Serotype Distribution, Antimicrobial Resistance, and Class 1 Integrons Profiles of Salmonella from Animals in Slaughterhouses in Shandong Province, China

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The current study aimed to analyze the prevalence and characterization of Salmonella enterica isolated from animals in slaughterhouses before slaughter. A total of 143 non-duplicate Salmonella were recovered from 1,000 fresh fecal swabs collected from four major pig slaughterhouses (49/600, 8.2%) and four major chicken slaughterhouses (94/400, 23.5%) between March and July 2016. Among Salmonella isolates from pigs, the predominant serovars were Salmonella Rissen (28/49, 57.1%) and Typhimurium (14/49, 28.6%), and high antimicrobial resistance rates were observed for tetracycline (44/49, 89.8%) and ampicillin (16/49, 32.7%). Class 1 integrons were detected in 10.2% (5/49) of these isolates and all contained gene cassettes aadA2 (0.65 kb). Two β-lactamase genes were detected among these isolates, and most of these isolates carried blaTEM-1 (46/49), followed by blaOXA-1 (4/49). Seven STs (MLST/ST, multilocus sequence typing) were detected in these isolates, and the predominant type was ST469 (19.6%). Among Salmonella isolates from chickens, the predominant serovars were Salmonella Indiana (67/94, 71.3%) and Enteritidis (23/94, 24.5%), and high antimicrobial resistance rates were observed for nalidixic acid (89/94, 94.7%), ampicillin (88/94, 93.6%) and tetracycline (81/94, 86.2%). Class 1 integrons were detected in 23 isolates (23/94, 24.5%), which contained empty integrons (0.15 kb, n = 6) or gene cassettes drfA17-aadA5 (1.7 kb, n = 6), aadA2 (1.2 kb, n = 5), drfA16-blaPSE-1-aadA2-ereA2 (1.6 kb, n = 5) or drfA1-aadA1 (1.4 kb, n = 1). Three β-lactamase genes were detected, and all 94 isolates carried blaTEM-1, followed by blaCTX-M-55 (n = 19) and blaSPE-1 (n = 3). Five STs were found in these isolates, and the predominant type was ST17 (71.3%). Our findings indicated that Salmonella was widespread in animals at slaughter and may be transmitted from animal to fork.

Keywords: Salmonella, animal slaughterhouses, antimicrobial resistance, class 1 integrons, MLST/ST

INTRODUCTION

Salmonella enterica is a major global foodborne pathogen (Chiu et al., 2010; Scallan et al., 2011). More than 2,600 different serovars have been identified worldwide (Guibourdenche et al., 2010). In China, Salmonella causes an estimated 22.2% of foodborne diseases, and the majority of diseases are associated with the ingestion of contaminated meat products (Wang et al., 2007). Both pigs and...
chickens have been recognized as an important reservoir for antibiotic resistant *Salmonella*, and the resistance genes can be transferred to other bacteria via mobile genetic elements, such as plasmids and gene cassettes (Vo et al., 2006).

Agents of different antimicrobial classes, such as β-lactams or fluoroquinolones, are frequently used in clinical practice for *Salmonella enterica* infections. Unfortunately, *Salmonella* has gradually developed a high resistance rate to these antimicrobials, leading to the increase of healthcare costs and even clinical treatment failure (Cui et al., 2009; Gonzalez-Sanz et al., 2009). To date, numerous studies have been conducted to monitor antimicrobial resistance and molecular epidemiology of *Salmonella* isolated from pigs and chickens in slaughterhouses (Van et al., 2013; Mohamed et al., 2014).

However, little information concerning prevalence and characterization of *Salmonella* from animals in slaughterhouses in China is available. Shandong province, as a major breeding region, is the main producer of meat products in China. Therefore, major animal slaughterhouses in Shandong province, China were singled out as sampling sites to analyze the prevalence and characterization of *Salmonella* from animals in slaughterhouses.

**MATERIALS AND METHODS**

**Description of Sampling Sites**

From March to July 2016, 1,000 fresh fecal swabs were collected from four major pig slaughterhouses with process capacity of 1,500–2,500 pigs per day (150 samples per pig slaughterhouse) and four major chicken slaughterhouses with process capacity of 10,000–40,000 chickens per day (100 samples per chicken slaughterhouse). The animal slaughterhouses are respectively located in Weihai, Ciyaoo, Zhucheng, and Yantai regions in Shandong province, China. Sampling was carried out before slaughter, and at the time animals from different farms has been mixed.

