




## Research Article

# Antimicrobial Resistance and Genomic Characterization of *Salmonella* Isolated from Pigeons in China

Yuhua Zhang,<sup>1</sup> Zheng Lu,<sup>1</sup> Haoyu Zhao,<sup>1</sup> Shuangyu Li,<sup>1</sup> Hong Zhuang,<sup>1</sup> Juan Wang,<sup>1</sup> Ruichao Li ,<sup>2</sup> Weibo Zheng,<sup>3</sup> Hongwei Zhu,<sup>3</sup> Peng Xie,<sup>1</sup> Yibin Hu,<sup>1</sup> Caiyuan Zhou,<sup>1</sup> Qian Mao,<sup>1</sup> Leilei Sun,<sup>1</sup> Shanshan Li,<sup>1</sup> Wenhui Wang,<sup>1</sup> Fang Wang,<sup>1</sup> Wei Pan ,<sup>4</sup> and Chengbao Wang <sup>1</sup>

<sup>1</sup>College of Veterinary Medicine, Northwest A&F University, Yangling 712100, Shaanxi, China

<sup>2</sup>College of Veterinary Medicine, Yangzhou University, Yangzhou 225009, Jiangsu, China

<sup>3</sup>School of Life Sciences, Ludong University, Yantai 264025, Shandong, China

<sup>4</sup>Jiangsu Key Laboratory of Immunity and Metabolism, Jiangsu International Laboratory of Immunity and Metabolism, Department of Pathogen Biology and Immunology, Xuzhou Medical University, Xuzhou 221004, Jiangsu, China

Correspondence should be addressed to Wei Pan; [panwei525@126.com](mailto:panwei525@126.com) and Chengbao Wang; [wangchengbao@nwsuaf.edu.cn](mailto:wangchengbao@nwsuaf.edu.cn)

Received 17 February 2024; Revised 9 April 2024; Accepted 16 April 2024; Published 6 May 2024

Academic Editor: Zongfu Wu

Copyright © 2024 Yuhua Zhang et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Salmonellosis is one of the important bacterial infectious diseases affecting the health of pigeons. Heretofore, the epidemiological characteristics of *Salmonella* in pigeon populations in China remain largely unclear. The present study investigated the antimicrobial resistance and genomic characteristics of *Salmonella* isolates in pigeons in different regions of China from 2022 to 2023. Thirty-two *Salmonella* isolates were collected and subjected to 24 different antimicrobial agents, representing nine categories. The results showed that these isolates were highly resistant to cefazolin (100%), gentamicin (100%), tobramycin (100%), and amikacin (100%). Three or more types of antimicrobial resistance were present in 90.62% of the isolates, indicating multidrug resistance. Furthermore, using whole genome sequencing technology, we analyzed the profiles of serotypes, multilocus sequence typing, virulence genes, antimicrobial resistance genes, and plasmid replicons and constructed phylogenetic genomics to determine the epidemiological correlation among these isolates. All strains belonged to *Salmonella* Typhimurium var. Copenhagen and exhibited five antimicrobial resistance genes and more than 150 *Salmonella* virulence genes. Moreover, each isolate contained both the IncFIB(S) and IncFII(S) plasmids. In addition, phylogenetic analysis showed that all isolates were very close to each other, and isolates from the same region clustered in the same branch. Overall, our findings provide the first evidence for the epidemiological characteristics of *Salmonella* in pigeons of China, highlighting the importance of preventing salmonellosis in pigeons.

## 1. Introduction

*Salmonella* is one of the main pathogens leading to the global outbreak of foodborne illness, which poses a serious threat to human health worldwide [1]. In China, 70%–80% of bacteria-related foodborne illnesses are caused by *Salmonella* infection [2]. Recent surveys of the disease burden in multiple Chinese provinces have shown that the annual incidence of *Salmonella* is roughly 245/100,000 [3]. Moreover, more than 2,610 *Salmonella* serotypes have been identified, many of which are pathogenic to both humans and animals [4, 5]. Notably, *Salmonella*-infected animals, including mice, wild

animals, fowl, and domestic livestock, are important sources of *Salmonella* contamination in humans. Thus, it is of great importance to monitor the epidemiological profiles of *Salmonella* infections in animals.

Due to a close contact with humans, pigeons present one important source where humans acquire *Salmonella* infections. For example, *Salmonella* in meat pigeons can infect humans *via* the food chain, while racing pigeons can transmit the pathogen through direct contact and feces [6, 7]. Salmonellosis can spread not only horizontally but also vertically. For instance, *Salmonella* can infect breeding pigeons and then spread to the eggs through vertical transmission,

TABLE 1: The source and prevalence statistics of *Salmonella* collected samples in this study.

Province	City	Breed	Sample	<i>Salmonella</i> isolates	Prevalence rate (%)
Hunan	Changde	Meat pigeon	30	6	20.0
Hebei	Shijiazhuang	Meat pigeon	6	3	50.0
Hebei	Hengshui	Meat pigeon	17	6	35.3
Hebei	Cangzhou	Meat pigeon	8	1	12.5
Henan	Pingdingshan	Meat pigeon	37	13	35.1
Shanghai	Shanghai	Meat pigeon	25	3	12.0
Hebei	Tangshan	Racing pigeon	5	0	0
Shaanxi	Xi'an	Racing pigeon	25	0	0
Shaanxi	Baoji	Racing pigeon	8	0	0
Jiangsu	Yancheng	Racing pigeon	30	0	0
Fujian	Fuzhou	Racing pigeon	15	0	0
Ningxia	Wuzhong	Racing pigeon	9	0	0

resulting in egg necrosis and a decrease in the hatching rate. It can also infect squabs *via* lactation, leading to infection or even death. Moreover, any age of pigeons can be infected with *Salmonella* [8], causing a large number of deaths [9]. In addition, *Salmonella* Typhimurium (*S. Typhimurium*) is one of the most important serotypes of *Salmonella* Enteritidis, which can infect both humans and a range of animal hosts due to its excellent survival ability under different dietary conditions [10].

In China, the industry for pigeon racing has grown, and meat pigeon consumption has increased in recent years. Accumulating evidence has shown the infection of *Salmonella* in pigeons, with *S. Typhimurium* infections as the majority species. For example, *S. Typhimurium* is reported to be the dominant serotype of pigeon *Salmonella* isolates in Guangdong and Shanghai, China, and Poland [11–13]. Infected pigeons often show single or multiple serious clinical symptoms, including diarrhea, weight loss, limps, head and neck skews, and other neurological symptoms [13, 14]. Furthermore, the misuse and abuse of antibiotics to treat salmonellosis has led to the emergence of multidrug resistance (MDR) bacteria [15, 16]. Therefore, a comprehensive epidemiological study and MDR profiles are needed to clarify the potential danger of *Salmonella* infection to pigeons and human health in China. Whole genome sequencing (WGS) can provide accurate information on population dynamics, genome epidemiology, and bacterial genomic characteristics [17]. A previous study has used WGS to analyze the serotype, antimicrobial resistance, multilocus sequence typing (MLST), virulence genes, and plasmid replicons of *Salmonella* strains in the United States [18]. However, the WGS profiles of *Salmonella* isolates in Chinese pigeon populations are still unknown.

In the present study, we investigated the prevalence of *Salmonella* in racing pigeon clubs and meat pigeon farms across various regions of China. A total of 32 *Salmonella* strains were isolated from pigeons. Phenotypic resistance results showed that 90.62% of the isolates were MDR. Furthermore, WGS in conjunction with bioinformatics analysis was used to investigate the genomic characteristics of *Salmonella* isolates. We found that all isolates were *Salmonella* Typhimurium var. Copenhagen, and the resistance genes, virulence genes, and

plasmid types carried by them were also very similar. Overall, these results contribute to a better understanding of the epidemiology and transmission mechanisms of *Salmonella* in pigeon populations in China.

