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Phenotypes and antimicrobial resistance genes in *Salmonella* isolated from retail chicken and pork in Changchun, China

Dayong Ren[1] | Ping Chen[1] | Ying Wang[1] | Jianan Wang[1] | Hongyan Liu[1] | Hongfeng Liu[2]

[1]Food Safety Department, College of Food Science and Engineering, Jilin Agriculture University, Changchun 130118, PR China

[2]Veterinary Science Department, College of Veterinary Medicine, Jilin University, Changchun 130062, PR China

Correspondence
Ping Chen, College of Food Science and Engineering, Jilin Agricultural University, Xincheng Street No. 2888, Changchun, Jilin Province 130122, PR China.
Email: ccchenping@sina.com

Abstract

The rate of the rise of drug resistance among pathogens in China is the fastest in the world. However, the resistance patterns for *Salmonella* serovars remain unknown. This study aimed to describe the resistance patterns for *Salmonella* serovars and confirm possible associations between antibiotic resistance and genes in connection to serovars. Five *Salmonella* serotypes were identified, with *S. typhi* (41.67%), *S. enteritidis* (20.83%) and *S. typhimurium* (18.75%) being the three most common strains. The majority of the *Salmonella* strains (93.75%) were resistant to one or more antimicrobial compounds. Multidrug resistance was only found in *Salmonella* from chicken, whereas isolates from pork were only resistant to tetracycline. Eight of 11 resistance genes were detected. Eight *Salmonella* isolates could transfer resistance genes to the recipient strain. These results indicated that the *Salmonella* isolates were resistant to more than one type of antibiotic, and resistance could be transmitted to humans through animal-based foods.

Practical applications

Salmonella is one of the world's most important food-borne pathogens. This study investigated *Salmonella* and drug resistance in fresh chicken and pork from the market to describe the resistance patterns for *Salmonella* serovars. The majority of the *Salmonella* strains were resistant to one or more antimicrobial compounds. Some *Salmonella* isolates could transfer resistance genes to *Escherichia coli*. Therefore, this study has significant application value for understanding the security situation of animal food and strengthening surveillance of food-borne zoonotic pathogens along the food chain.

KEYWORDS

animal food, antimicrobial resistance, gene transfer, resistance genes, *Salmonella*

1 | BACKGROUND

Salmonella is one of the world's most important food-borne pathogens. More than 2,500 serovars comprise the *Salmonella* genus (Evangelopoulou, Kritas, Govaris, & Burriel, 2013), and new serovars are regularly described (Guibourdenche et al., 2010). Despite the many measures currently implemented in food production, salmonellosis is the second most commonly reported zoonosis. In the United States, *S. enterica* has caused approximately one million cases of food-borne illness and 378 deaths per year (Scallan et al., 2011). The European Food Safety Authority (EFSA) has estimated that the overall economic burden caused by human cases in the EU can reach as high as 3 billion euros per year (Pires, Vigre, Makela, & Hald, 2010).

An increasing number of antimicrobial-resistant *Salmonella* has been reported from chicken, pork and other foods (Fearnley, Raupach, Lagala, & Cameron, 2011; Mąka, Maćkiw, Ścieżyńska, Pawłowska, & Popowska, 2014; Padungtod & Kaneene, 2006). Studies have shown that antibiotic treatment may prolong the course of illness of infected animals and release pathogenic bacteria (Weir, Martin, Poppe, Coombes, & Boerlin, 2008). According to epidemiological studies on

Dayong Ren and Ying Wang contributed equally to this work.

