International Journal of Food Microbiology 163 (2013) 14–18

Contents lists available at SciVerse ScienceDirect

International Journal of Food Microbiology

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

Short communication

# Prevalence and characterization of *Salmonella* species isolated from pigs, ducks and chickens in Sichuan Province, China

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ARTICLE INFO

*Article history:*
Received 30 August 2012
Received in revised form 7 January 2013
Accepted 27 January 2013
Available online 1 February 2013

*Keywords:*
*Salmonella*
Antimicrobial resistance
β-lactamase gene
Class 1 integron
Pulsed-field gel electrophoresis

ABSTRACT

This study aimed to analyze the prevalence of *Salmonella* isolated from different parts of the food production chain, and to characterize these isolates. A total of 165 *Salmonella enterica* isolates were identified from 1382 samples taken from conventional farms, abattoirs and retail markets from 2010 to 2011 in Sichuan, China. The *Salmonella* isolates were assayed for serotype, antimicrobial susceptibility, prevalence of class 1 integrons and β-lactamase genes, and subtyped using pulsed-field gel electrophoresis. Among these isolates, *S. enterica* serotypes Derby (76 isolates, 46%) and Typhimurium (16 isolates, 10%) were the most prevalent, and high antimicrobial resistance rates were observed for tetracycline (77%), sulfamethoxazole/trimethoprim (43%), nalidixic acid (41%) and spectinomycin (41%). Class 1 integrons were detected in 21% of these isolates, and contained gene cassettes *dfrA12–aadA2*, *dfrA1–aadA1*, *dfrA1*, $bla_{PSE-1}$ and *dfrA1/aadA2*. $bla_{OXA-1}$ was the most commonly identified β-lactamase gene (n = 14), followed by $bla_{TEM-1}$ (n = 6), $bla_{PSE-1}$ (n = 4) and $bla_{CMY-2}$ (n = 1). A *S. enterica* serotype Indiana isolate derived from chicken from a market was positive for both $bla_{OXA-1}$ and $bla_{CMY-2}$, and resistant to nine tested antibiotics. The PFGE patterns were diverse. Our findings indicated that most isolates from different sampling sites were phenotypically and genetically diverse, and *Salmonella* was widespread and may transmit along the food production chain from farm to market. Isolates with decreased susceptibility to fluoroquinolones and extended-spectrum cephalosporins, which are used to fight foodborne *Salmonella*, pose a serious threat to public health.



## 1. Introduction

Salmonellosis, caused by *Salmonella enterica*, is a global foodborne disease of humans and livestock. *Salmonella* remains the major cause of foodborne hospitalizations worldwide (Scallan et al., 2011; Shao et al., 2011). Although there is an absence of official surveillance data for *Salmonella* in China, it is estimated that 22.2% of foodborne diseases are caused by *Salmonella* (Wang et al., 2007). Domestic animals, especially poultry and pigs, are considered to be the major environmental reservoirs of *Salmonella* (Vo et al., 2006). The *Salmonella* serotypes isolated from farms have significant overlap with those causing illness in humans (Alcaine et al., 2006), suggesting that *Salmonella* from animals can be transmitted to humans via food chain.

The issue of antimicrobial resistance in *Salmonella* poses a significant threat to public health. β-lactams and fluoroquinolones are two important classes of antibiotics used to treat complicated cases of salmonellosis (Gonzalez-Sanz et al., 2009). Resistance to third-generation cephalosporins is generally a result of production of extended-spectrum β-lactamases, mostly derived from β-lactamases or AmpC-type enzymes. Various β-lactamase genes have been detected worldwide in various serotypes, located in plasmids or integrons, facilitating rapid transmission among serotypes (Batchelor et al., 2005; Gonzalez-Sanz et al., 2009; Rayamajhi et al., 2008; Yang et al., 2010). Integrons are DNA elements capable of capturing and mobilizing antimicrobial resistance genes among bacteria. The class I integron is the most common integron type identified in multidrug-resistant (MDR) *Salmonella*, and plays an important role in the dissemination of resistance genes among pathogens (Wannaprasat et al., 2011).

