frontiers in Microbiology

ORIGINAL RESEARCH
published: 04 October 2019
doi: 10.3389/fmicb.2019.02265

Incidence and Characterization of *Salmonella* Isolates From Raw Meat Products Sold at Small Markets in Hubei Province, China

Min Zhou[1], Xiaofang Li[1], Wenfu Hou[1], Hongxun Wang[1], George C. Paoli[2] and Xianming Shi[3]**

[1] School of Food Science and Engineering, Wuhan Polytechnic University, Wuhan, China, [2] Molecular Characterization of Foodborne Pathogens Research Unit, United States Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center (USDA-ARS-ERRC), USDA–MOST Joint Research Center for Food Safety, Wyndmoor, PA, United States, [3] State Key Laboratory of Microbial Metabolism, School of Agriculture and Biology, MOST-USDA Joint Research Center for Food Safety, Shanghai Jiao Tong University, Shanghai, China

OPEN ACCESS

Edited by:
Arun K. Bhunia, Purdue University, United States

Reviewed by:
Ashraf Khan, National Center for Toxicological Research (FDA), United States
Haiyan Zeng, Guangdong Academy of Science, China

***Correspondence:**
Hongxun Wang
wanghongxunhust@163.com
Xianming Shi
xmshi@sjtu.edu.cn

Specialty section:
This article was submitted to Food Microbiology, a section of the journal Frontiers in Microbiology

Received: *24 May 2019*
Accepted: *17 September 2019*
Published: *04 October 2019*

Citation:
Zhou M, Li X, Hou W, Wang H, Paoli GC and Shi X (2019) Incidence and Characterization of Salmonella Isolates From Raw Meat Products Sold at Small Markets in Hubei Province, China. Front. Microbiol. 10:2265. doi: 10.3389/fmicb.2019.02265

Salmonella is a leading cause of foodborne disease and is often associated with the consumption of foods of animal origin. In this study, sixty-six *Salmonella* isolates were obtained from 631 raw meat samples purchased at small retail suppliers in Hubei Province, China. The most prevalent *Salmonella* serotypes were Thompson (18.2%) and Agona (13.6%). Frequent antimicrobial resistance was observed for the sulfonamides (43.9%), tetracycline (43.9%), and the β-lactams amoxicillin and ampicillin (36.4% for each). Interestingly, a high incidence of resistance to cephazolin was observed in strains of the most common serotype, *S.* Thompson. Class I integrons were found in 27.3% (18/66) of the isolates and five of these integrons contained different gene cassettes (*aacA4C-arr-3-dfr2, dfrA12-aadA21, aadA2, dfrA12-aadA2, dfr17-aadA5*). Additional antimicrobial resistance genes, including $bla_{\text{TEM-1}}$, $bla_{\text{CTX-M-65}}$, $bla_{\text{CTX-M-15}}$, *qnrB,* and *qnrS,* were also identified among these *Salmonella* isolates. Results of replicon typing and conjugation experiments revealed that an integron with *qnrB* and $bla_{\text{CTX-M-15}}$ genes was present on incH12 mobile plasmid in *S.* Thompson strain. Multilocus sequence typing (MLST) analysis revealed 32 sequence types, indicating that these isolates were phenotypically and genetically diverse, among which ST26 (18.2%) and ST541 (12.1%) were the predominant sequence types. The integrons, along with multiple antimicrobial resistance genes on mobile plasmids, are likely contributors to the dissemination of multidrug resistance in *Salmonella*.

Keywords: *Salmonella*, MLST, class I integrons, $bla_{\text{CTX-M-65}}$, $bla_{\text{CTX-M-15}}$, *qnrB*

INTRODUCTION

Salmonella is an important source of foodborne disease, resulting in morbidity and mortality worldwide (Park et al., 2015). In China, 70–80% of bacterial food poisoning cases are due to *Salmonella* that originates in poultry, eggs, beef and pork (Yang et al., 2013). Direct and indirect spread of *Salmonella* between animals and humans are threats to human health. Although

more than 2610 serotypes of *Salmonella* have been identified, the majority of human *Salmonella* infections are caused by strains of only a few serotypes (Mancin et al., 2015). Therefore, serotype determination is an important aspect of epidemiological surveillance and disease assessment (Hung et al., 2017). Changes in the prevalence of specific serotypes can result from the movements of people, animals, and foods (Hendriksen et al., 2009). At present, serotypes Enteritidis and Typhimurium have been most frequently implicated in salmonellosis outbreaks from foods in Taiwan, Greece, Qatar and South Africa (Chang et al., 2016; Papadopoulos et al., 2016; Hung et al., 2017; Smith et al., 2017).

Though not as facile or rapid as serotyping, multilocus sequence typing (MLST) is a reliable and highly discriminatory molecular typing method. In this technique, the phylogenetic relationship between bacterial strains is determined based upon nucleotide sequence variations within several housekeeping and other conserved genes (Chang et al., 2016). MLST has many advantages compared with other genotyping techniques (Stepan et al., 2011). It is consistent and reproducible, and combines nucleotide sequencing and bioinformatics with a population genetics approach to produce accurate, reliable and highly portable results (Smith et al., 2017). Furthermore, it has been suggested that MLST is superior to serotyping for *Salmonella* surveillance and outbreak tracking (Achtman et al., 2012).

The widespread use of antibiotics poses problems for antimicrobial resistance, which leads to an increase in treatment costs and even to therapy failure (Hur et al., 2012). Multiple drug resistance (MDR) among *Salmonella* is prevalent. This applies not only to traditional antimicrobials such as sulfamethoxazole and chloramphenicol, but also against clinically important antimicrobials like the third generation β-lactams and fluoroquinolones (Agada et al., 2014). The selective pressure caused by the application of antimicrobials in poultry production and veterinary practice for growth promotion and prophylaxis has resulted in an increase in antibiotic resistance and an increase in the presence of genes conferring antimicrobial resistance to *Salmonella* (Zishiri et al., 2016). The spread of antimicrobial resistance in *Salmonella* is primarily due to integrons (Rajaei et al., 2014). Integrons are mobile DNA elements containing multiple antimicrobial resistant genes, which can move from one bacterium to another. This increases multi-drug resistance in bacterial populations (Asgharpour et al., 2014). Among various integrons, the class I integron is frequently observed among antimicrobial resistant *Salmonella* and plays a major role in the dissemination of resistance genes (Wright, 2010).

To the best of our knowledge, there is limited information regarding the incidence of *Salmonella* in retail raw meat products in Central China. Therefore, the objective of this study was to determine the prevalence, serotype distribution, genetic diversity, antimicrobial resistance, and the presence of class I integrons of *Salmonella* from retail raw meat products in Hubei Province, China. This data can be used for quantitative risk assessments of *Salmonella* isolates in retail meat and to help establish a series of prevention and control measures against foodborne pathogenic bacteria.

MATERIALS AND METHODS

Sample Collection and *Salmonella* Isolation and Serotyping

We collected 631 retail raw meat samples, including 263 chicken meat, 102 duck meat, 80 pork and 186 fish products from 20 small markets, in Wuhan City of Hubei Province, China, from 2014 to 2017. All samples were transported to our laboratory on ice within 6 h of collection. *Salmonella* isolation and identification methods were carried out according to the standard procedures of National Food Safety Standard Food Microbiological Examination: *Salmonella* (GB 4789.4-2016). Biochemical identification was done using an HBI microbial biochemical identification kit (Hope Biotechnology, Qingdao, China).

Serotyping of *Salmonella* isolates was done according to the Kauffmann-White scheme using an O and H antigen slide agglutination kit (Tianrun Biopharmaceutical, Ningbo, China) (Zhao et al., 2017).

