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Original Article

Prevalence and antimicrobial resistance patterns of monophasic *Salmonella typhimurium* in Hanzhong, China

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Abstract

Introduction: *Salmonella enterica*, particularly the monophasic variant of *Salmonella typhimurium*, is a significant foodborne pathogen with an increasing prevalence and alarming multidrug resistance profile. This study analyzed the prevalence, antimicrobial resistance, and genomic characteristics of *Salmonella typhimurium* monophasic variants in Hanzhong, China.

Methodology: Genomic analysis was conducted on 94 *Salmonella enterica* strains derived from diarrheal patients in Hanzhong, China, from 2021 to 2023. Serotyping was performed using microbial mass spectrometry and whole-genome sequencing. Drug susceptibility testing was conducted. Drug resistance genes were screened. Genomic characterization included core genome multi-locus sequence typing (cgMLST) and core genome single-nucleotide polymorphism (cgSNP) analysis.

Results: Out of 94 strains, 34 were confirmed as monophasic *Salmonella typhimurium*. Notably, 85.29% of cases were in children under five years. Multi-drug resistance was alarmingly high at 88.24%, particularly against streptomycin (85.29%), tetracycline (70.59%), ampicillin (61.76%), and sulfamethoxazole (61.76%). Additionally, only one strain was fully susceptible to all tested antibiotics. Genomic analysis identified 30 distinct drug-resistance genes across the strains. All 34 strains belonged to the ST34 type. The cgMLST generated six clusters. The largest cluster contained 14 strains, predominantly from the Hantai District. The cgSNP analysis identified eight distinct evolutionary branches, each containing isolates from different periods and regions.

Conclusions: This study highlights the alarming prevalence and high resistance rates of monophasic *Salmonella typhimurium* in Hanzhong, particularly among vulnerable populations such as young children. The findings underline the urgent need for public health interventions, including enhanced monitoring and antibiotic stewardship, to mitigate the risks associated with this pathogen.

Key words: Monophasic *Salmonella typhimurium*; whole-genome sequencing; antimicrobial resistance; cgMLST; cgSNP.

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Introduction

Salmonella enterica is an important foodborne pathogen with over 2600 reported serotypes, posing serious risks to food safety and public health worldwide. Among these, *Salmonella typhimurium* is one of the most prevalent serotypes associated with foodborne diseases [1]. Particularly concerning is the emergence of multidrug-resistant (MDR) strains of *Salmonella typhimurium*, which primarily resist commonly used antibiotics, including ampicillin (AMP), chloramphenicol (CHL), streptomycin (STR), sulfamethoxazole (SXT), and tetracycline (TET) [2]. Notably, the monophasic variant of *Salmonella typhimurium* (1,4,[5],12:i:-) is an atypical serotype that lacks the first-phase flagellar antigen (encoded by fljB) and shares similar antigenic and genotypic characteristics with *Salmonella typhimurium* [3]. It is believed to have evolved from this serotype.

The recent global emergence of the monophasic variant of *Salmonella Typhimurium* presents significant challenges for public health [4]. Its transmission routes are extensive, including not only foodborne pathways but also water sources and contact with infected animals, which complicates prevention and control efforts [5]. Alarmingly, the issue of antimicrobial resistance in monophasic *Salmonella typhimurium* has become increasingly prominent [6]. These bacteria exhibit varying levels of resistance to several commonly used antimicrobial agents, including AMP, CHL, and sulfonamides [5]. In certain regions, the proportion of resistant strains may exceed 50% [7]. The emergence of this resistance complicates clinical treatment, prolongs the duration of illness, elevates healthcare costs, and may contribute to the spread of infections [8]. This issue is particularly concerning for vulnerable populations with diminished immune defenses, such as children and the elderly [5]. Therefore, a comprehensive understanding of the prevalence and resistance patterns of monophasic *Salmonella typhimurium* is urgently needed to mitigate the misuse of antimicrobial drugs, decrease the incidence and mortality of foodborne diseases, and safeguard public health.

Given that the monophasic variant of *Salmonella typhimurium* has not yet been reported in Hanzhong, China, this study aims to evaluate its prevalence, antimicrobial resistance, and genomic characteristics in this region. By elucidating the specific characteristics and transmission dynamics of this strain in Hanzhong, our findings may provide a basis for developing preventive measures for this strain.

Methodology

Strain source

This study analyzed 94 strains of *Salmonella enterica*, which were obtained from human diarrhea samples of 94 patients. They were collected between 2021 and 2023 and maintained by the Hanzhong Center for Disease Control and Prevention. Ethical approval and informed consent were waived due to the anonymous nature of this study. A flowchart detailing the steps of the study methodology is presented in Figure 1.