## 2. Materials and Methods

**2.1. Ethics Statement.** This study was carried out in accordance with standard procedures without any operations on living animals. Dead pigeons with clinical symptoms of *Salmonella* infection were used as a sample source for this study.

**2.2. Sample Collection.** Between June 2022 and April 2023, 215 samples were gathered for this investigation, comprising 92 samples of racing pigeons and 123 samples of meat pigeons. This study was not a random sampling, but a sample of sick pigeons with clear clinical symptoms was selected through clinical diagnosis. These collected samples showed weight loss, head and neck deviation, arthritis, diarrhea, and other single or comprehensive symptoms. Details of the sample collection are shown in Table 1. These samples were collected from four cities in Hebei Province, two cities in Shaanxi Province, Henan Province, Hunan Province, Jiangsu Province, Fujian Province, Ningxia Hui Autonomous Region, and Shanghai (Figure 1).

**2.3. Isolation and Identification of *Salmonella* Isolates.** The procedure for *Salmonella* isolation is depicted as follows: Following aseptic necropsy, tissue samples from the liver, heart, kidney, lung, and intestine were collected for *Salmonella* isolation (Figure S1). To increase the number of bacteria in tissue samples, the proper amounts of the tissue samples were inoculated in a selenite cystine solution (Haibo biology, HB4085, China) and cultured for 18 hr at 37°C. Then, the red precipitate's culture solution was smeared on agar medium containing xylose–lysine–deoxycholic (XLD) (Haibo biology, HB4105, China) acid and cultured at 37°C for 24 hr to identify potential *Salmonella* colonies. Finally, characteristic black center colonies or colorless translucent colonies were chosen to identify *Salmonella* isolates using 16S rDNA PCR (Table S1 and Figure S2).

**2.4. Antimicrobial Susceptibility Testing.** According to the Clinical Laboratory Standard Institute (CLSI) guidelines,



FIGURE 1: Distribution of sampling locations in eight provinces and regions of China.

antimicrobial susceptibility profiles were assessed by broth microdilution using the Sensititre™ automated antimicrobial susceptibility system (Thermo Fisher Scientific, United Kingdom) and the Gram-negative GN4F plate. The 24 antimicrobial agents tested were as follows: penicillins (ampicillin: AMP, 8–16  $\mu\text{g}/\text{mL}$ ; piperacillin: PIP, 16–64  $\mu\text{g}/\text{mL}$ ; ticarcillin/clavulanic acid: TCC, 8/2–64/2  $\mu\text{g}/\text{mL}$ ; ampicillin/sulbactam: SAM, 4/2–16/8  $\mu\text{g}/\text{mL}$ ; piperacillin/tazobactam: TZP, 8/4–128/4  $\mu\text{g}/\text{mL}$ ), carbapenems (meropenem: MEM, 0.5–8  $\mu\text{g}/\text{mL}$ ; ertapenem: ETP, 0.25–8  $\mu\text{g}/\text{mL}$ ; imipenem: IPM, 0.5–8  $\mu\text{g}/\text{mL}$ ; doripenem: DOR, 0.5–4  $\mu\text{g}/\text{mL}$ ), cephalosporins (cefazolin: CZO, 1–16  $\mu\text{g}/\text{mL}$ ; cefepime: FEP, 4–32  $\mu\text{g}/\text{mL}$ ; ceftazidime: CAZ, 1–16  $\mu\text{g}/\text{mL}$ ; ceftriaxone: CRO, 0.5–32  $\mu\text{g}/\text{mL}$ ), monobactams (aztreonam: ATM, 1–16  $\mu\text{g}/\text{mL}$ ), aminoglycosides (gentamicin: GEN, 2–8  $\mu\text{g}/\text{mL}$ ; amikacin: AMK, 8–32  $\mu\text{g}/\text{mL}$ ; tobramycin: TOB 2–8  $\mu\text{g}/\text{mL}$ ), tetracyclines (tetracycline: TET, 4–8  $\mu\text{g}/\text{mL}$ ; minocycline: MIN, 1–8  $\mu\text{g}/\text{mL}$ ; tigecycline: TGC, 1–8  $\mu\text{g}/\text{mL}$ ), quinolones (ciprofloxacin: CIP, 0.5–2  $\mu\text{g}/\text{mL}$ ; levofloxacin: LVX, 1–8  $\mu\text{g}/\text{mL}$ ), folate pathway inhibitors (trimethoprim/sulfamethoxazole: TMS, 2/38–4/76  $\mu\text{g}/\text{mL}$ ), and nitrofurantoin (nitrofurantoin: NIT, 32–64  $\mu\text{g}/\text{mL}$ ). The minimum inhibitory concentration (MIC) for each antibiotic was interpreted using CLSI standards and NARMS breakpoints, and the MIC values were categorized as susceptible (S), intermediate (I), or resistant

(R). In order to facilitate the analysis of the results, the sensitive intermediate-resistant strains were identified as having antimicrobial resistance, and the strains with three or more antimicrobial resistances were identified as having MDR. The quality control strain utilized was *Escherichia coli* ATCC 25922.

**2.5. Genomic DNA Extraction and Whole Genome Sequencing.** The isolated strains were shaken overnight at 37°C in Luria–Bertani (LB) (Haibo biology, HB0128, China) liquid medium, and then centrifuged to obtain a bacterial precipitate. Bacterial genomic DNA was extracted using a commercial TIANmp Bacterial DNA kit (Tiangen Biotech, China) according to the manufacturer's instructions. After passing the DNA sample quality test, a sequencing library was generated using the Rapid Plus DNA Lib Prep Kit for Illumina (Cat. No. RK20208). WGS of all isolates was performed at Novogene Bioinformatics Technology Co., Ltd. (Tianjin, China) using Illumina platforms and the PE150 strategy.

**2.6. Bioinformatic Analyses.** After performing quality checks on the raw reads with Fastp (v0.23.1) [19], the reads were assembled with SPAdes 4.0.1 [20] using the “careful” command option. After genome assembly, SeqSero1.2 (<https://cge.food.dtu.dk/services/SeqSero/>) [21] and MLST2.0 (<https://cge.food.dtu.dk/se>

