Epidemiological investigation on drug resistance of *Salmonella* isolates from duck breeding farms in Shandong Province and surrounding areas, China

Xinyuan Zhao,[*,†,‡] Wei Li,[*,†,‡] Shaopeng Hou,[*,†,‡] Yanjun Wang,[*,†,‡] Shuyang Wang,[*,†,‡] Jing Gao,[*,†,‡] Ruihua Zhang,[*,†,‡] Shijin Jiang,[*,†,‡] and Yanli Zhu [*,†,‡,1]

[*]*Department of Preventive Veterinary Medicine, College of Veterinary Medicine, Shandong Agricultural University, Taian 201718, China;* [†]*Shandong Provincial Key Laboratory of Animal Biotechnology and Disease Control and Prevention, Taian 201718, China; and* [‡]*Shandong Provincial Engineering Technology Research Center of Animal Disease Control and Prevention, Taian 271018, China*

ABSTRACT Duck salmonellosis is a common acute septic infectious disease that spreads rapidly, with serious harm to the duck breeding industry and public health. To date, there are few reports about the epidemiological characteristics of drug resistance in *Salmonella* from ducks. In this study, an epidemiological investigation was conducted on drug resistance of 110 *Salmonella* strains isolated from multiple duck farms in Shandong Province and surrounding areas, China. The multidrug-resistant (**MDR**) rate for 110 *Salmonella* strains was up to 71.82% (79/110), and 12 types of drug resistance genes were detected in all isolates, including β-lactams, aminoglycosides, tetracycline, macrolides, and quinolones resistance genes. Using the multilocus sequence typing (**MLST**) based on 7 housekeeping genes, 13 various ST types were identified among all strains, and ST19 (32/110, 29.09%) was the primary type. As the dominant serotypes, *S. Kottbus* and *S. Typhimurium* were divided into multiple ST types. A total of 6 kinds of plasmid incompatibility groups were carried in the *Salmonella* strains, of which IncFIIs (29/110, 26.36%) was most prevalent, and the class I integrons were detected in 78.18% (86/110) of strains. Furthermore, we found that some drug resistance genes, plasmid incompatibility groups, and class I integrons coexist in the same strain. This phenomenon indicates that class I integrons and plasmids are important ways for the spread of drug resistance genes. Therefore, the spread of antibiotic resistance in *Salmonella* had been facilitated, especially erythromycin (108/110, 98.18%), streptomycin (93/110, 84.54%), and tetracycline (53/110, 48.18%). The above research results broadened ideas and provided directions for the transmission mechanism of *Salmonella* resistance.

Key words: *Salmonella*, drug resistance, multilocus sequence typing, plasmid typing, class I integron

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INTRODUCTION

Salmonella is a common zoonotic pathogen (Liu et al., 2018). Up to now, more than 2,600 *Salmonella* serovars have been discovered all over the world (Xiong et al., 2020). Duck salmonellosis can be transmitted vertically and horizontally, and it has a high fatality rate for ducklings (Adzitey et al., 2012; Soria et al., 2017; Wang et al., 2020). Over the past several decades, broad-spectrum antimicrobials such as aminoglycosides, β-lactams, and quinolones are often used as the first choice to treat bacterial infections (Wong et al., 2014; Kuang et al., 2015; Lin et al., 2015). However, due to the long-term abuse and misuse of antibiotics, the phenomenon of multidrug resistance has become more and more serious in recent years (dos Reis et al., 2018; Qiao et al., 2018). According to reports, the multidrug-resistant (**MDR**) rates of *Salmonella* isolated from patients, chicken, and swine all exceeded 70% (Kalonji et al., 2015; Song et al., 2020; de Azevedo et al., 2021).

At present, a host of molecular typing techniques have been widely used in the traceability analysis of pathogenic microorganisms (Achtman et al., 2012), such as multilocus sequence typing (**MLST**). MLST is convenient and has high efficiency, repeatability and feasibility, conducive to acquiring experimental data (Feijao et al., 2017; Jain et al., 2018). Drug resistance genes flowing between different bacteria strains closely related to bacterial plasmids and class I integrons. A PCR-based replicon typing (**PBRT**) method was successfully established to identify HI1, HI2, I1, X, L/M, N,

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[1]Corresponding author: ylz@sdau.edu.cn

FIA, FIB, W, Y, P, FIC, A/C, T, FIIs, FrepB, K, and B/O replicons through 5 multiplex PCR and 3 single PCR with 18 pairs of primers (Carattoli et al., 2005). Class I integrons are most commonly embedded in diverse and highly mobile elements and, thereby, have become broadly distributed drug-resistant genes amongst the Gram-negative bacteria (Hall, 2012).

Compared with other researches, the epidemiological investigations of antimicrobial resistance in *Salmonella* from ducks have rarely been reported systematically. This study used antimicrobial susceptibility testing, PCR, MLST, and plasmid typing to conduct epidemiological investigations about drug resistance and class I integrons on *Salmonella* isolated from duck farms in Shandong Province and surrounding areas, China. Consequently, this study is vital to reveal the drug resistance characteristics of *Salmonella* from ducks and will lay the foundation for the follow-up study of drug resistance mechanisms.

MATERIALS AND METHODS

Sample Collection

From June 2020 to June 2021, 110 *Salmonella* strains were isolated from ground embryonic tissues suspensions, cloacal cotton swabs of duck bodies, and the living environment (water, feed, soil, and manure) from multiple large-scale duck breeding farms in Shandong Province and surrounding areas, China, and their serotype distribution was shown in Table 1. All duck embryos were dead and weighed between 180.54 g and 190.33 g (average weight 186.75 g ± 5.4 g).

