

# Prevalence and characteristics of *Salmonella* isolates recovered from retail raw chickens in Shaanxi Province, China

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**ABSTRACT** In this study, we evaluated the prevalence of *Salmonella* in retail raw chickens in Shaanxi Province, China, on a monthly basis. In addition, we studied the antibiotic susceptibility, serotype, and genotype of *Salmonella* isolates and explored their relationships with sampling time, location, market type, and chicken type. The results showed that *Salmonella* was more prevalent in chickens sampled during the spring and summer than during the autumn and winter. Thirty-nine serotypes were identified from 406 *Salmonella* isolates, of which *Salmonella typhimurium* (16.7%) was the most prevalent. Other prevalent serotypes included *S. thompson* (12.8%), *S. essen* (9.1%), *S. infantis* (6.9%), *S. rissen* (5.7%), and *S. enteritidis* (5.4%). Approximately 71.4% of the 406 isolates were resistant to 3 or more antibiotics, 11.8% to 12 or more, and 1.7% to all 14 antibiotics tested. The most frequently detected resistance was to trimethoprim/sulfamethoxazole (82.0%), followed by nalidixic

acid (71.9%) and tetracycline (59.4%). The frequencies of resistance to ampicillin, chloramphenicol, and amoxicillin/clavulanic acid were moderately high (~50% each). Resistance to kanamycin, ceftiofur, streptomycin, gentamicin, and ciprofloxacin was less common (<40% each). Serotype distribution and antibiotic susceptibility of *Salmonella* isolates were related to sampling time, location, chicken type, and market type. Isolates recovered from the same sampling time, market type, location, and chicken type commonly exhibited identical or similar genotypes and antibiotic resistance profiles. However, DNA profiles and antibiotic resistance phenotypes of isolates within some serotypes were diverse. Our results revealed that multiple *Salmonella* subtypes with antibiotic resistance were prevalent in retail raw chickens in Shaanxi Province. Our study findings provide information for developing preventive measures against contamination of retail foods with *Salmonella*.

**Key words:** *Salmonella*, retail chicken, prevalence, antibiotic resistance, genotype

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## INTRODUCTION

Food safety and public health associated with antibiotic-resistant pathogens represent major challenges in China (Wang et al., 2019a,b). Among the foodborne pathogens, *Salmonella* causes serious human diseases and disseminates through the food supply, foods, humans, and animals. It has been estimated that ~1,000,000 *Salmonella* infections and 378 deaths occur each year in the

United States (Scallan et al., 2011). In China, 70%–80% of foodborne bacterial outbreaks are attributed to *Salmonella* infection (Wang and Zheng, 2007).

*Salmonella* has been detected in retail foods including chicken, pork, beef, ground meat, ice cream, dumplings, vegetables, powdered infant milk, fish, and ready-to-eat products. Among these foods, retail chicken is the most common carrier of *Salmonella* (White et al., 2001; Gatto et al., 2006; Nhung et al., 2018). Past studies have reported that chopping boards, butchers' hands, and knives used for retail chicken processing constitute potential sources for *Salmonella* cross-contamination (Olsen et al., 2003; Suresh et al., 2004).

Antibiotic resistance of *Salmonella*, which is closely linked to the abuse and misuse of antibiotics in food animal and humans, is increasing worldwide (Van et al., 2003; Gilchrist et al., 2007). Surveillance data have

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**Table 1.** Antibiotic resistance of *Salmonella* isolates recovered from retail raw chickens in different months.

| Antibiotic                     | Concentration range ( $\mu\text{g}/\text{mL}$ ) | Total no. (%) | % Resistance to individual antibiotics |                     |                   |                     |                     |                     |                   |                  |                     |                   |
|--------------------------------|---|---------------|--|---------------------|-------------------|---------------------|---------------------|---------------------|-------------------|------------------|---------------------|-------------------|
|                                |   |               | 2011                                   |                     |                   |                     |                     |                     | 2012              |                  |                     |                   |
|                                |   |               | April (n = 83)                         | May (n = 94)        | June (n = 13)     | July (n = 76)       | August (n = 32)     | September (n = 47)  | October (n = 4)   | January (n = 3)  | February (n = 4)    | March (n = 50)    |
| Trimethoprim/sulfa methoxazole | 0/0–8/152                                       | 333 (82.0)    | 45.8 <sup>d</sup>                      | 93.6 <sup>b</sup>   | 100 <sup>a</sup>  | 94.7 <sup>b</sup>   | 87.5 <sup>b</sup>   | 70.2 <sup>c</sup>   | 100 <sup>a</sup>  | 100 <sup>a</sup> | 100 <sup>a</sup>    | 100 <sup>a</sup>  |
| Nalidixic acid                 | 0–64  | 292 (71.9)    | 34.9 <sup>c</sup>                      | 99 <sup>a</sup>     | 100 <sup>a</sup>  | 90.8 <sup>b</sup>   | 43.8 <sup>c</sup>   | 38.3 <sup>c</sup>   | 100 <sup>a</sup>  | 100 <sup>a</sup> |                     | 98.0 <sup>a</sup> |
| Tetracycline                   | 0–32  | 241 (59.4)    | 57.8 <sup>c</sup>                      | 86.2 <sup>b</sup>   | 38.5              | 82.9 <sup>b</sup>   | 56.3 <sup>c</sup>   | 36.2                | 100 <sup>a</sup>  |                  | 75.0 <sup>b</sup>   | 4.0               |
| Ampicillin                     | 0–64  | 219 (53.9)    | 25.3                                   | 88.3 <sup>b</sup>   | 30.8              | 76.3 <sup>c</sup>   | 75.0 <sup>c</sup>   | 36.2                | 100 <sup>a</sup>  | 100 <sup>a</sup> | 75.0 <sup>c</sup>   | 4.0               |
| Amoxicillin/clavulanic acid    | 0/0–64/32                                       | 212 (52.2)    | 25.3                                   | 86.2 <sup>b</sup>   | 38.5              | 69.7 <sup>c</sup>   | 71.9 <sup>c</sup>   | 34.0                | 100 <sup>a</sup>  | 100 <sup>a</sup> | 100 <sup>a</sup>    | 4.0               |
| Chloramphenicol                | 0–64  | 212 (52.2)    | 21.7                                   | 89.4 <sup>b</sup>   | 15.4              | 68.4 <sup>c</sup>   | 43.8                | 72.3 <sup>c</sup>   | 100 <sup>a</sup>  |                  | 75.0 <sup>c</sup>   | 2.0               |
| Kanamycin                      | 0–128   | 158 (38.9)    | 22.9                                   | 76.6 <sup>a</sup>   | 23.1              | 32.9                | 65.6 <sup>a</sup>   | 34.0                | 50.0 <sup>b</sup> |                  |                     |                   |
| Ceftiofur                      | 0–128   | 154 (37.9)    | 20.5                                   | 69.1 <sup>c</sup>   |                   | 14.5                | 18.8                | 17.0                | 100 <sup>a</sup>  | 33.3             | 25.0                | 82.0 <sup>b</sup> |
| Streptomycin                   | 0–64  | 136 (33.5)    | 18.1 <sup>c,d</sup>                    | 83 <sup>b</sup>     | 15.4 <sup>d</sup> | 27.6 <sup>c</sup>   | 21.9 <sup>c,d</sup> | 17.0 <sup>c,d</sup> |                   | 100 <sup>a</sup> |                     | 2.0               |
| Gentamicin                     | 0–32  | 116 (28.6)    | 19.3 <sup>c</sup>                      | 41.5 <sup>a,b</sup> | 7.7               | 32.9 <sup>b</sup>   | 53.1 <sup>a</sup>   | 34.0 <sup>b</sup>   |                   |                  | 25.0 <sup>b,c</sup> | 2.0               |
| Ciprofloxacin                  | 0–16  | 90 (22.2)     | 25.3 <sup>b,c</sup>                    | 47.9 <sup>a</sup>   | 7.7               | 1.3                 | 18.8 <sup>c</sup>   | 31.9 <sup>b</sup>   |                   |                  | 25.0 <sup>b,c</sup> |                   |
| Amikacin                       | 0–128   | 80 (19.7)     | 15.7 <sup>c</sup>                      | 26.6 <sup>b,c</sup> |                   | 13.2 <sup>c</sup>   | 53.1 <sup>a</sup>   | 29.8 <sup>b</sup>   |                   |                  |                     | 2.0               |
| Ceftriaxone                    | 0–128   | 73 (18.0)     | 19.3 <sup>a,b</sup>                    | 29.9 <sup>a</sup>   |                   | 15.8 <sup>b</sup>   | 3.1                 | 29.8 <sup>a</sup>   |                   |                  |                     | 4.0               |
| Cefoxitin                      | 0–64  | 70 (17.2)     | 1.2                                    | 18.1 <sup>d</sup>   |                   | 23.7 <sup>c,d</sup> | 43.8 <sup>b</sup>   | 29.8 <sup>c</sup>   | 100 <sup>a</sup>  |                  |                     | 4.0               |

Values sharing the same superscript letter in each row are not significantly different ( $P > 0.05$ ).

