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Original Article

Prevalence, Antimicrobial Resistance, and Relatedness of *Salmonella* Isolated from Chickens and Pigs on Farms, Abattoirs, and Markets in Sichuan Province, China

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Abstract

This study aims at investigating the distribution, antimicrobial resistance, and genetic relationship of *Salmonella* isolated from 18 farms, their downstream abattoirs, and markets of chickens and pigs in Sichuan province, China. A total of 193 *Salmonella* isolates were identified from 693 samples with an isolation rate of 26.27% (88/335) in chickens and 29.33% (105/358) in pigs. *Salmonella* was isolated more frequently in abattoirs and markets than from farms. Serotypes were determined according to the White-Kauffmann-Le Minor scheme and 16 different serotypes were identified, with Derby being the most common, followed by Typhimurium and Meleagridis. Antimicrobial resistance phenotypes and genotypes were studied by using the disk diffusion method and polymerase chain reaction (PCR) amplification, respectively. Overall, 44.04% ($n=85$) of all isolates were multidrug resistant (MDR) and resistance to nalidixic acid (51.30%) was the most frequently observed. $bla_{\text{CTX-M-55}}$ was the most prevalent extended-spectrum β-lactamases gene, and polymyxin resistance gene *mcr-1* was present in strains with various serotypes. Multilocus sequence typing indicated that sequence type (ST) had a close relationship with serotype, and 34.20% of all strains were ST40, which was the most prevalent. The unweighted pair group method with arithmetic means (UPGMA) dendrogram of pulsed-field gel electrophoresis showed that *Salmonella* isolates belonging to the same serovar from different parts of the production chain were highly genetic related, indicating that *Salmonella* as well as resistance genes could potentially be transmitted from farms to markets. Our study highlights the fact that *Salmonella* isolates from chicken and pig production chain were frequently exhibiting MDR profiles, and the dissemination of MDR *Salmonella* from farm to market could pose significant threats to food safety and public health.

Keywords: *Salmonella*, serotype, antimicrobial resistance, MLST, PFGE

Introduction

Salmonella is an important foodborne pathogen that can cause severe infections in humans and animals throughout the world (Zhang *et al.*, 2016). It is estimated that ~1.2 million illnesses and 450 deaths annually in the United States (Scallan *et al.*, 2011) and 75% (30 million) of foodborne diseases in China are due to *Salmonella* infections (Wu *et al.*, 2013). The majority of clinical cases of salmonellosis are acquired by eating *Salmonella*-contaminated foods from animals (Kuang *et al.*, 2015). Therefore, food-producing animals, especially chickens and pigs, are recognized as the primary sources of *Salmonella* (Vo *et al.*, 2006; Zhang *et al.*, 2016). In China, previous studies show that *Salmonella* contamination varies from 10% to 40% in chickens and from 10% to 70% in pigs (Li *et al.*, 2013, 2016; Cai *et al.*, 2016; Meng *et al.*, 2016; Zhang *et al.*, 2016).

Although salmonellosis is always self-limiting, antimicrobial therapy is necessary in severe cases (Kim *et al.*, 2016). However, the use of antibiotics has led to selection of bacteria exhibiting antimicrobial resistance, especially multidrug resistance that is resistant to at least three antibiotic

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classes (Magiorakos *et al.*, 2011). This presents a new and significant threat to the public. Moreover, the infection by multidrug resistant (MDR) *Salmonella* can increase instances of morbidity and mortality (Bai *et al.*, 2016). Extended-spectrum β-lactams and fluoroquinolones are frequently used to treat *Salmonella* infections (Harrois *et al.*, 2014), so the resistance to these drugs has increased dramatically (Zhang *et al.*, 2016). The main contributor to extended-spectrum β-lactams resistance is $bla_{\text{CTX-M}}$, which is always located on transferable plasmids (Bonnet, 2004; Cantón and Coque, 2006). The major mechanisms mediating resistance to fluoroquinolones are the mutations of quinolone resistance-determining region (QRDR) and the presence of plasmid-mediated quinolone resistance (PMQR) genes (particularly *qnr* genes), which could provide an advantage of selection and facilitate the mutation of fluoroquinolone resistance genes (Hernández *et al.*, 2011). In addition, plasmid-mediated colistin resistance gene *mcr-1* has been reported in *Escherichia coli* (Liu *et al.*, 2016), as well as in *Salmonella* (Yang *et al.*, 2016).

The contamination and antimicrobial resistance of *Salmonella* isolated from food-producing animals has been particularly severe in China (Li *et al.*, 2013; Kuang *et al.*, 2015; Cai *et al.*, 2016; Meng *et al.*, 2016; Zhang *et al.*, 2016). Further, several studies have reported that *Salmonella* isolates recovered from pigs might be disseminated along the production chain from slaughterhouses to retail markets (Cai *et al.*, 2016; Li *et al.*, 2016). However, there are very few studies focusing on Sichuan province, which was one of the biggest producers of food-producing animals. Therefore, the intention of this study is to investigate the contamination, antimicrobial resistance, and genetic relationship of all *Salmonella* isolates recovered from chickens and pigs on farms, abattoirs, and markets located in Sichuan.

Materials and Methods

Isolation of Salmonella *and serotyping*

A total of 693 samples were collected from three cities in Sichuan between March 2015 and February 2016. For the purpose of this study, 18 sites were visited in each city, including 3 pig herds, 3 chicken farms, 6 related downstream abattoirs (3 for chickens and 3 for pigs), and 6 downstream markets (three for chicken and three for pork). Numeric details of the samples collected are illustrated in Table 1. The process of the selection of farms was based on their scale: Breeding stock of chickens and pigs consisted of more than 100,000 and 1000 heads, respectively. At farms, rectal swabs were randomly collected. At the downstream abattoirs, cotton swabs that had been premoistened with buffered peptone water (BPW; Luqiao, Beijing, China) were collected by swabbing the surface of carcasses from rump to cheek. There were no living animals sold in the sampled markets, although this was a traditional feature in China, and meat samples (pork and chicken) were purchased at markets. *Salmonella* isolation was carried out as previously described (Cai *et al.*, 2016). In brief, samples were cultured overnight in BPW, then inoculated into Rappaport-Vassiliadis (Luqiao) for 24 h. They were ultimately streaked onto xylose lysine tergitol 4 (XLT4; Luqiao) agar plates. Among suspected colonies, only one was picked up from a plate and confirmed by BD Diagnostic systems (Sparks, MD). Serotypes were conducted by slide agglutination according to the White-Kauffmann-Le Minor scheme (Issenhuth-Jeanjean *et al.*, 2014) (Tianrun Bio-Pharmaceutical, Ningbo, China).

