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From breeder flocks to processing plants: Multilocus sequence typing, antimicrobial resistance, and virulence gene profiles of Salmonella in the broiler production chain in Fujian, China

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Abstract

Salmonella is a major zoonotic pathogen that threatens public health through contaminated food and challenges the sustainability of China's rapidly expanding poultry industry. To investigate the prevalence of *Salmonella* along the broiler production in Fujian Province, 73 isolates recovered from different stages—including great-grandparent (GGP), grandparent (GP), parent stock (PS), commercial broiler (CB) flocks, and poultry processing plants (PPP)—were characterized by serotyping, multilocus sequence typing (MLST), virulence gene detection, and antimicrobial resistance profiling.

Serotyping identified *Salmonella* Enteritidis as the predominant serotype, detected across all stages, with a few isolates of *Salmonella* Gallinarum and *Salmonella* Pullorum. MLST revealed two sequence types: ST11 comprised 90.5% of isolates and was distributed throughout the five production stages, while ST13 accounted for 2.7% and was detected only in processing plants. Four isolates (5.4%) remained untypeable. Among 14 virulence genes tested, the core invasion genes (*invA*, *sipC*, *rck*) showed consistently high detection rates across all stages. The Class I was in GGP, GP, and PS flocks but absent in commercial broilers. The *sopB* gene exhibited a higher detection rate in processing plants, suggesting environmental adaptation. All isolates showed high resistance to ampicillin (72.6%). Resistance rates to doxycycline, tetracycline, and chloramphenicol in PPP samples (60.0%, 40.0%, and 60.0%, respectively) were significantly higher than in upstream breeding stages ($P < 0.05$). Carbapenem resistance was detected in GGP (5.4%) and PS (4.1%) flocks. The overall multidrug-resistant rate (MDR) was 36.9%, including three pandrug-resistant isolates.

These findings indicate that *Salmonella* Enteritidis ST11 is the dominant clone widely distributed across the broiler production chain, with the breeding stage serving as a primary reservoir of highly conserved virulence genes. The results provide a theoretical basis for improved farming practices, rational antimicrobial use, and *Salmonella* prevention and control in the poultry industry.

1. Introduction

Salmonella is a zoonotic pathogen that can be transmitted through multiple routes and is the leading causative agent of foodborne disease outbreaks linked to microbial pathogens in mainland China. *Salmonella* infection not only leads to decreased production performance and even death in poultry, causing severe economic losses to the poultry industry, but also contaminates the food chain, posing a serious threat to public health [1]. *Salmonella* contamination occurs predominantly in meat products, and chicken is particularly susceptible to *Salmonella* contamination during production and processing. Although various prevention and control measures have been implemented, including *Salmonella* eradication programs and the use of vaccines and antimicrobials [2], *Salmonella* contamination remains a major food safety concern worldwide.

The genus *Salmonella* primarily comprises two species: *Salmonella* bongori and *Salmonella* enterica. Based on serological differences, over 2,600 distinct serotypes have been identified to date [3]. Among these, *Salmonella* Enteritidis and *Salmonella* Typhimurium are classified as the most important foodborne pathogens. They are the most common worldwide and can infect both humans and various animals. *Salmonella* Typhimurium can cause systemic diseases similar to typhoid fever, while *Salmonella* Enteritidis is the primary pathogen responsible for acute gastroenteritis in humans [4]. *Salmonella* Enteritidis has host-specific variants, namely *Salmonella* Gallinarum and *Salmonella* Pullorum, which exclusively infect poultry, causing fowl typhoid and pullorum disease, respectively, and inflicting severe economic losses on the global poultry industry, including in China [5]. In China, *Salmonella* Enteritidis is one of the most frequently detected dominant serotypes in poultry meat products [6]. The primary sources of infection are poultry and poultry products; contaminated eggs have been confirmed as the main route of human infection [7]. Controlling this strain in poultry is challenging, as outbreaks often concentrate in chicks [8], while adult chickens frequently act as asymptomatic carriers, continuously shedding the pathogen through feces, leading to environmental contamination and widespread transmission within flocks. Other serotypes, such as *Salmonella* Kentucky (S. Kentucky), *Salmonella* Infantis (S. Infantis), and *Salmonella* Heidelberg (S. Heidelberg), have also been reported in chicken flocks [9].

