

Prevalence and antimicrobial resistance of *Salmonella* isolated from broilers in Shandong, China

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Abstract

Background *Salmonella* spp. are one of the most important foodborne bacterial pathogens in human beings and animals. The prevalence of *Salmonella* from broilers in Shandong, China and antimicrobial susceptibility of these isolates was determined.

Results From May to October 2018, 600 samples collected, 67 *Salmonella* isolates were recovered with an isolation rate of 11.2%. The most common serovars were *S. enteritidis* and *S. typhimurium*. The highest incidence of resistance observed were for PB (100%), and AMP (68.7%), and the MDR *Salmonella* isolate rate was 53.7%. Four β -lactamase genes were detected among the isolates, 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, the prevalent resistance genes was *aac*(6')-Ib-cr (18/67, 26.9%), followed by *oqx*B (9/67, 13.4%), *qnr*B (6/67, 9.0%), and *qnr*D (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 seven groups of resistance gene cassettes. MLST analysis revealed seven sequence types, and ST11 was the most frequent sequence types.

Conclusions This study indicated that reduction of *Salmonella* and strict control on the use of antibiotics in more than 5000 million broilers in Shandong are the vitally important measure to keep public health.

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 [1]. It has been widely reported that most of human salmonellosis is caused by infection derived from contaminated eggs, poultry meat and meat-products [1–4].

To date, more than 2600 *Salmonella* serovars have been reported [5]. Poultry, especially broilers, are well known reservoirs of various *Salmonella* serovars, most of which are able to infect humans [6]. Therefore, it is meaning that serovar determination is extremely important for effective epidemiological surveillance and disease assessment.

Antimicrobial resistance is increasingly becoming an important issue with salmonellosis infections in both animals and humans [7]. In animal husbandry, antibiotics are widely used for growth promotion, or treatment purposes which facilitated the emergence and dissemination of antibiotic resistance in *Salmonella* [8]. *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 [9]. The chicken can be used as a vehicle for spreading and distributing of these antimicrobial resistant strains to humans [10].

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. In China, broilers are widely reared and is an important sources of chicken meat [11]. Therefor, it is necessary to monitor Salmonella in broiler every year. The purpose of this study was to determine the prevalence and characteristics of Salmonella isolated from broilers in Shandong province, China.

Methods

Sampling

From May to October 2018, three large-scale intensive broiler farms in Tai'an, Jinan, and Weifang areas of Shandong Province were selected as sampling points. Farms were chosen based on their scale with the following requirements that the breeding stock was > 150,000 heads. For each farm, 200 fresh fecal swabs were randomly collected from different individual animals, a total of 600 samples, which were sent to the laboratory for bacterial isolation and identification at low temperature (Table 1).

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%)

Salmonella isolation and serotype identification

Salmonella isolation was conducted using previously published protocols [12], with some modification. Briefly, each swab sample was mixed with 9 mL of buffered peptone water (BPW) and incubated at 37°C for 16 to 18 h for pre-enrichment. 1 mL of suspensions was subcultured in 10 ml of selenite cysteine (SC) broth at 42°C for 24 h. One loopful SC broth culture was then streaked onto xylose lysine tergitol 4 agar plates, which were incubated at 37°C for 24–48 h. Putative Salmonella isolates were chosen by 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 [13] guidelines. Isolates were tested for sensitivity to amoxicillin/clavulanic acid (AC), ampicillin (AMP), ceftiofur (CEF), enrofloxacin (ENR), neomycin (NEO), doxycycline (DOX), florfenicol (FLO), gentamicin (GEN), and polymyxin (PB) which were commonly used in farms. An isolate was defined as multidrug-resistant (MDR) isolate if it was resistant to no less than three classes of antimicrobials. The *Escherichia coli* ATCC 25922 was used as a quality control in this study.

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 (ESBL) (*bla*_{TEM}, *bla*_{CTX-M}, *bla*_{OXA}, *bla*_{SHV} and *bla*_{PSE}), plasmid-mediated quinolone resistance genes (PMQR) (*aac*(6')-Ib-cr, *qnrA*, *qnrB*, *qnrC*, *qnrD*, *qepA*, *oqxA* and *oqxB*) and polymyxin resistance gene (*mcr-1*) was performed using previously reported primers and conditions [14–16]. 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.