With the growth in consumption of food products of animal origin in China, there is an increased potential for exposure to *Salmonella* via the food chain. Unfortunately, most studies on the prevalence and characterization of *Salmonella* have focused on isolates from farms, abattoirs or food products in markets separately, not from the combination of

*Abbreviations:* PFGE, pulsed-field gel electrophoresis; MDR, multidrug-resistant; MIC, minimum inhibitory concentration; GFN, Global Foodborne Infections Network.

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http://dx.doi.org/10.1016/j.ijfoodmicro.2013.01.020

these sites (Visscher et al., 2011; Yan et al., 2010; Yang et al., 2010). There is also very little research on the potential role of the food production chain in the dissemination of MDR *Salmonella* in China.

In this study, we analyzed the prevalence, serotypes, antimicrobial resistance, β-lactamase genes and class 1 integrons of *Salmonella* isolated from farms, abattoirs and markets in Sichuan, China. The objective of this study was to analyze the correlation between the MDR profiles, serotypes, β-lactamase genes, class 1 integrons and PFGE patterns of *Salmonella* isolated from different stages of the food production chain, and identify possible routes of *Salmonella* transmission.

## 2. Materials and methods

### 2.1. Isolation and identification of Salmonella

The sample collection was conducted in farms, abattoirs and markets of pig, chicken and duck from June 2010 to May 2011 in Sichuan Province, Southwest China. The number of farms, abattoirs and markets are described in Table 1, and the number of samples in each farm, abattoir or market was not constant and was proportional to the scale of production. At farms, rectal swabs were collected randomly from an individual animal of different pigpens or coops. At abattoirs, carcasses were sampled by swabbing from rump to cheek along the surface of the carcass. At markets, carcass rinse samples (25 mL buffered peptone water) were collected for chicken and duck, and grounded meat samples (25 g) were collected for pork. All the samples were collected randomly and every sampling site was visited only once. The sampling strategy was defined according to previous researches (Kirchner et al., 2011; Visscher et al., 2011; Yang et al., 2010). A total of 1382 samples were collected. The samples were analyzed for *Salmonella* according to the method as described by Yan et al. (2010) with some modifications. Briefly, 25 g (mL) of samples or swabbing cottons samples were placed into a sterile plastic bag containing 225 mL of buffered peptone water and shaken vigorously. The rinse was incubated at 37 °C in a shaking incubator at 120 rpm for 8 h, and then 1 mL of rinse was added to 10 mL selenite cysteine broth. Broths were incubated at 37 °C for 24 h, followed by streaking of inoculum from the selenite cysteine broth onto CHROMagar *Salmonella* plates (CHROMagar, Paris, France) and incubating at 37 °C for 24 h. Isolates with typical phenotype (mauve colony) were confirmed by PCR using a previously described method (Rahn et al., 1992).

### 2.2. Serotyping

*Salmonella* isolates were serotyped by slide agglutination using commercially available antiserum (Tianrun Bio-Pharmaceutical, Ningbo, China), according to manufacturer's instructions.

### 2.3. Antimicrobial susceptibility testing

A standard broth microdilution method, as described by the Clinical and Laboratory Standards Institute (CLSI, 2009), was carried out to determine the minimum inhibitory concentration (MIC) of the following ten antimicrobial agents: amoxicillin/clavulanic acid, ampicillin, ceftiofur, ciprofloxacin, florfenicol, gentamicin, nalidixic acid, spectinomycin, tetracycline and sulfamethoxazole/trimethoprim. *Escherichia coli* ATCC 25922 was used as the quality control strain. *Salmonella* isolates resistant to more than two classes of antimicrobials were defined as MDR isolates.

### 2.4. Detection of class I integrons and β-lactamase genes

The presence of the class I integron was detected by PCR targeting the class I integrase gene using previously described primers (Kerrn et al., 2002). Gene cassettes within the variable region of class I integron were then amplified as the described method (Sandvang et al., 1998). The PCR products were cloned into the pMD18-T vector using the pMD18-T cloning kit (Takara, Dalian, China), and submitted for sequencing (Invitrogen, Beijing, China).