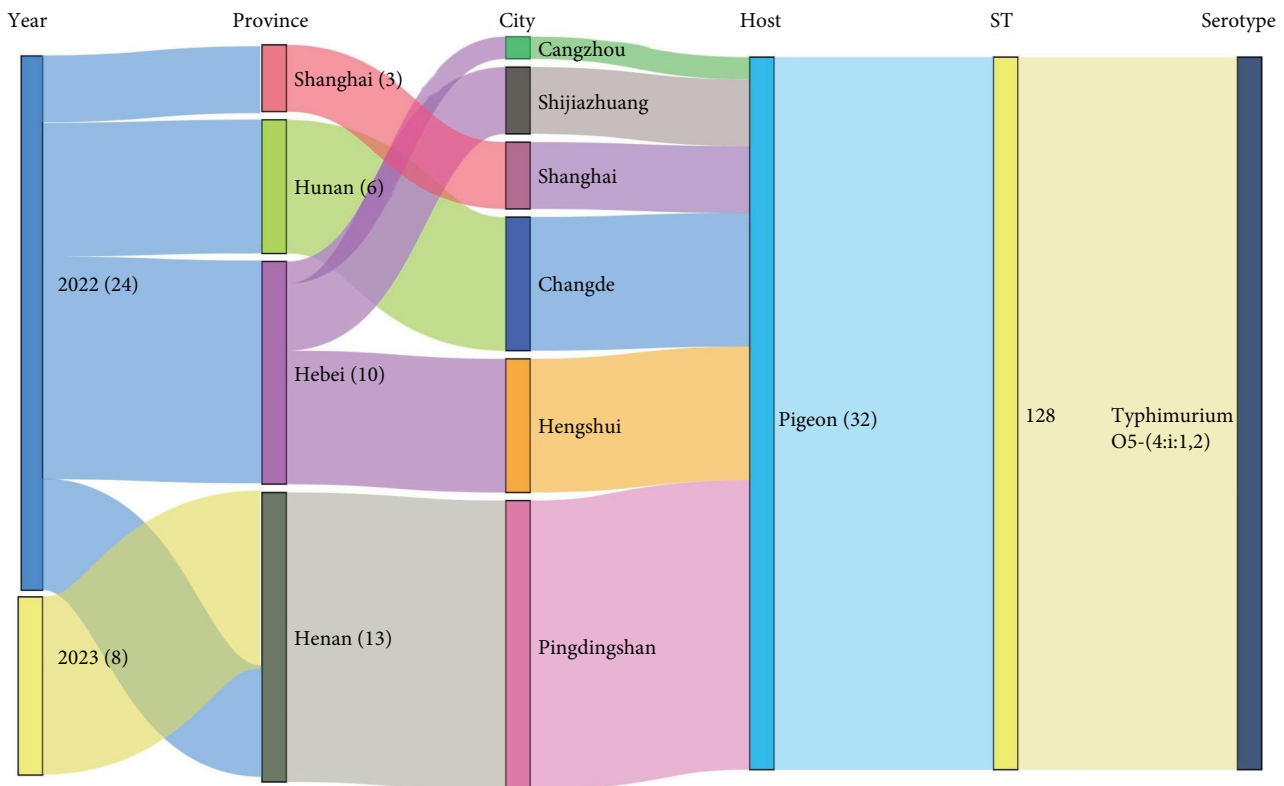


FIGURE 2: Distribution of *Salmonella* isolates studied according to sampling year, province, host, sequence type, and serotype.

rices/MLST/) were used to predict the serotype and sequence type of the isolated strains on the data platform of the Genome Epidemiology Center [22]. PlasmidFinder 2.1 (<https://cge.food.dtu.dk/services/PlasmidFinder/>) was additionally employed to find the plasmid type with a minimum coverage of 60% and a nucleotide identity of 95% [23]. In addition, the virulence factor database (VFDB) (<http://www.mgc.ac.cn/cgi-bin/VFs/v5/main.cgi?func=VFAnalyzer>) manages the virulence factors of bacterial pathogens, and we conducted virulence gene prediction for 32 *Salmonella* isolates here [24]. In order to conduct a comprehensive analysis of resistance genes as much as possible, the resistance genes were detected using ResFinder 4.4.1 (<https://cge.cbs.dtu.dk/services/ResFinder/>) with settings of threshold of 95% and minimum length of 60%. Moreover, using homology and single nucleotide polymorphism (SNP) model prediction of resistance groups on the comprehensive antibiotic resistance database platform, the Resistance Gene Identifier (<https://card.mcmaster.ca/analyze/rgi>) software was used to identify the AMR gene [25]. Additionally, an evolutionary tree based on the SNPs of the core genomes was built using the maximum likelihood method (Roary and FastTree). In this study, the phylogenetic tree, corresponding serotype, ST (sequence type), province, antimicrobial resistance genes, and insertion sequences were visualized using the Interactive Tree of Life (<https://itol.embl.de>) web server.

### 3. Results

**3.1. Prevalence and Serotypes of *Salmonella* in Pigeons.** Among all meat pigeon samples ( $n = 123$ ), 32 *Salmonella* isolates were identified, mainly distributed in Henan, Hebei,

Hunan, and Shanghai in China. Of the 96 racing pigeon samples collected from five provinces, no *Salmonella* isolate was found. In the entire pigeon population, the prevalence of *Salmonella* was 14.9%, while the prevalence in meat pigeons was 26%. Interestingly, every isolate shared the same serotype and MLST pattern and had similar copies of the seven housekeeping genes: *aroC*, *hemD*, *purE*, *thrA*, *dnaN*, *sucA*, and *hisD*. All isolates were *Salmonella* Typhimurium var. Copenhagen ST128 ( $n = 32$ ) (Figure 2).

**3.2. Phenotypic Antimicrobial Resistance.** All *Salmonella* isolates were subjected to testing for 24 different antimicrobial drugs in nine different categories, and the results are shown in Table S2 and Figure S3. In order to facilitate the statistical results, we classified intermediate drug-resistant strains as drug-resistant results. We found high resistance of isolates to gentamicin, tobramycin, cefazolin, and amikacin (100%; 32/32), followed by minocycline (84.375%; 27/32), tetracycline (78.125%; 25/32), and tigecycline (50%; 16/32). Moreover, all isolates were susceptible to cefepime, ceftriaxone, doripenem, ertapenem, aztreonam, and levofloxacin (Figure 3(a)). In addition, 90.62% of the strains were resistant to at least three types of antibiotics, indicating MDR (Figure 3(b)).

**3.3. Genotypic Antimicrobial Resistance.** Analyzing the antimicrobial resistance genes, 32 *Salmonella* isolates were identified to carry five identical antimicrobial resistance genes. All isolates contained aminoglycoside acetyltransferase *aac* (6′)-*Iaa* resistance genes, efflux mechanism genes *mdsA* and *mdsB*, genes encoding gold resistance *gols*, and biofilm-related genes



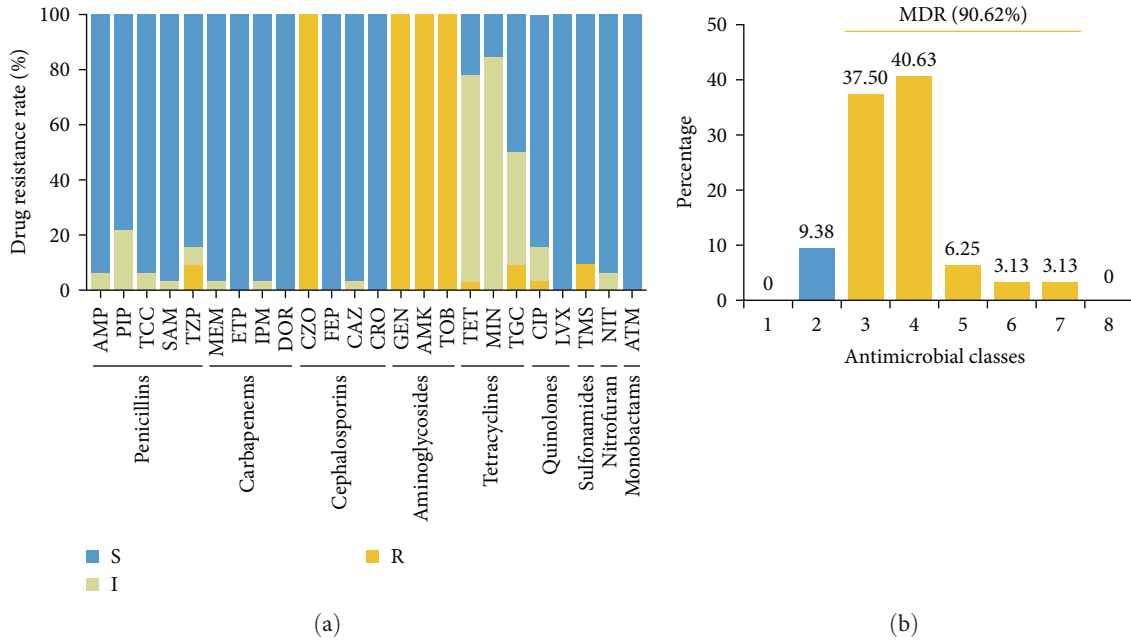


FIGURE 3: Phenotypic antimicrobial susceptibility (a) and distribution of multiple resistance among the studied *Salmonella* isolates (b). Abbreviations: AMP, ampicillin; PIP, piperacillin; TCC, ticarcillin/clavulanic acid; SAM, ampicillin/sulbactam; TZP, piperacillin/tazobactam; MEM, meropenem; ETP, ertapenem; IPM, imipenem; DOR, doripenem; CZO, cefazolin; FEP, cefepime; CAZ, ceftazidime; CRO, ceftriaxone; GEN, gentamicin; AMK, amikacin; TOB, tobramycin; TET, tetracycline; MIN, minocycline; TGC, tigecycline; CIP, ciprofloxacin; LVX, levofloxacin; TMS, trimethoprim/sulfamethoxazole; NIT, nitrofurantoin; and ATM, aztreonam.