**Identification and Sreotyping of Salmonella**

From each animal slaughterhouse, fresh fecal swabs were randomly collected from different individual animals, and transported in an ice box to our laboratory within 6 h for further bacteriological analysis. Each swab sample was added into 50 mL buffered peptone water (BPW) and was incubated at 37°C for 16 to 18 h. After that, 0.1 mL of the BPW suspensions was sub-cultured in 10 mL subpackaged Rappaport-Vassiliadis (RV) broth at 42°C for 24 h. One loopful of each RV broth culture was then plated onto xylose lysine tergitol 4 agar plates, and was incubated at 37°C for 24 to 48 h (Yan et al., 2010). Presumptive *Salmonella* colonies were identified using both the VITEK system (BioMerieux, Marcy l’Etoile, France) and polymerase chain reaction (PCR) amplification of the inherent gene *invA* (Malorny et al., 2003).

All *Salmonella* isolates were serotyped according to the Kauffmann-White scheme by slide agglutination with O and H antigen-specific sera (Tianrun Bio-Pharmaceutical, Ningbo, China) (Grimont and Weill, 2007).

**Antimicrobial Susceptibility Testing**

The Kirby-Bauer disk diffusion method was used in this study to examine resistance of *Salmonella* to 10 commonly used antibiotics, including amoxicillin/clavulanic acid (AMC, 20/10 µg), ampicillin (AMP, 10 µg), cefotaxime (CTX, 30 µg), ciprofloxacin (CIP, 5 µg), florfenicol (FFC, 30 µg), gentamicin (GEN, 10 µg), nalidixic acid (NAL, 10 µg), spectinomycin (SPT, 10 µg), tetracycline (TET, 30 µg), and sulfamethoxazole/trimethoprim (SXT, 1.25/23.75 µg). *Escherichia coli* (ATCC25922) was used as a quality control. The results were interpreted based on the Clinical and Laboratory Standards Institute (CLSI, 2013) *Salmonella* isolates resistant to more than three classes of antimicrobials were defined as multidrug resistance (MDR) isolates.

**Detection of Class I Integrons and β-Lactamase-Encoding Genes**

Bacterial DNA was extracted using a TIANamp bacteria DNA kit (Tiangen, Beijing, China) according to the manufacturer's instructions. The gene cassettes within the variable region of class I integrons were detected via polymerase chain reaction (PCR), using previously described primers and procedures (Kerrn et al., 2002). The PCR products were cloned into the pMD18-T vector using the pMD18-T cloning kit (Takara, Dalian, China).

PCR screening for β-lactamase-encoding genes *blaTEM*, *blaPSE-1*, *blaCMY-2*, *blaOXA-1*, and *blaACTX-M* was performed as previously described (Guerra et al., 2001; Chen et al., 2004; Batchelor et al., 2005; Hasman et al., 2005; Li et al., 2013). The PCR products were purified and subsequently sequenced.

**MLST**

The MLST analysis was performed by sequencing the fragments of seven housekeeping genes (*aroC, dnaN, hemD, hisD, purE, sucA*, and *thrA*), and the alleles and STs were assigned according to the MLST scheme at http://mlst.warwick.ac.uk/ mlst/dbs/Senterica. A minimum spanning tree was created using BioNumerics software 6.5 (Applied Maths, Kortrijk, Belgium), according to the instructions (the unweighted pair group method of arithmetic averages method).

**Statistical Analyses**

All statistical analyses were performed using package SPSS 15.0 (SPSS Inc., Chicago, IL, USA). The *chi-square* test was used to compare the prevalence, multidrug resistance rate and carriage of class 1 integron of *Salmonella* isolated from pigs and chickens, and *P* < 0.05 was considered difference significant.

**RESULTS**

**Prevalence and Serotypes of Salmonella**

From pig slaughterhouses, 49 *Salmonella* isolates were recovered (49/600, 8.2%), including 13 from Weihai (13/150, 8.7%), 9 from Ciyaoo (9/150, 6.0%), 11 from Yantai (11/150, 10.7%),...
and 16 from Zhucheng (16/150, 10.7%) (Table 1). In terms of isolation rate of Salmonella, no significant difference was found between the pig slaughterhouses (P > 0.05). The 49 Salmonella belonged to 6 serovars, including Salmonella Rissen (n = 28), Typhimurium (n = 14), Grampian (n = 3), Derby (n = 2), Indiana (n = 1), and Enteritidis (n = 1). The most common serovars were Salmonella Rissen (28/49, 57.1%) and Typhimurium (14/49, 28.6%) (Table 2).

From chicken slaughterhouses, 94 Salmonella isolates were recovered (94/400, 23.5%), including 23 from Weihai (23/100, 23.0%), 33 from Ciyao (33/100, 33.0%), 17 from Yantai (17/100, 17.0%), and 21 from Zhucheng (21/150, 21.0%) (Table 1). In terms of isolation rate of Salmonella, no significant difference was found between the chicken slaughterhouses (P > 0.05). These 94 Salmonella isolates belonged to 4 serovars, including Salmonella Indiana (n = 67), Enteritidis (n = 23), Typhimurium (n = 3), and Hadar (n = 1). The dominant serovars were Salmonella Indiana (67/94, 71.3%) and Enteritidis (23/94, 24.5%) (Table 2).

