

## Preplanned Studies

# Antimicrobial Resistance Analysis and Whole-Genome Sequencing of *Salmonella* Isolates from Environmental Sewage — Guangzhou City, Guangdong Province, China, 2022–2023

Bihui Su<sup>1</sup>; Guanghong Du<sup>2</sup>; Shuiping Hou<sup>3</sup>; Zongqiu Chen<sup>3</sup>; Xiaoying Wu<sup>1</sup>; Gang He<sup>1</sup>; Jun Yuan<sup>3,\*</sup>; Chaojun Xie<sup>4,\*</sup>

## Summary

### What is already known about this topic?

S.1,4,[5],12:i:- and *S. Rissen* are emerging serotypes of *Salmonella* that require close monitoring for antimicrobial resistance and containment of their spread.

### What is added by this report?

The study aimed to identify antimicrobial resistance genes (ARGs) in S.1,4,[5],12:i:- and *S. Rissen* strains isolated from environmental sewage in Guangzhou City, Guangdong Province, China. A phylogenetic tree was constructed using single nucleotide polymorphism data to assess genetic relatedness among strains, offering insights for *Salmonella* infection outbreak investigations in the future.

### What are the implications for public health practice?

It is crucial to implement strategies, such as integrating different networks, to control the spread of drug-resistant *Salmonella*. Novel technologies must be utilized to disinfect sewage and eliminate ARGs. Ensuring food safety and proper sewage disinfection are essential to curb the dissemination of *Salmonella*.

S.1,4,[5],12:i:- and *S. Rissen* are emerging *Salmonella* serotypes. Monitoring their antimicrobial resistance and controlling their spread is crucial. This study analyzed 35 S.1,4,[5],12:i:- and 6 *S. Rissen* isolates from untreated environmental sewage in Guangzhou. Resistance levels were tested, and whole-genome sequencing (WGS) was used to identify antimicrobial resistance genes (ARGs) and construct a phylogenetic tree to assess resistance and multi-drug resistance.

S.1,4,[5],12:i:- and *S. Rissen* were found to be more severe, carrying 183 ARGs related to various resistance mechanisms such as antibiotic efflux, target replacement, protection, inactivation, alteration, etc. It

is noteworthy that the rare plasmid-mediated colistin resistance gene *mcr-3.1* was detected. This research contributes to the understanding of resistance in S.1,4,[5],12:i:- and *S. Rissen*, indicating that *Salmonella* is prevalent on both domestic and international scales. These findings are essential for establishing effective epidemiological data, informing clinical management practices, and devising appropriate public health strategies.

Sewage samples were obtained from various locations in 11 districts of Guangzhou City, Guangdong Province, China, such as hospitals, communities, markets, hotels, sewage plants, restaurants, and schools. 660 sewage samples were collected between February 1, 2022, and January 31, 2023, resulting in the isolation of 35 S.1,4,[5],12:i:- and 6 *S. Rissen* strains. The study included a total of 35 S.1,4,[5],12:i:- and 6 *S. Rissen* isolates. Antibiotic susceptibility testing was performed using a Gram-negative aerobic bacterial susceptibility testing plate from Shanghai Fosun Pharmaceutical Company, evaluating 17 antibiotics:  $\beta$ -lactams [Ampicillin (AMP), Ceftazidime (CAZ), Cefotaxime (CTX), Meropenem (MEM), Ertapenem (ETP)],  $\beta$ -lactamase inhibitors [Ampicillin-Sulbactam (AMS), Ceftazidime/avibactam (CZA)], Tetracyclines [Tetracycline (TET), Tigecycline (TIG)], Polymyxin [Colistin (CT)], Quinolones [Ciprofloxacin (CIP), Nalidixic acid (NAL)], Macrolides [Azithromycin (AZI)], Chloramphenicol [Chloramphenicol (CHL)], Aminoglycosides [Streptomycin (STR), Amikacin (AMK)], and Sulfonamides [trimethoprim/sulfamethoxazole (SXT)].