Antimicrobial Susceptibility Testing

The sensitivity of 110 *Salmonella* strains to commonly used antibiotics was detected by the Kirby-Bauer disk diffusion method. The following 15 antimicrobials provided by Hangzhou Microbial Reagent Co., Ltd (Hangzhou City, Zhejiang Province, PR China) were used: amoxicillin (AMX, 20 μg), ampicillin (AMP, 10 μg), cefotaxime (CTX, 30 μg), amikacin (AMK, 30 μg), neomycin (NEO, 30 μg), streptomycin (STR, 10 μg), kanamycin (KAN, 30 μg), tetracycline (TET, 30 μg), erythromycin (ERY, 15 μg), ofloxacin (OFX, 5 μg), ciprofloxacin (CIP, 5 μg), norfloxacin (NOR, 10 μg), enrofloxacin (ENR, 10 μg), chloramphenicol (CHL, 30 μg), florfenicol (FFC, 30 μg). The quality control strain was *Escherichia coli* ATCC 25922. After measuring the diameters of the inhibition zones, all results were judged according to the Clinical and Laboratory Standards Institute (**CLSI**) guidelines (https://clsi.org/). If isolates were resistant to three or more categories of antibiotics, they were considered MDR strains.

Detection of Antibiotic Resistance Genes

This study used PCR to amplify the drug resistance genes of 110 *Salmonella* strains, including β-lactam resistance genes (bla_{TEM}, bla_{SHV}, $bla_{\text{CTX-M}}$, and $bla_{\text{CMY-2}}$), aminoglycoside resistance genes (*aac(6′)-Ib-cr, aph(3′)-Ia, aadA1, aac(3)-I, aac(3)-II, aac(3)-III, aac(3)-IV, rmtB*, and *armA*), tetracycline resistance genes (*tetA, tetC*), macrolide resistance genes (*ereA, mefA*), and quinolone resistance genes (*oqxAB, qnrA*, and *qnrD*). The related primer information can be found in Table S1(Ahmed et al., 2007; Phuc Nguyen et al., 2009; Zhang et al., 2009; Clemente et al., 2015; Ayad et al., 2016; Navajas-Benito et al., 2017; Tahbaz et al., 2019). After PCR amplification, positive DNA samples of antibiotic resistance genes were sequenced by Sangon Biotech in Shanghai, China.

MLST

In *Salmonella* MLST Database (https://enterobase.warwick.ac.uk), seven housekeeping genes were used in *Salmonella* MLST, including *aroC, dnaN, hemD, hisD, purE, sucA*, and *thrA*. According to the primer information and PCR conditions provided by *Salmonella* MLST Database (https://enterobase.warwick.ac.uk), seven housekeeping genes of *Salmonella* strains were amplified. PCR products were purified and sequenced by Sangon Biotech, and sequencing results were spliced. The sequences of *Salmonella* housekeeping genes were downloaded from Pubmlst (https://pubmlst.org/), and the spliced sequences were aligned with them to obtain the allele values. The corresponding ST types of different *Salmonella* strains can be queried on Pubmlst (https://pubmlst.org/). Bionumerics 8.0 was used to perform cluster analysis on the results of MLST and construct a minimum spanning tree.

Table 1. Serotype distribution of 110 *Salmonella* isolates.

		Serotype								
Area	Source	Kottbus	Typhimurium	Enteritidis	Newlands	Montevideo	Bonn	Potsdam	Unknown	Total
Taian	Duck embryos	26	11	3	1	3	2	1	13	60
Liaocheng	Duck embryos, duck bodies and living environment	13	9	3	3	0	0	0	0	28
Heze	Duck bodies, living environment	1	0	4	5	0	0	0	0	10
Jining	Duck embryos	0	9	0	0	0	0	0	0	9
Guantao	Duck embryos	0	3	0	0	0	0	0	0	3
Total		40	32	10	9	3	2	1	13	110

Plasmid Typing

The types of plasmid carried by *Salmonella* isolates were detected according to the method established by Carattoli et al. (Carattoli et al., 2005). PCR products were sent to Sangon Biotech for sequencing.

Detection of Class I Integrons

Referring to class I integronase gene *intI1* in NCBI (https://www.ncbi.nlm.nih.gov/), the primer sequence was designed (forward: 5′-CCGAGGATGCGAACCACTTC-3′; reverse: 5′-CCGCCAC TGCGCCGT TACCA-3′) by Primer Premier 6.0. Positive DNA samples of *intI1* were sent to Sangon Biotech for sequencing after PCR amplification.

RESULTS

Antibiotic Resistance and MDR Profiles

Among 15 antibiotics, the resistance rate of 110 *Salmonella* strains to ERY was 98.18% (108/110), followed by STR and TET, with resistance rates of 84.54% (93/110) and 48.18% (53/110), respectively (Table S2). On the contrary, the sensitivity rates of 110 isolates to OFX and AMK were 87.27% (96/110), which were highest in 15 antibacterials (Table S2). According to Table 2, 71.82% (79/110) of strains showed multidrug resistance, of which 17 strains (17/110, 15.45%) were resistant to 10 or more antibiotics, including 2 strains (2/110, 1.82%) resistant to all tested antibiotics. Interestingly, one strain of *Salmonella* (1/110, 0.91%) was not immune to 15 kinds of antibiotics. Moreover, 47 different antimicrobial resistance patterns were discovered in 110 isolates (Table 2). The MDR rates of *S. Newlands, S. Enteritidis*, and *S. Kottbus* varied between 77.50% and 88.89%, of which *S. Kottbus* was the dominant serotype (Figure 1). As shown in Table S3, the antibiotic resistance of *Salmonella* isolated from Heze was notably serious.