Detection of class 1 integrons

All *Salmonella* isolates were screened for the presence of class 1 integrons based on the primers previously described [17]. 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 performed using Basic Local Alignment Search Tool (BLAST) analysis (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>).

Multilocus sequence typing (MLST)

All *Salmonella* isolates were characterized by MLST, which was performed using seven housekeeping genes (*aroC*, *dnaN*, *hemD*, *hisD*, *purE*, *sucA*, and *thrA*) as described online (http://mlst.warwick.ac.uk/mlst/dbs/Senterica/documents/primers_Enterica_html). All amplification fragments were purified and sequenced (Invitrogen, Beijing, China). The alleles and 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 from Shandong is presented in Table 1. Overall, 600 fresh fecal swabs tested, 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, 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%) (Table 2).

Table 2

Resistance phenotype, ST, incidence of class 1 integron, and resistance genes in Salmonella isolated from broilers in Shandong province.

Source	Strain	ST	Serovar	Antimicrobial resistance	Integrans/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}

Source	Strain	ST	Serovar	Antimicrobial resistance	Integrans/resistance genes
	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}

Source	Strain	ST	Serovar	Antimicrobial resistance	Integrans/resistance genes
	J-2	17	Indiana	AC-AMP-DOX-ENR-FLO-NEO-PB	Class 1 (aadA2), bla _{TEM} , acc(6')-lb-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')-lb-cr, oqxB
	J-5	34	Typhimurium	AC-AMP-ENR-FLO-GEN-NEO-PB	bla _{TEM} , bla _{OXA} , acc(6')-lb-cr
	J-6	11	Enteritidis	AMP-ENR-FLO-GEN-NEO-PB	Class1(aadA2), bla _{TEM} , acc(6')-lb-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')-lb-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')-lb-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}

Source	Strain	ST	Serovar	Antimicrobial resistance	Integrans/resistance genes
	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')-lb-cr, mcr-1
	W-7	19	Typhimurium	AC-AMP-CEF-DOX-ENR-FLO-NEO-PB	Class1(aadA5-bla _{OXA}), bla _{TEM} , bla _{CTX-M} , acc(6')-lb-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')-lb-cr, qnrB
	W-17	11	Enteritidis	AC-AMP-CEF-DOX-ENR-FLO-NEO-PB	bla _{TEM} , acc(6')-lb-cr

Antimicrobial Susceptibility testing

The results of the antimicrobial susceptibility analysis of 67 Salmonella isolates are presented in Table 2. The highest incidence of resistance observed were for PB (67/67, 100%), and AMP (46/67, 68.7%). In

contrast, low level of resistance was found for GEN (10/67, 14.9%), and NEO (17/67, 25.4%). In this study, 36 out of 67 isolates (53.7%) showed multidrug resistance phenotypes to at least three classes of antimicrobials. 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 resistance genes revealed that four β -lactamase genes were detected among the isolates, 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, the prevalent resistance gene was $aac(6')-Ib-cr$ (18/67, 26.9%), followed by oqx_B (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) (Table 2).

Detection of class 1 integrons

The overall prevalence of class 1 integrons carrying *Salmonella* in tested samples was 26.9% (18/67) and contained seven 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%), $dfrA1-aadA1$ (1/18, 5.6%), $aadA5-bla_{OXA}$ (1/18, 5.6%) (Table 2).