The genes encoding β-lactamases were analyzed by PCR using primers listed in Table 2. The PCR conditions for the first five pairs of primers were 94 °C for 5 min, 30 cycles of 94 °C for 30 s, 55 °C for 30 s, 72 °C for 50 s, and a final extension at 72 °C for 7 min. β-lactamase gene amplicons were sequenced, and then compared to the sequence database using the BLAST tool (http://www.ncbi.nlm.nih.gov/BLAST/).

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

PFGE was performed using the protocol developed by the Centers for Disease Control and Prevention (Ribot et al., 2006). Briefly, agarose-embedded DNA was digested with 50 U of *Xba*I, using *Salmonella* Braenderup H9812 as a reference strain. The restriction fragments were separated using a CHEF MAPPER XA pulsed field electrophoresis system (Bio-Rad Laboratories, Hercules, CA, USA). 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 and discussion

### 3.1. Salmonella prevalence

A total of 165 (12.0%) *Salmonella* isolates were identified from 1382 samples (Table 1). Of these isolates, 71 (16.4%), 21 (10.7%) and 20 (10.0%) samples were positive for *Salmonella* at pig farms, abattoirs and markets; 30 (28.3%) were positive for *Salmonella* at chicken markets, no *Salmonella* was isolated from samples of chicken farms and abattoirs; and 6 (12.2%), 10 (21.7%) and 7 (26.9%) were positive for *Salmonella* at duck farms, abattoir and markets. The prevalence of *Salmonella* in contaminated food products in other regions of China was 9.4% in raw meat in 2001 (Wang et al., 2004), 13.1% in pork in 2002–2005 (Jiang et al., 2006), 18.1% in raw meat in 2005 (Yan et al., 2010), 36.1% in poultry meat in 2007 (Cui et al., 2009a) and 44.0% in retail meat in 2007–2008 (Yang et al., 2010). The isolation rate in other parts of the world was 12.4% in poultry meat in Spain (Alvarez-Fernandez et al., 2012), 29.3% in poultry meat in Turkey (Arslan and Eyi, 2010), 15.6% in broiler carcasses in EU (EFSA, 2010) and 35.3% in poultry meat in Mexico (Miranda et al., 2009). All those studies showed that *Salmonella* was widely distributed in retail meat globally, which increased salmonellosis

**Table 1**
Prevalence of *Salmonella* in the food production chain.

| Source of samples | Pig | | | Chicken | | | Duck | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|
| | Farm (n=3)[a] | Abattoir (n=3) | Market (n=10) | Farm (n=2) | Abattoir (n=2) | Market (n=5) | Farm (n=1) | Abattoir (n=1) | Market (n=1) | |
| No. of total samples | 433 | 196 | 200 | 199 | 127 | 106 | 49 | 46 | 26 | 1382 |
| No. of positive samples (%) | 71 (16) | 21 (11) | 20 (10) | 0 (0) | 0 (0) | 30 (28) | 6 (12) | 10 (22) | 7 (27) | 165 (12) |

[a] The numbers in parentheses indicate the number of farms, abattoirs and markets, respectively (n = No.).

**Table 2**
Primer sequences for β-lactamase resistance genes used in this study.

| Resistance gene | Forward primer (5′→3′) Reverse primer (5′→3′) | Amplicon (bp) | Source |
|---|---|---|---|
| $bla_{TEM}$ | CAGCGGTAAGATCCTTGAGA TTCATCCATAGTTGCCTGACT | 661 | This study |
| $bla_{PSE}$ | GCTCGTATAGGTGTTTCCGTTT CGATCCGCAATGTTCCATCC | 575 | This study |
| $bla_{CMY-2}$ | ACAGAACAACAGATTGCCGATA TGTCGCTGCCGTTGATGA | 856 | This study |
| $bla_{SHV}$ | TTCGCCTGTGTATTATCTCC TTTGCTGATTTCGCTCGG | 807 | This study |
| $bla_{DHA-1}$ | GCCGTCTCCGTAAAGGGTAAGC GCCAGAATCACAATCGCCACCT | 926 | This study |
| $bla_{OXA}$ | ACCAGATTCAACTTTCAA TCTTGGCTTTTATGCTTG | 590 | Guerra et al. (2001) |
| $bla_{CTX-M}$ universal | CGATGTGCAGTACCAGTAA AGTGACCAGAATCAGCGG | 585 | Batchelor et al. (2005) |

