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Antimicrobial resistance and molecular typing of *Salmonella* in the chicken production chain in Hubei Province, China

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Abstract

Salmonella is a significant foodborne zoonotic pathogen that endangers both human and animal health. The goal of this research is to gain a preliminary understanding of *Salmonella* contamination and antimicrobial resistance in the chicken production chain in Hubei Province, China. 1149 animal and environmental samples were collected from chicken farms, slaughterhouses, and retail markets in six cities across Hubei Province in China from 2019 to 2020, yielding *Salmonella* isolation rates of 4.68% (28/598), 12.21% (47/385), and 9.64% (16/166), respectively. Seventeen distinct serotypes were detected among 53 non-clonal *Salmonella* strains, of which Meleagridis (26.42%, 14/53) was the dominant serotype. Almost half of the strains (49.06%, 26/53) were multi-drug resistant (MDR). Whole-genome sequencing (WGS) showed that 10 resistance genes (*tetA*, bla_{TEM}, *parC*, *qnrS1*, *floR*, *aac(6')-Iy*, *aph(6)-Id*, *aph(3")-Ib*, *aac(6')-Iaa* and *sul2*) and 7 categories of virulence genes were present in all three links in 22 non-clonal dominant serotype strains. It was shown that *Salmonella* in the chicken production chain in Hubei Province had a high resistance rate to Tetracycline (TET, 73.58%), Ofloxacin (OFL, 69.81%), Florfenicol (FFC, 60.38%) and Ampicillin (AMP, 39.62%) which was consistent with the widespread use of these drugs in the husbandry industry in China. *Salmonella* ST types determined by MLST and serotypes determined by WGS had a one-to-one correlation. Minimum spanning tree analysis revealed that there was cross contamination of *Salmonella* in farms and slaughterhouses, slaughterhouses and markets, animal samples and environmental samples. This work provides useful information for the prevention and control of contamination and antimicrobial resistance of *Salmonella* in the chicken production chain, as well as demonstrating the dependable role of WGS in *Salmonella* molecular typing.

Keywords *Salmonella*, Serotype, Antimicrobial resistance, Multi-locus sequence typing, Whole genome sequencing

Introduction

Salmonella, a foodborne zoonotic pathogen, causes diarrhea, fever, stomach pains, and death in severe cases. Genus *Salmonella* include two species, *Bongori* and *Enterica*, and the latter includes six subspecies (*enterica*, *salamae*, *arizonae*, *diarizonae*, *houtenae* and *indica*). Subspecies *enterica*, which included more than 1500 serotypes, is often isolated from warm-blooded animals, while other five subspecies are generally isolated from cold-blooded animals and environments (Rincón-Gamboa et al. 2021b). *Salmonella* is the most common

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pathogen closely related to foodborne diseases in chickens. People can be infected with these pathogens through consumption of contaminated food or contact with animals and their environment. As a result, *Salmonella* contamination in the chicken production chain may pose a great risk to public health.

About 162,000 tons of antimicrobials are consumed annually in China, with animal husbandry accounting for 52.00% of the total usage and chicken production accounting for 19.60% (Chen et al. 2019). In 2020, 28.69 and 71.31% of the veterinary antimicrobials in China were used for growth promotion and therapeutic, respectively. Due to the widespread use and abuse of antimicrobials, the rapid spread of resistant *Salmonella*, especially multidrug-resistant (MDR) bacteria, seriously impairs the effectiveness of clinical antimicrobial therapy and poses a greater public health problem (Rincón-Gamboa et al. 2021b). *Salmonella* resistance can be transmitted from animals to humans *via* food production chain and related environment. Therefore, it is vital to study bacterial resistance throughout the food chain and the associated environment.

In recent years, chicken slaughterhouses are mostly used in some foreign countries for epidemic investigation and antimicrobial resistance monitoring of *Salmonella* (Procura et al. 2019; Asghar et al. 2018; Chuah et al. 2018). In China, the majority of current research focuses on one or two links (farm or slaughterhouse or market or slaughterhouse-market) in the chicken production chain (Zhou et al. 2019; Wang et al. 2021; Hu et al. 2018). Only a few studies evaluated *Salmonella* contamination in animal samples across the entire chicken production chain (Ma et al. 2017; Zhang et al. 2014), but without taking into account environmental samples. In addition to sampling links and sample sources, there are widespread shortcomings in sample collection methods at home and abroad,which may lead to many same sources in the same field and affect the accuracy of subsequent data.

Proper typing technologies for reliably tracing the source of contamination are critical for the control of *Salmonella*. Traditional *Salmonella* serotyping based on White-Kauffmann-Le Minor scheme has been used for more than 80 years, but it relies on the subjective judgment of the experimenter and may produce false-positive reactions due to weak agglutination or non-specific agglutination (Diep et al. 2019). Multi-locus sequence typing (MLST), which is based on sequence changes in numerous *Salmonella* housekeeping genes, is well-established and widely used as a replacement for traditional serotyping (Park et al. 2021). Whole-genome sequencing (WGS) captures DNA sequence changes across the complete genome of single microbial isolates, providing a precise description of the genetic relatedness of isolates. An increasing number of studies showed the usefulness of *Salmonella* subtyping using WGS in outbreak investigations and pathogen source tracing (Hoffmann et al. 2016; Inns et al. 2017).

In this study, animal and environmental samples were collected between July 2019 and September 2020 from chicken farms, slaughterhouses and retail markets in Hubei Province, China, to explore the prevalence and antimicrobial resistance of *Salmonella*. The evolutionary relationship between *Salmonella* strains in different links and sources of the chicken production chain was analyzed by MLST and WGS. This study will provide vital information for the prevention and control of *Salmonella* contamination in the local chicken production chain, as well as the risk assessment of antimicrobial resistance in *Salmonella*.

Results

Isolation and serotyping of *salmonella*

A total of 91 (7.92%, 91/1149) *Salmonella* were isolated from 1149 samples collected from 22 sites in 6 cities including Wuhan, Tianmen, Xiaogan, Jingzhou, Huanggang and Yichang of Hubei Province, China (Table S1). The overall isolation rates were 4.68% (28/598), 12.21% (47/385) and 9.64% (16/166) in chicken farms, slaughterhouses and retail markets, respectively. The isolation rates for animal and environmental samples were 11.25% (46/409) and 6.81% (45/740), respectively. In terms of animal samples, the total isolation rate in slaughterhouses (23.70%, 32/135) was substantially higher than those in farms (3.72%, 7/188) and retail markets (8.14%, 7/86). For environmental samples, the isolation rates of fence in farms, shower water and desk in slaughterhouse, and chopping board and knives in retail market were $\geq$ 10% (Table 1).