TABLE 1 PCR primers and gene targets for antimicrobial resistance

Gene	Nucleotide sequence (5′ to 3′)	Annealing temperature (T °C)	Size (bp)	Reference
bla_{TEM}	F-CATTTCCGTGTC GCCCTTATTC	60	800	Singh et al. (2010)
	R-CGTTCATCCAT AGTTGCCTGAC			
bla_{SHV}	F-AGCCGCTTGAG CAAATTAAAC	60	713	Singh et al. (2010)
	R-ATCCCGCAGAT AAATCACCAC			
$bla_{CTX\text{-}M\ group1}$ (includingCTX-M-1, CTX-M-3 and CTX-M-15)	F-TTAGGAARTGT GCCGCTGYA	60	668	Singh et al. (2010)
	R-CGATATCGTTG GTGGTRCCAT			
$bla_{CTX\text{-}M\text{-}2}$	F-CGTTAACGGCA CGATGAC	60	404	Singh et al. (2010)
	R-CGATATCGTTG GTGGTRCCAT			
$bla_{CTX\text{-}Mgroup8/25}$ (CTX-M-8,CTX-M-25,CTX-M-26 and CTX-M-39 to CTX-M-41)	F-AACRCRCAGAC GCTCTAC	60	326	Singh et al. (2010)
	R-TCGAGCCGGAA SGTGTYAT			
$bla_{CTX\text{-}M\ group9}$ (including CTX-M-9 and CTX-M-14)	F-TCAAGCCTGCC GATCTGGT	60	561	Singh et al. (2010)
	R-TGATTCTCGCC GCTGAAG			
bla_{IMP}	F-TTGACACTCCA TTTACDG	55	136	Singh et al. (2010)
	R-GATYGAGAATT AAGCCACYCT			
*qnr*A	F-AGAGGATTTCT CACGCCAGG	60	580	Thong et al. (2011)
	R-TGCCAGGCACA GATCTTGAC			
*qnr*S	F-GCAAGTTCATT GAACAGGGT	57	428	Thong et al. (2011)
	R-TCTAAACCGTC GAGTTCGGCG			
aac(6))-ib-cr	F-TTGCGATGCTCT ATGAGTGGCTA	55	482	Thong et al. (2011)
	R-CTCGAATGCCT GGCGTGTTT			
*tet*A	F-GCTACATCCTG CTTGCCTTC	58	210	Mąka et al. (2014)
	R-CATAGATCGCC GTGAAGAGG			

Salmonella, the isolates between humans and animals are difficult to distinguish on the molecular level of drug resistance, and multidrug resistance (MDR) in *Salmonella* may quickly increase after one kind of antibiotic is approved in animal treatment. Disturbance of the efficacy of antibacterial agents by MDR directly results in a severe threat to human health. The spread of resistance to other human pathogens also indirectly threatens human health. The increasing MDR of *Salmonella* has become a major public hazard to all humans. According to statistics, the rate of the rise of drug resistance among pathogens in China is the fastest in the world.

In recent years, besides genetic mutations, bacterial resistance to propagation by horizontal transfer of genes has become an important phenomenon. For example, the plasmid, an important mobile genetic element that is independent from the chromosome and DNA replication, is crucial in the spread of resistance genes. Therefore, analyses on antibiotic resistance and detection of resistance genes in *Salmonella*

TABLE 2 Incidence of *Salmonella* isolated from different retail chicken and pork

Source	No. of samples	No. of isolates	%
Chicken	200	42	21
Pork	85	6	7.06
Total	285	48	16.84

isolates from different sources can reveal the relationship between resistance genes and antibiotic-resistant strains.

The livestock and poultry breeding industry of Changchun is highly developed. Many livestock and poultry processing enterprises are found in this area, including the largest pig and chicken slaughtering and processing production base in China. Pork and chicken account for about 80% of meat consumption in Changchun. The city slaughters more than 2 million pigs and more than 130 million chickens per year. Pork and chicken are major ingredients in most of the local dishes in Changchun.

2 | OBJECTIVES

In this study, we investigated *Salmonella* and drug resistance in fresh chicken and pork from supermarkets and free markets in Changchun, Jilin Province, to describe the resistance patterns for *Salmonella* serovars. We also aimed to confirm possible statistical associations between antibiotic resistance patterns and genes in connection to serovars. We selected positive isolates to transfer resistance genes via conjugative transfer of plasmids to *Escherichia coli*; analyzed correlations between serotype, drug resistance phenotypes and drug resistance genes of *Salmonella*; located the resistance genes of *Salmonella*; and determined the relationship between resistance genes and mobile genetic elements. The obtained data were used to establish a scientific basis for risk assessment of *Salmonella* in food.

3 | MATERIALS AND METHODS

3.1 | Sampling

The isolates used in this study were collected monthly from pork ($n = 85$) and chicken ($n = 200$) at retail stores in Changchun, China. The pork and chicken samples were made by different processors and obtained from 23 markets. The sampling strategy used was established by the National Agricultural Sector Standard of China, Sampling criterion for the monitoring of veterinary drug residues in animals and animal products (NY/T 1897–2010). During each sampling time, only one retail store from each market was chosen for sampling. After the sample was purchased, it was placed in a single hermetic bag and used for *Salmonella* isolation within 24 h of collection.

3.2 | Isolation, identification and serotyping of *Salmonella*

Salmonella spp. were isolated according to the National Food Safety Standard of China, Food microbiological examination: General guidelines (GB 4789.1-2010). Samples (25 g each) were cut by sterile scissors, placed in a stomacher bag containing 225 mL of buffered peptone water, homogenized using a stomacher and incubated at 36 ± 1 °C for 8–18 h. Subsequently, 1 mL was transferred to 10 mL of selenite cystine broth and incubated at 36 ± 1 °C for 18–24 h. Plating was carried out on XLD agar, and plates were incubated at 36 ± 1 °C for 18–24 h. The typical colonies of *Salmonella* on the plates were pink with a black center. In this study, PCR was used to identify the isolates. The forward primesequence: 5′-gtgaaattatcgccacgttcgggcaa-3′, The reverse prime sequence: 5′-tcatcgcaccgtcaaaggaacc-3′. The length of product is 284 bp (El-Aziz, 2013). Slide agglutination was used for O and H antigens to serotype the *Salmonella* isolates according to the Kauffmann–White scheme (Kim et al., 2006).