Prevalence of Antibiotic Resistance Genes

As shown in Table S4, 12 types of drug resistance genes had been detected in 110 *Salmonella* strains, including β-lactam resistance genes (bla_{TEM}, $bla_{\text{CTX-M}}$), aminoglycoside resistance genes (*aac(6′)-Ib-cr, aph(3′)-Ia, aadA1, aac(3)-II, aac(3)-IV*, and *rmtB*), tetracycline resistance gene (*tetA*), macrolide resistance gene (*ereA*), and quinolone resistance genes (*oqxAB, qnrD*). Among β-lactam resistance genes, the most prevalent gene was bla_{TEM} (36/110, 32.73%). The positive rates of *aph(3′)-Ia* (36/110, 32.73%) and *aadA1* (33/110, 30.00%) were dominant in aminoglycoside resistance genes. The most common tetracycline resistance gene was *tetA* (37/110, 33.64%), and the highest carrying rate of macrolide resistance gene was *ereA* (38/110, 34.55%). *OqxAB* (7/110, 6.36%) and *qnrD* (9/110, 8.18%) were detected in quinolone resistance genes. Besides, the remaining 8 types of drug resistance genes were not detected. Among all serotypes, the above 12 types of drug resistance genes were all found only in *S. Kottbus* (Table S4).

Table 2. Antimicrobial resistance patterns of 110 *Salmonella* strains.

No. of drugs	Antimicrobial resistance pattern	No. of isolates
15	AMX-AMP-CTX-AMK-NEO-STR-KAN-TET-ERY-OFX-CIP-NOR-ENR-CHL-FFC	2
14	AMX-AMP-CTX-NEO-STR-KAN-TET-ERY-OFX-CIP-NOR-ENR-CHL-FFC	4
13	AMX-AMP-CTX-NEO-STR-KAN-TET-ERY-CIP-NOR-ENR-CHL-FFC	4
	AMX-AMP-NEO-STR-KAN-TET-ERY-OFX-CIP-NOR-ENR-CHL-FFC	1
12	AMX-AMP-CTX-NEO-STR-KAN-TET-ERY-CIP-ENR-CHL-FFC	2
11	AMX-AMP-CTX-NEO-STR-KAN-TET-ERY-ENR-CHL-FFC	2
10	NEO-STR-KAN-TET-ERY-CIP-NOR-ENR-CHL-FFC	1
	AMX-AMP-KAN-TET-ERY-CIP-NOR-ENR-CHL-FFC	1
9	NEO-STR-TET-ERY-CIP-NOR-ENR-CHL-FFC	1
	AMP-NEO-STR-KAN-TET-ERY-OFX-CIP-NOR	1
	AMX-AMP-STR-ERY-CIP-NOR-ENR-CHL-FFC	1
	AMX-AMP-AMK-NEO-TET-ERY-CIP-NOR-ENR	1
	AMX-AMP-NEO-STR-KAN-TET-ERY-CHL-FFC	1
8	STR-KAN-ERY-CIP-NOR-ENR-CHL-FFC	1
	CTX-AMK-NEO-STR-CIP-NOR-ENR-FFC	1
	AMP-NEO-STR-KAN-TET-ERY-CHL-FFC	1
7	STR-ERY-CIP-NOR-ENR-CHL-FFC	4
	NEO-STR-KAN-TET-ERY-CHL-FFC	2
	AMX-AMP-STR-TET-ERY-CHL-FFC	1
6	AMX-AMP-STR-TET-ERY-ENR	1
5	AMX-AMP-STR-TET-ERY	10
	AMP-STR-TET-ERY-ENR	1
	AMP-NEO-STR-TET-ERY	1
	NEO-STR-TET-ERY-ENR	1
	ERY-CIP-NOR-ENR-FFC	1
	AMX-AMP-STR-KAN-ERY	1
	STR-ERY-CIP-NOR-ENR	1
	AMX-AMP-CTX-STR-ERY	1
4	AMX-AMP-STR-ERY	6
	STR-TET-ERY-ENR	2
	AMX-AMP-TET-ERY	2
	CTX-KAN-TET-ERY	1
	AMX-STR-TET-ERY	1
	AMP-STR-TET-ERY	1
	AMK-NEO-STR-ERY	1
	STR-KAN-TET-ERY	1
	STR-KAN-ERY-ENR	1
3	STR-TET-ERY	4
	STR-ERY-ENR	3
	NEO-STR-ERY	2
	TET-ERY-ENR	1
	NEO-TET-ERY	1
	AMX-AMP-ERY	1
	STR-KAN-ERY	1
2	STR-ERY	23
1	ERY	7
0	None	1

Abbreviations: AMX, amoxicillin; AMP, ampicillin; AMK, amikacin; CIP, ciprofloxacin; CTX, cefotaxime; CHL, chloramphenicol; ENR, enrofloxacin; ERY, erythromycin; FFC, florfenicol; KAN, kanamycin; NEO, neomycin; NOR, norfloxacin; OFX, ofloxacin; STR, streptomycin; TET, tetracycline.