The micro broth dilution method was used to determine the susceptibility profiles of the isolates, classifying them as sensitive (S), intermediate (I), or resistant (R) in accordance with the standards set by the American Committee for Clinical Laboratory Standardization (CLSI). We defined multi-drug

resistance (MDR) as resistance to at least three different classes of antibiotics (1). The *Salmonella* isolates were submitted to Guangzhou Haotian Biotechnology Co., Ltd in China for WGS utilizing second-generation sequencing methods. Genome assembly of the sequencing data was performed using SPAdes (version 3.13.0; Algorithmic Biology Lab, St. Petersburg, Russia) software, allowing us to acquire the sequences of the *Salmonella* strains in FASTA format. To identify ARGs, we queried the assembled genomes against the CARD antibiotic resistance database (<https://card.mcmaster.ca/>). We constructed a whole-genome single nucleotide polymorphism (SNP) tree from the pan-SNPs generated by kSNP3.0, employing RAxML software with the General Time Reversible gamma substitution model and 1,000 bootstrap replicates for statistical support. This phylogenetic tree, annotated with antibiotic resistance genes, was visualized using the Interactive Tree of Life version 6 (iTOLv6; <http://itol.embl.de/>), including 12 reference *Salmonella* strains for comparison. The basic details of these reference strains are provided in Table 1.

For *S.1,4,[5],12:i:-* isolates, high rates of antimicrobial resistance were detected. Resistance was notably high against AMP (88.57%, 31/35), STR (88.57%, 31/35), TET (85.71%, 30/35), CHL (74.29%), SXT (71.43%, 26/35), and AMS (57.14%, 20/35). However, TIG (100%) and AMK (100%) showed complete sensitivity. MEM, ETP, CZA, CTX, CAZ, and AZI exhibited sensitivity rates exceeding 80%. All *S. Rissen* isolates displayed resistance to AMP (100%), TET (100%), CHL (100%), and SXT (100%), while being sensitive to MEM (100%), ETP

(100%), CZA (100%), TIG (100%), AZI (100%), and AMK (100%). Among the 35 *S.1,4,[5],12:i:-* isolates, 32 were MDR, resulting in an MDR rate of 91.43%. Interestingly, 6 *S.1,4,[5],12:i:-* isolates exhibited resistance to five antimicrobials and had an MDR pattern of AMP-TET-CHL-STR-SXT. The MDR rate for *S. Rissen* was 100%. Complete details of the antimicrobial resistance profiles of *Salmonella* isolates are presented in Figure 1.

A total of 183 ARGs were identified in the genomes of *Salmonella* isolates (Table 2), encompassing various gene families such as resistance-nodulation-cell division (RND) antibiotic efflux pump, major facilitator superfamily (MFS) antibiotic efflux pump, and ATP-binding cassette (ABC) antibiotic efflux pump. These genes provide resistance to fluoroquinolones, cephalosporins, tetracyclines, and other antibiotics through mechanisms like efflux, target protection, and target alteration. The presence of known ARGs showed differing correlations with phenotypic resistance, with rates of 95.24%, 92.86%, and 83.33% for polymyxins, macrolides, and aminoglycosides, respectively. The correlation rates were lower for chloramphenicol antibiotics at 47.62%. The rates for  $\beta$ -lactams, tetracyclines, sulfonamides,  $\beta$ -lactam inhibitors, and quinolones were 76.19%, 66.67%, 64.29%, 59.52%, and 50.00%, respectively.

The phylogenetic tree of SNP analysis presented in Figure 2 displayed a clustering pattern where a local strain of *S.1,4,[5],12:i:-* and a strain of *S. Muenster* from U.S. cows grouped together, as did a local strain of *S.1,4,[5],12:i:-* and a strain of *S. Enteritidis* from U.S. chicken meat, indicating significant genetic

TABLE 1. Basic information on the 12 reference strains included in the phylogeny from Environmental Sewage — Guangzhou City, Guangdong Province, China, 2022–2023.

Number	Area	Time	Serotype	Source	NCBI number
Se40	Nanjing	2018	<i>S. Enteritidis</i>	Bird droppings	CP067369.1
ASM842900v2	America	2016	<i>S. Muenster</i>	Cow	CP082453.1
ASM1148075v2	America	2019	<i>S. Typhimurium</i>	Chicken breast	CP082526.1
ASM786162v2	America	2018	<i>S. Enteritidis</i>	Chicken breast	CP082565.1
C629	Qingdao	2014	<i>S. Enteritidis</i>	Chicken	CP015724.1
ATCC14028	Qingdao	2022	<i>S. Typhimurium</i>	Chicken	CP102669.1
WW012	Beijing	2016	<i>S. Typhimurium</i>	Pork	CP022168.1
SH160	Shanghai	2016	<i>S. Typhimurium</i>	Pork	CP053294.1
S29	Guangzhou	2014	<i>S. Typhimurium</i>	Hospital patient stool	CP085699.1
S34	Guangzhou	2014	<i>S. Typhimurium</i>	Hospital patient stool	CP086118.1
81741	Guangzhou	2015	<i>S. Typhimurium</i>	Hospital patient stool	CP019442.1
KNP01	Guangzhou	2000	<i>S. Enteritidis</i>	Hospital patient stool	CP113364.1