3.3 | Antimicrobial susceptibility test

At present, ofloxacin (fluoroquinolone antibiotic), cephalosporin drugs (β-lactam antibiotics) and gentamicin (GEN; aminoglycoside antibiotic) are the domestic first-line drugs in the clinical treatment of *Salmonella* infection. Tetracyclines (TETs) are commonly used drugs for livestock and poultry feeding processes. All isolates used in this study were tested for susceptibility to four antimicrobial drugs on Mueller–Hinton agar by disk diffusion (Emilson, 1977). The following disks were used: cefotaxime sodium (CTX; 30 μg), GEN (10 μg), levofloxacin (LVX; 5 μg) and TET (30 μg). The zones of inhibition were divided into sensitive, intermediate susceptibility or resistant according to the CLSI recommendations (Williamson et al., 2012). The results were explained according to the CLSI recommendations. The quality control strain was *E. coli* ATCC 25922.

3.4 | PCR detection of resistance genes

A single colony of each isolate on the agar plates was retrieved and suspended in 100 μL of distilled water. After centrifugation for 1 min at 13,000 rpm, the suspension was boiled for 10 min. The supernatant

TABLE 3 Antibiogram sensitivity/resistance pattern of *Salmonella* isolates

Antimicrobial agent	Total no. of *Salmonella* isolates tested	Pattern of antibiogram of *Salmonella* isolates		
		Resistant (%)	Intermediate (%)	Sensitive (%)
Cefotaxime sodium	48	12 (25)	6 (12.5)	30 (62.5)
Gentamicin	48	19 (39.58)	3 (6.25)	26 (54.17)
Levofloxacin	48	8 (16.67)	12 (25)	28 (58.33)
Tetracycline	48	42 (87.5)	4 (8.33)	2 (4.17)

TABLE 4 Multiresistance and antibiotic resistance pattern of *Salmonella* isolates

No. of antimicrobials	Antibiotic resistance pattern	No. of *Salmonella* isolates
0	No resistance	3
1	GEN	1
	TET	22
2	CEX-GEN	1
	GEN-TET	7
	CEX-TET	1
	LVX-TET	1
3	CEX-GEN-TET	5
	LVX-GEN -TET	2
4	CEX-GEN-LVX-TET	5

was collected and used as the DNA template for PCR. The PCR condition was one cycle of pre-denaturation at 94°C for 5 min, 35 cycles of denaturation at 94°C for 40 s, and elongation at 72°C for 90 s, and one cycle of post-elongation at 72°C for 7 min. The annealing temperature and the primers for the detection of the 11 antimicrobial resistance genes are listed in Table 1.

3.5 | Conjugation experiments

The donors were eight *Salmonella* isolates with multiple resistance gene (>6) cassettes. TET-resistant derivatives of *E. coli* DH5α were used as receivers. Integron transfer was confirmed in transconjugants as described above. The primers used to confirm the transfer of the resistance genes are shown in Table 1.

4 | RESULTS

4.1 | Isolation, identification and serotyping of *Salmonella*

A total of 285 samples were tested for *Salmonella*, and 16.8% ($n = 48$) of these samples were contaminated. The studied samples were chicken ($n = 200$) and pork ($n = 85$). The results are shown in Table 2.

Most of the 48 *Salmonella* strains were isolated from chicken meat ($n = 42$). The incidence of *Salmonella* isolation was significantly higher in chicken than in pork. Among the 48 *Salmonella* isolates, five serotypes were identified as follows: *S. enteritidis* (20.83%, 10/48), *S. newport* (16.67%, 8/48), *S. typhi* (41.67%, 20/48), *S. gallinarum* (4.17%, 2/48) and *S. typhimurium* (18.75%, 9/48).

4.2 | Antimicrobial susceptibility

All the 48 isolates were examined for their antimicrobial susceptibility over the study period. Results on the resistance profile of *Salmonella* strains against the four antimicrobial agents evaluated in this study are presented in Tables 3–5. Resistance to CEX (25%), GEN (39.58%), LVX (16.67%) and TET (87.5%) was observed (Table 3).