Fifty-three non-clonal *Salmonella* strains were serotyped into 17 serotypes by the slide agglutination method (Fig.1A). We believe that *Salmonella* from the same field, belonging to the same serotype, and the MIC difference is within two times have cloning relationship. Meleagridis (26.42%, 14/53) was the dominant serotype, followed by Agona (11.32%, 6/53), Mbandaka (11.32%, 6/53) and Corvallis (9.43%, 5/53). *Salmonella* isolated from the farm, slaughterhouse and market included 6, 12 and 6 serotypes, with Meleagridis (73.68%, 14/19), Agona (18.52%, 5/27) and Indiana (28.57%, 2/7) being the most prevalent, respectively. Thirteen and 12 serotypes were identified from animal-derived ($n=26$) and environment-derived ($n=27$) strains, respectively, with Meleagridis, Agona, Mbandaka, Corvallis, Braenderup, Indiana, Rissen and Schwarzengrund serotypes included in both sample sources (Fig. 1B). In addition, an unclassified serotype

Table 1 Isolation of *Salmonella* from different sample sources in the chicken production chain in Hubei Province, China

Sampling link	Sampling source	Sample No.	Isolate No.	Isolation rate
Farm	**Animal samples**	188	7	**3.72%**
	Chicken cloaca swab	188	7	3.72%
	Environmental samples	410	21	**5.12%**
	Sewage	85	6	7.06%
	Feed	85	2	2.35%
	Chicken manure	85	1	1.18%
	Fence	70	9	12.86%
	Soil	85	3	3.53%
	Total	**598**	**28**	**4.68%**
Slaughterhouse	**Animal samples**	135	32	**23.70%**
	Chicken cloaca swab	83	23	27.71%
	Caecum sample	52	9	17.31%
	Environmental samples	250	15	**6%**
	Desk	50	5	10%
	Ground	50	3	6%
	Shower water	50	5	10%
	Instrument	50	2	4%
	Knife	50	0	0%
	Total	**385**	**47**	**12.21%**
Market	**Animal samples**	86	7	**8.14%**
	Chicken	86	7	8.14%
	Environmental samples	80	9	**11.25%**
	Egg surface	10	0	0%
	Chopping board	40	5	12.5%
	Knife	30	4	13.33%
	Total	**166**	**16**	**9.64%**
Total		**1149**	**91**	**7.92%**

Marmande were isolated in isolates from farm animal samples by slide agglutination.

WYS slaughterhouse in Wuhan was chosen for particular analysis due to its large sample volume and high isolation rate, which included 8 strains from animal samples ($n=53$) and 7 strains from environmental samples ($n=50$). The 15 *Salmonella* isolates were divided into eight serotypes, with Agona being the most common. Seven environment-derived strains were classified into six serotypes, suggesting that environmental-derived *Salmonella* serotypes are more abundant than those of animal-derived. Agona and Mbandaka serotypes existed in the shower water and animal samples at the same time, suggesting cross-contamination between animal and environmental samples (Fig. 1C).

Antimicrobial susceptibility of *salmonella*

Fifty-three non-clonal Salmonella strains exhibited variable resistance rates to 16 different antimicrobials. The resistance rate of tetracycline (TET) was 73.58%, followed by ofloxacin (OFL, 69.81%), florfenicol (FFC, 60.38%), trimethoprim-sulfamethoxazole (SXT, 41.51%) and ampicillin (AMP, 39.62%). Only 1.89% of isolates were resistance to amikacin (AK), and all 53 *Salmonella* strains were susceptible to meropenem(MEM) and tigecycline (TIG) (Fig. 2A; Table S2).

Antimicrobials resistance of *Salmonella* varied depending on the sources. Resistance rates were significantly higher in slaughterhouses and markets than in farms. Antimicrobial resistances to AMP, Ceftiofur (CEF), Aztreonam (ATM), Gentamicin (GEN), Spectinomycin (SPT), Enrofloxacin (ENR), OFL, FFC, SXT and TET were found in all three links of farms, slaughterhouses and markets (Fig. 2B). In general, isolates from animal samples had greater resistance rates than those of isolates from environmental samples (Fig. 2C). Except OFL, animal-originated isolates from the farm had higher resistance rates to various antimicrobials compared with the environment-originated ones (Fig. 2D). Antimicrobial resistance rates of animal and environmental isolates to AMP, SPT, ENR, OFL, FFC, SXT and TET were close in slaughterhouses (Fig. 2E),