The prevention and control of *Salmonella* infections have long relied on antimicrobial drugs; the frequent and inappropriate use of these drugs has directly led to the continuous increase in drug resistance and the constant emergence of multidrug-resistant (MDR) strains. This issue of bacterial resistance has become a serious threat to global public health and animal health, imposing a heavy economic burden [10]. The mechanisms underlying the emergence and transmission of bacterial resistance are complex, and resistance can be horizontally transmitted between different strains via mobile genetic elements such as plasmids and transposons [11]. Human activities have significantly accelerated this process, transforming the problem of resistance in animal husbandry into a severe public health crisis. Currently, reports of *Salmonella* strains resistant to key clinical drugs such as fluoroquinolones and broad-spectrum cephalosporins are frequent worldwide, and the trend toward multidrug resistance is becoming increasingly severe. In particular, in some regions of Asia, the resistance rate of *Salmonella* in Vietnam to ampicillin has reached as high as 80.4% [12]. The widespread use of antibiotics has exposed food-producing animals to infections caused by multidrug-resistant bacteria [13]. Recently, major human clinical resistance genes, such as mcr-1, have even been detected in zoonotic pathogens of animal origin [14]. Although the Chinese government is actively promoting policies to “reduce and restrict antibiotic use,” antimicrobial agents remain a key option in the current prevention and control system [15], which further exacerbates the global pressure for the proliferation of MDR strains.

Salmonella not only comprises numerous serotypes but also exhibits significant variations in resistance profiles across different serotypes [16], making the prevention and control of *Salmonella* infections particularly challenging. Multilocus sequence typing (MLST) facilitates cross-temporal and geographical comparisons and is a commonly used typing method for monitoring *Salmonella* both domestically and internationally [17–19]. To address the increasingly severe challenge of *Salmonella* resistance, and given the significant differences among serotypes in terms of pathogenicity, host adaptation, and transmission characteristics, systematically elucidating the serotype distribution, virulence features, and resistance mechanisms of circulating strains throughout the entire poultry production chain holds critical scientific significance for formulating precise prevention and control strategies and ensuring the safety of food of animal origin. Therefore, in this study, *Salmonella* isolates were recovered from different stages of the chicken production (ranging from grandparent flocks to slaughtering stages). MLST was performed to identify the predominant sequence types (STs). Through serotyping, detection of virulence factors, and antimicrobial susceptibility testing, the genetic relatedness, antimicrobial resistance profiles, and potential pathogenic characteristics of the isolates were systematically evaluated, aiming to provide a scientific basis for the establishment of targeted surveillance systems and effective intervention measures.

2. Materials and Methods

2.1 Identification and serotyping of Salmonella isolates

In 2025, a total of 137 samples were collected from great-grandparent (GGP), grandparent (GP), parent stock (PS), and commercial broiler (CB) flocks in large-scale poultry farms in Fujian Province, China. All birds were euthanized humanely by intramuscular injection of sodium pentobarbital at a dosage of 100 mg/kg body weight.This method was approved by the institutional ethics committee (Approval No.: PZCASFAFU24011) and is consistent with the AVMA Guidelines for the Euthanasia of Animals. The samples included liver, intestine, oviduct, cloaca, live embryos, and dead embryos from sick and healthy-looking chickens suspected of *Salmonella* infection, as well as meat samples from poultry processing plants (PPP). From these, 73 *Salmonella* isolates were recovered and purified. To isolate *Salmonella*, the collected disease samples or cloacal swabs were transported directly to the laboratory. Each sample was inoculated into 4.5 mL of buffered peptone water (BPW; Land Bridge, China). After incubation at 37°C for 16 h, 0.1 mL and 0.5 mL of the BPW culture were transferred into 4.5 mL of selenite cystine (SC) broth and incubated at 37°C for an additional 18–24 h. The enriched cultures were streaked onto xylose-lysine-deoxycholate (XLD; Land Bridge, China) agar. After 16–24 h of incubation, single colonies were selected, inoculated into LB broth, and incubated at 37°C for 16 h. Typical *Salmonella* colonies were then confirmed by PCR amplification of the *stn* gene. The primers used were: stn-F, 5'-CTTTGGTCGTAAAATAAGGCG-3' and stn-R, 5'-TGCCCAAAGCAGAGAGATTC-3'. Serotypes were determined by slide agglutination tests using O-antigen and H-antigen antisera.

2.2 Antimicrobial resistance analysis

A total of 73 *Salmonella* isolates collected from great-grandparent (GGP, n = 34), grandparent (GP, n = 10), parent stock (PS, n = 11), commercial broiler (CB, n = 13) flocks, and poultry processing plants (PPP, n = 5) were tested for susceptibility to 10 antimicrobial agents. The following antibiotics were provided by Hangzhou Microbial Reagent Co., Ltd. (Hangzhou, Zhejiang, PR China): penicillins (ampicillin, AMP, 10 μg); cephems (cefotaxime, CTX, 30 μg); carbapenems (ertapenem, ETP, 10 μg; meropenem, MEM, 10 μg); macrolides (azithromycin, AZM, 15 μg); tetracyclines (tetracycline, TCY, 30 μg; doxycycline, DOX, 30 μg); quinolones (ciprofloxacin, CIP, 5 μg); amphenicols (chloramphenicol, CHL, 30 μg); and a folate pathway inhibitor (trimethoprim-sulfamethoxazole, TMP, 23.75 μg). *Escherichia coli* ATCC 25922 was used for quality control.After measuring the diameters of the inhibition zones, the results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (https://clsi.org/). Isolates resistant to three or more classes of antimicrobial agents were defined as multidrug-resistant (MDR) strains.