Serotype identification

The strains were inoculated onto Columbia blood agar plates (Guangzhou Huankai Biology, China), incubated at 37°C for 18 hours, and subsequently identified using a microbial mass spectrometer (Autof ms600: Autobio Labtec Instruments Co., Ltd, Zhengzhou, China). Serum agglutination tests for the O and H antigens of *Salmonella* were performed, and serotype interpretations were made according to the Kauffmann-White Scheme. Normal saline served as a self-coagulation control. The monophasic variant of *Salmonella typhimurium* was identified through gene sequencing.

Drug susceptibility test

The drug susceptibility test was performed using the broth microdilution method, following the CLSI (Clinical and Laboratory Standards Institute) guidelines [9]. A commercial Gram-negative bacteria susceptibility identification plate (Fosun Diagnostics Technology (Shanghai) Co., Ltd, China) was used. *Salmonella typhimurium* was tested for susceptibility to the following 17 antibiotics: CHL, SXT, polymyxin E (CT), ertapenem (ETP), meropenem (MEM), cefotaxime, ceftazidime, ceftazidime/avibactam (CZA), TET, tigecycline, ciprofloxacin, nalidixic acid, azithromycin, amikacin, STR, AMP, and AMP/sulbactam.

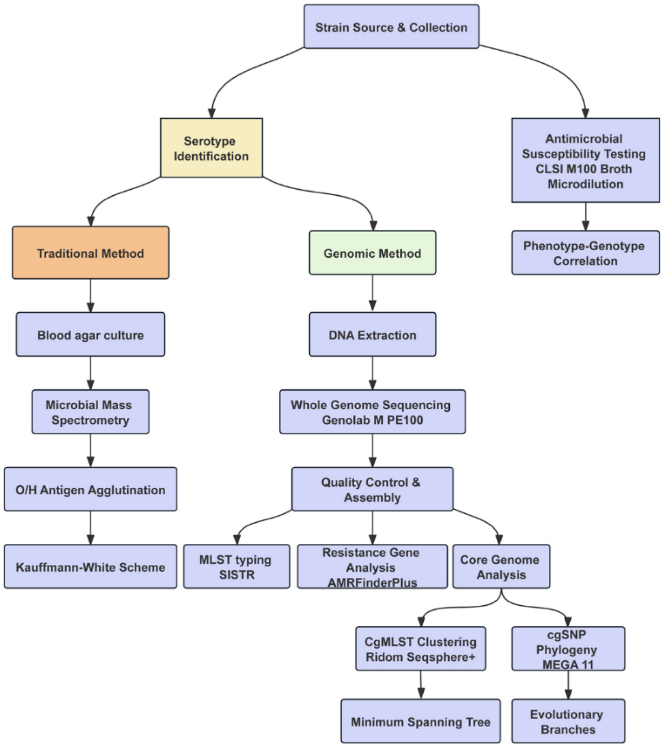
Gene sequencing

The genomic DNA of *Salmonella* was isolated using a bacterial genome extraction kit (Xi'an Tianlong Technology Co., Ltd., China). Whole genome sequencing was subsequently conducted by the Shaanxi Yunshen Pharmaceutical Technology Co., Ltd (Xi'an, China) utilizing the GenoLab M (GeneMind Biosciences Co., Ltd., Shenzhen, China) with PE100 dual-end sequencing and a sequencing depth of 100. The original sequence data underwent quality trimming, quality control, and statistical analysis. Subsequently, the genome sequence was assembled using the data processing program from the identification network.

Gene serotyping and drug resistance gene analysis

The sequencing data were imported into Ridom Seqsphere+ version 10.0.5 [10] and the serogroups and Multilocus Sequence Typing (ST) were analyzed using SISTR software version 1.1.1, which incorporates integrated *Salmonella* serotyping capabilities [11]. For strains confirmed as monophasic variants of *Salmonella typhimurium*, *Salmonella* drug-resistance genes were

Figure 1. Flowchart of the research methodology.



screened using AMRFinderPlus software version 3.11.26 [12].

The core genome multi-locus sequence typing (cgMLST) and core genome single-nucleotide polymorphism (cgSNP) analysis

The comparison table of the *Salmonella* core genome was established using Ridom Seqsphere + 10.0.5. In the cgMLST analysis of the single variant strain [13], the maximum distance for the minimum spanning tree (MST) cluster was set to 7, while the minimum sample size was set to 3. Subsequently, an MST was generated. The cgSNPs were identified using the SNV search function to construct the cgSNP phylogenetic tree based on maximum likelihood, using Mega 11.0.13.

Results

Strain distribution and demographic characteristics

A total of 94 *Salmonella* strains were isolated from human diarrhea samples obtained from 94 patients. These patients included 48 males and 46 females. The age distribution of the patients included 60 individuals under 5 years, 7 individuals aged 5-10 years, 2 aged 11-20 years, 7 aged 21-30 years, 5 aged 31-40 years, 4 aged 41-50 years, 4 aged 51-60 years, and 5 individuals over 61 years. Geographically, the strains were distributed as follows: 53 strains from Hantai District, 14 from Nanzheng District, 8 from Mianxian County, 6 from Chenggu County, 4 from Xixiang County, 4 from Yangxian County, 3 from Ningqiang County, and 2 from Foping County. The annual distribution was recorded as 37 strains in 2021, 37 strains in 2022, and 20 strains in 2023.