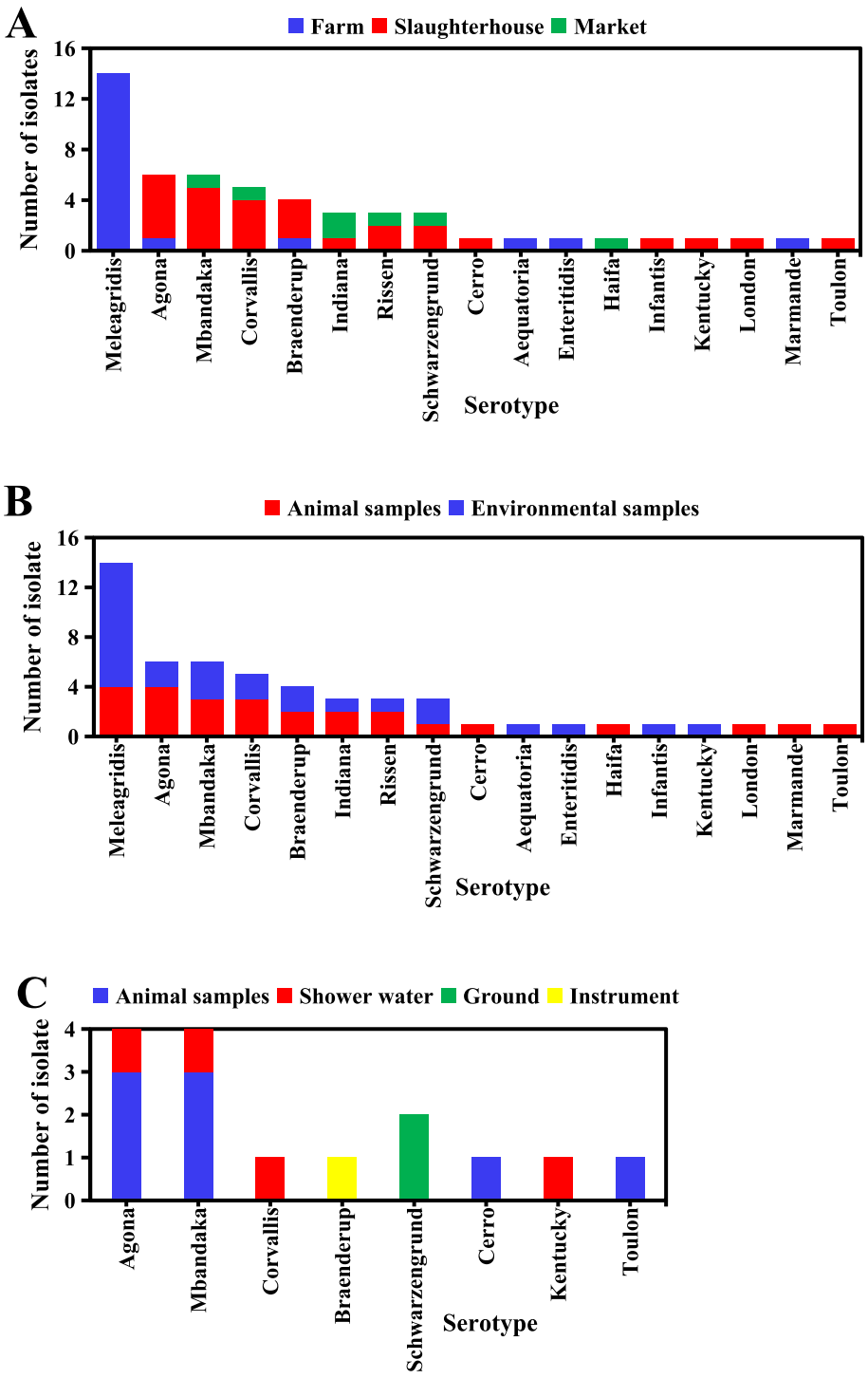

Fig. 1 Serotypes of 53 *Salmonella* isolated in the chicken production chain in Hubei Province, China. **A** Serotype distribution in different links. **B** Serotype distribution according to animal and environmental samples. **C** Serotype distribution in WYS slaughterhouse

while resistance rates for AMP, ENR, OFL, FFC and TET were close in the retail markets (Fig. 2F). The major serotype Meleagridis had diverse resistance patterns to 16 antimicrobials, with the highest rate of OFL resistance (Fig. 2G). In WYS slaughterhouses, animal derived strains and environmentally derived strains had a relatively close resistance rate to AC, SPT and ENR. Only shower water samples were resistant

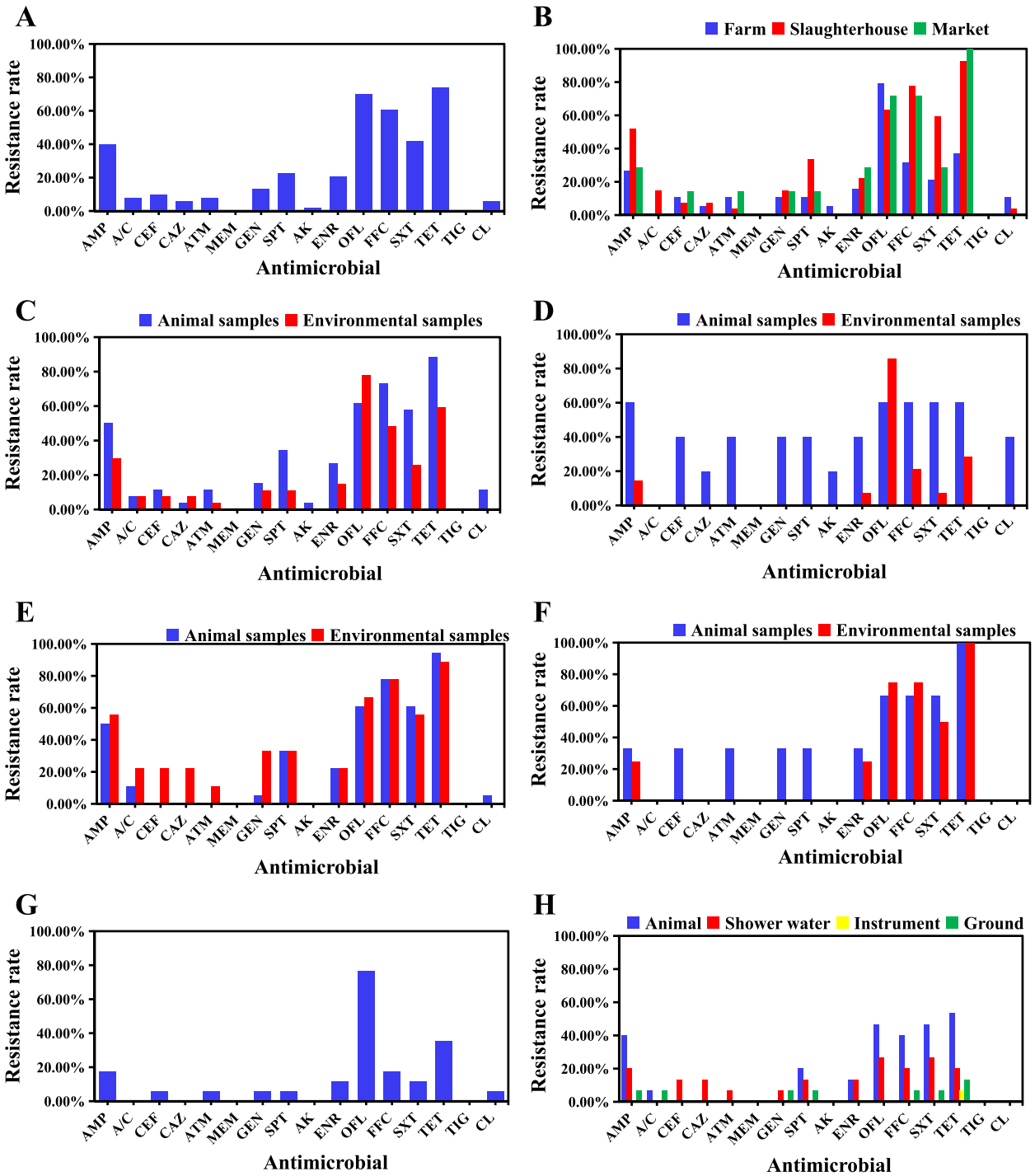

Fig. 2 Antimicrobial resistance rates of *Salmonella* isolates. **A** The overall antimicrobial resistance rates of 53 *Salmonella* strains isolated from the chicken production chain in Hubei Province, China. **B** The antimicrobial resistance rates of *Salmonella* isolated from different links. **C** The overall antimicrobial resistance rates of *Salmonella* isolated in different sample sources. **D, E, F** Antimicrobial resistance rates of *Salmonella* isolated from animal and environmental samples in farms (**D**), slaughterhouses (**E**), and retail markets (**F**). **G** Resistance rates of *Salmonella* Meleagridis. **H** Resistance rates of *Salmonella* isolated in WYS slaughterhouse

