Food Control 62 (2016) 270–276

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Short communication

# Prevalence and antimicrobial resistance of *Salmonella* isolated from an integrated broiler chicken supply chain in Qingdao, China

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ARTICLE INFO

*Article history:*
Received 9 June 2015
Received in revised form
24 October 2015
Accepted 26 October 2015
Available online 11 November 2015

*Keywords:*
*Salmonella*
Serotypes
Antimicrobial resistance
Pulsed-field gel electrophoresis

ABSTRACT

The present study analyzed the prevalence and antimicrobial resistance of *Salmonella* along an integrated broiler chicken supply chain. A total of 172 *Salmonella* isolates were recovered from 1148 samples collected from four sample sources (breeder farms, broiler farms, abattoir, and retail markets), representing nine production stages. These *Salmonella* isolates were examined for antimicrobial susceptibility to 12 different antimicrobial agents using a disk diffusion assay. Among them, 168 were identified as six different serotypes of *Salmonella enterica.* The predominant serotype was *S.* Enteritidis (n = 116), followed by *S.* Infantis (n = 18), *S.* Gueuletapee (n = 16), *S.* Derby (n = 12), *S.* Meleagridis (n = 4), and *S.* London (n = 2). The remaining four isolates were serogroup-untypeable. A majority of the 172 isolates (96.51%) was resistant to one or more antibiotics and 61.05% of the *Salmonella* isolates showed a multidrug resistance phenotype. Statistical analysis indicated the one risk product stage for *Salmonella* contamination occurred in the sample source at the abattoir, specifically the stage of Carcasses after chilling. The majority of *S.* Enteritidis isolates shared the same pulsed-field gel electrophoresis (PFGE) cluster, suggesting that the *S.* Enteritidis strain might spread along the broiler chicken supply chain. The prevalence and antimicrobial resistance of *Salmonella* in different production stages suggest the importance of controlling *Salmonella* in the broiler chicken supply chain for public health, underlying the need for improved measures of reducing carcass contamination in abattoirs and the appropriate use of antimicrobials in broiler flocks.



## 1. Introduction

Salmonellosis, caused by *Salmonella enterica*, is one of the most frequently reported foodborne illnesses worldwide (Scallan et al., 2011; Shao, Shi, Wei, & Ma, 2011). Oral transmission is one of the most common routes of *Salmonella* infection; humans are infected through the ingestion of contaminated animal-derived food, indicating that *Salmonella* from animals can be transmitted to humans via the food chain (Fearnley, Raupach, Lagala, & Cameron, 2011).

The increase of antibiotic resistance in *Salmonella* has become a worldwide problem in recent decades (White, Zhao, Simjee, Wagner, & McDermott, 2002). Food contamination with multidrug-resistant (MDR) bacteria poses a major threat to public health, as there is an abundance of evidence showing that antibiotic-resistant bacteria of animal origin can be transmitted to humans (Khemtong & Chuanchuen, 2008).

Chicken is one of the most widely consumed animal meats in the world; however, it is also recognized as an important reservoir of *Salmonella* (Adu-Gyamfi, Torgby-Tetteh, & Appiah, 2012; Thai &

*Abbreviations:* PFGE, Pulsed-field gel electrophoresis; MDR, multidrug resistant; DOX, doxycycline; GEN, gentamicin; CHL, chloramphenicol; NAD, nalidixic acid; CIP, ciprofloxacin; AMP, ampicillin; CAZ, ceftazidime; CFZ, cefazolin; AMC, amoxicillin/clavulanic acid; MEM, meropenem; SXT, trimethoprim/sulfamethoxazole; PB, polymyxinB; HACCP, hazard analysis and critical control point.

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http://dx.doi.org/10.1016/j.foodcont.2015.10.036
0956-7135/

Yamaguchi, 2012). In addition, previous research has indicated the importance of considering slaughter and other stages in the meat supply chain for preventing *Salmonella* contamination (Arguello, Alvarez-Ordonez, Carvajal, Rubio, & Prieto, 2013; Marin, Balasch, Vega, & Lainez, 2011; Schmidt et al., 2012). During the various stages of chicken slaughter and processing, all potentially edible tissues are at risk of contamination from sources both within and outside the animal, including the environment, equipment, and operators. Thus, due to the high consumption of chicken meat and increased occurrence and invasiveness of MDR *Salmonella*, the prevalence and antibacterial resistance of *Salmonella* spp. in the broiler chicken supply chain need to be monitored.

In order to collect information on *Salmonella* cross-contamination along the broiler chicken supply chain in Qingdao City, China, the prevalence of *Salmonella* spp. in nine distinct stages of the chicken supply chain and antimicrobial resistance were investigated. Pulsed-field gel electrophoresis (PFGE) was performed to study genetic relatedness of the dominant serotype *S.* Enteritidis isolates from different production stages. The study describes the characteristics of *Salmonella* in the broiler chicken supply chain, which contributes to the understanding of antimicrobial resistance in this supply chain and may aid the creation of strategies to prevent *Salmonella* contamination.

