Characterization of integrons and antimicrobial resistance in *Salmonella* from broilers in Shandong, China

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ABSTRACT *Salmonella* spp. are one of the most important foodborne bacterial pathogens in human beings and animals. This study aimed to analyze the prevalence and characterization of *Salmonella* from broilers in Shandong, China. A total of 67 *Salmonella* were recovered from 600 rectal swabs collected from 3 large-scale intensive broiler farms (67/600, 11.2%) between May and October 2018. Among *Salmonella* isolates, the most common serovars were *S. enteritidis* and *S. typhimurium*. The highest occurrence of resistance observed was for polymyxin (100%), followed by ampicillin (68.7%). The multidrug-resistant *Salmonella* isolation rate was observed to be 53.7%. Four β-lactamase genes were detected among the isolates, and all the isolates carried bla_{TEM} (67/67, 100%), followed by bla_{OXA} (19/67, 28.4%), $bla_{\text{CTX-M}}$ (17/67, 25.4%), and bla_{PSE} (7/67, 10.4%). Four plasmid-mediated quinolone resistance gene were detected among the isolates; the prevalent resistance genes was *aac(6′)-Ib-cr* (18/67, 26.9%), followed by *oqxB* (9/67, 13.4%), *qnrB* (6/67, 9.0%), and *qnrD* (1/67, 1.5%). The prevalent rate of *mcr-1* was 6.0% (4/67). Class 1 integrons were detected in 26.9% of these isolates and contained 7 groups of resistance gene cassettes. Multilocus sequence typing analysis revealed 7 sequence types, and ST11 was the most frequent sequence types. This study indicated that reduction of *Salmonella* and strict control on the use of antibiotics in more than 5,000 million broilers in Shandong are the vitally important measures to keep public health.

Key words: *Salmonella*, broiler, antimicrobial susceptibility, class 1 integron, MLST

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BACKGROUND

Salmonella is a notorious human pathogen that can lead to an estimated 153 million enteric infections and 56,969 diarrheal deaths each year worldwide (Kirk et al., 2015). In China, a study based on the literature review estimated that the incidence of nontyphoidal salmonellosis was 626.6 cases per 100,000 persons (Yue et al., 2020). It has been widely reported that most of human salmonellosis is caused by infection derived from contaminated eggs, poultry meat, and meat products (Omwandho and Kubota, 2010; Kirk et al., 2015; Gonçalves-Tenório et al., 2018). Previous studies have reported the consistency relationship between *Salmonella* causing human diseases and those isolated at farms or food products (Pan et al., 2019; Paudyal et al., 2019).

Serovar determination is extremely important for epidemiological surveillance and disease assessment because different serovars may have different host ranges or cause different diseases. To date, more than 2,600 *Salmonella* serovars have been reported (Achtman et al., 2012). Poultry, especially broilers, are well-known reservoirs of various *Salmonella* serovars, most of which are able to infect humans (Nógrády et al., 2012).

Antimicrobial resistance is increasingly becoming an important issue with salmonellosis infections in both animals and humans (Duc et al., 2019). In animal husbandry, antibiotics are widely used for growth promotion or treatment purposes which facilitated the emergence and dissemination of antibiotic resistance in *Salmonella* (Thomas et al., 2020). Currently, the increasing prevalence of multidrug resistance (**MDR**) in *Salmonella*

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Table 1. Prevalence of *Salmonella* isolates from broilers in Shandong province.

Locations	No. of samples	No. of positive samples (%)
Tai'an	200	35 (17.5%)
Jinan	200	15 (7.5%)
Weifang	200	17 (8.5%)
Total	600	67 (11.2%)

to clinically important antimicrobial agents such as fluoroquinolones and β-lactams has been an emerging problem in China (Xu et al., 2019). This phenomenon of multiple resistance represents a worldwide problem for both veterinary and public health sectors. *Salmonella* can acquire resistance genes through mobile elements such as integrons, which contributes to the spread and distribution of antibiotic resistance genes across diverse bacterial populations (Blair et al., 2015). The chicken can be used as a vehicle for spreading and distributing these antimicrobial-resistant strains to humans (Gong et al., 2016).

Research on the prevalence and antimicrobial resistance of *Salmonella* isolated from broilers is important for determining the specific distribution patterns of antimicrobial resistance for this pathogen and developing effective treatment strategies to control and prevent *Salmonella* infections in humans and animals. Routine monitoring of antimicrobial-resistant *Salmonella* is undertaken in most developed countries and is an integral part of health risk assessment programs (WHO, 2014). To date, numerous studies reported the prevalence and characterization of *Salmonella* from broilers, the contamination of *Salmonella* was 6.5 to 38.5% in broilers, and the major serotypes were *Salmonella* infantis and enteritidis (Kumar et al., 2019; İncili et al., 2019; Firouzabadi et al., 2020; Yang et al., 2020). In China, broilers are widely reared and is an important sources of chicken meat (Yue et al., 2020). Therefore, it is necessary to monitor *Salmonella* in broilers every year. The purpose of this study was to determine the prevalence and characteristics of *Salmonella* isolated from broilers in Shandong province, China. It will help to define guidelines for improving salmonellosis control which in turn might lead to fewer human foodborne salmonellosis cases.