MLST analysis

An interlinked dataset with partial sequencing of seven housekeeping genes at 399 bp to 501 bp revealed that all the *Salmonella* isolates were grouped into seven STs: ST11, ST14, ST17, ST19, ST34, ST92, ST198 (Table 2). ST11 was the most common ST in this study involved with 37 *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 [18]. In this study, The *Salmonella* isolation rate of 11.2% from broilers showed a lower prevalence than that from poultry slaughterhouses in Shandong province (23.5%) [19] and in Ireland (26.4%) from the poultry carcasses [20], and was similar to the previous reports from poultry slaughterhouses that in Sichuan province (10.7%) [14], and in Germany (13.2%) [21]. Although different sampling procedures, sample sizes, collection seasons, region difference and bacteria isolation and identification methods could affect the isolation rates of *Salmonella*, this level of contamination indicates a potential breakdown of hygiene at broiler farms. In many countries all over the world, a wide range of different *Salmonella* serotypes have been found to contaminate the broilers [22]. Additionally, *S. enteritidis* was the most common serotype identified in broilers, which was similar to the previous reports from Sichuan province and Henan province [23, 24], and has been isolated in chicken eggs and chicken

meat (11), but contrasts with our previous report from chickens that the most common serovar was *S. Indiana* [25]. This difference may be associated with geographic variation and the chicken breeds. Of note, high isolation rates of *S. typhimurium* was also noticed. *S. typhimurium* remains one of the main serotype that can lead to severe human and animal diseases [26].

Antibiotic resistant in *Salmonella* has been a major problem in animal farms especially in broilers. Though 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 resistance to PB, which was much higher than that previously reported in *Salmonella* from food-producing animals (5.2%) at slaughter in Europe [27]. This high resistant rate of PB in broilers may be attributed to the widely use of this antibiotic in animals during breeding, and disease control and it suggesting that farm managers should reduce antibiotic usage. Resistance to AMP (68.7%) frequently observed in *Salmonella* isolates, which was higher than that previously reported in Jiangsu province (19.3%) [28] and in the South Africa and Brazil (47.0%) [29]. In this study, the MDR *Salmonella* isolate rate was 53.7% which was lower than that our previously reported from Shandong province (80.8%) in 2016 [25] and similar to the report from pigs in Southern Brazil [30]. In addition, *S. enteritidis* showed a high multidrug resistance rate, contrasts with the report that most of *S. Indiana* showed multidrug resistance [24].

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 [31]. In this study, all the *Salmonella* isolates carried bla_{TEM} which was higher than the report in Egypt (41.5%) [32]. 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 [33], which was different from our result.

Quinolones are commonly used in veterinary practice worldwide for *Salmonella* infections [34]. 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 [35]. 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 [36].

Colistin are often used to treat food-producing animals and has been considered the last resort antibiotics for the rapidly increasing MDR gram-negative pathogens [37]. However, colistin resistance mediated by $mcr-1$ -harbouring plasmids is an emerging threat in Enterobacteriaceae, like *Salmonella* [38]. In this study, the prevalent rate of $mcr-1$ was 6.0% which was higher than that in Europe (0.1%). In this study, 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 [39].

The presence of genetic element such as integrons is often associated with multi-resistant phenotypes among Salmonella isolates and plays an important role in spread of antimicrobial resistance genes among gram-negative bacteria [40]. 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%) [36] and from poultry in Korea (9.1%) [41], but lower when compared with a report from meat and dairy products in Egypt (39.1%) [32]. The predominant gene cassette was *dfrA17-aadA5* which conferring resistance to trimethoprim (*dfrA*) and spectinomycin (*aadA*) and has been reported worldwide in isolates from different origins, 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 the reports that all the class 1 integron exhibited resistance to at least three classes of antimicrobials [42, 43].

MLST results showed that seven STs were generated from all Salmonella isolates belonging to six serotypes. ST11 was the most frequent genotype that was recovered in this study and this ST corresponded to *S. enteritidis*, which was coincide with our previously report from chickens in Shandong [25]. ST19 and ST 34 corresponded to *S. typhimurium* have continually been reported to cause human salmonellosis in recent years [44]. Another case that should be considered is ST92, a rarely reported ST which appeared in Shandong province that corresponds to *S. pullorum* which often reported to cause pullorum disease of chickens in China [42].

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. *S. enteritidis* and *S. typhimurium* were the most common serotypes that reported to cause human salmonellosis, and the high rate of MDR Salmonella were recovered in this study. This finding highlights the fact that broilers remain to be a potential reservoir of antimicrobial-resistant Salmonella. Therefore, prudent use of antibiotics and management in broilers breeding should be enacted by the authorities to ensure food safety.