## 2. Materials and methods

### 2.1. Sample collection

A total of 1148 samples (detailed in Table 1) were collected during August and September, 2013, from a vertically-integrated commercial broiler chicken supply chain in Qingdao City, China, in which more than 90,000,000 broiler chickens are reared, slaughtered, and sold per year. In this study, we selected related breeder and broiler farms that constitute a vertically integrated broiler supply chain; all animals were part of the same cohort through this chain. Samples were collected from four types of sources (three breeder farms (approximately 8000 birds/flock), two broiler farms (approximately 15,000 birds/flock), one abattoir, and three retail markets), including nine production stages (Breeder, 5-day-old broiler, 20-day-old broiler, Adult broiler: 45 days old, sampled after slaughter but prior to evisceration, Pre-cleaning carcasses: carcasses sampled after evisceration but prior to cleaning, Post-cleaning carcasses: carcasses sampled after evisceration and cleaning, Carcasses after chilling, Segmented chicken: leg or breast fillet of butchered carcass, and Retail chicken: whole carcass packaged and sold to consumers, purchased the same day or day after delivery to the market.

One sample was collected from each animal or meat product as appropriate. At farms, rectal swabs were collected from randomly selected individual animals at three of the stages (Breeder, 5-day-old broiler, and 20-day-old broiler). Broiler cecal samples, representing samples of the Adult broiler stage, were collected from random animals at the abattoir. We chose the swab sampling method because previous reference noted that whole-carcass sampling by swabbing is necessary for optimum *Salmonella* recovery (McEvoy, Nde, Sherwood, & Logue, 2005). Additionally, *Salmonella* contamination at abbatoirs often occurs on the surface of the carcass. We used large swabs moistened with buffered peptone water and swabbed the entire surface of the carcass. Whole carcasses or meat from the next four stages (Pre-cleaning carcasses, Post-cleaning carcasses, Carcasses after chilling, and Segmented chicken) were sampled in the chicken processing chain, using cotton swabs across the surface of the meat. Carcasses from the Retail chicken stage were collected from three markets, and were swabbed in the same manner. Sampling was timed to follow folks through rearing and processing, and sampling on farms included numerous locations to ensure a representative sample. All samples obtained were immediately transported to the laboratory in an insulated ice chest containing ice packs. Microbial analysis was performed immediately upon arrival at the laboratory.

### 2.2. Isolation and serotyping of Salmonella

Pre-enrichment of the sample for *Salmonella* was performed according to a method described previously (Li et al., 2013), with modifications. Briefly, cotton swab samples or 5 g of fecal matter were placed into sterile 5 mL plastic tubes containing 2 mL of buffered peptone water and incubated at 37 °C for 6–12 h. A 0.2-mL aliquot of these pre-enriched cultures were then inoculated into 2 mL of selenite cysteine broth, which was incubated at 37 °C for 24 h. Selenite cysteine broth cultures were then streaked onto CHROMagar *Salmonella* plates (CHROMagar, Paris, France) and incubated at 37 °C for 24 h. Isolates with a typical phenotype (mauve colony) were confirmed by PCR using a previously described method (Rahn et al., 1992). *Salmonella* isolates were serotyped by slide agglutination for O and H antigens using

**Table 1**
Prevalence of *Salmonella* isolated from nine stages of the broiler chicken supply chain.

| Source of isolates | Stages of isolates | Total no. of samples | No. of *Salmonella*-positive isolates (%) | Serotypes (no. Of isolates) |
|---|---|---|---|---|
| Breeder farms | Breeder | 150 | 2 (1.33) | *S.* Infantis (2) |
| Broiler farms | 5-day-old broiler | 100 | 8 (8.00) ↑↑ | *S.* Enteritidis (8) |
| | 20-day-old broiler | 100 | 13 (13.00) | *S.* Enteritidis (12); untypeable (1) |
| | Adult broiler | 290 | 33 (11.38) | *S.* Enteritidis (14); *S.* Infatis (15); *S.* Meleagridis (4) |
| Abattoir | Pre-cleaning carcasses | 100 | 13 (13.00) | *S.* Enteritidis (8); *S.* Derby (4); untypeable (1) |
| | Post-cleaning carcasses | 103 | 15 (14.56) | *S.* Enteritidis (12); *S.* Gueuletapee (2); *S.* London (1) |
| | Carcasses after chilling | 99 | 26 (26.26) ↑ | *S.* Enteritidis (25); untypeable (1) |
| | Segmented chicken | 80[a] | 33 (41.25) | *S.* Enteritidis (18); *S.* Derby (8); *S.* Gueuletapee (5); *S.* London (1); non-serogroup (1) |
| Retail markets | Retail chicken | 126 | 29 (23.02) ↓↓ | *S.* Enteritidis (19); *S.* Infatis (1); *S.* Gueuletapee (9) |
| Total | | 1148 | 172 (14.98) | *S.* Enteritidis (116); *S.* Infatis (18); *S.* Gueuletapee (16); *S.* London (2); *S.* Meleagridis (4); *S.* Derby (12); untypeable (4) |

"↑" indicates a significant increase ($p \leq 0.008$).
"↓↓" and "↑↑" indicate highly significant decrease and increase ($p \leq 0.002$).
[a] 40 samples were collected from raw chicken leg. 40 samples were collected from raw chicken breast fillet.