METHODS

Sampling

From May to October 2018, 3 large-scale intensive broiler farms in Tai'an, Jinan, and Weifang areas of Shandong province were selected as sampling points (Figure 1). During sampling time, all broilers in each farm were well. The broiler flocks had the capacity ranging from 150,000 to 200,000 birds at the average age of 25 d. All the commercial farms used cage farming. We selected 5 broiler houses for each farm, and 20 rectal swabs from different individual animals were collected from each broiler houses. All the sampling sites were visited only once. To prevent cross-contamination, gloves were worn during sampling and changed after each sample. A total of 600 rectal swab samples (200 per farm) were collected and transported under aseptic conditions and cold chain to our laboratory for bacterial isolation and identification (Table 1).

Salmonella *Isolation and Serotype Identification*

Salmonella isolation was conducted using previously published protocols (Yan et al., 2010), with some modification. Briefly, each swab sample was mixed with 9 mL of buffered peptone water (Hopebiol, Qingdao, China)

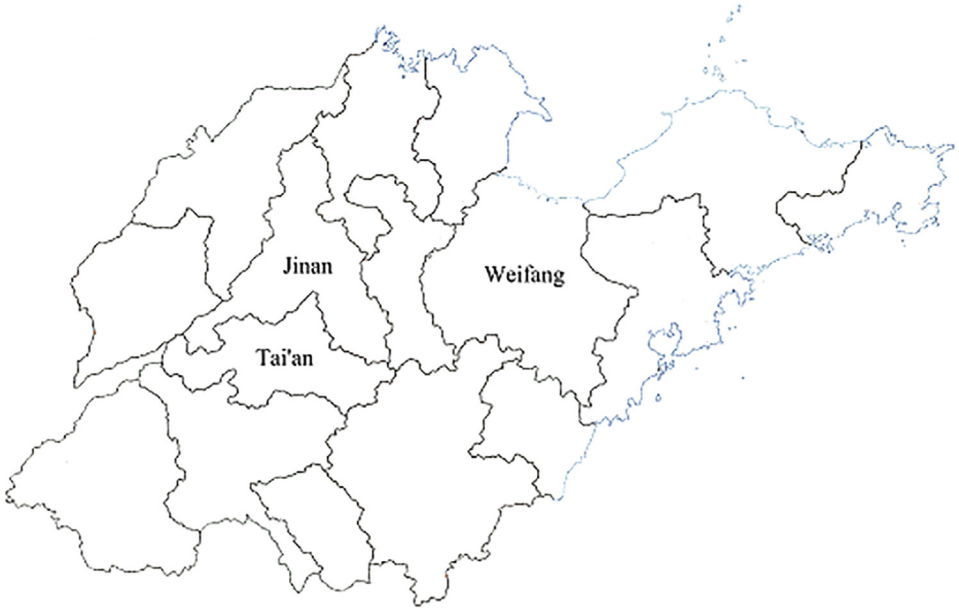

Figure 1. Map of sampling locations. The 3 regions are the main broiler-producing areas in Shandong.

and incubated at 37°C for 16 to 18 h for preenrichment. After incubation, 1-mL aliquots of buffered peptone water suspensions were inoculated in 10-mL volumes of selenite cysteine broth (Hopebiol, Qingdao, China) at 42°C and 10-mL volumes of tetrathionate brilliant green broth (Hopebiol, Qingdao, China) at 37°C for 24 h, respectively. Loopful of selenite cysteine broth and tetrathionate brilliant green broth cultures were then streaked onto xylose lysine tergitol 4 agar plates (Hopebiol, Qingdao, China), which were incubated at 37°C for 24 to 48 h. A single isolated colony was picked from the plates, based on typical *Salmonella* colony characteristics for microscopical examination, and further confirmed using the API 20E system (Sysmex bioMerieux, Tokyo, Japan).

For all confirmed *Salmonella* isolates, their serovars were determined according to the Kauffmann-White scheme by slide agglutination with O and H antigens (Tianrun Bio-Pharmaceutical, Ningbo, China) following the manufacturer's instructions.