### Antimicrobial Susceptibility Testing

All 49 isolates from pig slaughterhouses were susceptible to amoxicillin/clavulanic acid and ceftaxime. But most isolates were resistance to tetracycline (44/49, 89.8%) and ampicillin (16/49, 32.7%). In addition, 7 isolates (7/49, 14.3%) exhibited MDR (Table 2). In addition, 4 isolates were susceptible to all antibiotics used in this study.

All 94 isolates from chicken slaughterhouses were susceptible to amoxicillin/clavulanic acid and sulfamethoxazole/trimethoprim. But most isolates were resistant to nalidixic acid (89/94, 94.7%), ampicillin (87/94, 92.6%), and tetracycline (81/94, 86.2%). Eighty-six isolates (86/94, 91.5%) exhibited MDR (Table 2). Of note, MDR rate of Salmonella from chickens was higher than that from pigs (P < 0.05). In addition, 2 isolates were susceptible to all antibiotics used in this study.

### Characteristics of Class 1 Integrons and β-Lactamase-Encoding Genes

Among the 49 isolates recovered from pigs, class 1 integrons were found in 5 isolates (5/49, 10.2%), including 4 Salmonella Typhimurium and 1 Enteritidis. The 5 isolates only contained the single resistance gene cassette aadA2 (0.65 kb). Two β-lactamase genes were detected among the isolates, most of the isolates carried bla_{spe}-1 (n = 46) and bla_{oxa-1} (n = 4) (Table 2).

Among the 94 isolates recovered from chicken, class 1 integrons were found in 23 isolates (23/94, 24.5%), including 16 Salmonella Indiana, 5 Enteritidis and 2 Typhimurium. Of these isolates, 5 groups of resistance gene cassettes were detected: empty integrons (0.15 kb, n = 6), dfrA17-aadA5 (1.6 kb, n = 6), aadA2 (1.2 kb, n = 5), dfrA16-bla_{spe}-1-aadA2-ereA2 (1.7 kb, n = 5), and dfrA1-aadA1 (1.4 kb, n = 1). Three β-lactamase genes were detected among these isolates. Most of the isolates carried bla_{TEM}-1 (n = 94), followed by bla_{CTX-M-55} (n = 19) and bla_{spe}-1 (n = 3) (Table 2).

### DISCUSSION

In this study, Salmonella isolation rate from pigs (8.2%) was much lower than that (71.8%) in Jiangsu province, China (Cai et al., 2016), and the most common serotype in pigs was Salmonella Rissen, which in consistent with the result from the retail pork products in Thailand (Prapas et al., 2016). However, this finding was different from that reported in EU in which Salmonella Typhimurium was the most common serotype (European Food Safety Authority, 2014). Of note, Salmonella Rissen isolates from pigs only showed resistant to tetracycline (85.7%), which may be associated with the fact that the antimicrobial is frequently used in pig farms in China (Bai et al., 2015).

The Salmonella isolation rate from chickens (23.5%) was similar to the result reported for frozen chicken meat in Shandong province (26.3%), China (Cui et al., 2016). However, the result in this study was much lower than that (45.2%) from chickens in Henan province, China (Bai et al., 2015) and was higher than that (4.5%) from large-scale chicken farms in Shanghai, China (Liu et al., 2010). The difference of these isolation rates may be related with collection seasons, culture methods, and local environments. In the present study, the most common serotypes identified in chickens were

### Summary

This study evaluated the prevalence and characteristics of Salmonella isolates from pigs and chickens in Shandong province, China. The results showed that Salmonella was prevalent in both species, with a higher prevalence in chickens compared to pigs. The most common serotypes identified were Salmonella Indiana and Enteritidis. The antimicrobial susceptibility testing revealed that most isolates were resistant to tetracycline, ampicillin, and sulfamethoxazole/trimethoprim, indicating the need for targeted antimicrobial strategies to control Salmonella infections in these animals.
### Table 2: Resistance phenotype, incidence of class 1 integron, and resistance genes in Salmonella isolated from animals in slaughterhouses.