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was performed using the Kirby-Bauer disk diffusion method as described by the Clinical and Laboratory Standards Institute (Clinical and Laboratory Standards Institute [CLSI], 2016). Antimicrobial disks (Oxoid Ltd., Basingstoke, United Kingdom) used for testing contained 10 μg amoxicillin, 10 μg ampicillin, 30 μg cefotaxime, 30 μg cefuroxime sodium, 30 μg cephazolin, 30 μg chloramphenicol, 5 μg ciprofloxacin, 30 μg nalidixic acid, 10 μg norfloxacin, 10 μg streptomycin, 10 μg gentamicin, 300 μg nitrofurantoin, 300 μg sulfonamide and 30 μg tetracycline. *Escherichia coli* strain ATCC 25922 was used as a reference strain. The standard break points were interpreted according to the guidelines of the Clinical and Laboratory Standards Institute (Clinical and Laboratory Standards Institute [CLSI], 2016).

Antimicrobial Resistance Genes and Class I Integron Identification

Bacterial DNA was extracted from *Salmonella* isolates cultured overnight in Luria–Bertani broth using the MiniBEST Bacteria Genomic DNA Extraction Kit Ver.3.0 (Takara, Beijing, China), according to the manufacturer's instructions. Eighteen genes known to be associated with resistance to the antibiotics as well as Class I integrons were tested by PCR using the oligonucleotide primers listed in **Table 1**. The PCRs were carried out using a MyCyler Thermocycler (BioRad, Hercules, CA, United States) in a 25 μL mixture that contained 0.5 mM of each primer, 1 × PCR buffer (Takara), 250 μM of dNTP, 0.5 U of Taq DNA polymerase (Takara), 1.5 mM $MgCl_2$, and 0.5 μL of template DNA. The cycling conditions were 94°C for 5 min, followed by 30 cycles of 94°C for 30 s, 54–61°C for 30 s and 72°C for 1 min, and a final extension of 72°C for 10 min. PCR amplification products were separated on agarose gels and the DNA was purified from agarose plugs using the Takara Agarose Gel DNA Purification Kit (Takara). BGI Tech Solutions (Wuhan, China) performed DNA

TABLE 1 | Primer sequences used for gene screening.

Resistance gene type	Gene	Primer Sequence (5′ → 3′)	Amplicon size (bp)	Annealing Temperature (°C)	References
Chloramphenicol	*catA1*	F: GCAAGATGTGGCGTGTTACGGTGAA	258	56	This study
		R: TCATTAAGCATTCTGCCGACATGGA			
	floR	F: AACCCGCCCTCTGGATCAAGTCAA	549	56	Ghoddusi et al., 2015
		R: CAAATCACGGGCCACGCTGTATC			
	cmlA	F: ATTTTAGTTGGCGGTACTCCCT	307	61	This study
		R: CCCAGAAAGACTTCAGCCGAT			
Tetracycline	*tetA*	F: GCTACATCCTGCTTGCCTTC	210	56	This study
		R: CATAGATCGCCGTGAAGAGG			
	tetB	F: TTGGTTAGGGGCAAGTTTTG	659	56	This study
		R: GTAATGGGCCAATAACACCG			
	tetC	F: AAAGGCTATCAAGCTGTTTC	311	56	This study
		R: CCAGTAATGTATTCAAGGCTAA			
Sulfonamide	*sul1*	F: CTTCGATGAGAGCCGGCGGC	437	55	Aarestrup et al., 2003
		R: GCAAGGCGGAAACCCGCGCC			
	sul2	F: GCGCTCAAGGCAGATGGCATT	285	55	Aarestrup et al., 2003
		R:GCGTTTGATACCGGCACCCGT			
	sul3	F: AGATGTGATTGATTTGGGAGC	443	55	Zhang et al., 2009
		R: TAGTTGTTTCTGGATTAGAGCCT			
Quinolone	*qnrA*	F: AGAGGATTTCTCACGCCAGG	580	60	Cattoir et al., 2007
		R: TGCCAGGCACAGATCTTGAC			
	qnrB	F: GGMATHGAAATTCGCCACTG	264	56	Cattoir et al., 2007
		R: TTTGCYGYYCGCCAGTCGAA			
	qnrS	F: GCAAGTTCATTGAACAGGGT	428	57	Cattoir et al., 2007
		R: TCTAAACCGTCGAGTTCGGCG			
	qnrD	F: ACAGGAATAGCTTGGAAGGGTG	329	58	This study
		R: AAGATCGGAGCCACGAAACA			
β-lactamase	bla_{TEM}	F: GAGTACTCACCATCACAGAAAAGC	489	58	Lu et al., 2010
		R: GACTTCCCGTCGTGTAGATAAC			
	bla_{PSE}	F: AATGGCAATCAGCGCTTCCC	598	55	Shahada et al., 2006
		R: GGGGCTTGATGCTCACTACA			
	bla_{CTX-M}	F: CGATGTGCAGCACCAGTAA	584	58	This study
		R: AGTGACCAGAATCAGCGG			
	bla_{CMY-2}	F: GACAGCCTCTTTCTCCACA	1015	57	This study
		R: TGGAACGAAGGCTACGTA			
	bla_{SHV}	F: TTCGCCTGTGTATTATCTCC	807	60	This study
		R: TTTGCTGATTTCGCTCGG			
Class I integron	*qacEΔ1-F*	F: ATCGCAATAGTTGGCGAAGT	800	54	Zhou et al., 2014
	sul1-B	R: GCAAGGCGGAAACCCGCGCC			
	intI1	F: TGTCCACTGGGTTCGTGCCT	707	56	This study
		R: GCTTCGTGATGCCTGCTTGTT			
	5′-CS	F: GGCATCCAAGCAGCAAGC	Random	54.5	Meng et al., 2011
	3′-CS	R: AAGCAGACTTGACCTGAT			

sequencing, and this data was aligned and analyzed using the Basic Local Alignment Search Tool (BLAST).

Conjugation Experiments

To determine whether resistance determinants were present in transferable genetic elements in *Salmonella* isolates, conjugation experiments were carried out using the broth mating method as previously described (Olsen et al., 2004). The *Salmonella* isolates positive for bla_{CTX-M} and/or integrons served as donors, and *E. coli* C600 was used as a recipient. Transconjugants were selected on Mueller–Hinton agar (Beijing Land Bridge Technology Co., Ltd.), containing 750 μg/ml rifampin and 100 μg/ml ampicillin. Susceptibility patterns of the transconjugants were determined by the methods described above. Co-transfer of resistance determinants was identified by amplifying the relevant genes from the transconjugants by PCR and determining the nucleotide sequencing of the PCR products. Plasmid DNA from transconjugants and donor strains was extracted utilizing a Tiangen Plasmid Purification Mini Kit (Tiangen Biotech, China) according to

the manufacturer's instructions. Replicon typing of plasmids was performed by a PCR-based method as previously described (Carattoli et al., 2005).

MLST

Seven housekeeping genes (*aroC*, *dnaN*, *hemD*, *hisD*, *purE*, *sucA*, and *thrA*) were used for MLST as described online[1]. All PCR products were purified from agarose gels and sequenced by BGI Tech Solutions as described above. Alleles and STs were assigned according to the MLST scheme.

RESULTS AND DISCUSSION

Salmonella Serotype Prevalence Among the Animal Products

Out of the 631 meat samples, 66 (10.5%) tested positive for *Salmonella*, and chicken had the highest prevalence (14.8%; 39/263), followed by duck meat (11.8%; 12/102), pork (8.8%; 7/80), and fish (4.3%; 8/186).

A total of 19 serotypes were identified among 66 *Salmonella* isolates and one isolate from chicken was un-typeable (**Table 2**). The 19 serotypes included 11 from chicken, and 8, 5, and 6 from duck meat, pork and fish, respectively. *S.* Thompson was the serotype most frequently identified (18.2%; 12/66), followed by *S.* Agona (13.6%; 9/66), *S.* Takoradi (12.1%; 8/66), and *S.* Typhimurium (10.6%; 7/66). *S.* Thompson was not isolated from pork but it was the most common serotype isolated from both duck meat and fish with 33.3% (4/12) and 37.5% (3/8), respectively. Although *S.* Thompson was the most prevalent serotype overall and chicken was the most likely to be contaminated with *Salmonella*, *S.* Thompson was not the most common serotype isolated from chicken. Both *S.* Agona and *S.* Takoradi were isolated from chicken at a higher rate (20.5%; 8/39 for each of the two serotypes) than S. Thompson (12.8%; 5/39) (**Table 2**).