**Table 2**
Comparison of antimicrobial resistance of *Salmonella* isolated from nine stages of the broiler chicken supply chain.

| Source of isolates (no. of isolates) | No. (%) of isolates resistant to drug[a] | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | DOX | GEN | CHL | NAD | CIP | AMP | CAZ | CFZ | AMC | MEM | SXT | PB |
| Breeder (n = 2) | – | **2 (100.00)** | **1 (50.00)** | **2 (100.00)** | – | **2 (100.00)** | – | **2 (100.00)** | – | – | **2 (100.00)** | – |
| 5-day-old broiler (n = 8) | **5 (62.50)**[b] | 2 (25.00) | 2 (25.00) | **8 (100.00)** | 2 (25.00) | **8 (100.00)** | – | 2 (25.00) | – | – | 2 (25.00) | – |
| 20-day-old broiler (n = 13) | **13 (100.00)** | – | – | **13 (100.00)** | – | **13 (100.00)** | – | – | – | – | 1 (7.69) | – |
| Adult broiler (n = 33) | 7 (21.21) | **18 (54.55)** | **18 (54.55)** | **27 (81.82)** | – | **32 (96.97)** | – | **18 (54.55)** | – | – | **21 (63.64)** | – |
| Pre-cleaning carcasses (n = 13) | 6 (46.15) | 1 (7.69 ) | — | **12 (92.31)** | – | **11 (84.62)** | – | 2 (15.38) | — | — | 4 (30.77) | – |
| Post-cleaning carcasses (n = 15) | **9 (60.00)** | – | – | **15 (100.00)** | — | **13 (86.67)** | – | **8 (53.33)** | **8 (53.33)** | – | – | – |
| Carcasses after chilling (n = 26) | 5 (19.23) | – | – | **26 (100.00)** | – | 11 (42.31) | – | – | – | – | – | – |
| Segmented chicken (n = 33) | 11 (33.33) | 6 (18.18) | 3 (9.09) | **21 (63.64)** | – | **18 (54.55)** | **1 (3.03)** | 8 (24.24) | — | — | 6 (18.18) | — |
| Retail chicken (n = 29) | 4 (13.79) | 7 (24.14) | 1 (3.45) | **29 (100.00)** | – | **23 (79.31)** | 6 (20.69) | 10 (34.48) | 6 (20.69) | 1 (3.45) | 3 (10.34) | — |
| Total (n = 172) | 60 (34.88) | 36 (20.93) | 25 (14.53) | **153 (88.95)** | 2 (1.16) | **131 (76.16)** | 7 (4.07) | 50 (29.07) | 14 (8.14) | 1 (0.58) | 39 (22.67) | — |

[a] DOX, doxycycline; GEN, gentamicin; CHL, chloramphenicol; NAD, nalidixic acid; CIP, ciprofloxacin; AMP, ampicillin; CAZ, ceftazidime; CFZ, cefazolin; AMC, amoxicillin/clavulanic acid; MEM, meropenem; SXT, trimethoprim/sulfamethoxazole; PB, polymyxinB; –, not found.
[b] Boldface type indicates a high resistance rate (≥50%).

commercially available antiserum (Tianrun Bio-Pharmaceutical, Ningbo, China), according to manufacturer's instructions.

SAS software (version 9.0, SAS Institute, Cary, NC, USA) was used to analyze the prevalence of *Salmonella* isolates obtained from different stages. Mantel-Haenszel chi-square test was used to test for an association between processing stage and the prevalence of *Salmonella* isolates, which tells us whether the processing stage affects the likelihood of contamination; that is, if contamination is likely to enter the processing chain at one or more specific points rather than randomly. If there was a significant association ($p \leq 0.05$) between stages and *Salmonella* prevalence, then multiple comparisons of two related stages were performed by partitioning chi-squares to determine if prevalence of *Salmonella* at one stage was correlated with the prevalence at the next. Raw p values were corrected using the Bonferroni method. Differences were considered significant when adjusted $p \leq 0.008$ and highly significant when adjusted $p \leq 0.002$.

### 2.3. Antimicrobial susceptibility testing

The disk diffusion test, as described by the Clinical and Laboratory Standards Institute (CLSI, 2013), was performed using Mueller-Hinton agar plates with disks containing the following 12 antimicrobial agents (Oxoid, Basingstoke, Hampshire, UK): amoxicillin/clavulanic acid (AMC), 20 μg/10 μg; nalidixic acid (NAD), 30 μg; ampicillin (AMP), 10 μg; cefazolin (CFZ), 30 μg; doxycycline (DOX), 30 μg; gentamicin (GEN), 10 μg; trimethoprim/sulfamethoxazole (SXT) 1.25 μg/23.75 μg; ceftazidime (CAZ), 30 μg; chloramphenicol (CHL), 30 μg; ciprofloxacin (CIP), 5 μg; meropenem (MEM), 10 μg; polymyxinB (PB), 300 units. The interpretive category for each isolate (susceptible, intermediate, or resistant) was determined according to the CLSI guidelines (CLSI, 2013). *Escherichia coli* ATCC 25922 was used as a quality control strain. *Salmonella* isolates showing resistance to three or more antibiotics were defined as MDR isolates.