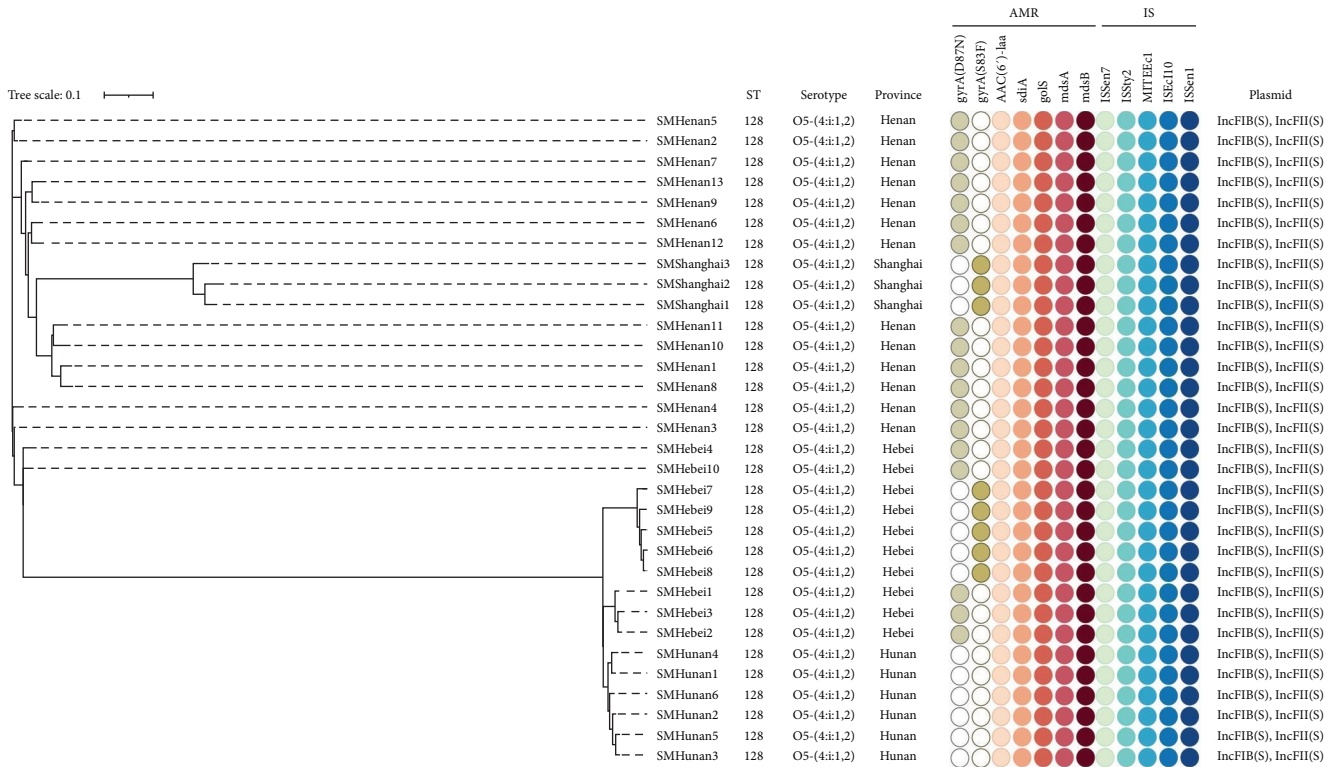


FIGURE 4: Phylogenetic tree and statistical combination of 32 strains of *Salmonella* from pigeons in China based on SNP analysis.

*sdhA* (Figure 4). Moreover, genomic mutations conferring quinolone resistance were detected in 26 (81.25%) isolates, and two single mutations of the *gyrA* gene were observed: *gyrA* (D87N) ( $n = 18$ ) and *gyrA* (S83F) ( $n = 8$ ) (Figure 4).

**3.4. Plasmid Replicons.** Using the PlasmidFinder tool, we detected the plasmids carried by *Salmonella* strains. These isolates carried the same plasmid replicon, and a total of two plasmids were detected in 32 isolates. The plasmids of

isolates were IncFII(S) and IncFIB(S) (Figure 4). Interestingly, the IncFII(S) and IncFIB(S) plasmids were carried by different isolates from pigeons from different provinces, indicating that these plasmids have widespread distribution across different geographical regions.

**3.5. *Salmonella* Virulence Genes.** In order to investigate the virulence gene profile of *Salmonella* isolates, we analyzed the genome with the complete VFDB dataset. A total of 156 genes associated with the virulence and pathogenicity mechanisms of *Salmonella* were found in the 32 *Salmonella* strains. The number of virulence genes in each isolate ranged from 151 to 156, and there was little difference in virulence genes among isolates (Figure 5). Moreover, the distribution of *S. Typhimurium* virulence genes isolated from various geographical locations was nearly the same, with only one to six virulence gene differences. There were 1–6 virulence gene differences among different isolates, including *safD*, *stdC*, *ssaI*, *ssaM*, *sseG*, and *sspH2*. All *Salmonella* isolates had plasmids harboring the *spvB* gene, the stress adaptation gene *sodCI*, the enterotoxin gene *stn*, and the serum resistance gene *rck*, all of which are critical components of the virulence system of *Salmonella*. In addition, the typical virulence genes from *Salmonella* pathogenic islands 1 and 2 (SPI-1 and SPI-2) were present in all *Salmonella* strains analyzed.

#### 4. Discussion

As an important zoonotic pathogen, *Salmonella* is seriously harmful to humans and animals, causing huge economic losses. The pigeon breeding industry is the fourth major poultry breeding industry in China [26]. However, so far, the pigeon industry has not yet established a strict biosafety management system, and there are also defects in disease prevention and immunization procedures. Therefore, once a *Salmonella* infection occurs, it will cause serious damage to pigeons. The present study investigated the epidemiological, antimicrobial resistance, and genomic characteristics of *Salmonella* isolated from pigeons in various parts of China using WGS data in conjunction with accurate bioinformatics techniques, which provides a scientific basis for the spread of *Salmonella* in pigeons in China.

In this study, 32 strains of *Salmonella* from pigeons were all *Salmonella* Typhimurium var. Copenhagen. MLST analysis showed that all strains belong to ST128. Previous studies have reported that *S. Typhimurium* is the most common serotype of *Salmonella* in animals [27]. Moreover, the Copenhagen variant is the most prevalent variant of *S. Typhimurium* in pigeons [28]. It is reported that the sequence type of *S. Typhimurium* isolated from pigeons from China and Poland was also ST128. These results, including ours, indicate that the serotype and sequence type of *Salmonella* isolates may be a stable lineage in the pigeon population. Furthermore, we showed that the prevalence of *S. Typhimurium* in the overall pigeon population was 14.9%, and in meat pigeons, it was 26%. In line with our finding, two previous studies reported 26.1% and 21% prevalence of *S. Typhimurium* in the meat pigeon population in the markets of Shanghai and Beijing, respectively [12, 29].