following consumption of contaminated meat or cross-contamination in household. Data on the prevalence of *Salmonella* in different studies were difficult to compare because the observed prevalence may be biased by diversity in sampling methods, sampling seasons and technique. According to previous studies (Cui et al., 2009a; Yan et al., 2010; Yang et al., 2010), prevalence of *Salmonella* in China was widespread. In this study, the prevalence of *Salmonella* in chicken (28.3%) from markets was higher than that from abattoirs, indicating that poor hygiene can result in wide-spreading of *Salmonella* in retail meat, the hygiene of markets was very important.

### 3.2. Serotyping and antimicrobial susceptibility testing

Fourteen serotypes were identified among 129 *Salmonella* isolates, and 36 isolates were untypeable (Table 3). The top seven serotypes were Derby (n = 76), Typhimurium (n = 16), Potsdam (n = 9), Give (n = 6), Tshiongwe (n = 4), Kottbus (n = 4) and Saintpaul (n = 3). The most commonly isolated serotype from different origins was Derby, which was the most common serotype isolated from infants and toddlers in China (Cui et al., 2009b). This suggests an association between *Salmonella*-contaminated food and salmonellosis. According to the Global Foodborne Infections Network (GFN) Country Databank of the World Health Organisation (http://www.who.int/gfn/activities/en/), *Salmonella* Derby was the main serotype identified in foodstuffs in China, which differed from findings in other countries, e.g. *Salmonella* Typhimurium in USA and *Salmonella* Infantis in Japan. According to the GFN and previous reports (Wang et al., 2004), *Salmonella* Derby was the predominant serotype isolated from foods of animal origin in China, while the main serotype isolated from humans was *Salmonella* Typhimurium (Deng et al., 2012). The difference in dominant serotypes between food producing animals and humans may be due to differences in pathogenicity of the two serotypes and their respective resistance profiles (Volf et al., 2010).

Antimicrobial susceptibility testing of 165 *S. enterica* strains to ten antimicrobials was also performed (Table 4). High resistance rates were observed against tetracycline (MIC ≥ 16 μg/mL, 77%), sulfamethoxazole/trimethoprim (MIC ≥ 76/4 μg/mL, 43%), nalidixic acid (MIC ≥ 32 μg/mL, 41%), spectinomycin (MIC ≥ 64 μg/mL, 41%) and ampicillin (MIC ≥ 32 μg/mL, 25%). The percentages of resistance to gentamicin (MIC ≥ 16 μg/mL, 15%), amoxicillin/clavulanic acid (MIC ≥ 32/16 μg/mL, 14%), ciprofloxacin (MIC ≥ 4 μg/mL, 12%) and florfenicol (MIC ≥ 16 μg/mL, 10%) were low, and most isolates were susceptible to ceftiofur (MIC ≥ 8 μg/mL, 98%). Decreased susceptibility to both ciprofloxacin (MIC > 0.12 μg/mL) and ceftiofur (MIC > 2 μg/mL) was detected in four isolates from chickens (*Salmonella* Indiana and *Salmonella* Give) and ducks (*Salmonella* Indiana and untyped), and two *Salmonella* Indiana isolates showed resistance to nine antimicrobials. Co-resistance to fluoroquinolones and extended-spectrum cephalosporins in these isolates would limit therapeutic options for *Salmonella* infections (Whichard et al., 2007).

When resistance profiles and serovars were compared (Table 5), there appeared to be an association between antimicrobial resistance and particular serotypes. *Salmonella* Typhimurium and *Salmonella* Indiana were more likely to be resistant to seven antimicrobials or more, but other serotypes showed diverse resistance profiles. This indicated that the spread of MDR isolates of *Salmonella* Typhimurium and *Salmonella* Indiana is potentially serious in China, which is in agreement with previously described findings (Yang et al., 2010).