### 2.4. Pulsed-field gel electrophoresis (PFGE)

Among the 116 *S.* Enteritidis isolates from the eight broiler production stages, 40 *S.* Enteritidis isolates were selected from 73 *Salmonella* spp. isolates with the predominant antimicrobial resistance pattern for each stage (numbers with boldface type indicated in Table 3) for PFGE analysis. In addition, six *S.* Enteritidis isolates with an NAD-AMP pattern and two *S.* Enteritidis with an NAD-AMP-DOX pattern from the Adult broiler stage were chosen for PFGE analysis, as *S.* Enteritidis isolates did not have the predominant antimicrobial resistance pattern for this stage (Table 3). PFGE analysis was performed according to the PulseNet standardized protocol for subtyping *Salmonella* (Hunter et al., 2005; Ribot, Fitzgerald, Kubota, Swaminathan, & Barrett, 2001), using 50 U of *Xba*I restriction endonuclease and the reference strain *Salmonella* Braenderup strain H9812. The restriction fragments were separated using a CHEF Mapper XA pulsed field electrophoresis system (Bio-Rad Laboratories, Hercules, CA, USA) according to the manufacturer's instructions. Gel images were analyzed using InfoQuest FP software (Bio-Rad Laboratories). Isolates were considered genetically related if the Dice correlation coefficient was 80% or greater, according to Tenover's criterion (Tenover et al., 1995).

## 3. Results

### 3.1. Prevalence and serotyping of Salmonella isolates

The prevalence of *Salmonella* isolates from the broiler chicken supply chain is summarized in Table 1. A total of 172 *Salmonella* isolates were recovered from 1148 samples (14.98%). Approximately 41.25% of Segmented chicken samples were contaminated with *Salmonella*, followed by Carcasses after chilling (26.26%) at the abattoir. The lowest prevalence of *Salmonella* was observed in breeder animals (1.33%). The prevalence of *Salmonella* at the other sampling stages varied from 8.00% to 23.02%. Notably, while the highest prevalence of *Salmonella* contamination was observed in the Segmented chicken stage (41.25%), the level of *Salmonella* contamination at the Retail chicken stage was significantly lower (23.02%). In addition, of the 172 *Salmonella* isolates from the nine stages, 168 were identified as belonging to one of six serotypes, while the remaining four isolates were untypeable.

Mantel-Haenszel chi-square test showed a significant association ($p < 0.01$) between stage and the prevalence of *Salmonella* isolates. Table 1 shows the distribution of *Salmonella* isolates among the nine stages. There is a trend of increasing rates of *Salmonella* from the Breeder stage on breeder farms to the Segmented chicken stage at the abattoir. Further statistical analysis revealed that the *Salmonella* infection in the 5-day-broiler stage showed a highly significant increase ($p < 0.0001$) over the Breeder stage, and the *Salmonella* contamination in Carcasses after chilling presented a significant increase ($p < 0.0001$) from Post-cleaning carcasses. Consequently, these two stages were determined as two important risk stages. However, the detection rate of *Salmonella* at the stage of Retail meat presented a significant decline ($p = 0.004$), when compared to that in the stage of Segmented chicken.

### 3.2. Characterization of antimicrobial susceptibility

All 172 *Salmonella* isolates were tested for susceptibility to 12 antimicrobial agents. Resistance is summarized in terms of

**Table 3**
Antimicrobial resistance patterns of *Salmonella* isolated from the nine stages of the broiler chicken supply chain.

| Resistance pattern | Source of isolates (no. of isolates) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Breeder (n = 2) | 5-day-old broiler (n = 8) | 20-day-old broiler (n = 13) | Adult broiler (n = 33) | Pre-cleaning carcasses (n = 13) | Post-cleaning carcasses (n = 15) | Carcasses after chilling (n = 26) | Segmented chicken (n = 33) | Retail chicken (n = 29) |
| NAD-AMP-CFZ-GEN-CHL-CIP-SXT | — | 2 (25.00) | — | — | — | — | — | — | — |
| NAD-AMP-CFZ-AMC-CHL-SXT-MEM | — | — | — | — | — | — | — | — | 1 (3.45) |
| NAD-AMP-CFZ-GEN-CHL-SXT | 1 (50.00[a]) | — | — | **13 (39.39)** | — | — | — | — | — |
| NAD-AMP-CFZ-GEN-CAZ-AMC | —[b] | — | — | — | — | — | — | — | 5 (17.24) |
| NAD-AMP-CFZ-GEN-SXT | 1 (50.00) | — | — | — | — | — | — | — | — |
| NAD-AMP-CFZ-GEN-AMC | — | — | — | — | — | — | — | — | 2 (6.90) |
| NAD-AMP-CFZ-CAZ-AMC | — | — | — | — | — | — | — | — | 1 (3.45) |
| NAD-AMP-CFZ-AMC-DOX | — | — | — | — | — | **7 (46.67)** | — | — | — |
| NAD-AMP-CAZ-DOX-SXT | — | — | — | — | — | — | — | 1 (3.03) | — |
| AMP-CFZ-GEN-CHL-SXT | — | — | — | 5 (15.15) | — | — | — | 3 (9.09) | — |
| NAD-AMP-DOX-SXT | — | — | 1 (7.69) | 2 (6.06) | **4 (30.77)** | — | — | 1 (3.03) | 2 (6.90) |
| NAD-AMP-CFZ-GEN | — | — | — | — | 1 (7.69) | — | — | 2 (6.06) | — |
| NAD-AMP-CFZ-AMC | — | — | — | — | | 1 (6.67) | — | 1 (3.03) | — |
| AMP-CFZ-DOX-SXT | — | — | — | — | | | — | 1 (3.03) | — |
| NAD-AMP-CFZ | — | — | — | — | 1 (7.69) | | — | 2 (6.06) | 1 (3.45) |
| NAD-AMP-GEN | — | — | — | — | — | — | — | 1 (3.03) | |
| NAD-AMP-DOX | — | **5 (62.50)**[c] | **12 (92.31)** | 5 (15.15) | 2 (15.38) | 2 (13.33) | 6 (23.08) | **8 (24.24)** | 2 (6.90) |
| AMP-CFZ | — | — | — | — | — | — | — | 1 (3.03) | — |
| NAD-AMP | — | 1 (12.50) | — | 7 (21.21) | 3 (23.08) | 3 (20.00) | 5 (19.23) | 2 (6.06) | **9(31.03)** |
| NAD | — | — | — | — | 1 (7.69) | 2 (13.33) | **15(57.69)** | 4 (12.12) | 6 (20.69) |
| SXT | — | — | — | 1 (3.03) | — | — | — | — | — |
| CFZ | — | — | — | — | 1 (7.69) | — | — | — | — |