Characterization of Salmonella strains through serotyping and sequence type analysis

Traditional serotyping: A total of 94 *Salmonella* strains were classified into 19 serotypes. This included 57 strains of *Salmonella typhimurium*, 13 strains of *Salmonella enteritidis*, 4 strains of *Salmonella newport*, and 3 strains of *Salmonella montevideo*. Additionally, there were 2 strains each of *Salmonella kentucky* and *Salmonella manhattan*, and one strain each of *Salmonella aberdeen, Salmonella bareilly, Salmonella derby, Salmonella dublin, Salmonella rissen, Salmonella london, Salmonella rosen, Salmonella muenster, Salmonella stanley, Salmonella thompson, Salmonella salford, Salmonella chichiyu,* and *Salmonella swine.*

Gene serotyping: Whole genome sequencing identified a total of 20 serotypes. Among these, *Typhimurium* (1,4,[5],12:i:-) represented 34 strains, accounting for 36.17% of the total, making it the predominant serotype. The distribution of other serotypes was as follows: *Salmonella typhimurium* at 17.02% (16/94), *Salmonella enteritidis* at 15.96% (15/94), *Salmonella stanley* at 4.26% (4/94), and *Salmonella muenchen* at 3.19% (3/94). Other serotypes, including *Salmonella agona*, *Salmonella goldcoast*, *Salmonella london*, *Salmonella montevideo*, *Salmonella rissen*, *Salmonella thompson*, *Salmonella aberdeen*, *Salmonella derby*, *Salmonella arizonae* (IIIa 18:z4,z23:-), *Salmonella infantis, Salmonella litchfield, Salmonella muenster, Salmonella newport, Salmonella oranienburg, Salmonella poona,* and *Salmonella singapore*, were also identified.

ST typing: A total of 34 monomorphic *Salmonella typhimurium* strains were classified as ST34, 16 *Salmonella typhimurium* strains were classified as ST19, and 15 *Salmonella enteritidis* strains were

Table 1. Drug resistance results of 34 strains of *Salmonella typhimurium*.

Antibiotics	**Number of strains (%)**		
	Resistance	**Intermediate**	**Sensitive**
Ampicillin (AMP)	21 (61.76)	0 (0.00)	13 (38.24)
Ampicillin / sulbactam (AMS)	11 (32.35)	7 (20.59)	16 (47.06)
Ceftazidime / avibactam (CZA)	0 (0.00)	0 (0.00)	34 (100.00)
Ceftazidime (CTZ)	3 (8.82)	0 (0.00)	31 (91.18)
Cefotaxime (CTX)	8 (23.53)	2 (5.88)	24 (70.59)
Meropenem (MEM)	0 (0.00)	0 (0.00)	34 (100.00)
Ertapenem (ETP)	0 (0.00)	0 (0.00)	34 (100.00)
Streptomycin (STR)	29 (85.29)	0 (0.00)	5 (14.71)
Amikacin (AMK)	0 (0.00)	0 (0.00)	34 (100.00)
Polymyxin E (CT)	11 (32.35)	0 (0.00)	23 (67.65)
Azithromycin (AZM)	5 (14.71)	-	29 (85.29)
Tetracycline **(TET)**	24 (70.59)	0 (0.00)	10 (29.41)
Tigecycline (TIG)	0 (0.00)	1 (2.94)	33 (97.06)
Nalidixic acid (NAL)	11 (32.35)	-	23 (67.65)
Ciprofloxacin (CIP)	1 (2.94)	21 (61.76)	12 (35.29)
Chloramphenicol (CHL)	19 (55.88)	1 (2.94)	14 (35.29)
Sulfamethoxazole (SXT)	21 (61.76)	-	13 (38.24)

classified as ST11.

Distribution of monophasic variants of Salmonella typhimurium

A total of 34 monophasic strains of *Salmonella typhimurium* were isolated from patients with diarrhea. There were 20 males and 14 females, resulting in a sex ratio of 1.4:1. Among these strains, 29 were from individuals aged 5 years or younger, accounting for 85.29%, while 5 strains were from individuals over 10 years old. The distribution of the strains was as follows: 20 strains in Hantai District, 7 strains in Nanzheng District, 3 strains in Mianxian County, and 1 strain each in Chenggu County, Ningqiang County, Xixiang County, and Yang County.

Drug resistance of monophasic Salmonella typhimurium

Of the 34 monophasic *Salmonella typhimurium* strains, only one was susceptible to all tested antimicrobial agents, while the remaining 97.06% exhibited varying degrees of resistance (Table 1). Resistance rates were highest for STR (85.29%), followed by TET (70.59%), AMP (61.76%), and SXT (61.76%). Resistance to three cephalosporins was observed at 23.53%, while the resistance rate for ciprofloxacin was 2.94%, and reduced susceptibility was noted at 64.71%. Additionally, the resistance rate

Table 2. Resistance spectra of 34 strains of *Salmonella typhimurium*.

