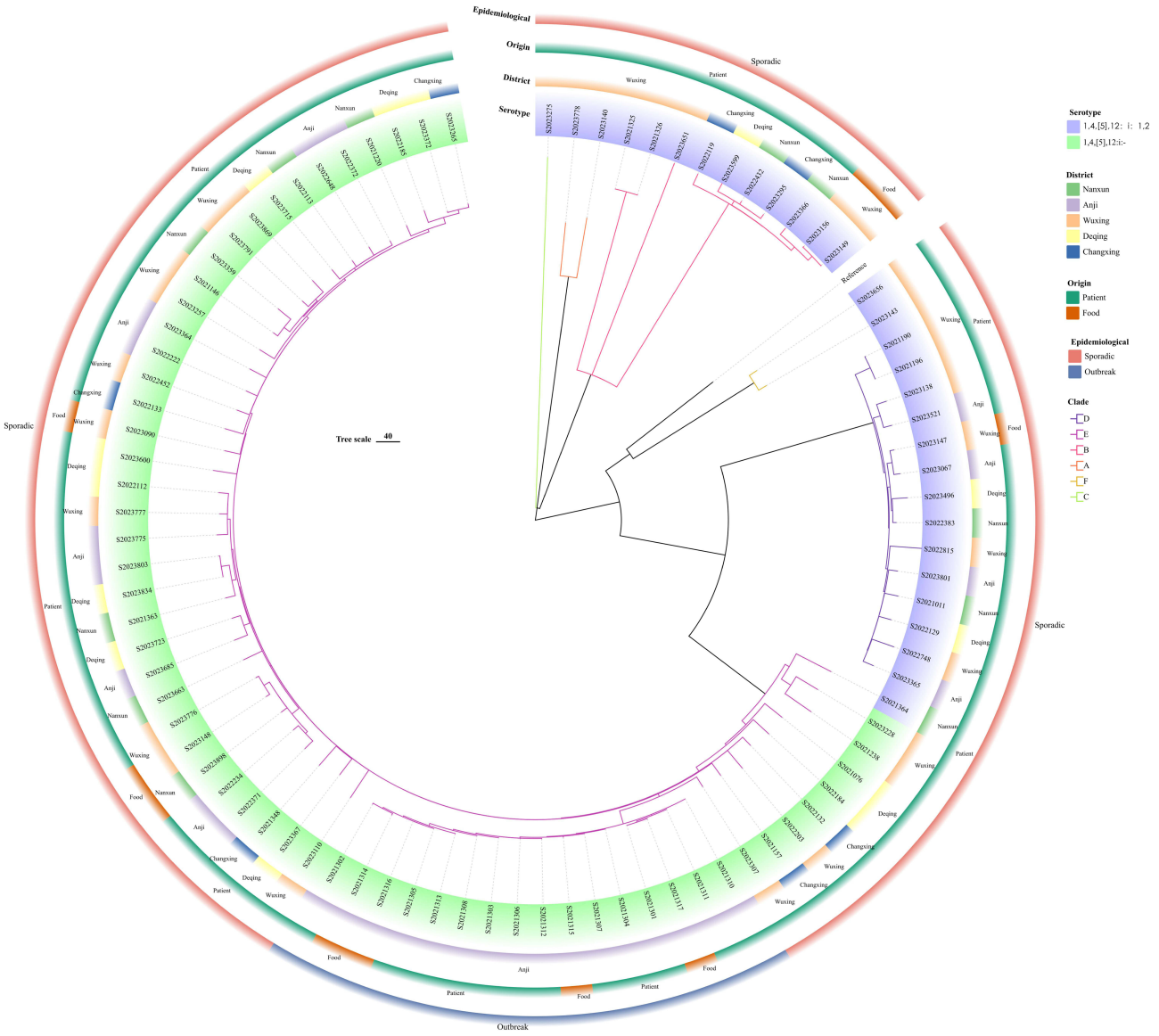

Figure 4 Core genome SNP-based phylogenomic tree of all 90 *Salmonella* isolates.

Discussion

S. Typhimurium and monophasic *Salmonella* 4,[5],12:i:- are common causes of foodborne illness. With increasing antibiotic resistance, especially MDR, they have become more and more prevalent in the world, posing a serious public health problem. *Salmonella* 4,[5],12:i:- has become a global new epidemic clone associated with human food and environmental samples.[13,33] Similar to these studies, *S.* Typhimurium isolated from food was the top serotype in Huzhou;[34] however, the prevalence of *S.* Typhimurium and *Salmonella* 4,[5],12:i:- with human infections in Huzhou were unknown.