| No. | Location | Slaughterhouse | Serovar | Resistance phenotype | Integrons/resistance genes |
|-----|----------|----------------|---------|----------------------|----------------------------|
| 1   | Weihai   | Pig            | S. Typhimurium | AMP, TET             | blTEM-1 |
| 2   | Weihai   | Pig            | S. Typhimurium | AMP, TET             | blTEM-1 |
| 3   | Weihai   | Pig            | S. Typhimurium | AMP, TET             | blTEM-1 |
| 4   | Weihai   | Pig            | S. Typhimurium | AMP, TET             | blTEM-1 |
| 5   | Weihai   | Pig            | S. Enteritidis  | AMP, GEN, NAL        | Class 1 (aadA2), blTEM-1,  |
| 6   | Weihai   | Pig            | S. Typhimurium | AMP, TET             | blTEM-1 |
| 7   | Weihai   | Pig            | S. Derby       | AMP, TET             | blTEM-1 |
| 8   | Weihai   | Pig            | S. Rissen     | TET                  | blTEM-1 |
| 9   | Weihai   | Pig            | S. Rissen     | TET                  | blTEM-1 |
| 10  | Weihai   | Pig            | S. Derby       | TET                  | blTEM-1 |
| 11  | Weihai   | Pig            | S. Rissen     | TET                  | blTEM-1 |
| 12  | Weihai   | Pig            | S. Typhimurium | AMP, TET             | blTEM-1, blaOXA-1 |
| 13  | Weihai   | Pig            | S. Typhimurium | AMP, TET             | blTEM-1, blaOXA-1 |
| 14  | Ciyao    | Pig            | S. Typhimurium | AMP, TET             | blTEM-1 |
| 15  | Ciyao    | Pig            | S. Typhimurium | AMP, TET             | blTEM-1 |
| 16  | Ciyao    | Pig            | S. Typhimurium | AMP, TET             | blTEM-1 |
| 17  | Ciyao    | Pig            | S. Grampian    | TET                  | blTEM-1 |
| 18  | Ciyao    | Pig            | S. Indiana     | CIP, FFC, NAL, SXT, TET | Class 1 (aadA2), blTEM-1,  |
| 19  | Ciyao    | Pig            | S. Grampian    | AMP, FFC, SPT, SXT, TET | blTEM-1 |
| 20  | Ciyao    | Pig            | S. Rissen     | TET                  | blTEM-1 |
| 21  | Ciyao    | Pig            | S. Grampian    | TET                  | blTEM-1 |
| 22  | Ciyao    | Pig            | S. Typhimurium | AMP, GEN, FFC, NAL, SPT, SXT, TET | Class 1 (aadA2), blaTEM-1,  |
| 23  | Yantai   | Pig            | S. Typhimurium | AMP, GEN, FFC, SPT, SXT, TET | blTEM-1 |
| 24  | Yantai   | Pig            | S. Typhimurium | AMP, GEN, FFC, NAL, SPT, SXT, TET | Class 1 (aadA2), blTEM-1,  |
| 25  | Yantai   | Pig            | S. Typhimurium | AMP, GEN, FFC, NAL, SPT, SXT, TET | Class 1 (aadA2), blTEM-1,  |
| 26  | Yantai   | Pig            | S. Rissen     | GEN, TET             | blTEM-1 |
| 27  | Yantai   | Pig            | S. Rissen     | TET                  | blTEM-1 |
| 28  | Yantai   | Pig            | S. Rissen     | TET                  | blTEM-1 |
| 29  | Yantai   | Pig            | S. Rissen     | TET                  | blTEM-1 |
| 30  | Yantai   | Pig            | S. Rissen     | TET                  | blTEM-1 |
| 31  | Yantai   | Pig            | S. Rissen     | TET                  | blTEM-1 |
| 32  | Yantai   | Pig            | S. Rissen     | TET                  | blTEM-1 |
| 33  | Yantai   | Pig            | S. Rissen     | GEN, TET             | blTEM-1 |
| 34  | Zhucheng | Pig            | S. Rissen     | TET                  | blTEM-1 |
| 35  | Zhucheng | Pig            | S. Rissen     | TET                  | blTEM-1 |
| 36  | Zhucheng | Pig            | S. Rissen     | TET                  | blTEM-1 |
| 37  | Zhucheng | Pig            | S. Rissen     | TET                  | blTEM-1 |
| 38  | Zhucheng | Pig            | S. Rissen     | TET                  | blTEM-1 |
| 39  | Zhucheng | Pig            | S. Rissen     | TET                  | blTEM-1 |
| 40  | Zhucheng | Pig            | S. Rissen     | TET                  | blTEM-1 |
| 41  | Zhucheng | Pig            | S. Rissen     | TET                  | blTEM-1 |
| 42  | Zhucheng | Pig            | S. Rissen     | TET                  | blTEM-1 |
| 43  | Zhucheng | Pig            | S. Rissen     | TET                  | blTEM-1 |
| 44  | Zhucheng | Pig            | S. Rissen     | TET                  | blTEM-1 |
| 45  | Zhucheng | Pig            | S. Rissen     | TET                  | blTEM-1 |
| 46  | Zhucheng | Pig            | S. Rissen     | TET                  | blTEM-1 |
| 47  | Zhucheng | Pig            | S. Rissen     | TET                  | blTEM-1 |
| 48  | Zhucheng | Pig            | S. Rissen     | TET                  | blTEM-1 |
| 49  | Zhucheng | Pig            | S. Rissen     | TET                  | blTEM-1 |
| 50  | Weihai   | Chicken        | S. Enteritidis | AMP, CTX, NAL        | blTEM-1 |
| 51  | Weihai   | Chicken        | S. Indiana    | AMP, CIP, CTX, NAL, TET | blTEM-1 |