Antimicrobial susceptibility testing

Antimicrobial susceptibilities of *Salmonella* isolates to 18 antibiotics were tested by the Kirby-Bauer disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) recommendations (CLSI, 2013). Antimicrobials were selected as follows: amikacin (AMK, 30 μg), amoxicillin-clavulanic acid (AMC, 30 μg), ampicillin (AMP, 10 μg), ampicillin-sulbactam 1:1 (SAM, 20 μg), aztreonam (ATM, 30 μg), cefotaxime (CTX, 30 μg), cefoxitin (FOX, 30 μg), ceftazidime (CAZ, 30 μg), ceftriaxone (CRO, 30 μg), ciprofloxacin (CIP, 5 μg), doxycycline (DOX, 30 μg), florfenicol (FFC, 30 μg), gentamicin (GEN, 10 μg), imipenem (IPM, 10 μg), nalidixic acid (NAL, 30 μg), norfloxacin (NOR, 10 μg), polymyxin B (PB, 300 U), and sulfamethoxazole-trimethoprim19:1 (SXT, 25 μg). *E. coli* ATCC 25922 was used as a quantity control strain.

Investigation of antimicrobial resistance genes

Antimicrobial resistance genes, including extended-spectrum β-lactamases (ESBLs) genes (bla_{TEM}, bla_{SHV}, $bla_{\text{CTX-M}}$), AmpC-encoding genes (bla_{MOX}, bla_{CMY}, bla_{DHA}, bla_{ACC}, bla_{ATC}, bla_{FOX}), sulfonamides resistance genes (*sul1, sul2, sul3*), tetracycline resistance genes (*tetA, tetC, tetM*), 16S rRNA methylase genes (*armA*, *rmtB*), the florfenicol resistance gene *floR,* the polymyxin resistance gene *mcr-1*, and carbapenemases genes (bla_{NDM}, bla_{KPC}, bla_{VIM}), were screened by PCR with primers listed in Supplementary Table S1 (Supplementary Data are available online at www.liebertpub.com/fpd). Nalidixic acid-resistant isolates were further screened for mutations of QRDR genes (*gyrA, gyrB, parC*, *and parE*) and the presence of PMQR

Table 1. Contamination Frequency of Chickens and Pigs by *Salmonella* on Farms, Abattoirs, and Markets

	Chicken				*Pig*				
City	*Farm*	*Abattoir*	*Market*	*Total*	*Farm*	*Abattoir*	*Market*	*Total*	*Total*
Chengdu	8[a]/37[b]	13/37	14/38	35/112	8/37	15/45	11/37	34/119	69/231
Mianyang	7/34	8/35	10/39	25/108	8/37	15/43	12/39	35/119	60/227
Guangyuan	9/38	7/38	12/39	28/115	11/38	10/40	15/42	36/120	64/235
Total	24/109	28/110	36/116	88/335	27/112	40/128	38/118	105/358	193/693

[a]Number of samples positive for *Salmonella*.
[b]Total number of samples.

genes (*qnrA-D, qnrS, aac(6′)-Ib-cr, qepA, oqxAB*). The PCR products were sequenced by the Tsingke Biological Technology Company (Chengdu, China).

Multilocus sequence typing

Multilocus sequence typing (MLST) was carried out with seven housekeeping genes. This was accomplished with the application of the primers and conditions stipulated on the following site: http://mlst.warwick.ac.uk/mlst/dbs/Senterica/documents/primersEnterica_html. The PCR products were sequenced, and the sequence data were submitted to: http://mlst.warwick.ac.uk/mlst/dbs/Senterica to define sequence type (ST).

Pulsed-field gel electrophoresis

Pulsed-field gel electrophoresis (PFGE) was performed by following the standards formulated by the Centers for Disease Control and Prevention (Ribot *et al.*, 2006) with some modifications. Genomic DNA was digested by 50 U *Xba*I (Takara, Dalian, China) for 3 h, and the electrophoresis run time was 18.5 h with an initial switch time of 2.2 s and a final switch time of 63.8 s. *Salmonella* Braenderup H9812 was used as a reference strain. A dendrogram was developed with 1.5% tolerances by BioNumerics software (Applied Maths) by employing the unweighted pair group method with arithmetic means (UPGMA).

Results

Isolation of Salmonella *and serotyping*

A total of 193 (27.85%) *Salmonella* strains were isolated from 693 samples, with 88 (45.60%) from chickens and 105 (55.40%) from pigs (Table 1). All isolates belonged to 16 distinct serotypes, except 3 untypable isolates (Table 2). Derby, Typhimurium, and Meleagridis were isolated most frequently. These three serovars and Mbandaka were shared by all sources and origins. By contrast, Give, Indiana, Worthington, Riseen, and Goldcoast were only found in the samples of pigs and Anatum was only detected in chicken samples.

Antimicrobial susceptibility testing

Among 193 *Salmonella* isolates, more than half (51.30%) were resistant to nalidixic acid whereas the rates of resistance to ciprofloxacin (9.84%) and norfloxacin (8.29%) were much lower. Resistance to florfenicol (38.34%), ampicillin (36.27%), doxycycline (34.72%), and sulfamethoxazole-trimethoprim (33.68%) were frequently observed but no isolate showed resistance to imipenem (Table 3).

Based on the results of antimicrobial susceptibility testing, seven isolates belonging to different serotypes (two Albany, one Newport, one Stanley, one Indiana, one Thompson, and one Mbandaka) were resistant to ciprofloxacin and ceftriaxone concomitantly. Overall, 124 (64.25%) strains were resistant to at least one antibiotic and 85 (44.04%) isolates were MDR (Fig. 1) but displayed different resistance phenotypes (Supplementary Table S2).

Prevalence of antimicrobial resistance genes

For most of the isolated strains, there was a close relationship between antibiotic resistance phenotypes and genotypes. Among 28 aminoglycoside-resistant isolates, one carried *armA* and the other 27 strains harbored *rmtB* (Table 4).