2.3 Multilocus sequence typing (MLST) and phylogenetic analysis

To analyze the genetic diversity, MLST was performed. The isolates were grown overnight in Luria-Bertani (LB) broth at 37°C, and genomic DNA was extracted using a magnetic bead-based nucleic acid extraction kit (Tianlong Technology, Xi'an, China). Seven housekeeping genes (*aroC, dnaN, hemD, hisD, purE, sucA, and thrA*) were amplified by PCR for each isolate [20]. The PCR products were sequenced using bidirectional DNA sequencing. The obtained sequences were analyzed using the *Salmonella* MLST database (http://enterobase.warwick.ac.uk/species/senterica/allele_st_search), and allele STs were assigned accordingly.A phylogenetic tree was constructed using MEGA 11 cluster analysis.

3. Results

3.1 Serotyping of Salmonella isolates from chickens

A total of 73 isolates were confirmed as *Salmonella*, including 34 from GGP flocks, 10 from GP flocks, 11 from PS flocks, 13 from CB flocks, and 5 from PPP. All isolates were further serotyped by agglutination tests. All isolates belonged to serogroup D. Among them, two isolates were identified as *Salmonella* Gallinarum from CB flocks and a processing plant, four isolates as *Salmonella* Pullorum from GP flocks and a processing plant, and the remaining 68 isolates as *Salmonella* Enteritidis (Table 1).

Table 1 *Salmonella* sources and their corresponding serotypes and STs

Group	GGP (n=34)	GP（n=10）	PS (n=11)	CB (n=13)	PPP (n=5)
ST	11	11	11	11\ND	11\13\ND
Serovar	SE	SE	SE	SE	SE
Source	Cloaca (n=11)	Healthy Chick (n=10)	Cloaca (n=1)	Cloaca (n=10)	Frozen Meat (n=5)
	Hatcher (n=2)		Liver (n=1)	Liver (n=1)	
	Liver (n=7)		Dead Embryo (n=1)	Dead Embryo (n=1)	
	Oviduct (n=1)		Live Embryo (n=8)	Live Embryo (n=1)	
	Healthy Chick (n=2)				
	Tracheal Swab (n=6)				
	Skeleton (n=1)				
	Intestine (n=4)				

Group：GGP, great-grandparent; GP, grandparent; PS, parent stock; CB, commercial broiler; PPP, poultry processing plant. Samples were collected from each production stage.

3.2 Antimicrobial resistance of *Salmonella* isolates from different production stages

The susceptibility of the *Salmonella* isolates to 10 antimicrobial agents was determined using the disk diffusion method. The antimicrobial susceptibility results are shown in (Table 2). Overall, the isolates exhibited the highest resistance rate to ampicillin (72.6%), which was observed across all production stages. This was followed by resistance to doxycycline (41.0%), chloramphenicol (23.2%), trimethoprim (21.9%), azithromycin (21.9%), tetracycline (19.1%), ciprofloxacin (16.4%), cefotaxime (13.6%), ertapenem (8.2%), and meropenem (8.2%). Ampicillin was the antibiotic with the highest resistance rate at each production stage (GP, 100%; GGP, 64.7%; PS, 81.8%; CB, 76.9%; PPP, 40%). Significantly elevated resistance rates to doxycycline and tetracycline were observed in PS and PPP stages. Ciprofloxacin also showed higher resistance rates in PS (27.3%) and PPP (20%). Ertapenem resistance was detected in all stages except CB, with the highest rate reaching 20%. Chloramphenicol exhibited a resistance rate as high as 80% in PPP, substantially exceeding those in other stages (Figure 1). A total of seven pan-susceptible isolates were identified, and 27 distinct antimicrobial resistance patterns were observed. Three pandrug-resistant (PDR) isolates were detected, all of which originated from the GGP stage. MDR rate was 36.9% (Table 3).

Table 2 Overall antimicrobial resistance profile of the 73 *Salmonella* isolates.