(Continued)
| No. | Location | Slaughterhouse | Serovar        | Resistance phenotype | Integrons/Resistence genes |
|-----|----------|----------------|----------------|----------------------|---------------------------|
| 52  | Weihai   | Chicken        | S. Enteritidis | AMP, GEN, CTX, FFC, NAL, TET | Class 1 (aadA2), \(\beta\)TEM-1 |
| 53  | Weihai   | Chicken        | S. Enteritidis | AMP, GEN, CTX, FFC, NAL, TET | \(\beta\)TEM-1, \(\beta\)CTX-M-55 |
| 54  | Weihai   | Chicken        | S. Enteritidis | AMP, GEN, CTX, FFC, NAL, TET | \(\beta\)TEM-1, \(\beta\)CTX-M-55 |
| 55  | Weihai   | Chicken        | S. Enteritidis | AMP, GEN, CTX, FFC, NAL, TET | \(\beta\)TEM-1, \(\beta\)CTX-M-55 |
| 56  | Weihai   | Chicken        | S. Indiana     | AMP, CTX, NAL         | Class 1 (drfA17-aadA5), \(\beta\)TEM-1 |
| 57  | Weihai   | Chicken        | S. Enteritidis | AMP, GEN, CTX, FFC, NAL, TET | \(\beta\)TEM-1, \(\beta\)CTX-M-55 |
| 58  | Weihai   | Chicken        | S. Typhimurium | AMP, GEN, SPT         | Class 1 (aadA2), \(\beta\)TEM-1 |
| 59  | Weihai   | Chicken        | S. Typhimurium | AMP, SPT              | \(\beta\)TEM-1 |
| 60  | Weihai   | Chicken        | S. Enteritidis | AMP, GEN, CTX, FFC, NAL, TET | \(\beta\)TEM-1, \(\beta\)CTX-M-55 |
| 61  | Weihai   | Chicken        | S. Enteritidis | AMP, CTX, NAL         | \(\beta\)TEM-1 |
| 62  | Weihai   | Chicken        | S. Enteritidis | AMP, GEN, CTX, FFC, NAL, TET | \(\beta\)TEM-1, \(\beta\)CTX-M-55 |
| 63  | Weihai   | Chicken        | S. Enteritidis | AMP, CTX, FFC, NAL, TET | \(\beta\)TEM-1, \(\beta\)CTX-M-55 |
| 64  | Weihai   | Chicken        | S. Enteritidis | AMP, GEN, CTX, FFC, NAL, TET | \(\beta\)TEM-1, \(\beta\)CTX-M-55 |
| 65  | Weihai   | Chicken        | S. Enteritidis | AMP, GEN, CTX, FFC, NAL, TET | Class 1 (aadA2), \(\beta\)TEM-1 |
| 66  | Weihai   | Chicken        | S. Enteritidis | AMP, GEN, CTX, FFC, NAL, TET | \(\beta\)TEM-1, \(\beta\)CTX-M-55 |
| 67  | Weihai   | Chicken        | S. Enteritidis | AMP, GEN, CTX, FFC, NAL, TET | \(\beta\)TEM-1, \(\beta\)CTX-M-55 |
| 68  | Weihai   | Chicken        | S. Typhimurium | AMP, SPT              | Class 1 (aadA2), \(\beta\)TEM-1 |
| 69  | Weihai   | Chicken        | S. Enteritidis | NAL                   | \(\beta\)TEM-1, \(\beta\)CTX-M-55 |
| 70  | Weihai   | Chicken        | S. Enteritidis | AMP, GEN, CTX, FFC, NAL, TET | \(\beta\)TEM-1, \(\beta\)CTX-M-55 |
| 71  | Weihai   | Chicken        | S. Enteritidis | AMP, CTX, NAL         | \(\beta\)TEM-1, \(\beta\)CTX-M-55 |
| 72  | Weihai   | Chicken        | S. Enteritidis | AMP, CTX, NAL         | \(\beta\)TEM-1, \(\beta\)CTX-M-55 |
| 73  | Ciyao    | Chicken        | S. Indiana     | AMP, CTX, NAL         | \(\beta\)TEM-1, \(\beta\)CTX-M-55 |