Table 2. Serovars and Sequence Type Distribution of *Salmonella* Isolated from Chickens and Pigs on Farms, Abattoirs, and Markets

		Chicken				*Pig*				
Serovar	*ST*	*Farm*	*Abattoir*	*Market*	*Total*	*Farm*	*Abattoir*	*Market*	*Total*	*Total*
Derby	40	6[a] (25.00[b])	11 (39.29)	13 (36.11)	30 (34.09)	9 (33.33)	11 (27.50)	16 (42.11)	36 (34.29)	66 (34.20)
Typhimurium	19	1 (4.17)	0	0	1 (1.14)	0	0	0	0	1 (0.52)
	34	1 (4.17)	1 (3.57)	7 (19.44)	9 (10.23)	2 (7.41)	6 (15.00)	1 (2.63)	9 (8.57)	18 (9.33)
	36	0	0	1 (2.78)	1 (1.14)	0	0	0	0	1 (0.52)
	3021	1 (4.17)	0	1 (2.78)	2 (2.27)	0	0	0	0	2 (1.04)
Meleagridis	463	6 (25.00)	1 (3.57)	4 (11.11)	11 (12.50)	2 (7.41)	2 (5.00)	7 (18.42)	11 (10.48)	22 (11.40)
Albany	292	0	5 (17.86)	3 (8.33)	8 (9.09)	2 (7.41)	6 (15.00)	3 (7.89)	11 (10.48)	19 (9.84)
Mbandaka	413	9 (37.50)	1 (3.57)	2 (5.56)	12 (13.64)	3 (11.11)	1 (2.50)	3 (7.89)	7 (6.67)	19 (9.84)
Newport	31	0	1 (3.57)	2 (5.56)	3 (3.41)	1 (3.70)	4 (10.00)	3 (7.89)	8 (7.62)	11 (5.70)
Infantis	32	0	5 (17.86)	1 (2.78)	6 (6.82)	0	2 (5.00)	0	2 (1.90)	8 (4.15)
Give	516	0	0	0	0	0	7 (17.50)	0	7 (6.67)	7 (3.63)
Schwarzengrund	96	0	1 (3.57)	0	1 (1.14)	5 (18.52)	0	0	5 (4.76)	6 (3.11)
Stanley	29	0	1 (3.57)	1 (2.78)	2 (2.27)	1 (3.70)	0	0	1 (0.95)	3 (1.55)
Worthington	592	0	0	0	0	0	0	2 (5.26)	2 (1.90)	2 (1.04)
Anatum	2441	0	1 (3.57)	0	1 (1.14)	0	0	0	0	1 (0.52)
Indiana	17	0	0	0	0	1 (3.70)	0	0	1 (0.95)	1 (0.52)
Rissen	469	0	0	0	0	0	0	1 (2.63)	1 (0.95)	1 (0.52)
Goldcoast	358	0	0	0	0	1 (3.70)	0	0	1 (0.95)	1 (0.52)
Thompson	26	0	0	0	0	0	1 (2.50)	0	1 (0.95)	1 (0.52)
Untyped	2709	0	0	1 (2.78)	1 (1.14)	0	0	2 (5.26)	2 (1.90)	3 (1.55)
Total		24	28	36	88	27	40	38	105	193

[a]Number of *Salmonella* isolates.
[b]Percentage of this serovar, unit was: %.
ST, sequence type.

Table 3. Antibiotic Resistance of *Salmonella* Isolated from Chickens and Pigs on Farms, Abattoirs, and Markets

	Chicken				*Pig*				
Antibiotic	*Farm*	*Abattoir*	*Market*	*Total*	*Farm*	*Abattoir*	*Market*	*Total*	*Total*
AMK	2[a] (8.33[b])	4 (14.29)	2 (5.56)	8 (9.09)	6 (22.22)	4 (10.00)	7 (18.42)	17 (16.19)	25 (12.95)
GEN	3 (12.50)	5 (17.86)	6 (16.67)	14 (15.91)	5 (18.52)	6 (15.00)	3 (7.89)	14 (13.33)	28 (14.51)
DOX	6 (25.00)	11 (39.29)	10 (27.78)	27 (30.68)	15 (55.56)	13 (32.50)	12 (31.58)	40 (38.10)	67 (34.72)
FFC	5 (20.83)	12 (42.86)	12 (33.33)	29 (32.95)	14 (51.85)	18 (45.00)	13 (34.21)	45 (42.86)	74 (38.34)
PB	2 (8.33)	4 (14.29)	5 (13.89)	11 (12.50)	5 (18.52)	1 (2.50)	5 (13.16)	11 (10.48)	22 (11.40)
SXT	1 (4.17)	8 (28.57)	14 (38.89)	23 (26.14)	11 (40.74)	20 (50.00)	11 (28.95)	42 (40.00)	65 (33.68)
AMP	5 (20.83)	10 (35.71)	14 (38.89)	29 (32.95)	13 (48.15)	13 (32.50)	15 (39.47)	41 (39.05)	70 (36.27)
AMC	1 (4.17)	2 (7.14)	1 (2.78)	4 (4.55)	3 (11.11)	4 (10.00)	3 (7.89)	10 (9.52)	14 (7.25)
SAM	1 (4.17)	7 (25.00)	4 (11.11)	12 (13.64)	5 (18.52)	4 (10.00)	7 (18.42)	16 (15.24)	28 (14.51)
CTX	4 (16.67)	7 (25.00)	6 (16.67)	17 (19.32)	8 (29.63)	4 (10.00)	8 (21.05)	20 (19.05)	37 (19.17)
CRO	4 (16.67)	6 (21.43)	6 (16.67)	16 (18.18)	8 (29.63)	4 (10.00)	8 (21.05)	20 (19.05)	36 (18.65)
CAZ	2 (8.33)	7 (25.00)	6 (16.67)	15 (17.05)	6 (22.22)	4 (10.00)	8 (21.05)	18 (17.14)	33 (17.10)
FOX	0	1 (3.57)	0	1 (1.14)	0	1 (2.50)	0	1 (0.95)	2 (1.04)
ATM	1 (4.17)	5 (17.86)	5 (13.89)	11 (12.50)	9 (33.33)	3 (7.50)	7 (18.42)	19 (18.10)	30 (15.54)
NAL	9 (37.50)	16 (57.14)	19 (52.78)	44 (50.00)	14 (51.85)	25 (62.50)	16 (42.11)	55 (52.38)	99 (51.30)
CIP	2 (8.33)	3 (10.71)	5 (13.89)	10 (11.36)	4 (14.81)	4 (10.00)	1 (2.63)	9 (8.57)	19 (9.84)
NOR	1 (4.17)	3 (10.71)	4 (11.11)	8 (9.09)	3 (11.11)	5 (12.50)	0	8 (7.62)	16 (8.29)
IPM	0	0	0	0	0	0	0	0	0

[a]Number of *Salmonella* isolates.
[b]Percentage of resistance isolates, unit was: %.
AMC, amoxicillin-clavulanic acid; AMK, amikacin; AMP, ampicillin; ATM, aztreonam; CAZ, ceftazidime; CIP, ciprofloxacin; CRO, ceftriaxone; CTX, cefotaxime; DOX, doxycycline; FFC, florfenicol; FOX, cefoxitin; GEN, gentamicin; IPM, imipenem.; NAL, nalidixic acid; NOR, norfloxacin; PB, polymyxin B; SAM, ampicillin-sulbactam 1:1; SXT, sulfamethoxazole-trimethoprim 19:1.

