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ORIGINAL RESEARCH

Dynamic Serotype Distribution and Antimicrobial Resistance of *Salmonella* Isolates from 2019 to 2023 in Guizhou, China

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Introduction: *Salmonella*, a leading cause of human infectious diarrhea diseases, foodborne illness, and zoonotic infections, poses a significant health burden.

Methods: A retrospective screening was performed to elucidate the serotype distribution and antimicrobial resistance of 933 human *Salmonella* isolates from nine cities (prefectures) in Guizhou province of southwestern China between 2019 and 2023 through slide agglutination and antimicrobial resistance testing.

Results: Fifty-four different serotypes were identified in this study, with *S.* Typhimurium (44.4%) and *S.* Enteritidis (20.7%) being the predominant serotypes, followed by *S.* London (3.1%), *S.* Derby (2.8%), and *S.* Rissen (2.0%). A total of 39 serotypes were reported for the first time in Guizhou province, and 121 isolates (13.0%) could not be classified. The diversity of *Salmonella* serotypes in Guizhou has increased from 8 in 2019 to 39 in 2023. In addition, the detection rate of *S.* Enteritidis showed a decreasing trend over time, while the detection rate of *S.* Typhimurium demonstrated an annual increase since 2020. For 933 isolates, a significant majority (94.0%) exhibited resistance to at least one class of antimicrobial agents. The highest resistance observed was to ampicillin (86.4%), followed by resistance to tetracycline (76.3%) and streptomycin (72.8%). Notably, we discovered that the resistance rate to colistin was 4.7%, with 93.2% of these isolates being *S.* Enteritidis. Meanwhile, 78.5% of isolates were demonstrated multidrug resistance (MDR), with the MDR rates for *S.* Rissen and *S.* Typhimurium exceeding 90%. Additionally, 5.7% of *Salmonella* isolates were extensively drug-resistant (XDR), with *S.* Typhimurium and *S.* Enteritidis exhibiting XDR rates of 5.1% and 4.1%, respectively. The rate of MDR and XDR in *Salmonella* peaked in 2019 and then gradually declined from 2020 to 2022, rising again in 2023.

Conclusion: Our research revealed an increasing diversity in *Salmonella* serotypes within Guizhou province, alongside significant challenges posed by MDR and a rising XDR rate. Therefore, it is essential to continuously improve the surveillance of *Salmonella*, keep track of changes in serotype distribution and dynamic shifts, and strengthen the persistent monitoring of antimicrobial agents.

Keywords: *Salmonella*, serotype, antimicrobial resistance, colistin, multidrug resistance

Introduction

Salmonella is one of the most common intestinal pathogens causing infectious diarrhea, foodborne illnesses, and zoonotic infections in humans.[1] Within the European Union, salmonellosis was the second most common foodborne zoonotic disease and a leading cause of foodborne outbreaks, with approximately 65,000 cases reported yearly.[2] Surveillance data in Guizhou province from 2018 to 2022 indicated that *Salmonella* infections were responsible for 92.0% of all diagnosed cases of infectious diarrhea, a rate significantly higher than that of other bacterial infections, including those caused by *Escherichia coli*.[3]

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Furthermore, the prevalence of *Salmonella* serotypes showed variation across different regions and over time. A study from Israel revealed that *S*. Enteritidis was the prevalent serotype from 2015 to 2018, yet by 2020, *S*. Virginia and *S*. Muenchen emerged as the dominant serotypes.[4] Between 2013 and 2018, the predominant *Salmonella* serotype in Guizhou province was *S*. Enteritidis, similar to the serotype frequently observed in the European Union, the United States, Guangdong and Shandong of China.[5–9] However, the primary serotype was *S*. Agona, found in Maanshan City, Anhui province of China.[10] From 2015 to 2020, 82 serotypes across 980 *Salmonella* isolates were discovered in Anhui province, whereas 36 serotypes from 1499 *Salmonella* isolates between 2015 and 2019 were identified in Jiangsu province.[11,12] The above monitoring data indicated the diversity of serotypes and the difference in different regions. Our previous study found 24 serotypes within 363 *Salmonella* isolates from 2013 to 2018 in Guizhou province.[5] The change of *Salmonella* serotypes in Guizhou province from 2019 to 2023 remains fully understood, highlighting the importance of ongoing surveillance and detailed analysis of *Salmonella* serotypes in Guizhou province to help prevent and manage *Salmonella*.

The growing antimicrobial resistance (AMR) in *Salmonella* has become a global concern, posing a significant threat to public health.[13] In addition, the rise in AMR rates, including the profile of multidrug resistance (MDR) and extensively drug-resistant (XDR) isolates, has complicated efforts to prevent and control infections. This issue has been observed to be a worrying trend across the globe.[14] More importantly, the AMR, AMR patterns and MDR rates varied across years, serotypes and regions. Research conducted from 2013 to 2018 indicated that the MDR rate in *Salmonella* isolates from Guizhou province was over 80%, significantly higher than that observed in other regions of China.[5,11,15] Additionally, there was an observed increase in resistance to ampicillin, tetracycline, and streptomycin over time. In contrast, a reduction in resistance to tetracycline, ampicillin, and cefotaxime was reported in northwestern Italy between the period of 2012–2016 and 2017–2021.[16] The previous study showed that the azithromycin resistance rate of *Salmonella* in Guizhou province had reached 9.1%, much higher than that reported in Xi'an.[5,17] Reports on *Salmonella* in Beijing and Shaoxing showed that the resistance rate of colistin, the last line of defense for antibiotic treatment, was 60.0% and 52.0%, respectively.[18,19] Nonetheless, colistin resistance of human *Salmonella* cases in Guizhou province has not been tracked, leaving its resistance levels unknown. Moreover, antimicrobial resistance varies across different regions and changes over time. As a result, it is crucial to implement ongoing, dynamic surveillance of *Salmonella* resistance to prevent and control salmonellosis.

By collecting *Salmonella* isolates from the Chinese Pathogen Identification Net (CPIN) sentinel sites in Guizhou province over the past five years, this aim was to assess the distribution of *Salmonella* serotypes and the patterns of antimicrobial resistance. The results of this study will contribute to a better understanding of *Salmonella*'s prevalence and resistance trends, enabling more effective strategies to combat this pathogen. By monitoring and analyzing the data, public health officials and medical professionals can work together to reduce the incidence of *Salmonella* infections and improve patient outcomes. This research plays a crucial role in safeguarding the health of the population and promoting the rational use of antibiotics, which is essential for mitigating the growing problem of antibiotic resistance.