| 74  | Ciyao    | Chicken        | S. Enteritidis | NAL                   | \(\beta\)TEM-1, \(\beta\)CTX-M-55 |
| 75  | Ciyao    | Chicken        | S. Indiana     | AMP, NAL, TET         | \(\beta\)TEM-1, \(\beta\)CTX-M-55 |
| 76  | Ciyao    | Chicken        | S. Indiana     | AMP, CIP, NAL, TET    | \(\beta\)TEM-1, \(\beta\)CTX-M-55 |
| 77  | Ciyao    | Chicken        | S. Enteritidis | NAL                   | \(\beta\)TEM-1, \(\beta\)CTX-M-55 |
| 78  | Ciyao    | Chicken        | S. Indiana     | AMP, CIP, NAL, TET    | \(\beta\)TEM-1, \(\beta\)CTX-M-55 |
| 79  | Ciyao    | Chicken        | S. Indiana     | AMP, NAL, TET         | \(\beta\)TEM-1, \(\beta\)CTX-M-55 |
| 80  | Ciyao    | Chicken        | S. Indiana     | AMP, NAL, TET         | \(\beta\)TEM-1, \(\beta\)CTX-M-55 |
| 81  | Ciyao    | Chicken        | S. Indiana     | AMP, CIP, NAL, TET    | \(\beta\)TEM-1, \(\beta\)CTX-M-55 |
| 82  | Ciyao    | Chicken        | S. Enteritidis | AMP, CIP, NAL, TET    | \(\beta\)TEM-1, \(\beta\)CTX-M-55 |
| 83  | Ciyao    | Chicken        | S. Indiana     | AMP, CIP, NAL, TET    | \(\beta\)TEM-1, \(\beta\)CTX-M-55 |
| 84  | Ciyao    | Chicken        | S. Indiana     | AMP, CIP, NAL, TET    | \(\beta\)TEM-1, \(\beta\)CTX-M-55 |
| 85  | Ciyao    | Chicken        | S. Indiana     | AMP, NAL, TET         | \(\beta\)TEM-1, \(\beta\)CTX-M-55 |
| 86  | Ciyao    | Chicken        | S. Indiana     | AMP, CIP, NAL, TET    | \(\beta\)TEM-1, \(\beta\)CTX-M-55 |
| 87  | Ciyao    | Chicken        | S. Indiana     | AMP, CIP, NAL, TET    | \(\beta\)TEM-1, \(\beta\)CTX-M-55 |
| 88  | Ciyao    | Chicken        | S. Indiana     | AMP, NAL, TET         | \(\beta\)TEM-1, \(\beta\)CTX-M-55 |
| 89  | Ciyao    | Chicken        | S. Indiana     | AMP, CIP, NAL, TET    | \(\beta\)TEM-1, \(\beta\)CTX-M-55 |
| 90  | Ciyao    | Chicken        | S. Indiana     | AMP, CIP, GEN, NAL, TET | \(\beta\)TEM-1, \(\beta\)CTX-M-55 |
| 91  | Ciyao    | Chicken        | S. Indiana     | AMP, CIP, NAL, TET    | \(\beta\)TEM-1, \(\beta\)CTX-M-55 |
| 92  | Ciyao    | Chicken        | S. Indiana     | AMP, CIP, NAL, TET    | \(\beta\)TEM-1, \(\beta\)CTX-M-55 |
| 93  | Ciyao    | Chicken        | S. Indiana     | AMP, CIP, NAL, TET    | \(\beta\)TEM-1, \(\beta\)CTX-M-55 |
| 94  | Ciyao    | Chicken        | S. Indiana     | AMP, CIP, NAL, TET    | \(\beta\)TEM-1, \(\beta\)CTX-M-55 |
| 95  | Ciyao    | Chicken        | S. Indiana     | AMP, CIP, NAL, TET    | \(\beta\)TEM-1, \(\beta\)CTX-M-55 |
| 96  | Ciyao    | Chicken        | S. Indiana     | AMP, CIP, NAL, TET    | \(\beta\)TEM-1, \(\beta\)CTX-M-55 |
| 97  | Ciyao    | Chicken        | S. Indiana     | AMP, NAL, TET         | \(\beta\)TEM-1, \(\beta\)CTX-M-55 |
| 98  | Ciyao    | Chicken        | S. Indiana     | AMP, CIP, CTX, NAL, TET | \(\beta\)TEM-1, \(\beta\)CTX-M-55 |