[a] Numbers in parentheses indicate the percentage of antimicrobial resistance patterns relative to the whole stage.
[b] —, not found.
[c] Boldface type indicates the predominant antimicrobial resistance pattern.

sampling stages in Table 2. In total, 166 (96.51%) of the 172 isolates were resistant to one or more antimicrobial agents. All six susceptible *Salmonella* isolates came from the Segmented chicken stage. The highest rate of resistance was for nalidixic acid (88.95%), followed by ampicillin (76.16%), while resistance to the other antimicrobial agents was relatively low: doxycycline, 34.88%; cefazolin, 29.07%; trimethoprim/sulfamethoxazole, 22.67%; gentamicin, 20.93%; chloramphenicol, 14.53%; amoxicillin/clavulanic acid, 8.14%; ceftazidime, 4.07%; ciprofloxacin, 1.16%; and meropenem, 0.58%.

Antimicrobial resistance patterns of the *Salmonella* isolates are outlined in Table 3. Among 172 tested isolates, 22 different patterns of susceptibility were observed in 166 isolates, in which 105 exhibited 17 MDR phenotypes, covering all nine broiler supply stages. All stages, apart from the Carcasses after chilling and Retail chicken stages, showed a high prevalence (≥50%) of MDR *Salmonella* isolates. The two isolates derived from the Breeder stage both exhibited an MDR phenotype. In addition, the result of relationship between serotype and MDR phenotype (Table 4) showed that MDR phenotypes were most commonly associated with *S.* Enteritidis isolates (64; 37.20%), followed by *S.* Infantis isolates (18; 10.47%).

### 3.3. PFGE analysis of S. Enteritidis from the broiler chicken supply chain

The 48 *S.* Enteritidis isolates were subdivided into 14 different clusters by PFGE analysis. The clusters were named X1–X14, and consisted of 32 *Xba*I profiles with coefficient of similarity values of 36.59%–100% (Fig. 1). The majority of *Xba*I profiles were assigned to cluster X1, which represented 20 isolates from the Adult broiler, Pre-cleaning carcasses, Post-cleaning carcasses, Carcasses after chilling, and Retail chicken stages. Both the isolates with different PFGE clusters representing the same antimicrobial resistance patterns and the isolates with identical cluster representing the same or different antimicrobial resistance patterns were observed (Fig. 1), indicating no correlation between PFGE pattern and antimicrobial resistance pattern in *S.* Enteritidis isolates.

## 4. Discussion

Many *S. enterica* serotypes can cause disease in humans. The serotypes *S.* Enteritidis, *S.* Typhimurium, and *S.* Heidelberg contributed to the majority of salmonellosis cases reported in Canada and the United States (Sivaramalingam, Pearl, McEwen, Ojkic, & Guerin, 2013). Notably, *S.* Enteritidis was the most common serotype isolated along the broiler supply chain in the current study, which agrees with European Food Safety Authority reports (EFSA, 2007), and with previous research from Eastern Spain and China (Marin et al., 2011; Yang et al., 2014). However, this result differs from findings in other countries such as South Korea, Vietnam, and Cambodia, where *S.* Hadar, *S.* Infantis, and *S.* Anatum are most prevalent, respectively (Choi et al., 2014; Lay, Vuthy, Song, Phol, & Sarthou, 2011; Thai & Yamaguchi, 2012). Most interestingly, previous investigations of *Salmonella* contamination of