Antimicrobial Susceptibility Testing

The antimicrobial susceptibility of all the *Salmonella* isolates was assessed by the Kirby-Bauer disc diffusion method on Mueller-Hinton agar plates according to the Clinical and Laboratory Standard Institute (CLSI, 2019) guidelines. Isolates were tested for sensitivity to amoxicillin/clavulanic acid (**AC**), ampicillin (**AMP**), ceftiofur (**CEF**), enrofloxacin (**ENR**), neomycin (**NEO**), doxycycline (**DOX**), florfenicol (**FLO**), gentamicin, and polymyxin (**PB**), which were commonly used in farms. An isolate was defined as MDR isolate if it was resistant to no less than 3 classes of antimicrobials. The *Escherichia coli* ATCC 25922 was used as a quality control and was purchased from Beina Biotechnology Co., Ltd (Beijing, China).

Detection of Antimicrobial Resistance Genes

The DNA for all experiments was extracted by the TIANamp Bacteria DNA Kit (Tiangen, Beijing, China) according to the manufacturer's instructions, and DNA templates were stored at −20°C until used. PCR screening for extended-spectrum β-lactamase genes (*bla*$_{\text{TEM}}$, *bla*$_{\text{CTX-M}}$, *bla*$_{\text{OXA}}$, *bla*$_{\text{SHV}}$ and *bla*$_{\text{PSE}}$), plasmid-mediated quinolone resistance genes (*aac(6′)-Ib-cr*, *qnrA*, *qnrB*, *qnrC*, *qnrD*, *qepA*, *oqxA* and *oqxB*), and PB resistance gene (*mcr*-1) was performed using previously reported primers (Table 2) (Ahmed et al., 2013; Li et al., 2013; Liu et al., 2016). The amplification consisted of an initial denaturation at 94°C for 10 min, followed by 32 cycles of 94°C for 30 s, 50°C to 64°C (depending on the primer) for 30 s, and 72°C for 15 s, and final extension at 72°C for 10 min. Amplified products were identified by their molecular weights after electrophoresis on 1.0% agarose gel at 180 V for 90 min and staining with ethidium bromide. The PCR products were sequenced (Sangon Shanghai, China), and the sequences were analyzed and aligned using the NCBI Basic Local Alignment Search Tool program (http://blast.ncbi.nlm.nih.gov/).

Detection of Class 1 Integrons

All *Salmonella* isolates were screened for the presence of class 1 integrons based on the primers previously

Table 2. PCR primers and annealing temperatures in this study.

Gene	Oligonucleotide sequence (5′→ 3′)	Annealing temperature (°C)	Reference
bla$_{\text{TEM}}$	F: ATTCTTGAAGACGAAAGGGC R: ACGCTCAGTGGAACGAAAAC	60	Li et al., 2013
bla$_{\text{SHV}}$	F: CACTCAAGGATGTATTGTG R: TTAGCGTTGCCAGTGCTCG	60	Li et al., 2013
bla$_{\text{OXA}}$	F: ACACAATACATATCAACTTCGC R: AGTGTGTTTAGAATGGTGATC	60	Li et al., 2013
bla$_{\text{PSE}}$	F: TTT GGT TCCGCG CTA TCT G R: TAC TCC GAG CAC CAA ATC CG	58	Li et al., 2013
bla$_{\text{CTX-M}}$	F: CGCTTTGCGATGTGCAG R: ACCGCGATATCGTTGGT	60	Li et al., 2013
qnrA	F: ATTTCTCACGCCAGGATTTG R: TGCCAGGCACAGATCTTGAC	60	Ahmed et al., 2013
qnrB	F: CGACCTKAGCGGCACTGAAT R: GAGCAACGAYGCCTGGTAGYTG	50	Ahmed et al., 2013
qnrC	F: GGGTTGTACATTTATTGAATC R: TCCACTTTACGAGGTTCT	50	Ahmed et al., 2013
qnrD	F: CGAGATCAATTTACGGGGAATA R: AACAAGCTGAAGCGCCTG	50	Ahmed et al., 2013
aac(6′)-Ib-cr	F: TTGCGATGCTCTATGAGTGGCTA R: CTCGAATGCCTGGCGTGTTT	55	Ahmed et al., 2013
qepA	F: GCAGGTCCAGCAGCGGGTAG R: CTTCCTGCCCGAGTATCGTG	60	Ahmed et al., 2013
oqxA	F: GATCAGTCAGTGGGATAGTTT R: TACTCGGCGTTAACTGATTA	55	Ahmed et al., 2013
oqxB	F: TTCTCCCCCGGCGGGAAGTAC R: CTCGGCCATTTTGGCGCGTA	64	Ahmed et al., 2013
mcr-1	F: CGGTCAGTCCGTTT R: CTTGGTCGGTCTGTAGGG	55	Liu et al., 2016

Table 3. Resistance phenotype, sequence type, incidence of class 1 integron, and resistance genes in *Salmonellla* isolated from broilers in Shandong province.