(Continued)
| No. | Location | Slaughterhouse | Serovar | Resistance phenotype | Integrons/Resistance genes |
|-----|----------|----------------|---------|----------------------|-----------------------------|
| 99  | Ciyao    | Chicken        | S. Indiana | AMP, CIP, NAL, TET | blaTEM-1 |
| 100 | Ciyao    | Chicken        | S. Indiana | AMP, CIP, NAL, TET | empty integron, blaTEM-1 |
| 101 | Ciyao    | Chicken        | S. Indiana | AMP, CIP, NAL, TET | blaTEM-1 |
| 102 | Ciyao    | Chicken        | S. Indiana | AMP, CIP, NAL, TET | blaTEM-1 |
| 103 | Ciyao    | Chicken        | S. Indiana | AMP, CIP, CTX, NAL, TET | blaTEM-1 |
| 104 | Ciyao    | Chicken        | S. Indiana | AMP, CIP, CTX, NAL, TET | blaTEM-1, blaCTX-M-55 |
| 105 | Ciyao    | Chicken        | S. Indiana | AMP, CIP, CTX, NAL, TET | empty integron, blaTEM-1 |
| 106 | Yantai   | Chicken        | S. Indiana | AMP, CIP, CTX, NAL, TET | blaTEM-1 |
| 107 | Yantai   | Chicken        | S. Hadar   | NAL, TET             | blaTEM-1, blaCTX-M-55 |
| 108 | Yantai   | Chicken        | S. Indiana | AMP, CIP, CTX, NAL, TET | empty integron, blaTEM-1 |
| 109 | Yantai   | Chicken        | S. Indiana | AMP, CIP, CTX, NAL, TET | blaTEM-1 |
| 110 | Yantai   | Chicken        | S. Indiana | AMP, CIP, CTX, NAL, TET | blaTEM-1, blaCTX-M-55 |
| 111 | Yantai   | Chicken        | S. Indiana | AMP, CIP, CTX, NAL, TET | empty integron, blaTEM-1 |
| 112 | Yantai   | Chicken        | S. Indiana | AMP, CIP, NAL, TET  | blaTEM-1 |
| 113 | Yantai   | Chicken        | S. Indiana | AMP, CIP, NAL, TET  | empty integron, blaTEM-1 |
| 114 | Yantai   | Chicken        | S. Indiana | AMP, CIP, CTX, NAL, TET | blaTEM-1, blaCTX-M-55 |
| 115 | Yantai   | Chicken        | S. Indiana | AMP, CIP, NAL, TET  | empty integron, blaTEM-1 |
| 116 | Yantai   | Chicken        | S. Indiana | AMP, CIP, CTX, NAL, TET | blaTEM-1, blaCTX-M-55 |
| 117 | Yantai   | Chicken        | S. Indiana | AMP, CIP, GEN, CTX, NAL, TET | blaTEM-1, blaCTX-M-55 |
| 118 | Yantai   | Chicken        | S. Indiana | AMP, CIP, NAL, TET  | empty integron, blaTEM-1 |
| 119 | Yantai   | Chicken        | S. Indiana | AMP, CIP, NAL, TET  | empty integron, blaTEM-1 |
| 120 | Yantai   | Chicken        | S. Indiana | AMP, CIP, NAL, TET  | empty integron, blaTEM-1 |
| 121 | Yantai   | Chicken        | S. Indiana | AMP, CIP, NAL, TET  | empty integron, blaTEM-1 |
| 122 | Yantai   | Chicken        | S. Indiana | AMP, CIP, NAL, TET  | empty integron, blaTEM-1 |
| 123 | Zhucheng | Chicken        | S. Indiana | AMP, CIP, NAL, TET  | blaTEM-1 |
| 124 | Zhucheng | Chicken        | S. Indiana | AMP, CIP, NAL, TET  | empty integron, blaTEM-1 |
| 125 | Zhucheng | Chicken        | S. Enteritidis | AMP, CIP, NAL, TET | blaTEM-1 |
| 126 | Zhucheng | Chicken        | S. Indiana | AMP, CIP, NAL, TET  | empty integron, blaTEM-1 |
| 127 | Zhucheng | Chicken        | S. Indiana | AMP, CIP, NAL, TET  | empty integron, blaTEM-1 |
| 128 | Zhucheng | Chicken        | S. Indiana | AMP, CIP, NAL, TET  | empty integron, blaTEM-1 |
| 129 | Zhucheng | Chicken        | S. Enteritidis | AMP, CIP, NAL, TET | blaTEM-1 |
| 130 | Zhucheng | Chicken        | S. Indiana | AMP, CIP, NAL, TET  | empty integron, blaTEM-1 |
| 131 | Zhucheng | Chicken        | S. Indiana | AMP, CIP, NAL, TET  | empty integron, blaTEM-1 |
| 132 | Zhucheng | Chicken        | S. Indiana | AMP, CIP, NAL, TET  | empty integron, blaTEM-1 |
| 133 | Zhucheng | Chicken        | S. Indiana | AMP, CIP, NAL, TET  | empty integron, blaTEM-1 |
| 134 | Zhucheng | Chicken        | S. Indiana | AMP, CIP, NAL, TET  | empty integron, blaTEM-1 |
| 135 | Zhucheng | Chicken        | S. Indiana | AMP, CIP, CTX, NAL, TET | empty integron, blaTEM-1 |