**Table 4**
Antimicrobial resistance patterns of *Salmonella* serotypes from the broiler chicken supply chain.

| Resistance pattern | *Salmonella* serovars (no. of isolates) | | | | | |
|---|---|---|---|---|---|---|
| | *S.* Enteritidis (116) | *S.* Infatis (18) | *S.* Gueuletapee (16) | *S.* Derby (12) | *S.* Meleagridis (4) | *S.* London (2) |
| NAD-AMP-CFZ-AMC-CHL-SXT-MEM | – | 1 (5.56) | – | – | – | – |
| NAD-AMP-CFZ-GEN-CHL-CIP-SXT | 2 (1.72[a]) | – | – | – | – | – |
| NAD-AMP-CFZ-GEN-CHL-SXT | –[b] | 14 (77.78) | – | – | – | – |
| NAD-AMP-CFZ-GEN-CAZ-AMC | – | – | 5 (31.25) | – | – | – |
| NAD-AMP-CFZ-GEN-SXT | – | 1 (5.56) | – | – | – | – |
| NAD-AMP-CFZ-GEN-AMC | – | – | 2 (12.50) | – | – | – |
| NAD-AMP-CFZ-CAZ-AMC | 1 (0.86) | – | – | – | – | – |
| NAD-AMP-CFZ-AMC-DOX | 7 (6.03) | – | – | – | – | – |
| NAD-AMP-CAZ-DOX-SXT | – | – | 1 (6.25) | – | – | – |
| AMP-CFZ-GEN-CHL-SXT | 2 (1.72) | 2 (11.11) | – | – | 3 (75.00) | 1 (50.00) |
| NAD-AMP-DOX-SXT | 9 (7.76) | – | – | – | – | – |
| NAD-AMP-CFZ-GEN | 2 (1.72) | – | – | 1 (8.33) | – | – |
| NAD-AMP-CFZ-AMC | 1 (0.86) | – | – | 1 (8.33) | – | – |
| AMP-CFZ-DOX-SXT | 1 (0.86) | – | – | – | – | – |
| NAD-AMP-CFZ | 4 (3.45) | – | – | – | – | – |
| NAD-AMP-GEN | 1 (0.86) | – | – | – | – | – |
| NAD-AMP-DOX | 36 (31.03) | – | 4 (25.00) | 1 (8.33) | – | – |
| AMP-CFZ | – | – | – | 1 (8.33) | – | – |
| NAD-AMP | 26 (22.41) | – | 4 (25.00) | 1 (8.33) | – | – |
| NAD | 24 (20.69) | – | – | – | – | 1 (50.00) |
| SXT | – | – | – | – | 1 (25.00) | – |
| CFZ | – | – | – | 1 (8.33) | – | – |

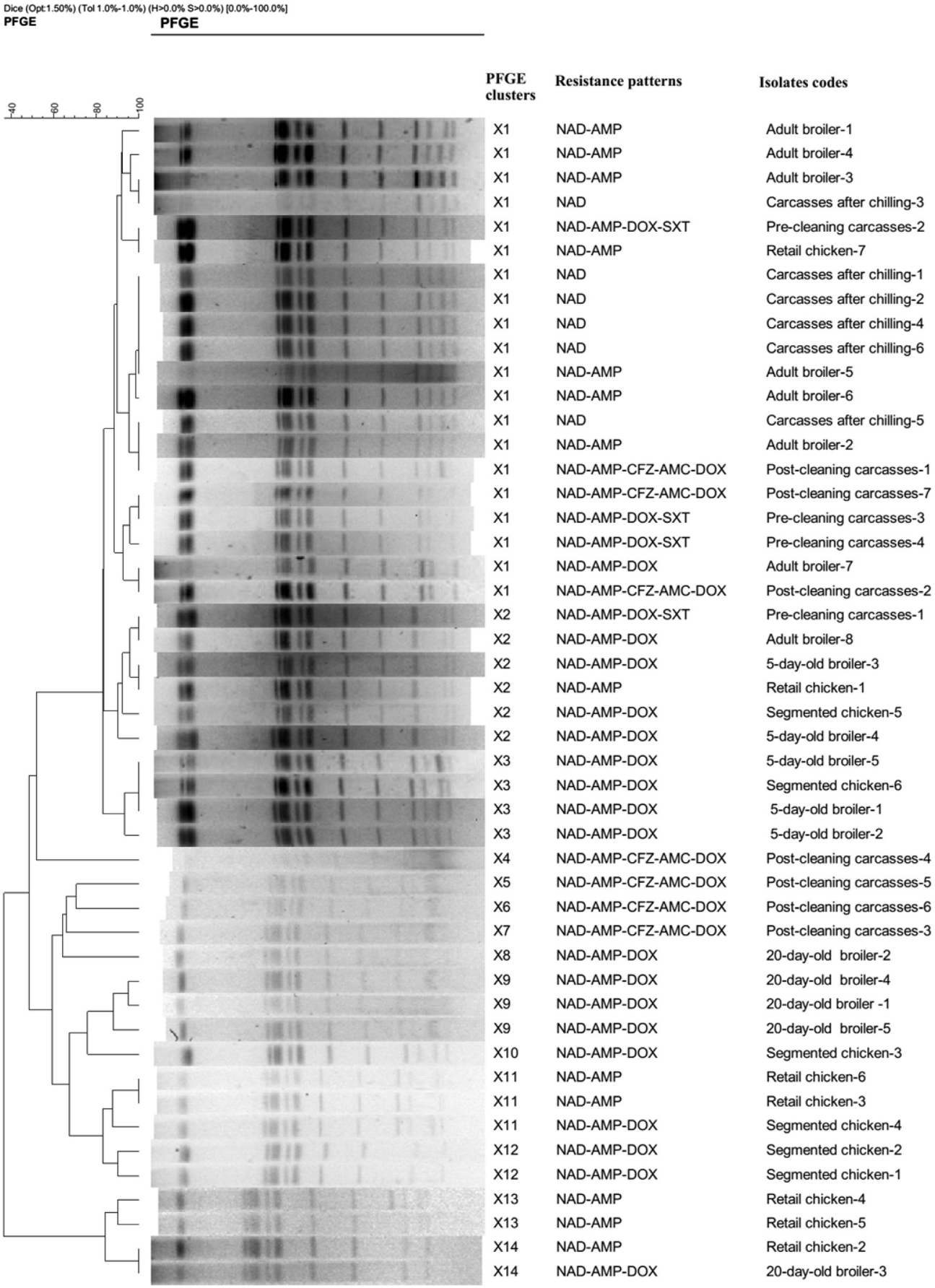
[a] Numbers in parentheses indicate the percentage of antimicrobial resistance patterns relative to the serotype.
[b] –, not found.