The prevalence of salmonellosis in pigeons in Italy, Egypt, and Poland was reported to be 0.9%, 13.3%, and 5.5%, respectively [13, 30, 31]. The prevalence of salmonellosis in pigeons varies in different countries, which may be related to different sampling methods. But it is worth noting that the situation of *Salmonella* infection in meat pigeon populations in China may be more severe, which needs extensive investigation in pigeon populations.

Antibiotics are the most commonly therapy for the treatment of bacterial diseases, including pigeon salmonellosis. In the field of veterinary medicine, the widespread and sustained use of antimicrobial agents has led to the development of AMR in animals and livestock [32]. The present study evaluated the phenotypic and genotypic AMR profiles of 32 *Salmonella* strains in pigeons. In order to identify accurate epidemiological information about the antimicrobial susceptibility of *Salmonella*, all isolates were tested for 24 different antimicrobial agents. In the present study, the phenotypic resistance results of amikacin, gentamicin, and tobramycin were consistent with the aminoglycoside resistance gene *aac* (6′)-*Iaa*, and there was no significant correlation between the resistance of other types of antibiotics and the resistance genes carried. Antimicrobial resistance genes do not necessarily mean phenotypic resistance and vice versa. The occurrence of antimicrobial resistance is not only determined by the presence of antimicrobial resistance genes but also affected by various factors, such as enzyme activation, target modification or protection, and biofilm formation [33]. We found that all strains exhibited a common resistance to gentamicin, amikacin, tobramycin (aminoglycosides), and cefazolin (first-generation cephalosporins), although they come from different regions. The result was also observed in the antimicrobial susceptibility test of *Salmonella* isolated from humans [34]. We also found that some strains were resistant to tigecycline, but no plasmid-mediated tigecycline resistance genes *tet* (X3) and *tet* (X4) were found. It is speculated that the causes of antimicrobial resistance are related to other tigecycline resistance mechanisms, such as overexpression of efflux pumps, mutation of cell membrane porin, and ribosome protection mechanisms [35]. Notably, we showed that 90.62% of *Salmonella* isolates in pigeons were MDR and could resist at least three types of antimicrobial agents. Considering the limited antibiotics available to pigeons at present, infection with MDR strains may lead to ineffective treatment. This reminds us to continuously monitor antimicrobial resistance in pigeon populations and use antibiotics reasonably.

The antimicrobial resistance genes predicted by the two data platforms are slightly different. Therefore, we combined the antimicrobial resistance genes and chromosome mutation results with a threshold consistency higher than 95% in the two data platforms for statistics. Antimicrobial resistance can be more accurately predicted when the two methods are combined. We detected five antimicrobial resistance genes in *Salmonella* isolates from pigeons. Among them, the most common resistance gene is *aac* (6′)-*Iaa*, which encodes aminoglycoside drugs. Moreover, phenotypic resistance studies confirmed that all strains were resistant to aminoglycosides

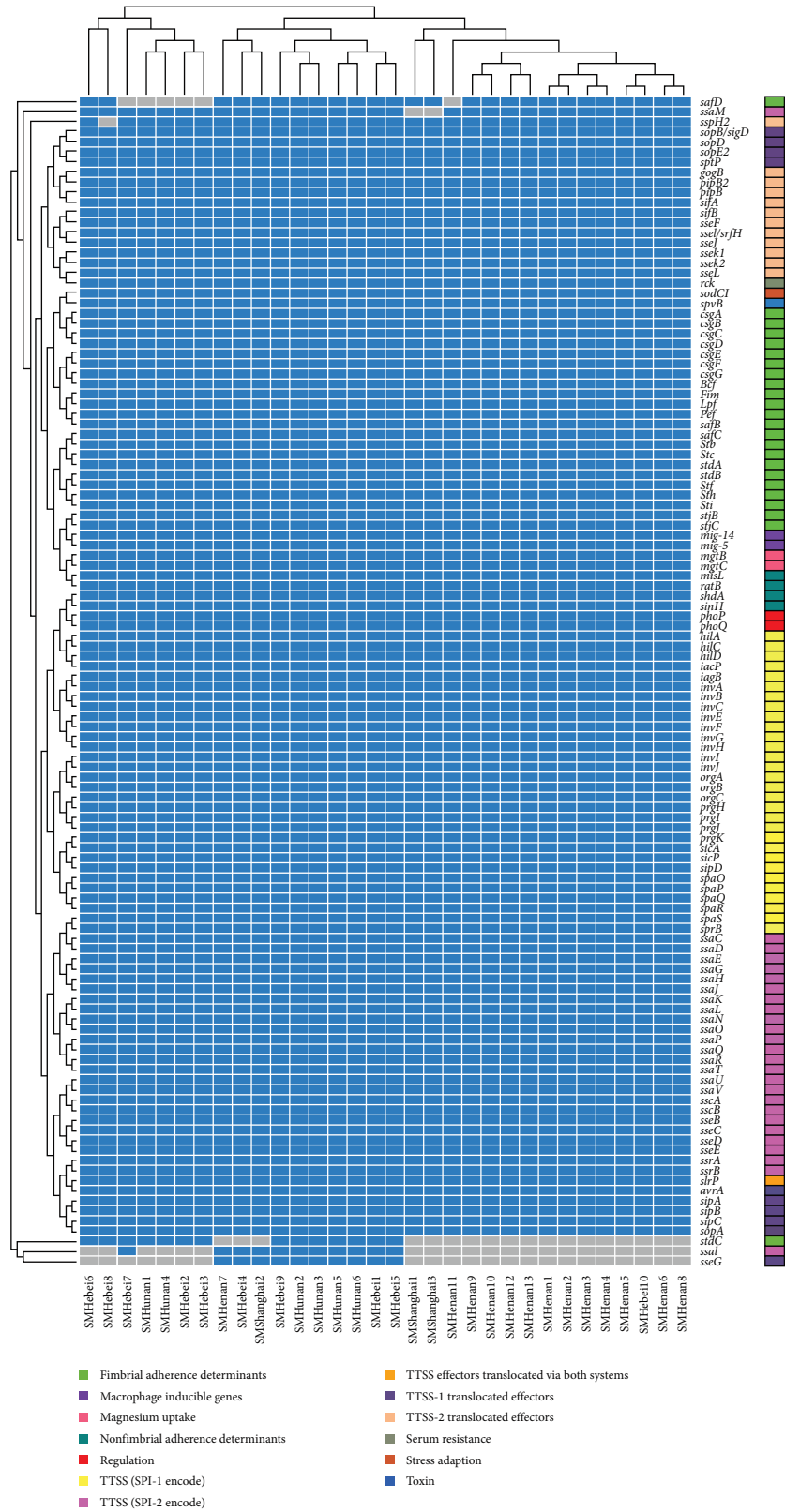


FIGURE 5: Heatmap of tree hierarchical clustering of virulence genes and isolated strains. This figure shows the prediction of the virulence gene profile of the studied isolates. The x-axis shows the isolate ID numbers, and the y-axis shows the identified selected virulence genes. Blue cells indicate the presence of a gene; gray cells indicate the absence of a gene.