Multidrug resistance	Drug resistance spectrum	Number of strains
0	-	1
1	CT	1
2	TET-AMP	1
	STR-CT	1
3	TET-STR-AMP	1
	TET-AMP-NAL	1
	STR-SXT-CHL	3
4	TET-STR-SXT-NAL	1
	TET-STR-AMP-NAL	1
	TET-STR-AMP-CTX	1
	TET-STR-AMP-CHL	1
	STR-SXT-CHL-CT	3
5	TET-STR-AMP-AMS-NAL	1
	TET-AMP-CTX-AMS-CTZ	1
	TET-STR-AMP-CTX-AMS	1
	TET-STR-AMP-CTX-CTZ	1
	STR-SXT-CHL-CT-AZM	1
6	TET-STR-AMP-SXT-CTX-AMS	1
	TET-STR-AMP-SXT-CHL-NAL	1
	TET-STR-SXT-CHL-CT-AZM	1
7	TET-STR-AMP-SXT-CTX-AMS-CHL	1
	TET-STR-AMP-SXT-CTX-CHL-NAL	1
	TET-STR-AMP-SXT-AMS-CT-NAL	1
	TET-STR-AMP-SXT-CHL-CT-NAL	1
	TET-STR-AMP-SXT-CTX-AMS-CHL	1
	TET-STR-AMP-SXT-AMS-CHL-NAL	1
	TET-STR-SXT-CHL-CT-NAL-AZM	1
8	TET-STR-AMP-SXT-AMS-CHL-CT-NAL	1
9	TET-STR-AMP-SXT-CTX-AMS-CHL-CTZ-AZM	1
10	TET-STR-AMP-SXT-CTX-AMS-CHL-CIP-NAL-AZM	1
Total		**34**

AMP: Ampicillin; AMS: Ampicillin / Sulbactam; CZA: Ceftazidime / Avibactam; CTZ: Ceftazidime; CTX: Cefotaxime; MEM: Meropenem; ETP: Ertapenem; STR: Streptomycin; AMK: Amikacin; CT: polymyxin E; AZM: Azithromycin; TET: Tetracycline; TIG: Tigecycline; NAL: Nalidixic Acid; CIP: Ciprofloxacin; CHL: Chloramphenicol; SXT: sulfamethoxazole.

Table 3. Analysis of drug-resistance genes of *Salmonella typhimurium*.

Type of antibiotics	Antibiotics	Genotypes of drug resistance	n	Total
Aminoglycoside	Amikacin	aac(6)-Ib-cr5	1	34
	Gentamicin	aph(4)-Ia	3	
		aph(3)-Ia	3	
	Streptomycin	aadA1 / aph(3)-Ib / aph(6)-Id	1	
		aadA16 / aph(3)-Ib / aph(6)-Id	1	
		aph(3)-Ib	1	
		aph(3)-Ib / aph(6)-Id	21	
	Tobramycin	aac(3)-IVa	3	
β-lactams	Beta-lactam	blaTEM-1	20	20
Cephalosporins	Cephalosporin	blaCTX-M-14 (ESBL)	1	8
		blaCTX-M-55 (ESBL)	3	
		blaCTX-M-65 (ESBL) / blaOXA-10	2	
		blaOXA-10	2	
Lincosamides	Lincosamide	lnu(F)	4	4
Chloramphenicols	Chloramphenicol	cmlA5 / floR	4	11
		floR	7	
Quinolones	Quinolone	gyrA_S83F	4	17
		qnrS1	13	
Rifomycins	Rifamycin	arr-2	5	5
Sulfanilamides	Sulfonamide	sul1 / sul2	2	27
		sul2	24	
		sul2 / sul3	1	
Tetracyclines	Tetracycline	tet(A)	1	27
		tet(A) / tet(B)	2	
		tet(B)	23	
		tet(B) / tet(M)	1	
Trimethoprim	Trimethoprim	dfrA14	3	5
		dfrA15	1	
		dfrA27	1	

to CT was 32.35%. All strains were sensitive to CZA, MEM, ETP, and amikacin, as well as ceftazidime (91.18%), tigecycline (97.06%), and azithromycin (85.29%) (Table 1).

The 34 monophasic strains of *Salmonella typhimurium* exhibited 29 resistance profiles against 17 different antimicrobial agents, 25 of which were classified as multiple resistance profiles (Table 2). The overall MDR rate was 88.24% (30 out of 34 strains). The multiple resistance profiles were polymorphic, with the most common profiles being STR-SXT-CHL and STR-SXT-CHL-CT, while the remaining resistant strains each exhibited unique profiles. The multiple resistance profiles consisted of 23 strains with TET, 24 with STR, 21 with AMP, 16 with SXT, 15 with CHL, and 11 with nalidixic acid.