In our study, the younger age group (aged 0–5 years) was the main population infected by *S.* Typhimurium and *Salmonella* 4,[5],12:i:-, which was consistent with previous findings.[10] The underdeveloped immune systems of children under 5 years old render them particularly susceptible to *Salmonella* infections, which may account for the disproportionately high incidence of severe gastroenteritis in this age group.[35] In terms of serotype, monophasic *Salmonella* 4,[5],12:i:- replaced *S.* Typhimurium as the most prevalent serotype of *Salmonell*a.[23] ST34 and ST19 were the most frequent sequence type in our study, accounting for 97.8%, which was consistent with several previous studies in China.[36,37] Enhanced surveillance targeting *S.* Typhimurium sequence type 34 (ST34) was imperative to curb the dissemination of

https://doi.org/10.2147/IDR.S521802

multidrug-resistant strains, given that ST34 isolates exhibit heightened adaptive capabilities.[38] The frequency and severity of *Salmonella* infections vary between men and women, and we observed that males below 30 and over 60 years of age and females 30–60 years of age were more vulnerable. The different incidences of *Salmonella* according to gender could be attributed to pathogen exposure, eating habits, genetic factors, and so on.[39]

With the irrational clinical and veterinary use of antibiotics, the problem of resistance is becoming more and more serious.[40] The 90 isolates from different sources were tested against 17 antimicrobials, and 49 different patterns of resistance were recorded, confirming the wide diversity of resistance profiles in *Salmonella* strains. Cephalosporins and fluoroquinolones are considered the first treatment for severe salmonellosis;[10] however, our findings indicate potential therapeutic failure: 35.6% of isolates were resistant to cefotaxime, and 56.7% were resistant to nalidixic acid, so that is a serious problem in Huzhou. High levels of resistance were observed to tetracycline, ampicillin, and trimethoprim/sulfamethoxazole, consistent with previous reports,[23,41,42] indicating that these antibiotics may be ineffective in the treatment of *Salmonella* infections.

Effectively treating the MDR of salmonellosis requires costly antibiotics, which is an added burden for developing countries.[43] We found that 100.0% and 92.5% of food and patient samples with diarrhea-associated isolates were multidrug resistant in this study, respectively. However, previous studies in Shenzhen showed higher MDR in clinical isolates (81.6%) than in food isolates (66.7%),[44] which indicated the diversity of multidrug-resistant isolates in different regions. Similarly, high percentages of MDR were also observed in *Salmonella* isolated from food[45] and patient[41] samples in various regions of China. The surge in multidrug-resistant *Salmonella* isolates is recognized as a crucial public health issue,[46] which may lead to therapeutic failure, especially with the limited number of alternative antibiotics available.

Previous studies have reported some concordance between antimicrobial resistance genes and phenotypes;[47,48] however, they did not consider confounders in bacterial genome-wide associations such as population structure.[49] In this study, we found a wide distribution of the aminoglycoside-encoding gene *aac(6')-Iaa* in all 90 isolates, which were not consistent with resistance phenotypes of aminoglycoside (67.8% were resistant to STR, 1.1% to AMI). *aac (6')-Iaa* usually are transcriptionally silent and rarely become transcriptionally active, which means the mere presence of *aac(6')-Iaa* can not confer aminoglycoside resistance to *Salmonella*.[50] Antimicrobial resistance genes were not usually sufficient to cause changes in resistance phenotypes unless isolates had mutations that could increase their expression.[51] ESBLs (encoded by *balCTX-M-14, balCTX-M-65, balTEM-1and balOXA-1*) are modified broad-spectrumbeta-lactamases that hydrolyze beta-lactam antibiotics. We found a wide distribution of the ESBL-encoding gene *balTEM-1* (33.3%) in these two serotypes. It has been reported that *balTEM-1* is mostly linked with ampicillin resistance[52] (72.2%). Resistance to tetracyclines and chloramphenicol is often associated with efflux pumps by *tet* and *floR*, while resistance to sulfonamides is associated with antibiotic target replacement mechanism by *sul* and *dfrA*.[53]