Materials and Methods

Ethics Statement

The present study was reviewed and approved by the Ethics Review Committee of Guizhou Provincial Center for Disease Control and Prevention. We ensured that all data were fully anonymized and did not collect any information on individual participants during or after data collection.

Isolates Collection and Identification

This study was a retrospective analysis of archived isolates, with 933 *Salmonella* isolates from human feces, all sporadic cases, collected from January 1, 2019, to December 31, 2023. All *Salmonella* isolates were obtained from the CPIN sentinel sites (https://139.9.117.189/CPIN/login), including 109 sentinel hospitals in nine cities (prefectures) in Guizhou province, with Guiyang (the center of Guizhou, n=164), Zunyi (north of Guizhou, n=149), Tongren (northeast of Guizhou, n=259), Bijie (northwest of Guizhou, n=33), Liupanshui (south of Guizhou, n=90), Anshun (central and

west of Guizhou, n=45), Qianxinan (southwest of Guizhou, n=32), Qiannan (south of Guizhou, n=89), and Qiandongnan (southeast of Guizhou, n=72). These collected isolates were inoculated on the *Salmonella* chromogenic medium (CHROMagar, France) and incubated at 36°C for 18–24 h. Pale purple or purple colonies were selected and inoculated onto Krebs disaccharide iron medium (KIA) and motility indole urea iron medium (MIU) at 36°C for 18–24 h (Cyclokay Biological, China). The isolates that adhered to the initial biochemistry of *Salmonella* were further identified by MALDI-TOF MS system MS1000 (Autobio Diagnostics CO., Ltd, China).

Serotype Testing

According to the White-Kaufmann-Le Minor Scheme, the confirmed *Salmonella* isolates were serotyped by slide agglutination test for O and H antigens (SSI, Denmark).[20] The identified *Salmonella* isolates were transferred to the soft agar plates and incubated at 36°C for 18–24 h. A few bacteria were picked for the polyvalent and monovalent slide serum agglutination test for the O and H phases of *Salmonella*. Saline was used as a negative control for this test.

Antimicrobial Resistance Test

Salmonella isolates were examined for antimicrobial resistance tests using the micro-broth dilution recommended by the Clinical and Laboratory Standards Institute (CLSI M100). The minimal inhibit concentration (MIC) was determined using Customized AST plate CHNENF (Thermo Fisher Scientific, America). Seventeen antimicrobial agents of 11 classes were tested, including Penicillin (ampicillin-AM), Phenicol (chloramphenicol-C), Aminoglycosides (streptomycin-STS, amikacin-AN), Carbapenems (ertapenem-ETP, meropenem-MEM), β-lactamase inhibitor (ceftazidime/avibactam-CZA, ampicillin/sulbactam-SAM), Cephems (cefotaxime-CTX, ceftazidime-CAZ), Sulfonamides (trimethoprim-sulfamethoxazole-SXT), Tetracyclines (tetracycline-TE, tigecycline-TGC), Quinolones and Fluoroquinolones (nalidixic acid-NA, ciprofloxacin-CIP), Macrolides (azithromycin-AZM), and Lipopeptide (colistin-CL). Different antimicrobial breakpoints were interpreted by Clinical Laboratory Standards Institute guidelines.[21] Streptomycin (STS) was interpreted by the National Antimicrobial Resistance Monitoring System for enteric bacteria (NARMS) established breakpoints for *Salmonella* isolates (https://www.cdc.gov/narms/antibiotics-tested.html) and tigecycline was interpreted by the Food and Drug Administration (FAD) established breakpoints for Enterobacteria (https://www.fda.gov/drugs/development-resources/tigecycline-injection-products). *Escherichia coli* ATCC 25922 was used as the control isolate. MDR *Salmonella* was defined as being resistant to at least three different classes of antimicrobial drugs, and XDR was defined as remaining susceptible to only one or two classes.[22]

Data Analysis

Tables and figures were generated using Microsoft Excel 2020 (Microsoft Corporation, New York, NY, USA). SPSS 22.0 software was used for the statistical analysis. The Multivariable chi-square, Trend chi-square, and Fisher's exact test were used to analyze data. Results with a p-value of <0.05 were considered statistically significant. Antimicrobial resistance, including MIC values, antimicrobial resistance rates, resistance profiles, and intermediate resistance rates of *Salmonella* isolates, were analyzed using Whonet 2023 software (https://whonet.org/software.html).

Results

Serotypes Distribution

A total of 933 *Salmonella* isolates were collected between 2019 and 2023 in Guizhou province. Among these isolates, 53 isolates (5.7%) were detected in 2019, 122 (13.1%) in 2020, 244 (26.2%) in 2021, 231 (24.8%) in 2022, and 283 (30.3%) in 2023. Additionally, 54 distinct serotypes were discovered, of which the most common serotype was *S*. Typhimurium (44.4%), followed by *S*. Enteritidis (20.7%), *S*. London (3.1%), *S*. Derby (2.8%), and *S*. Rissen (2.0%). Nevertheless, 121 isolates (13.0%) could not be serotyped, including 45 isolates of serogroup B, 31 of C2, 13 of E1, 12 of C3, 7 of C1, 2 of D1, and 2 of D. In addition, serogroups C, I, G, and E each had one isolate, and four isolates had not been classified.

The serotypes of *Salmonella* isolates in Guizhou from 2019 to 2023 are shown in Figure 1A. The diversity of *Salmonella* serotypes increased, with 8, 15, 21, 22, and 39 serotypes detected from 2019 to 2023, respectively. In