**Table 3**
Distribution of *Salmonella* serotypes of isolates from food production chain.

| Serotypes | No. of isolates | Source of isolates[a] | | |
|---|---|---|---|---|
| | | Pig | Chicken | Duck |
| Derby | 76 | 45F, 13A, 7M | 8M | 2F, 1M |
| Typhimurium | 16 | 12F, 2A | 2M | 0 |
| Potsdam | 9 | 4F, 3M | 1M | 1A |
| Give | 6 | 2A, 3M | 1M | 0 |
| Tshiongwe | 4 | 1F, 1A | 1M | 1F |
| Kottbus | 4 | 0 | 4M | 0 |
| Saintpaul | 3 | 2M | 1M | 0 |
| Agona | 2 | 1F | 1M | 0 |
| Okefoko | 2 | 2F | 0 | 0 |
| Indiana | 2 | 0 | 1M | 1A |
| Choleraesuis | 2 | 2M | 0 | 0 |
| Bury | 1 | 0 | 0 | 1M |
| Braenderup | 1 | 1F | 0 | 0 |
| Anatum | 1 | 1M | 0 | 0 |
| Untyped | 36 | 5F, 3A, 2M | 10M | 3F, 8A, 5M |

[a] F, A and M stand for the strains isolated from farm, abattoir and market, respectively.

### 3.3. Prevalence of class 1 integrons and β-lactamase genes

Class 1 integrons were detected in 34 *Salmonella* isolates, nine of which were negative for the resistance gene cassette. The remaining 25 *IntI1*-positive *Salmonella* isolates contained five groups of resistance gene cassettes, consisting of *dfrA*12–*aadA*2 (2 k, n = 12), *dfrA*1–*aadA*1 (1.7 k, n = 1), *dfrA*1 (1.2 k, n = 4), $bla_{PSE-1}$ (1.2 k, n = 3) and *dfrA*1/*aadA*2 (1.2 k/1 k, n = 5). Four β-lactamase genes were detected among the isolates. $bla_{OXA-1}$ was the most commonly isolated β-lactamase gene (n = 14), followed by $bla_{TEM-1}$ (n = 6), $bla_{PSE-1}$(n = 4) and $bla_{CMY-2}$(n = 1).

All *IntI1*-positive isolates showed resistance to two or more classes of antibiotics. This high frequency of MDR among *IntI1*-positive isolates supports the hypothesis of an association between the presence of class 1 integrons and emerging MDR in *Salmonella* (Firoozeh et al., 2012; O'Mahony et al., 2006; Wannaprasat et al., 2011). The two main *IntI1*-positive serotypes were Typhimurium and Derby. All isolates containing a β-lactamase gene were resistant to ampicillin.

Although $bla_{CMY-2}$-producing *Salmonella* have been found in many countries (Li et al., 2007), the incidence of $bla_{CMY-2}$-positive *Salmonella* in China was thought to be very low, with positive isolates previously only reported in Shandong and Shanxi Provinces (Yang et al., 2010; Zhang et al., 2008). In the current survey, a MDR *Salmonella* Indiana isolate harboring both $bla_{OXA-1}$ and $bla_{CMY-2}$ exhibited resistance to all tested antimicrobials, except ciprofloxacin. To the best of our knowledge, this is the first report of $bla_{CMY-2}$-positive *Salmonella* in Sichuan, China. As one type of AmpC β-lactamase gene, $bla_{CMY-2}$ encodes resistance to third-generation cephalosporins, which is an important class of antibiotics used to treat complicated cases of salmonellosis (Gonzalez-Sanz et al., 2009). The spread of $bla_{CMY-2}$-harboring *Salmonella* through food, especially animal-derived food, has important public health implications.