FIGURE 1. Resistance of *Salmonella* to 17 antibiotics ( $n=41$ ) from Environmental Sewage—Guangzhou City, Guangdong Province, China, 2022–2023.

Abbreviation: CHL=chloramphenicol; SXT=trimethoprim/sulfamethoxazole; AMS=ampicillin-sulbactam; TET=tetracycline; AMP=ampicillin; STR=streptomycin; NAL=nalidixic acid; CT=colistin; CIP=ciprofloxacin; CTX=cefotaxime; CZA=ceftazidime; ETP=ertapenem; MEM=meropenem; TIG=tigecycline; AMK=amikacin; CAZ=ceftazidime; AZI=azithromycin.

similarity. Moreover, four S.1,4,[5],12:i:- isolates from Guangzhou wastewater were closely genetically linked to three *Salmonella* Typhimurium isolates from patient feces in Guangzhou hospitals during 2014 and 2015. Additionally, three strains of S.1,4,[5],12:i:- showed close genetic relationships with *Salmonella* Typhimurium, *Salmonella* Muenster, and *Salmonella* Enteritidis strains from the United States.

## DISCUSSION

*Salmonella* represents a prevalent foodborne pathogen globally. The emergent trend of MDR, exacerbated by the misuse and overuse of antibiotics,

has compromised treatment effectiveness and led to therapeutic failures (2). It is, therefore, vital to examine the resistance patterns and the genetic basis of antimicrobial resistance in *Salmonella*, with a focus on MDR, to better manage and contain infections. Notably, the serovars S.1,4,[5],12:i:- and *S. Rissen* have gained recognition as emerging threats to human health in various countries (3–4). There remains, however, a substantial gap in the understanding of these serovars' resistance profiles in China, particularly in Guangzhou. Our study aims to fill this crucial knowledge void. We conducted our research on *Salmonella* isolates obtained from environmental sewage, which offers distinctive insights. Conventional

TABLE 2. Predicted ARGs and resistance mechanisms in the genomes of *Salmonella* isolated (n=41) from Environmental Sewage — Guangzhou City, Guangdong Province, China, 2022–2023.