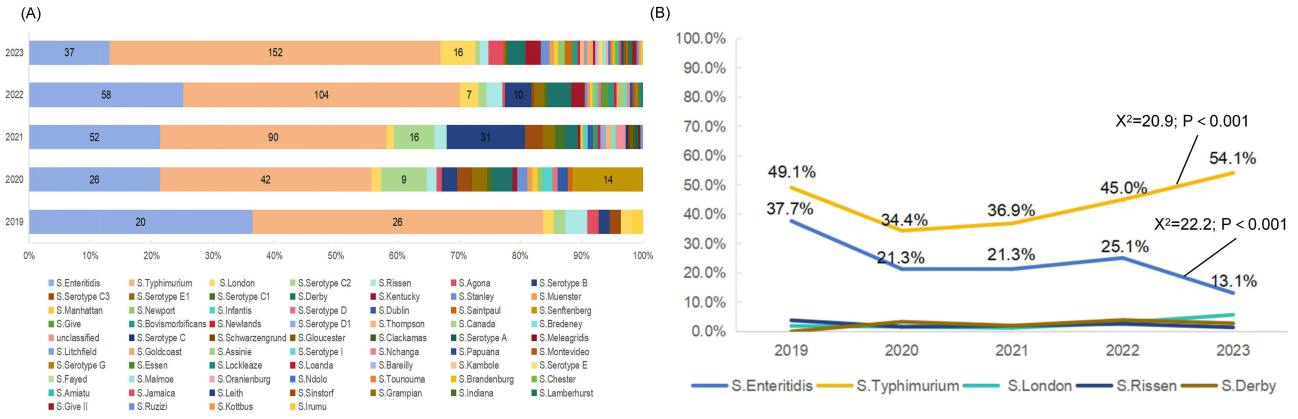

Figure 1 (**A**) The serotype distribution of *Salmonella* isolates in Guizhou from 2019 to 2023. (**B**) The detection rate of the top five serotypes of *Salmonella* isolates in Guizhou from 2019 to 2023.

addition, the detection rate of *S*. Enteritidis showed a decreasing trend over time, while the detection rate of *S*. Typhimurium exhibited an upward trend beginning in 2020 ($P < 0.05$) (Figure 1B).

The geographical distribution of *Salmonella* serotypes is shown in Table 1. *S*. Enteritidis and *S*. Typhimurium were the predominant serotypes in four cities (prefectures), including Qiannan, Qianxinan, Liupanshui, and Guiyang. *S*. Typhimurium was the dominant serotype in Bijie, Tongren, Anshun, and Zunyi. However, Qiandongnan was mainly dominated by *S*. Typhimurium, followed by *S*. Senftenberg and *S*. Enteritidis. Notably, the *Salmonella* serotypes found in Tongren, Guiyang, and Zunyi exhibited a greater degree of diversity, with each comprising over 20 distinct serotypes. Of the 36 serotypes identified, only one isolate was detected for each. Additionally, five isolates were grouped but not classified. The highest detection rate was observed in Zunyi (6.7%), followed by Guiyang (4.9%) and Qiandongnan (4.2%).

Antimicrobial Resistance

The antimicrobial resistance testing on 933 *Salmonella* isolates revealed that 877 (94.0%) exhibited resistance to at least one class of antimicrobial agent. The most common resistance was to ampicillin (86.4%), followed by tetracycline (76.3%) and streptomycin (72.8%). Furthermore, three antimicrobials showed relatively high levels of resistance, including chloramphenicol (54.9%), nalidixic acid (52.4%), and trimethoprim-sulfamethoxazole (51.9%). Antimicrobial resistance of human *Salmonella* isolates in Guizhou province from 2019 to 2023 was analyzed (Table S1). The results showed that the resistance rate to ciprofloxacin increased significantly ($P < 0.05$), while the resistance rates to chloramphenicol, trimethoprim-sulfamethoxazole, cefotaxime, ceftazidime, tetracycline, nalidixic acid, azithromycin, ampicillin, and streptomycin decreased ($P < 0.05$).

In addition, statistically significant differences were observed in the resistance rates of the top five dominant serotypes, exception for ertapenem, meropenem, ceftazidime/avibactam, ceftazidime, amikacin, and tigecycline (Table 2). Ampicillin resistance was found in over 90% of *S*. Enteritidis and *S*. Typhimurium isolates among the top five dominant serotypes. Specifically, *S*. Typhimurium presented the highest resistance to tetracycline (93.5%), whereas *S*. Enteritidis displayed the highest resistance to nalidixic acid (96.8%). *S*. Rissen exhibited markedly high resistance levels to ampicillin, tetracycline, and trimethoprim-sulfamethoxazole, with rates of 100%, 94.1%, and 94.1%, respectively. For cefotaxime and ceftazidime, the resistance rates were 33.3% and 19.7%, respectively. Moreover, the resistance to tigecycline, ertapenem, and meropenem in *Salmonella* isolates was below 1%. The alternative first-line drug, azithromycin, showed a resistance rate of 14.3%. Notably, 34.5% of *S*. London were resistant to azithromycin, whereas the resistance rates for other dominant serotypes ranged from 4.0% to 17.6%. It was essential to highlight that 4.7% of *Salmonella* isolates in Guizhou were resistant to colistin, with 93.2% being *S*. Enteritidis. Furthermore, 18.5% of *Salmonella* isolates showed intermediate resistance to colistin, with 48.2% of *S*. Enteritidis being intermediate resistant to colistin. The detailed resistance of *Salmonella* to various antimicrobials was shown in Table S2.

Table 1 The Serotype Distribution of *Salmonella* Isolates from Nine Cities (Prefectures) in Guizhou