Source	Strain	Sequence type	Serovar	Antimicrobial resistance	Integrons/resistance genes
Tai'an	T-1	34	Typhimurium	PB	bla_{TEM}, bla_{SPE}, bla_{OXA}, *acc(6′)-Ib-cr*
	T-2	11	Enteritidis	AC-AMP-DOX-PB	bla_{TEM}
	T-3	11	Enteritidis	AC-AMP-PB	bla_{TEM}
	T-4	11	Enteritidis	AC-AMP-DOX-PB	Class 1 (*aadA2*), bla_{TEM}, bla_{SPE}
	T-5	19	Typhimurium	CEF-DOX-ENR-PB	Class 1 (*dfrA1-aadA1*), bla_{TEM}, bla_{SPE}
	T-6	19	Typhimurium	DOX-ENR-PB	bla_{TEM}
	T-7	11	Enteritidis	DOX-ENR-PB	bla_{TEM}, bla_{SPE}
	T-8	11	Enteritidis	ENR-FLO-PB	Class1 (*aacA4-*bla_{OXA}*-CatB3-aar*-3), bla_{TEM}, bla_{SPE}, bla_{OXA}
	T-9	11	Enteritidis	PB	bla_{TEM}, bla_{OXA}
	T-10	11	Enteritidis	FLO-PB	bla_{TEM}
	T-11	14	Senftenberg	AC-AMP-CEF-DOX-ENR-FLO-GEN-PB	bla_{TEM}, bla_{CTX-M}, bla_{OXA}, *acc(6′)-Ib-cr*
	T-12	17	Indiana	AC-AMP-CEF-DOX-ENR-FLO-PB	bla_{TEM}, bla_{CTX-M}
	T-13	11	Enteritidis	ENR-FLO-PB	bla_{TEM}, bla_{OXA}, *acc(6′)-Ib-cr*
	T-14	34	Typhimurium	PB	bla_{TEM}
	T-15	14	Senftenberg	AC-AMP-CEF-DOX-ENR-GEN-FLO-PB	bla_{TEM}, bla_{CTX-M}, bla_{OXA}, *acc(6′)-Ib-cr*, *oqxB*
	T-16	19	Typhimurium	PB	bla_{TEM}
	T-17	19	Typhimurium	PB	bla_{TEM}
	T-18	198	Kentucky	AC-AMP-CEF-DOX-ENR-GEN-FLO-PB	Class1 (*aadA7*), bla_{TEM}, bla_{CTX-M}, bla_{OXA}, *acc(6′)-Ib-cr*, *mcr*-1
	T-19	11	Enteritidis	AC-AMP-PB	bla_{TEM}
	T-20	19	Typhimurium	PB	bla_{TEM}
	T-21	11	Enteritidis	AC-AMP-FLO-PB	bla_{TEM}
	T-22	11	Enteritidis	AC-AMP-CEF-DOX-ENR-FLO-PB	bla_{TEM}
	T-23	11	Enteritidis	CEF-PB	bla_{TEM}
	T-24	11	Enteritidis	AMP-CEF-PB	bla_{TEM}, bla_{OXA}
	T-25	11	Enteritidis	AC-AMP-DOX-PB	bla_{TEM}
	T-26	11	Enteritidis	AMP-CEF-PB	bla_{TEM}
	T-27	11	Enteritidis	AMP-ENR-PB	bla_{TEM}
	T-28	11	Enteritidis	AMP-CEF-PB	bla_{TEM}, bla_{OXA}
	T-29	19	Typhimurium	PB	bla_{TEM}
	T-30	11	Enteritidis	AC-AMP-DOX-PB	bla_{TEM}
	T-31	19	Typhimurium	PB	bla_{TEM}
	T-32	19	Typhimurium	PB	bla_{TEM}
	T-33	198	Kentucky	AC-AMP-CEF-DOX-ENR-FLO-GEN-NEO-PB	bla_{TEM}, bla_{CTX-M}, bla_{OXA}, *acc(6′)-Ib-cr, qnrB, mcr*-1
	T-34	198	Kentucky	AC-AMP-CEF-DOX-ENR-FLO-GEN-NEO-PB	bla_{TEM}, bla_{CTX-M}, bla_{OXA}, *acc(6′)-Ib-cr*
	T-35	198	Kentucky	AC-AMP-CEF-DOX-ENR-FLO-NEO-PB	bla_{TEM}, bla_{CTX-M}, bla_{OXA}, *acc(6′)-Ib-cr*, *mcr*-1
Jinan	J-1	19	Typhimurium	DOX-ENR-GEN-NEO-PB	Class 1 (*dfrA17-aadA5*), bla_{TEM}
	J-2	17	Indiana	AC-AMP-DOX-ENR-FLO-NEO-PB	Class 1 (*aadA2*), bla_{TEM}, *acc(6′)-Ib-cr*
	J-3	92	Pullorum	AMP-CEF-PB	bla_{TEM}
	J-4	17	Indiana	AC-AMP-DOX-FLO-GEN-NEO-PB	Class 1 (*aadA2*), bla_{TEM}, *acc(6′)-Ib-cr*, *oqxB*
	J-5	34	Typhimurium	AC-AMP-ENR-FLO-GEN-NEO-PB	bla_{TEM}, bla_{OXA}, *acc(6′)-Ib-cr*
	J-6	11	Enteritidis	AMP-ENR-FLO-GEN-NEO-PB	Class1 (*aadA2*), bla_{TEM}, *acc(6′)-Ib-cr, qnrB*, *oqxB*
	J-7	92	Pullorum	AC-AMP-CEF-DOX-ENR-FLO-NEO-PB	bla_{TEM}, bla_{CTX-M}, *oqxB*
	J-8	92	Pullorum	AC-AMP-CEF-DOX-ENR-FLO-NEO-PB	Class1 (*aacA4-*bla_{OXA}*-CatB3-aar-3*), bla_{TEM}, bla_{CTX-M}, *qnrB*, *oqxB*
	J-9	11	Enteritidis	AC-AMP-DOX-ENR-GEN-NEO-PB	bla_{TEM}
	J-10	19	Typhimurium	AMP-PB	bla_{TEM}, bla_{OXA}
	J-11	19	Typhimurium	PB	Class1 (*aacA4-*bla_{OXA}*-CatB3-aar-3*), bla_{TEM}, bla_{CTX-M}, bla_{SPE}, bla_{OXA}, *acc(6′)-Ib-cr*, *oqxB*
	J-12	11	Enteritidis	AMP-PB	Class1 (*dfrA17-aadA5*), bla_{TEM}, bla_{CTX-M}, *oqxB*
	J-13	19	Typhimurium	PB	Class1 (*aadA22*), bla_{TEM}
	J-14	19	Typhimurium	PB	Class1 (*dfrA17-aadA5*), bla_{TEM}, bla_{CTX-M}, bla_{OXA}, *acc(6′)-Ib-cr*, *qnrB, oqxB*
	J-15	34	Typhimurium	PB	bla_{TEM}, bla_{OXA}
Weifang	W-1	11	Enteritidis	AC-AMP-PB	Class1 (*dfrA17-aadA5*), bla_{TEM}
	W-2	11	Enteritidis	AC-AMP-PB	bla_{TEM}
	W-3	11	Enteritidis	AC-AMP-PB	bla_{TEM}, bla_{CTX-M}
	W-4	11	Enteritidis	AC-AMP-PB	bla_{TEM}
	W-5	11	Enteritidis	AC-AMP-CEF-NEO-PB	bla_{TEM}, bla_{CTX-M}
	W-6	11	Enteritidis	AC-AMP-CEF-DOX-ENR-FLO-NEO-PB	bla_{TEM}, bla_{CTX-M}, bla_{OXA}, *acc(6′)-Ib-cr*, *mcr*-1