Salmonella is a major cause of foodborne illness worldwide. In this study, *Salmonella* were recovered from retail raw meat products in Hubei Provence, China. The overall prevalence of *Salmonella* in retail meat products sampled in this study (10.5%) agreed with that reported in Henan Province, China (9.7%) (Yu et al., 2014) but was higher than that reported from Xinjiang, China (6.8%) (Yin et al., 2016). However, a simple conclusion could not be made from direct comparisons among studies due to regional differences, sampling seasons and the types of products sampled. In this study, the higher contamination rate found may be related to fewer biosecurity and hygiene measures inside these small markets.

We also identified 19 serotypes among the 66 isolates. The most common serotype in retail raw meat products was *S.* Thompson. While this result was consistent to two previous studies conducted in Iran (Soltan-Dallal et al., 2010; Sodagari et al., 2015), it differed from other reports in which the dominant serotype was *S.* Hadar in Xinjiang, China (Yin et al., 2016), *S.* Enteritidis in Henan Province, China (Yang et al., 2013) and *S.* Typhimurium in Brazil (Ristori et al., 2017). Other serotypes recovered in this study included *S.* Agona, *S.* Typhimurium, *S.* Enteritidis, *S.* Kentucky, and *S.* Derby, which have all been previously found in food products (Mąka et al., 2014; Shah et al., 2017; Wang et al., 2017). The differences in dominant serotypes may be due to geographical differences, sample types or seasons and serotype pathogenicity.

[1]http://mlst.warwick.ac.uk/mlst/dbs/Senterica

TABLE 2 | Distribution of *Salmonella* serotypes in retail meat.

Serotype	Number (%) of isolates				
	Chicken	Duck	Pork	Fish	Total
Thompson	5 (12.8)	4 (33.3)		3 (37.5)	12 (18.2)
Agona	8 (20.5)			1 (12.5)	9 (13.6)
Takoradi	8 (20.5)				8 (12.1)
Typhimurium	3 (7.7)	2 (16.7)	2 (28.6)		7 (10.6)
Enteritidis	4 (10.3)	1 (8.3)		1 (12.5)	6 (9.1)
Kentucky	5 (12.8)				5 (7.6)
Derby	1 (2.6)		2 (28.6)		3 (4.5)
Lindenburg	1 (2.6)	1 (8.3)		1 (12.5)	3 (4.5)
London	1 (2.6)		1 (14.3)		2 (3.0)
Anatum	1 (2.6)				1 (1.5)
Indiana	1 (2.6)				1 (1.5)
Galiema				1 (12.5)	1 (1.5)
Madelia				1 (12.5)	1 (1.5)
Paratyphi A			1 (14.3)		1 (1.5)
Senftenberg			1 (14.3)		1 (1.5)
Brunei		1 (8.3)			1 (1.5)
Brijbhumi		1 (8.3)			1 (1.5)
Muenchen		1 (8.3)			1 (1.5)
Rissen		1 (8.3)			1 (1.5)
Untypable	1 (2.6)				1 (1.5)
Total	39 (59.1)	12 (18.2)	7 (10.6)	8 (12.1)	66 (100)

Antimicrobial Susceptibility

Among 66 *Salmonella* isolates, a high percentage of the isolates were resistant to sulfonamides (43.9%), tetracycline (43.9%), amoxicillin (36.4%), ampicillin (36.4%), chloramphenicol (27.3%), nalidixic acid (21.2%), streptomycin (18.2%), and cephazolin (15.2%) (**Table 3**). It was found that 17 (25.8%) of the 66 isolates were resistant to 1 antibiotic, 10 isolates (15.2%) were resistant to 2–3 antibiotics, 20 isolates (30.3%) were resistant to 4–6 antibiotics, 3 isolates (4.5%) were resistant to 7–9 antibiotics and a single isolate (1.5%) was resistant to 11 antibiotics (**Table 4**). Thus, 51 of the 66 *Salmonella* isolates (77.3%) were resistant to at least one antibiotic. *Salmonella* isolated from chicken were most likely to be resistant with 92.3% (36/39) of the isolates showing resistance to at least one antibiotic, and a single isolate exhibiting resistance to 11 antibiotics. *Salmonella* isolates from fish were the most susceptible to the antimicrobials tested with only 2 strains (25%) showing any resistance (**Table 4**).

The increasing frequency of antimicrobial resistance among *Salmonella* has become an emerging challenge to public health.

TABLE 3 | Antimicrobial resistance of *Salmonella* isolates from retail meat.

Antimicrobial agent	Number of isolates (%)				
	Chicken (*n* = 39)	Duck (*n* = 12)	Pork (*n* = 7)	Fish (*n* = 8)	Total (*n* = 66)
Sulfonamides	21 (53.8)	4 (33.3)	4 (57.1)		29 (43.9)
Tetracycline	20 (51.3)	3 (25)	6 (85.7)		29 (43.9)
Amoxicillin	16 (41.0)	4 (33.3)	3 (42.9)	1 (12.5)	24 (36.4)
Ampicillin	16 (41.0)	4 (33.3)	3 (42.9)	1 (12.5)	24 (36.4)
Chloramphenicol	15 (38.5)		3 (42.9)		18 (27.3)
Nalidixic acid	9 (23.1)	2 (16.7)	1 (14.3)	2 (25)	14 (21.2)
Streptomycin	10 (25.6)		2 (28.6)		12 (18.2)
Cephazolin	7 (17.9)	2 (16.7)	1 (14.3)		10 (15.2)
Gentamicin	3 (7.7)	1 (8.3)	1 (14.3)		5 (7.6)
Cefuroxime	2 (5.1)				2 (3.0)
Ciprofloxacin	1 (2.6)	1 (8.3)			2 (3.0)
Norfloxacin	1 (2.6)				1 (1.5)
Nitrofurantoin			1 (14.3)		1 (1.5)

Our results indicated that 30.3% of isolates were resistant to 4–6 antimicrobial agents and 4.5% were resistant to 7–9 antimicrobials. This indicated that antimicrobial resistance among *Salmonella* isolates from retail meat in Hubei Province was lower than that in other areas of China (Zhu et al., 2014; Wang et al., 2017). In our study, the highest rates of antimicrobial resistance were to the sulfonamides, tetracycline, amoxicillin, ampicillin and chloramphenicol. These results were consistent to studies from other regions in China and other countries (Mąka et al., 2014; Yu et al., 2014; Abdi et al., 2017). The reasons for this may lie in the continuous and extensive use of these antimicrobials in food animals for disease treatment and growth promotion.

Cephalosporins and fluoroquinolones have been effectively used for the treatment of invasive and systemic salmonellosis in humans and animals (Bhan et al., 2005). Our study revealed that 15.2 and 3.0% of *Salmonella* isolates were resistant to first-generation (cephazolin) and second-generation cephalosporins (cefuroxime), respectively, and two isolates showed intermediate resistance to third-generation cephalosporins (cefotaxime) (**Table 5**). Previous work has described a decreasing susceptibility of *Salmonella* from retail foods to these antimicrobials (Hindermann et al., 2017; Kim et al., 2017). Interestingly, our results indicated that a high prevalence of cephazolin resistance was observed in isolates of *S.* Thompson, the predominant serotype. In this study, *Salmonella* resistance to nalidixic acid (21.2%) was not very common, however, 31.8% of the isolates also showed intermediate resistance to nalidixic acid (**Table 5**). Only 3.0 and 1.5% of the isolates were resistant to ciprofloxacin and norfloxacin, respectively. These rates were lower than those reported in other studies from China (Pan et al., 2009; Yin et al., 2016).