TABLE 5 The antibiotic resistance pattern of *Salmonella* serovars isolated from retail chicken and pork

Source	Serovar (n)	Antibiotic resistance pattern	No. of isolates
Chicken	*S.* typhi (15)	No resistance	1
		TET	7
		CEX-TET	1
		CEX-GEN	1
		GEN-TET	3
		LVX-GEN-TET	1
		CEX-GEN-TET	1
	S. Newport (8)	No resistance	1
		TET	5
		CEX-LVX-GEN-TET	2
	S. Enteritidis (9)	TET	2
		GEN-TET	3
		CEX-GEN-TET	1
		CEX-LVX-GEN-TET	3
	S. Typhimurium (8)	TET	2
		GEN	1
		LVX-TET	1
		CEX-GEN-TET	3
		LVX-GEN-TET	1
	S. gallinarum (2)	No resistance	1
		GEN-TET	1
Pork	*S. S.*typhi (5)	TET	5
	S. Enteritidis (1)	TET	1

Among the 48 isolates, 45 strains (93.75%) showed resistance to the antimicrobial agents, and three strains demonstrated no resistance to the four antibiotics. The frequency of resistance among isolates was high. Of the 45 resistant *Salmonella* isolates, 23 strains were resistant to one antibiotic and 10 were resistant to two antibiotics. Multiresistance (resistance to at least three antimicrobials) was observed in this study. The percentage of multiresistant strains among the antibiotic-resistant isolates was 25%. Approximately 10.42% of the strains were resistant to the four antibiotics (Table 4).

TABLE 6 Distribution of antimicrobial resistance genes in *Salmonella* isolates

Resistance genes	No. of isolates	%
bla_{TEM}	26	54.17
bla_{SHV}	17	35.42
$bla_{CTX\text{-}M\ group1}$	13	27.08
$bla_{CTX\text{-}M\text{-}2}$	23	47.92
$bla_{CTX\text{-}M\ group9}$	30	62.5
aac(6)-ib-cr	38	79.17
*qnr*S	36	75
*tet*A	44	91.67

TABLE 7 Distribution of multiple resistance genes in *Salmonella* isolates

No. of genes	No. of isolates	%
0	2	4.17
1	1	2.83
2	6	12.5
3	3	6.75
4	5	10.42
5	12	22.92
6	11	22.92
7	5	12.5
8	3	6.75

Seven strains were resistant to both GEN and TET, and only one strain was resistant to both CEX and GEN. Only one isolate was found to be resistant to both CEX and TET. Twelve strains demonstrated multiresistance. Five of them were resistant to CEX, GEN and TET, whereas two were resistant to GEN, LVX and TET (Table 4).

Meanwhile, MDR was observed in *S. typhimurium* ($n = 4$), *S. typhi* ($n = 2$), *S. enteritidis* ($n = 4$) and *S. newport* ($n = 2$) isolated from chicken (Table 5). *S. typhi* and *S. enteritidis* from pork were only resistant to TET. Approximately 12.5% (1/8 isolates) of *S. newport* isolates from chicken were susceptible to all the tested antibiotics, and multiresistance to CEX, LVX, GEN and TET was discovered in 25% of *S. newport* isolates. All 15 isolates of *S. typhi* from chicken showed resistance, and multiresistance (three antimicrobials) was found in only 13.3% (2/15 isolates). MDR to LVX, GEN and TET was found in 6.67% (1/15 of isolates) of the *S. typhi* isolates and 12.5% (1/8 of isolates) of the *S. typhimurium* isolates. All the *S. enteritidis* isolates were resistant to TET. One of the *S. gallinarum* isolates was resistant to GEN and TET, and the others were susceptible to all the tested antimicrobials.

4.3 | Prevalence of resistance genes

Eight of the 11 resistance genes (*tet*A, *qnr*S, $bla_{\text{CTX-Mgroup1}}$, $bla_{\text{CTX-M-2}}$, $bla_{\text{CTX-M group9}}$, bla_{TEM}, bla_{SHV} and *aac(6)-ib-cr*) were found in resistant *Salmonella* isolates by PCR. Two of the isolates did not carry any resistance genes. The $bla_{\text{CTX-Mgroup8/25}}$, *qnr*A and bla_{IMP} genes were not found in any of the *Salmonella* isolates (Table 6). Table 7 shows that the *tet*A gene was the most common gene (91.67% of isolates; 44/48) found in *Salmonella* from pork and chicken, followed by the *aac(6)-ib-cr* (79.17%) and *qnr*S (75%) genes. More than two resistance genes were detected in approximately 81.25% of the isolates (39/48).

Table 8 shows the resistance gene patterns found in different serovars isolated from pork and chicken. Thirty-one resistance gene patterns were found in this study. The majority of the isolates showed patterns containing more than two resistance genes. The distribution of resistance genes differed among various sources (Table 8). All kinds of genes were found in *S. typhimurium*, *S. typhi*, *S. enteritidis* and *S. newport* from chicken. The gene patterns *tet*A and *qnr*S were the most common gene pattern in *S. typhi* from chicken.