(continued on next page)

Table 3. (*continued*)

Source	Strain	Sequence type	Serovar	Antimicrobial resistance	Integrons/resistance genes
	W-7	19	Typhimurium	AC-AMP-CEF-DOX-ENR-FLO-NEO-PB	Class1 (*aadA5*-bla_{OXA}), bla_{TEM}, bla_{CTX-M}, *acc(6′)-Ib-cr*, *qnrD*
	W-8	11	Enteritidis	AMP-CEF-DOX-ENR-FLO-NEO-PB	Class1 (*aacA4*-bla_{OXA}-*CatB3-aar-3*), bla_{TEM}
	W-9	11	Enteritidis	AMP-CEF-DOX-ENR-FLO-PB	bla_{TEM}, bla_{CTX-M}, *qnrB*, *oqxB*
	W-10	11	Enteritidis	AC-AMP-PB	Class1 (*dfrA17-aadA5*), bla_{TEM}
	W-11	11	Enteritidis	AC-AMP-PB	Class1 (*dfrA17-aadA5*), bla_{TEM}, bla_{OXA}
	W-12	11	Enteritidis	AC-AMP-PB	bla_{TEM}
	W-13	11	Enteritidis	AC-AMP-PB	bla_{TEM}, bla_{SPE}
	W-14	11	Enteritidis	AC-AMP-DOX-PB	bla_{TEM}
	W-15	11	Enteritidis	AC-AMP-DOX-ENR-PB	bla_{TEM}
	W-16	11	Enteritidis	AC-AMP-CEF-DOX-ENR-FLO-NEO-PB	bla_{TEM}, bla_{CTX-M}, *acc(6′)-Ib-cr*, *qnrB*
	W-17	11	Enteritidis	AC-AMP-CEF-DOX-ENR-FLO-NEO-PB	bla_{TEM}, *acc(6′)-Ib-cr*

described (Kerrnet et al., 2002). The amplification fragments were purified from the agarose gel using a gel extraction kit (Tiangen, Beijing, China) and subsequently sequenced (Invitrogen, Beijing, China). Gene cassette homology searches were preformed using a Basic Local Alignment Search Tool analysis (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