The Occurrence of Antimicrobial Resistance Genes and Class I Integrons

All 66 *Salmonella* isolates were screened for the presence of antimicrobial resistance genes and class 1 integrons. Numerous oligonucleotide primer pairs (**Table 1**) were used to screen the 66 *Salmonella* isolates by PCR for the presence of genes encoding resistance to chloramphenicol, tetracycline, sulfonamides, quinolones, and β-lactam antibiotics. Overall, the results showed a strong correlation between antimicrobial resistance genotypes and phenotypes (**Table 5**).

Among 26 β-lactam-resistant isolates in this study, four different β-lactamase genes were identified, including $bla_{\text{TEM-1}}$, $bla_{\text{CTX-M-65}}$, $bla_{\text{CTX-M-15}}$, and $bla_{\text{PSE}-1}$ (**Table 5**). The $bla_{\text{TEM-1}}$ gene had the highest occurrence (69.2%, 18/26) among β-lactam-resistant isolates; a rate similar to that previously reported for *Salmonella* isolated from meat and dairy products in Egypt (Ahmed et al., 2014). The extended-spectrum β-lactamase (ESBL) genes, $bla_{\text{CTX-M-65}}$ and $bla_{\text{CTX-M-15}}$, were each observed in a single *S.* Indiana and *S.* Thompson isolate, respectively. The $bla_{\text{PSE}-1}$ gene was also identified in only one isolate of *S.* Anatum. A cephalosporin resistant *S.* Thompson isolate carried the gene encoding CTX-M-15, which is the most prevalent ESBL worldwide (Chon et al., 2015). In this study, the rarely observed $bla_{\text{CTX-M-65}}$ was present in a ciprofloxacin- and cefuroxime-resistant isolate of *S.* Indiana. Similarly, it was reported that the $bla_{\text{CTX-M-65}}$ gene was harbored in ciprofloxacin- and cefotaxime-resistant *S.* Indiana isolates in Henan Province of China (Bai et al., 2016). In addition, the $bla_{\text{CTX-M-65}}$ gene was identified previously in an outbreak of *S.* Infantis in Ecuador (Cartelle Gestal et al., 2016).

The *qnr* family of genes encodes a pentapeptide repeat protein that protects DNA gyrase and topoisomerase IV from quinolone and fluoroquinolone inactivation. Plasmids containing *qnr* can be transferred among bacteria *via* conjugation and this is a very important emerging public health concern (García-Fernández et al., 2009). Although *qnr* confers only low-level quinolone resistance, mutations in the quinolone

TABLE 4 | Multidrug resistance strains identified among *Salmonella* isolates recovered from retail meat.

Sources	Number (%) of isolates resistant to indicated number of antimicrobials					
	1	2–3	4–6	7–9	11	Total (≥1)
Chicken (*n* = 39)	11 (28.2)	8 (20.5)	14 (35.9)	2 (5.1)	1 (2.6)	36 (92.3)
Duck (*n* = 12)	3 (25)		4 (33.3)			7 (58.3)
Pork (*n* = 7)	2 (28.6)	1 (14.3)	2 (28.6)	1 (14.3)		6 (85.7)
fish (*n* = 8)	1 (12.5)	1 (12.5)				2 (25)
Total (*n* = 66)	17 (25.8)	10 (15.2)	20 (30.3)	3 (4.5)	1 (1.5)	51 (77.3)

TABLE 5 | Resistance phenotypes and antibiotic resistance genes from *Salmonella* isolates recovered from retail meat.

Strain no.	Source	Serotype	Sequence Type	Resistance phenotype	Intermediate phenotype	Integrons/resistance genes
1	Chicken	Not detected	463	AML, AMP, C, NA, TE		**Class I,** *qnrS, sul2, tetA*
2	Chicken	*S.* Agona	13		ST	bla_{TEM-1}, *sul1*
3	Chicken	*S.* Agona	13	AML, AMP, ST, S3, TE		bla_{TEM-1},*sul3, tetA*
4	Chicken	*S.* Agona	13	AML, AMP, ST, S3, TE	NA	bla_{TEM-1}, *qnrS, sul3, tetA*
5	Chicken	*S.* Agona	13	AML, AMP, ST, S3, TE	NA	bla_{TEM-1}, *qnrS,sul3,tetA, floR*
6	Chicken	*S.* Agona	13	C	NA, ST	*qnrS, floR*
7	Chicken	*S.* Agona	13	AML, AMP, NA		bla_{TEM-1}, *qnrB, sul1, sul2*
8	Chicken	*S.* Agona	1215	C	NA, ST	*qnrS, floR*
9	Chicken	*S.* Agona	1328	C	NA,ST	*qnrS, floR*
10	Chicken	*S.* Anatum	516	NA		bla_{TEM-1}, bla_{PSE-1}, *sul1*
11	Chicken	*S.* Derby	20	S3, TE	NA, ST	bla_{TEM-1},*sul1,sul2,tetA*
12	Chicken	*S.* Enteritidis	11	AML, AMP, NA, ST, S3		**Class I,** bla_{TEM-1}, *sul1*
13	Chicken	*S.* Enteritidis	11	AML, AMP, NA		**Class I,** *qnrB, qnrS, sul1, sul2, tetA*
14	Chicken	*S.* Enteritidis	11	AML, AMP, NA		**Class I,** bla_{TEM-1}, *qnrB, sul1, sul2*
15	Chicken	*S.* Enteritidis	11	NA		bla_{TEM-1}, *sul1*
16	Chicken	*S.* Indiana	17	AML, AMP, CXM, KZ, C, CIP, NA, NOR, ST, CN, S3	CTX, TE	bla_{TEM-1}, $bla_{CTX-M-65}$, *sul1, sul2, tetA*
17	Chicken	*S.* Kentucky	314	C, S3, TE	NA, ST	**class I,** *qnrB, sul2, tetA*
18	Chicken	*S.* Kentucky	314	C, S3, TE	NA, ST	*qnrB, sul3, tetA*
19	Chicken	*S.* Kentucky	314	C, S3, TE	NA, ST	**Class I,** *qnrB, sul4, tetA*
20	Chicken	*S.* Kentucky	314		ST	
21	Chicken	*S.* Kentucky	314		ST	*floR*
22	Chicken	*S.* Lindenburg	46	S3		bla_{TEM-1}, *sul1*
23	Chicken	*S.* London	155	AML, AMP, C, S, CN, S3, TE		**Class I,** bla_{TEM-1}, *qnrB, sul1,floR,*
24	Chicken	*S.* Takoradi	541	TE	NA, ST	*qnrS, tetA*
25	Chicken	*S.* Takoradi	541	TE	NA	*qnrS, tetA*
26	Chicken	*S.* Takoradi	541	C, ST, S3, TE		*sul2, tetA*
27	Chicken	*S.* Takoradi	541	TE	NA	*qnrS, tetA*
28	Chicken	*S.* Takoradi	541	C, ST, S3, TE	NA	*qnrS, sul2,tetA, floR,*
29	Chicken	*S.* Takoradi	541	C, ST, S3, TE	NA	*sul2,tetA, floR*
30	Chicken	*S.* Takoradi	541	C, ST, S3, TE		*sul2,tetA,floR*
31	Chicken	*S.* Takoradi	541	C, ST, S3, TE		*floR, sul3, tetA*
32	Chicken	*S.* Thompson	26	AML, AMP, KZ, S3, TE	CXM, NA, ST	**class I,** bla_{TEM-1}, *qnrB, sul1, tetA*
33	Chicken	*S.* Thompson	26	AML, AMP, KZ, S3	CXM, NA	*qnrB, tetA*
34	Chicken	*S.* Thompson	26	AML, AMP, CXM, KZ, C, CN, S3, TE	CTX, ST	**Class I:** ***dfr17-aadA5,*** bla_{TEM-1}, $bla_{CTX-M-15}$, *qnrB, sul1*
35	Chicken	*S.* Thompson	26	AML, AMP, KZ, S3	ST	**Class I,** bla_{TEM-1}, *sul1*
36	Chicken	*S.* Thompson	26	AML, AMP, KZ, S3	ST	**Class I,** *sul1*
37	Chicken	*S.* Typhimurium	19	NA		bla_{TEM-1}, *sul1*
38	Chicken	*S.* Typhimurium	1682	KZ	AML	bla_{TEM-1}
39	Chicken	*S.* Typhimurium	1682	AML, AMP, TE	S3	bla_{TEM-1},*sul1*
40	Duck	*S.* Braenderup	22	NA		bla_{TEM-1}
41	Duck	*S.* Brunei	2256			
42	Duck	*S.* Enteritidis	11	AML, AMP, CIP, CN		**Class I,** *sul1*
43	Duck	*S.* Lindenburg	46	S3		bla_{TEM-1}, *sul1*
44	Duck	*S.* Muenchen	82			
45	Duck	*S.* Rissen	469	AML, AMP, S3, TE		*sul1, sul3, tetA*
46	Duck	*S.* Thompson	26		ST	bla_{TEM-1}, *sul1*
47	Duck	*S.* Thompson	26	AML, AMP, KZ, S3, TE	NA	**Class I:** ***dfrA12-aadA2,*** *qnrB, sul1, tetA*
48	Duck	*S.* Thompson	26	AML, AMP, KZ, S3, TE	CXM, NA, ST	**Class I,** bla_{TEM-1}, *qnrB, sul1, tetA*
49	Duck	*S.* Thompson	26			
50	Duck	*S.* Typhimurium	19	NA		bla_{TEM-1}, *sul1*