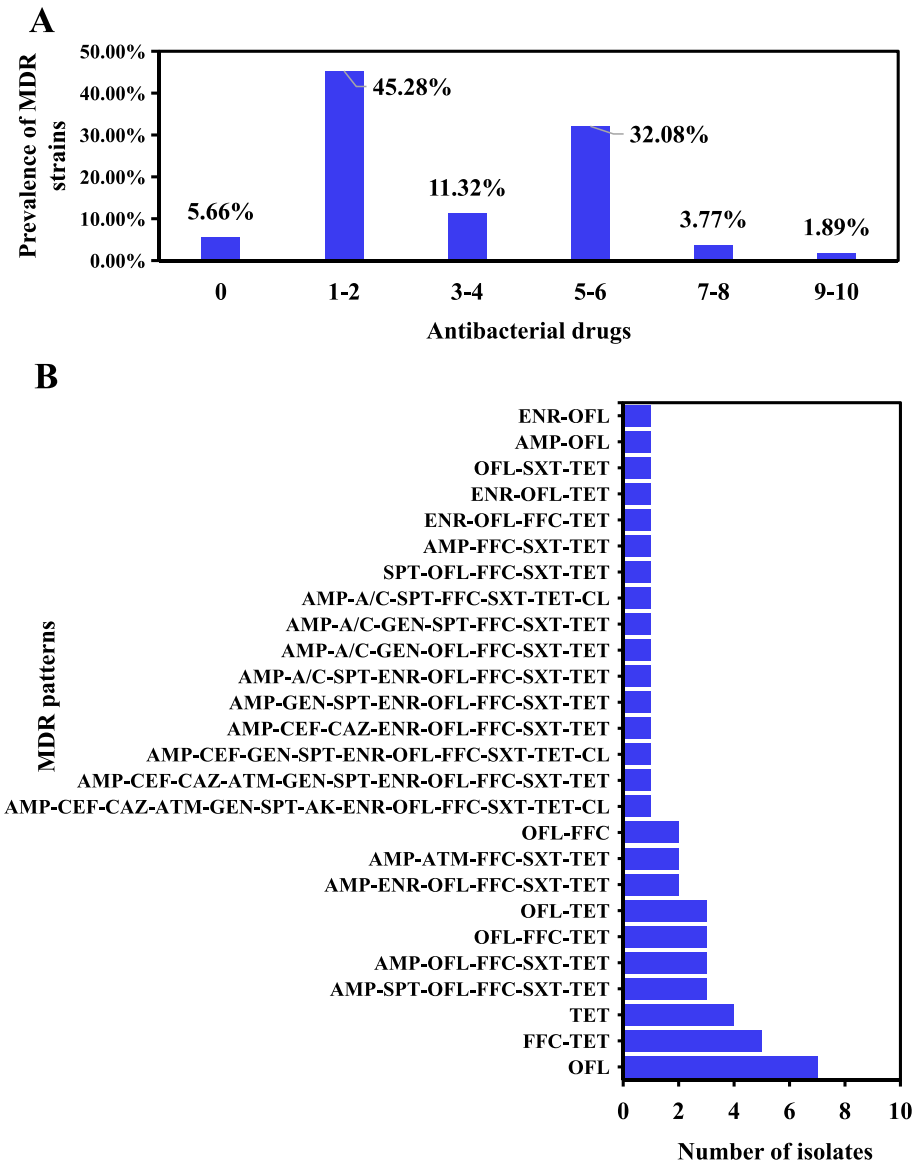

Fig. 3 Prevalence (**A**) and patterns (**B**) of multi-drug resistance of 53 *Salmonella* strains isolated from the chicken production chain in Hubei Province, China

to CEF, ceftazidime (CAZ) and ATM, whereas the isolates of animal, shower water, instruments, and ground samples were resistant to TET (Fig. 2H). The higher number of animal-derived isolates resistant to CEF, ATM and GEN in the market compared to abattoir animal-derived isolates is potentially explained by the fact that chicken meat in the market is flushed through water sources (Fig. 2E, Fig. 2F). Totally 49.06% (26/53) *Salmonella* strains were multi-drug resistant (MDR) strains. Among them, the number of strains resistant to 5–6 drug classes was the highest, accounting for 32.08% (17/53), a fewer were resistant to 9–10 drugs (1.89%, 1/53); only 5.66% (3/53) strains were susceptible or intermediate to all antimicrobials (Fig. 3A). There were 26 drug resistance patterns in 53 *Salmonella* strains, with OFL accounting for the largest proportion (13.21%, 7/53), followed by TET-FFC (9.43%, 5/53) and TET (7.55%, 4/53) (Fig. 3B).

Distribution of resistance genes and virulence genes of *salmonella*

A total of 32 antimicrobial resistance genes were detected in 22 *Salmonella* strains, including four β-lactam resistance genes ($bla_{\text{CTX-M}}$, bla_{TEM}, bla_{CARB} and $bla_{\text{OXA-1}}$),

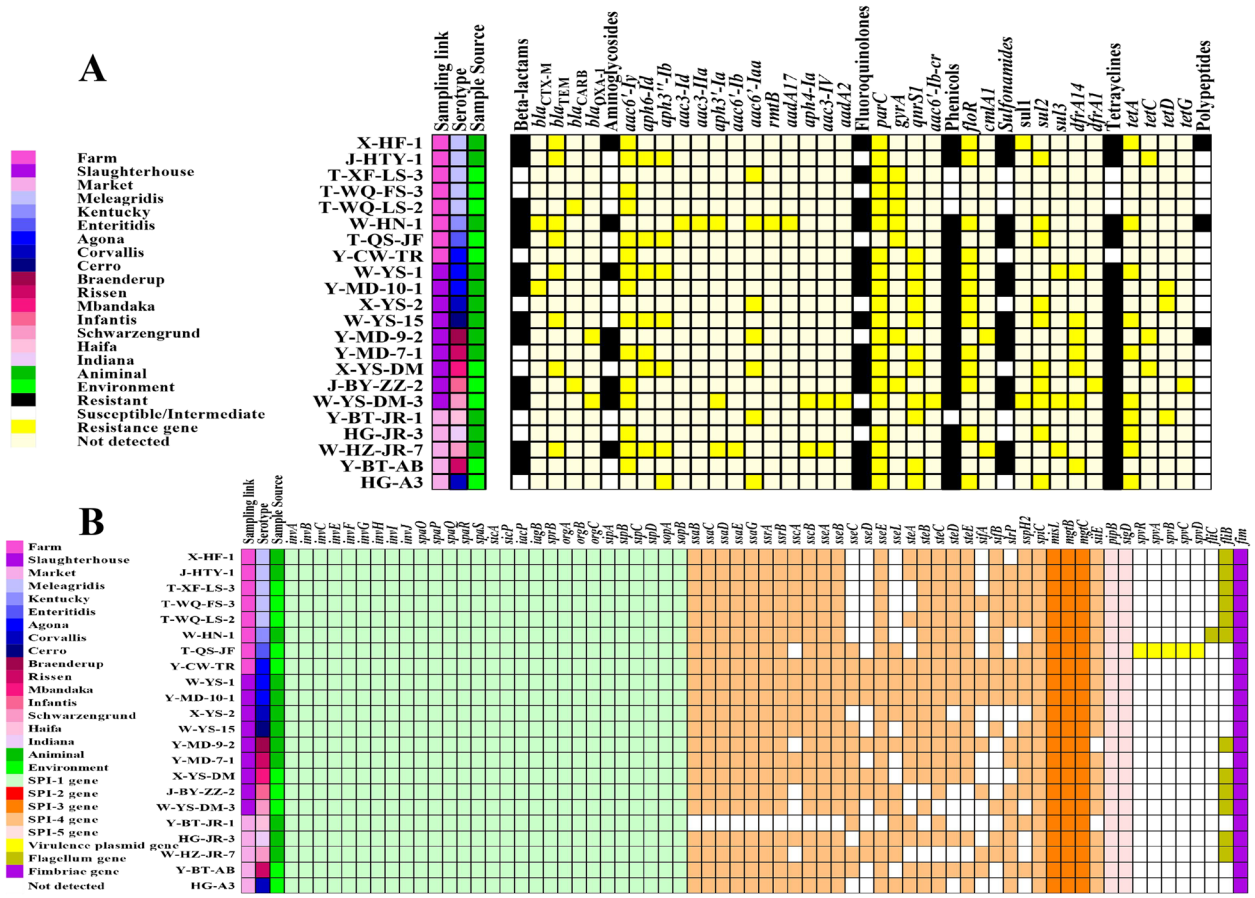

Fig. 4 Distribution of resistance genes (**A**) and virulence genes (**B**) of 22 whole-genome sequenced *Salmonella* strains