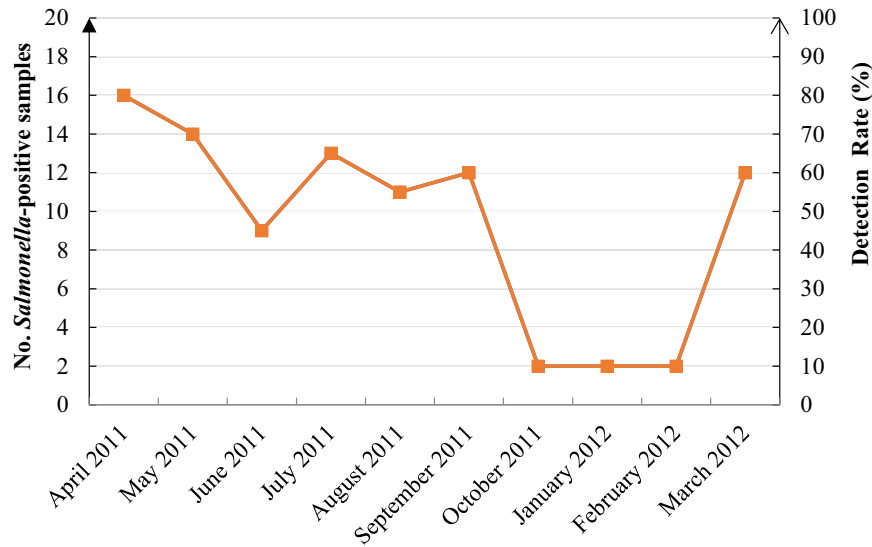


Figure 1. Prevalence of *Salmonella* in retail raw chickens in Shaanxi province, China (2011 and 2012).

demonstrated a noticeable increase in overall antibiotic resistance of *Salmonella* from 20% to 30% in the early 1990s to nearly 70% in the 2000s (Su et al., 2004). In recent years, an alarming emergence of multidrug-resistant (MDR) *Salmonella* has been reported in several European and Asian countries including China (Isenbarger et al., 2002; Cailhol et al., 2006; Xia et al., 2009; Feasey et al., 2015; Wong et al., 2015).

The prevalence and antibiotic susceptibility of *Salmonella* in retail foods such as meats, dumplings, and cold dishes have been reported in different regions of China (Yang et al., 2011, 2014; Hu et al., 2017; Wang et al., 2017). Moreover, *Salmonella* isolates recovered from retail meats in Shaanxi Province, China, have been characterized (Yang et al., 2010), but the monthly prevalence of *Salmonella* in retail raw chickens in this region remains poorly understood. In the present study, we evaluated the prevalence of *Salmonella* in retail chickens in Shaanxi Province on a monthly basis. In addition, we characterized 406 *Salmonella* isolates to better understand their serotype distribution and antibiotic susceptibility in retail foods.

## MATERIALS AND METHODS

### Retail Raw Chicken Sampling

We collected 20 retail raw chicken carcasses each month from April to October of 2011 and from January to March of 2012 in Shaanxi Province, China (Supplementary Table 1). The 20 samples included 10 freshly slaughtered chickens from 4 different wet markets (2 slaughter houses were selected in each wet market in each location, and 1 to 2 chicken carcasses were randomly sampled in each slaughter house); and 10 chickens (5 chilled and 5 frozen) from 4 different supermarkets (one supermarket was selected in each location, and 1 to 2 chilled/frozen chicken carcasses were selected in each supermarket without consideration of the brand of the

chicken) across 4 different locations (Yangling, Zhouzhi, Wugong, and Baoji). A total of 200 retail raw chicken carcasses were sampled over a 10-month period. Each sample was aseptically placed in a sterile stomacher bag (Seward, UAC House, London, UK), labeled, and transported in an ice-cooler to the laboratory within 4 h.

### *Salmonella* Isolation and Identification

The prevalence of *Salmonella* in the samples was quantitatively assessed using the most probable number method developed by the Food Safety and Inspection Service of the United States Department of Agriculture with minor modifications (USDA/FSIS, 2014). Briefly, after the chicken carcass was weighed (ranged from 1.0 kg to 1.8 kg), 400-fold (mL) of sterilized buffered peptone water (BPW; BD, Sparks, MD) of the chicken's weight (kg) was added to the stomacher bag. The carcass was manually rinsed for at least 10 min to ensure BPW was in contact with the external and internal surfaces of the carcass. Subsequently, 1.0 mL of the BPW rinse was transferred to 3 tubes containing 9.0 mL of BPW each, and 0.1 mL of the BPW rinse was transferred to 3 other tubes containing 9.9 mL of BPW each.

The inoculated tubes were incubated at  $35 \pm 2^\circ\text{C}$  for 20–24 h. Then 10 mL of tetrathionate broth (TT; BD) and 10 mL of modified Rappaport Vassiliadis broth (mRV; BD) were inoculated with  $0.5 \pm 0.05$  mL and  $0.1 \pm 0.02$  mL of the cultures, respectively. The TT and mRV broths were incubated at  $42 \pm 0.5^\circ\text{C}$  under constant shaking (100 rpm) for 22–24 h. After incubation, a loopful of TT and mRV cultures were streaked onto xylose-lysine-tergitol-4 agar (XLT4; BD) plates. The XLT4 plates were incubated at  $35 \pm 2^\circ\text{C}$  for 22–24 h. One presumptive *Salmonella* colony from each XLT4 plate was transferred onto a fresh XLT4 plate. *Salmonella* colonies were identified and confirmed using the API20 E test kit (bioMérieux, Inc., Hazelwood, MO).