Serotype	Number of Isolates (%)									
	Qianan (n=89)	Qiandongnan (n=72)	Qianxinan (n=32)	Liupanshui (n=90)	Bijie (n=33)	Tongren (n=259)	Guiyang (n=164)	Anshun (n=45)	Zunyi (n=149)	Total (n=933)
***S*.Typhimurium**	39(43.8)	25(34.7)	9(28.1)	32(35.6)	20(60.6)	137(52.9)	54(32.9)	27(60.0)	71(47.7)	414(44.4)
***S*.Enteritidis**	28(31.5)	13(18.1)	8(25.0)	22(24.4)	5(15.2)	35(13.5)	48(29.3)	7(15.6)	27(18.1)	193(20.7)
***S*.Serotype B**	2(2.2)	0(0.0)	2(6.3)	18(20.0)	0(0.0)	11(4.2)	9(5.5)	0(0.0)	3(2.0)	45(4.8)
***S*.Serotype C2**	2(2.2)	1(1.4)	1(3.1)	3(3.3)	2(6.1)	13(5.0)	4(2.4)	2(4.4)	3(2.0)	31(3.3)
***S*.London**	2(2.2)	5(6.9)	0(0.0)	4(4.4)	1(3.0)	4(1.5)	8(4.9)	3(6.7)	2(1.3)	29(3.1)
***S*.Derby**	2(2.2)	1(1.4)	1(3.1)	0(0.0)	0(0.0)	10(3.9)	6(3.7)	1(2.2)	5(3.4)	26(2.8)
***S*.Rissen**	4(4.5)	3(4.2)	0(0.0)	3(3.3)	0(0.0)	4(1.5)	3(1.8)	0(0.0)	2(1.3)	19(2.0)
***S*.Kentucky**	2(2.2)	1(1.4)	2(6.3)	0(0.0)	0(0.0)	2(0.8)	2(1.2)	0(0.0)	5(3.4)	14(1.5)
***S*.Senftenberg**	0(0.0)	14(19.4)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	14(1.5)
***S*.Serotype E1**	0(0.0)	1(1.4)	3(9.4)	0(0.0)	1(3.0)	3(1.2)	1(0.6)	1(2.2)	3(2.0)	13(1.4)
***S*.Agona**	2(2.2)	0(0.0)	0(0.0)	0(0.0)	1(3.0)	2(0.8)	3(1.8)	0(0.0)	3(2.0)	10(1.1)
***S*.Serotype C3**	1(1.1)	0(0.0)	0(0.0)	1(1.1)	0(0.0)	6(2.3)	2(1.2)	0(0.0)	2(1.3)	12(1.3)
***S*.Serotype C1**	1(1.1)	0(0.0)	0(0.0)	1(1.1)	0(0.0)	3(1.2)	1(0.6)	0(0.0)	1(0.7)	7(0.8)
***S*.Stanley**	1(1.1)	0(0.0)	2(6.3)	0(0.0)	0(0.0)	0(0.0)	1(0.6)	0(0.0)	3(2.0)	7(0.8)
***S*.Give**	0(0.0)	2(2.8)	0(0.0)	0(0.0)	0(0.0)	1(0.4)	1(0.6)	1(2.2)	0(0.0)	5(0.5)
***S*.Manhattan**	1(1.1)	0(0.0)	2(6.3)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	2(1.3)	5(0.5)
***S*.Newport**	0(0.0)	0(0.0)	0(0.0)	1(1.1)	0(0.0)	2(0.8)	0(0.0)	0(0.0)	2(1.3)	5(0.5)
***S*.Infantis**	0(0.0)	0(0.0)	0(0.0)	1(1.1)	0(0.0)	3(1.2)	1(0.6)	0(0.0)	0(0.0)	5(0.5)
***S*.Saintpaul**	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	2(0.8)	2(1.2)	0(0.0)	0(0.0)	4(0.4)
***S*.Bovismorbificans**	0(0.0)	1(1.4)	0(0.0)	0(0.0)	2(6.1)	1(0.4)	0(0.0)	1(2.2)	0(0.0)	5(0.5)
***S*.Muenster**	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(0.4)	2(1.2)	0(0.0)	1(0.7)	4(0.4)
***S*.Dublin**	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	4(1.5)	0(0.0)	0(0.0)	0(0.0)	4(0.4)

(*Continued*)

https://doi.org/10.2147/IDR.S492042

Table 1 (Continued).

Serotype	Number of Isolates (%)									
	Qianan (n=89)	Qiandongnan (n=72)	Qianxinan (n=32)	Liupanshui (n=90)	Bijie (n=33)	Tongren (n=259)	Guiyang (n=164)	Anshun (n=45)	Zunyi (n=149)	Total (n=933)
***S*.Thompson**	0(0.0)	0(0.0)	0(0.0)	1(1.1)	0(0.0)	2(0.8)	1(0.6)	0(0.0)	0(0.0)	4(0.4)
Unclassified	0(0.0)	2(2.8)	0(0.0)	0(0.0)	0(0.0)	2(0.8)	0(0.0)	0(0.0)	0(0.0)	4(0.4)
***S*.Goldcoast**	1(1.1)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(0.6)	1(2.2)	1(0.7)	4(0.4)
***S*.Newlands**	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	2(1.2)	0(0.0)	1(0.7)	3(0.3)
***S*.Serotype D**	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(0.4)	1(0.6)	0(0.0)	0(0.0)	2(0.2)
***S*.Serotype D1**	0(0.0)	0(0.0)	1(3.1)	0(0.0)	0(0.0)	1(0.4)	0(0.0)	0(0.0)	0(0.0)	2(0.2)
***S*.Assinie**	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	2(1.3)	2(0.2)
***S*.Give II**	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(0.4)	1(0.6)	0(0.0)	0(0.0)	2(0.2)
***S*.Litchfield**	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	2(1.2)	0(0.0)	0(0.0)	2(0.2)
Rare serotypes	1(1.1)	3(4.2)	1(3.1)	3(3.3)	1(3.0)	8(3.1)	8(4.9)	1(2.2)	10(6.7)	36(3.9)

Notes: Rare serotypes: *S*. Irumu, *S*. Canada, *S*. Bredeney, *S*. Serotype C, *S*. Schwarzengrund, *S*. Gloucester, *S*. Clackamas, *S*. Serotype A, *S*. Meleagridis, *S*. Serotype I, *S*. Nchanga, *S*. Papuana, *S*. Montevideo, *S*. Serotype G, *S*. Essen, *S*. Lockleaze, *S*. Loanda, *S*. Bareilly, *S*. Kambole, *S*. Serotype E, *S*. Fayed, *S*. Malmoe, *S*. Oranienburg, *S*. Ndolo, *S*. Tounouma, *S*. Brandenburg, *S*. Chester, *S*. Amiatu, *S*. Jamaica, *S*. Leith, *S*. Sinstorf, *S*. Grampian, *S*. Indiana, *S*. Lamberhurst, *S*. Ruzizi, *S*. Kottbus.