13 aminoglycoside resistance genes (*aac6'-Iy*, *aph6-Id*, *aph3"-Ib*, *aac3-Id*, *aac3-IIa*, *aph3'-Ia*, *aac6'-Ib*, *aac6'-Iaa*, *rmtB*, *aadA17*, *aph4-Ia*, *aac3-IV* and *aadA2*), four fluoroquinolones resistance genes (*parC*, *gyrA*, *qnrS1* and *aac6'-Ib-cr*), two chloramphenicol resistance genes (*floR* and *cmlA1*), five sulfonamide resistance genes (*sul1*, *sul2*, *sul3*, *dfrA14* and *dfrA1*) and four tetracycline resistance genes (*tetA*, *tetC*, *tetD* and *tetG*) (Fig. 4A). However, polypeptide resistant genes were not detected. The detection rates of bla_{TEM} (36.36%, 8/22), *aac6'-Iy* (68.18%, 15/22), *parC* (90.91%, 20/22), *floR* (59.09%, 13/22), *sul2* (45.45%, 10/22) and *tetA* (45.45%, 10/22) were significantly greater than those of other resistance genes for the same drug class (Fig. 4A). A total of 73 virulence genes were detected in 22 *Salmonella* strains, including 28 *SPI-1* genes, 28 *SPI-2* genes, three *SPI-3* genes, one *SPI-4* gene, three *SPI-5* genes, two *SPI-6* genes, five virulence plasmid genes, two flagella genes and one fimbriae gene (Fig. 4B). The isolation rates of *SPI-1* gene, *SPI-3* gene, *SPI-5* gene and flagella gene were 100.00%. In the *SPI-2* gene, the isolation rates of *spiC*, *ssaB*, *ssaC*, *ssaD*, *ssaE*, *ssaG*, *ssrA*, *ssrB*, *sscB*, *sseA*, *sseB*, *sseE*, *steC*, *steB* and *steE* were higher than 80.00%; the *sscA*, *steD*, *slrP*, *sspH2*, *steA* and *sifB* were 60–80%, while the *sseC*, *sseL*, *sseD* and *sifA* were lower than 60%. In addition, the isolation of virulence plasmid genes was low (all 4.55%) (Fig. 4B).

Except polypeptide class, the coincidence rate between the resistance phenotype and genotype of other six class of antimicrobials in resistant strains was above 83%, of which the coincidence rate of aminoglycosides and fluoroquinolones was as high as 100%, followed by β-lactams (92.31%), sulfonamides (92.31%), tetracyclines (88.89%) and phenicols (83.33%) (Table 2).

Totally 95.45% ($n=21$) of 22 *Salmonella* strains shared common QRDR mutations in GyrA and/or ParC, but none of the strains had mutations in GyrB and ParE. Additionally we detected the *qnr* gene (*qnrS1*) in a strain that did not detect any QRDR mutation.

There are 66.67% (10/15) of the fluoroquinolone-resistant strains had a single ParC Thr57Ser mutation, with 90.00% (9/10) having low-level antimicrobial resistance (ENR:2 μg/mL, OFL:2–8 μg/mL); 20.00% (3/15) fluoroquinolone-resistant strains had double mutations of GyrA Ser83Phe and ParC Tyr57Ser,

Table 2 The coincidence rates of resistance phenotypes and resistance genes

Antimicrobial class	Susceptible strains			Resistant strains		
	No. of strains	No. of antibiotic resistance gene-free strains	Coincidence rate (%)	No. of strains	No. of antibiotic resistance gene-carrying strains	Coincidence rate (%)
β-lactams	9	9	100.00	13	12	92.31
Aminoglycosides	14	0	0.00	8	8	**100.00**
Fluoroquinolones	7	0	0.00	15	15	**100.00**
Phenicols	4	4	100.00	18	15	83.33
Sulfonamides	9	5	55.56	13	12	92.31
Tetracyclines	4	4	100.00	18	16	88.89
Polypeptides	19	19	100.00	3	0	0.00

with low-level of antimicrobial resistance (ENR:1 μg/mL, OFL:2 μg/mL); 6.67% (1/15) strains had quadruple mutations of GyrA Ser83Phe/Asp87Asn and ParC Thr57Ser/Ser80Ile, with high-level antimicrobial resistance (ENR:32 μg/mL, OFL:64 μg/mL) (Table S3)

Molecular typing of *salmonella*

In 53 non-clonal isolates, 14 distinct sequence types (STs) were discovered, and ST463 was the most dominant type (28.30%, 15/53), followed by ST413 and ST13 (11.32%, 6/53) (Table S4). The 53 *Salmonella* strains were classified into 14 different serotypes, with 100% concordance with ST types (Fig. 5). The minimum spanning tree revealed that there was no significant correlation between *Salmonella* strains isolated from six cities (Fig. 6A). The slaughterhouse had the most ST types among the three links in the chicken production chain, while ST types from the farm were rather simple, with ST463 being the predominant (Fig. 6B). Isolates from farms and slaughterhouses shared the same ST types, as did slaughterhouses and markets, but farms and markets did not have the same ST types. Except desk and chicken manure samples, isolates from all other environment samples shared the same ST types as from animal samples (Fig. 6C).

Discussions

Our investigation revealed that slaughterhouses and markets were important sources of contamination of *Salmonella*, which was consistent with previous findings on chicken production chains in Sichuan Province (Ma et al. 2017). The isolation rate of *Salmonella* of animal origin in farms was much lower than that in slaughterhouses, possible due to the use of purification measures in farms. In this study, Meleagridis serotype dominated in farms, including animal samples and environmental samples. In China, *Meleagridis* had previously been reported as the dominant serotype in cloaca swab samples from chicken farms in Sichuan Province (Ma et al. 2017). *Salmonella* serotypes were abundant in WYS slaughterhouse, and cross-over of *Salmonella* serotypes was only detected in shower water of environmental samples and animal samples, suggesting potential harm of *Salmonella* presence in the water to food safety (Kilonzo-Nthenge et al. 2018). In this study, the coincidence rate of serotyping by WGS and slide agglutination was 86.36% (19/22), which was similar to 84.16% (170/202) reported by Mark Achtman (Moran et al. 2017), suggesting that WGS is accurate and useful for epidemiological tracking.