(gentamicin, tobramycin, and amikacin). Consistent with a previous study [36], all isolates were positive for the gene *aac* (6)-*Iaa* encoding aminoglycoside resistance. In addition, we found that the efflux pump of the *mdsABC* complex encodes the antimicrobial resistance genes *mdsA* and *mdsB*. *MdsABC* is a unique multidrug transport factor for *Salmonella*. *MdsA* and *mdsB* can promote resistance to various antibacterial drugs, including phenicol antibiotic, cephalomycin, monobactam, carbapenem, and cephalosporin. This indicates that its presence can lead to resistance to multiple antibiotics [37–40]. Furthermore, partial regulatory systems contribute to bacterial resistance to heavy metals. In *Salmonella*, the response to gold ions is mediated by a specific metal regulatory factor *gols*, which controls the expression of the RND (resistance–nodulation–division) efflux pump *gesABC*. The *CpxR/CpxA* system (cell-envelope stress-responding system) promotes the gold resistance of *Salmonella* by controlling *gols*-dependent *gesABC* transcription, enabling microorganisms to resist contaminated environments [41–43]. Notably, the *sdiA* gene exists in all isolated strains. To our best knowledge, there is no study to clarify the role of the *sdiA* gene in antimicrobial resistance. The *sdiA* gene of *Salmonella* regulates the *rck* gene, which mediates the adhesion and invasion of *Salmonella* to epithelial cells and the body's resistance to complement [44, 45]. In addition, we observed mutations caused by amino acid substitutions in *gyrA*, from serine to phenylalanine at codon 83 (S83F) and from aspartic acid to asparagine at codon 87 (D87N). Single point mutations in the quinolone resistance-determining region of *gyrA* reduce the sensitivity of fluoroquinolones (e.g., ciprofloxacin). However, antimicrobial resistance may require two or more mutations in the quinolone-determining region of *gyrA*, *gyrB*, *parC*, and *parE* [46–48].

Plasmids, transferred between different bacterial species and clones, can carry antimicrobial resistance or virulence genes. Obtaining plasmids carrying antimicrobial resistance or virulence genes is transmitted to each other between bacteria of different and geographical origins, which may alter the prevalence of virulence or multidrug-resistant bacterial cloning [49, 50]. The present study found IncFIB(S) and IncFII(S) plasmids in all isolates. IncFIB(S) and IncFII(S) plasmids are *Salmonella* virulence plasmids that may carry virulence-related genes *spv*, *rck* (resistance to complement killing), and *pef* (plasmid-encoded fimbriae) [51]. However, antimicrobial genes were not found on the contigs carrying the IncFIB(S) and IncFII(S) sequence replicons. This may have been due to the absence of these genes on virulence plasmids [23].

Virulence genes are closely related to the ability of *Salmonella* to invade host cells and spread *in vivo* [52]. In this study, we found 156 genes related to *Salmonella* virulence and pathogenic mechanisms in the genomes of 32 *Salmonella* isolates in pigeons. All isolates carried the typical virulence factors of *Salmonella* pathogenicity islands 1 and 2, indicating the strong pathogenicity of these isolates. There were 1–6 virulence gene differences among different *Salmonella* isolates; however, the serum resistance gene *rck*, the

stress adaptation gene *sodCI*, the enterotoxin gene *stn*, and plasmid-carrying *spvB* gene were detected in each *Salmonella* isolate. It is reported that *rck* functions as a “gene toxin” that affects the DNA integrity of epithelial cells, in addition to its roles as a “cyclomodulin” that affects the cell cycle machinery and as an invasion factor [53]. The *sodCI* gene decreases external oxidative damage to host cells by encoding phagocytic superoxide dismutase [54]. The enterotoxin *stn* gene has a significant role in the pathogenicity of *S. Typhimurium* infection, which can maintain the composition and integrity of the bacterial cell membrane by regulating the localization of the OmpA membrane [55]. The *spvB* virulence gene of *Salmonella* acts as an intracellular ADP-ribosylation toxin, which can rearrange the host cytoskeleton to increase *Salmonella* invasion and promote systemic transmission [56, 57].

Overall, the *Salmonella* strains prevalent in Chinese pigeon populations were *Salmonella* Typhimurium var. Copenhagen, although these isolates were isolated in different regions and at different times. The analysis of the whole genome sequence characteristics showed that these strains had highly similar antimicrobial resistance genes and virulence genes along with the same sequence type, serotype and plasmid replicons. In addition, the genetic relationship between isolates from different regions is very close, indicating that there may be a pandemic clonal transmission of pigeon salmonellosis in China. Studies have reported that the *Salmonella* Typhimurium var. Copenhagen can be transmitted to humans and other animals [7, 9, 58]. This suggests that *Salmonella* originating from pigeons may pose a threat to human health. Therefore, the prevention and control of salmonellosis should be strengthened in the pigeon industry.

## 5. Conclusions

Using WGS combined with bioinformatics analysis methods, this study provides accurate information on the epidemiology of *Salmonella* in pigeon flocks from different regions of China. Even if there are several limitations to the present study, such as a possible insufficient sample size, the results nevertheless carry significant reference value. We found that *Salmonella* typhimurium Copenhagen variants are the common serotype prevalent in pigeons in different regions of China. Notably, 90.62% of the isolates showed multidrug resistant, which deserves more investigations. Meanwhile, there are large number of virulence genes carried by the isolated strains, indicating a strong pathogenicity and virulence and consequently, a serious harm to pigeon industry. Overall, the present study reveals the urgency of strengthening the research and monitoring of *Salmonella* epidemiology in pigeons.

## Data Availability

The data used to support the findings of this study are included within the article.

## Conflicts of Interest

The authors declare that they have no conflicts of interest.



## Authors' Contributions

Yuhua Zhang, Zheng Lu, Haoyu Zhao, Shuangyu Li, and Hong Zhuang contributed equally to this study.

## Acknowledgments

This study was supported by grants from Xi'an IRIS Livestock Technology Co. Ltd. (K4030218170), China Postdoctoral Science Foundation (2017M610659, 2018T111113), Shaanxi Provincial Natural Science Foundation (2022JQ-158), the State Key Laboratory of Veterinary Etiological Biology, Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences (grant numbers: SKLVEB2016KFKT014), and the Fundamental Research Funds for the Central Universities (2452019053).

## Supplementary Materials

Figure S1: part of the samples in this study were dissected for clinical symptoms. Figure S2: the results of PCR amplification of 16S rDNA gene of some *Salmonella* isolates. Figure S3: phenotypic antimicrobial resistance heat map of *Salmonella* isolates studied. Table S1: PCR primers used in this study. Table S2: summary of detailed data involved in this study. (*Supplementary Materials*)