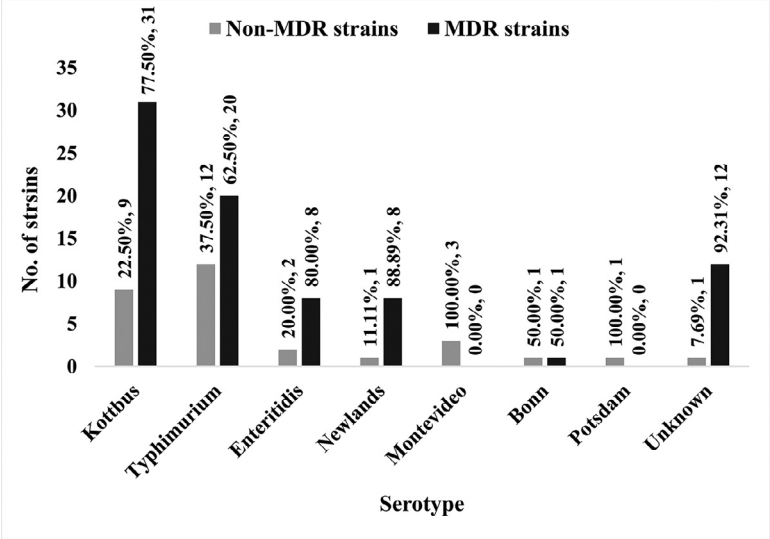

Figure 1. MDR profiles of *Salmonella* in different serotypes.

Relevance of Drug Resistance Phenotypes and Drug Resistance Genes

Coincidence rate = No. of strains with resistance phenotypes/No. of strains carrying resistance genes*100%. Table 3 showed that the coincidence rates of macrolides (37/38, 97.37%), aminoglycosides (59/62, 95.16%), β-lactams (40/47, 85.11%), and tetracyclines (27/37, 72.97%) were more than 70%. Only quinolones had a low coincidence rate of 25.00% (4/16).

MLST

The results of MLST showed that 110 *Salmonella* strains were divided into 13 various ST types, including ST19 (32/110, 29.09%), ST1546 (24/110, 21.82%), ST808 (21/110, 19.09%), ST11 (9/110, 8.18%), ST321 (8/110, 7.27%), ST305 (4/110, 3.63%), ST1690 (3/110, 2.73%), ST198 (2/110, 1.82%), ST2441 (2/110, 1.82%), ST358 (1/110, 0.91%), ST367 (1/110, 0.91%), ST1544 (1/110, 0.91%), and ST2309 (1/110, 0.91%) (Figure 2). In addition, only one strain could not be identified ST type (Figure 4). Among the 13 ST types, ST19, ST1546, and ST808 were the main types (Figure 2). As shown in Figure 2, the most closely related ST types to genetic evolution were ST19 and ST1544, ST808, and ST1690, and they all had 6 identical alleles. Based on the results in Figure 4 and *Salmonella* MLST Database, a few relationships between ST types and *Salmonella* serotypes were established, involving ST19 with *S. Typhimurium*, ST808 and ST1690 with *S. Kottbus*, ST11 with *S. Enteritidis*, ST305 with *S. Montevideo*, and ST2309 with *S. Potsdam*. Except for *S. Montevideo* and *S. Potsdam*, other serotypes were all divided into multiple ST types (Figure 4).

Table 3. The coincidence rates of drug resistance phenotype and drug resistance gene.

Category	No. of strains with resistance phenotypes	No. of strains carrying resistance genes	Coincidence rate (%)
β-lactams	40	47	85.11
Aminoglycosides	59	62	95.16
Tetracyclines	27	37	72.97
Macrolides	37	38	97.37
Quinolones	4	16	25.00

Plasmid Typing

As shown in Table 4, 110 *Salmonella* strains carried 6 types of plasmid incompatibility groups, namely IncFIIs (29/110, 26.36%), IncHI2 (10/110, 9.09%), IncI1 (7/110, 6.36%), IncFrepB (7/110, 6.36%), IncFIB (6/110, 5.45%), and IncY (1/110, 0.91%). As can be seen in Table 4, one strain (1/110, 0.91%) carried 3 different types of plasmids, 11 strains (11/110, 10.00%) carried two different types of plasmids, and 35 strains (35/110, 31.82%) carried one type of plasmids. Besides, no plasmid incompatibility group was detected in the other 63 strains (63/110, 57.27%). Based on Table 4, the carrying rates of plasmid incompatibility groups in *S. Typhimurium, S. Enteritidis*, and *S. Newlands* were far higher than those in other serotypes. In addition to IncY, all detected drug resistance genes almost presented in *Salmonella* holding the other five types of plasmid incompatibility groups (Table S5).

Detection of Class I Integrons

PCR results showed that class I integronase gene *intI1* was detected in 86 *Salmonella* strains (78.18%, 86/110). According to Table 5, the carrying rates of all detected drug resistance genes in class I integrons-positive strains were much higher than those in class I integrons-negative strains. The carrying rate of class I integrons in MDR strains was considerably higher than that in non-MDR stains (Figure 3).