Official Veterinary Announcement have also shown that tetracycline, β-lactams and phenicol antimicrobials ranked first, second and fourth respectively in the usage amount of veterinary drugs in 2020 in China, respectively (Abate and Assefa 2021). The high resistance rate of strains to OFL suggests that there may be antimicrobial abuse in this area. Fluoroquinolones and third-generation cephalosporins are currently the primary treatment options for invasive salmonellosis (Rincón-Gamboa et al. 2021a). The strains had relatively low resistance rates to CEF (9.43%) and CAZ (5.66%) compared with ENR (20.75%) and OFL (69.81%). Similarly, the resistance pattern containing FFC-OFL-TET accounted for a relatively large proportion in MDR strains. The resistance rate of isolates from animal samples in the farm was significantly higher than that of environmental samples, suggesting that antimicrobials were used in animal breeding process, whereas the resistance rates from animal samples and environmental samples in the slaughterhouse and the market were relatively consistent, suggesting cross contamination of resistant bacteria in these two links. The low resistance rate of CL (5.66%) may be due to the prohibition of CL use as animal growth promoter in China starting in 2016 (Wang et al. 2020). Furthermore, despite the fact that the overall resistance rate of CL was low, the resistance rate of animal-originated isolates in the farm was as high as 40.00% (2/5), which may be caused by irrational use of antibacterial in farms.

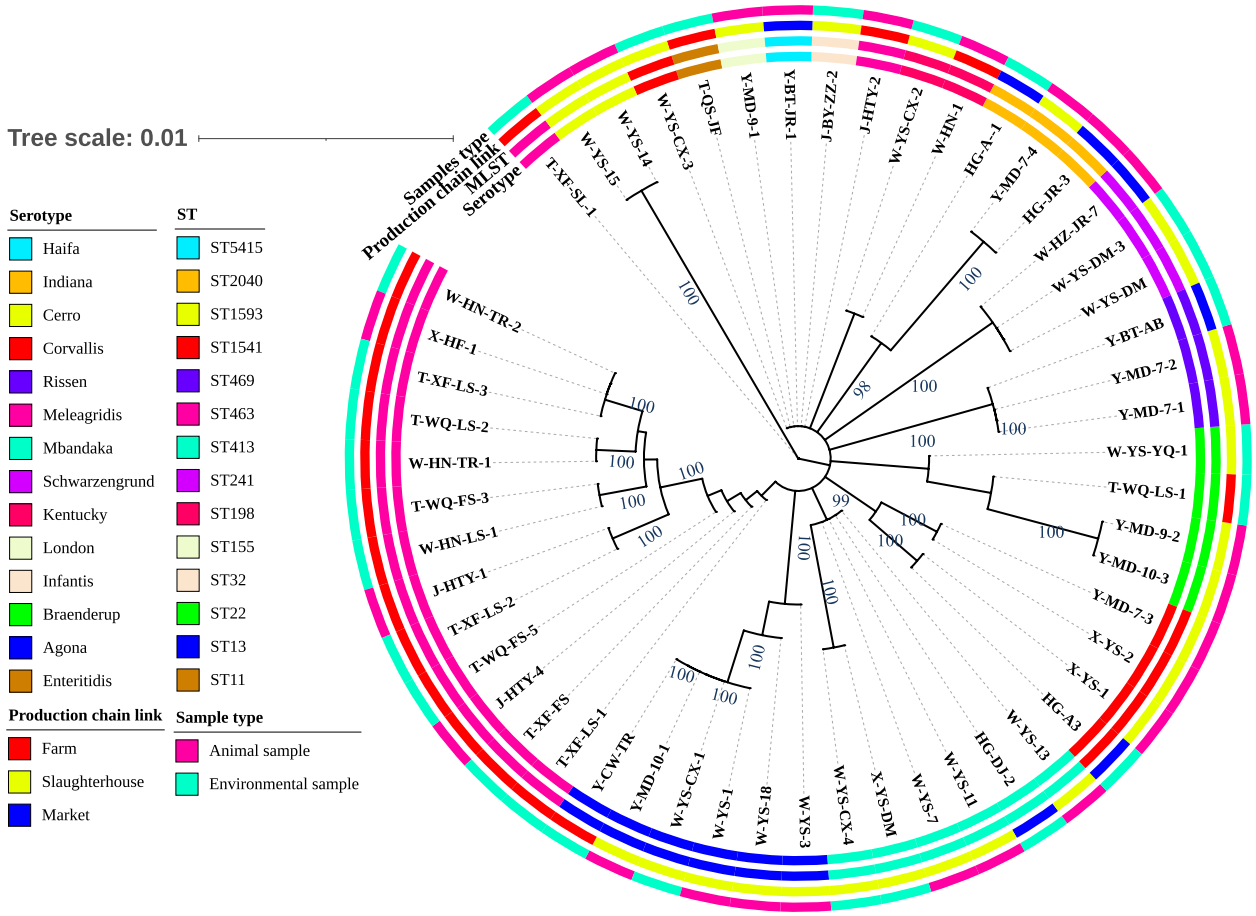

Fig. 5 Cluster of ST types based on MLST analysis and serotypes of 53 *Salmonella* strains

There was a certain correlation between resistance phenotypes and resistance genes of resistant strains in this study (Monistero et al. 2020). The case that resistance was observed but no related resistance genes were found may be due to some unknown resistance genes or gaps in draft genome sequence (Walker et al. 2015). Another case that resistance genes were detected but it was not resistant may be related to the resistance determination criteria such as the alternative use of breakpoints (Abate and Assefa 2021). Additionally, our study showed that 59.10% (13/22) strains carried different types of ESBL genes, e.g., $bla_{\text{CTX-M}}$, bla_{TEM}, bla_{CARB} and $bla_{\text{OXA-1}}$, which suggests a potential threat of ESBL-producing *Salmonella* in the chicken production chain .

QRDR mutations have been observed more frequently in GyrA and ParC than in GyrB and ParE in this study. Previous study also showed that only GyrA and ParC mutations occurred in the ciprofloxacin resistant strains from chickens, with ParC Thr57Ser mutation being the most common (71.6%, 53/74) (Chen et al. 2021). Furthermore, MIC values for ENR and OFL of strains with GyrA and ParC quadruple mutations in this study was much higher than that of single mutation or double mutation strains, due to that the mutation of both type II enzymes can result in medium and high-level resistance. It is important to note that strains with double mutations in QRDR but without *qnr* gene were found to be more susceptible than strains with single mutations and *qnr* gene, which can be explained by the synergistic effect of *qnr* gene with QRDR mutations (Van Doren et al. 2013). For fluoroquinolone-resistant strains in which no QRDR mutation but the *qnr* gene was detected, it suggests that *qnr* gene alone may also mediate the development of fluoroquinolone resistance.