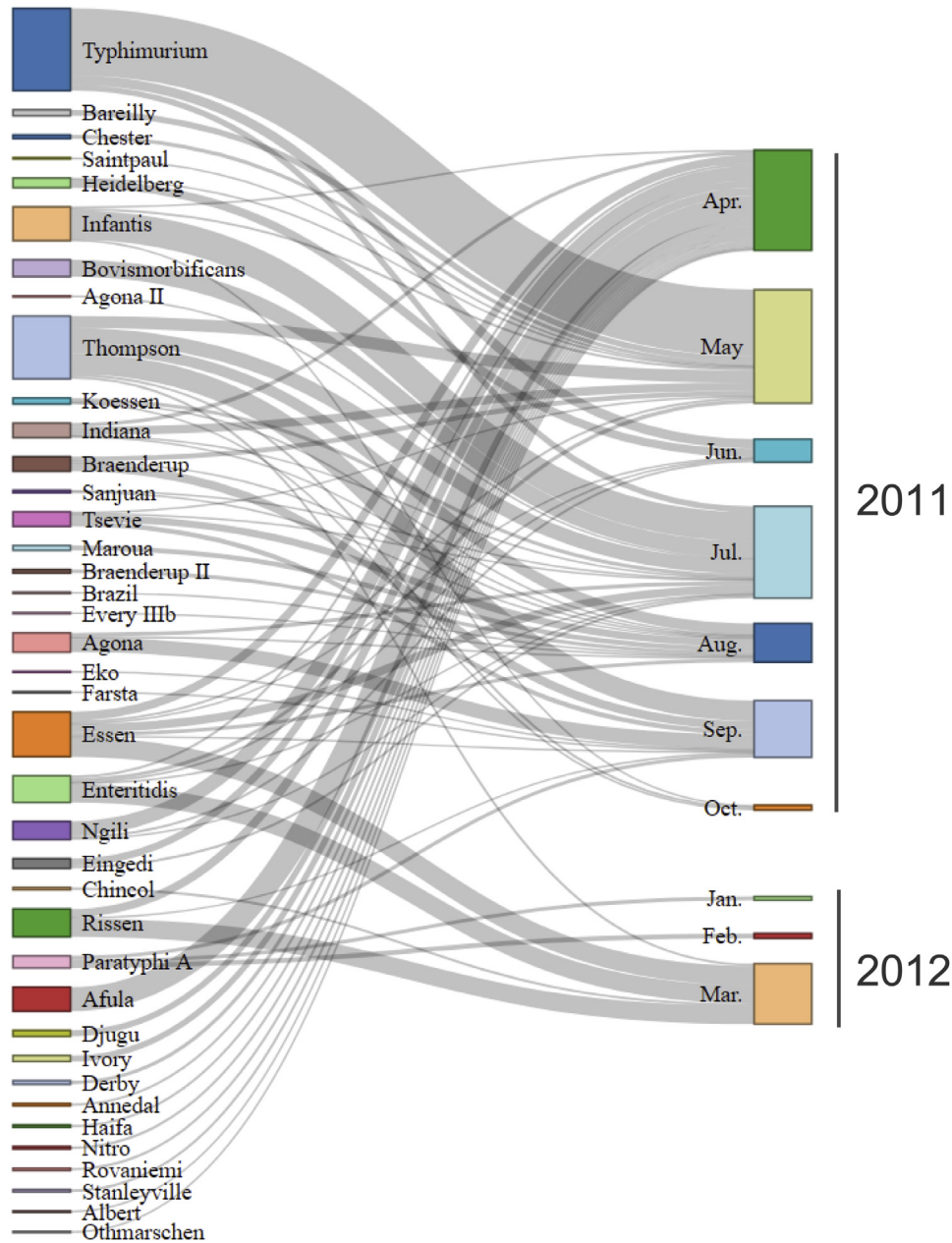


Figure 2. Distribution of 39 *Salmonella* serotypes identified in retail raw chickens in different months.

## Salmonella Serotyping

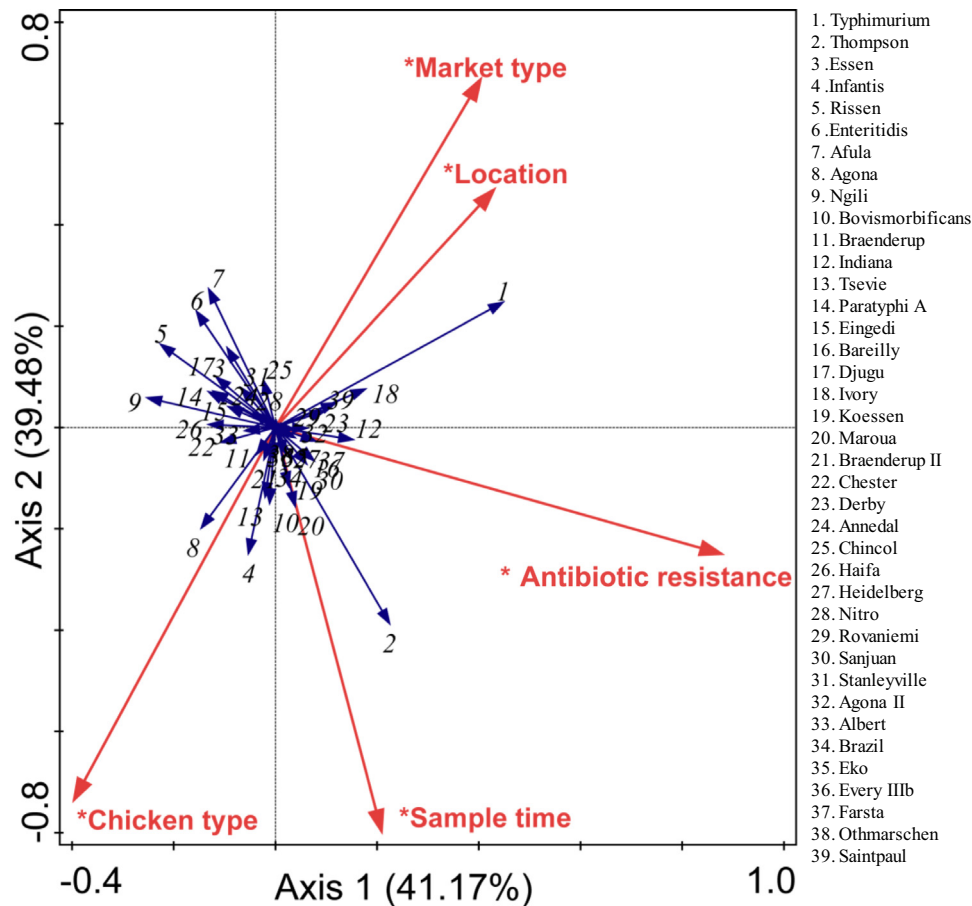
*Salmonella* isolates were serotyped in the Henan Center for Disease Control and Prevention (Zhengzhou, Henan, China). O and H antigens were detected by the slide agglutination method using *Salmonella*-specific hyperimmune sera (Statens Serum Institute, Artillerivej, Denmark). Serotype was determined following the manufacturer's instructions and White-Kauffmann classification scheme (Kauffmann, 2010).

## Antibiotic Susceptibility Test

Antibiotic susceptibility of *Salmonella* isolates was evaluated by the agar dilution method using Mueller-Hinton agar (Land Bridge Technology Co., Ltd., Beijing, China) according to the protocol reported by the Clinical

and Laboratory Standard Institute (CLSI, 2014). Fourteen antibiotics that are monitored by the National Antimicrobial Resistance Monitoring System ([http://www.fda.gov/downloads/Animal\\_Veterinary/Safety\\_Health/Antimicrobial\\_Resistance/National\\_Antimicrobial\\_Resistance\\_Monitoring\\_System/UCM334834.pdf](http://www.fda.gov/downloads/Animal_Veterinary/Safety_Health/Antimicrobial_Resistance/National_Antimicrobial_Resistance_Monitoring_System/UCM334834.pdf)) were tested (National Antimicrobial Resistance Monitoring System, 2014). The antibiotics were nalidixic acid, ciprofloxacin, ceftriaxone, ceftiofur, gentamicin, kanamycin, amikacin, streptomycin, ampicillin, amoxicillin/clavulanic acid, chloramphenicol, trimethoprim/sulfamethoxazole, and tetracycline. Their concentration ranges are listed in Table 1. All antibiotics were acquired from Sigma St. (Louis, MO).

*Escherichia coli* ATCC 25922 and *Enterococcus faecalis* ATCC 29212 were used as quality-control microorganisms for the determination of minimum



**Figure 3.** Effects of sampling time, location, market type, and chicken type on *Salmonella* serotype distribution in retail raw chickens (\* $P < 0.01$  in “mantel test”).

inhibitory concentrations of antibiotics. For the interpretative breakpoints of resistance and susceptibility, we followed CLSI standards (CLSI, 2014) for most antibiotics. An exception was streptomycin, for which we used that in National Antimicrobial Resistance Monitoring System.