$bla_{\text{TEM-1}}$ was present in 57 out of 70 ampicillin-resistant isolates and $bla_{\text{CTX-M}}$ was found in 36 third-generation cephalosporin-resistant isolates with $bla_{\text{CTX-M-55}}$ ($n=18$), $bla_{\text{CTX-M-164}}$ ($n=7$), $bla_{\text{CTX-M-79}}$ ($n=5$), $bla_{\text{CTX-M-15}}$ ($n=4$), $bla_{\text{CTX-M-65}}$ ($n=1$), and $bla_{\text{CTX-M-14}}$ ($n=1$). $bla_{\text{CMY-2}}$ was detected in two amoxicillin-clavulanic acid-resistant isolates, one of which coexisted with $bla_{\text{CTX-M-55}}$ (Supplementary Table S2). *mcr-1* was present in 22 polymyxin B-resistant isolates, and 20 were concurrent with ESBLs genes. Sixty-two out of 67 doxycycline-resistant isolates carried tetracycline resistance genes, of which 49 and 1 were detected in *tetA* and *tetM*, respectively, and 12 carried *tetA* and *tetM* simultaneously. Among 74 strains carrying sulfonamides resistance gene, *sul1* was the most common (43.24%). All florfenicol-resistant strains ($n=74$) were positive for *floR*.

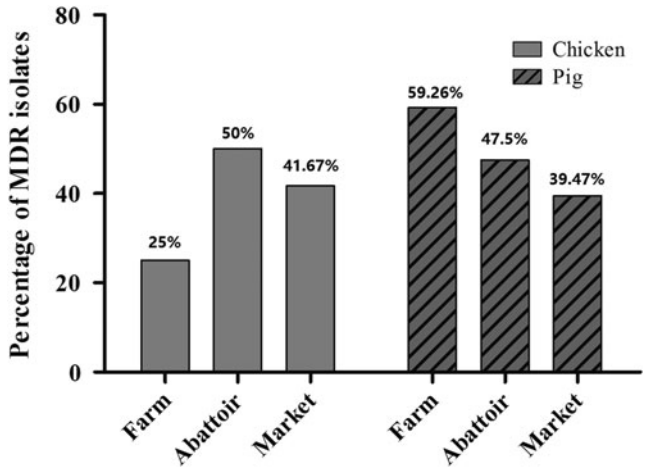


FIG. 1. Proportions of multidrug-resistant (MDR) *Salmonella* isolated from chickens and pigs on farms, abattoirs, and markets.

Among the 99 isolates that were resistant to nalidixic acid, 95 (95.96%) carried PMQR genes, of which *aac(6′)-Ib-cr* (54.74%) and *qnrS1* (44.21%) were the most prevalent. But all tested strains were negative for *qnrA*, *qnrC*, and *qnrD*. The mutations of QRDR were found in 22 strains, of which a single mutation was found in 17 isolates (S83F, $n=9$; D87N, $n=6$; D87N, $n=2$) and double mutations were found in 5 isolates (S83F and D87N, $n=3$; S83L and D87N, $n=2$). In contrast, mutations in *gyrB*, *parC*, and *parE* were not detected.

Multilocus sequence typing

We found 20 different ST (Table 2), of which 14 ST were from chickens and 16 ST were from pigs. Sixty-six isolates were ST40 as the most prevalent, with 30 and 36 from chickens and pigs, respectively. ST463, ST292, ST413, and ST34 were also widespread. ST34, ST40, ST413, and ST463 were shared by all origins and sources.

Pulsed-field gel electrophoresis

According to the similarity of 80%, 193 *Salmonella* isolates could be grouped into 39 clusters (Fig. 2A–C). Some *Salmonella* isolates recovered from different stages of production chain could generate similar PFGE fingerprint profiles and belong to same serovar. For example, 21 out of 22 Typhimurium isolates occupied the same cluster and 22 *Salmonella* Meleagridis strains belonged to a single cluster. However, the antibiotic susceptibility of the same serotype in

Table 4. Number of Isolates Harboring Antibiotic Resistance Genes Among *Salmonella* Isolated from Chickens and Pigs on Farms, Abattoirs, and Markets

	Chicken				*Pig*				
Resistance gene	*Farm*	*Abattoir*	*Market*	*Total*	*Farm*	*Abattoir*	*Market*	*Total*	*Total*
armA	0	0	0	0	1	0	0	1	1
rmtB	3	10	9	22	6	8	8	22	44
tetA	5	12	9	26	12	12	11	35	61
tetM	2	2	1	5	2	3	3	8	13
floR	6	14	13	33	14	20	13	47	80
mcr-1	2	4	5	11	5	1	5	11	22
sul1	1	7	17	25	9	12	10	31	56
sul2	1	2	7	10	9	12	10	31	41
sul3	1	0	2	3	5	6	5	16	19
$bla_{\text{TEM-1}}$	3	9	10	22	11	13	11	35	57
$bla_{\text{CTX-M-55}}$	1	4	2	7	2	3	6	11	18
$bla_{\text{CTX-M-164}}$	0	2	2	4	2	0	1	3	7
$bla_{\text{CTX-M-79}}$	0	0	2	2	2	0	1	3	5
$bla_{\text{CTX-M-15}}$	2	1	0	3	1	0	0	1	4
$bla_{\text{CTX-M-65}}$	0	0	0	0	1	0	0	1	1
$bla_{\text{CTX-M-14}}$	1	0	0	1	0	0	0	0	1
$bla_{\text{CMY-2}}$	0	0	0	0	0	1	0	1	2
aac(6)-Ib-cr	4	8	10	22	5	17	8	30	52
qnrB	0	3	2	5	2	1	5	8	13
qnrS	4	8	8	20	6	9	7	22	42
qepA	0	0	0	0	0	1	0	1	1
oqxAB	3	4	6	13	6	7	2	15	28

one cluster was not always identical such as *Salmonella* Meleagridis, of which 16 resistant isolates showed 13 different antibiotic resistance patterns.