Table 9 shows the distribution of resistance genes relative to antibiotic resistance patterns. Three strains did not exhibit phenotypic resistance; one isolate did not have a resistance gene, and the other two isolates showed no phenotypic resistance but harbored resistance genes. Moreover, one isolate had no resistance genes, but it was resistant to TET. Notably, the phenotype with CEX resistance always carried the β-lactamase genes $bla_{\text{CTX-M group1}}$, $bla_{\text{CTX-M-2}}$ and $bla_{\text{CTX-M group9}}$, either individually or concurrently.

4.4 | Conjugative transfer of resistance genes

Conjugation experiments were performed on eight *Salmonella* isolates, which harbored more than seven resistance genes. Six of the eight resistance genes (*tet*A, *qnr*S, *aac(6)-ib-cr*, bla_{TEM}, bla_{SHV} and $bla_{\text{CTX-M group9}}$) were successfully transferred into *E. coli* DH5α recipient cells. The $Bla_{\text{CTX-M-2}}$ and $bla_{\text{CTX-M group9}}$ genes were not found in the transconjugants. All eight isolates successfully transferred their resistance genes into the recipient cells.

5 | DISCUSSION

Chicken and pork are the most common retail meat in Changchun, because they are major ingredients in most local dishes. Thus, *Salmonella* in pork and chicken are very easily transfected to human beings in Changchun. The *Salmonella* search was positive in 16.8% of the 285 animal-based foods in this region. Approximately 87.5% of the positive *Salmonella* samples (42/48) were from chicken, and 12.5% (6/48) were from pork. A high *Salmonella* prevalence was observed by Abbassi-Ghozzi et al. (2012), who reported 48.3% of *Salmonella* recovery from 60 chicken samples from outlet stores in the area of "Grand Tunis," Tunisia. In a study performed in Mexico, Zaidi et al. (2006) found high *Salmonella* spp. prevalence in pork (58.1%) and poultry (39.7%).

In the present study, five and two serovars were recovered from chicken and pork, respectively. *S. typhi* was the most common serovar in both chicken and pork. In a previous study, a total of 21, 8 and 3 different serovars were recovered from chicken, turkey and pork, respectively, and *S. hadar* was found to be the most common in chicken (Aslam et al., 2012). Singh, Yadav, Singh, and Bharti (2010) reported that 14 of 26 isolates were serotyped as *S. typhimurium*, indicating a prevalence rate of 53.85%. The prevalence of *Salmonella* serovars from chicken may be distinguished by region (Parveen et al., 2007).

In this study, resistance to TET was observed in 87.5% of all the strains. This frequency was the highest of all the antimicrobials used in this test. Resistance to CEX, GEN and LVX was observed in 25, 39.58 and 16.67%, respectively. The results indicated that TET resistance in *Salmonella* was very common, and resistances to CEX and LVX were low in Changchun, China. The isolates from pork were found to be resistant only to TET. In Bacci et al. (2012)'s report, 86.1% of the *Salmonella* isolates showed resistance to TET, 80.5% demonstrated resistance to sulfamethoxazole, and 33.3% were resistant to ampicillin; moreover, GEN inhibited the growth of all the isolates. Wannaprasat, Padungtod, and Chuanchuen (2011) reported that 89% of the isolates

TABLE 8 Resistance gene patterns of *Salmonella* serovars recovered from retail chicken and pork