Multilocus Sequence Typing

All *Salmonella* isolates were characterized by multilocus sequence typing (**MLST**), which was performed using 7 housekeeping genes (*aroC*, *dnaN*, *hemD*, *hisD*, *purE*, *sucA*, and *thrA*) as described online (http://mlst.warwick.ac.uk/mlst/dbs/Senterica/documents/primersEnterica_html). All amplification fragments were purified and sequenced (Invitrogen, Beijing, China). The alleles and sequence types (**STs**) were assigned according to the MLST database (http://mlst.warwick.ac.uk/mlst/dbs/Senterica) criteria.

RESULTS

Prevalence and Serovars of Salmonella

The prevalence of *Salmonella* in broilers form Shandong is presented in Table 1. Overall, 600 fresh fecal swabs were tested, and 67 (67/600, 11.2%) samples were positive for *Salmonella*, corresponding to 35/200 (17.5%) samples in Tai'an, 15/200 (7.5%) samples in Jinan, and 17/200 (8.5%) samples in Weifang.

The 67 *Salmonella* isolates were serotyped into 6 distinct serovars. These serotypes were including *Salmonella enteritidis* (*S. enteritidis*), *Salmonella typhimurium* (*S. typhimurium*), *Salmonella kentucky* (*S. kentucky*), *Salmonella indiana* (*S. indiana*), *Salmonella pullorum* (*S. pullorum*), and *Salmonella senftenberg* (*S. senftenberg*). The most common serovars were *S. enteritidis* (37/67, 55.2%) and *S. typhimurium* (18/67, 26.9%). In addition, there were 5 distinct serovars from Tai'an, 4 from Jinan, and 2 from Weifang. *S. pullorum* was detected only in Jinan, while *S. senftenberg* and *S. kentucky* were isolated only from Tai'an (Table 3).

Antimicrobial Susceptibility Testing

The results of the antimicrobial susceptibility analysis of 67 *Salmonella* isolates are presented in Table 3. The highest occurrence of resistance observed was for PB (67/67, 100%), followed by AMP (46/67, 68.7%). In contrast, low level of resistance was found for gentamicin (10/67, 14.9%)and NEO (17/67, 25.4%). In addition, 36 isolates (36/67, 53.7%) exhibited MDR. Of the isolates from Tai'an, the most frequent MDR pattern was AC, AMP, DOX, and PB, which was represented by 4 *S. enteritidis* isolates; the isolates from Jinan were AC, AMP, CEF, DOX, ENR, FLO, NEO, and PB, which were represented by 2 *S. pullorum* isolates; the isolates from Weifang was AC, AMP, CEF, DOX, ENR, FLO, NEO, and PB, which was represented by one *S. typhimurium* isolates and 3 *S. enteritidis* isolates. The MDR *Salmonella serovars* were *S. enteritidis* (20/36, 55.6%), *S. typhimurium* (5/36, 13.9%), *S. kentucky* (4/36, 11.1%), *S. indiana* (3/36, 8.3%), *S. pullorum* (2/36, 5.6%), and *S. senftenberg* (2/36, 5.6%).

Detection of Antimicrobial Resistance Genes

PCR analysis for antimicrobial resistance revealed that 4 β-lactamase genes were detected among the isolates, and all the isolates carried bla_{TEM} (67/67, 100%), followed by bla_{OXA} (19/67, 28.4%), bla_{CTX-M} (17/67, 25.4%), and bla_{PSE} (7/67, 10.4%). Four plasmid-mediated quinolone resistance genes were detected among the isolates, and the prevalent resistance gene was *aac(6′)-Ib-cr* (18/67, 26.9%), followed by *oqxB* (9/67, 13.4%), *qnrB* (6/67, 9.0%), and *qnrD* (1/67, 1.5%). The prevalent rate of *mcr*-1 was 6.0% (4/67). Of note, bla_{OXA} was commonly found in *Salmonella* isolates from Tai'an, *oqxB* was commonly found in Jinan, and bla_{CTX-M} was commonly found in Weifang (Table 3).