Table 2 Antimicrobial Resistance of the Top Five *Salmonella* Serotypes Isolates from Guizhou Province, China, from 2019 to 2023

Antibiotics	Number of Isolates (%)						X^2	P
	Salmonella (n=933)	S.Typhimurium (n=414)	S.Enteritidis (n=193)	S.London (n=29)	S.Derby (n=25)	S.Rissen (n=17)		
C	512(54.9%)	271(65.5%)	53(27.5%)	16(55.2%)	17(68.0%)	9(52.9%)	78.6	<0.001
SXT	484(51.9%)	247(59.7%)	56(29.0%)	19(65.5%)	15(60.0%)	16(94.1%)	65.4	<0.001
CL	44 (4.7%)	3(0.7%)	41(21.2%)	0(0.0%)	0(0.0%)	0(0.0%)	83	<0.001
ETP	2 (0.2%)	1(0.2%)	1(0.5%)	0(0.0%)	0(0.0%)	0(0.0%)	3.9	0.627
MEM	0 (0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	/	/
CTX	311(33.3%)	162(39.1%)	43(22.3%)	2(6.9%)	3(12.0%)	4(23.5%)	31.8	<0.001
CAZ	184(19.7%)	80 (19.3%)	26(13.5)	2(6.9%)	3(12.0%)	4(23.5%)	6.2	0.176
CZA	1 (0.1%)	0 (0.0%)	1(0.5)	0(0.0%)	0(0.0%)	0(0.0%)	7.1	0.389
TE	712(76.3%)	387(93.5%)	89(46.1)	19(65.5%)	19(76.0%)	16(94.1%)	178.4	<0.001
TGC	2(0.2%)	2 (0.5%)	0(0.0)	0(0.0%)	0(0.0%)	0(0.0%)	3.8	1
CIP	196(21.0%)	93 (22.5%)	18(9.3)	9(31.0%)	3(12.0%)	0(0.0%)	23	<0.001
NA	489(52.4%)	174(42.0%	187(96.9)	7(24.1%)	7(28.0%)	5(29.4%)	188.6	<0.001
AZM	133(14.3%)	50 (12.1%)	14(7.3)	10(34.5%)	1(4.0%)	3(17.6%)	16.9	0.001
AN	13 (1.4%)	3 (0.7%)	3(1.6)	0(0.0%)	0(0.0%)	0(0.0%)	1.9	0.686
STS	679(72.8%)	352(85.0%)	123(63.7)	17(58.6%)	12(48.0%)	11(64.7%)	51.1	<0.001
AM	806(86.4%)	383(92.5%)	177(91.7%)	20(69.0%)	17(68.0%)	17(100%)	25.3	<0.001
SAM	411(44.1%)	177(42.8%)	104(53.9%)	14(48.3%)	5(20.0%)	2(11.8%)	21.0	<0.001

Abbreviations: C, chloramphenicol; SXT, trimethoprim sulfamethoxazole; CL, colistin; ETP, ertapenem; MEM, meropenem; CTX, cefotaxime; CAZ, ceftazidime; CZA, ceftazidime/avibactam; TE, tetracycline; TGC, tigecycline; CIP, ciprofloxacin; NA, nalidixic acid; AZM, azithromycin; AN, amikacin; STS, streptomycin; AM, ampicillin; SAM, ampicillin/sulbactam.

Trend of Multidrug Resistance

Of the 933 *Salmonella* isolates, 732 isolates (78.5%) showed MDR. The prevalence of MDR *Salmonella* peaked at 96.2% in 2019, followed by a steady decline from 2020 to 2022. However, this trend reversed in 2023, with MDR prevalence rising to 85.5%. In addition, 53 isolates (5.7%) showed resistance to at least nine classes of antibiotics, exhibiting XDR. The MDR and XDR rates trends closely mirrored each other over this period (Figure 2A).

The prevalence of MDR isolates among the top five serotypes was notably high. The highest MDR rate was observed in *S*. Rissen (94.1%), followed by *S*. Typhimurium (92.8%). Additionally, the MDR rates of *S*. Enteritidis, *S*. London, and *S*. Derby ranged from 65.5% to 80.0%. Notably, *S*. Typhimurium and *S*. Enteritidis exhibited XDR rates of 5.1% and 4.1%, respectively, while the XDR rates of the other dominant serotypes were zero (Figure 2B).

The MDR rates of *Salmonella* isolates from nine cities (prefectures) in Guizhou between 2019 and 2023 were analyzed. The results revealed that the highest rate of MDR was observed in Bijie (96.9%), followed by Anshun (91.1%). The other cities (prefectures) also showed higher rates of MDR, ranging from 73.6% to 86.9%. Concurrently, the cities of Zunyi and Liupanshui displayed high XDR rates of 12.8% and 8.9%, respectively (Figure 2C).

Distribution of AMR Patterns

A total of 933 *Salmonella* isolates showed resistance to 11 distinct classes of antimicrobials, and 223 unique AMR patterns were identified. The most prevalent AMR pattern was TE+STS+AM (5.8%), followed by C+SXT+TE+STS