Drug-resistance genes of monophasic Salmonella typhimurium

In the analysis of 34 monophasic *Salmonella typhimurium* strains, we identified 30 drug-resistance genes. As shown in Table 3, all 34 strains (100%) carried 8 aminoglycoside resistance genes, while 27 strains (79.41%) harbored 3 resistance genes related to sulfonamides and TET. Furthermore, 20 strains (58.82%) possessed β-lactam resistance genes (blaTEM-1), and 17 strains (50.00%) carried two quinolone resistance genes. Among the analyzed strains, 2 presented TET resistance. The resistance phenotypes exhibited high rates of resistance to aminoglycosides, fluoroquinolones, TET, and β-lactams, along with high carriage rates for the

Figure 2. Relationship between drug resistance phenotypes and drug resistance genes in the 34 strains of *Salmonella typhimurium*.

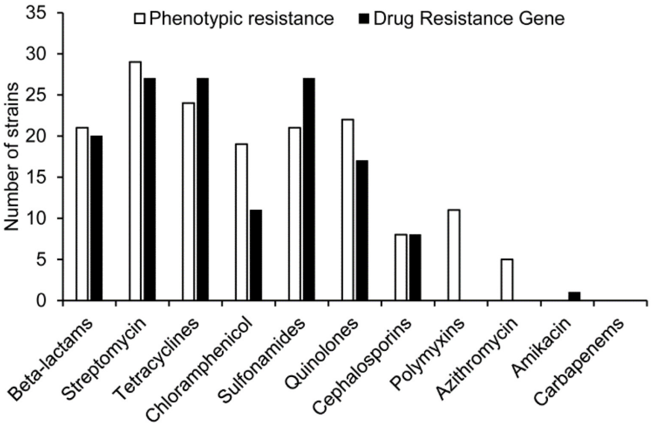
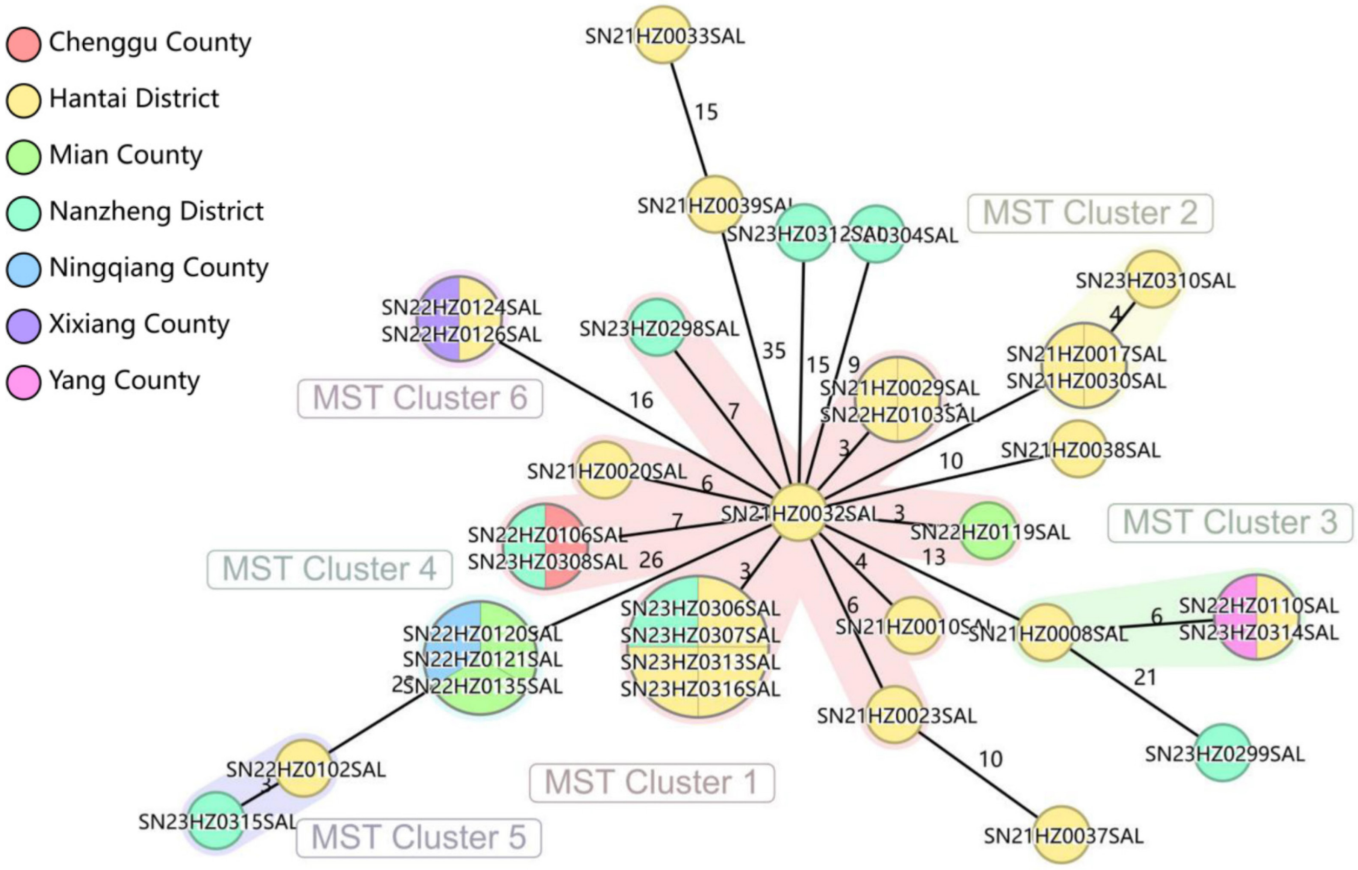