## References

- [1] S. Yan, Z. Jiang, W. Zhang et al., "Genomes-based MLST, cgMLST, wgMLST and SNP analysis of *Salmonella* Typhimurium from animals and humans," *Comparative Immunology Microbiology and Infectious Diseases*, vol. 96, Article ID 101973, 2023.
- [2] B. Tang, M. Elbediwi, R. B. Nambiar et al., "Genomic characterization of antimicrobial-resistant *Salmonella* enterica in duck, chicken, and pig farms and retail markets in Eastern China," *Microbiology Spectrum*, vol. 10, no. 5, Article ID e0125722, 2022.
- [3] Y.-J. Li, Y.-F. Yang, Y.-J. Zhou et al., "Estimating the burden of foodborne gastroenteritis due to nontyphoidal *Salmonella* enterica, shigella and vibrio parahaemolyticus in China," *Plos One*, vol. 17, no. 11, Article ID e0277203, 2022.
- [4] J. Gong, P. Kelly, and C. Wang, "Prevalence and antimicrobial resistance of *Salmonella* enterica Serovar Indiana in China (1984–2016)," *Zoonoses and Public Health*, vol. 64, no. 4, pp. 239–251, 2017.
- [5] D. Monte, M. A. Nethery, R. Barrangou, M. Landgraf, and P. J. Fedorka-Cray, "Whole-genome sequencing analysis and CRISPR genotyping of rare antibiotic-resistant *Salmonella* enterica serovars isolated from food and related sources," *Food Microbiology*, vol. 93, Article ID 103601, 2021.
- [6] M. C. V. de Oliveira, B. Q. Camargo, M. P. V. Cunha et al., "Free-ranging synanthropic birds (*Ardea alba* and *Columba livia domestica*) as carriers of *Salmonella* spp. and diarrheagenic *Escherichia coli* in the vicinity of an Urban Zoo," *Vector-Borne and Zoonotic Diseases*, vol. 18, no. 1, pp. 65–69, 2018.
- [7] L. Teske, M. Ryll, D. Rubbenstroth et al., "Epidemiological investigations on the possible risk of distribution of zoonotic bacteria through apparently healthy homing pigeons," *Avian Pathology*, vol. 42, no. 5, pp. 397–407, 2013.
- [8] S. Yousef and R. Mamdouh, "Class I integron and beta-lactamase encoding genes of multidrug resistance *Salmonella* isolated from pigeons and their environments," *Cellular and Molecular Biology*, vol. 62, no. 14, pp. 48–54, 2016.
- [9] W. Rabsch, H. L. Andrews, R. A. Kingsley et al., "*Salmonella* enterica serotype Typhimurium and its host-adapted variants," *Infection and Immunity*, vol. 70, no. 5, pp. 2249–2255, 2002.
- [10] C. V. Jawale and J. H. Lee, "An immunogenic *Salmonella* ghost confers protection against internal organ colonization and egg contamination," *Veterinary Immunology and Immunopathology*, vol. 162, no. 1-2, pp. 41–50, 2014.
- [11] M. L. Yang, S. Q. Zheng, K. Peng, W. Wang, Y. H. Huang, and J. Peng, "Isolation and Identification of pigeon-derived *Salmonella* typhimurium and pathogenic analysis," *Acta Veterinaria et Zootechnica Sinica*, vol. 54, no. 11, pp. 4880–4888, 2023.
- [12] D. M. Liang, X. B. Xu, W. F. Wang et al., "Isolation, identification and analysis of serotype and drug resistance of *Salmonella* from pigeon in Shanghai," *China Animal Husbandry & Veterinary Medicine*, vol. 2016, no. 43, pp. 3322–3328, 2016.
- [13] E. Kaczorek-Lukowska, P. Sowinska, A. Franaszek, D. Dziewulska, J. Malaczewska, and T. Stenzel, "Can domestic pigeon be a potential carrier of zoonotic *Salmonella*?" *Transboundary and Emerging Diseases*, vol. 68, no. 4, pp. 2321–2333, 2021.
- [14] M. Vereecken, P. De Herdt, R. Ducatelle, and F. Haesebrouck, "The effect of vaccination on the course of an experimental *Salmonella* typhimurium infection in racing pigeons," *Avian Pathology*, vol. 29, no. 5, pp. 465–471, 2010.
- [15] B. Walther, K. Tedin, and A. Lübke-Becker, "Multidrug-resistant opportunistic pathogens challenging veterinary infection control," *Veterinary Microbiology*, vol. 200, pp. 71–78, 2017.
- [16] Y. Xu, X. Zhou, Z. Jiang, Y. Qi, A. Ed-Dra, and M. Yue, "Antimicrobial resistance profiles and genetic typing of *Salmonella* serovars from chicken embryos in China," *Antibiotics-Basel*, vol. 10, no. 10, Article ID 1156, 2021.
- [17] Y. Wang, Y. Liu, N. Lyu et al., "The temporal dynamics of antimicrobial-resistant *Salmonella* enterica and predominant serovars in China," *National Science Review*, vol. 10, no. 3, 2023.
- [18] S. Pornsukarom, A. H. M. van Vliet, and S. Thakur, "Whole genome sequencing analysis of multiple *Salmonella* serovars provides insights into phylogenetic relatedness, antimicrobial resistance, and virulence markers across humans, food animals and agriculture environmental sources," *Bmc Genomics*, vol. 19, no. 1, Article ID 801, 2018.
- [19] S. Chen, Y. Zhou, Y. Chen, and J. Gu, "fastp: an ultra-fast all-in-one FASTQ preprocessor," *Bioinformatics*, vol. 34, no. 17, pp. i884–i890, 2018.
- [20] A. Bankevich, S. Nurk, D. Antipov et al., "SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing," *Journal of Computational Biology*, vol. 19, no. 5, pp. 455–477, 2012.
- [21] S. Zhang, Y. Yin, M. B. Jones et al., "*Salmonella* serotype determination utilizing high-throughput genome sequencing data," *Journal of Clinical Microbiology*, vol. 53, no. 5, pp. 1685–1692, 2015.
- [22] M. V. Larsen, S. Cosentino, S. Rasmussen et al., "Multilocus sequence typing of total-genome-sequenced bacteria," *Journal of Clinical Microbiology*, vol. 50, no. 4, pp. 1355–1361, 2012.
- [23] A. Carattoli, E. Zankari, A. Garcia-Fernandez et al., "In silico detection and typing of plasmids using plasmid finder and plasmid multilocus sequence typing," *Antimicrobial Agents and Chemotherapy*, vol. 58, no. 7, pp. 3895–3903, 2014.
- [24] B. Liu, D. Zheng, Q. Jin, L. Chen, and J. Yang, "VFDB 2019: a comparative pathogenomic platform with an interactive web