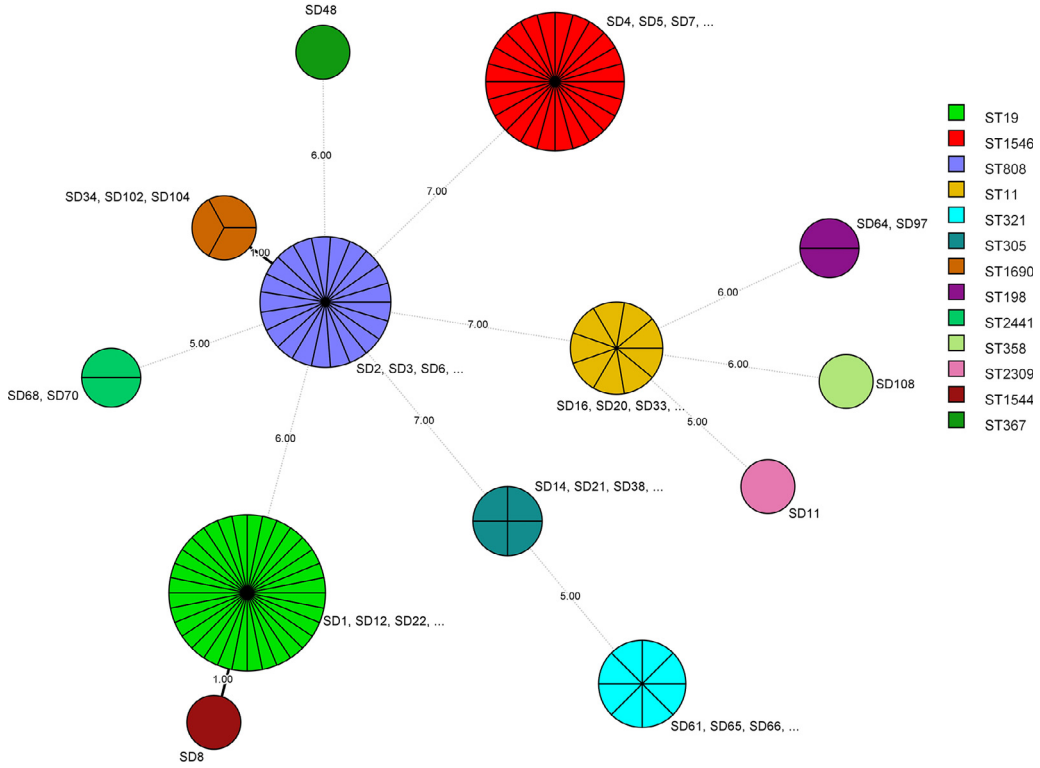

Figure 2. Minimum spanning tree of different ST types. Different colored circles represent different ST types. The larger the circle indicates that the ST type contains more strains. "SD..." stands for the strain number. The number on the line represents the number of alleles that differ between different ST types.

Table 4. The carrying rates of plasmid incompatibility groups in different serotypes of *Salmonella*.

Plasmid incompatibility group	Positive detection rate (%)	Serotype							
		Kottbus	Typhimurium	Enteritidis	Newlands	Montevideo	Bonn	Potsdam	Unknown
IncFIIs	24.54 (27/110)	0.00 (0/40)	65.62 (21/32)	60.00 (6/10)	0.00 (0/9)	0.00 (0/3)	0.00 (0/2)	0.00 (0/1)	0.00 (0/13)
IncHI2	2.73 (3/110)	0.00 (0/40)	0.00 (0/32)	0.00 (0/10)	33.33 (3/9)	0.00 (0/3)	0.00 (0/2)	0.00 (0/1)	0.00 (0/13)
IncI1	1.82 (2/110)	5.00 (2/40)	0.00 (0/32)	0.00 (0/10)	0.00 (0/9)	0.00 (0/3)	0.00 (0/2)	0.00 (0/1)	0.00 (0/13)
IncFrepB	1.82 (2/110)	0.00 (0/40)	0.00 (0/32)	0.00 (0/10)	0.00 (0/9)	0.00 (0/3)	0.00 (0/2)	0.00 (0/1)	15.38 (2/13)
IncFIB	0.91 (1/110)	2.50 (1/40)	0.00 (0/32)	0.00 (0/10)	0.00 (0/9)	0.00 (0/3)	0.00 (0/2)	0.00 (0/1)	0.00 (0/13)
IncFIIs+IncFrepB	0.91 (1/110)	0.00 (0/40)	0.00 (0/32)	10.00 (1/10)	0.00 (0/9)	0.00 (0/3)	0.00 (0/2)	0.00 (0/1)	0.00 (0/13)
IncFIIs+IncI1	0.91 (1/110)	0.00 (0/40)	3.13 (1/32)	0.00 (0/10)	0.00 (0/9)	0.00 (0/3)	0.00 (0/2)	0.00 (0/1)	0.00 (0/13)
IncHI2+IncI1	3.63 (4/110)	5.00 (2/40)	0.00 (0/32)	10.00 (1/10)	11.11 (1/9)	0.00 (0/3)	0.00 (0/2)	0.00 (0/1)	0.00 (0/13)
IncHI2+IncY	0.91 (1/110)	0.00 (0/40)	0.00 (0/32)	0.00 (0/10)	11.11 (1/9)	0.00 (0/3)	0.00 (0/2)	0.00 (0/1)	0.00 (0/13)
IncHI2+IncFIB	0.91 (1/110)	2.50 (1/40)	0.00 (0/32)	0.00 (0/10)	0.00 (0/9)	0.00 (0/3)	0.00 (0/2)	0.00 (0/1)	0.00 (0/13)
IncFrepB+IncFIB	2.73 (3/110)	2.50 (1/40)	0.00 (0/32)	0.00 (0/10)	0.00 (0/9)	0.00 (0/3)	0.00 (0/2)	0.00 (0/1)	15.38 (2/13)
IncHI2+IncFIB+IncFrepB	0.91 (1/110)	0.00 (0/40)	0.00 (0/32)	0.00 (0/10)	11.11 (1/9)	0.00 (0/3)	0.00 (0/2)	0.00 (0/1)	0.00 (0/13)
Total	42.73 (47/110)	17.50 (7/40)	68.75 (22/32)	80.00 (8/10)	66.66 (6/9)	0.00 (0/3)	0.00 (0/2)	0.00 (0/1)	30.76 (4/13)

Table 5. The carrying rates of resistance genes in positive strains with class I integrons and negative strains without class I integrons.