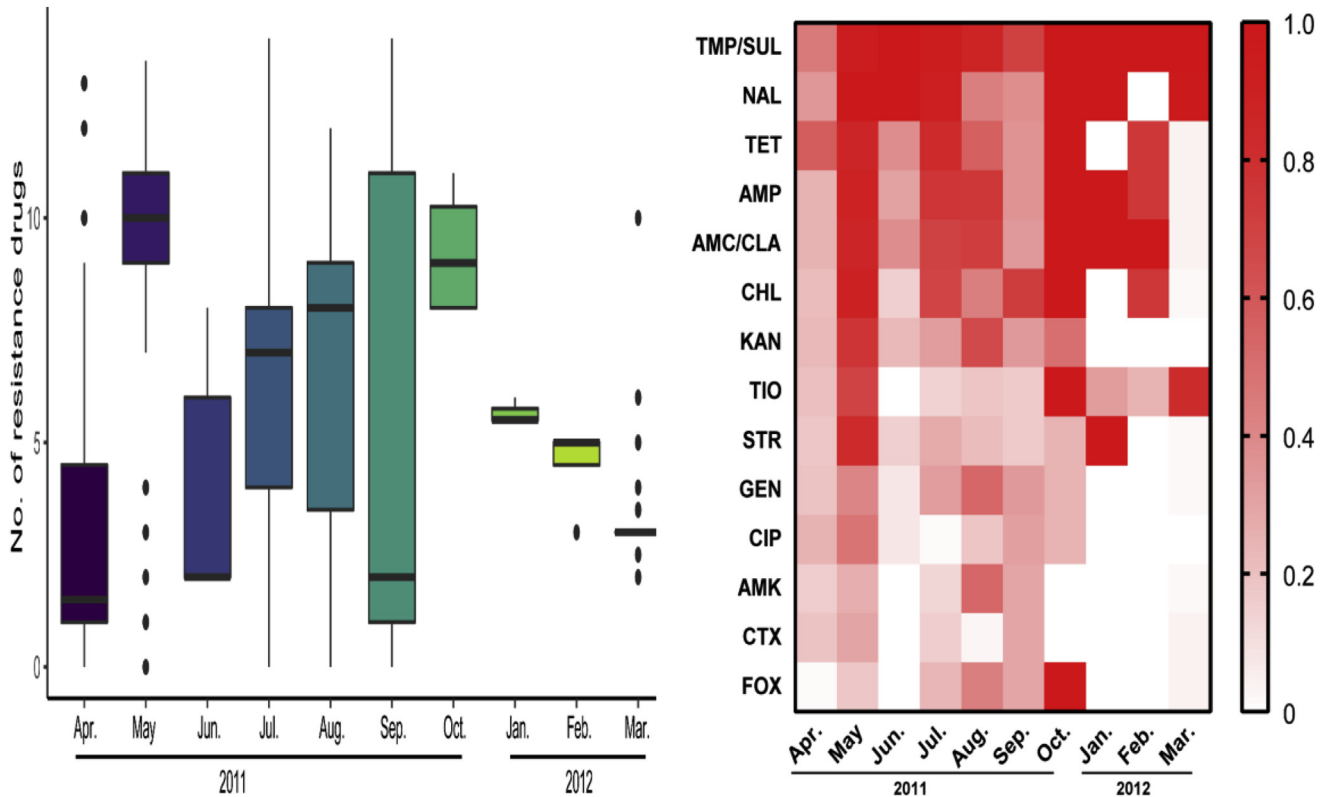
### Pulsed-Field Gel Electrophoresis

The genetic relatedness of *Salmonella* isolates was determined using pulsed-field gel electrophoresis (PFGE) combined with restricted enzyme digestion according to the protocol reported by the United States Center for Disease Control and Prevention (Ribot et al., 2006). Briefly, *Salmonella* cells were embedded in agarose and lysed. The genomic DNAs were digested using XbaI (TaKaRa, Dalian, China) for 1.5–2 h at 37°C in a water bath. DNA digests in 0.5 × Tris-borate-EDTA buffer were subsequently separated at 14°C for 19 h using a Chef Mapper electrophoresis system (Bio-Rad, Hercules, CA) with a pulse time of 2.16–63.8 s. After staining of the PFGE gel with ethidium bromide, the DNA bands were visualized in an UV trans-illumination imaging system (Bio-Rad). The genetic relatedness of the isolates was assessed using the BioNumerics software (version 3.0; Applied-Maths, Kortrijk, Belgium) with different ban

coefficients and single linkage clustering. *Salmonella* Braenderup H9812 was used as a positive standard control for PFGE.

### Statistical Analysis

Differences among the detection rates of *Salmonella*-positive samples, serotypes, and antibiotic-resistant isolates in different months were determined by chi-square test using Minitab v16.2.3 (Minitab Inc., State College, PA). Significant difference and extreme significant difference were set at  $P < 0.05$  and  $P < 0.01$ , respectively. The relationship between *Salmonella* serotype and antibiotic resistance, sampling time, location, market type, and chicken type was determined by redundancy analysis using CANOCO v5.0 (Microcomputer Power, Ithaca, NY). The relationship between antibiotic resistance and the aforementioned factors was also determined by redundancy analysis. Descriptive and comparative analyses of the total number of serotypes and antibiotic resistance phenotypes in each month were performed using Graph Pad Prism v7.0 (GraphPad Software, La Jolla, CA). A Sankey plot was generated to visualize the distribution of *Salmonella* serotypes in retail raw chickens using the networkD3 library in R v3.6.2 (network D3:D3 JavaScript Network GRAPHS from R; <https://rdocumentation.org/>).



**Figure 4.** Antibiotic susceptibility of *Salmonella* isolates recovered from retail raw chickens in different months. Abbreviations: AMC/CLA, amoxicillin/clavulanic acid; AMK, amikacin; AMP, ampicillin; CHL, chloramphenicol; CIP, ciprofloxacin; CTX, ceftriaxone; FOX, cefoxitin; GEN, gentamicin; KAN, kanamycin; NAL, nalidixic acid; TET, tetracycline; TIO, ceftiofur; TMP/SUL, trimethoprim/sulfamethoxazole; STR, streptomycin.

## RESULTS

### *Salmonella* Prevalence and Isolate Recovery

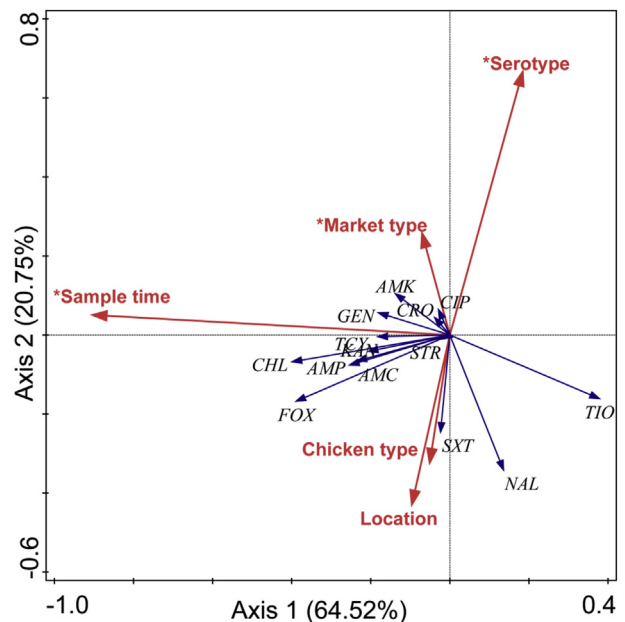
Among the 200 retail raw chicken samples, 93 (46.5%) were *Salmonella*-positive. In general, *Salmonella*-positive chickens were not prevalent in October, January, or February but were frequently detected in April and May. The detection rates of *Salmonella*-positive chickens were higher in April ( $P < 0.01$ ) and May ( $P < 0.05$ ) than in June, October, January, and February (Figure 1, Supplementary Table 1). Using the most probable number procedure, a total of 406 *Salmonella*-positive TT and

**Table 2.** Multidrug resistance monthly observed among *Salmonella* isolates recovered from retail raw chickens and their main antibiotic resistance profile.