broiler chicken supply chains in the Netherlands and Eastern Spain (Marin et al., 2011; van Asselt, Thissen, & van der Fels-Klerx, 2009) revealed a much greater diversity in the serotypes of the *Salmonella* isolates identified compared with the present case. This suggests that geographical differences exist in the occurrence and dominance of various *Salmonella* serotypes.

Prevention of *Salmonella* contamination at any stage requires detailed knowledge of the most important risk factors associated with its presence in the broiler chicken supply chain. In our case, the data about *Salmonella*-positive isolates has provided detailed information about contamination along an integrated broiler chicken supply chain. The riskiest stage for *Salmonella* contamination of the flock was the 5-day-old broiler stage, in agreement with previous findings about risk factors in Eastern Spain (Marin et al., 2011). The second important risk stage (Carcasses after chilling) was found in the abattoir, which may be related to cooling equipment contamination. Similarly, the evidence for contamination of equipment on a slaughter line and subsequent cross-contamination to non-infected chickens has been reported previously (Olsen, Brown, Madsen, & Bisgaard, 2003), which may partly support our finding that the contamination becomes more serious with the increasing degree of mixing in the abattoir. To minimize cross-contamination, slaughtering equipment is sterilized once a day, the pool of water used to cool the carcasses is disinfected with sodium hypochlorite, and organs are conveyed to a separate production chain immediately after evisceration. However, these protocols are not always followed and thus cross-contamination is allowed. Another major source of Salmonella contamination and cross-contamination is the hatchery (Davies et al., 1997). Unfortunately, we were unable to obtain permits to collect chicken or egg samples at the hatcheries in this supply chain.

Antimicrobial resistance represents a serious public health problem. Increasing antibiotic resistance in *S. enterica* has led to a shift in the choice of antibiotics used against this organism, from chloramphenicol and ampicillin to trimethoprim-sulfamethoxazole, fluoroquinolones, and extended-spectrum cephalosporins in clinical settings (Roy, Rawat, & Malik, 2015). Currently, the primary antimicrobial treatment options for salmonellosis include fluoroquinolones or extended-spectrum cephalosporins (Folster et al., 2015). In the study, a very high rate (88.95%) of nalidixic acid resistance was observed in *Salmonella* isolates, which is similar to the rate (89.28%) found in Turkey (Siriken, Türk, Yildirim, Durupinar, & Erol, 2015). Nalidixic acid resistance plays a role in the initial steps of the development of ciprofloxacin resistance, although a low rate (1.16%) of ciprofloxacin resistance was observed in the isolates. It is worth noting that third-generation cephalosporins have become the primary drugs for the treatment of salmonellosis because of the recent increase in fluoroquinolone resistance (Mawatari et al., 2013). In this study, there was a high prevalence (29.07%) of resistance to cefazolin, while a relatively low rate of resistance was observed for ceftazidime (4.07%), which is a third-generation cephalosporin. Farm managers provided information about the antibiotic usage. Penicillins, amphenicols, sulfanilamide fluoroquinolones, cephalosporins, tetracycline, and polymyxin were used to treat and prevent bacterial diseases during the poultry-rearing period. This study showed high prevalence of antibiotic-resistant *Salmonella* isolates and MDR *Salmonella* isolates along the integrated broiler chicken supply chain, which can likely be attributed to the extensive use of antibiotics during intensive rearing (Economou & Gousia, 2015). In addition, to some extent, antimicrobial resistance in *Salmonella* is serotype-dependent (Clemente et al., 2014). The data also provided evidence that *S.* Enteritidis, the dominant serotype, was strongly associated with MDR phenotypes. However, these findings were contrary to a previous study showing that *S.* Enteritidis rarely displays multiple resistance while *S.* Derby is commonly associated with multidrug resistance (Newell et al., 2010).

Molecular characterization of 48 *S.* Enteritidis isolates from eight different stages (all except Breeder) demonstrated that genotype diversity is associated with broiler chicken production stages. PFGE pattern diversity within a serotype is useful in assessing the effectiveness of control measures (Sandt et al., 2013). PFGE is the most widely used molecular subtyping method for *Salmonella*; previous studies using this method have shown that *S.* Infantis could be transmitted along the food chain and represent a hazard to human health (Hauser et al., 2012; Nogrady et al., 2008).