Resistance gene	No. of strains	Detection rate (%)	
		intI1 (+)[a]	*intI1* (-)[b]
bla_{TEM}	36	86.11 (31/36)	13.89 (5/36)
bla_{CTX-M}	14	100.00 (14/14)	0.00 (0/14)
aac(6′)-Ib-cr	29	100.00 (29/29)	0.00 (0/29)
aph(3′)-Ia	36	97.22 (35/36)	2.78 (1/36)
aadA1	33	100.00 (33/33)	0.00 (0/33)
aac(3)-II	10	100.00 (10/10)	0.00 (0/10)
aac(3)-IV	31	100.00 (31/31)	0.00 (0/31)
rmtB	4	100.00 (4/4)	0.00 (0/4)
tetA	37	81.08 (30/37)	18.92 (7/37)
ereA	38	100.00 (38/38)	0.00 (0/38)
oqxAB	7	71.43 (5/7)	28.57 (2/7)
qnrD	9	77.78 (7/9)	22.22 (2/9)

[a]Class I integrons-positive strains.
[b]Class I integrons-negative strains.

Drug Resistance Epidemiological Investigation Heatmap for 110 Salmonella Strains

Figure 4 provided the complete information of 110 *Salmonella* strains, including cluster analysis, drug resistance phenotype, drug resistance gene, plasmid typing, class I integron, strain number, corresponding ST type, serotype, isolation area, and source.

DISCUSSION

In this study, 110 *Salmonella* strains had 47 antimicrobial resistance patterns to 15 different antibacterial drugs. Among them, the resistance rates to ERY, STR, and TET reached 98.18%, 84.54%, and 48.18%,

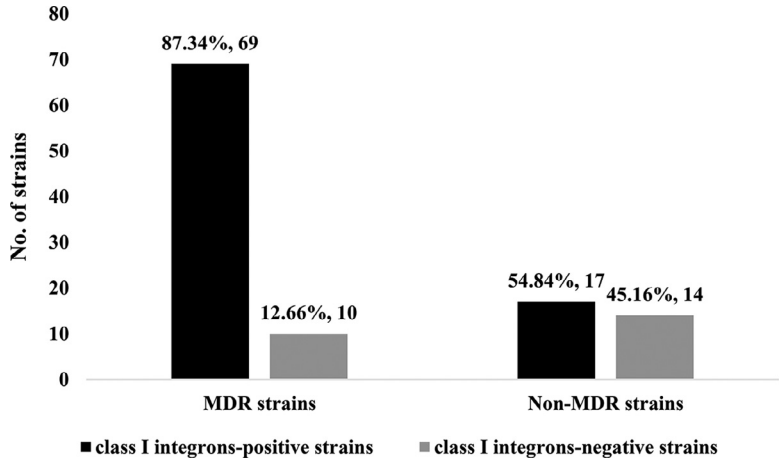

Figure 3. The distribution of class I integrons in MDR strains and non-MDR stains.

respectively. These results are consistent with previous reports from China (Wang et al., 2014; Yang et al., 2014; Zhou et al., 2018; Song et al., 2020; Yang et al., 2020b), Turkey (Yildirim et al., 2011), and Greece (Zdragas et al., 2012). Of all, 71.82% isolates were MDR strains, which is similar to the results from former surveys (Ziech et al., 2016; Zhou et al., 2018; Yang et al., 2020a). Of particular concern, the multidrug resistance rate of *S. Kottbus* as the dominant serotype was 77.50%. Still, the same serotype showed low resistance levels to the tested antimicrobial drugs in other areas of China (Yang et al., 2020a). The MDR phenomenon of strains isolated from living environment was more serious than other sources, and the drug resistance rate of *Salmonella* isolated from Heze was vastly higher than other regions. Owing to the different types of drugs and medication habits in different areas, numerous poultry breeding farms also have certain geographical and time discrepancies in *Salmonella* resistance (Foley and Lynne, 2008; Lai et al., 2014). According to the results of antibiotic susceptibility tests, we knew that 110 *Salmonella* strains were most sensitive to OFX and AMK, which are third-generation antibiotics with a broad antibacterial spectrum to solve the above problems.

The present investigation has detected 12 types of drug resistance genes with multitudes of varieties and only in the living environment, nine types of drug resistance genes existed in *Salmonella*, reflecting the potential hazard to duck breeding farms. Of note, among all serotypes, only *S. Kottbus* contained all 12 types of drug resistance genes, which further exhibits that *S. Kottbus* is a significant risk to the duck industry. Among β-lactam resistance genes, the most prevalent gene was bla_{TEM}, which is coherent with the detection in other areas of Shandong (Yang et al., 2019). The positive rates of *aph(3′)-Ia* and *aadA1* were dominant in aminoglycoside genes. Likewise, we can get similar results in another article (Li et al., 2015). Compared with the other 4 categories of resistance genes, the general detection rate of quinolone resistance genes (16/110, 14.55%) was extremely low, which corresponds to the reports from Guangdong Province (Xiong et al., 2020). It is worth noting that quinolones had the lowest coincidence rate (4/16, 25.00%) of drug-resistant phenotype and genotype. This data shows that the generation of antimicrobial resistance is not only caused by drug resistance genes, which is also relevant to complex antibiotic resistance mechanisms (Hooper and Jacoby, 2015).