Figure 3. The minimum spanning tree of the 34 strains of *Salmonella typhimurium*.



corresponding resistance genes. This was consistent with the MDR characteristics of monophasic *Salmonella typhimurium*. Conversely, no resistance genes for polymyxin and azithromycin were detected; however, 11 strains were resistant to polymyxin and 5 strains to azithromycin, revealing a discrepancy between resistance phenotypes and genotypic profiles, as illustrated in Figure 2.

The cgMLST analysis of monophasic Salmonella typhimurium

We performed cluster analysis on the 34 monophasic *Salmonella typhimurium* strains using cgMLST. As presented in Figure 3, we identified 6 MST clusters. MST Cluster 1 was the largest, comprising 14 strains: 5 from 2021, 3 from 2022, and 6 from 2023. Geographically, 9 strains were found in Hantai District, 3 in Nanzheng District, and 1 each in Chenggu County and Mianxian County. MST Cluster 2 consisted of three strains, with 1 strain recorded in each year from 2021 to 2023, including 2 from Hantai District and 1 from Yangxian County. MST Cluster 3 also comprised three strains, two from 2021 and one from 2023, all originating from Hantai District. In MST Cluster 4, all 3 strains were recorded in 2022, with 2 from Mianxian County and 1 from Ningqiang County. MST Cluster 5 comprised two strains, one from Hantai District in 2022 and the other from Nanzheng District in 2023. Lastly, MST Cluster 6 included two strains isolated from Hantai District and Xixiang County in 2022. The various MST clusters demonstrated a distribution across different periods, with regional representation observed in each county (or district), revealing no significant differences.

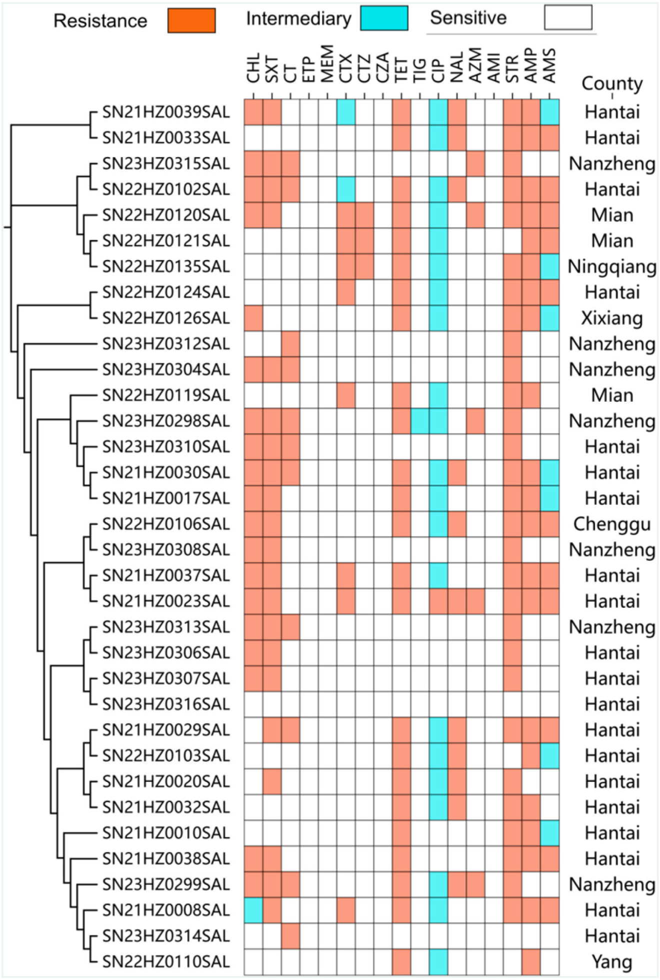
The cgSNP analysis of monophasic Salmonella typhimurium

The cgSNP analysis of 34 monophyletic *Salmonella typhimurium* strains was conducted using Ridom software, and a total of 374 cgSNP sites were identified, as illustrated in Figure 4. There were eight evolutionary branches in the cgSNP phylogenetic tree. The largest branch included 6 strains, 4 from Hantai District and 1 each from Nanzheng District and Yang County. Two branches each contained 5 strains, 3 branches comprised 4 strains, and 2 branches consisted of 2 strains. No significant differences were observed in the epidemiological information among the strains within each clade. The strains SN22HZ102SAL, SN22HZ120SAL, and SN22HZ121SAL originated from Mianxian County, while 1 strain came from Ningqiang County. Additionally, the SNP profiles of the strains SN22HZ124SAL and SN22HZ126SAL were from Hantai District and Xixiang County, respectively. This suggests the potential presence of a common exposure source within these two groups. Furthermore, some strains exhibited SNP differences at one site, indicating a possible scenario of homologous exposure.