Virulome analysis detected the typical genes implicated in the virulence and pathogenicity mechanisms of *Salmonella*. Virulence factors (VFs) can overcome host defense mechanisms and cause disease in a host.[54] The fimbrial genes (eg, *bcf, lpf, fim*) may influence isolates' tropism to interact with host epithelial cells, and enhance ability to adhere to different host cell surfaces, aiding in the colonization and infection process.[55] In fact, 50.4% of VFs (93/184) belong to the effector delivery system, involving the Type VI secretion system (T6SS) and Type III secretion system (T3SS). With the exception of*fljB*, the differences in VFs between *S.* Typhimurium and *Salmonella* 4,[5],12:i:- were *spvB, rck* and *mig-5* (see Figure 3). These genes were detected in only some isolates of *S.* Typhimurium, while the overall presence rates of the other genes were not significantly different between the serovars.[56] The *spv* is associated with the survival and proliferation of *Salmonella* within macrophages.[57]

Whole genome sequencing can reveal the complete DNA make-up of an organism and facilitate the detection of variations both within and between species. Phylogenomic analysis of 90 strains, which were categorized into six main clades (A to F), showed that these strains were closely related and clustered together with serovars. Sixteen strains from outbreaks were clustered in clade E. In addition, the isolate of S2021364 was clustered in clade E while its serotype was 1,4,[5],12:i:1,2; however, the serotype of the remaining 60 strains in clade E were 1,4,[5],12:i:-. Between S2021364 and S2023228(1,4,[5],12:i:-), there were 116 differences in SNP compared to S2023365(1,4,[5],12:i: 1,2), in

which there were 468. The classification of S2021364 into clade E was driven by its distinct SNP, rather than the serotype. The clades of a phylogenetic tree did not exhibit particular geographic distributions, origin, or years of isolation.

Conclusion

In conclusion, the data presented here offer an overview of the distribution, antibiotic resistance profile and genome characteristics of *S*. Typhimurium and its monophasic variant (*Salmonella* 4,[5],12:i:-) strains isolated in Huzhou City, China. A high prevalence of multidrug-resistant *S*. Typhimurium and *Salmonella* 4,[5],12:i:- strains (93.3%) was found and these isolates showed a worrying resistance to TET, AMP and SXT, which are currently used as first-line treatment in human infections. A total of eight virulence factor categories were detected, with effector delivery system being the most abundant. The relationships between resistance phenotypes and resistance genes warrent further investigation. Hence, continuous monitoring of multidrug-resistant isolates using WGS is necessary for public health.

Data Sharing Statement

The data generated in this study is confidential. For any inquiries, please contact the corresponding author. Sequencing data are available at https://www.ncbi.nlm.nih.gov/datasets/genome/?bioproject=PRJNA1195539

Ethics Approval and Informed Consent

This study was approved by the human research ethics committee of the Huzhou Center for Disease Control and Prevention (HZ2020007). Informed consent for the anal swab samples was obtained from patients or their guardians. The provision of verbal consent is recorded in patients' notes. All patient data were anonymized prior to analysis. Study procedures were conducted in accordance with the ethical standards of the Declaration of Helsinki.

Funding

This study was supported by Zhejiang Province Disease Prevention and Control Science and Technology program (2025JK098), Huzhou Science and Technology Bureau Project (2023GY08).

Disclosure

The authors declare no competing interests in this work.

References

1. Issenhuth-Jeanjean S, Roggentin P, Mikoleit M, et al. Supplement 2008-2010 (no. 48) to the White-Kauffmann-Le Minor scheme. *Res Microbiol*. 2014;165(7):526–530. doi:10.1016/j.resmic.2014.07.004
2. Buckle GC, Walker CL, Black RE. Typhoid fever and paratyphoid fever: systematic review to estimate global morbidity and mortality for 2010. *J Glob Health*. 2012;2(1):010401. doi:10.7189/jogh.02.010401
3. Hohmann EL, Hohmann EL. Nontyphoidal salmonellosis. *Clin Infect Dis*. 2001;32(2):263–269. doi:10.1086/318457
4. Ohl ME, Miller SI. Salmonella: a model for bacterial pathogenesis. *Annu Rev Med*. 2001;52(1):259–274. doi:10.1146/annurev.med.52.1.259
5. Xu Y, Zhou X, Jiang Z, et al. Epidemiological Investigation and Antimicrobial Resistance Profiles of *Salmonella* Isolated From Breeder Chicken Hatcheries in Henan, China. *Front Cell Infect Microbiol*. 2020;10:497. doi:10.3389/fcimb.2020.00497
6. Wu-Chen RA, Feng J, Elhadidy M, et al. Long-term exposure to food-grade disinfectants causes cross-resistance to antibiotics in Salmonella enterica serovar Typhimurium strains with different antibiograms and sequence types. *Antimicrob Resist Infect Control*. 2023;12(1):145. doi:10.1186/s13756-023-01333-w
7. Stevens MP, Kingsley RA. Salmonella pathogenesis and host-adaptation in farmed animals. *Curr OpinMicrobiol*. 2021;63:52–58. doi:10.1016/j.mib.2021.05.013
8. 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
9. Wang Z, Gu D, Hong Y, et al. Microevolution of Salmonella 4,[5],12:i:- derived from Salmonella enterica serovar Typhimurium through complicated transpositions. *Cell Rep*. 2023;42(10):113227. doi:10.1016/j.celrep.2023.113227
10. Nambiar RB, Elbediwi M, Ed-Dra A, et al. 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
11. Napoleoni M, Villa L, Barco L, et al. Monophasic Variant of *Salmonella* Typhimurium 4,[5],12:i:- (ACSSuGmTmpSxt Type) Outbreak in Central Italy Linked to the Consumption of a Roasted Pork Product (Porchetta). *Microorganisms*. 2023;11(10):2567. doi:10.3390/microorganisms11102567
12. Sun H, Wan Y, Du P, et al. The Epidemiology of Monophasic *Salmonella* Typhimurium. *Foodborne Pathog Dis*. 2020;17(2):87–97. doi:10.1089/fpd.2019.2676