Source	Serovar (n)	Gene pattern	No. of isolates
Chicken	*S.* typhi (15)	No resistant genes	1
		tetA bla_{TEM} $bla_{CTX\text{-}M\text{-}2}$ $bla_{CTX\text{-}Mgroup9}$ aac(6)-ib-cr	1
		tetA qnrS bla_{TEM} $bla_{CTX\text{-}M\text{-}2}$ $bla_{CTX\text{-}Mgroup9}$ aac(6)-ib-cr	3
		tetA qnrS bla_{TEM} $bla_{CTX\text{-}Mgroup1}bla_{CTX\text{-}M\text{-}2}$ $bla_{CTX\text{-}Mgroup9}$ aac(6)-ib-cr	1
		tetA qnrS bla_{TEM} aac(6)-ib-cr	1
		tetA bla_{TEM} $bla_{CTX\text{-}M\text{-}2}$ $bla_{CTX\text{-}Mgroup9}$ aac(6)-ib-cr	1
		tetA qnrS bla_{TEM} bla_{SHV} $bla_{CTX\text{-}M\text{-}2}$ $bla_{CTX\text{-}Mgroup9}$ aac(6)-ib-cr	1
		tetA qnrS bla_{TEM} bla $_{CTX\text{-}Mgroup9}$ aac(6)-ib-cr	1
		tetA bla $_{CTX\text{-}Mgroup9}$ bla_{SHV} aac(6)-ib-cr	1
		tetA qnrS bla_{SHV} $bla_{CTX\text{-}Mgroup9}$ aac(6)-ib-cr	1
		tetA qnrS bla_{TEM} bla_{SHV} $bla_{CTX\text{-}Mgroup1}$ $bla_{CTX\text{-}M\text{-}2}$ $bla_{CTX\text{-}Mgroup9}$ aac(6)-ib-cr	1
		tetA bla_{SHV} $bla_{CTX\text{-}Mgroup1}$ $bla_{CTX\text{-}M\text{-}2}$ $bla_{CTX\text{-}Mgroup9}$	1
		tetA qnrS bla_{TEM} bla_{SHV} $bla_{CTX\text{-}Mgroup9}$ aac(6)-ib-cr	1
	S. Newport (8)	tetA qnrS bla_{TEM} $bla_{CTX\text{-}M\text{-}2}$ $bla_{CTX\text{-}Mgroup9}$ aac(6)-ib-cr	2
		tetA qnrS $bla_{CTX\text{-}M\text{-}2}$ aac(6)-ib-cr	1
		tetA bla_{TEM} bla_{SHV} $bla_{CTX\text{-}Mgroup1}$ $bla_{CTX\text{-}M\text{-}2}$ $bla_{CTX\text{-}Mgroup9}$ aac(6)-ib-cr	1
		tetA qnrS bla_{TEM} $bla_{CTX\text{-}Mgroup1}$ aac(6)-ib-cr	1
		tetA qnrS bla_{TEM} bla_{SHV} aac(6)-ib-cr	1
		tetA qnrS bla_{SHV} aac(6)-ib-cr	1
		tetA qnrS $bla_{CTX\text{-}M\text{-}2}$ $bla_{CTX\text{-}Mgroup9}$ aac(6)-ib-cr	1
		tetA $bla_{CTX\text{-}Mgroup9}$ aac(6)-ib-cr	1
	S. Enteritidis (9)	tetA qnrS bla_{TEM} bla_{SHV} $bla_{CTX\text{-}Mgroup1}$ $bla_{CTX\text{-}Mgroup9}$ aac(6)-ib-cr	1
		tetA	1
		tetA bla_{SHV}	1
		tetA qnrS $bla_{CTX\text{-}Mgroup1}bla_{CTX\text{-}M\text{-}2}$ $bla_{CTX\text{-}Mgroup9}$ aac(6)-ib-cr	2
		tetA qnrS $bla_{CTX\text{-}M\text{-}2}$ $bla_{CTX\text{-}Mgroup9}$ aac(6)-ib-cr	1
		tetA qnrS $bla_{CTX\text{-}Mgroup1}$ $bla_{CTX\text{-}Mgroup9}$ aac(6)-ib-cr	1
		tetA qnrS bla_{TEM} bla_{SHV} $bla_{CTX\text{-}Mgroup1}$ $bla_{CTX\text{-}M\text{-}2}$ $bla_{CTX\text{-}Mgroup9}$ aac(6)-ib-cr	1
	S. Typhimurium (8)	tetA qnrS bla_{TEM} $bla_{CTX\text{-}M\text{-}2}$ $bla_{CTX\text{-}Mgroup9}$ aac(6)-ib-cr	1
		tetA bla_{TEM}	1
		tetA bla_{TEM} bla_{SHV} $bla_{CTX\text{-}Mgroup1}bla_{CTX\text{-}M\text{-}2}$ $bla_{CTX\text{-}Mgroup9}$ aac(6)-ib-cr	1
		tetA qnrS bla_{TEM} bla_{SHV} aac(6)-ib-cr	1
		tetA qnrS bla_{SHV} $bla_{CTX\text{-}Mgroup9}$ aac(6)-ib-cr	1
		tetA qnrS $bla_{CTX\text{-}Mgroup1}$ aac(6)-ib-cr	1
		tetA qnrS bla_{SHV} $bla_{CTX\text{-}M\text{-}2}$ $bla_{CTX\text{-}Mgroup9}$ aac(6)-ib-cr	1
		tetA qnrS bla_{TEM} $bla_{CTX\text{-}Mgroup1}bla_{CTX\text{-}M\text{-}2}$ $bla_{CTX\text{-}Mgroup9}$ aac(6)-ib-cr	1
	S. gallinarum (2)	bla_{TEM} aac(6)-ib-cr	1
		No resistant genes	1
Pork	*S. S.*typhi (5)	tetA qnrS bla_{SHV}	1
		tetA qnrS bla_{TEM}	1
		tetA qnrS	3
	S. Enteritidis (1)	tetA qnrS bla_{TEM} $bla_{CTX\text{-}Mgroup9}$ aac(6)-ib-cr $bla_{CTX\text{-}M\text{-}2}$	1

are resistant to at least one antibiotic, with TET resistance (74%) being the most common. Thus, the *Salmonella* isolates from meat were commonly resistant to TET, and TET resistance was the most common form of antimicrobial resistance.