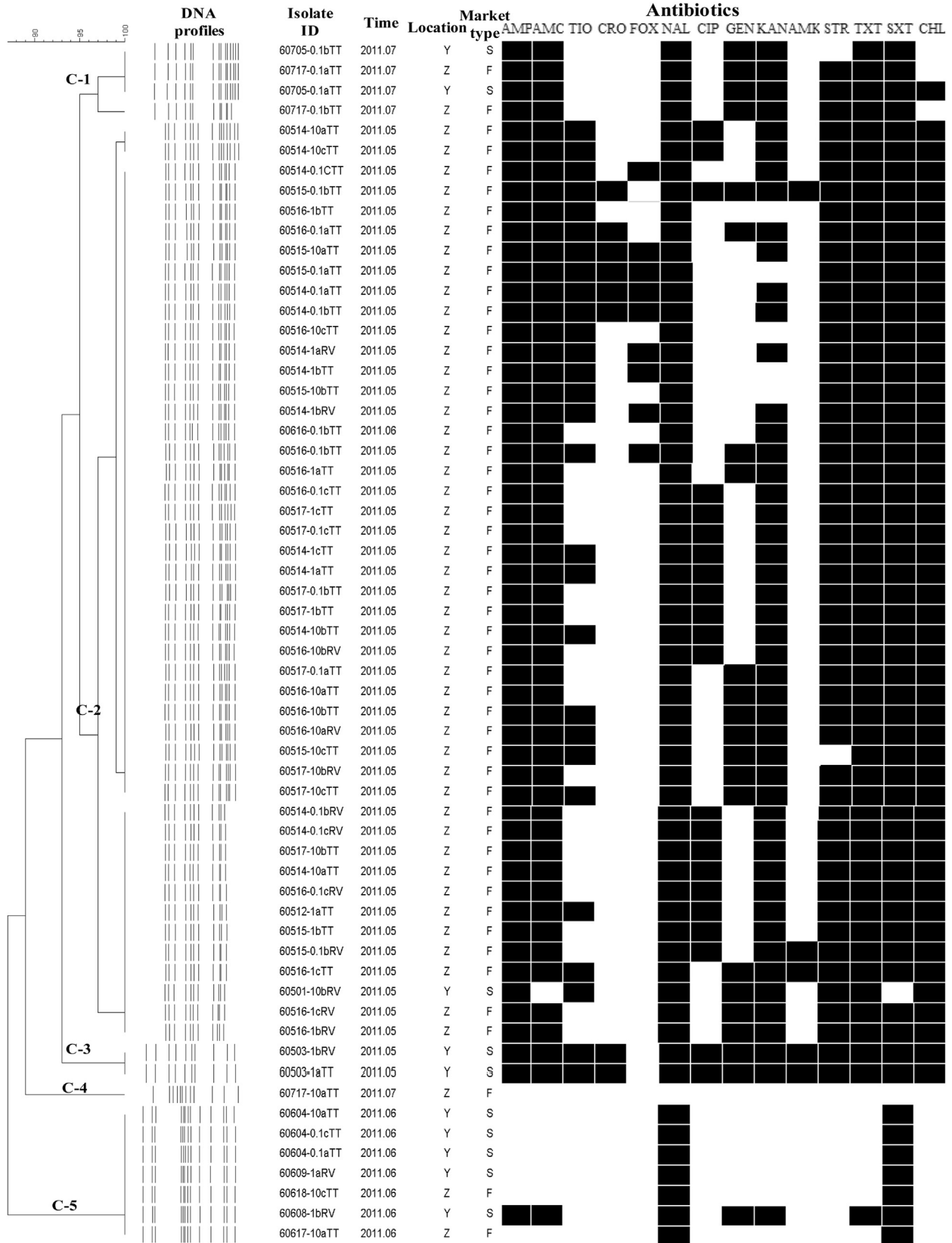
| Year | Month (isolate no.) | % Resistance to indicated category of antibiotics |                   |                     |                     |
|------|---------------------|---|-------------------|---------------------|---------------------|
|      |                     | <3  | 3–5               | 6–7                 | Total ( $\geq 3$ )  |
| 2011 | April (n = 83)      | 72.3 <sup>a</sup>                                 | 6.0 <sup>e</sup>  | 21.7 <sup>d,e</sup> | 27.7 <sup>e</sup>   |
|      | May (n = 94)        | 4.3 <sup>d</sup>                                  | 6.4 <sup>e</sup>  | 89.4 <sup>b</sup>   | 95.7 <sup>b</sup>   |
|      | June (n = 13)       | 61.5 <sup>a,b</sup>                               | 23.1 <sup>d</sup> | 15.4 <sup>e</sup>   | 38.5 <sup>d,e</sup> |
|      | July (n = 76)       | 3.9 <sup>d</sup>                                  | 51.3 <sup>c</sup> | 44.7 <sup>c,d</sup> | 96.1 <sup>b</sup>   |
|      | August (n = 32)     | 25.0 <sup>c</sup>                                 | 28.1 <sup>d</sup> | 46.9 <sup>c</sup>   | 75.0 <sup>c</sup>   |
|      | September (n = 47)  | 57.4 <sup>b</sup>                                 | 10.6 <sup>e</sup> | 31.9 <sup>d</sup>   | 42.6 <sup>d</sup>   |
|      | October (n = 4)     |   |                   | 100 <sup>a</sup>    | 100 <sup>a</sup>    |
| 2012 | January (n = 3)     |   | 100 <sup>a</sup>  |                     | 100 <sup>a</sup>    |
|      | February (n = 4)    |   | 100 <sup>a</sup>  |                     | 100 <sup>a</sup>    |
|      | March (n = 50)      | 16.0 <sup>c</sup>                                 | 82.0 <sup>b</sup> | 2.0 <sup>f</sup>    | 84.0 <sup>c</sup>   |

Values sharing the same superscript letter in each column are not significantly different ( $P > 0.05$ ).

mRV cultures were obtained from 3,600 TT and mRV tubes. From each TT- and mRV-XLT4 plate, one isolate



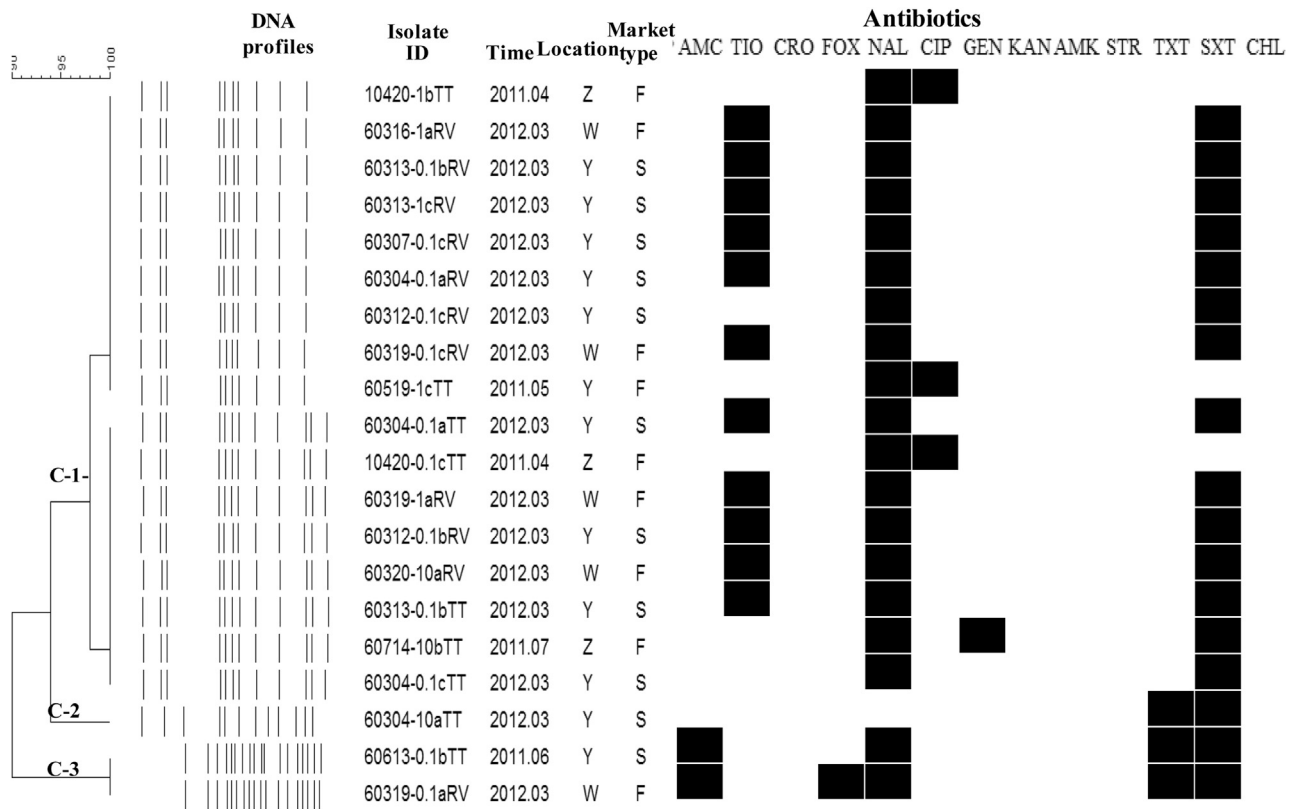
**Figure 5.** Effects of sampling time, location, market type, and chicken type on antibiotic resistance of *Salmonella* in retail raw chickens (\* $P < 0.01$  in “mantel test”). Abbreviations: AMC/CLA, amoxicillin/clavulanic acid; AMK, amikacin; AMP, ampicillin; CHL, chloramphenicol; CIP, ciprofloxacin; CTX, ceftriaxone; FOX, cefoxitin; GEN, gentamicin; KAN, kanamycin; NAL, nalidixic acid; TET, tetracycline; TIO, ceftiofur; TMP/SUL, trimethoprim/sulfamethoxazole; STR, streptomycin.