Abbreviations

AC

Amoxicillin/clavulanic acid; AMP:Ampicillin; BPW:Buffered peptone water; CEF:Ceftiofur; ENR:Enrofloxacin; NEO:Neomycin; DOX:Doxycycline; FLO:Florfenicol; GEN:Gentamicin; MDR:Multidrug-resistant; MLST:Multilocus sequence typing; PB:Polymyxin; SC:Selenite cysteine; STs:Sequence types.

Declarations

Acknowledgments

Not Applicable.

Authors' contributions

Yuqing Liu and Xiaonan Zhao conceived the project and designed the study. All authors performed the experiments and analyzed the results. Xiaonan Zhao wrote the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The data used and analyzed during the present study are accessible from the corresponding author on request.

Ethics approval and consent to participate

Verbal consent for all the sampling procedures was obtained from the owners of the animals. All procedures were approved by the Animal Care and Use of Shandong Academy of Agricultural Sciences (SAAS-2019-021).

Consent for publication

Not Applicable.

Conflict of interest

All the authors declare no conflict of interest.

References

1. Kirk MD, Pires SM, Black RE, Caipo M, Crump JA, Devleeschauwer B, et al. World health organization estimates of the global and regional disease burden of foodborne bacterial, protozoal, and viral diseases, 2010: a data synthesis. *PLoS Med.* 2015;12(12):e1001921.
2. Hedican E, Smith K, Jawahir S, Scheftel J, Kruger K, Birk R, et al. Multistate outbreaks of Salmonella infections associated with live poultry-United States, 2007. *Morb Mortal Wkly Rep.* 2009;58:25–9.
- 3.

- Omwandho C, Kubota T. *Salmonella enterica* serovar Enteritidis: a mini review of contamination routes and limitations to effective control. *Jpn Agric Res Q.* 2010;44:7–16.
4. Hope BK, Baker AR, Edel ED, Hogue AT, Schlosser WD, Whiting E, et al. An overview of the *Salmonella* Enteritidis risk assessment for shell eggs and egg products. *Risk Anal.* 2002;22:203–18.
 5. Achtman M, Wain J, Weill F, Nair S, Zhou Z, Sangal V, et al. Multilocus sequence typing as a replacement for serotyping in *Salmonella enterica*. *PLoS Pathog.* 2012;8:e1002776.
 6. Nógrády N, Király M, Davies R, Nagy B. Multidrug resistant clone of *Salmonella* Infantis of broiler in Europe. *Int J Food Microbiol.* 2012;157:108–12.
 7. Duc VM, Nakamoto Y, Fujiwara A, Toyofuku H, Obi T, Chuma T. Prevalence of *Salmonella* in broiler chickens in Kagoshima, Japan in 2009 to 2012 and the relationship between serovars changing and antimicrobial resistance. *BMC Vet Res.* 2019;15:108.
 8. Gyles CL. Antimicrobial resistance in selected bacteria from poultry. *Anim Health Res Rev.* 2008;9:149–58.
 9. Blair J, Webber M, Baylay A, Ogbolu D, Piddock L. Molecular mechanisms of antibiotic resistance. *Nat Rev Microbiol.* 2015;13:42–51.
 10. Vo AT, Van DE, Fluit AC, Heck ME, Verbruggen A, Maas HM, et al. Distribution of *Salmonella enterica* serovars from humans, livestock and meat in Vietnam and the dominance of *Salmonella* Typhimurium phage type 90. *Vet Microbiol.* 2006;113:153–8.
 11. Long M, Lai H, Deng W, Zhou K, Li B, Liu S, et al. Disinfectant susceptibility of different *Salmonella* serotypes isolated from chicken and egg production chains. *J Appl Microbiol.* 2016;121:672–81.
 12. Yan H, Li L, Alam MJ, Shinoda S, Miyoshi S, Shi L. Prevalence and antimicrobial resistance of *Salmonella* in retail foods in northern China. *Int J Food Microbiol.* 2010;143:230–4.
 13. CLSI. Performance standards for antimicrobial susceptibility testing: twentieth-third informational supplement M100-S23. Wayne: Clinical and Laboratory Standards Institute; 2013.
 14. Li RC, Lai J, Wang Y, Liu SL, Li Y, Liu KY, et al. Prevalence and characterization of *Salmonella* species isolated from pigs, ducks and chickens in Sichuan Province, China. *Int J Food Microbiol.* 2013;163:14–8.
 15. Liu YY, Wang Y, Walsh TR, Yi LX, Zhang R, Spencer J, et al. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular

biological study. *Lancet Infect Dis.* 2016;16:161–8.

16.

Ahmed AM, Shimamoto T, Shimamoto T. Molecular characterization of multidrug-resistant avian pathogenic *Escherichia coli* isolated from septicemic broilers. *Int J Med Microbiol.* 2013;303:475–83.

17.

Kernnet MB, Klemmensen T, Frimodt-Moller N, Espersen F. Susceptibility of Danish *Escherichia coli* strains isolated from urinary tract infections and bacteraemia, and distribution of sul genes conferring sulphonamide resistance. *J Antimicrob Chemother.* 2002;50:513–6.

18.

Scallan E, Hoekstra RM, Angulo FJ, Tauxe RV, Widdowson MA, Roy SL, et al. Foodborne illness acquired in the United States-major pathogens. *Emerg Infect Dis.* 2011;17:7–15.

19.

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

20.

Duffy G, Cloak OM, O'Sullivan MG, Guillet A, Sheridan JJ, Blair IS, et al. The incidence and antibiotic resistance profiles of *Salmonella* spp. on Irish retail meat products. *Food Microbiol.* 1999;16:623–31.

21.

Visscher CF, Klein G, Verspohl J, Beyerbach M, Stratmann-Selke J, Kamphues J. Serodiversity and serological as well as cultural distribution of *Salmonella* on farms and in abattoirs in Lower Saxony, Germany. *Int J Food Microbiol.* 2011;146:44–51.

22.

Guibourdenche M, Roggentin P, Mikoletit M, Fields PI, Bockemuhl J, Grimont P, et al. Supplement 2003–2007 (No.47) to the white-Kauffmann-16 minor scheme. *Res Microbiol.* 2010;161:26–9.

23.

Bai L, Lan RT, Zhang XL, Cui SH, Xu J, Guo YC, et al. Prevalence of *Salmonella* Isolates from Chicken and Pig Slaughter houses and Emergence of Ciprofloxacin and Cefotaxime Co-Resistant *S. enterica* Serovar Indiana in Henan, China. *Plos One.* 2015;10:e0144532.

24.

Lu Y, Wu CM, Wu GJ, Zhao HY, He T, Cao XY, et al. Prevalence of antimicrobial resistance among *Salmonella* isolates from chicken in China. *Foodborne Pathog Dis.* 2011;8:45–53.

25.

Zhao X, Yang J, Zhang B, Sun S, Chang W. Characterization of Integrons and Resistance Genes in *Salmonella* Isolates from Farm Animals in Shandong Province, China. *Front Microbiol.* 2017;8:1300.

26.

Deng X, Ran L, Wu S, Ke B, He D, Yang X, et al. Laboratory-based surveillance of non-typhoidal *Salmonella* infections in Guangdong Province, China. *Foodborne Pathog Dis.* 2012;9:305–12.

27.