- interface," *Nucleic Acids Research*, vol. 47, no. D1, pp. D687–D692, 2019.
- [25] B. P. Alcock, W. Huynh, R. Chalil et al., "CARD 2023: expanded curation, support for machine learning, and resistance prediction at the comprehensive antibiotic resistance database," *Nucleic Acids Research*, vol. 51, pp. D690–D699, 2023.
- [26] X. Li, X. M. Wang, Y. X. Shao, Z. Wang, F. Ji, and J. G. Huang, "Research progress on pigeon salmonellosis and prevention and control measures of its antibiotic substitutes," *China Animal Husbandry & Veterinary Medicine*, vol. 48, no. 7, pp. 2584–2593, 2021.
- [27] Y. Li, Q. Yang, C. Cao et al., "Prevalence and characteristics of *Salmonella* isolates recovered from retail raw chickens in Shaanxi Province, China," *Poultry Science*, vol. 99, no. 11, pp. 6031–6044, 2020.
- [28] C. Dorn, A. Schroeter, and R. Helmuth, "Report on *Salmonella* isolates submitted to the German National Veterinary Salmonella reference laboratory in the year 1999," *Berliner Und Munchener Tierarztliche Wochenschrift*, vol. 115, no. 7-8, pp. 252–258, 2002.
- [29] G. D. Xie, H. P. Luo, X. Ren, Y. W. Chen, W. Yu, and S. H. Cui, "Isolation, identification and drug resistance analysis of *Escherichia coli* and *Salmonella* in Beijing pigeon meat," *Journal of Food Safety and Quality*, vol. 10, no. 1, pp. 48–53, 2019.
- [30] A. Gargiulo, T. P. Russo, R. Schettini et al., "Occurrence of enteropathogenic bacteria in urban pigeons (*Columba livia*) in Italy," *Vector Borne and Zoonotic Diseases (Larchmont, N.Y.)*, vol. 14, no. 4, pp. 251–255, 2014.
- [31] K. M. Osman, M. Mehrez, A. M. Erfan, and A. Nayerah, "*Salmonella enterica* isolated from pigeon (*Columba livia*) in Egypt," *Foodborne Pathogens and Disease*, vol. 10, no. 5, pp. 481–483, 2013.
- [32] B. Tang, A. Siddique, C. Jia et al., "Genome-based risk assessment for foodborne *Salmonella enterica* from food animals in China: a one health perspective," *International Journal of Food Microbiology*, vol. 390, Article ID 110120, 2023.
- [33] Z. Xu, M. Wang, C. Zhou et al., "Prevalence and antimicrobial resistance of retail-meat-borne *Salmonella* in southern China during the years 2009–2016: the diversity of contamination and the resistance evolution of multidrug-resistant isolates," *International Journal of Food Microbiology*, vol. 333, Article ID 108790, 2020.
- [34] L. J. Wu, Y. Luo, G. L. Shi, and Z. Y. Li, "Prevalence, clinical characteristics and changes of antibiotic resistance in children with nontyphoidal *Salmonella* Infections from 2009–2018 in Chongqing, China," *Infection and Drug Resistance*, vol. 14, pp. 1403–1413, 2021.
- [35] J. Zhu, J. Lv, Z. Zhu et al., "Identification of TMexCD-TOprJ-producing carbapenem-resistant gram-negative bacteria from hospital sewage," *Drug Resistance Updates: Reviews and Commentaries in Antimicrobial and Anticancer Chemotherapy*, vol. 70, Article ID 100989, 2023.
- [36] J. Chen, A. Ed-Dra, H. Zhou, B. Wu, Y. Zhang, and M. Yue, "Antimicrobial resistance and genomic investigation of nontyphoidal *Salmonella* isolated from outpatients in Shaoxing city, China," *Frontiers in Public Health*, vol. 10, Article ID 988317, 2022.
- [37] F. Campioni, F. P. Vilela, G. Cao et al., "Whole genome sequencing analyses revealed that *Salmonella enterica* serovar Dublin strains from Brazil belonged to two predominant clades," *Scientific Reports*, vol. 12, no. 1, Article ID 10555, 2022.
- [38] I. M. Martins, A. A. Seribelli, R. T. Machado et al., "Invasive non-typhoidal *Salmonella* (iNTS) aminoglycoside-resistant ST313 isolates feature unique pathogenic mechanisms to reach the bloodstream," *Infection Genetics and Evolution*, vol. 116, Article ID 105519, 2023.
- [39] S. Song, S. Hwang, S. Lee, N. C. Ha, and K. Lee, "Interaction mediated by the putative tip regions of MdsA and MdsC in the formation of a *Salmonella*-specific tripartite efflux pump," *Plos One*, vol. 9, no. 6, Article ID e100881, 2014.
- [40] F. P. Vilela, P. R. D. Dos, M. W. Allard, and J. P. Falcao, "Genomic analyses of drug-resistant *Salmonella enterica* serovar Heidelberg strains isolated from meat and related sources between 2013 and 2017 in the south region of Brazil," *Current Genetics*, vol. 69, no. 2-3, pp. 141–152, 2023.
- [41] K. O. Akinyemi, C. O. Fakorede, J. Linde et al., "Whole genome sequencing of *Salmonella enterica* serovars isolated from humans, animals, and the environment in Lagos, Nigeria," *Bmc Microbiology*, vol. 23, no. 1, Article ID 164, 2023.
- [42] S. K. Checa, M. Espariz, M. E. Audero, P. E. Botta, S. V. Spinelli, and F. C. Soncini, "Bacterial sensing of and resistance to gold salts," *Molecular Microbiology*, vol. 63, no. 5, pp. 1307–1318, 2007.
- [43] S. Cerminati, G. F. Giri, J. I. Mendoza, F. C. Soncini, and S. K. Checa, "The CpxR/CpxA system contributes to *Salmonella* gold-resistance by controlling the *GolS*-dependent *gesABC* transcription," *Environmental Microbiology*, vol. 19, no. 10, pp. 4035–4044, 2017.
- [44] J. L. Smith, P. M. Fratamico, and X. Yan, "Eavesdropping by bacteria: the role of *SdiA* in *Escherichia coli* and *Salmonella enterica* serovar Typhimurium quorum sensing," *Foodborne Pathogens and Disease*, vol. 8, no. 2, pp. 169–178, 2011.
- [45] J. Volf, M. Sevcik, H. Havlickova, F. Sisak, J. Damborsky, and I. Rychlik, "Role of *SdiA* in *Salmonella enterica* serovar Typhimurium physiology and virulence," *Archives of Microbiology*, vol. 178, no. 2, pp. 94–101, 2002.
- [46] D. J. Eaves, L. Randall, D. T. Gray et al., "Prevalence of mutations within the quinolone resistance-determining region of *gyrA*, *gyrB*, *parC*, and *parE* and association with antibiotic resistance in quinolone-resistant *Salmonella enterica*," *Antimicrobial Agents and Chemotherapy*, vol. 48, no. 10, pp. 4012–4015, 2004.
- [47] G. P. Macias-Farrera, J. R. de Oca, J. Varela-Guerrero et al., "Antibiotics susceptibility of quinolones against *Salmonella* spp. strains isolated and molecularly sequenced for *gyrA* gene," *Microbial Pathogenesis*, vol. 114, pp. 286–290, 2018.
- [48] L. M. Weigel, C. D. Steward, and F. C. Tenover, "*gyrA* mutations associated with fluoroquinolone resistance in eight species of Enterobacteriaceae," *Antimicrobial Agents and Chemotherapy*, vol. 42, no. 10, pp. 2661–2667, 1998.
- [49] K. T. Aung, W. C. Khor, K. H. Ong et al., "Characterisation of *Salmonella* Enteritidis ST11 and ST1925 Associated with Human Intestinal and Extra-Intestinal Infections in Singapore," *International Journal of Environmental Research and Public Health*, vol. 19, no. 9, Article ID 5671, 2022.
- [50] B. K. Khajanchi, N. A. Hasan, S. Y. Choi et al., "Comparative genomic analysis and characterization of incompatibility group FIB plasmid encoded virulence factors of *Salmonella enterica* isolated from food sources," *Bmc Genomics*, vol. 18, no. 1, Article ID 570, 2017.
- [51] I. Rychlik, D. Gregorova, and H. Hradecka, "Distribution and function of plasmids in *Salmonella enterica*," *Veterinary Microbiology*, vol. 112, no. 1, pp. 1–10, 2006.

- [52] S. A. Dos, R. G. Ferrari, and C. A. Conte-Junior, "Virulence factors in *Salmonella* Typhimurium: the sagacity of a Bacterium," *Current Microbiology*, vol. 76, no. 6, pp. 762–773, 2019.
- [53] J. Mambu, E. Barilleau, L. Fragnet-Trapp et al., "Rck of *Salmonella* typhimurium delays the host cell cycle to facilitate bacterial invasion," *Frontiers in Cellular and Infection Microbiology*, vol. 10, Article ID 586934, 2020.
- [54] F. C. Fang, M. A. DeGroote, J. W. Foster et al., "Virulent *Salmonella* typhimurium has two periplasmic Cu, Zn-superoxide dismutases," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 96, no. 13, pp. 7502–7507, 1999.
- [55] M. Nakano, E. Yamasaki, A. Ichinose et al., "*Salmonella* enterotoxin (*Stn*) regulates membrane composition and integrity," *Disease Models & Mechanisms*, vol. 5, no. 4, pp. 515–521, 2012.
- [56] M. L. Lesnick, N. E. Reiner, J. Fierer, and D. G. Guiney, "The *Salmonella* *spvB* virulence gene encodes an enzyme that ADP-ribosylates actin and destabilizes the cytoskeleton of eukaryotic cells," *Molecular Microbiology*, vol. 39, no. 6, pp. 1464–1470, 2001.
- [57] L. Sun, S. Yang, Q. Deng et al., "*Salmonella* Effector SpvB disrupts intestinal epithelial barrier integrity for bacterial translocation," *Frontiers in Cellular and Infection Microbiology*, vol. 10, Article ID 606541, 2020.
- [58] D. Haag-Wackernagel and H. Moch, "Health hazards posed by feral pigeons," *The Journal of Infection*, vol. 48, no. 4, pp. 307–313, 2004.