**Figure 6.** Dendrogram of PFGE profiles and antibiotic resistance phenotypes of 60 *Salmonella typhimurium* isolates recovered from retail raw chickens. Abbreviations: Location–Y: Yangling; Z: Zhouzhi. Market type–S: supermarket; F: wet market. Antibiotic–AMC/CLA, amoxicillin/clavulanic acid; AMK, amikacin; AMP, ampicillin; CHL, chloramphenicol; CIP, ciprofloxacin; CTX, ceftriaxone; FOX, ceftiofur; GEN, gentamicin; KAN, kanamycin; NAL, nalidixic acid; PFGE, pulsed-field gel electrophoresis; TET, tetracycline; TIO, ceftiofur; TMP/SUL, trimethoprim/sulfamethoxazole; STR, streptomycin.







**Figure 8.** Dendrogram of PFGE profiles and antibiotic resistance phenotypes of 20 *Salmonella enteritidis* isolates recovered from retail raw chickens. Abbreviations: Location—Y: Yangling; Z: Zhouzhi; W: Wugong. Market type—S: supermarket; F: wet market. Antibiotic—AMC/CLA, amoxicillin/clavulanic acid; AMK, amikacin; AMP, ampicillin; CHL, chloramphenicol; CIP, ciprofloxacin; CTX, ceftriaxone; FOX, ceftiofur; GEN, gentamicin; KAN, kanamycin; NAL, nalidixic acid; PFGE, pulsed-field gel electrophoresis; TET, tetracycline; TIO, ceftiofur; TMP/SUL, trimethoprim/sulfamethoxazole; STR, streptomycin.

those recovered from the other 4 mo were relatively susceptible to the antibiotics tested (Figure 4, Table 1). The detection rates of isolates resistant to  $\geq 3$  categories of antibiotics in May (95.7%), July (96.1%), August (75.0%) of 2011 and March (84.0%) of 2012 were higher ( $P < 0.01$ ) than those in April (27.7%), June (38.5%), and September (42.6%) of 2011 and lower ( $P < 0.05$ ) than those in October (100%) of 2011 and January (100%) and February (100%) of 2012 (Table 2).

Resistance to different antibiotics was closely related to various factors. For example, resistance to ceftiofur, amoxicillin/clavulanic acid, ampicillin, chloramphenicol, kanamycin, streptomycin, and tetracycline was most correlated with sampling time and location, followed by chicken type. In addition, resistance to gentamicin, ceftriaxone, amikacin, and ciprofloxacin was most correlated with sampling time, followed by market type. However, resistance to nalidixic acid and ceftiofur was not correlated with any factors (Figure 5).

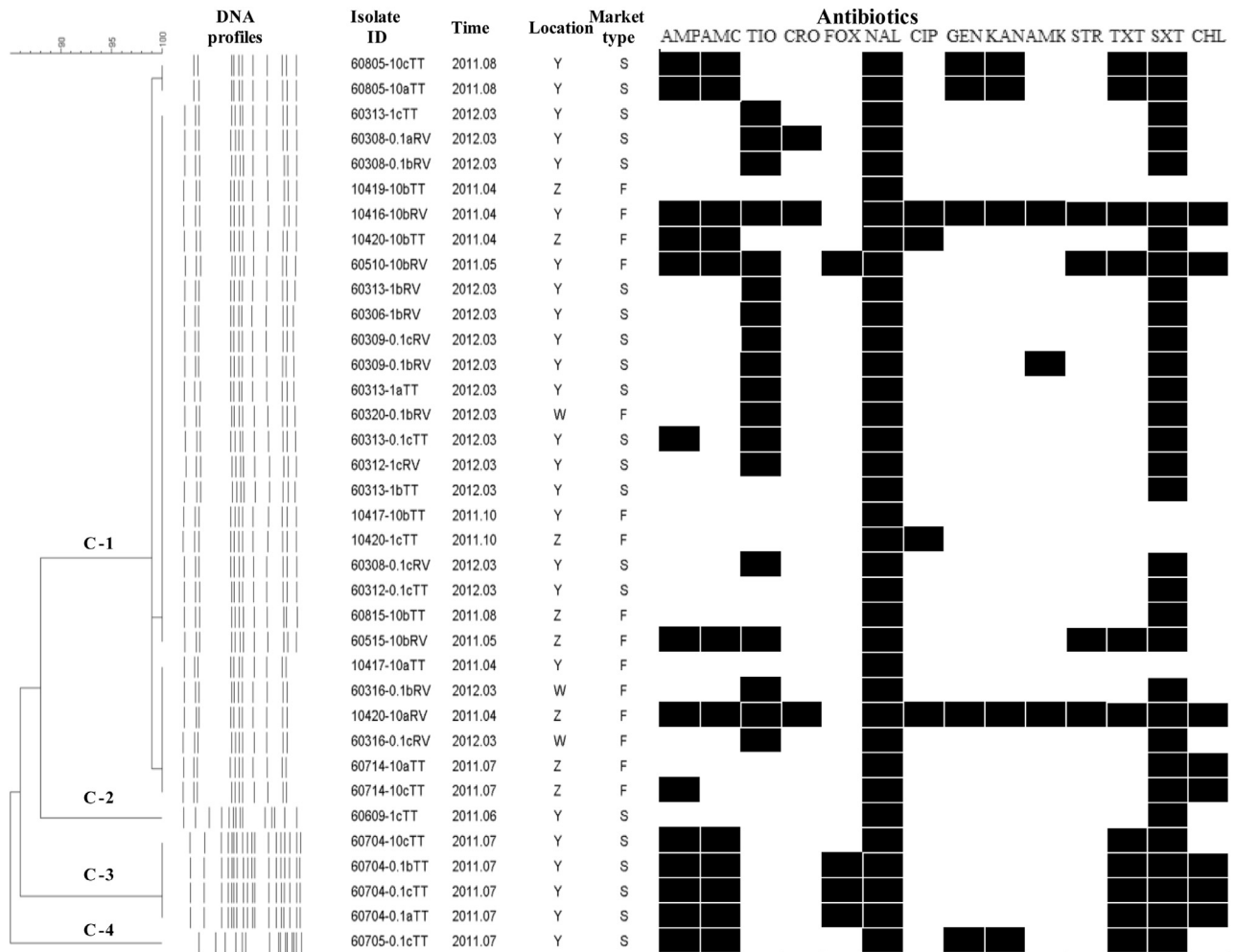
### PFGE Subtyping

In total, 203 isolates belonged to the 6 most prevalent *Salmonella* serotypes, *S. typhimurium*, *S. thompson*, *S. essen*, *S. infantis*, *S. rissen*, and *S. enteritidis*. These 6 serotypes were further subtyped using PFGE. Sixty *S. typhimurium* isolates, which presented eight different PFGE patterns (DNA profiles), were grouped into 5 clusters

(C-1 to C-5) with 96% similarity. Among them, isolates recovered from samples collected at the same location and sampling time were grouped in the same cluster. For example, 32 isolates in C-2, which were derived from samples collected in wet markets from Zhouzhi in May of 2011, shared similar PFGE patterns (Figure 6).

Even though some *S. typhimurium* isolates were recovered from samples collected from different locations and market types in the same month, they commonly presented the same or similar DNA profiles. For example, 4 isolates in C-1 were recovered from 2 samples collected from different locations and market types in July; 3 of them showed the same DNA profiles, and the other isolate was similar to the first 3 isolates. Similar findings were obtained for C-5 (Figure 6). Moreover, *S. typhimurium* isolates with the same or similar DNA profiles commonly exhibited the same or similar antibiotic resistance phenotypes except for some isolates in C-5 (Figure 6). The same or similar PFGE patterns and antibiotic susceptibility of isolates recovered from different locations and months indicated the potential long-term existence or cross-contamination of *S. typhimurium* in raw chickens and the possibility of foodborne outbreaks.

The same or similar DNA profiles and antibiotic resistance phenotypes were obtained for *S. rissen* (Figure 7) and *S. enteritidis* isolates (Figure 8). DNA profiles of *S. essen* isolates recovered from the same month, location, and market type were also the same or similar, but their



**Figure 9.** Dendrogram of PFGE profiles and antibiotic resistance phenotypes of 36 *Salmonella essen* isolates recovered from retail raw chickens. Abbreviations: Location—Y: Yangling; Z: Zhouzhi. Market type—S: supermarket; F: wet market. Antibiotic—AMC/CLA, amoxicillin/clavulanic acid; AMK, amikacin; AMP, ampicillin; CHL, chloramphenicol; CIP, ciprofloxacin; CTX, ceftriaxone; FOX, cefoxitin; GEN, gentamicin; KAN, kanamycin; NAL, nalidixic acid; PFGE, pulsed-field gel electrophoresis; TET, tetracycline; TIO, ceftiofur; TMP/SUL, trimethoprim/sulfamethoxazole; STR, streptomycin.