In previous research, we found as many as 13 ST types in 110 isolates from multiple duck breeding farms. Yang et al. discovered seven ST types in *Salmonella* isolated from duck farms and a slaughterhouse in Shandong Province (Yang et al., 2019). Also in Shandong Province, Zhao et al. found nine ST types in 154 *Salmonella* strains isolated from farm animals (Zhao et al., 2017). In 2019, only 5 ST types were identified in 95 *Salmonella* strains isolated from chicken breeder flocks in nine Chinese provinces (Song et al., 2020). Compared with previous reports, the ST type is more abundant in our study. Among all ST types we have detected, the leading ST type was ST19, the most frequent sequence type in *Salmonella* isolated from humankind and food products derived from animals throughout the globe (Mandomando et al., 2015; Murgia et al., 2015; Ktari et al., 2016; Panzenhagen et al., 2018). Accordingly to the results of MLST, the proportion of ST11 in all strains was only 8.18%. However, ST11 was the most common ST type in other reports (Cha et al., 2013; Yang et al., 2019). This phenomenon reflects that MLST has prominent geographical distribution characteristics. Additionally, 110 *Salmonella* strains were divided into seven serotypes, but 13 different ST types were distinguished in them. This data indicated that MLST is more accurate than serotype identification. At the same time, the relationships between ST types and *Salmonella* serotypes showed that MLST is usually related to serotypes and can predict the serovars of *Salmonella* to a certain degree, some of which is agreement features (Achtman et al., 2012; Zhao et al., 2017). As the main serotypes, both *S. Kottbus* and *S. Typhimurium* were divided into multiple ST types, which were similar with another report (Liu et al., 2011). In contrast, nondominant serotypes *S. Montevideo* and *S. Potsdam* were matched with a single ST type. These results indicated that organisms frequently evolve specialized phenotypes to adapt to local environmental

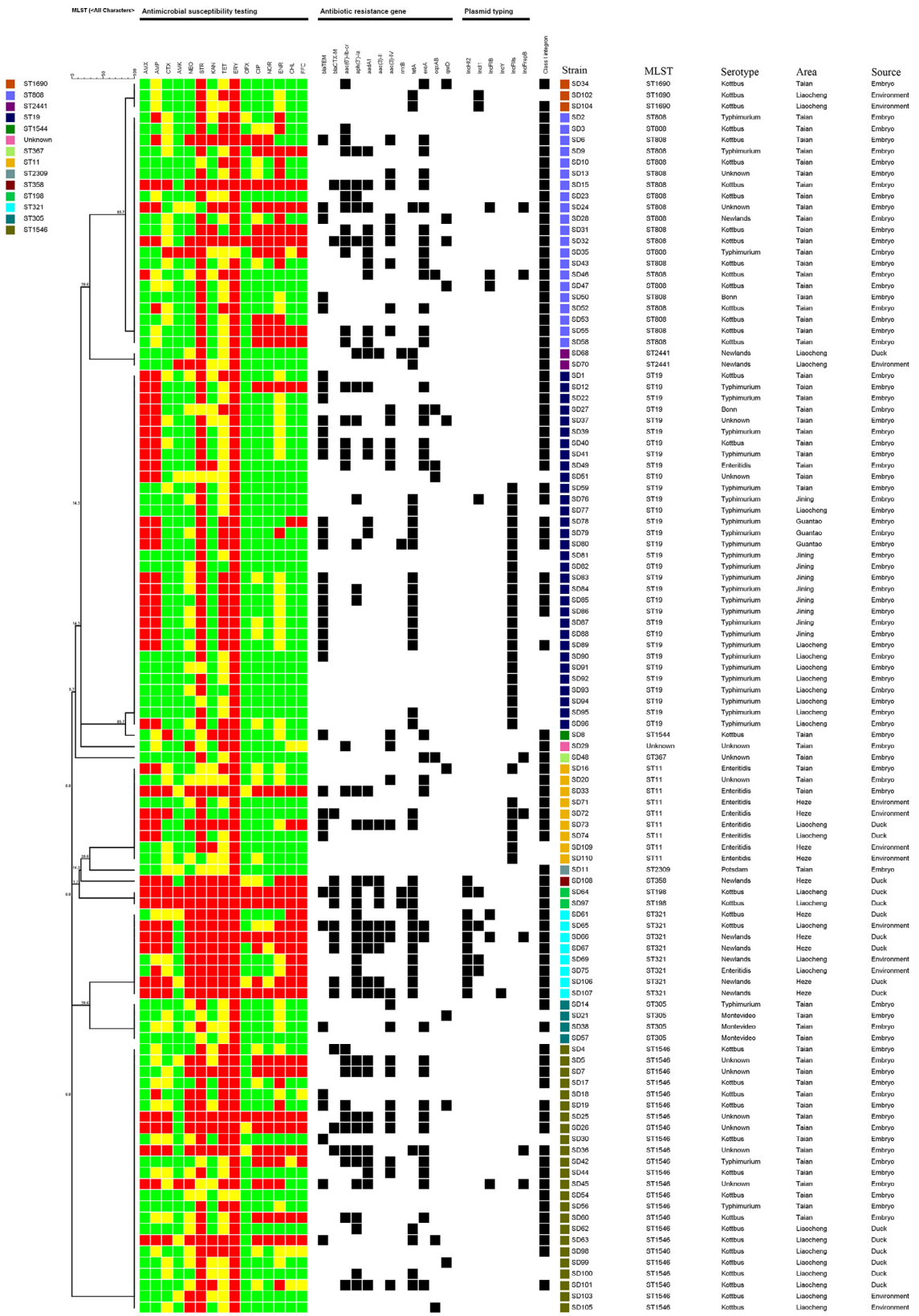

Figure 4. Drug resistance epidemiological investigation heatmap for 110 *Salmonella* strains. From left to right followed by cluster analysis of 110 *Salmonella* strains, drug resistance phenotype (green for sensitivity, yellow for intermediary, red for resistance), drug resistance gene (black for positivity, white for negativity), plasmid typing (black for positivity, white for negativity), class I integron (black for positivity, white for negativity), strain number, corresponding ST type, serotype, isolation area, and source.