Antimicrobial agents	Resistance rate（%）
AMP	72.6 (n=53)
DOX	41.0 (n=30)
CHL	23.2 (n=17)
TMP	21.9 (n=16)
AZM	21.9(n=16)
TCY	19.1(n=14)
CIP	16.4 (n=12)
CTX	13.6(n=10)
ETP	8.2(n=6)
MEM	8.2(n=6)

Abbreviations: AMP, ampicillin; DOX, doxycycline; CHL, chloramphenicol; TMP, trimethoprim-sulfamethoxazole; AZM, azithromycin; TCY, tetracycline; CIP, ciprofloxacin; CTX, cefotaxime; ETP, ertapenem; MEM, meropenem.

Table 3 Resistance patterns among the 73 *Salmonella* isolates.

Resistance patterns	Group					
	GP	GGP	PS	CB	PPP	Total
Pan-S	0	4	0	3	0	7
AMP	6	11	1	4	0	22
AMP-DOX	1	2	1	2	0	6
AMP-TMP	0	0	0	1	0	1
AMP-AZM	0	0	0	2	0	2
AMP- CHL	1	0	0	1	0	2
AMP-CIP-CHL	0	1	0	0	0	1
AMP-CIP-DOX	0	0	3	0	0	3
AMP-DOX-TCY	0	0	1	0	0	1
CIP-DOX-CTX	0	1	0	0	0	1
AMP-CTX-AZM	0	0	0	1	0	1
TPM-DOX-AZM	0	1	0	0	0	1
DOX-TCY-CHL	0	0	0	0	2	2
AMP-TMP-CIP-DOX	0	1	0	0	0	1
AMP-TPM-DOX-TCY	0	0	1	0	0	1
AMP-DOX-CTY-AZM	0	1	0	0	0	1
TMP-DOX-CHL-AZM	0	1	0	0	0	1
TMP-CIP-DOX-TCY-ETP	0	0	0	0	1	1
AMP-CIP-DOX-AZM-MEM	0	0	1	0	0	1
AMP-DOX-TCY-CTX-AZM	0	1	0	0	0	1
AMP-DOX-TCY-CHL-CTX	0	0	0	0	1	1
AMP-TMP-DOX-TCY-CHL	0	0	0	0	1	1
AMP-CIP-DOX-TCY-CHL-CTX	0	1	0	0	0	1
AMP-TMP-DOX-TCY-CHL-CTX	0	0	0	1	0	1
AMP-DOX-TCY-CHL-AZM-MEM	0	0	1	0	0	1
XDR	1	0	0	0	0	1
PDR	0	3	0	0	0	3

Abbreviations: AMP, ampicillin; DOX, doxycycline; TMP, trimethoprim-sulfamethoxazole; CTX, cefotaxime; CHL, chloramphenicol; CIP, ciprofloxacin; TCY, tetracycline; AZM, azithromycin; ETP, ertapenem; MEM, meropenem; Pan-S, pan-susceptible strain; XDR, extensively drug-resistant (resistant to all but one or two classes); PDR, pandrug-resistant;A total of 27 MDR strains (resistant to ≥3 antimicrobial classes).

3.3 MLST genotypes of Salmonella isolates

The MLST results, shown in (Figure 2), identified two distinct sequence types (STs). ST11 comprised 68 isolates (91.8%) and was distributed across all five production stages. ST13 accounted for only two isolates (2.7%) and was detected exclusively in

the processing stage. Four isolates (5.4%) remained untypeable, which were recovered from commercial broilers (n=2) and processing plants (n=2). ST11 isolates originated from diverse sources, including live embryos, dead embryos, healthy chicks, cloacal swabs, tracheal swabs, liver, intestine, and processing environment samples.In the GGP stage, ST11 isolates were widely detected in cloacal swabs, visceral organs, embryos, and environmental samples from breeder birds, suggesting that this clone has established deep colonization in grandparent flocks. The simultaneous detection of ST11 and ST13 in the slaughtering stage indicates the possibility of contamination from multiple sources at this stage. The persistent presence of ST11 across all production stages suggests that this clone is capable of both vertical and horizontal transmission along the production chain, exhibiting strong environmental adaptability and survivability.

3.4 Virulence gene distribution in Salmonella by production stage

A total of 14 virulence-associated genes were detected in the 73 *Salmonella* isolates. Among these, the detection rates of *invA*, *hilA*, *stnp1*, *sipA*, *rck*, *sipC*, *spvC*, *sopE*, and *ssrA* exceeded 85%. The lowest detection rate was observed for *ssaR* (20.5%), followed by *sopB* (35.6%) , Class I integrons (12.2%). In the GGP, GP, PS, and CB groups, most virulence genes showed high and similar detection rates. (Table 4).

Table 4 Prevalence of virulence-associated genes among 73 *Salmonella* isolates.