(Continued)

TABLE 5 | Continued

Strain no.	Source	Serotype	Sequence Type	Resistance phenotype	Intermediate phenotype	Integrons/resistance genes
51	Duck	*S.* Typhimurium	99		ST	bla_{TEM-1}, *sul1*
52	Pork	*S.* Derby	40	TE	C, NA, S3	*sul1, tetA*
53	Pork	*S.* Derby	40	TE	C, NA	*sul1, sul2, tetA*
54	Pork	*S.* London	155	AML, AMP, C, ST, S3, TE	NA	**Class I: *aacA4C- arr-3- dfr2*,** bla_{TEM-1}, *qnrB, sul1, sul2, tetA*
55	Pork	*S.* ParatyphiA	463	KZ, S3, TE		*sul1, tetC*
56	Pork	*S.* Senftenberg	14			
56	Pork	*S.* Typhimurium	313	AML, AMP, C, ST, S3, TE		**Class I:*aadA2,*** bla_{TEM-1}, *qnrB, sul1, sul2*
58	Pork	*S.* Typhimurium	19	AML, AMP, C, NA, CN, F, S3, TE	CIP, NOR, ST	**Class I:*dfrA12-aadA21,*** bla_{TEM-1}, *sul1, tetB, tetC*
59	fish	*S.* Agona	13			
60	fish	*S.* Enteritidis	11	AML, AMP, NA		**Class I,** bla_{TEM-1}, *qnrB, sul1*
61	fish	*S.* Galiema	501		ST	bla_{TEM-1}, *sul1*
62	fish	*S.* Lindenburg	45		S3	bla_{TEM-1}, *sul1*
63	fish	*S.* Madelia	226	NA		bla_{TEM-1}, *sul1*
64	fish	*S.* Thompson	26			
65	fish	*S.* Thompson	26			
66	fish	*S.* Thompson	26			

Class I, class I integrons which did not possess antibiotic resistance gene cassettes; AML, Amoxycillin; AMP, Ampicillin; KZ, Cephazolin; CXM, Cefuroxime sodium; CTX, Cefotaxime; C, Chloramphenicol; NA, Nalidixic acid; NOR, Norfloxacin; CIP, Ciprofloxacin; ST, Streptomycin; CN, Gentamicin; F, Nitrofurantoin; S3, Sulfonamides compound; TE, Tetracycline.

resistance-determining region (QRDR) could result in higher-level resistance to fluoroquinolones, especially to ciprofloxacin. The existence of *qnr* genes has been reported in *Salmonella* from other regions in China and in other countries (Yang et al., 2013; Wasyl et al., 2014). In the current study, 62.9% (22/35) of the 14 nalidixic acid-resistant and 21 intermediate resistant *Salmonella* isolates harbored *qnrB* and/or *qnrS* genes (**Table 5**). The *qnrB* gene was present in 34.3% (12/35) of the nalidixic acid resistant or intermediate resistant *Salmonella* isolates while *qnrS* was present in 31.4% (11/35) of the isolates. Both *qnrB* and *qnrS* were found in only a single isolate of *S.* Enteritidis. The genes *qnrA* and *qnrD* were not found among any of the isolates. The $bla_{CTX-M-15}$ gene was found in combination with *qnrB*, while the isolate with $bla_{CTX-M-65}$ carried no quinolone resistance genes.

The tetracycline resistance genes (*tetA*, *tetB,* and *tetC*) and sulfonamide resistance genes (*sul1*, *sul2*, and *sul3*) were identified in 86.7 and 96.9% of the strains resistant or intermediate resistant to the cognate antibiotic. The *floR* gene was identified in 40% (8/20) of chloramphenicol-resistant isolates ($n = 18$) and intermediate resistant isolates ($n = 2$) (**Table 5**).

An integron is a mobile DNA element, which usually contains one or more antimicrobial resistance genes that can be transferred among bacteria. In this study, Class I integrons (intI1) were found in 18 isolates (27.3%) and 13 of them did not possess antimicrobial resistance gene cassettes. The remaining 5 intI1-positive isolates contained five groups of resistance gene cassettes including *aacA4C-arr-3-dfr2*, *dfrA12-aadA21*, *aadA2*, *dfrA12-aadA2,* and *dfr17-aadA5* (**Table 5**). Our study also indicated that all of the class I integron-positive isolates were resistant to at least three classes of antimicrobials. This supports the hypothesis of the association between class I integrons and MDR in *Salmonella* isolates (Abraham et al., 2016; Meng et al., 2016).

Previous investigators identified class I integrons in foodborne *Salmonella* isolated in China at rates that ranged from 20 to 61% (Zhu et al., 2014; Meng et al., 2016; Li et al., 2017). In this study, the gene cassettes *aac*A*4C-arr-3-dfr2* from *S.* London exhibited >98.0% nucleotide sequence identity to that of the variable region of Integron In1021 from a clinical *E. coli* isolated in China (accession no. KR338349), suggesting that some of its components may have originated from other species of Enterobacteriaceae such as *Escherichia*. The existence of class I integrons containing genes for antimicrobial resistance in *Salmonella* isolates poses a threat to public health and food safety.

Transferability of bla_{CTX-M} Genes and Integrons

Six *Salmonella* strains harboring the ESBL bla_{CTX-M} gene and integrons were selected as donor strains in conjugation experiments. These donor strains were mated with an *E. coli* C600 recipient strain. Four transconjugants were obtained and characterized. PCR based replicon typing revealed that plasmids belonging to incompatibility types H12 and N were transferred from *Salmonella* to the *E. coli* C600 (**Table 6**). However, an incA/C plasmid in a *S.* Indiana isolate was not transferred. Genes present in the transconjugants included class I integrons, bla_{TEM-1}, $bla_{CTX-M-65}$, $bla_{CTX-M-15}$, *qnrB, sul1, sul2*, *tetA,* and *tetB*. The co-transfer of $bla_{CTX-M-15}$ and *qnrB* was observed with an integron from an *S.* Thompson isolate to *E. coli* strain C600 (**Table 6**). When mated with the *S.* London donor, the

TABLE 6 | Characteristics of *Salmonella* isolates and *E. coli* transconjugants.