amoxicillin/clavulanic acid (AMC), ampicillin (AMP), cefotaxime (CTX), ciprofloxacin (CIP), florfenicol (FFC), gentamicin (GEN), nalidixic acid (NAL), spectinomycin (SPT), tetracycline (TET), and sulfamethoxazole/trimethoprim (SXT).
Salmonella Indiana and Enteritidis, consistent with findings reported in Henan, China (Bai et al., 2015). However, this finding differed from the result reported in Sichuan province, China, in which Salmonella Derby and Typhimurium were the most common serotypes (Li et al., 2013). Additionally, Salmonella Kentucky and Enteritidis were the most common serotypes in the USA (National Antimicrobial Resistance Monitoring System, 2011), and Salmonella Typhimurium in the EU (European Food Safety Authority, 2014). This difference may be associated with geographical regions. In the present study, Salmonella Indiana showed a high MDR rate (61/68, 89.7%), similar with the result conducted in China (Lu et al., 2011), which demonstrated that most of Salmonella Indiana showed MDR, and these bacteria were not only resistant to streptomycin and tetracycline but also were resistant to chloramphenicol, fluoroquinolones and cephalosporin antibiotics.

In the current study, most Salmonella isolates showed high resistance to tetracycline, ampicillin, and nalidixic acid, similar to the report on slaughterhouses in Italy (Piras et al., 2011), suggesting that these drugs may have been widely used on animals during disease control and prevention. A high resistance rate (63.4%) of nalidixic acid was observed in Salmonella isolates, consistent with other reports (Piras et al., 2011; Siriken et al., 2015). The resistance rate to ciprofloxacin was up to 42.7%. The results may be related with the fact that fluoroquinolone antibiotics are the most common treatment for Salmonella infections. A relatively high resistance rate to cefotaxime (29.1%) was observed in this study, which may be associated with the fact that third-generation cephalosporins have become the primary drugs for the treatment of salmonellosis because of the increase in fluoroquinolone resistance. In addition, the results of the present study showed the high prevalence of multidrug resistant Salmonella isolates in chickens (91.5%), much higher than those reported in Henan province (46.0%), China (Bai et al., 2015) and in central China (34.7%) (Kuang et al., 2015). In this study, MDR isolate rate of Salmonella (91.5%) from chickens were higher than that (14.3%) from pigs, and the higher occurrence of MDR Salmonella isolates from chickens likely reflects the extensive use of antibiotics during intensive rearing. In addition, MDR Salmonella is serotype-dependent (Clemente et al., 2014); the data provided evidence that Salmonella Indiana, Typhimurium and Enteritidis were strongly associated with MDR phenotypes. However, these findings were different from a previous study showing that Salmonella Derby is commonly associated with MDR (Newell et al., 2010).

In the present study, PCR identified class 1 integrons in 19.6% of Salmonella isolates, which was similar to the 15.0% reported from retail meat products in the USA (Zhao et al., 2009) but higher than that of (2.8%) reported from milk products (Van et al., 2013). In the present study, the incidence of class 1 integrons was higher in Salmonella from chickens (24.5%) than Salmonella from pigs (10.2%) ($P < 0.05$). Class 1 integrons are often associated with MDR Salmonella isolates, consistent with the result of the present study. In addition, the Salmonella isolates carrying class 1 integrons included Salmonella Typhimurium, Enteritidis, and Indiana.

Four β-lactamase genes were detected among Salmonella isolates recovered from pigs and chickens: blaTEM-1, blaPSE-1, blaOXA-1, and blaCTX-M-55. Most isolates carried blaTEM-1, consistent with the report from meat and milk products in Egypt (Ashraf et al., 2014), but different from the report from animal slaughterhouses and retail meat products in Sichuan, China, which showed the dominant β-lactamase gene was blaOXA-1, followed by blaTEM-1, blaPSE-1, and blaCMY-2 (Li et al., 2013). The fact that 46 Salmonella from pigs carried blaTEM-1 whereas only 16 were resistant to ampicillin, and only 88 out of 94 Salmonella carrying blaTEM-1 from chickens showed resistant to ampicillin may be associated with the expression status of blaTEM-1 genes and is needed to be further studied.

In addition, blaCMY-2 encodes resistance to third-generation cephalosporins, an important class of antibiotics used to treat complicated cases of salmonellosis (Gonzalez-Sanz et al., 2009). The incidence of blaCMY-2-positive Salmonella in China was low and was only reported in Shanxi and Sichuan (Yang et al., 2010; Li et al., 2013).

The MLST results revealed 9 STs identified in Salmonella from pigs and chickens. ST19 and ST34 have continually been reported to cause human salmonellosis in recent years, and these bacteria belong to the same serotype, Salmonella Typhimurium (Cai et al., 2016), and this circumstance was also true for Salmonella Enteritidis, represented by ST11 and ST3172. These findings suggested that serovars and STs were tightly coupled (Sukhnanand et al., 2005). ST358 is rare in China and corresponds to Salmonella Grampian, which causes an unusual increase in human cases of Salmonella Grampian infections (Horvath et al., 2013). This observation indicates that Salmonella could spread from animals to humans through the food chain and could also be transmitted from animals to humans through casual contact.
CONCLUSIONS

Collectively, our findings exhibit the prevalence and characteristics of Salmonella isolated from animals in slaughterhouses in Shandong province, China. In addition, this study highlights the necessity to carry out the long-term surveillance for Salmonella recovered from food-producing animals.

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AUTHOR CONTRIBUTIONS

WC and SS: conceived and designed the study. XZ and CY: performed the experiments and analyzed the data. WC, and SS: wrote and revised the manuscript.

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