Discussion

The detection rate of monophasic *Salmonella typhimurium* has gradually increased. This serotype has emerged as a primary cause of infection in both animals and humans. In this study, 57 strains of *Salmonella typhimurium* were identified through traditional serotyping among 94 strains isolated from Hanzhong between 2021 and 2023. The identification of *Salmonella* serotypes necessitates flagellar induction when the second phase of the H antigen is negative. Factors such as freezing and heating can damage flagellar structures, thus complicating the identification

Figure 4. The phylogenetic tree of cgSNP of 34 strains of *Salmonella typhimurium*.



process due to the attenuation of the H antigen [14]. With advancements in sequencing technology and the continuous enhancement of reference genome databases, serotype prediction based on whole genome sequencing data is anticipated to become the new gold standard for *Salmonella* serotype identification in the future [15]. In this study, 34 strains of monophasic *Salmonella typhimurium* serotype, representing 36.17%, were the dominant serotype. All these strains were identified as MLST type ST34, consistent with the previous finding [15]. The majority of these strains were isolated from children, with 29 strains from individuals under 5 years old, accounting for 85.29%. Moreover, a higher number of strains were found in urban areas (Hantai District), but they were also distributed across other districts and counties, suggesting that this serotype has become widely prevalent in Hanzhong City.

An important factor contributing to the increasing emphasis on monophasic *Salmonella typhimurium* is its MDR. In our study, resistance test results from 34 monophasic *Salmonella typhimurium* strains revealed a resistance rate of 97.06%, with the highest resistance observed against STR (85.29%), followed by TET (70.59%), AMP (61.76%), and SXT (61.76%). These findings are largely consistent with previous reports [16,17]. Polymyxins are recognized as the last line of defense for treating gram-negative bacterial infections; however, it is noteworthy that the resistance rate to CT reached 32.35% [18]. Generally, resistance to more than three antibiotics is classified as MDR. In this study, we identified 30 multiresistant strains, resulting in an MDR rate of 88.24%, which is more than the general MDR rate of *Salmonella* [18] and aligns with the MDR rate of monophasic *Salmonella typhimurium* reported in Guangxi [19]. Among the resistance profiles, there were 25 distinct patterns with polymorphisms observed. The most prevalent resistance profiles included STR-SXT-CHL and STR-SXT-CHL-CT. Most of the resistance profiles included 11 strains resistant to TET, STR, AMP, SXT, CHL, and nalidixic acid. This is consistent with the study in Yulin, Guangxi [20]. Additionally, it is encouraging that all 34 strains were found to be susceptible to CZA, MEM, ETP, and amikacin.

The resistance genes identified included aminoglycoside resistance genes (100%), sulfonamides (79.41%), tetracyclines (79.41%), β-lactam resistance genes (blaTEM-1) (58.82%), and quinolones (50.00%), which corresponded closely with the results of drug sensitivity tests. From an epidemiological perspective, *Salmonella* outbreaks associated with pork can proliferate through the food chain. The prevalence of highly resistant antibiotics during the breeding process is concerning [21]. Some banned antibiotics, such as sulfonamides and amide alcohols, may still be illegally used due to their low cost [21]. The high resistance rate of strains may be attributed to the inappropriate use of these antibiotics [21]. In this study, although resistance genes for polymyxin and azithromycin were not detected, 11 and 5 strains showed resistance, respectively. This discrepancy suggests that resistance phenotypes may not always correlate with the presence of specific resistance genes. In particular, polymyxin resistance appears to be primarily mediated by SNPs in the two-component regulatory systems located on the chromosome [22]. Several mechanisms may contribute to this discrepancy, including the action of efflux pumps that actively expel antibiotics from bacterial cells, thereby reducing intracellular concentrations [23]. Additionally, mutations in chromosomal genes may alter the antibiotic targets, diminishing the drug's efficacy without any specific resistance genes being present [24]. Biofilm formation is another factor that can contribute to reduced susceptibility, as it creates a protective environment for bacteria, making them less accessible to antibiotics [25]. Thus, while certain resistance genes can predict phenotypic resistance accurately, the presence of multiple resistance mechanisms, such as those described above, introduces variability and complexity in this relationship.