13. Hopkins KL, Kirchner M, Guerra B, et al. Multiresistant Salmonella enterica serovar 4,[5],12:i:- in Europe: a new pandemic strain? *Euro Surveill*. 2010;15(22):19580. doi:10.2807/ese.15.22.19580-en
14. Elnekave E, Hong S, Mather AE, et al. Salmonella enterica Serotype 4,[5],12:i:- in Swine in the United States Midwest: an Emerging Multidrug-Resistant Clade. *Clin Infect Dis*. 2018;66(6):877–885. doi:10.1093/cid/cix909
15. Mulvey MR, Bharat A, Boyd DA, et al. Characterization of a colistin-resistant Salmonella enterica 4,[5],12:i:- harbouring mcr-3.2 on a variant IncHI-2 plasmid identified in Canada. *J Med Microbiol*. 2018;67(12):1673–1675. doi:10.1099/jmm.0.000854
16. Biswas S, Li Y, Elbediwi M, et al. Emergence and Dissemination of *mcr*-Carrying Clinically Relevant *Salmonella* Typhimurium Monophasic Clone ST34. *Microorganisms*. 2019;7(9):298. doi:10.3390/microorganisms7090298
17. European Food Safety Authority and European Centre for Disease Prevention and Control (EFSA and ECDC). The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2017. *EFSA J*. 2018;16(12):e05500. doi:10.2903/j.efsa.2018.5500.
18. Huang JM, Ke BX, Li BS, et al. Molecular epidemiological characteristics and antibiotic resistance of multi-drug resistant Salmonella 1,4,[5],12:i:- in Guangdong. *Disease Surveillance*. 2021;36:501–508. doi:10.3784/jbjc.202012020405
19. Lamichhane B, Mawad AMM, Saleh M, et al. Salmonellosis: an Overview of Epidemiology, Pathogenesis, and Innovative Approaches to Mitigate the Antimicrobial Resistant Infections. *Antibiotics*. 2024;13(1):76. doi:10.3390/antibiotics13010076
20. Mather AE, Phuong TLT, Gao Y, et al. New Variant of Multidrug-Resistant *Salmonella enterica* Serovar Typhimurium Associated with Invasive Disease in Immunocompromised Patients in Vietnam. *mBio*. 2018;9(5):e01056–18. doi:10.1128/mBio.01056-18
21. Monte DF, Nelson V, Cerdeira L, et al. Corrigendum: multidrug- and colistin-resistant *Salmonella enterica* 4,[5],12:i:- sequence type 34 carrying the *mcr-3.1* gene on the IncHI2 plasmid recovered from a human. *J Med Microbiol*. 2019;68(11):1694. doi:10.1099/jmm.0.001057
22. Xu Y, Zhou X, Jiang Z, et al. Antimicrobial Resistance Profiles and Genetic Typing of *Salmonella* Serovars from Chicken Embryos in China. *Antibiotics*. 2021;10(10):1156. doi:10.3390/antibiotics10101156
23. Li P, Zhan L, Wang H, et al. Prevalence and Antimicrobial Resistance Diversity of *Salmonella* Isolates in Jiaxing City, China. *Antibiotic*. 2024;13(5):443. doi:10.3390/antibiotics13050443
24. Yan W, Ji L, Dong F, et al. Antimicrobial resistance and genomic analysis of *Vibrio parahaemolyticus* isolates from foodborne outbreaks, Huzhou, China, 2019–2023. *Front Microbiol*. 2024;15:1439522. doi:10.3389/fmicb.2024.1439522
25. Tang B, Elbediwi M, Nambiar RB, et al. Genomic Characterization of Antimicrobial-Resistant Salmonella enterica in Duck, Chicken, and Pig Farms and Retail Markets in Eastern China. *Microbiol Spectr*. 2022;10(5):e0125722. doi:10.1128/spectrum.01257-22
26. Tang B, Chang J, Zhang L, et al. Carriage of Distinct mcr-1-Harboring Plasmids by Unusual Serotypes of Salmonella. *Adv Biosyst*. 2020;4(3):e1900219. doi:10.1002/adbi.201900219
27. Yan W, Xu D, Shen Y, et al. Molecular epidemiology of string test-positive Klebsiella pneumoniae isolates in Huzhou, China, 2020-2023. *Front Cell Infect Microbiol*. 2024;14:1411658. doi:10.3389/fcimb.2024.1411658
28. Yan W, Xu D, Chen L, et al. Antimicrobial resistance and genome characteristics of Salmonella enteritidis from Huzhou, China. *PLoS One*. 2024;19(6):e0304621. doi:10.1371/journal.pone.0304621