Gene	GGP (n=34)	GP (n=10)	PS (n=11)	CB (n=13)	PPP (n=5)	Total (%)
invA	34	9	+	12	+	97.2
rck	31	9	+	11	+	91.7
sipC	29	9	+	11	+	89.0
sopE	32	7	+	12	3	89.0
hilA	32	+	+	+	+	98.6
pefA	30	8	9	7	4	79.4
sipA	31	+	+	+	+	95.8
ssaR	8	2	1	3	1	20.5
spvC	30	8	10	11	+	87.6
ssrA	30	9	10	12	4	89.0
stnp1	33	+	10	12	+	95.8
sefA	30	8	8	11	+	84.9
sopB	11	2	4	8	1	35.6
Class I	3	1	1	4	-	12.2

[+] indicates the presence of the gene; - indicates that the gene does not exist

4. Discussion

Numerous serotypes of *Salmonella* are currently recognized, and different serotypes cause distinct diseases in humans and animals. Furthermore, the distribution of *Salmonella* serotypes varies considerably by country and region. In Egypt and India, *Salmonella* Enteritidis and *Salmonella* Typhimurium are the most commonly reported serotypes, whereas *Salmonella* Infantis, *Salmonella* Manhattan, and *Salmonella* Schwarzengrund have been detected in the cecal samples of broiler chickens in Japan [21]. In China, *Salmonella* Enteritidis and *Salmonella* Typhimurium are the most prevalent serotypes, with *Salmonella* accounting for approximately 70%–80% of foodborne disease outbreaks and causing an estimated 9.87 million cases of gastroenteritis annually [22]. Consistent with these reports, all serotypes identified in the present study belonged to serogroup

D, including *Salmonella* Pullorum, *Salmonella* Gallinarum, and *Salmonella* Enteritidis, with the vast majority being *Salmonella* Enteritidis. *Salmonella* Pullorum primarily infects chicks up to 20 days of age, causing acute septicemia [23], with high morbidity and mortality, whereas adult chickens often remain asymptomatic carriers [24]. As broiler chickens are an introduced breed, many European and North American countries have successfully achieved the eradication of pullorum disease in commercial breeder flocks, and China is also implementing pullorum disease eradication programs. In this study, four *Salmonella* Pullorum isolates were recovered from cloacal swabs of GGP birds (28.5%) and from the processing stage. The detection of *Salmonella* Gallinarum in the processing stage is suspected to result from exogenous contamination at this stage. In contrast, *Salmonella* Enteritidis was detected throughout all production and breeding stages and across all sample types, indicating its high epidemic potential and host adaptability. This may also be attributable to the current lack of effective control measures against *Salmonella* Enteritidis infection in China. *Salmonella* Enteritidis is one of the major foodborne pathogens and causes gastroenteritis in humans [25]. We therefore propose that the detection of *Salmonella* Enteritidis at all production stages underscores the urgent need to strengthen prevention and control measures to minimize its entry into the human food chain.

Antimicrobial resistance in *Salmonella* is continuously increasing, and MDR strains are emerging at an alarming rate, with a growing number of reports on *Salmonella* resistance [26-28]. This has not only made the prevention and control of *Salmonella* more challenging but has also posed a serious threat to public health. In the present study, *Salmonella* isolates from all production and breeding stages in this region exhibited a high resistance rate to ampicillin (72.6%), indicating the extensive use of penicillins in animal husbandry. The resistance rates to tetracyclines in PS flocks and slaughterhouses were significantly higher than those in upstream breeding stages, which may be associated with regional antimicrobial use practices, drug accumulation, or environmental factors. Carbapenems are strictly prohibited for use in animal husbandry worldwide. Therefore, the detection of carbapenem resistance in GGP, GP, and PS stages suggests exogenous introduction. Such introduction likely originates from human healthcare settings, where carbapenemase-producing strains enter the farming environment. The genes encoding these enzymes are typically located on mobile genetic elements, facilitating their cross-species and cross-host transmission [29]. The emergence of such resistance at the breeding stage implies that contamination or resistance genes may be present at the genetic stock level and could be transmitted vertically through hatching eggs. This situation urgently requires enhanced prevention and control measures. Of note, the present study revealed a trend of increasing MDR along the production chain, indicating progressive accumulation of resistance traits throughout the breeding and processing continuum.