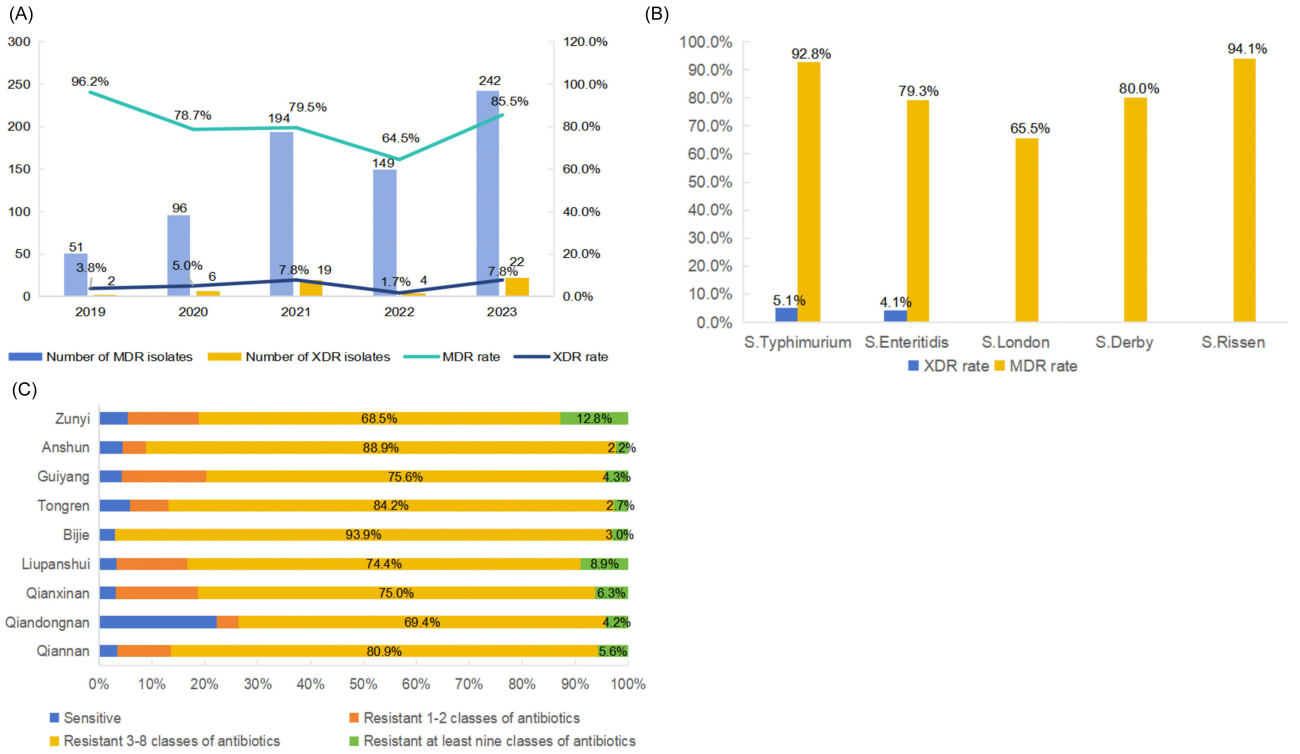

Figure 2 (**A**) MDR and XDR rates of *Salmonella* isolates in Guizhou from 2019 to 2023. (**B**) MDR and XDR of the top five serotypes of *Salmonella* isolates in Guizhou. (**C**) Antimicrobial resistance of *Salmonella* in different cities (prefectures) of Guizhou from 2019 to 2023.

+AM (4.5%). In addition, the prevalent AMR patterns of *Salmonella* in Guizhou province from 2019 to 2023 are shown in Table 3. In 2019, the predominant AMR pattern was C+SXT+CTX+CAZ+TE+NA+AZM+STS+AM (9.4%). However, the dominant AMR pattern shifted in 2020, with NA+STS+AM+SAM emerging as the predominant pattern (8.2%). In 2021, TE+STS+AM (7.8%) was the most common AMR pattern. In 2022, NA+AM (9.1%) became the dominant AMR pattern, and in 2023, C+SXT+TE+STS+AM (6.4%) was the most prevalent pattern.

This study analyzed the AMR patterns associated with the top five serotypes (Table 4). A total of 135 distinct AMR patterns were identified in *S.* Typhimurium, with the dominant AMR pattern being TE+STS+AM (10.4%). Additionally, the pattern of C+SXT+TE+STS+AM was identified as the most common in both *S.* Typhimurium and *S.* Rissen. Among

Table 3 Dominant AMR Patterns of *Salmonella* Isolates in Guizhou Province from 2019 to 2023

Year	AMR Pattern	Number of Isolates	Percent (%)
2019	C+SXT+TE+NA+STS+AM+SAM	3	5.7
	C+SXT+CTX+TE+NA+STS+AM+SAM	3	5.7
	C+SXT+CTX+CAZ+TE+NA+AZM+STS+AM	5	9.4
2020	NA	5	4.1
	NA+STS+AM+SAM	10	8.2
	C+SXT+TE+NA+STS+AM+SAM	6	4.9
	C+SXT+TE+AM+SAM	5	4.1
	C+SXT+CTX+CAZ+TE+NA+STS+AM	5	4.1

(*Continued*)

Table 3 (Continued).

Year	AMR Pattern	Number of Isolates	Percent (%)
2021	C+SXT+TE+STS+AM	15	6.1
	C+SXT+TE+AM	8	3.3
	TE+STS+AM	19	7.8
	C+SXT+CTX+CAZ+TE+CIP+NA+AZM+STS+AM+SAM	8	3.3
2022	TE+STS+AM	15	6.5
	NA+STS+AM+SAM	9	3.9
	NA+AM	21	9.1
	C+SXT+CTX+TE+CIP+NA+STS+AM+SAM	9	3.9
2023	TE+STS+AM	13	4.6
	C+SXT+TE+STS+AM	18	6.4
	C+SXT+CTX+CAZ+TE+CIP+NA+AZM+STS+AM+SAM	14	4.9

Abbreviations: C, chloramphenicol; SXT, trimethoprim sulfamethoxazole; CL,colistin; ETP, ertapenem; MEM, meropenem; CTX, cefotaxime; CAZ, ceftazidime; CZA, ceftazidime/avibactam; TE, tetracycline; TGC, tigecycline; CIP, ciprofloxacin; NA, nalidixic acid; AZM, azithromycin; AN, amikacin; STS, streptomycin; AM, ampicillin; SAM, ampicillin/sulbactam.

Table 4 Dominant AMR Patterns of the Top Five Serotypes of *Salmonella* Isolates in Guizhou

Serotype	AMR Pattern	Number of Isolates	Percent (%)
***S*.Typhimurium**	TE+STS+AM	43	10.4
	C+SXT+TE+STS+AM	27	6.5
***S*.Enteritidis**	NA+AM	25	13.2
	NA+STS+AM+SAM	22	11.6
***S*.London**	C+SXT+TE+CIP+STS+AM+SAM	3	10.3
***S*.Derby**	C+SXT+TE+NA+STS+AM	2	8.0
	C+TE+AM	2	8.0
	C+SXT+TE	2	8.0
***S*.Rissen**	C+SXT+TE+STS+AM	4	23.5
	C+SXT+CTX+CAZ+TE+NA+AZM+STS+AM	3	17.6
	SXT+TE+AM	3	17.6

Abbreviations: C, chloramphenicol; SXT, trimethoprim sulfamethoxazole; CL,colistin; ETP, ertapenem; MEM, meropenem; CTX, cefotaxime; CAZ, ceftazidime; CZA, ceftazidime/avibactam; TE, tetracycline; TGC, tigecycline; CIP, ciprofloxacin; NA, nalidixic acid; AZM, azithromycin; AN, amikacin; STS, streptomycin; AM, ampicillin; SAM, ampicillin/sulbactam.

the 190 *S*. Enteritidis isolates, 66 different patterns of AMR were observed. The dominant patterns were NA+AM (13.2%) and NA+STS+AM+SAM (11.6%). The *S*. London, *S*. Derby, and *S*. Rissen isolates exhibited 17, 19, and 9 different AMR patterns, respectively, with each serotype displaying a unique dominant pattern.