29. Punina NV, Makridakis NM, Remnev MA, et al. Whole-genome sequencing targets drug-resistant bacterial infections. *Hum Genomics*. 2015;9:19. doi:10.1186/s40246-015-0037-z
30. GB 4789.4-2016. *National Food Safety Standard for Microbiological Examination of Food–Salmonella Examination*. Beijing, China: China Foodand Drug Administration; 2016.
31. CLSI. *Performance Standards for Antimicrobial Susceptibility Testing*. 34th edition. Wayne, PA, USA: Clinical and Laboratory Standard Institute; 2024. CLSI Supplement M100. Available from https://clsi.org/standards/products/microbiology/documents/m100/.
32. Xie J, Chen Y, Cai G, et al. Tree Visualization By One Table (tvBOT): a web application for visualizing, modifying and annotating phylogenetic trees. *Nucleic Acids Res*. 2023;51(W1):W587–W592. doi:10.1093/nar/gkad359
33. Zhou L, Zhang TJ, Zhang W, et al. Prevalence and genetic diversity of multidrug-resistant *Salmonella* Typhimurium monophasic variant in a swine farm from China. *Front Microbiol*. 2023;14:1200088. doi:10.3389/fmicb.2023.1200088
34. Xu D, Chen L, Lu Z, et al. Prevalence and Serotyping of Salmonella in Retail Food in Huzhou China. *J Food Prot*. 2024;87(2):100219. doi:10.1016/j.jfp.2024.100219
35. Das R, Haque MA, Chisti MJ, et al. Association between Non-Typhoidal Salmonella Infection and Growth in Children under 5 Years of Age: analyzing Data from the Global Enteric Multicenter Study. *Nutrients*. 2021;13(2):392. doi:10.3390/nu13020392
36. Zhang K, Ge H, He J, et al. *Salmonella* Typhimurium ST34 Isolate Was More Resistant than the ST19 Isolate in China, 2007– 2019. *Foodborne Pathog Dis*. 2022;19(1):62–69. doi:10.1089/fpd.2021.0047
37. Dong N, Li Y, Zhao J, et al. The phenotypic and molecular characteristics of antimicrobial resistance of Salmonella enterica subsp. enterica serovar Typhimurium in Henan Province, China. *BMC Infect Dis*. 2020;20(1):511. doi:10.1186/s12879-020-05203-3
38. Kirkwood M, Vohra P, Bawn M, et al. Ecological niche adaptation of Salmonella Typhimurium U288 is associated with altered pathogenicity and reduced zoonotic potential. *Commun Biol*. 2021;4(1):498. doi:10.1038/s42003-021-02013-4
39. Peer V, Schwartz N, Green MS. Sex Differences in Salmonellosis Incidence Rates-An Eight-Country National Data-Pooled Analysis. *J Clin Med*. 2021;10(24):5767. doi:10.3390/jcm10245767
40. Arias CA, Murray BE. Antibiotic-resistant bugs in the 21st century--a clinical super-challenge. *N Engl J Med*. 2009;360(5):439–443. doi:10.1056/NEJMp0804651
41. Chen J, Ed-Dra A, Zhou H, et al. Antimicrobial resistance and genomic investigation of non-typhoidal *Salmonella* isolated from outpatients in Shaoxing city, China. *Front Public Health*. 2022;10:988317. doi:10.3389/fpubh.2022.988317
42. Nazari MM, Rahimi E, Shakerian A, et al. Prevalence of Salmonella Typhimurium and Salmonella Enteritidis isolated from poultry meat: virulence and antimicrobial-resistant genes. *BMC Microbiol*. 2023;23(1):168. doi:10.1186/s12866-023-02908-8
43. Zhou X, Kang X, Chen J, et al. Genome degradation promotes *Salmonella* pathoadaptation by remodeling fimbriae-mediated proinflammatory response. *Natl Sci Rev*. 2023;10(10):nwad228. doi:10.1093/nsr/nwad228
44. Li W, Li Y, Liu Y, et al. Clonal Expansion of Biofilm-Forming *Salmonella* Typhimurium ST34 with Multidrug-Resistance Phenotype in the Southern Coastal Region of China. *Front Microbiol*. 2017;8:2090. doi:10.3389/fmicb.2017.02090