Resistance mechanism	ARG family	ARG
Antibiotic efflux	RND antibiotic efflux pump	<i>golS, mdsA, mdsB, YajC, sdiA, acrB Escherichia coli acrA, Shigella flexneri acrA; acrD, mdtA, mdtC, mdtB, CRP, mdtE, mdtF, gadX, rsmA, adeF, rsmA, OprN, OprJ, rsmA, OpmH, TriB, TriC, TriA, OpmD, OpmB, mdtB, cpxA, mdtM, baeR, baeS, OprM, Pseudomonas aeruginosa CpxR; MuxC, MuxB, MuxA, opmE; AcrF, AcrE, AcrS</i>
	MFS antibiotic efflux pump, RND antibiotic efflux pump	<i>H-NS, evgS</i>
	MATE transporter	<i>MdtK, PmpM</i>
	MFS antibiotic efflux pump	<i>mdtG, leuO, MexB, mdtN, mdtO, mdtP, Escherichia coli mdfA, emrY, mdtH, emrB, emrR, emrA, emrK, Escherichia coli mdfA, floR, cmlA1, cmlA5, cmlA6, tetR, tet (A), tet (B), tet (M), bcr-1, qacEdelta1</i>
	ABC antibiotic efflux pump	<i>msbA; YojI</i>
	SMR antibiotic efflux pump	<i>Klebsiella pneumoniae KpnF, Klebsiella pneumoniae KpnE, Klebsiella pneumoniae Kpn, Klebsiella pneumoniae KpnH; qacL</i>
	kdpDE	<i>kdpE, Type A NfxB</i>
Antibiotic target replacement and antibiotic target protection	Sulfonamide resistant sul; trimethoprim resistant dihydrofolate reductase dfr qnr; msr-type ABC-F protein	<i>sul1, sul2, sul3; dfrA1, dfrA12, dfrA14, dfrA27, QnrB6, QnrD1, QnrS1; msrE</i>
Antibiotic inactivation	ANT (3"); AAC (3); TEM beta-lactamase; AAC (6'); PDC beta-lactamase; fosfomycin thiol transferase; OXA beta-lactamase; CTX-M beta-lactamase; APH (6); APH (4); APH (3'); APH (3"); CAT; EC beta-lactamase; CARB beta-lactamase; CMH beta-lactamase; MPH; LNU; rifampin ADP-ribosyltransferase (Arr); DHA beta-lactamase;	<i>aadA2, aadA, aadA22, aadA16, aadA3, ANT (3")-IIa; AAC (3)-IId, AAC (3)-IVa; TEM-1, TEM-169; AAC (6')-Iy, AAC(6')-Iaa, AAC (6')-Ib-cr6; PDC-11, PDC-3; FosA, FosA8, FosA2, FosA7; OXA-846, OXA-904, OXA-1, OXA-10; CTX-M-55, CTX-M-65; CTX-M-3; APH (6)-Id; APH (4)-Ia; APH (3')-IIb, APH (3')-Ia; APH (3")-Ib; Pseudomonas aeruginosa catB7catB3; EC-13; Escherichia coli ampC beta-lactamase, CARB-3; catA4; CMH-3; mphA, Mrx; linG, lnuF; arr-2, arr-3; DHA-1</i>
Antibiotic target alteration	Undecaprenyl pyrophosphate related proteins; glycopeptide resistance gene cluster, Van ligase; pmr phosphoethanolamine transferase; antibiotic-resistant UhpT; Penicillin-binding protein mutations conferring resistance to beta-lactam antibiotics; antibiotic-resistant GlpT; elfamycin resistant EF-Tu; vanW, glycopeptide resistance gene cluster; pmr phosphoethanolamine transferase; pmr phosphoethanolamine transferase; MCR phosphoethanolamine transferase	<i>bacA; vanG; PmrF, ArnT, arnA, cprR, cprS, basR; Escherichia coli UhpT with mutation conferring resistance to fosfomycin; Haemophilus influenzae PBP3 conferring resistance to beta-lactam antibiotics; Escherichia coli GlpT with mutation conferring resistance to fosfomycin; Escherichia coli EF-Tu mutants conferring resistance to Pulvomycin; vanW gene in vanG cluster; eptA; ugd; MCR-3.1</i>
Antibiotic efflux, reduced permeability to antibiotic	RND antibiotic efflux pump, General Bacterial Porin with reduced permeability to beta-lactams; RND antibiotic efflux pump, Opr	<i>marA, ramA; ParS, ParR</i>
Antibiotic target alteration, antibiotic efflux	RND antibiotic efflux pump; pmr phosphoethanolamine transferase	<i>Escherichia coli AcrAB-TolC with MarR mutations conferring resistance to ciprofloxacin and tetracycline; cprS, basS</i>

Abbreviation: ARG=antimicrobial resistance gene; RND=resistance-nodulation-cell division; MFS=major facilitator superfamily; MATE=multidrug and toxic compound extrusion; ABC=ATP-binding cassette; SMR=small multidrug resistance; qnr=quinolone resistance protein; CAT=chloramphenicol acetyltransferase; CARB beta-lactamase=ampC-type beta-lactamase; MPH=macrolide phosphotransferase; LNU=lincosamide nucleotidyltransferase; Arr=rifampin ADP-ribosyltransferase; CMH=neutral glycosphingolipids; OXA=oxidase assembly; ADP=adenosine diphosphate; DHA=dhahran; MCR=mobile colistin resistance; Opr=outer membrane porin; PDC=pseudomonas-derived cephalosporinase; EF-Tu=elongation factor thermo-unstable; msr=methionine sulfoxide reductase; ANT=aminoglycoside nucleotidyl transferase; qnr=quinolone resistance; MCR=mobile colistin resistance.

antimicrobial resistance surveillance primarily targets symptomatic clinical cases, thereby overlooking asymptomatic carriers and key environmental reservoirs including livestock, vegetables, and water bodies. Conversely, environmental sewage likely harbors *Salmonella* strains shed from multiple sources, offering

a more comprehensive overview of the strains present. Additionally, we utilized SNP analysis to elucidate the genetic relationships among isolates, providing valuable data for tracing the origins of potential *Salmonella* outbreaks in the future.