The dominant AMR patterns of *Salmonella* isolates from nine cities (prefectures) in Guizhou province also differed (Table 5). The dominant AMR pattern observed in Qiannan, Qiandongnan, and Tongren was TE+STS+AM. On the other

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Table 5 Distribution of Dominant AMR Patterns of *Salmonella* in Different Cities (Prefectures) of Guizhou

Region	AMR Pattern	Number of Isolates	Percent (%)
Qiannan	C+SXT+CTX+CAZ+TE+CIP+NA+AZM+STS+AM+SAM	4	4.5
	TE+STS+AM	7	7.9
Qiandongnan	TE+STS+AM	4	5.6
Qianxinan	NA	3	9.4
Liupanshui	C+SXT+TE+STS+AM	9	10
	NA+AM	6	6.7
Bijie	C+SXT+CTX+CAZ+TE+CIP+NA+STS+AM+SAM	5	15.2
	C+SXT+TE+STS+AM	5	15.2
Tongren	C+SXT+TE+STS+AM	9	3.5
	TE+STS+AM	24	9.3
Guiyang	NA+AM	6	3.7
	C+SXT+CTX+CAZ+TE+NA+STS+AM	5	3
	NA	6	3.7
Anshun	C+SXT+TE+NA+STS+AM+SAM	5	11.1
Zunyi	C+SXT+CTX+TE+CIP+AZM+STS+AM+SAM	7	4.7
	C+SXT+CTX+TE+CIP+NA+STS+AM+SAM	7	4.7
	C+SXT+CTX+CAZ+TE+CIP+NA+AZM+STS+AM+SAM	9	6
	NA+AM	7	4.7

Abbreviations: C, chloramphenicol; SXT, trimethoprim sulfamethoxazole; CL,colistin; ETP, ertapenem; MEM, meropenem; CTX, cefotaxime; CAZ, ceftazidime; CZA, ceftazidime/avibactam; TE, tetracycline; TGC, tigecycline; CIP, ciprofloxacin; NA, nalidixic acid; AZM, azithromycin; AN, amikacin; STS, streptomycin; AM, ampicillin; SAM, ampicillin/sulbactam.

hand, the pattern of C+SXT+TE+STS+AM was the dominant AMR pattern in Liupanshui, Bijie, and Tongren. The pattern of NA+AM was the dominant AMR pattern in Guiyang and Zunyi.

Discussion

Between 2019 and 2023, an increasing trend was observed in the collection of 933 *Salmonella* isolates in this study, which might be attributed to the gradual improvements in the CPIN initiated in 2019, along with advancements in detection technology and a national focus on pathogenicity testing. Serotype testing showed that *S*. Typhimurium and *S*. Enteritidis were the main serotypes of *Salmonella* isolates, followed by S. London, S. Derby, and S. Rissen, which was consistent with the top five dominant serotypes of *Salmonella* isolated from animal food in Guizhou province in the previous study.[23] Meanwhile, we found different dominant serotypes in different cities in this study. Given the diverse geographical distribution of various *Salmonella* serotypes in Guizhou province, it is essential to develop local public health policies tailored to effectively address the specific isolates of *Salmonella* that were most prevalent in each city or prefecture. This approach should involve comprehensive epidemiological studies to identify the predominant serotypes in each region, understand the environmental and socio-economic factors contributing to their transmission, and create targeted interventions.

Moreover, 54 serotypes were discovered in Guizhou province, and the number of *Salmonella* serotypes increased significantly from 8 in 2019 to 39 in 2023. Of 54 serotypes, 39 serotypes were detected for the first time in Guizhou province compared with the previous study conducted from 2013 to 2018.[5] In recent years, Guizhou province has

experienced rapid economic growth and increased participation in foreign trade, which has led to a diversification of dietary types and a rise in interpersonal interactions, increasing the varieties of *Salmonella* found in the region. Notably, the greater diversity of *Salmonella* serotypes found in cities like Tongren, Guiyang, and Zunyi can be attributed to higher population density, urbanization, and increased tourism. These factors can facilitate the spread of pathogens in crowded living conditions. Additionally, various dietary habits can expose individuals to different sources of *Salmonella*. Understanding these factors is essential for informing public health strategies to control *Salmonella* transmission in different cities. Importantly, different serotypes of *Salmonella* can cause various clinical diseases, which depend on the virulence of the bacteria and the susceptibility of the host.[24] While some serotypes lead to gastroenteritis in healthy individuals, others can result in more severe conditions such as systemic enteric fever or invasive nontyphoidal salmonellosis.[25] From a "One Health" perspective, vaccines against multiple serotypes of NTS with protective efficacy in multiple susceptible host species are desirable. However, the vaccines currently licensed for use generally only protect against a single serovar, limiting their efficacy in environments where many serotypes are circulating.[26]

More importantly, we newly identified serogroups and serotypes (eg, *S*. Senftenberg) in Guizhou, which might contribute to changes in the incidence and prevalence of *Salmonella* infections, as they may indicate the emergence of new isolates that could possess different virulence factors or resistance profiles. Typically, specific serotypes are often associated with particular sources, which can aid in tracing the origins of outbreaks.[27] In March 2020, the first outbreak of foodborne illness caused by *Salmonella* Senftenberg-contaminated food was consumed by nearly 200 students in Guizhou province, linked to cross-contamination of meat and vegetables.[28] To enhance food safety, it is crucial for relevant authorities to strengthen guidance and supervision. Training for canteen staff on managing high-risk foods and preventing cross-contamination is essential to reduce foodborne disease occurrences.

The results of antimicrobial resistance testing in this study showed that nearly 94.0% of the isolates were resistant to at least one class of antimicrobial agent. The highest resistance rate was observed for ampicillin, followed by tetracycline and streptomycin. This result was similar to those reported in some regions of China.[29,30] At the same time, a report on animal food in Guizhou from 2017 to 2021 showed that more than 80% of *Salmonella* isolates separated from meat and eggs were resistant to at least one class of antimicrobial, with the highest resistance rates to ampicillin and tetracycline, which was consistent with the results of our study.[23,31] We detected the β-lactams resistance gene in *Salmonella* isolates collected from 2013 to 2018 in Guizhou and found a specific correlation between the bla_{TEM} gene and ampicillin.[5] We should further strengthen the β-lactams resistance gene surveillance of *Salmonella* isolates in recent years. In 2021, the Ministry of Agriculture and Rural Affairs also reported that the top three veterinary drugs used in China were tetracyclines, sulfonamides, and β-lactams, which implied that high resistance rates of human *Salmonella* in our study might be caused by transmission from animal food.[32] Therefore, relevant agricultural and animal husbandry departments should strengthen supervision for antimicrobials to prevent further generation and spread of animal-derived drug-resistant isolates.