Isolate No.	Serotype	Resistance profile	Resistance genes	Replicon typing
16	*S.* Indiana	AML, AMP, CXM, KZ, C, CIP, NA, NOR, ST, CN, S3	bla_{TEM-1}, $bla_{CTX-M-65}$, *sul1*, *sul2*, *tetA*	H12, A/C
16-T		AML, AMP, CXM, KZ, C, CIP, NA, NOR, ST, CN,	bla_{TEM-1}, $bla_{CTX-M-65}$, *tetA*	H12
34	*S.* Thompson	AML, AMP, CXM, KZ, C, CN, S3, TE	Class 1: *dfr17-aadA5,* bla_{TEM-1}, $bla_{CTX-M-15}$, *qnrB, sul1*	H12
34-T		AML, AMP, CXM, KZ, CN	Class 1: *dfr17-aadA5,* bla_{TEM-1}, $bla_{CTX-M-15}$, *qnrB*	H12
54	*S.* London	AML, AMP, C, ST, S3, TE	Class 1: *aacA4C-arr-3-dfr2*, bla_{TEM-1}, *qnrB*, *sul1*, *sul2*, *tetA*	N
54-T		AML, AMP, C, ST, S3, TE	Class 1:*aacA4C-arr-3-dfr2*, bla_{TEM-1}, *qnrB*, *sul1*, *tetA*	N
58	*S.* Typhimurium	AML, AMP, C, NA, CN, F, S3, TE	Class 1:*dfrA12-aadA21,* bla_{TEM-1}, *sul1*, *tetB*, *tetC*	H12
58-T		AML, AMP, C, NA, CN, F, TE	Class 1:*dfrA12-aadA21,* bla_{TEM-1}, *tetB*	H12

AML, Amoxycillin; AMP, Ampicillin; KZ, Cephazolin; CXM, Cefuroxime sodium; C, Chloramphenicol; NA, Nalidixic acid; NOR, Norfloxacin; CIP, Ciprofloxacin; ST, Streptomycin; CN, Gentamicin; F, Nitrofurantoin; S3, Sulfonamides compound; TE, Tetracycline.

E. coli C600 transconjugants showed an identical antimicrobial resistance phenotype to the donor. For the other *Salmonella* donors, the *E. coli* C600 recipients showed similar, but not identical antimicrobial resistances (**Table 6**).

Of concern, the findings of conjugation tests demonstrated the presence of genes including bla_{TEM-1}, $bla_{CTX-M-15}$, and *qnrB* on mobile plasmids. These resistance genes and integron were conjugally transferred from a *Salmonella* donor strain and conferred a MDR phenotype to a naïve *E. coli* recipient. Few studies have shown the co-existence of integron with plasmid-mediated quinolone resistance (PMQR) determinants and ESBL genes on transferable plasmids (Lai et al., 2013). It has been suggested that the transmissible elements containing these genes could be selected and transmitted during exposure to any of the antimicrobials (González and Araque, 2013; Jiang et al., 2014).

MLST Analysis

Thirty-two STs were identified among the 66 isolates, and ST26 (n = 12) was the most common followed by ST541 (n = 8), ST13 (n = 7), ST11 (n = 6), and ST314 (n = 5) (**Table 5**). The STs in this study were correlated with specific serotypes, such as ST11 with *S.* Enteritidis, ST13 with *S.* Agona, ST19 with *S.* Typhimurium, ST314 with *S.* Kentucky and ST26 with *S.* Thompson. Though a particular ST was always associated with the same serotype, some serotypes could be represented by multiple STs. For example, the isolates characterized as *S.* Derby belonged to ST20 and ST40.

The diversity of samples we collected was also reflected in a diversity of the 32 STs that we identified. ST26 was the most common especially among poultry samples and this ST corresponded to *S.* Thompson that has a low rate of occurrence in China (Ni et al., 2018). ST11 and ST19 were the most frequent sequence types recovered from meat samples and these STs are commonly associated with human salmonellosis outbreaks (Gunel et al., 2015; Ashton et al., 2016). Additionally, our results revealed that the serotypes and STs were highly correlated, which is consistent with previous research (Achtman et al., 2012). We did find sequence type differentiation within serotypes such as with *S.* Typhimurium and *S.* Derby. Therefore, MLST analysis provided a greater discriminatory power than serotype determination.

CONCLUSION

This study identified *S.* Thompson and *S.* Agona as the most common serotypes among *Salmonella* isolates recovered from retail raw meat products in Hubei Province, China. Antimicrobial resistance was prevalent and correlated well with the presence of cognate resistance genes. *Salmonella* isolates containing class I integrons showed broader antimicrobial resistance than those without them. In addition, conjugation experiments revealed a co-transfer of class I integron with PMQR and ESBL genes. The reasonable use of antibiotics in livestock breeding and management of the food supply chain is of importance to ensure food safety.

DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study. This data can be found here: http://enterobase.warwick.ac.uk/species/senterica/allele_st_search.

AUTHOR CONTRIBUTIONS

XS and HW designed the study and revised the manuscript. MZ conducted the experiments and interpreted the results. XL and WH assisted in the completion of the experiments. GP rewrote the discussion and revised the manuscript.

FUNDING

This study was supported by the National Key R&D Program of China (Grant No. 2017YFC1600100), a grant from the Hubei Provincial Department of Education, China (Grant No. D20161702) and the National Natural Science Foundation of China (Grant No. 31471660).

REFERENCES

Aarestrup, F. M., Lertworapreecha, M., Evans, M. C., Bangtrakulnonth, A., Chalermchaikit, T., Hendriksen, R. S., et al. (2003). Antimicrobial susceptibility and occurrence of resistance genes among *Salmonella enterica* serovar weltevreden from different countries. *J. Antimicrob. Chemother.* 52, 715–718. doi: 10.1093/jac/dkg426

Abdi, R. D., Mengstie, F., Beyi, A. F., Beyene, T., Waktole, H., Mammo, B., et al. (2017). Determination of the sources and antimicrobial resistance patterns of *Salmonella*, isolated from the poultry industry in southern ethiopia. *BMC Infect. Dis.* 17:352. doi: 10.1186/s12879-017-2437-2

Abraham, S., O'Dea, M., Trott, D. J., Abraham, R. J., Hughes, D., Pang, S., et al. (2016). Isolation and plasmid characterization of carbapenemase (imp-4) producing *Salmonella enterica* typhimurium from cats. *Sci. Rep.* 6:35527. doi: 10.1038/srep35527

Achtman, M., Wain, J., Weill, F. X., Nair, S., Zhou, Z., Sangal, V., et al. (2012). Multilocus sequence typing as a replacement for serotyping in *Salmonella enterica*. *PLoS Pathog.* 8:e1002776. doi: 10.1371/journal.ppat.1002776

Agada, G. O. A., Abdullahi, I. O., Aminu, M., Odugbo, M., Chollom, S. C., Kumbish, P. R., et al. (2014). Prevalence and antibiotic resistance profile of *Salmonella* isolates from commercial poultry and poultry farm-handlers in Jos. *Plateau State Nigeria. Br. Microbiol. Res. J.* 4, 462–479. doi: 10.9734/BMRJ/2014/5872

Ahmed, A. M., Shimamoto, T., and Shimamoto, T. (2014). Characterization of integrons and resistance genes in multidrug-resistant *Salmonella enterica* isolated from meat and dairy products in Egypt. *Int. J. Food Microbiol.* 189, 39–44. doi: 10.1016/j.ijfoodmicro.2014.07.031

Asgharpour, F., Rajabnia, R., Ferdosi Shahandashti, E., Marashi, M. A., Khalilian, M., and Moulana, Z. (2014). Investigation of class I integron in *Salmonella infantis* and its association with drug resistance. *Jundishapur J. Microbiol.* 7:e10019. doi: 10.5812/jjm.10019

Ashton, P. M., Nair, S., Peters, T. M., Bale, J. A., Powell, D. G., Painset, A., et al. (2016). Identification of *Salmonella* for public health surveillance using whole genome sequencing. *Peer J.* 4, e1752. doi: 10.7717/peerj.1752

Bai, L., Zhao, J., Gan, X., Gan, X., Wang, J., Zhang, X., et al. (2016). Emergence and diversity of *Salmonella enterica* serovar Indiana isolates with concurrent resistance to ciprofloxacin and cefotaxime from patients and food-producing animals in China. *Antimicrob. Agents Chemother* 60, 3365–3371. doi: 10.1128/AAC.02849-15

Bhan, M. K., Bahl, R., and Bhatnagar, S. (2005). Typhoid and paratyphoid fever. *Lancet* 366, 749–762.