Detection of Class 1 Integrons

The overall occurrence of class 1 integrons carrying *Salmonella* in tested samples was 26.9% (18/67) and contained 7 groups of resistance gene cassettes. The

gene cassette *dfrA17-aadA5* (6/18, 33.3%) was the most prevalent in class 1 integrons-carrying *Salmonella* in this study, followed by *aadA2* (4/18, 22.2%), *aacA4-bla*$_{OXA}$-*CatB3-aar-3* (4/18, 22.2%), *aadA7* (1/18, 5.6%), *aadA22* (1/18, 5.6%), d*frA1-aadA1* (1/18, 5.6%), and *aadA5-bla*$_{OXA}$ (1/18, 5.6%) (Table 3). *aadA7* and d*frA1-aadA1* were found only in Tai'an, *aadA22* was found only in Jinan, and *aadA5-bla*$_{OXA}$ was found only in Weifang.

MLST Analysis

An interlinked data set with partial sequencing of 7 housekeeping genes at 399 bp to 501 bp revealed that all the *Salmonella* isolates were grouped into 7 STs: ST11, ST14, ST17, ST19, ST34, ST92, and ST198 (Table 3). ST11 was the most common ST in this study both in Tai'an and Weifang and involved with 37 *Salmonella* isolates. ST19 was the most prevalent ST in Jinan and involved with 5 *Salmonella* isolates. In addition, most of the isolates with similar STs belonged to the same serovars, such as ST11 with *S. enteritidis*, ST19 and ST34 with *S. typhimurium*, and ST17 with *S. indiana*.

DISCUSSION

The prevalence and distribution of *Salmonella* constitute a threat to human health and present a major financial burden (Moawad et al., 2017). In the present study, the prevalence of *Salmonella* was 11.2%, which was similar to that in the previous reports from poultry slaughterhouses in Sichuan province (10.7%) (Li et al., 2013) and Germany (13.2%) but was lower than that reported from poultry slaughterhouses in Shandong province (23.5%) (Zhao et al., 2017a, Zhao et al., 2017b) and Thailand-Cambodia border provinces (35.8%) (Trongjit et al., 2017). Data on the prevalence of *Salmonella* in different studies were difficult to compare based on differences in regions, sampling procedures, sample sizes, collection seasons, and bacteria isolation and identification methods. In this study, the prevalence of *Salmonella* in broilers (17.5%) from Tai'an was higher than that in other regions. This may be the main reason for the poor farm management which can result in widespreading of *Salmonella* in farms; farm management was very important. The source of *Salmonlla* infection to broilers may be the feed, drinking water, and rodents.

In many countries all over the world, a wide range of different *Salmonella* serotypes have been found to contaminate the broilers (Abdeen et al., 2018). In addition, *S. enteritidis* was the most common serotype identified in broilers, which was similar to the previous reports from Sichuan and Henan province (Lu et al., 2011; Bai et al., 2015). However, other studies have reported the dominance of other *Salmonella* serovars: *S. indiana* in Chicken farms in Shandong, China, and Kagoshima, Japan (Zhao et al., 2017a, Zhao et al., 2017b; Duc et al., 2019), and *S. typhimurium* in Latvia (Terentjeva et al., 2017). This difference may be associated with geographic variation and the chicken breeds. Of note, high isolation rates of *S. typhimurium* were also noticed. *S. typhimurium* remains one of the main serotype that can lead to sever human and animal diseases (Amanda et al., 2020). Therefore, it is necessary to continuously monitor the local serovar variation of *Salmonella* and formulate a reasonable prevention and control strategy accordingly.

Antibiotic resistance in *Salmonella* has been a major problem in animal farms especially in broilers. Although relevant departments have been emphasizing the limited use of antibiotics in animal feeding, the effect was small. In this study, all the *Salmonella* isolates were resistant to PB, which was much higher than that previously reported in *Salmonella* from food-producing animals (5.2%) at slaughter in Europe (Farid et al., 2018). This high resistant rate of PB in broilers may be attributed to the wide use of this antibiotic in animals during breeding and disease control, and it suggests that farm managers should reduce antibiotic usage. Resistance to AMP (68.7%) was frequently observed in *Salmonella* isolates, which was higher than that previously reported in Jiangsu province (19.3%) (Cai et al., 2016) and in South Africa (47.0%) (Zishiri et al., 2016). In this study, the MDR *Salmonella* isolate rate was 53.7% which was lower than that previously reported from Shandong province (80.8%) in 2016 (Zhao et al., 2017a, Zhao et al., 2017b) and similar to the report from pigs in Southern Brazil (Tamang et al., 2015). In addition, *S. enteritidis* showed a high MDR rate, in contrast with the report that most of *S. indiana* showed MDR (Zhang et al., 2020). Considering these results, the prudent use of antibacterial agents should be strongly recommended in clinical, veterinary, and agricultural settings to preserve antibiotic activity and avoid the development of cross-resistance.