A gradual preference for azithromycin as a primary alternative antimicrobial in clinical settings due to the high resistance to the traditional first-line antibiotics.[33] In this study, the resistance rate of azithromycin was 14.3%, marking a 5.2% increase from the previous data, which was similar to the results reported in Gansu and Jiangsu provinces, but significantly higher than those *Salmonella* isolates in Shandong province, Zhuhai city and the European Union.[2,5,8,9,12,29] In addition, this study also reported high resistance rates for cefotaxime and ceftazidime at 33.3% and 19.7%, respectively, figures that were consistent with Jiangsu province and lower than in Henan province, but considerably above those found in human *Salmonella* cases in the European Union.[2,12,34] These findings showed that the choice of therapeutic drugs should be determined based on the results of antimicrobial resistance testing to avoid clinical treatment failure. Notably, *Salmonella* isolates in this study showed higher sensitivity to ertapenem and meropenem, with a resistance rate of only 0.2%, suggesting carbapenems as a potential treatment reference when resistance outcomes of patients with acute and serious illnesses were inconclusive.

Colistin is considered the "last line drug" for treating MDR isolate infections, particularly carbapenem-resistant Enterobacteria infections.[35] The results showed that the resistance rate was 4.7%, with 93.2% of these isolates being *S*. Enteritidis, similar to the results of the European Union and animal food in China.[2,36] However, the rate was significantly lower than reports from Shaoxing City and a district of Beijing.[18,19] The study also noted an 18.2% intermediate

resistance rate to colistin, indicating a heightened antimicrobial resistance risk and challenging clinical treatment efficacy. Colistin-resistant *Salmonella* in Guizhou province hinted at potential resistance genes like *mcr and pmrA/B*.[37–39] In 2015, a new gene resistant to colistin, known as mcr-1, was first identified in China. The research showed that mcr-1 could be horizontally transferred between different isolates through plasmids, which poses a significant threat to public health.[38] Therefore, it is crucial for future efforts in Guizhou province not only to continuously strengthen the surveillance of colistin resistance in *Salmonella* but also to delve into the resistance mechanism at the molecular level, which would provide a scientific foundation for controlling the spread of colistin-resistant isolates and developing effective prevention and control measures.

The emergence of MDR and XDR *Salmonella* isolates represented a burgeoning global challenge, posing a significant threat to therapeutic efficacy, thus increasing the morbidity and mortality of patients. This study showed that the MDR rate of *Salmonella* isolates was 78.5%, and the detection rate of XDR isolates had significantly increased from 4.4% in 2013–2018 to 5.7% in recent years.[5] The MDR rate was highest in 2019 at 96.2%, gradually decreasing from 2020 to 2022, but recovered to 85.5% in 2023, while the trend in XDR and MDR rates was the same. The COVID-19 pandemic influenced AMR trends in *Salmonella*, decreasing MDR and XDR rates from 2020 to 2022 in Guizhou province. This decline can be attributed to changes in human behavior, such as improved hygiene practices and less dining out, which reduced the transmission of foodborne illnesses. The focus on managing COVID-19 decreased unnecessary antibiotic prescriptions, reducing selection pressure on bacteria. These factors might contribute to the observed changes in AMR trends during the COVID-19 pandemic. These changes highlighted human mobility's critical role in spreading resistant pathogens. Ongoing research is essential to fully understand these dynamics and their long-term implications for public health and AMR management. The rise of MDR and XDR *Salmonella* isolates poses an increasingly serious global challenge that poses a significant threat to treatment efficacy, thereby increasing morbidity and mortality in patients. Addressing this issue requires a coordinated effort that integrates improved diagnostics, treatment protocols, and robust prevention measures, which are essential in curbing the spread of resistant *Salmonella* isolates in Guizhou. Therefore, "Veterinary Antimicrobial Drug Use Reduction Action" and "Antimicrobial Drug Clinical Application Grading Management" were implemented to reduce the inappropriate use of antibiotics in Guizhou province, which may be the reason of the decreased resistance rates to multiple antibiotics in this study. Furthermore, we should continue to monitor MDR and XDR isolates based on whole-genome sequencing (WGS) to discover the possible emergence of new clones that may replace current high-risk clones, which is essential for predicting global epidemic isolates.[40]

The expression of resistance genes offers insights into resistance mechanisms. Genomic islands containing clusters of these genes can be identified through comparative genomic analysis, helping to understand resistance patterns.[41] Integrons are important as they capture and express resistance genes, playing a critical role in the spread of antibiotic resistance.[42] Advanced techniques like WGS and bioinformatics can enhance the identification of these molecular markers, aiding rapid diagnostics, which allows clinicians to make informed treatment decisions and may help curb the spread of resistant *Salmonella* isolates.[24] Meanwhile, genomic surveillance is vital for tracking the emergence and spread of drug-resistant *Salmonella* isolates in Guizhou. By employing WGS, researchers can monitor genetic mutations and resistance patterns in real-time, enabling early detection of outbreaks. In essence, genomic surveillance enhances our capacity to respond to drug-resistant *Salmonella*, ensuring effective control and treatment measures. We should further analyze the in-depth characterization of *Salmonella* isolates from Guizhou through WGS to delve into the resistance mechanisms and molecular epidemiology.

Conclusion

Our study systematically examined the serotype distribution and development trends of antimicrobial resistance in *Salmonella* isolates from Guizhou province, China, between 2019 and 2023. The results revealed a diversification of *Salmonella* serotypes over time, with some types emerging for the first time in the region. However, the level of MDR remained high, and the rate of XDR increased significantly, accompanied by a surge in the diversity of AMR patterns. Therefore, it is essential to continue to monitor *Salmonella* serotypes and antimicrobial resistance. Further investigation into the mechanisms of antimicrobial resistance and transmission is crucial for developing evidence-based prevention and control strategies for *Salmonella* infections. This study had certain limitations. We only analyzed *Salmonella* isolated

from human feces, excluding food and environment samples. Moreover, the current data only focused on the phenotypes of antimicrobial resistance, lacking a comprehensive understanding of the underlying genetic mechanisms. Future studies should address these gaps to provide a more complete picture of *Salmonella* resistance and inform more effective public health interventions.

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Disclosure

The authors report no conflicts of interest in this work.

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