Carattoli, A., Bertini, A., Villa, L., Falbo, V., Hopkins, K. L., and Threlfall, E. J. (2005). Identification of plasmids by PCR-based replicon typing. *J. Microbiol. Methods* 63, 219–228. doi: 10.1016/j.mimet.2005.03.018

Cartelle Gestal, M., Zurita, J., Paz, Y., Mino, A., Ortega-Paredes, D., and Alcocer, I. (2016). Characterization of a small outbreak of *Salmonella enterica* serovar Infantis that harbour CTX-M-65 in Ecuador. *Braz. J. Infect. Dis.* 20, 406–407. doi: 10.1016/j.bjid.2016.03.007

Cattoir, V., Poirel, L., Rotimi, V., Soussy, C. J., and Nordmann, P. (2007). Multiplex PCR for detection of plasmid-mediated quinolone resistance *qnr* genes in ESBL-producing enterobacterial isolates. *J. Antimicrob. Chemother.* 60, 394–397. doi: 10.1093/jac/dkm204

Chang, Y. C., Scaria, J., Ibraham, M., Doiphode, S., Chang, Y. F., Sultan, A., et al. (2016). Distribution and factors associated with *Salmonella enterica* genotypes in a diverse population of humans and animals in Qatar using multi-locus sequence typing (MLST). *J.Infect. Public Health* 9, 315–323. doi: 10.1016/j.jiph.2015.10.013

Chon, J. W., Jung, H. I., Kuk, M., Kim, Y. J., Seo, K. H., and Kim, S. K. (2015). High occurrence of extended-spectrum β-lactamase-producing *Salmonella* in broiler carcasses from poultry slaughterhouses in South Korea. *Foodborne Pathog. Dis.* 12, 190–196. doi: 10.1089/fpd.2014.1847

Clinical and Laboratory Standards Institute [CLSI] (2016). *Performance Standards for Antimicrobial Susceptibility Testing: Twenty-sixth Informational Supplement, M100-S26*. Wayne, PA: Clinical and Laboratory Standards Institute.

García-Fernández, A., Fortini, D., Veldman, K., Mevius, D., and Carattoli, A. (2009). Characterization of plasmids harbouring *qnrS1*, *qnrB2* and *qnrB19* genes in *Salmonella*. *J. Antimicrob. Chemother.* 63, 274–281. doi: 10.1093/jac/dkn470

Ghoddusi, A., Nayeri Fasaei, B., Karimi, V., Ashrafi Tamai, I., Moulana, Z., and Zahraei Salehi, T. (2015). Molecular identification of *Salmonella* Infantis isolated from backyard chickens and detection of their resistance genes by PCR. *Iran. J. Vet. Res.* 16, 293–297.

González, F., and Araque, M. (2013). Association of transferable quinolone resistance determinant *qnr*B19 with extended-spectrumβ-lactamases in *Salmonella* Give and *Salmonella* Heidelberg in Venezuela. *Int. J. Microbiol.* 2013:628185. doi: 10.1155/2013/628185

Gunel, E., Polat Kilic, G., Bulut, E., Durul, B., Acar, S., Alpas, H., et al. (2015). *Salmonella* surveillance on fresh produce in retail in Turkey. *Int.J. Food Microbiol.* 199, 72–77. doi: 10.1016/j.ijfoodmicro.2015.01.010

Hendriksen, R. S., Mikoleit, M., Carlson, V. P., Karlamose, S., Vieira, A. R., Jensen, A. B., et al. (2009). WHO Global Salm-Surv external quality assurance system for serotyping of *Salmonella* isolates from 2000 to 2007. *J. Clin. Microbiol.* 47, 2729–2736. doi: 10.1128/JCM.02437-08

Hindermann, D., Gopinath, G., Chase, H., Negrete, F., Althaus, D., Zurfluh, K., et al. (2017). *Salmonella enterica* serovar Infantis from food and human infections, Switzerland, 2010-2015: poultry-related multidrug resistant clones and an emerging ESBL producing clonal lineage. *Front. Microbiol.* 8:1322. doi: 10.3389/fmicb.2017.01322

Hung, Y. T., Lay, C. J., Wang, C. L., and Koo, M. (2017). Characteristics of nontyphoidal *Salmonella* gastroenteritis in Taiwanese children: a 9-year period retrospective medical record review. *J. Infect. Public Health* 10, 518–521. doi: 10.1016/j.jiph.2016.09.018

Hur, J., Jawale, C., and Lee, J. H. (2012). Antimicrobial resistance of *Salmonella*, isolated from food animals: a review. *Food Res. Int.* 45, 819–830. doi: 10.1016/j.foodres.2011.05.014

Jiang, H., Song, L., Liu, J., Zhang, X., Ren, Y., Zhang, W., et al. (2014). Multiple transmissible genes encoding fluoroquinolone and third-generation cephalosporin resistance co-located in non-typhoidal *Salmonella* isolated from food-producing animals in China. *Int. J. Antimicrob. Agents* 43, 242–247. doi: 10.1016/j.ijantimicag.2013.12.005

Kim, J. S., Kim, S., Park, J., Shin, E., Yun, Y. S., Lee, D. Y., et al. (2017). Plasmid-mediated transfer of CTX-M-55 extended-spectrum beta-lactamase among different strains of *Salmonella* and *Shigella* spp. in the republic of korea. *Diagn. Microbiol. Infect. Dis.* 89, 86–88. doi: 10.1016/j.diagmicrobio.2017.03.014

Lai, J., Wang, Y., Shen, J., Li, R., Han, J., Foley, S. L., et al. (2013). Unique class 1 integron and multiple resistance genes co-located on IncHI2 plasmid is associated with the emerging multidrug resistance of *Salmonella* Indiana isolated from Chicken in China. *Foodborne Pathog. Dis.* 10, 581–588. doi: 10.1089/fpd.2012.1455

Li, S., Zhou, Y., and Miao, Z. (2017). Prevalence and antibiotic resistance of non-typhoidal *Salmonella* isolated from raw chicken carcasses of commercial broilers and spent hens in Tai'an. *China. Front. Microbiol.* 8:2106. doi: 10.3389/fmicb.2017.02106

Lu, H., Wang, X., Lang, X., Wang, Y., Dang, Y., Zhang, F., et al. (2010). Preparation and application of microarrays for the detection of antibiotic resistance genes in samples isolated from Changchun. *China. Mol. Biol. Rep.* 37, 1857–1865. doi: 10.1007/s11033-009-9621-4

Mąka, Ł., Mackiw, E., Sciezynska, H., Pawłowska, K., and Popowska, M. (2014). Antimicrobial susceptibility of *Salmonella* strains isolated from retail meat products in Poland between 2008 and 2012. *Food Control* 36, 199–204. doi: 10.1016/j.foodcont.2013.08.025

Mancin, M., Barco, L., Saccardin, C., and Ricci, A. (2015). Proposed statistical analysis to evaluate qualitative profificiency testing of *Salmonella* serotyping. *Accred. Qual. Assur.* 20, 305–310. doi: 10.1007/s00769-015-1129-0

Meng, H., Zhang, Z., Chen, M., Su, Y., Li, L., Miyoshi, S., et al. (2011). Characterization and horizontal transfer of class 1 integrons in *Salmonella* strains isolated from food products of animal origin. *Int. J. Food Microbiol.* 149, 274–277. doi: 10.1016/j.ijfoodmicro.2011.07.006

Meng, X., Zhang, Z., Li, K., Wang, Y., Xia, X., Wang, X., et al. (2016). Antibiotic susceptibility and molecular screening of class I integron in *Salmonella* isolates recovered from retail raw chicken carcasses in China. *Microb. Drug Resist.* 23, 230–235. doi: 10.1089/mdr.2015.0359

Ni, P., Xu, Q., Yin, Y., Liu, D., Zhang, J., Wu, Q., et al. (2018). Prevalence and characterization of *Salmonella* serovars isolated from farm products in Shanghai. *Food Control* 85, 269–275. doi: 10.1016/j.foodcont.2017.10.009