In addition to serotyping, *Salmonella* can also undergo molecular typing. MLST typing, based on seven housekeeping genes, demonstrates a certain correlation with serotype [26]. In this study, all *Salmonella typhimurium* isolates were classified as ST34. However, the presence of additional ST types beyond ST34 has also been reported. While ST classification serves as a useful tool, it has limitations in tracing the origin of *Salmonella* transmission [20]. Whole-genome sequencing of the *Salmonella* core genome allows for a more accurate description of genetic evolution and variation among strains [16]. Ridom software serves as an analytical tool for bacterial genomes. The *Salmonella enterica* cgMLST protocol uses 3,002 core genome alleles, enabling the division of 34 isolates into 11 cgMLST Complex types [11]. The strains identified in this study were visualized in the MST generated through cluster analysis, revealing that the largest cluster contained 14 bacterial strains. Furthermore, MLST site variations among strains ranged up to seven, indicating clustering that includes strains from diverse regions. Notably, there were no obvious regional or temporal clusters, suggesting that these strains have become dominant in various locations.

The cgSNP analysis provides insights into the genetic evolutionary relationships among strains, offering a slightly higher resolution than cgMLST through examination of single-nucleotide variations within the core genome [27]. The cgSNP analysis of 34 monophasic *Salmonella typhimurium* revealed eight evolutionary clades, each containing isolates from different periods and regions. Among these, two strains from Mianxian County and one strain from Ningqiang County exhibited identical SNPs, while one strain from Hantai District and another from Xixiang County also shared the same SNP sites. Some strains differed by 1 to 3 SNPs, with their isolation times being relatively close. Payne *et al.* have emphasized that in the study of *Salmonella* transmission traceability, setting a dynamic SNP threshold can help identify outbreak events; specifically, a threshold of 0 SNPs is applicable for suspected outbreaks occurring within four weeks, while a dynamic threshold of 0 to 5 SNPs should be used for events extending beyond four weeks [28]. The findings of this study suggest a potential common source of exposure for the strains, indicating that these strains have circulated across different regions. Additionally, monophasic *Salmonella typhimurium* isolated from Hanzhong is distributed across various counties, becoming the predominant serotype with significant drug resistance. However, the data from this study are limited, making continuous monitoring of monophasic *Salmonella typhimurium* essential. Focused efforts on animal food detection, including understanding changes in serotype and drug resistance characteristics, are necessary to prevent illegal use in livestock and guide rational clinical drug usage. By mastering the genetic variation through gene sequencing, we can trace the sources of aggregation events and provide a scientific basis for prevention and control efforts.

To mitigate the growing concerns of antimicrobial resistance and the illegal use of banned antibiotics in food production and agriculture, we recommend several interventions. First, there is an urgent need to strengthen regulatory frameworks that prohibit the use of certain antibiotics. This includes increased monitoring and enforcement of existing policies to deter illegal antibiotic use. Second, promoting alternative farming practices such as improved animal husbandry, biosecurity measures, and vaccination can reduce the reliance on antibiotics as a growth promoter or preventive measure. Third, raising awareness among farmers, food producers, and consumers about the dangers of antibiotic misuse and its impact on public health is essential. Education campaigns can play a vital role in shifting perceptions and behaviors surrounding antibiotic use. Additionally, embracing a One Health approach, which unites human health, animal health, and environmental health, is vital for effectively combating antimicrobial resistance. This strategy involves coordinated efforts among healthcare providers, veterinarians, and environmental scientists to develop holistic interventions that address antimicrobial resistance comprehensively, thus ensuring the health of both humans and animals within the ecosystems they share.

This study provides critical insights into the epidemiology of monophasic *Salmonella typhimurium* in Hanzhong, China, from 2021 to 2023. The findings reveal that this variant has become the predominant serotype in diarrheal cases, particularly affecting young children, a group that represents a significant vulnerability. Alarmingly, we identified a high MDR rate of 88.24%, with notable resistance to essential antibiotics such as STR, TET, AMP, and SXT. The genomic analysis provides a deeper understanding of the transmission dynamics, highlighting distinct clusters and potential common exposure sources within the community. These results underscore the urgent need for enhanced public health strategies, including improved antibiotic stewardship and monitoring measures to manage this growing threat effectively. Overall, this study serves as a significant contribution to understanding the genetic diversity and resistance patterns of monophasic *Salmonella typhimurium*, informing future interventions aimed at safeguarding public health against foodborne pathogens.

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Authors' contributions

Yali Chen: Data collection, Statistical Analysis, Data Interpretation, Manuscript Preparation; Ning Zou: Data collection, Manuscript Revision; Yang Bai: Data collection, Manuscript Revision; Pengpeng Li: Data collection, Manuscript Revision; Shen Li: Data collection, Manuscript Revision; Yifei Han: Statistical Analysis, Manuscript Revision; Zhiqiang Zhang: Literature Search, Manuscript Revision; Quanmin Ding: Literature Search, Manuscript Revision; Jianjun Wei: Literature Search, Manuscript Revision; Dongli Liu: Study Design, Supervision, Funds

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Conflict of interest

No conflict of interest is declared.

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