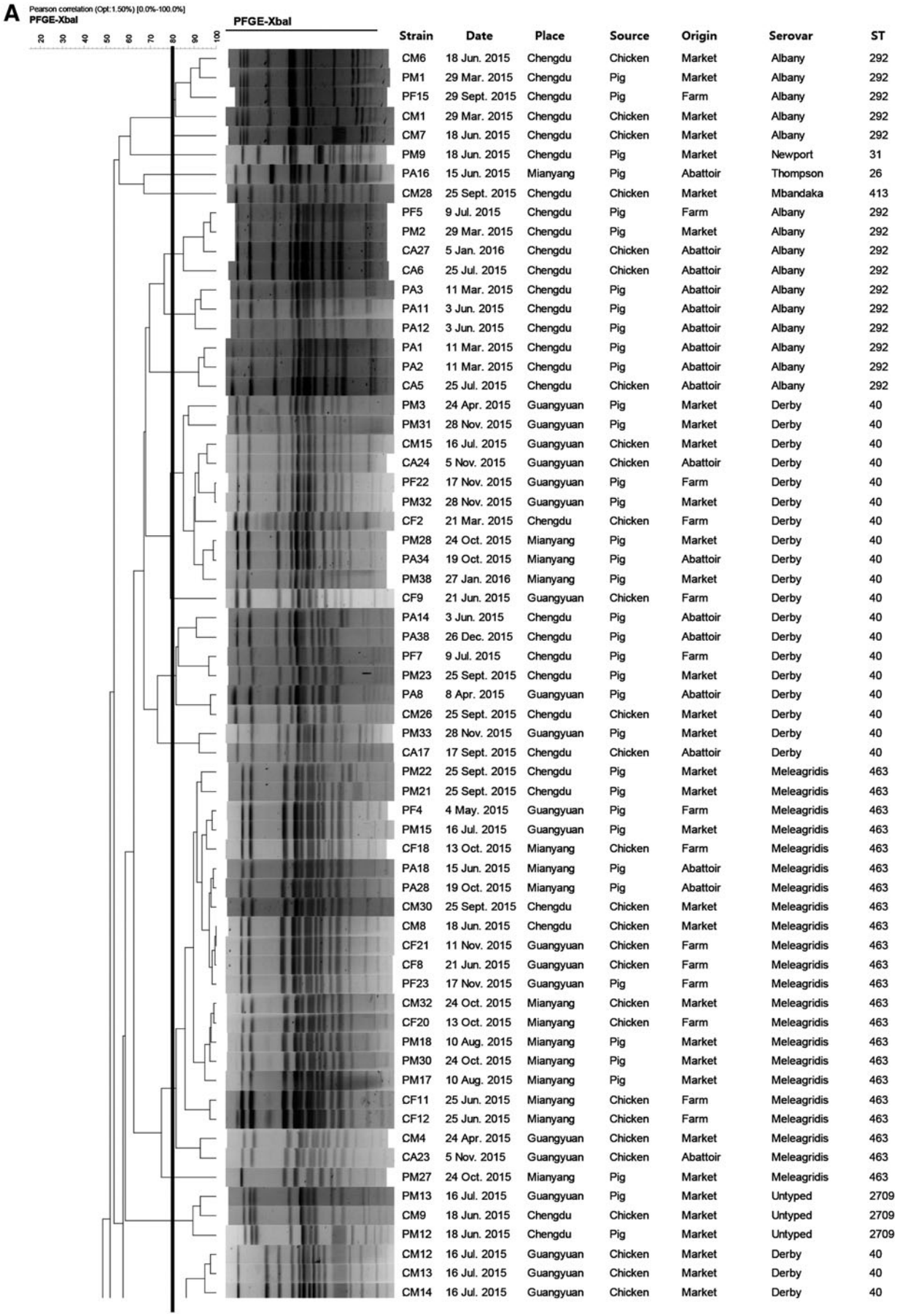
Discussion

For the purpose of this study, *Salmonella* isolates were recovered from a number of sites, including farms, abattoirs, and markets of chickens and pigs. The levels of antimicrobial resistance and serotype were assessed. All strains were then subtyped by PFGE to determine their genetic relationships. The results showed that *Salmonella* strains from different parts of the food production chain were genetically related. This leads to the conclusion that *Salmonella* isolates may proliferate through the processing of food products, which poses a potential challenge to food safety and public health.

In total, the isolation rate of *Salmonella* was 27.85%, which was higher than the average of pigs, chickens, and ducks (12.0%) in 2011 (Li *et al.*, 2013). It was found that the rate of prevalence was variable for *Salmonella* samples derived from food-producing animals. In central China, it was rated at 11.35% (Kuang *et al.*, 2015); whereas in Yangzhou, occurrences were higher than 70% (Cai *et al.*, 2016). The differences between different regions of China were related to collection areas, samples, seasons, and isolation methods. The isolation rate of *Salmonella* was higher in abattoirs and markets than in farms. One reason might be that *Salmonella* is likely to colonize in bile or liver other than gut in healthy-looking animals, but they might frequently shed in the stool. The other reason might be that *Salmonella* spread more readily in slaughterhouses and markets. The stress of transportation and poor control measures during the slaughter process could increase the shedding and thus the likelihood of *Salmonella* contamination (Li *et al.*, 2016). Moreover, improper storage conditions for retail meat in markets could also foster conditions that encourage the survival of *Salmonella*.

Derby was the most prevalent serotype of all *Salmonella* isolates. For chickens, Derby and Typhimurium were the dominant serotypes, which was in line with the investigation conducted in Sichuan (Li *et al.*, 2013), but differed from that conducted in Shandong (Lai *et al.*, 2014). The diversity of *Salmonella* serovars is likely to be associated with specific geographical areas (Zhang *et al.*, 2016). In addition, they were also the dominant serotypes of *Salmonella* isolated from humans (Cui *et al.*, 2009; Xia *et al.*, 2009). Besides Derby, the dominant serotype detected in pigs was Meleagridis, which has never been reported in this region, whereas it has been found to be the most common serovar in humans and retail meats in Yucatan (Zaidi *et al.*, 2006). Great attention should be paid to this serotype as 68.18% of isolates (15/22) were MDR in this study. Typhimurium was also prevalent in pigs. This serovar usually exhibits high-level resistance to antibiotics, and the mortality associated with infection by Typhimurium was much higher than with other serovars according to previous studies (Wong *et al.*, 2013).