- Farid EG, Anno DJ, Xavier B, Didier H, Marlene S. *mcr-1*-like detection in commensal *Escherichia coli* and *Salmonella* spp. From food-producing animals at slaughter in Europe. *Vet Microbiol.* 2018;213:42–6. 28.
- Cai YQ, Tao J, Jiao Y, Fei X, Zhou L, Wang Y, et al. Phenotypic characteristics and genotypic correlation between *Salmonella* isolates from a slaughterhouse and retail markets in Yangzhou, China. *Int J Food Microb.* 2016;222:56–64. 29.
- Zishiri O, Mkhize N, Mukaratirwa S. 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.* 2016;83:1–11. 30.
- Tamang MD, Gurung M, Nam HM, Moon DC, Kim SR, Jang GC, et al. Prevalence and characterization of *Salmonella* in pigs from conventional and organic farms and first report of *S. serovar* 1,4,[5],12: i:- from Korea. *Vet Microbiol.* 2015;178:119–124. 31.
- Rhouma M, Letellier A. Extended-spectrum β -lactamases, carbapenemases and the *mcr-1* gene: is there a historical link? *Int J Antimicrob Agents.* 2017;49:269–71. 32.
- Ashraf MA, Toshi S, Tadashi S. Characterization of integrons and resistance genes in multidrug-resistant *Salmonella enterica* isolated from meat and dairy products in Egypt. *Int J Food Microbiol.* 2014;189:39–44. 33.
- Clemente L, Manageiro V, Ferreira E, Jones-Dias D, Correia I, Themudo P, et al. Occurrence of extended-spectrum β -lactamases among isolates of *Salmonella enterica* subsp. *enterica* from food-producing animals and food products, in Portugal. *Int J Food Microbiol.* 2013;167:221–8. 34.
- Dimitrov T, Udo EE, Albaksami O, Kilani AA, Shehab EMR. Ciprofloxacin treatment failure in a case of typhoid fever caused by *Salmonella enterica* serotype Paratyphi A with reduced susceptibility to ciprofloxacin. *J Med Microbiol.* 2007;56:277–9. 35.
- Yang B, Qiao L, Zhang X, Cui Y, Xia X, Cui S, et al. Serotyping, antimicrobial susceptibility, pulse field gel electrophoresis analysis of *Salmonella* isolates from retail foods in Henan Province, China. *Food Control.* 2013;32:228–35. 36.
- Moawad AA, Hotzel H, Awad O, Tomaso H, Neubauer H, Hafe MH, et al. Occurrence of *Salmonella enterica* and *Escherichia coli* in raw chicken and beef meat in northern Egypt and dissemination of their antibiotic resistance markers. *Gut Pathog.* 2017;9:57. 37.
- Kaye KS, Pogue JM, Tran TB, Nation RL, Li J. Agents of last resort: polymyxin resistance. *Infect Dis Clin N Am.* 2016;30:391–414.

38.

Sun J, Zhang H, Liu YH, Feng Y. Towards understanding MCR-like colistin resistance. *Trends Microbiol.* 2018;26:794–808.

39.

Zhuge XK, Ji YM, Tang F, Sun Y, Jang M, Hu WH, et al. Population structure and antimicrobial resistance traits of avian-origin mcr-1-positive *Escherichia coli* in Eastern China, 2015 to 2017. *Transbound Emerg Dis.* 2019;00:1–10.

40.

Mazel D. Integrons: agents of bacterial evolution. *Nat Rev Microbiol.* 2006;4:608–20.

41.

Dessie HK, Bae DH, Lee YJ. Characterization of integrons and their cassettes in *Escherichia coli* and *Salmonella* isolates from poultry in Korea. *Poultry Sci.* 2013;92:3036–43.

42.

Wang J, Dai D, Zhang HJ, Wu SG, Han YM, Wu YY, et al. Organic Acids Modulate Systemic Metabolic Perturbation Caused by *Salmonella Pullorum* Challenge in Early-Stage Broilers. *Front Physiol.* 2019;10:1418.

43.

Firoozeh F, Zahraei-Salehi T, Shahcheraghi F, Karimi V, Aslani MM. Characterization of class I integrons among *Salmonella enterica* serovar Enteritidis isolated from humans and poultry. *FEMS Immunol Med Microbiol.* 2012;64:237–43.

44.

Garvey P, McKeown P, Kelly P, Cormican M, Anderson W, Flack A, et al. Investigation and management of an outbreak of *Salmonella* Typhimurium DT8 associated with duck eggs, Ireland 2009 to 2011. *Eurosurveillance.* 2013;18:17–23.