In this study, we found that both S.1,4,[5],12:i:- and

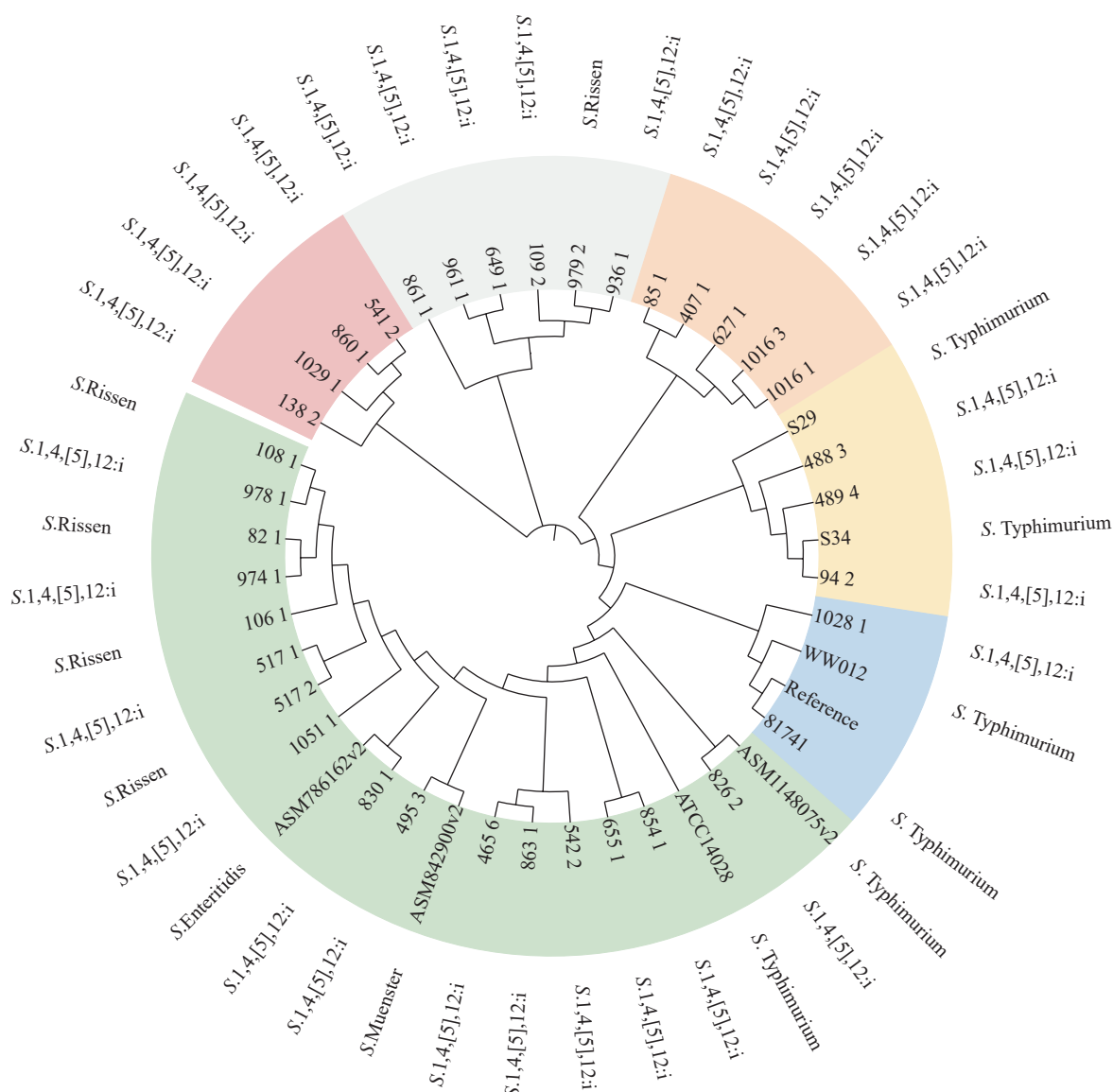


FIGURE 2. A phylogenetic tree illustrating the evolutionary relationship of *Salmonella* strains isolated from Guangzhou's environmental wastewater using whole-genome SNPs. Abbreviation: SNP=single nucleotide polymorphism.