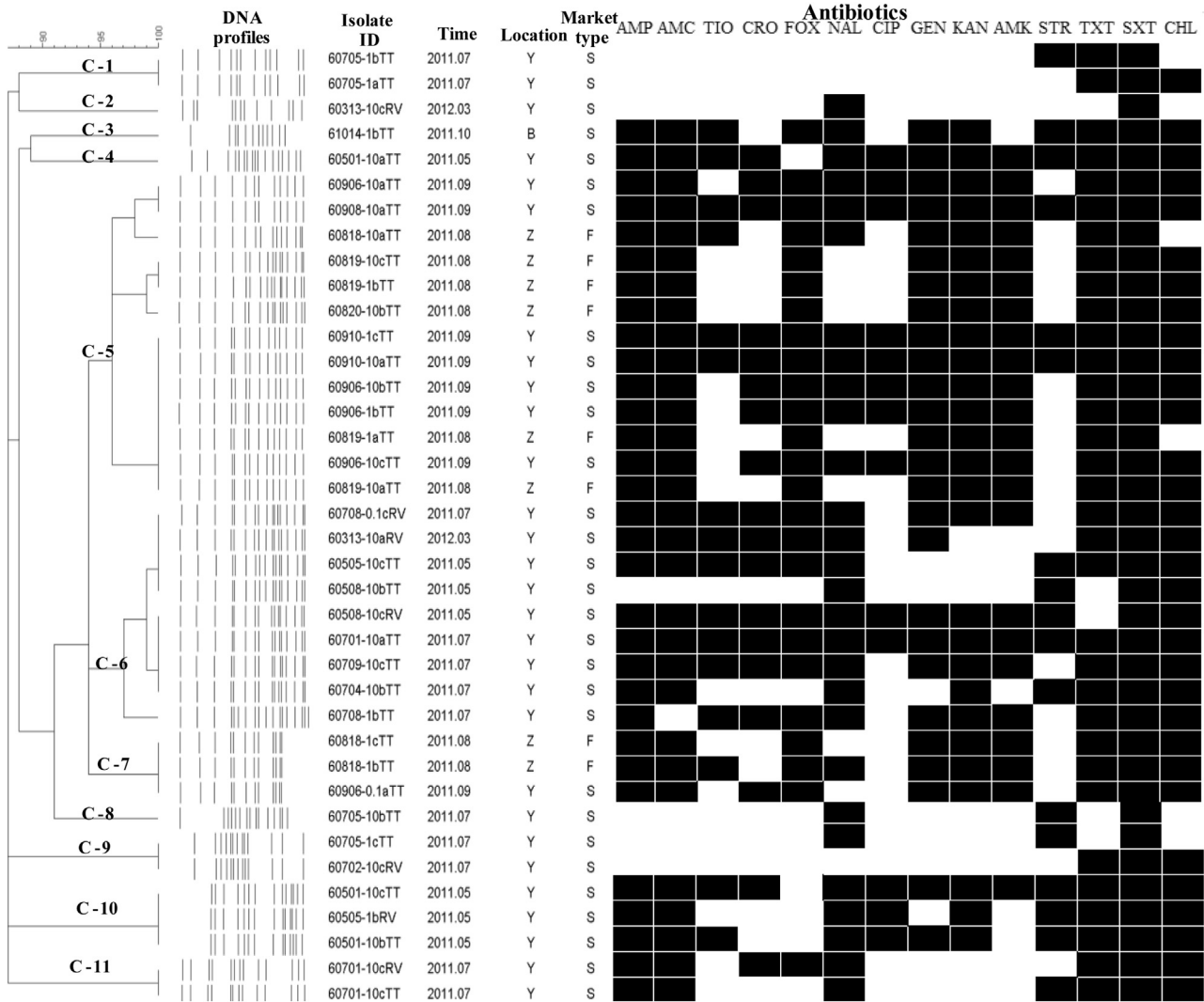
antibiotic resistance phenotypes were not consistent (Figure 9). *S. thompson* and *S. infantis* isolates (Figures 10 and 11) had more diverse DNA profiles and antibiotic resistance profiles than *S. typhimurium*, *S. rissen*, *S. enteritidis*, and *S. essen* isolates (Figures 6–9).

## DISCUSSION

In this study, we characterized *Salmonella* isolates recovered from retail raw chickens purchased in wet markets (freshly slaughtered) and supermarkets (chilled and frozen) in Shaanxi Province on a monthly basis. We found that *Salmonella* was more prevalent in chicken carcasses in spring and summer than during autumn and winter and considered the possible reasons: 1) *Salmonella* can grow in a wide temperature range (5–47°C), as the temperature gradually reaches to the optimal growth temperature of *Salmonella*, its reproduction rate will accelerate, and it is easier to survive on the surface of chicken carcass (Oscar, 2009; Smadi et al., 2012). In spring, the temperature in Shaanxi is around

28°C to 35°C; in summer, it is around 35°C to 40°C, that is, the optimal temperature for *Salmonella* to grow; 2) in Shaanxi, it is often windy in spring and facilitated the prevalence of *Salmonella* in the environment. Meanwhile, we obtained 406 *Salmonella* isolates that belonged to 39 serotypes. Among them, *S. typhimurium* was the most prevalent. This serotype, one of the most important worldwide, contributes to deaths in young broiler chickens and salmonellosis in humans (Padron, 1990; Alban et al., 2002; Loharikar et al., 2013). *S. typhimurium* has been detected in a wide range of poultry- and animal-derived foods such as retail chickens and pigs from various market types (i.e., wet markets and supermarkets) and animal products stored at various temperatures (i.e., ambient, chilled, and frozen; Humphrey et al., 2000; Yang et al., 2010; Yang et al., 2011; Campos et al., 2012; Yang et al., 2014).

Prior studies conducted in Africa and North America have revealed that *S. typhimurium* is the most common serotype in cattle and chickens (Thung et al., 2016; Gutema et al., 2019). In our study region, *S. typhimurium* was also the predominant serotype in retail raw