MLST genotyping revealed the distribution of *Salmonella* STs along the chicken meat production chain, identifying *Salmonella* Enteritidis ST11 as the dominant clone, present throughout the entire continuum from breeding flocks to final chicken meat products. This finding is consistent with numerous national and international studies [30-32]. ST11 is widely recognized as the major epidemiological type of avian-derived *Salmonella* worldwide and has been circulating in Japan, Iran, and Brazil for decades [33, 34], demonstrating strong host adaptability and transmissibility. The high detection rate of ST11 in GGP flocks suggests that breeding birds may serve as the primary reservoir and source of dissemination for this clone, which can spread to downstream stages through both vertical and horizontal transmission, ultimately contaminating end products. ST11 strains were isolated from a wide range of sample types, including live embryos, dead embryos, healthy chicks, cloacal swabs, and visceral organs.This indicates that this clone can not only be asymptomatically carried in chicken flocks but also cause embryonic death and visceral colonization, demonstrating strong pathogenic potential and tissue invasiveness. These findings provide a basis for developing targeted eradication measures, underscoring the need for enhanced monitoring and culling of ST11-positive birds in breeding flocks. ST13 strains have been reported in China, Pakistan, and Lahore, among other regions, and in public databases they primarily correspond to *Salmonella* Agona [31, 35, 36]. Furthermore, it is noteworthy that two ST13 strains identified during the slaughter process were serologically typed as *Salmonella* Enteritidis. This may indicate that rare gene transfer or recombination events have occurred among local strains, resulting in the integration of serotype antigen genes into the ST13 clonal background. This finding demonstrates that in practical monitoring and traceability efforts, one cannot rely solely on MLST-based ST typing to definitively predict serotypes; traditional serotyping or serotype prediction via whole-genome sequencing remains indispensable. The present study found that ST13 was present only in the processing stage, whereas ST11 was also detected at this stage, suggesting that the processing stage may serve as a cross-

contamination node for *Salmonella* of different origins. Therefore, hygiene management in the processing environment should be strengthened to prevent the spread and genetic recombination of different clones.

Salmonella possesses a variety of virulence factors, including pathogenicity islands, virulence plasmids, structural virulence factors, and enterotoxins, all of which are closely associated with its pathogenicity. The present study systematically analyzed the distribution of *Salmonella* virulence genes along the chicken meat production chain, revealing patterns of gene retention and variation from the breeding source to the processing stage. The pathogenicity of *Salmonella* is primarily reflected in its ability to invade non-phagocytic cells, survive within phagocytes, and replicate within these cells; these processes are closely associated with virulence factors encoded in *Salmonella* pathogenicity islands (*SPI*s) [37]. The high detection rates of *invA*, *hilA*, and *sipA* (all >93%) indicate that the isolates possess strong capabilities for cell invasion, intracellular survival, and immune evasion.This finding is highly consistent with the fundamental pathogenic mechanisms of *Salmonella* and suggests a risk of continued transmission of these strains within the production chain. When *Salmonella* enters intestinal epithelial cells, environmental factors such as high osmotic pressure and neutral pH activate *hilD*, thereby inducing the expression of the *hilA* and *invF* genes [38]. As a transcriptional regulator, *hil*A further activates the translocation of all *SPI* 1 genes required for T3SS assembly [39], along with various *SPI*1 effector proteins, host-interacting proteins (*Sop* proteins, *Sip*A), and *Dks*A, to coordinate the NAD(P)H/NAD(P)+ redox balance under nutrient-limited conditions [40]. Differences in the distribution of virulence genes across different production stages reflect the adaptive evolution of strains during transmission.The *sefA* gene is a major component of the *Salmonella sef* operon and encodes the major subunit protein of the *sef*14 fimbria [41], which enhances the virulence of *Salmonella* Enteritidis [42]. Both *Salmonella* Pullorum and *Salmonella* Typhimurium possess complete *sef* operons but do not express the *sef*14 fimbria [43]. The absence of *sefA* in downstream stages may be related to this phenomenon and could also be associated with reduced selective pressure on fimbriae in non-host environments. The *Sop*B protein alters host cell exocytosis [44], and the relative enrichment of *sopB* in CB may be linked to its role in biofilm formation or responses to environmental stress, warranting further investigation into the relationship between its function and environmental adaptability. Studies have shown that the *SPI1-T3SS* effector protein *SopE* increases *Salmonella* invasiveness and induces a strong inflammatory host response [45]. Class I integrons are mobile genetic elements. Although their overall detection rate was low (12.2%), they were distributed across multiple stages and were also detected in multidrug-resistant strains, suggesting that antibiotic resistance genes may be transmitted between different strains via mobile genetic elements, indicating the potential co-transfer of virulence and resistance determinants. The present study found that the virulence gene profile of *Salmonella* detected GGP breeding flocks encompassed nearly all high-frequency virulence factors. This indicates that source control is of critical importance for blocking the transmission of virulence genes within the production chain. We therefore recommend that *Salmonella* surveillance programs not only focus on strain detection rates but also incorporate virulence gene profiling, particularly in high-risk segments such as breeding flocks and processing environments, to enable the implementation of targeted prevention and control measures.