| PFGE clusters | Resistance patterns | Isolates codes |
|---|---|---|
| X1 | NAD-AMP | Adult broiler-1 |
| X1 | NAD-AMP | Adult broiler-4 |
| X1 | NAD-AMP | Adult broiler-3 |
| X1 | NAD | Carcasses after chilling-3 |
| X1 | NAD-AMP-DOX-SXT | Pre-cleaning carcasses-2 |
| X1 | NAD-AMP | Retail chicken-7 |
| X1 | NAD | Carcasses after chilling-1 |
| X1 | NAD | Carcasses after chilling-2 |
| X1 | NAD | Carcasses after chilling-4 |
| X1 | NAD | Carcasses after chilling-6 |
| X1 | NAD-AMP | Adult broiler-5 |
| X1 | NAD-AMP | Adult broiler-6 |
| X1 | NAD | Carcasses after chilling-5 |
| X1 | NAD-AMP | Adult broiler-2 |
| X1 | NAD-AMP-CFZ-AMC-DOX | Post-cleaning carcasses-1 |
| X1 | NAD-AMP-CFZ-AMC-DOX | Post-cleaning carcasses-7 |
| X1 | NAD-AMP-DOX-SXT | Pre-cleaning carcasses-3 |
| X1 | NAD-AMP-DOX-SXT | Pre-cleaning carcasses-4 |
| X1 | NAD-AMP-DOX | Adult broiler-7 |
| X1 | NAD-AMP-CFZ-AMC-DOX | Post-cleaning carcasses-2 |
| X2 | NAD-AMP-DOX-SXT | Pre-cleaning carcasses-1 |
| X2 | NAD-AMP-DOX | Adult broiler-8 |
| X2 | NAD-AMP-DOX | 5-day-old broiler-3 |
| X2 | NAD-AMP | Retail chicken-1 |
| X2 | NAD-AMP-DOX | Segmented chicken-5 |
| X2 | NAD-AMP-DOX | 5-day-old broiler-4 |
| X3 | NAD-AMP-DOX | 5-day-old broiler-5 |
| X3 | NAD-AMP-DOX | Segmented chicken-6 |
| X3 | NAD-AMP-DOX | 5-day-old broiler-1 |
| X3 | NAD-AMP-DOX | 5-day-old broiler-2 |
| X4 | NAD-AMP-CFZ-AMC-DOX | Post-cleaning carcasses-4 |
| X5 | NAD-AMP-CFZ-AMC-DOX | Post-cleaning carcasses-5 |
| X6 | NAD-AMP-CFZ-AMC-DOX | Post-cleaning carcasses-6 |
| X7 | NAD-AMP-CFZ-AMC-DOX | Post-cleaning carcasses-3 |
| X8 | NAD-AMP-DOX | 20-day-old broiler-2 |
| X9 | NAD-AMP-DOX | 20-day-old broiler-4 |
| X9 | NAD-AMP-DOX | 20-day-old broiler -1 |
| X9 | NAD-AMP-DOX | 20-day-old broiler-5 |
| X10 | NAD-AMP-DOX | Segmented chicken-3 |
| X11 | NAD-AMP | Retail chicken-6 |
| X11 | NAD-AMP | Retail chicken-3 |
| X11 | NAD-AMP-DOX | Segmented chicken-4 |
| X12 | NAD-AMP-DOX | Segmented chicken-2 |
| X12 | NAD-AMP-DOX | Segmented chicken-1 |
| X13 | NAD-AMP | Retail chicken-4 |
| X13 | NAD-AMP | Retail chicken-5 |
| X14 | NAD-AMP | Retail chicken-2 |
| X14 | NAD-AMP-DOX | 20-day-old broiler-3 |

**Fig. 1.** PFGE profiles of *S.* Enteritidis isolated from the broiler chicken supply chain. Isolate codes indicate the stage of broiler chicken supply chain and the isolate number. Abbreviations of antimicrobial agents: NAD, nalidixic acid; AMP, ampicillin; DOX, doxycycline; CFZ, cefazolin; AMC, amoxicillin/clavulanic acid; SXT, trimethoprim/sulfamethoxazole.

In the present study, we did not find a single PFGE pattern covering all investigated stages, however, 20 of 48 *S.* Enteritidis strains from five of nine different stages (Adult broiler, Pre-cleaning carcasses, Post-cleaning carcasses, Carcasses after chilling, and Retail chicken) assigned to the dominant cluster X1, suggesting that the *S.* Enteritidis strain may spread along the broiler chicken chain. In addition, the diversity of PFGE patterns in the current study may be attributed to either different *S.* Enteritidis clones or no correlation among the samples along the broiler chicken supply chain.

## 5. Conclusions

The prevalence of *Salmonella* and its antimicrobial resistance during the chicken supply chain are always potential risks for human health. The data presented in this study demonstrate that samples of a broiler chicken supply chain in Qingdao City, China were commonly contaminated with *Salmonella*, especially with the serotype *S.* Enteritidis. The result of PFGE suggested that *S.* Enteritidis might spread along the broiler chicken supply chain. Cleaning procedures on abattoir lines should be reinforced to control *Salmonella* contamination in the broiler chicken supply chain. In addition, characterization of *Salmonella* isolates and determination of high-risk stages will contribute to the implementation of hazard analysis and critical control point (HACCP) measures for preventing *Salmonella* infection of chicken products. The high levels of antibiotic resistance and multiple MDR patterns observed amongst *Salmonella* isolates suggest that control measures for antimicrobial usage and surveillance should be put into place to avoid the inappropriate use of antibiotics in animal husbandry.

## Acknowledgments

This work was supported by the Key Projects in the National Science & Technology Pillar Program during the Twelfth Five-year Plan Period (2012BAK01B02), the Special Fund for Agro-scientific Research in the Public Interest (201203040) and the National Natural Science Foundation of China (no. 30972216).

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