Olsen, J. E., Brown, D. J., Thomsen, L. E., Platt, D. J., and Chadfield, M. S. (2004). Differences in the carriage and the ability to utilize the serotype associated virulence plasmid in strains of *Salmonella enterica* serotype typhimurium investigated by use of a self-transferable virulence plasmid, pOG669. *Microb. Pathog.* 36, 337–347. doi: 10.1016/j.micpath.2004.02.005

Pan, Z., Wang, X., Zhang, X., Geng, S., Chen, X., Pan, W., et al. (2009). Changes in antimicrobial resistance among *Salmonella enterica* subspecies enterica serovar Pullorum isolates in China from 1962 to 2007. *Vet. Microbiol.* 136, 387–392. doi: 10.1016/j.vetmic.2008.11.015

Papadopoulos, T., Petridou, E., Zdragas, A., Mandilara, G., Nair, S., Peters, T., et al. (2016). Comparative study of all *Salmonella enterica*, serovar Enteritidis strains isolated from food and food animals in Greece from 2008 to 2010 with clinical isolates. *Eur. J. Clin. Microbiol. Infect. Dis.* 35, 741–746. doi: 10.1007/s10096-016-2591-2

Park, S., Choi, S., Kim, H., Kim, Y., Kim, B. S., Beuchat, L. R., et al. (2015). Fate of mesophilic aerobic bacteria and *Salmonella enterica* on the surface of eggs as affected by chicken feces, storage temperature, and relative humidity. *Food Microbiol.* 48, 200–205. doi: 10.1016/j.fm.2015.01.003

Rajaei, B., Siadat, S. D., Rad, N. S., Badmasti, F., Razavi, M. R., Aghasadeghi, M. R., et al. (2014). Molecular detection of antimicrobial resistance gene cassettes associated with class 2 integron in *Salmonella* serovars isolated in Iran. *Br. Microbiol. Res. J.* 4, 132–141. doi: 10.9734/BMRJ/2014/4639

Ristori, C. A., Rowlands, R. E. G., Martins, C. G., Barbosa, M. L., Dos Santos, L. F., Jakabi, M., et al. (2017). Assessment of consumer exposure to *Salmonella* spp., *Campylobacter* spp., and Shiga Toxin-Producing *Escherichia coli* in meat products at retail in the city of Sao Paulo, Brazil. . *Foodborne Pathog. Dis.* 14, 447–453. doi: 10.1089/fpd.2016.2270

Shah, D. H., Paul, N. C., Sischo, W. C., Crespo, R., and Guard, J. (2017). Population dynamics and antimicrobial resistance of the most prevalent poultry-associated *Salmonella* serotypes. *Poult. Sci.* 96, 687–702. doi: 10.3382/ps/pew342

Shahada, F., Chuma, T., Tobata, T., Okamoto, K., Sueyoshi, M., and Takase, K. (2006). Molecular epidemiology of antimicrobial resistance among *Salmonella enterica* serovar Infantis from poultry in Kagoshima. *Japan. Int. J. Antimicrob. Agents* 28, 302–307. doi: 10.1016/j.ijantimicag.2006.07.003

Smith, A. M., Smouse, S. L., Tau, N. P., Bamford, C., Moodley, V. M., Jacobs, C., et al. (2017). Laboratory-acquired infections of *Salmonella enterica* serotype typhi in South Africa: phenotypic and genotypic analysis of isolates. *Bmc Infect. Dis.* 17:656. doi: 10.1186/s12879-017-2757-2

Sodagari, H. R., Mashak, Z., and Ghadimianazar, A. (2015). Prevalence and antimicrobial resistance of *Salmonella* serotypes isolated from retail chicken meat and giblets in Iran. *J. Infect. Dev. Ctries.* 9, 463–469. doi: 10.3855/jidc.5945

Soltan-Dallal, M., Doyle, M., Rezadehbashi, M., Dabiri, H., Sanael, M., Modarresi, S., et al. (2010). Prevalence and antimicrobial resistance profiles of *Salmonella* serotypes, *Campylobacter* and *Yersinia* spp. isolated from retail chicken and beef, Tehran, Iran. *Food Control* 21, 388–392. doi: 10.1016/j.foodcont.2009.06.001

Stepan, R. M., Sherwood, J. S., Petermann, S. R., and Logue, C. M. (2011). Molecular and comparative analysis of *Salmonella enterica* Senftenberg from humans and animals using PFGE. *MLST and NARMS. BMC Microbiol.* 11:153. doi: 10.1186/1471-2180-11-153

Wang, Y., Cao, C., Alali, W. Q., Cui, S., Li, F., Zhu, J., et al. (2017). Distribution and antimicrobial susceptibility of foodborne *Salmonella* serovars in eight provinces in China from 2007 to 2012 (Except 2009). *Foodborne Pathog. Dis.* 14, 393–399. doi: 10.1089/fpd.2016.2237

Wasyl, D., Hoszowski, A., and Zając, M. (2014). Prevalence and characterisation of quinolone resistance mechanisms in *Salmonella* spp. *Vet. Microbiol.* 171, 307–314. doi: 10.1016/j.vetmic.2014.01.040

Wright, G. D. (2010). Antibiotic resistance in the environment: a link to the clinic? *Curr. Opin. Microbiol.* 13, 589–594. doi: 10.1016/j.mib.2010.08.005

Yang, B., Qiao, L., Zhang, X., Cui, Y., Xia, X., and Cui, S. (2013). Serotyping, antimicrobial susceptibility, pulse field gel electrophoresis analysis of *Salmonella* isolates from retail foods in Henan Province. *China. Food Control* 32, 228–235. doi: 10.1016/j.foodcont.2012.11.022

Yin, M., Yang, B., Wu, Y., Wang, L., Wu, H., Zhang, T., et al. (2016). Prevalence and characterization of *Salmonella enterica* serovar in retail meats in market place in Uighur, Xinjiang, China. *Food Control* 64, 165–172. doi: 10.1016/j.foodcont.2015.12.029

Yu, T., Jiang, X., Zhou, Q., Wu, J., and Wu, Z. (2014). Antimicrobial resistance, class I integrons, and horizontal transfer in *Salmonella* isolated from retail food in Henan. *China. J. Infect. Dev. Ctries.* 8, 705–711. doi: 10.3855/jidc.4190

Zhang, A., Wang, H., Tian, G., Zhang, Y., Yang, X., Xia, Q., et al. (2009). Phenotypic and genotypic characterisation of antimicrobial resistance in faecal bacteria from 30 Giant pandas. *Int. J. Antimicrob. Agents* 33, 456–460. doi: 10.1016/j.ijantimicag.2008.10.030

Zhao, X., Ye, C., Chang, W., and Sun, S. (2017). Serotype distribution, antimicrobial resistance, and class 1 integrons profiles of *Salmonella* from animals in slaughterhouses in Shandong province, China. *Front. Microbiol* 8:1049. doi: 10.3389/fmicb.2017.01049

Zhou, R., Li, L., Su, J., Li, B., and Xu, Z. (2014). Detection of class I integron from foodborne *Salmonella* and analysis on the drug resistance gene cassettes. *Sci. Technol. Food Ind.* 35, 279–278.

Zhu, Y., Yi, Y., Liu, F., Lv, N., Yang, X., Li, J., et al. (2014). Distribution and molecular profiling of class 1 integrons in MDR *Acinetobacter baumannii* isolates and whole genome-based analysis of antibiotic resistance mechanisms in a representative strain. *Microbiol. Res.* 169, 811–816. doi: 10.1016/j.micres.2014.04.002

Zishiri, O. T., Mkhize, N., and Mukaratirwa, S. (2016). Prevalence of virulence and antimicrobial resistance genes in *Salmonella* spp. isolated from commercial chickens and human clinical isolates from South Africa and Brazil. *Onderstepoort. J. Vet. Res.* 83:a1067. doi: 10.4102/ojvr.v83i1.1067

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.