45. 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. doi:10.1016/j.ijfoodmicro.2023.110120
46. Eng SK, Pusparajah P, Ab Mutalib NS, et al. Salmonella: a review on pathogenesis, epidemiology and antibiotic resistance. *Front Life Sci*. 2015;8 (3):284–293. doi:10.1080/21553769.2015.1051243
47. Eyler AB, M'ikanatha NM, Xiaoli L, et al. Whole-genome sequencing reveals resistome of highly drug-resistant retail meat and human Salmonella Dublin. *Zoonoses Public Health*. 2020;67(3):251–262. doi:10.1111/zph.12680
48. Srednik ME, Morningstar-Shaw BR, Hicks JA, et al. Antimicrobial resistance and genomic characterization of *Salmonella enterica* serovar Senftenberg isolates in production animals from the United States. *Front Microbiol*. 2022;13:979790. doi:10.3389/fmicb.2022.979790
49. Power RA, Parkhill J, de Oliveira T. Microbial genome-wide association studies: lessons from human GWAS. *Nat Rev Genet*. 2017;18(1):41–50. doi:10.1038/nrg.2016.132
50. Neuert S, Nair S, Day MR, et al. Prediction of phenotypic antimicrobial resistance profiles from whole genome sequences of non-typhoidal Salmonella enterica. *Front Microbiol*. 2018;9:592. doi:10.3389/fmicb.2018.00592
51. Salipante SJ, Hall BG. Determining the limits of the evolutionary potential of an antibiotic resistance gene. *Mol Biol Evol*. 2003;20(4):653–659. doi:10.1093/molbev/msg074
52. Eguale T, Birungi J, Asrat D, et al. Genetic markers associated with resistance to beta-lactam and quinolone antimicrobials in non-typhoidal Salmonella isolates from humans and animals in central Ethiopia. *Antimicrob Resist Infect Control*. 2017;6(1):1–10. doi:10.1186/s13756-017-0171-6
53. Alcock BP, Huynh W, Chalil R, et al. CARD 2023: expanded curation, support for machine learning, and resistome prediction at the Comprehensive Antibiotic Resistance Database. *Nucleic Acids Res*. 2023;51(D1):D690–D699. doi:10.1093/nar/gkac920
54. Sayers S, Li L, Ong E, et al. Victors: a web-based knowledge base of virulence factors in human and animal pathogens. *Nucleic Acids Res*. 2019;47 (D1):D693–D700. doi:10.1093/nar/gky999
55. Cheng RA, Wiedmann M. Recent Advances in Our Understanding of the Diversity and Roles of Chaperone-Usher Fimbriae in Facilitating *Salmonella* Host and Tissue Tropism. *Front Cell Infect Microbiol*. 2021;10:628043. doi:10.3389/fcimb.2020.628043
56. Han J, Tang H, Zhao S, et al. Salmonella enterica virulence databases and bioinformatic analysis tools development. *Sci Rep*. 2024;14(1):25228. doi:10.1038/s41598-024-74124-x
57. Rychlik I, Gregorova D, Hradecka H. Distribution and function of plasmids in Salmonella enterica. *Vet Microbiol*. 2006;112(1):1–10. doi:10.1016/j.vetmic.2005.10.030