Non-typhoidal Salmonella (NTS) virulence factors include flagella, fimbriae, toxins, SPIs and virulence plasmids, *etc.* which do not occur in all NTS serotypes, so their presence or absence affects the virulence of a particular isolate or serotype. This study showed that all 22 strains carried different types of virulence genes, of which *SPI-1*, *SPI-3*, *SPI-5* and fimbriae genes had high consistency, while *SPI-2*, *SPI-4*, virulence plasmid, flagellum

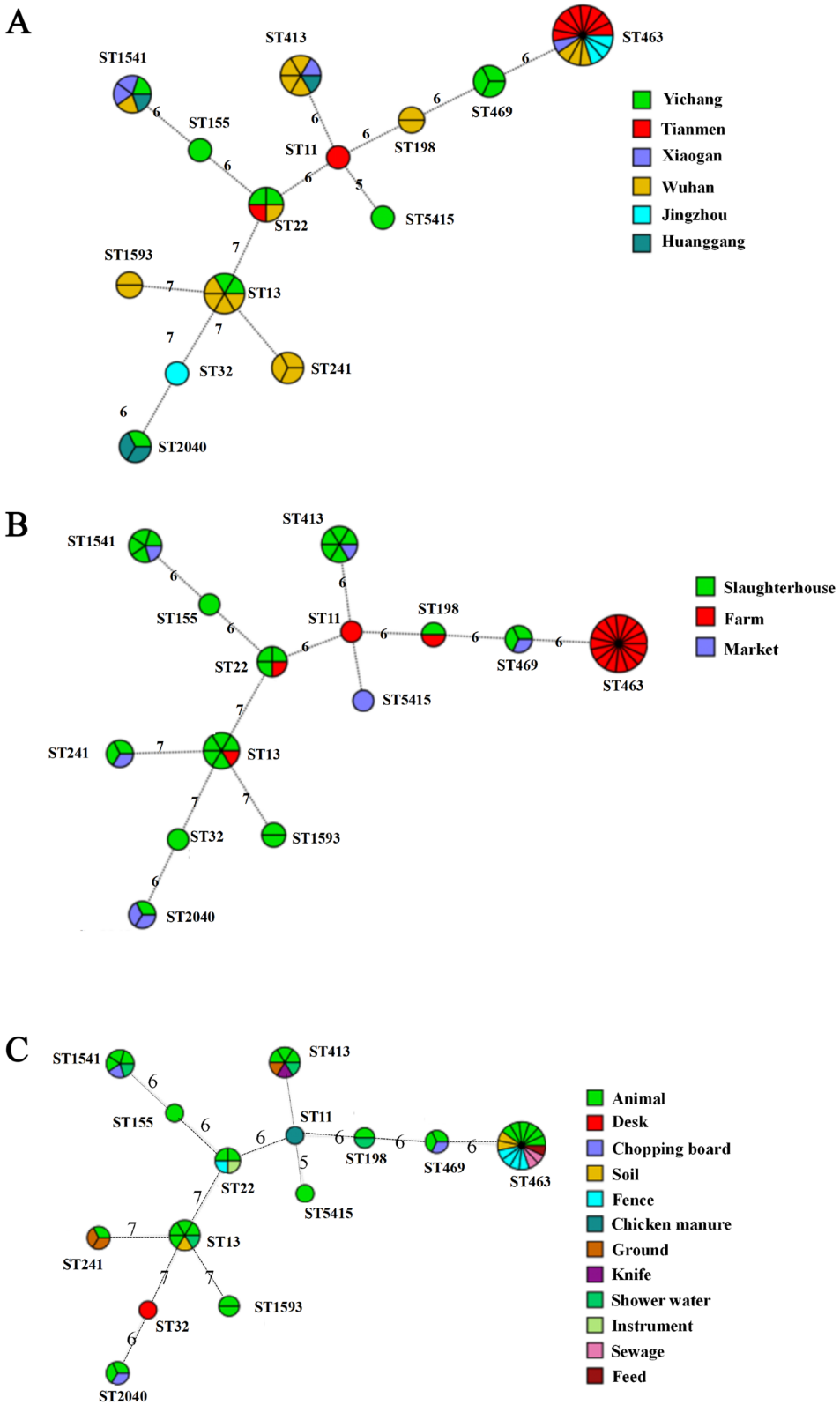

Fig. 6 MLST-based minimum spanning trees of 53 *Salmonella* strains classified by cities (**A**), links of the production chain (**B**) and animal or environmental sample sources (**C**). The number on the line represents the number of mutation sites of allele between the two ST types

genes had great variability. *SPI-1* were most abundant which was consistent with other finding (Qiao et al. 2018) (Fig. 4B). This study also showed that the virulence gene profiles of strains in the same serotype were similar.

Results showed that ST463, ST413 and ST13 were more prevalent among *Salmonella* in chicken production chain in six cities of Hubei Province. Cluster analysis revealed a substantial relationship between ST type and serotype, which was in line with previous finding (Lee et al. 2022). We found four interesting strains. The serotype, ST and resistance phenotype (within 2-fold difference) of HG-JR3 from animal sample in Huanggang market and Y-MD-7-4 from animal sample in Yichang slaughterhouse were shown to be the same. Genomic relatedness of W-YS-1 from animal sample and W-YS-CX-1 from environmental sample in the WYS slaughterhouse was shown on the phylogenetic tree and the antimicrobial resistance profiles was comparable, suggesting that *Salmonella* might spread or cross-contaminate throughout the chicken production chain. In view of the large number of pollution sources in WYS slaughterhouse, HACCP procedures should be strictly implemented (Shepelin et al. 2018). *Salmonella* strains varied by location, which could be attributed to the spread of strain clones by a variables such as food chain, trade in animals and animal products (Yan et al. 2021). It is worth noting that we found cross-contamination with animal samples in all environmental samples except chicken manure and tabletop, demonstrating that the disinfection work in our chicken production chain is not comprehensive.

Conclusions

This research elucidate the contamination and antimicrobial resistance characteristics of *Salmonella* in chicken production chain in Hubei Province, China. The diversity and high prevalence of serotypes, resistance profiles, resistance and virulence genes found in the isolates also emphasize the risk of the three links in chicken production chain as resistance and virulence reservoirs and pipelines. The results presented here also demonstrate the complementary role of WGS and phenotypic assays in high-resolution mapping analysis of foodborne pathogens, and the role of MLST typing in displaying genetic diversity of *Salmonella*. In addition, this study illustrates that standard and effective monitoring are very important to prevent and control the emergence and spread of antimicrobial resistance of foodborne pathogens.

Materials and methods

Sample collection

From July 2019 to September 2020, 1149 animal and environment samples were collected in 11 farms, five slaughterhouses and six retail markets throughout six cities in Hubei province, China. Animal samples were derived from healthy chicken cloaca swab in farms, caecum and cloaca swab in slaughterhouses and chicken meat in retail markets. The sampling size for animal samples in farms was determined according to the size of the farm and the number of fences (30–50 samples for large-scale farms, 10–30 samples for medium or small-sized farms, five samples for each fence of the farm). In slaughterhouses, 5–10 animal samples were collected from each farm sources. In the retail markets, 5–10 animal samples were obtained from each vendor. For environmental samples, 40–70 samples were gathered from each farm and slaughterhouse, and 10–15 samples were obtained from each market, with 3–5 samples from each type of environmental sample. All obtained samples were maintained in iceboxes and send to laboratory within 24 h for immediate processing.