In this study, the resistance phenotype to GEN, TET, LVX and CEX was clearly present, and isolates from pork were only resistant to TET. Data in this study showed that retail chicken had a higher prevalence of *Salmonella* serovars presenting resistance to antimicrobials used in

TABLE 9 Comparison of antibiotic resistance patterns and resistance genes patterns of *Salmonella* isolates recovered from chicken and pork

Antibiotic resistance pattern	Resistance genes	No. of isolates
No resistance	No genes	1
	$bla_{CTX\text{-}Mgroup9}$ bla_{SHV} tetA aac(6)-ib-cr	1
	qnrS bla_{TEM} tetA $bla_{CTX\text{-}Mgroup1}$ aac(6)-ib-cr	1
GEN	bla_{TEM} aac(6)-ib-cr	1
TET	tetA qnrS bla_{TEM} bla $_{CTX\text{-}Mgroup9}$ aac(6)-ib-cr	1
	tetA qnrS bla_{TEM} aac(6)-ib-cr	1
	tetA qnrS $bla_{CTX\text{-}Mgroup1}$ aac(6)-ib-cr	1
	tetA qnrS bla_{SHV}	1
	tetA qnrS bla_{TEM}	1
	tetA qnrS bla_{SHV} $bla_{CTX\text{-}Mgroup9}$ aac(6)-ib-cr	1
	tetA qnrS $bla_{CTX\text{-}M\text{-}2}$ aac(6)-ib-cr	1
	tetA qnrS	3
	No genes	1
	tetA qnrS bla_{SHV} aac(6)-ib-cr	1
	tetA qnrS $bla_{CTX\text{-}M\text{-}2}$ $bla_{CTX\text{-}Mgroup9}$ aac(6)-ib-cr	1
	qnrS bla_{TEM} tetA $bla_{CTX\text{-}Mgroup9}$ aac(6)-ib-cr $bla_{CTX\text{-}M\text{-}2}$	1
	tetA	1
	tetA bla_{SHV}	1
	tetA qnrS $bla_{CTX\text{-}Mgroup1}$$bla_{CTX\text{-}M\text{-}2}$ $bla_{CTX\text{-}Mgroup9}$ aac(6)-ib-cr	1
	tetA qnrS bla_{TEM} $bla_{CTX\text{-}M\text{-}2}$ $bla_{CTX\text{-}Mgroup9}$ aac(6)-ib-cr	5
CEX-GEN	tetA bla_{SHV} $bla_{CTX\text{-}Mgroup1}$ $bla_{CTX\text{-}M\text{-}2}$ $bla_{CTX\text{-}Mgroup9}$	1
GEN-TET	tetA bla_{TEM} $bla_{CTX\text{-}M\text{-}2}$ $bla_{CTX\text{-}Mgroup9}$ aac(6)-ib-cr	1
	tetA qnrS bla_{TEM} $bla_{CTX\text{-}Mgroup1}$$bla_{CTX\text{-}M\text{-}2}$ $bla_{CTX\text{-}Mgroup9}$	1
	aac(6)-ib-cr	
	tetA bla_{TEM} $bla_{CTX\text{-}M\text{-}2}$ $bla_{CTX\text{-}Mgroup9}$ aac(6)-ib-cr	1
	tetA $bla_{CTX\text{-}Mgroup9}$ aac(6)-ib-cr	1
	tetA qnrS bla_{TEM} bla_{SHV} $bla_{CTX\text{-}Mgroup1}$$bla_{CTX\text{-}Mgroup9}$ aac(6)-ib-cr	1
	bla_{TEM} aac(6)-ib-cr	1
	tetA qnrS $bla_{CTX\text{-}M\text{-}2}$ $bla_{CTX\text{-}Mgroup9}$ aac(6)-ib-cr	1
CEX-TET	tetA qnrS bla_{TEM} bla_{SHV} $bla_{CTX\text{-}Mgroup1}$ $bla_{CTX\text{-}M\text{-}2}$ $bla_{CTX\text{-}Mgroup9}$ aac(6)-ib-cr	1
LVX-TET	tetA qnrS bla_{TEM} bla_{SHV} aac(6)-ib-cr	1
CEX-GEN-TET	tetA qnrS bla_{TEM} bla_{SHV} $bla_{CTX\text{-}M\text{-}2}$ $bla_{CTX\text{-}Mgroup9}$	1
	aac(6)-ib-cr	
	tetA qnrS bla_{TEM} bla_{SHV} $bla_{CTX\text{-}Mgroup1}$$bla_{CTX\text{-}M\text{-}2}$ $bla_{CTX\text{-}Mgroup9}$ aac(6)-ib-cr	1
	tetA qnrS bla_{SHV} $bla_{CTX\text{-}Mgroup9}$ aac(6)-ib-cr	1
	tetA qnrS bla_{SHV} $bla_{CTX\text{-}M\text{-}2}$ $bla_{CTX\text{-}Mgroup9}$ aac(6)-ib-cr	1
	tetA qnrS $bla_{CTX\text{-}Mgroup1}$$bla_{CTX\text{-}M\text{-}2}$ $bla_{CTX\text{-}Mgroup9}$ aac(6)-ib-cr	1
GEN-LVX-TET	tetA qnrS bla_{TEM} $bla_{CTX\text{-}Mgroup1}$$bla_{CTX\text{-}M\text{-}2}$ $bla_{CTX\text{-}Mgroup9}$	1
	aac(6)-ib-cr	
	tetA qnrS bla_{TEM} bla_{SHV} $bla_{CTX\text{-}Mgroup9}$ aac(6)-ib-cr	1
CEX-GEN-LVX-TET	tetA qnrS bla_{TEM} $bla_{CTX\text{-}M\text{-}2}$ $bla_{CTX\text{-}Mgroup9}$ aac(6)-ib-cr	1
	tetA bla_{TEM} bla_{SHV} $bla_{CTX\text{-}Mgroup1}$ $bla_{CTX\text{-}M\text{-}2}$ $bla_{CTX\text{-}Mgroup9}$ aac(6)-ib-cr	1
	tetA qnrS $bla_{CTX\text{-}M\text{-}2}$ $bla_{CTX\text{-}Mgroup9}$ aac(6)-ib-cr	1
	tetA qnrS $bla_{CTX\text{-}Mgroup1}$$bla_{CTX\text{-}Mgroup9}$ aac(6)-ib-cr	1
	tetA qnrS bla_{TEM} bla_{SHV} $bla_{CTX\text{-}Mgroup1}$$bla_{CTX\text{-}M\text{-}2}$ $bla_{CTX\text{-}Mgroup9}$ aac(6)-ib-cr	1