Antimicrobial resistance limits the choice for useful antibiotics and may lead to discount or even failure to treat salmonellosis (Liang *et al.*, 2015). Some isolated strains showed resistance to nearly all tested antibiotics except carbapenem, which remains as a choice to treat fluoroquinolone- and cephalosporin-resistant *Salmonella* infections. As a result, resistance to carbapenems linked to carbapemases has been widely reported in recent years, particularly among *E. coli*. However, to date, it has never been found in *Salmonella* of animal origin in our country. Therefore, long-term supervision and monitoring are required. Colistin has been



Strain	Date	Place	Source	Origin	Serovar	ST
CM6	18 Jun. 2015	Chengdu	Chicken	Market	Albany	292
PM1	29 Mar. 2015	Chengdu	Pig	Market	Albany	292
PF15	29 Sept. 2015	Chengdu	Pig	Farm	Albany	292
CM1	29 Mar. 2015	Chengdu	Chicken	Market	Albany	292
CM7	18 Jun. 2015	Chengdu	Chicken	Market	Albany	292
PM9	18 Jun. 2015	Chengdu	Pig	Market	Newport	31
PA16	15 Jun. 2015	Mianyang	Pig	Abattoir	Thompson	26
CM28	25 Sept. 2015	Chengdu	Chicken	Market	Mbandaka	413
PF5	9 Jul. 2015	Chengdu	Pig	Farm	Albany	292
PM2	29 Mar. 2015	Chengdu	Pig	Market	Albany	292
CA27	5 Jan. 2016	Chengdu	Chicken	Abattoir	Albany	292
CA6	25 Jul. 2015	Chengdu	Chicken	Abattoir	Albany	292
PA3	11 Mar. 2015	Chengdu	Pig	Abattoir	Albany	292
PA11	3 Jun. 2015	Chengdu	Pig	Abattoir	Albany	292
PA12	3 Jun. 2015	Chengdu	Pig	Abattoir	Albany	292
PA1	11 Mar. 2015	Chengdu	Pig	Abattoir	Albany	292
PA2	11 Mar. 2015	Chengdu	Pig	Abattoir	Albany	292
CA5	25 Jul. 2015	Chengdu	Chicken	Abattoir	Albany	292
PM3	24 Apr. 2015	Guangyuan	Pig	Market	Derby	40
PM31	28 Nov. 2015	Guangyuan	Pig	Market	Derby	40
CM15	16 Jul. 2015	Guangyuan	Chicken	Market	Derby	40
CA24	5 Nov. 2015	Guangyuan	Chicken	Abattoir	Derby	40
PF22	17 Nov. 2015	Guangyuan	Pig	Farm	Derby	40
PM32	28 Nov. 2015	Guangyuan	Pig	Market	Derby	40
CF2	21 Mar. 2015	Chengdu	Chicken	Farm	Derby	40
PM28	24 Oct. 2015	Mianyang	Pig	Market	Derby	40
PA34	19 Oct. 2015	Mianyang	Pig	Abattoir	Derby	40
PM38	27 Jan. 2016	Mianyang	Pig	Market	Derby	40
CF9	21 Jun. 2015	Guangyuan	Chicken	Farm	Derby	40
PA14	3 Jun. 2015	Chengdu	Pig	Abattoir	Derby	40
PA38	26 Dec. 2015	Chengdu	Pig	Abattoir	Derby	40
PF7	9 Jul. 2015	Chengdu	Pig	Farm	Derby	40
PM23	25 Sept. 2015	Chengdu	Pig	Market	Derby	40
PA8	8 Apr. 2015	Guangyuan	Pig	Abattoir	Derby	40
CM26	25 Sept. 2015	Chengdu	Chicken	Market	Derby	40
PM33	28 Nov. 2015	Guangyuan	Pig	Market	Derby	40
CA17	17 Sept. 2015	Chengdu	Chicken	Abattoir	Derby	40
PM22	25 Sept. 2015	Chengdu	Pig	Market	Meleagridis	463
PM21	25 Sept. 2015	Chengdu	Pig	Market	Meleagridis	463
PF4	4 May. 2015	Guangyuan	Pig	Farm	Meleagridis	463
PM15	16 Jul. 2015	Guangyuan	Pig	Market	Meleagridis	463
CF18	13 Oct. 2015	Mianyang	Chicken	Farm	Meleagridis	463
PA18	15 Jun. 2015	Mianyang	Pig	Abattoir	Meleagridis	463
PA28	19 Oct. 2015	Mianyang	Pig	Abattoir	Meleagridis	463
CM30	25 Sept. 2015	Chengdu	Chicken	Market	Meleagridis	463
CM8	18 Jun. 2015	Chengdu	Chicken	Market	Meleagridis	463
CF21	11 Nov. 2015	Guangyuan	Chicken	Farm	Meleagridis	463
CF8	21 Jun. 2015	Guangyuan	Chicken	Farm	Meleagridis	463
PF23	17 Nov. 2015	Guangyuan	Pig	Farm	Meleagridis	463
CM32	24 Oct. 2015	Mianyang	Chicken	Market	Meleagridis	463
CF20	13 Oct. 2015	Mianyang	Chicken	Farm	Meleagridis	463
PM18	10 Aug. 2015	Mianyang	Pig	Market	Meleagridis	463
PM30	24 Oct. 2015	Mianyang	Pig	Market	Meleagridis	463
PM17	10 Aug. 2015	Mianyang	Pig	Market	Meleagridis	463
CF11	25 Jun. 2015	Mianyang	Chicken	Farm	Meleagridis	463
CF12	25 Jun. 2015	Mianyang	Chicken	Farm	Meleagridis	463
CM4	24 Apr. 2015	Guangyuan	Chicken	Market	Meleagridis	463
CA23	5 Nov. 2015	Guangyuan	Chicken	Abattoir	Meleagridis	463
PM27	24 Oct. 2015	Mianyang	Pig	Market	Meleagridis	463
PM13	16 Jul. 2015	Guangyuan	Pig	Market	Untyped	2709
CM9	18 Jun. 2015	Chengdu	Chicken	Market	Untyped	2709
PM12	18 Jun. 2015	Chengdu	Pig	Market	Untyped	2709
CM12	16 Jul. 2015	Guangyuan	Chicken	Market	Derby	40
CM13	16 Jul. 2015	Guangyuan	Chicken	Market	Derby	40
CM14	16 Jul. 2015	Guangyuan	Chicken	Market	Derby	40

FIG. 2. Unweighted pair group method with arithmetic means (UPGMA) dendrogram based on *Xba*I-pulsed-field gel electrophoresis (PFGE) profiles of the 193 *Salmonella* isolated from chickens and pigs on farms, abattoirs, and markets.