**Table 4**
Antimicrobial resistance prevalence (%) of *Salmonella* isolated from the food production chain.

| Antibiotics[a] | All sources (165) | Pig | | | Chicken | Duck | | |
|---|---|---|---|---|---|---|---|---|
| | | Farm (71) | Abattoir (21) | Market (20) | Market (30) | Farm (6) | Abattoir (10) | Market (7) |
| AMC | 14 | 21 | 10 | 0 | 13 | 0 | 0 | 29 |
| AMP | 26 | 25 | 33 | 10 | 23 | 33 | 20 | 57 |
| CEF | 2 | 0 | 0 | 0 | 3 | 0 | 10 | 14 |
| CIP | 12 | 20 | 5 | 0 | 0 | 17 | 10 | 29 |
| FFC | 10 | 9 | 14 | 5 | 10 | 17 | 10 | 29 |
| GEN | 15 | 23 | 10 | 5 | 3 | 17 | 10 | 29 |
| NAL | 41 | 35 | 29 | 35 | 47 | 67 | 70 | 71 |
| SPE | 41 | 34 | 33 | 50 | 57 | 33 | 30 | 57 |
| SXT | 43 | 34 | 38 | 40 | 47 | 83 | 70 | 71 |
| TET | 77 | 89 | 95 | 85 | 50 | 83 | 20 | 71 |
| Percentage of MDR | 55 | 47 | 48 | 55 | 67 | 83 | 70 | 71 |

[a] AMC, amoxicillin/clavulanic acid; AMP, ampicillin; CEF, ceftiofur; CIP, ciprofloxacin; FFC, florfenicol; GEN, gentamicin; NAL, nalidixic acid; SPE, spectinomycin; SXT, sulfamethoxazole/trimethoprim; TET, tetracycline.

**Table 5**
Multidrug resistance (MDR) observed among *Salmonella* serotypes recovered from food production chain.

| Serotypes (no. of isolates) | % Resistant to indicated number of antimicrobials | | | | |
|---|---|---|---|---|---|
| | 1–3 | 4–6 | 7–8 | ≥9 | Total resistance (≥1) |
| Derby (n=76) | 73 | 14 | 3 | 0 | 90 |
| Typhimurium (n=16) | 19 | 0 | 69 | 12 | 100 |
| Potsdam (n=9) | 67 | 11 | 11 | 0 | 89 |
| Give (n=6) | 33 | 0 | 33 | 0 | 66 |
| Tshiongwe (n=4) | 25 | 75 | 0 | 0 | 100 |
| Kottbus (n=4) | 50 | 0 | 0 | 0 | 50 |
| Saintpaul (n=3) | 33 | 0 | 33 | 0 | 66 |
| Agona (n=2) | 50 | 0 | 0 | 0 | 50 |
| Okefoko (n=2) | 100 | 0 | 0 | 0 | 100 |
| Indiana (n=2) | 0 | 0 | 0 | 100 | 100 |
| Choleraesuis (n=2) | 100 | 0 | 0 | 0 | 100 |
| Other serovars (n=39) | 46 | 10 | 21 | 10 | 87 |

### 3.4. PFGE

All 165 isolates in this study were subjected to PFGE analysis. The resultant dendrogram of isolate patterns exhibited clusters with a high level of diversity; only the PFGE patterns of a small number of isolates from different parts in the food chain were genetically related (data not shown). Several reports have shown that clonal groups could be transmitted along the food chain and represent a hazard to human health (Hauser et al., 2011, 2012; Nogrady et al., 2008). The current study indicated that the phenomenon of *Salmonella* transmission along the food chain does exist in Sichuan Province. The diversity of PFGE patterns in this study may be due to either of the two reasons: first, different clones of *Salmonella* are distributed across different parts of the food chain in Sichuan Province; and second, that the samples collected were not correlative along the food chain.