5. Conclusion

Salmonella serogroup D was the major subgroup, and Salmonella Enteritidis was the most common serotype in this region. The overall resistance rate was highest for ampicillin. Resistance rates to tetracyclines and chloramphenicol were higher in the processing stage than in upstream breeding stages, and the MDR rate showed an increasing trend along the production chain. MLST analysis revealed that the vast majority of Salmonella isolates belonged to ST11, which was detected in a wide range of sample types, whereas ST13 was found only in the processing stage. These findings suggest that ST11 possesses strong pathogenic potential and tissue invasiveness, and that cross-contamination with Salmonella from different sources may occur at the processing stage.These findings indicate that the breeding stage serves as a major reservoir of antimicrobial resistance and virulence genes.

Abbreviations

GGP

great-grandparent
GP
grandparent
PS
parent stock
CB
commercial broiler
PPP
poultry processing plant
MDR
multidrug-resistant
MLST
Multilocus Sequence Typing
STs
Sequence types.

Declarations

Ethics approval and consent to participate

Informed consent was obtained from all farm owners for the collection of samples from their flocks. This study was approved by the Academic Committee of Fujian Agriculture and Forestry University (Approval No.: PZCASFAFU24011), which serves as the institutional ethics review body for animal experiments at Fujian Agriculture and Forestry University. All animal procedures were conducted in accordance with the institutional guidelines for the care and use of laboratory animals and the regulations of Fujian Province.

Consent for publication

Not applicable This manuscript does not contain any individual person's data, clinical images, or case reports. No human participants, identifiable human tissue, or individual animal data are included in this study.

Availability of data and materials

The 16S rRNA sequences sequencing data have been deposited in the NCBI SRA database under BioProject accession number PRJNA1474247.The others datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declaration of Competing Interest

The authors declare that they have no conflicts of interest related to this study. Specifically, they have no financial, personal, or professional affiliations that could influence or bias the research outcomes presented in this manuscript.

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Not applicable.

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Figures

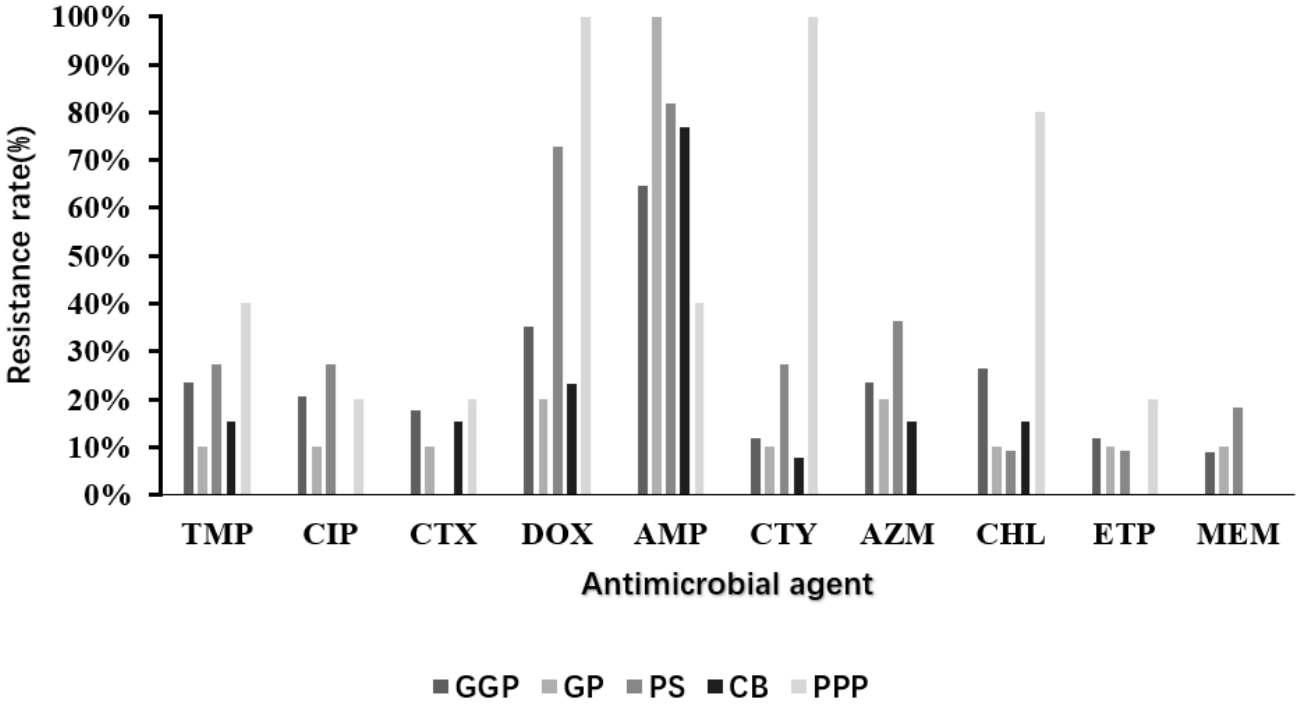

Figure 1

Antimicrobial resistance profiles of *Salmonella* isolates by production stage.Ertapenem resistance was detected in all stages except CB, with the highest rate reaching 20%. Chloramphenicol exhibited a resistance rate as high as 80% in processing plants (PPP), substantially exceeding those in other stages