*S. Rissen* isolates exhibited high resistance levels to commonly prescribed clinical antibiotics, such as AMP, STR, CHL, SXT, AMS, as well as to TET, an antibiotic critically important in veterinary medicine. These findings underscore the need for judicious use of these antibiotics in both human medicine and animal farming to prevent treatment failures. However, antibiotics such as TIG, AMK and CZA have demonstrated high antimicrobial sensitivity and thus may offer effective treatment options. The extremely high prevalence of multidrug resistance observed in these isolates is alarming, with potential severe implications for both human health and life. A comprehensive strategy that integrates bacterial and

fungal resistance surveillance, clinical prescription monitoring, and hospital infection control is essential to combat the spread of drug-resistant pathogens (5).

Drug efflux pumps are integral membrane proteins that actively expel antibiotics from the cell, representing a significant mechanism contributing to the MDR observed in Gram-negative bacteria (1). Our investigation identified six types of efflux pumps in both *S.1,4,[5],12:i:-* and *S. Rissen* isolates: the RND family, ABC superfamily, MFS, SMR family, MATE, and kdpDE. These systems are likely involved in the extensive antibiotic resistance demonstrated by *S.1,4,[5],12:i:-* and *S. Rissen* isolates. In addition, we discovered the *mcr-3.1* subtype of the plasmid-



mediated colistin resistance gene *mcr-3*, which has been infrequently reported in China (6). Literature suggests that *mcr-3.1* is instrumental in propagating drug resistance via both plasmid transfer, or horizontal transmission, and chromosomal insertion, or vertical transmission (7). Ongoing surveillance of *mcr-3.1* is vital to controlling its dissemination within China. Furthermore, our WGS analysis predicted genes responsible for resistance to fluoroquinolones, aminoglycosides, and tetracyclines, among others. Notably, there was a discernible correlation between the presence of these ARGs and the corresponding resistance phenotypes, underscoring the importance of ARGs in *Salmonella* resistance and the reduction in antibiotic efficacy.

Antibiotic-resistant bacteria (ARB), ARGs, and mobile genetic elements (MGEs), such as plasmids, are present in sewage and promote the horizontal transfer of ARGs among various microorganisms, leading to increased bacterial resistance (8). This study has identified numerous ARGs; consequently, their eradication from sewage is of paramount importance. Traditional disinfection methods, including chlorine, ozone, and ultraviolet light, exhibit minimal effectiveness in eliminating ARGs. While the combined photocatalytic oxidation-membrane bioreactor (MBR) process has proven effective at removing ARGs in laboratory studies, its application in real-world settings remains impractical (9). Therefore, there is an urgent need to develop innovative disinfection technologies suited for the efficient removal of ARGs from sewage.

The phylogenetic analysis indicates that *Salmonella* possesses the capability to disseminate various sources and geographical areas, including across international borders. The primary transmission vectors for *Salmonella* include contaminated food, water, and the international trade of animal feed (10). To mitigate the dissemination of *Salmonella*, it is imperative to enforce stringent food safety inspection protocols that encompass both food products and animal feed. Concurrently, the disinfection of environmental wastewater is of paramount importance.

This study is subject to some limitations due to the small sample size, which comprised only 35 *S.* 1,4,[5],12:i:- isolates and 6 *S.* Rissen isolates. Consequently, these numbers may not sufficiently reflect the overall resistance features of these two *Salmonella* serotypes. In addition, owing to the limitations of second-generation genome sequencing methods for WGS, it's impossible to obtain the

location of resistance genes, such as whether it's on the chromosomes or plasmids, which will limit further study on the resistance mechanism of *Salmonella*.

Nonetheless, the breadth of the sewage sample sources — from seven locations across eleven districts in Guangzhou city — lends some degree of representativeness to the findings. Additionally, the collection of sewage samples was carried out by trained professionals and the samples were transported to the laboratory for analysis within 48 hours, stored at 4 °C. The methodological procedures, including selective enrichment, isolation, morphological examination, biochemical testing, and serological typing, were methodically performed to ensure the isolation of *Salmonella* strains. The thoroughness of the experimental protocol supports the accuracy of the results.