Production of β-lactamases has been identified as the main plasmid-mediated mechanisms of resistance to third-generation cephalosporins and is currently considered a major concern both in human and veterinary medicine (Rhouma and Letellier, 2017). In this study, all the *Salmonella* isolates carried *bla*$_{TEM}$, which was higher than that in the report from Egypt (41.5%) (Ashraf et al., 2014). Of note, all the *Salmonella* isolates carried *bla*$_{TEM}$, but 68.7% showed phenotypical resistance to AMP, indicating that there existed another resistance mechanism. It has been reported that *bla*$_{OXA}$ was considered to be the most commonly identified β-lactamase gene in *Salmonella* isolates from China, and in portugal, *bla*$_{CTX-M}$ was commonly detected in *Salmonella* isolates from poultry, swine, and food products of animal origin (Clemente et al., 2013), which was different from our result.

Quinolones are commonly used in veterinary practice worldwide for *Salmonella* infections (Mehdi et al., 2018). In this study, *aac(6′)-Ib-cr*, *oqxB*, *qnrB*, and *qnrD* genes were detected in 26.9, 13.4, 9.0, and 1.5%, respectively, in all *Salmonella* isolates, which was different from the report in Henan province, *qnrA*, *qnrB*, *qnrS*, and *aac(6′)-Ib-cr* genes were identified in *Salmonella* strains isolated from retail foods with the incidence of 46.6, 12.7,

19.5, and 13.6%, respectively (Yang et al., 2013). In other study, in Egypt, *qnrA*, *qnrB*, and *qnrS* genes were detected in 33.3, 20.0, and 6.7%, respectively, in all *Salmonella* isolates from raw chicken and beef meat (Moawad et al., 2017). In addition, most *Salmonella* isolates containing a quinolone resistance gene displayed resistance to ENR, suggesting the chromosomal quinolone resistance determining region point mutation might be present in these strains and conferring the high-level quinolone resistance.

Colistin is often used to treat food-producing animals and has been considered the last resort antibiotics for the rapidly increasing MDR gram-negative pathogens (Kaye et al., 2016). However, colistin resistance mediated by *mcr*-1–harboring plasmids is an emerging threat in Enterobacteriaceae, similar to *Salmonella* (Sun et al., 2018). In this study, the prevalent rate of *mcr*-1 was 6.0%, which was higher than that in Europe (0.1%). In this study, of all the *Salmonella* resistance to PB, however, only 6.0% of the isolates carried *mcr*-1, which was different from the report that there was a close positive correlation between the resistance phenotypes and genotypes of the isolates.

The presence of genetic element such as integrons is often associated with multiresistant phenotypes among *Salmonella* isolates and plays an important role in the spread of antimicrobial resistance genes among gram-negative bacteria (Marathe et al., 2019). In this study, class 1 integron detected in 26.9% of *Salmonella* isolates, which was higher than that previously study from raw chicken and beef meat in Egypt (13.3%) (Moawad et al., 2017) and from poultry in Korea (9.1%) (Dessie et al., 2013) but lower than a report from meat and dairy products in Egypt (39.1%) (Ashraf et al., 2014). The predominant gene cassette was d*frA17-aadA5*, which confers resistance to trimethoprim (*dfrA*) and spectinomycin (*aadA*) and has been reported worldwide in isolates from different origins; it might be associated with the extensive use of trimethoprim and spectinomycin in broiler breeding. In addition, 61.1% class 1 integron was detected in MDR *Salmonella* isolates, which was different from the report that all the class 1 integron exhibited resistance to at least 3 classes of antimicrobials (Firoozeh et al., 2012; Wang et al., 2020).

MLST results showed that 7 STs were generated from all *Salmonella* isolates belonging to 6 serotypes. ST11 was the most frequent genotype that was recovered in this study, and this ST corresponded to *S. enteritidis*, which coincides with our previous report from chickens in Shandong (Zhao et al., 2017a, Zhao et al., 2017b). ST19 and ST34 corresponded to *S. typhimurium*, which have continually been reported to cause human salmonellosis in recent years (Garvey et al., 2013). Another case that should be considered is ST92, a rarely reported ST which appeared in Shandong province that corresponds to *S. pullorum* and often reported to cause pullorum disease of chickens in China (Wang et al., 2020). Our results revealed that the MLST patterns were generally associated with serotypes and provided a reliable prediction of the *Salmonella* serovars (Cai et al., 2016).

CONCLUSION

In summary, we examined the epidemiology of *Salmonella* from broilers in Shandong, China. The results showed that the prevalence of *Salmonella* was found to be high in the broilers. Clinically important serovars *S. enteritidis* and *S. typhimurium* dominated the isolates recovered in this study, with almost all other identified serovars also linked to human salmonellosis. In addition, the high rate of MDR *Salmonella* is alarming which requires the prudent use of antibiotics in broilers. Therefore, continuous surveillance of *Salmonella* in broilers is essential to detect emerging *Salmonella* serovar, antimicrobial resistance trends, and associated resistance genes.

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DISCLOSURES

The authors declare that they have no conflict of interest.

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