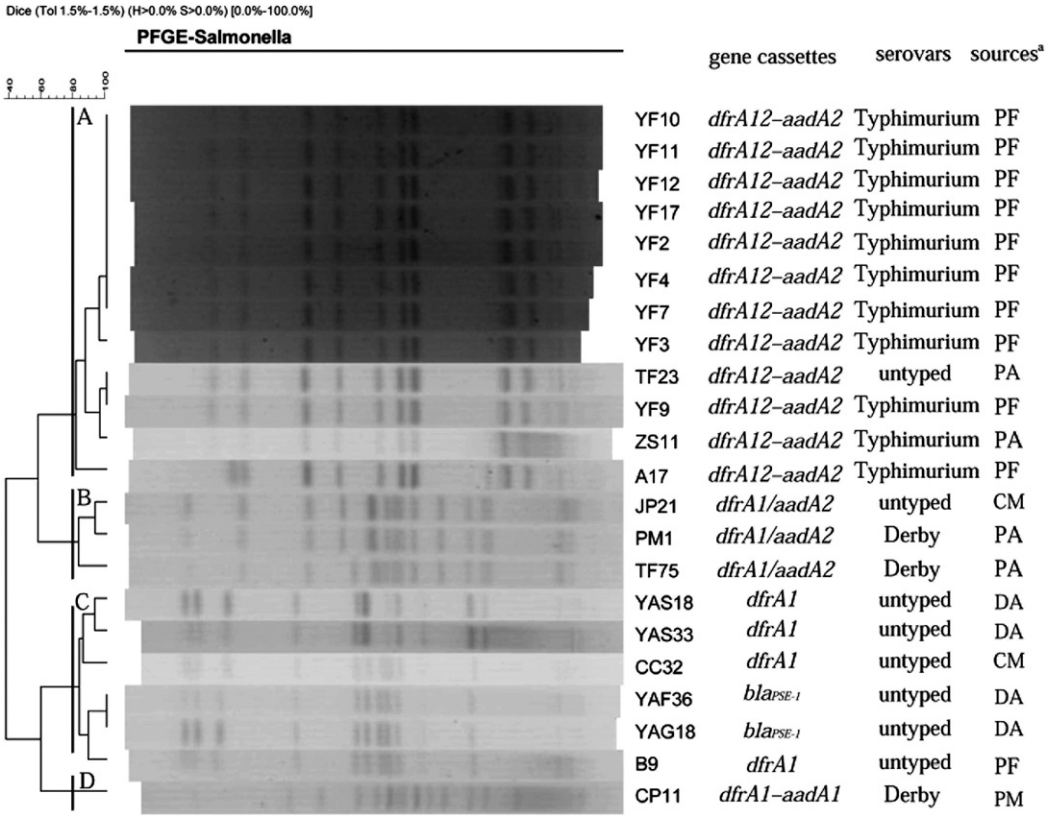


**Fig. 1.** Dendrogram of PFGE patterns of 22 *Salmonella* isolates containing gene cassettes. [a]P, C and D stand for the strains isolated from pig, chicken and duck, respectively. F, A and M stand for the strains isolated from farm, abattoir and market, respectively.

Four clusters of PFGE patterns for 22 *Salmonella* isolates (the other three isolates were untypeable) containing gene cassettes are presented in Fig. 1. Clusters A, B and D corresponded to *dfrA*12–*aadA*2, *dfrA*1/*aadA*2 and *dfrA*1–*aadA*1, respectively. Cluster C included two types of cassettes, *dfrA*1 and $bla_{PSE-1}$. Isolate TF23 was isolated from a pig abattoir and belonged to cluster A, which mainly contained isolates from a pig farm. Isolate JP21 was isolated from a chicken market and belonged to cluster B, others of which were isolated from a pig abattoir. The PFGE results demonstrated that *Salmonella* can spread along the food production chain.

In conclusion, food products of animal origin in markets in Sichuan Province, China, are contaminated with MDR *Salmonella*, which may originate from farms further up the food chain, or from horizontal contamination. Keeping the food chain free of *Salmonella* is vital for preventing food infection caused by *Salmonella*, and the hygiene of retail meat is critical. The high rates of MDR *Salmonella* detected, *IntI1* and *bla*-positive isolates suggest that measures to facilitate the reasonable use of antimicrobials in animal husbandry must be taken. Moreover, more research into the characteristics of dissemination of *Salmonella* and antibiotic resistance genes is warranted.

## Acknowledgments

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

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