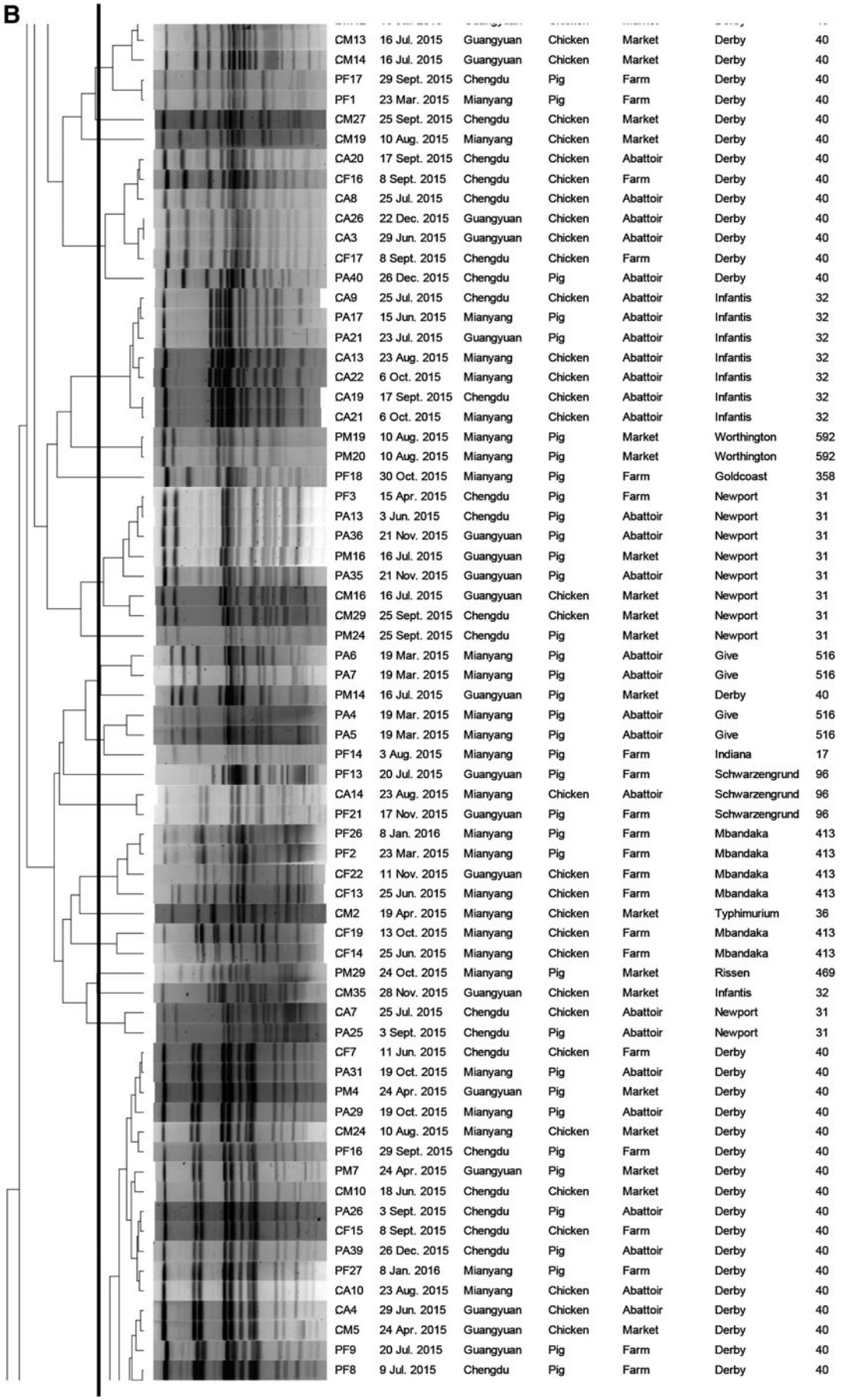


FIG. 2. (Continued).