conditions. The minimum spanning tree showed that the strains from duck embryos belonging to ST19 and ST1544, ST808, and ST1690 had a close genetic evolution relationship to each other. This phenomenon indicates that strains with different ST types may come from the same clonal ancestor in the vertical transmission process of *Salmonella*. Nevertheless, ST types holding remote evolutionary relationships were widely

distributed in isolates from duck embryos, duck bodies, and the living environment.

Plasmids are ubiquitous mobile genetic elements in *Salmonella*, facilitating the spread of resistance genes in bacteria (Lopatkin et al., 2017). Therefore, plasmid families are related to the emergence and spread of specific antibiotic resistance (Carattoli, 2013). Accordingly, the identification of plasmids helps track the transfer of resistance between various bacterial species. 74.47% of the 47 strains possessing plasmid incompatibility groups carried only one plasmid incompatibility group. This finding verifies that different plasmid incompatibility groups cannot coexist in most strains. IncFIIs, IncHI2, and IncN which can be extensively spread have attracted widespread attention worldwide and they can provide drug resistance genes, bacteriocins, siderophores, cytotoxins, adhesion factors, and other substances to promote the survival of bacteria (Pereira et al., 2015; Campos et al., 2016; Mansour et al., 2020). However, IncFIIs and IncHI2 were both detected except IncN in our research. Interestingly, we found that *tetA* (16/29, 55.17%) and bla_{TEM} (14/29, 48.28%) were predominant genes in the strains containing IncFIIs, and similar results were discovered in other articles (Yang et al., 2016; Oliva et al., 2018). At the same time, the results of antimicrobial susceptibility tests showed that the *Salmonella* stains isolated from duck embryos had high resistance to β-lactams and tetracyclines antibiotics. Combining the above findings, vertical transmission is more conducive to the spread of drug resistance with IncFIIs as the main carrier in this study. Moreover, the aminoglycosides and tetracyclines resistance genes were all detected in the strains containing IncHI2, and the resistance rates of these strains to aminoglycosides and tetracyclines antibacterials were 100%. These *Salmonella* strains were isolated from duck bodies and living environment. Consequently, the horizontal transmission may help spread drug resistance with IncHI2 as the major carrier. Other than IncY, all detected drug resistance genes almost existed in *Salmonella* carrying the other 5 types of plasmid incompatibility groups, which were isolated from duck embryos, duck bodies, and the living environment. This phenomenon highlights the importance of plasmids for the transfer of drug-resistant genes.

Integrons are a genetic platform that allows bacteria to evolve rapidly by acquiring, storing, removing, and rearranging gene cassettes. So far, class I integrons are the most common integrons, associating with antibiotic resistance genes, transposons, and plasmids (Escudero et al., 2015; Partridge et al., 2018). Class I integrons can integrate multiple drug resistance genes harboring plasmids or chromosomes, especially β-lactams, aminoglycosides, and sulfonamides, leading to severe drug resistance in bacteria (Firoozeh et al., 2011). Of all, 68.09% of the 47 *Salmonella* strains possessing plasmid incompatibility groups carried class I integrons, and the detection rates of β-lactams, aminoglycosides, and tetracyclines resistance genes were all over 40% in them. At the same time, the resistance rates of these strains to β-lactams, aminoglycosides, and tetracyclines antibiotics were exceedingly high. These results suggested that class I integrons integrate drug resistance genes into plasmids to promote the horizontal spread of antimicrobial resistance. However, some strains carrying class I integrons were also resistant to antibiotics, but no plasmid incompatibility group had been detected in them. This phenomenon indicates that class I integrons may recombine drug resistance genes into chromosomes to promote antibiotic resistance by vertical transmission.

Unlike many previous reports, the dominant serotype was *S. Kottbus* isolated from duck embryos, duck bodies, and the living environment and they had 7 ST types in this study. The MDR rate for *S. Kottbus* was 77.50% and they carried all detected drug resistance genes. Moreover, the positive rate of class I integrons in *S. Kottbus* was 82.50% and 4 types of plasmid incompatibility groups existed in them. The above results indicated that *S. Kottbus* evolved from different ancestors and they greatly contributed to the generation and spread of drug resistance. Therefore, *S. Kottbus* from ducks is very worthy of attention.

CONCLUSIONS

The MDR rate for 110 *Salmonella* strains isolated from multiple duck breeding farms was up to 71.82%. We found as many as 13 ST types in all strains and ST19 was the leading type. As the dominant serotypes, both *S. Kottbus* and *S. Typhimurium* were divided into multiple ST types. Twelve types of drug resistance genes and 6 kinds of plasmid incompatibility groups had been detected in 110 *Salmonella* strains. Moreover, the carrying rate of class I integrons in all isolates was 78.18%. Class I integrons integrated drug resistance genes into plasmids or chromosomes to promote the spread of antibiotic resistance, especially ERY, STR, and TET. Hence, it is essential to continuously monitor the drug resistance related to *Salmonella* isolated from ducks.

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DISCLOSURES

The authors declare no conflict of interest.

SUPPLEMENTARY MATERIALS

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