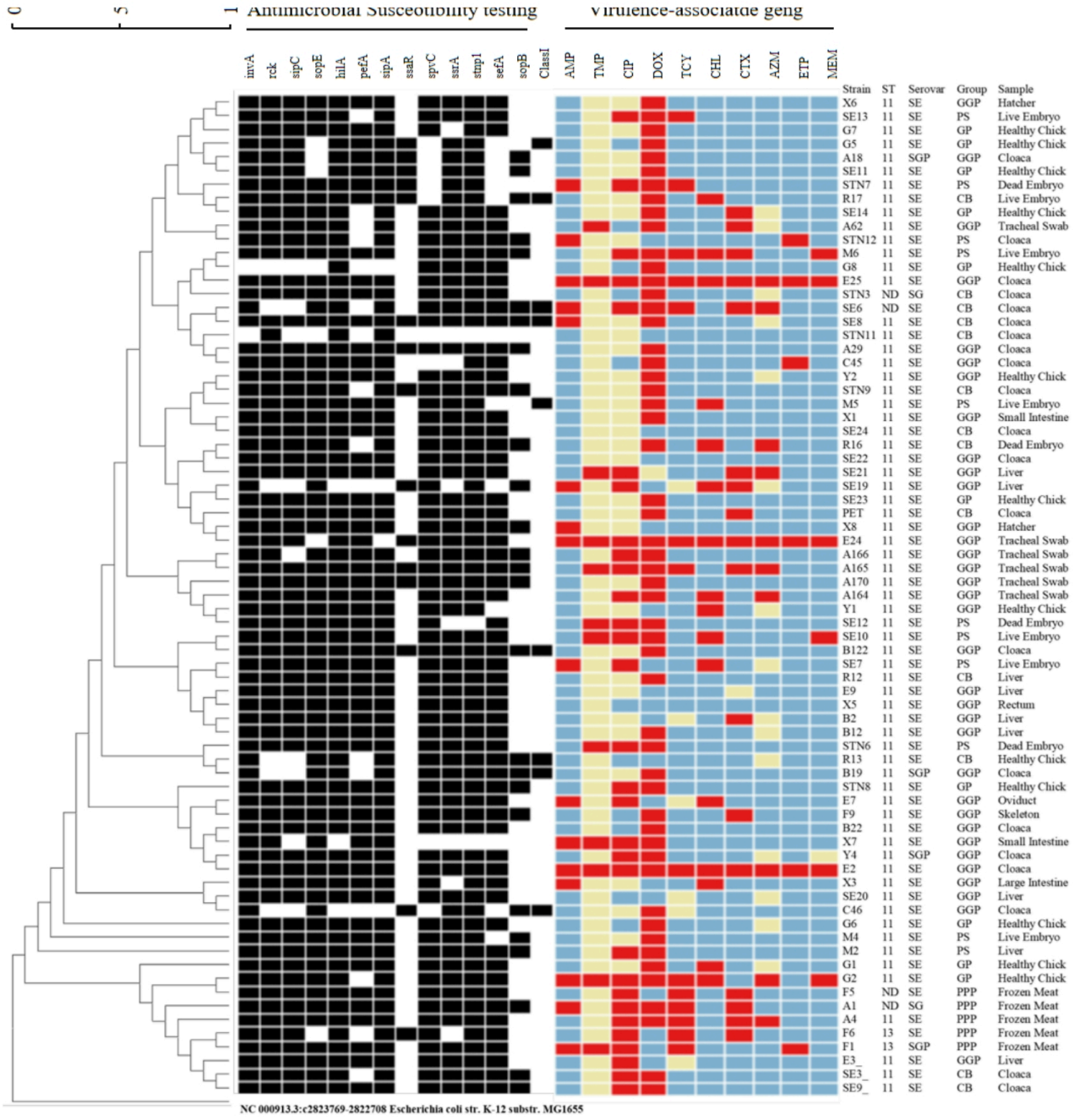

Figure 2

Epidemiological heatmap of 73 *Salmonella* isolates. From left to right: cluster analysis of the isolates; virulence gene profiles (black, positive; white, negative); antimicrobial resistance phenotypes (blue, susceptible; yellow, intermediate; red, resistant); strain ID; corresponding sequence type (ST); serotype; production stage; and sample source.