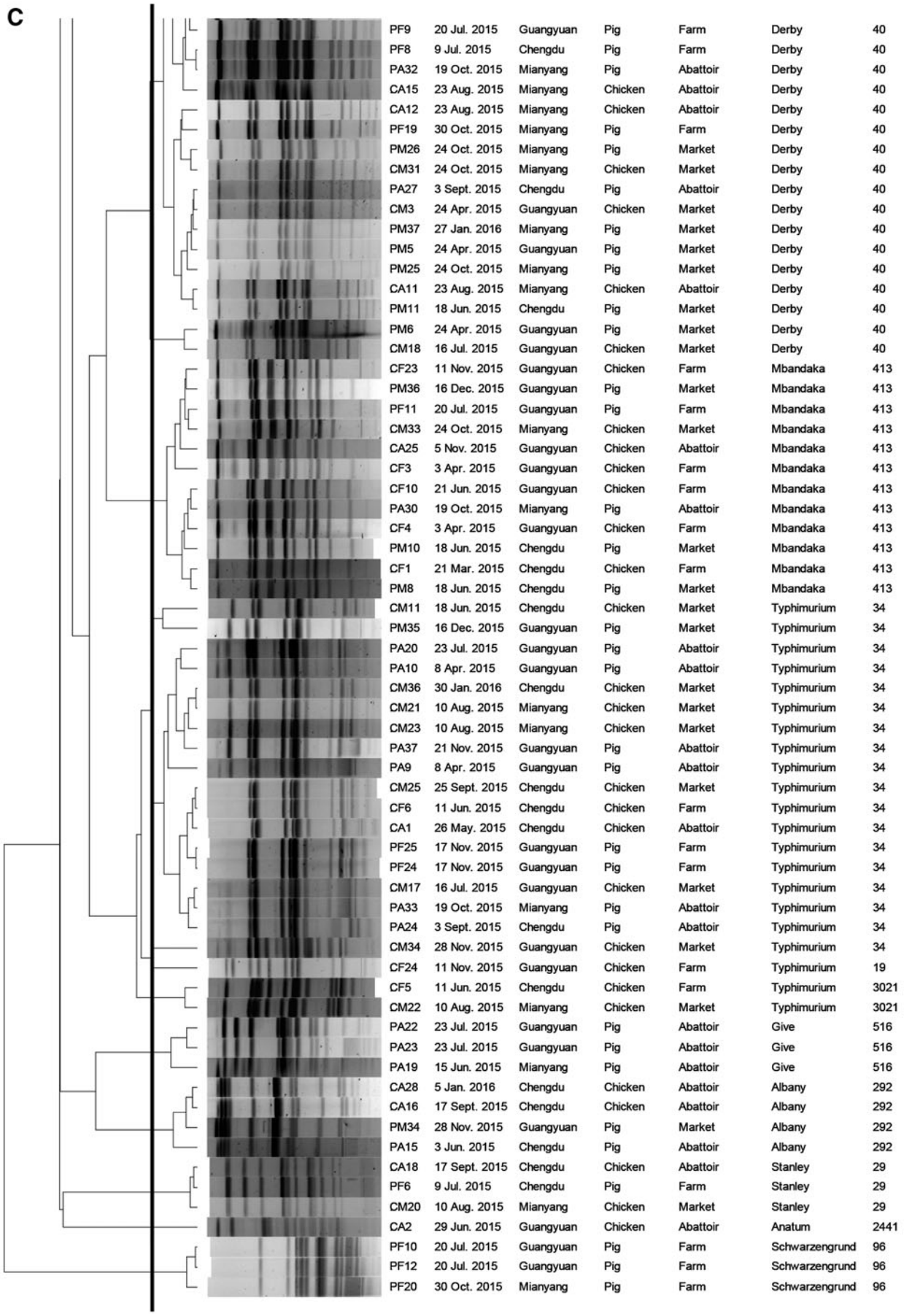


FIG. 2. (Continued).

recognized as the last-resort antibiotic for a number of bacterial infections (Falagas *et al.*, 2011; Halaby *et al.*, 2013) but bacteria that are resistant to colistin have been in connection with acquisition of *mcr-1* increasingly reported worldwide (Liu *et al.*, 2016; Yao *et al.*, 2016).

Above all, antibiotic resistance is frequently a common feature within a serotype such as serovar Indiana, which is related to MDR and has a broad distribution in China (Bai *et al.*, 2016; Zhang *et al.*, 2016). Similarly, in this study, this serotype was resistant to eight antibiotic classes. Moreover, it carried *armA*, $bla_{\text{TEM-1}}$, and $bla_{\text{CTX-M-65}}$. Besides this strain, the other amikacin-resistant isolates were all positive for *rmtB*, which was the most common of 16S rRNA methyltransferase genes among *E. coli* in China (Deng *et al.*, 2013; Xia *et al.*, 2016). In line with other studies (Xia *et al.*, 2016), nearly all (90.91%) *rmtB*-positive isolates harbored other resistance genes. Importantly, *rmtB* was usually located on different plasmids accelerating the dissemination of resistance genes (Xia *et al.*, 2016). All isolates carrying *mcr-1* genes displayed MDR patterns and 90.91% (20/22) harbored ESBLs genes. Moreover, the serotype of *mcr-1*-positive isolates was various (Albany, Derby, Newport, Mbandaka and Stanley), suggesting the horizontal transmission of this gene. *mcr-1* was located on transmissible plasmid and could spread rapidly between Gram-negative strains, causing some untreatable infections (Liu *et al.*, 2016). Consistent with our previous study (Yang *et al.*, 2016), serotype Albany harbored *mcr-1* and $bla_{\text{CTX-M-55}}$ at the same time. $bla_{\text{CTX-M-55}}$ was the most prevalent ESBLs gene in this study. It was not common in *Salmonella* as reported and differed from the $bla_{\text{CTX-M-27}}$ and $bla_{\text{CTX-M-65}}$ that were dominant in Guangdong and Henan, respectively (Bai *et al.*, 2016; Zhang *et al.*, 2016). $bla_{\text{CTX-M-55}}$ was initially found in *E. coli* (Rao *et al.*, 2014) and has been widely reported in humans (Hu *et al.*, 2013; Zhang *et al.*, 2014). In addition, it was always located on conjugative plasmids (Bai *et al.*, 2016), which indicated that $bla_{\text{CTX-M-55}}$ of *Salmonella* might originate from *E. coli* (Wong *et al.*, 2015).

Co-occurrence of ESBLs, especially CTX-M genes and PMQR genes, has been widely reported (Bai *et al.*, 2016). In this study, 28 $bla_{\text{CTX-M}}$-encoding isolates (77.78%) harbored PMQR genes. To the best of our knowledge, this is the first report of serovar Thompson carrying *qepA* and it was the third serovar after Typhimurium and Indiana (Al-Gallas *et al.*, 2013; Bai *et al.*, 2016). Contrary to previous studies (Eaves *et al.*, 2004), substitutions of amino acid in *gyrA* at codon 83 or 87 did not differ between serotypes. There was no significant association between fluoroquinolone resistance genes and serotypes.

It was obvious that ST was related to serotype such as ST40 with Derby and ST463 with Meleagridis. It is worth noting that 18 out of 22 Typhimurium isolates were ST34, which was frequently isolated in Europe and Spain with ACSSuT (ampicillin, chloramphenicol, streptomycin, sulfamethoxazole, and tetracycline) resistance type and could expand quickly through environments and animals (Antunes *et al.*, 2011; Wong *et al.*, 2013). We found that all of the ST34 were resistant strains and 14 isolates (77.78%) showed MDR in this study. To our knowledge, this is the first time that *Salmonella* serovar Rissen ST469 was isolated in China, which had only previously been reported in a few European countries and could also affect humans through travels and the consumptions of pig products (Hendriksen *et al.*, 2008). Strains with identical ST and serovar were isolated from different parts of the production chain, suggesting that *Salmonella* might spread during the process.

PFGE has an advantage of discriminating the genetic relatedness of strains, particularly for the same serotype and ST isolates (Soyer *et al.*, 2010; Cai *et al.*, 2016). The PFGE patterns of isolates from different stages of food production such as strains PF19, PA32, and PM26 were similar, which indicated that those isolates were clonally related and *Salmonella* isolates could disseminate from farm to abattoir and then to market. The antimicrobial resistance of isolates generating the same pulsotypes may be different, implying that resistance genes can be acquired or lost through horizontal transmission depending on the environment.

Conclusion

This investigation emphasizes the fact that pigs and chickens may be identified as potential sources of *Salmonella* for humans. And there are possible cross-contaminations in the slaughterhouses and on the markets, which can increase the possibility of *Salmonella* infection. In addition, *Salmonella* isolates from food-producing animals frequently exhibited MDR patterns and antimicrobial resistance genes $bla_{\text{CTX-M}}$, *mcr-1*, and *rmtB* were prevalent. Importantly, *Salmonella* isolates from different sections of production chains were genetically homologous and may be liable to spread along processing routes from farms to markets. Therefore, measures should be taken to normalize the processing of food-producing animals and the use of antibiotics to reduce the chance of *Salmonella* contamination and antimicrobial resistance.

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Disclosure Statement

No competing financial interests exist.

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