**Conflicts of interests:** No conflicts of interest.

**Funding:** Supported by Guangdong Basic and Applied Basic Research Foundation (2021A1515012539), Science and Technology Program of Guangzhou, China (202102080295), Guangzhou Key Medical Discipline (2021-2023-11), 2023 Huadu District Medical and Health General Scientific Research Special Project of Guangzhou Huadu District Bureau of Science, Technology, Industry, Commerce, and Information Technology (23-HDWS-079), Key R&D Plan of Guangzhou Science and Technology Project (202206080003), and Guangzhou Science and Technology Plan Project (2023A03J0938).

doi: 10.46234/ccdcw2024.050

# Corresponding authors: Jun Yuan, [yuanjuncom@163.com](mailto:yuanjuncom@163.com); Chaojun Xie, [35451900@qq.com](mailto:35451900@qq.com).

<sup>1</sup> Tuberculosis Management and Treatment Department, Guangzhou Chest Hospital, Guangzhou City, Guangdong Province, China;

<sup>2</sup> School of Public Health, Guangzhou Medical University, Guangzhou City, Guangdong Province, China; <sup>3</sup> Guangzhou Center for Disease Control and Prevention, Guangzhou City, Guangdong Province, China; <sup>4</sup> Office of the Director, Huadu District Center for Disease Control and Prevention, Guangzhou City, Guangdong Province, China.

Submitted: December 28, 2023; Accepted: February 29, 2024

## REFERENCES

1. Nang SC, Li J, Velkov T. The rise and spread of *mcr* plasmid-mediated polymyxin resistance. *Crit Rev Microbiol* 2019;45(2):131 – 61. <https://doi.org/10.1080/1040841X.2018.1492902>.
2. Borah P, Dutta R, Das L, Hazarika G, Choudhury M, Deka NK, et al. Prevalence, antimicrobial resistance and virulence genes of *Salmonella* serovars isolated from humans and animals. *Vet Res Commun* 2022;46 (3):799 – 810. <https://doi.org/10.1007/s11259-022-09900-z>.

3. Qin XJ, Yang MZ, Cai H, Liu YT, Gorris L, Aslam MZ, et al. Antibiotic resistance of *Salmonella* typhimurium monophasic variant 1, 4,[5],12:i:-in China: a systematic review and meta-analysis. *Antibiotics (Basel)* 2022;11(4):532. <https://doi.org/10.3390/antibiotics11040532>.
4. Elbediwi M, Shi DW, Biswas S, Xu XB, Yue M. Changing patterns of *Salmonella* enterica serovar rissen from humans, food animals, and animal-derived foods in China, 1995-2019. *Front Microbiol* 2021;12:702909. <https://doi.org/10.3389/fmicb.2021.702909>.
5. Guo Y, Ding L, Yang Y, Han RR, Yin DD, Wu S, et al. Multicenter antimicrobial resistance surveillance of clinical isolates from major hospitals - China, 2022. *China CDC Wkly* 2023;5(52):1155 – 60. <https://doi.org/10.46234/ccdcw2023.217>.
6. Yu K, Wang HY, Cao ZZ, Gai YD, Liu M, Li GQ, et al. Antimicrobial resistance analysis and whole-genome sequencing of *Salmonella* enterica serovar Indiana isolate from ducks. *J Glob Antimicrob Resist* 2022;28:78 – 83. <https://doi.org/10.1016/j.jgar.2021.12.013>.
7. Lee S, An JU, Kim WH, Yi S, Lee J, Cho S. Different threats posed by two major mobilized colistin resistance genes— *mcr-1.1* and *mcr-3.1* — revealed through comparative genomic analysis. *J Glob Antimicrob Resist* 2023;32:50 – 7. <https://doi.org/10.1016/j.jgar.2022.12.007>.
8. Wang JQ, Xu SQ, Zhao K, Song G, Zhao SN, Liu RP. Risk control of antibiotics, antibiotic resistance genes (ARGs) and antibiotic resistant bacteria (ARB) during sewage sludge treatment and disposal: a review. *Sci Total Environ* 2023;877:162772. <https://doi.org/10.1016/j.scitotenv.2023.162772>.
9. Pazda M, Kumirska J, Stepnowski P, Mulikiewicz E. Antibiotic resistance genes identified in wastewater treatment plant systems - a review. *Sci Total Environ* 2019;697:134023. <https://doi.org/10.1016/j.scitotenv.2019.134023>.
10. Li Y, Teng L, Xu XB, Li XM, Peng XQ, Zhou X, et al. A nontyphoidal *Salmonella* serovar domestication accompanying enhanced niche adaptation. *EMBO Mol Med* 2022;14(11):e16366. <https://doi.org/10.15252/emmm.202216366>.