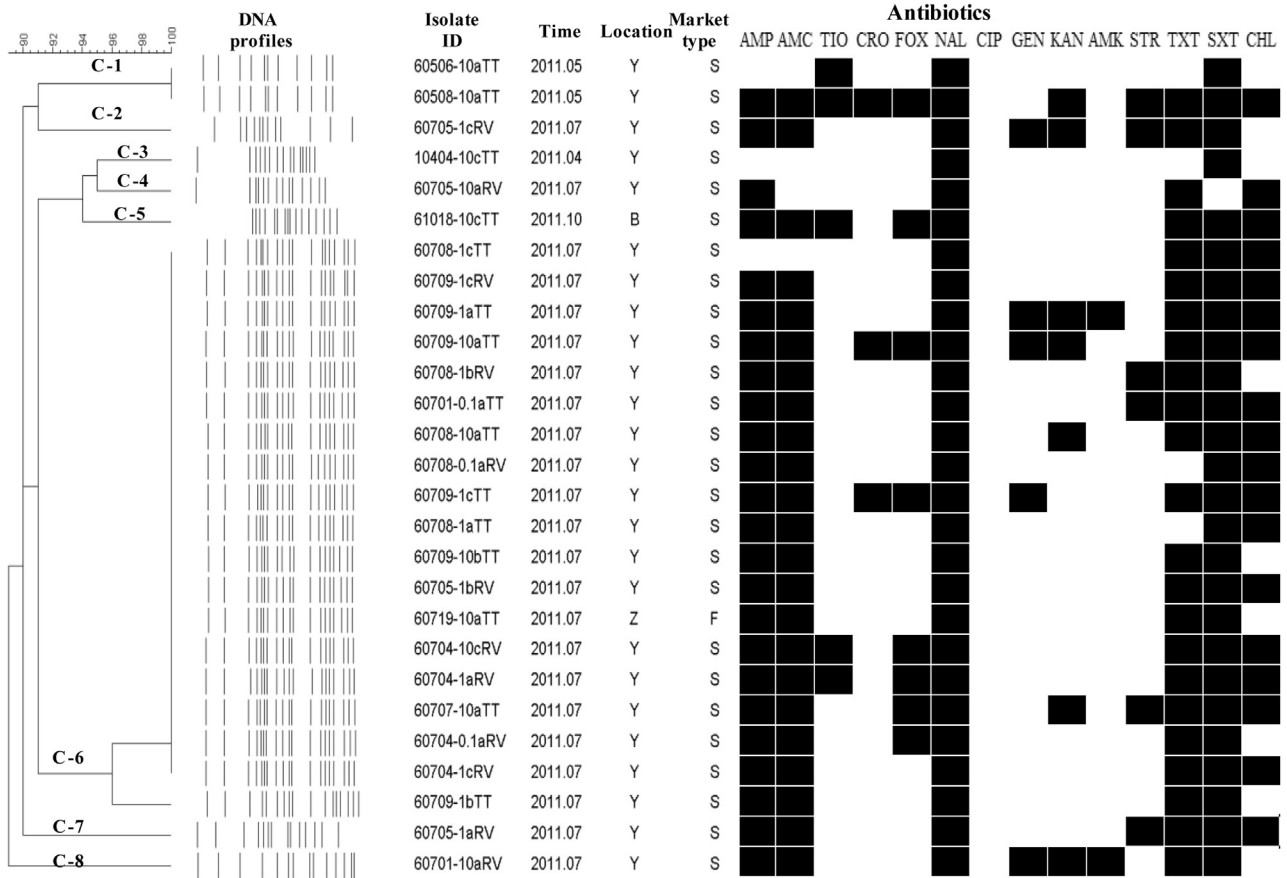


**Figure 10.** Dendrogram of PFGE profiles and antibiotic resistance phenotypes of 38 *Salmonella thompson* isolates recovered from retail raw chickens. Abbreviations: Location–Y: Yangling; Z: Zhouzhi; W: Wugong. Market type–S: supermarket; F: wet market. Antibiotic–AMC/CLA, amoxicillin/clavulanic acid; AMK, amikacin; AMP, ampicillin; CHL, chloramphenicol; CIP, ciprofloxacin; CTX, ceftriaxone; FOX, cefoxitin; GEN, gentamicin; KAN, kanamycin; NAL, nalidixic acid; PFGE, pulsed-field gel electrophoresis; TET, tetracycline; TIO, ceftiofur; TMP/SUL, trimethoprim/sulfamethoxazole; STR, streptomycin.

chickens, followed by *S. thompson*, *S. essen*, *S. infantis*, *S. riseen*, and *S. enteritidis*. Our results were in accordance with the findings of several studies carried out in Henan and Shaanxi, China (Xia et al., 2009; Yang et al., 2011). However, *S. thompson* (48.7%) has been frequently detected in chickens and giblets in Iran (Sodagari et al., 2015). In Asia, Europe, and Latin America, *S. enteritidis* is more prevalent than *S. typhimurium* and other serotypes in chickens (Humphrey et al., 2000; Olsen et al., 2001; Herikstad et al., 2002; Bangtrakulnonth et al., 2004; Galanis et al., 2006). In addition, several serotypes including *S. derby* and *S. heidelberg* have been detected in chickens and other foods in several countries of Southeast Asia, while these are rarely identified in the Shaanxi province (Humphrey et al., 2000; Olsen et al., 2001; Herikstad et al., 2002; Bangtrakulnonth et al., 2004; Galanis et al., 2006). It is possible that the lack of agreement between our results and those of previous studies are due to the differences in

sample type, location, detection method, sampling time, and sample size.

In recent years, there has been an increasing emergence and spread of antibiotic-resistant *Salmonella* in several countries, particularly in Asia. This is a global problem caused by numerous interconnected factors, especially the abuse and misuse of antibiotics (Hoge et al., 1998; Davis et al., 1999; Cailhol et al., 2006; Lauderdale et al., 2006; Wang et al., 2019a,b). A high prevalence of antibiotic-resistant *Salmonella* in retail meats has been reported in Greece, United States, Canada, and China (Daoust et al., 1992; Arvanitidou et al., 1998; Chen et al., 2004). In the present study, 7.1% of 406 *Salmonella* isolates were susceptible to 14 antibiotics, whereas 92.8% of the isolates resisted at least one antibiotic and 1.7% resisted all 14 antibiotics. The rate of trimethoprim/sulfamethoxazole-resistant *Salmonella* was significantly higher in our study (82.0%) than that in Shaanxi province, 2007–2008 (58.0%) (Yang et al., 2011).



**Figure 11.** Dendrogram of PFGE profiles and antibiotic resistance phenotypes of 27 *Salmonella infantis* isolates recovered from retail raw chickens. Abbreviations: Location–Y: Yangling; Z: Zhouzhi; W: Wugong. Market type–S: supermarket; F: wet market. Antibiotic–AMC/CLA, amoxicillin/clavulanic acid; AMK, amikacin; AMP, ampicillin; CHL, chloramphenicol; CIP, ciprofloxacin; CTX, ceftriaxone; FOX, cefoxitin; GEN, gentamicin; KAN, kanamycin; NAL, nalidixic acid; PFGE, pulsed-field gel electrophoresis; TET, tetracycline; TIO, ceftiofur; TMP/SUL, trimethoprim/sulfamethoxazole; STR, streptomycin.

Trimethoprim/sulfamethoxazole, fluoroquinolones, and cephalosporins are frontline therapeutic drugs for most bacterial infections; therefore, the high rates of *Salmonella* with resistance to these antibiotics are of significant concern (Fey et al., 2000; Bradford, 2001).

When the relationship between sampling time and antibiotic resistance of *Salmonella* was determined, we found that the rates of MDR *Salmonella* in March, May, July, and August were substantially higher than those in April, June, and September and lower than those in October, January, and February. Previous studies have reported that March through May and July through September are high-incidence seasons for foodborne *Salmonella* because of the direct influence of epidemic diseases on pathogen prevalence. The abuse and misuse of antibiotics in animals and humans have therefore prompted the emergence of antibiotic-resistant *Salmonella* (Xu et al., 2009; Lu et al., 2014). The highest rates of MDR *Salmonella* in October, January, and February (100%) might be overestimated because of low *Salmonella* prevalence (10%).

We further subtyped the 6 most prevalent *Salmonella* serotypes using PFGE and found that some *Salmonella* isolates within specific serotypes shared identical PFGE patterns. For example, even though the retail

chickens were collected from different months, locations, and chicken types, *S. typhimurium* isolates in clusters C-1, C-3, and C-5 exhibited high genomic similarities. In addition, some *S. typhimurium* (C-2 and C-5), *S. rissen* (C-1, C-2, and C-3), *S. enteritidis* (C-1), *S. essen* (C-1 and C-3), and *S. infantis* (C-1 and C-6) isolates from samples collected from the same month and location shared identical or similar PFGE profiles. Prior studies found that *Salmonella* isolates in certain outbreaks almost shared similar PFGE patterns (Yang et al., 2011; Salwani et al., 2014; Wang et al., 2017; Qi et al., 2019). Taken together, our results revealed that these *Salmonella* serotypes might be potential risks for salmonellosis outbreaks in the study years because of their identical or similar PFGE profiles, apart from the wide range and long duration of prevalence, even though genomic diversities were found among some of the *Salmonella* serotypes. According to our PFGE results, the potential risk of *S. typhimurium* outbreaks in May, *S. infantis* in July, and *S. rissen* and *S. enteritidis* in March could be higher than that of other *Salmonella* serotypes in Shaanxi province during the sampling time.

In some cases, *S. typhimurium* in retail raw chickens is more prevalent in wet markets than in supermarkets (Thung et al., 2016; Nhung et al., 2018). In the present

study, only 11 of 68 *S. typhimurium* isolates were obtained from retail chickens in supermarkets in May of 2011; the remaining isolates were recovered from wet markets and exhibited 100% genetic similarity. Outdoor sales, consumer contamination, shared chopping boards, butchers' unclean hands, and unclean knives have contributed to a wide prevalence of *S. typhimurium* in wet markets (Chen et al., 2011; Nidaullah et al., 2017).

In conclusion, our results strongly suggest that *Salmonella* contamination in retail raw chickens is common in Shaanxi province, China. Most *Salmonella* isolates of the same serotype exhibited identical or highly similar genetic relationships. MDR isolates were identified, with specific MDR phenotypes closely associated with *Salmonella* serotypes and sampling seasons. Preventive measures such as hazard analysis of critical control points and consumer education on the proper handling of raw poultry during preparation and cooking should be implemented to ensure food safety.

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## SUPPLEMENTARY DATA

Supplementary data associated with this article can be found in the online version at <http://doi.org/10.1016/j.psj.2020.07.038>.

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