Isolation and serotyping

Salmonella was isolated according to the Chinese National standard: national food safety standard food microbiological examination:*salmonella* (GB4789.4–2010). Generally, after pre-enrichment of each sample in 3 mL sterile selenite cystine broth (SC), the pre-enriched suspension was diluted at 1:1000, then streaked over *Salmonella* chromogenic medium (Hopebio, Qingdao, China) and incubated for 24 h at 37°C, with typical *Salmonella* appearing purple. *Salmonella* housekeeping gene *invA* was amplified by PCR (Primer F: 5′-GTG AAATTA TCGCCACGTTCGGGCAA-3′; Primer R: 5′-TCATCGCACCGTCAAAGGAACC-3′) for identification of the strains after preliminary purification. The PCR reaction conditions were: 94°C for 5 min, followed by 34 cycles of 94°C for 30 s, 64°C for 30 s, and 72°C for 30 s, and finally extension at 72°C for 5 min. *Salmonella* Serotype was identified using commercial Serodiagnosis kits (Statens Serum Institute, Copenhagen, Denmark) based on the slide agglutination method and interpreted according to the White-Kauffmann-Le Minor scheme.

Antimicrobial susceptibility testing (AST)

A total of 10 categories and 16 types of antibacterial agents were chosen for AST, including penicillins [AMP and amoxicillin/clavulanic acid (A/C)], cephalosporins (CEF and CAZ), and monocyclic β-lactams: ATM, carbapenems: MEM, aminoglycosides (GEN, SPT and AK), fluoroquinolones (ENR and OFL), phenicols:FFC, sulfonamides: SXT, tetracyclines (TET and TIG) and polypeptides:CL.Minimum inhibitory concentrations (MICs) were determined by the microbroth dilution method according to CLSI M07, and then interpreted in accordance with CLSI M100 31th, CLSI VET 08 and EUCAST 2016. *Escherichia coli* ATCC25922 was used as

the quality control strain. Salmonella isolates exhibited resistance to at least three different antimicrobial classes was defined as MDR. In the same sampling site, clonal strains were defined as showing the same serotypes and the MIC variations was within 2 folds. After excluding 38 clonal strains, 53 strains were remained and subjected to the subsequent study.

Whole genome sequencing (WGS)

Twenty two strains of dominant serotype among 53 non-clonal strains were subjected to WGS on an Illumina HiSeq PE150 platform (Allwegene Tech., Beijing, China). The genomic DNA of *Salmonella* isolates was extracted using the TIANamp Bacteria DNA Mini Kit (TIANGEN, Beijing, China) to construct the library. Qubit 2.0 was used for preliminary quantification, Agilent 5400 was used to detect the insertion size of the library diluted to 2 ng/μL, and Q-PCR was utilized to accurately quantify the effective concentration of the library and ensure the quality of the library. Trimmatic (v0.36) software was used to control the data quality of the qualified library, Spades (v3.13.0) software was used to assemble and statistically analyze the sequencing data of each strain after quality control, and finally, the scaffold sequence greater than 500 bp was chosen for further analysis. The SeqSero2 database (https://cge.food.dtu.dk/services/SeqSero/) was used to predict *Salmonella* serotypes, which were then compared to the results from the slide agglutination approach. The CARD database (https://card.mcmaster.ca/) was used to annotate drug resistance genes, with coverage ≥90% and identity≥90% (Shen et al. 2018; Ma et al. 2018). The VFDB database (http://www.mgc.ac.cn/VFs/) was used for virulence gene annotation, with coverage≥95% and identity≥95% (Gupta et al. 2019). Additionally, quinolone resistance determining regions (QRDR) were analyzed by SnapGene Viewer V. 1.5.2 software (Kang et al. 2020). Graphpad prism V. 8.0.2 was used for heat map mapping of antimicrobial resistance genes and virulence genes.

Multi locus sequence typing (MLST)

Seven housekeeping genes (*aroC*, *dnaN*, *hemD*, *hisD*, *purE*, *sucA* and *thrA*) were amplified by PCR (Zhu et al. 2020) and sequenced by Allwegene Tech (Beijing, China) in 31 non-clonal strains that were not submitted to WGS. The sequences of the seven housekeeping genes were also acquired in 22 WGS-sequenced strains. The PubMLST database (https://pubmlst.org/mlst) was used to determine the sequence type (ST) for 53 isolates based on the seven-gene legacy multilocus sequence typing (MLST) loci for *Salmonella*: *aroC*, *dnaN*, *hemD*, *hisD*, *purE*, *sucA* and *thrA*. MEGA (V. 11) (Aslanyan et al. 2020) was used to create a phylogenetic tree. The iTOL (V. 3) was used to edit and visualize the resulting phylogenetic tree. The BioNumerics V. 8.0 was used to create an MLST-based Minimum Spanning Tree according to Yao (Yao et al. 2014).

Nucleotide sequence accession number

The FASTQ reads from 22 genomes of *Salmonella* presented in this study were deposited in National Center for Biotechnology Information and assigned under SRA accession NO. PRJNA743595.

Abbreviations

AMP	Ampicillin
A/C	Amoxicillin/Claevulanic Acid
ATM	Aztreonam
AK	Amikacin
CL	Colistin
CLSI	Clinicaland and Laboratory Standards Intitute
CEF	Ceftiofur
CAZ	Ceftazidime
CARD	Comprehensive Antibiotic Research Database
ENR	Enrofloxacin
FFC	Florfenicol
GEN	Gentamicin
MIC	Minimal inhibitory concentration
MLST	Multi Locus Sequence Typing
MEM	Meropenem
NCBI	National Center for Biotechnology Information
OFL	Ofloxacin
SC	Selenite cystine broth
SXT	Trimethoprim-Sulfamethoxazole
SPT	Spectinomycin
TET	Tetracycline
TIG	Tigecycline
VFDB	Virulence Factors Database
WGS	Whole Genome Sequencing

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s44149-022-00063-7.

Additional file 1: Table S1. Isolation rates of *Salmonella* in different cities in Hubei Province, China. **Table S2.** MICs (μg/ml) of 91 *Salmonella* strains isolated in the chicken production chain in Hubei Province, China. **Table S3.** Gene mutation sites in QRDR of 22 whole-genome sequenced *Salmonella* isolated. **Table S4.** Typing results of 53 non-clonal *Salmonella* strains. **Table S5.** Comparison of serotype results of 22 *Salmonella* strains by slide agglutination method and whole genome sequencing method.

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Authors' contributions

CW and GC conceived and designed the progect. CW, LL,YS, LH and YC performed the experiments. CW, YS, LL and GC analyzed the data. GC, HH, YP and ZL contributed reagents/materials/analysis tools. CW, LL, YS and GC wrote the paper. All authors read and approved the final manuscript.

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Availability of data and materials
All data generated or analysed during this study are included in this published article [and its supplementary information files].

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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