clinical treatment. This finding may be due to early large-scale breeding of poultry and the use of antibiotics as chicken feed additives to prevent disease and promote growth.

The analysis of resistance genes in *Salmonella* was an important aspect of this study. To further study the mechanism of antimicrobial resistance in *Salmonella*, we amplified the strains' resistance genes. The results showed that *tet*A was the most common gene followed by *aac (6)-ib-cr*. In some reports, the *tet*A gene was very common if the isolate was resistant to TET (Hur, Kim, Park, Lee, & Lee, 2011). The β-lactamase genes are very important, because the β-lactam antibiotic utilization rate among patients has reached 70% in China. Antibiotic resistance genes of β-lactam have lower resistance rates than other antibiotic resistance genes of several antibiotics. $Bla_{CTX\text{ -m group9}}$ exhibited the highest detection rate (62.5%), whereas $bla_{CTX\text{ -m group1}}$ had the lowest detection rate (27.08%). This study is the first report on the presence of resistance genes in *Salmonella* isolated from chicken and pork in Changchun. The presence of such genes is one of the main causes of drug resistance. Resistance to TET, GEN, CEX and ofloxacin is closely related to the *tet*A gene, *aac*(*6*)*-ib-cr*, *qnr*S and bla_{CTX-M}, respectively.

Notably, some isolates carry resistance genes but do not encode the corresponding resistant phenotype. Thus, detecting the genes that code for drug resistance will not necessarily result in a resistant phenotype. Some gene expression levels were relatively low and did not have any effect on treatment, or the resistant phenotypes encoded by the detected drug-resistant genes did not match the antibiotics in our experiments. Moreover, an inactivated enzyme or purified enzyme was only one factor of resistance mechanisms. It was also closely related to changes in the cell wall permeability active efflux mechanism and changes in the drug target.

Plasmid transduction was performed on the isolates, and *E. coli* DH5α was used as the receiver. The resistance genes of the strains were detected by PCR. Six of the eight resistance genes (*tet*A, *qnr*S, *aac*(*6*)*-ib-cr*, bla_{TEM}, bla_{SHV} and $bla_{CTX\text{-M group9}}$) were successfully transferred into *E. coli* DH5α recipient cells. Resistance of *Salmonella* could spread to other microorganisms by horizontal gene transfer. This finding was similar to the results reported by Thong & Modarressi (2011), but the genes differed.

In conclusion, this study demonstrated the role of food-producing animals as an origin of MDR *Salmonella* and underscored the necessity of continuing surveillance of food-borne zoonotic pathogens along the food chain.

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