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Serotype distribution and antibiotic resistance of *Salmonella* in food-producing animals in Shandong province of China, 2009 and 2012

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ABSTRACT

The aims of this study were to investigate the serotype distribution, genetic relationships and antibiotic resistance of *Salmonella* from food-producing animals in Shandong province of China in 2009 and 2012. A total of 362 out of 1825 samples from chickens, 53 out of 445 samples from ducks, and 50 out of 692 samples from pigs were positive for *Salmonella*. Isolates were subjected to serotyping, antibiotic susceptibility testing (15 antibiotics) and pulsed-field gel electrophoresis (PFGE). The most common serotypes recovered in the chicken samples were Enteritidis (n = 294, 81.2%) and Indiana (n = 45, 12.4%). For ducks, Cremieu (n = 25, 47.2%), Indiana (n = 13, 24.5%) and Typhimurium (n = 9, 17%) were frequently isolated. In the pig samples, Derby (n = 29, 58%), Typhimurium (n = 9, 18%), and Enteritidis (n = 6, 12%) were the most common serovars. PFGE results indicated that clonal dissemination of each serovar was prevalent, and that the *Salmonella* found on the poultry carcasses was caused by cross-contamination in the abattoirs. More than 99% of the *Salmonella* isolates collected were resistant to at least one antibiotic. The *Salmonella* resistance rates for 15 antibiotics in 2012 were significantly higher than those in 2009. In 2012, the highest resistance was to nalidixic acid (95.9%), followed by sulphafurazole (78.2%) and ampicillin (72.3%); the lowest levels of resistance were to kanamycin (40.1%) and amikacin (38.7%). Additionally, 41.5% and 42.2% of the *Salmonella* were resistant to ciprofloxacin and ceftiofur, respectively. Noticeably, 25% of the serovar Enteritidis and all of the serovar Indiana were resistant to at least 10 antibiotics in 2012. The increasing trend of antibiotic resistance in Shandong province indicates the need for more careful use of antibiotics.

1. Introduction

Non-typhoidal *Salmonella* is an important food-borne pathogen, which can cause gastroenteritis and septicaemia in humans. Food-producing animals play a role as a primary reservoir of non-typhoid *Salmonella*, and most human *Salmonella* infections are a consequence of eating food of animal origin that is contaminated with *Salmonella* (Ribot et al., 2002). Slaughter is potentially the key time for contamination of food-producing animal carcasses and subsequently meat products with *Salmonella* (Jong et al., 2009).

The use of antibiotics in food-producing animals has raised concerns regarding their potential impact on human health (Anderson et al., 2003). Resistant *Salmonella* may be transmitted through the food chain to humans (Barza, 2002). Patients infected with *Salmonella* at the extremes of age and those who are immunocompromised need appropriate antimicrobial therapy (White et al., 2001). Because resistance to conventional antibiotics has increased, extended-spectrum cephalosporins and fluoroquinolones have been chosen to treat infections caused by multidrug resistant *Salmonella* (Su et al., 2004). In recent years, *Salmonella* strains that are resistant to extended-spectrum cephalosporins or fluoroquinolones have been observed in some countries, and surveillance data have indicated an increasing trend for drug-resistance (Gupta et al., 2003; Biedenbach et al., 2006; Xia et al., 2008).

Because of the widespread commercial trade in animal derived food products, surveillance of *Salmonella* serotype distribution and antibiotic resistance levels in food-producing animals in individual countries is of global importance. Knowledge about the epidemiology of different serovars in different geographic regions may assist in recognising and tracing new emerging pathogens (Bangtrakulnonth et al., 2004).

Shandong is located in the northeast of China, has a population of 95 million, and is the main producer of food of animal origin in China. In this area, chicken, duck and pork farming is intensive, and the processed animal products are shipped to different markets all over China. The objectives of this study were to determine the prevalence, serotype distribution, genetic relatedness and antibiotic susceptibility of *Salmonella* isolated from chickens, ducks and pigs in Shandong, China. Additionally, the current study investigated the *Salmonella* contamination rate for

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chicken and duck carcasses in abattoirs, and compared the antimicrobial resistance levels in 2009 and 2012.

2. Material and methods

2.1. Sample collection and Salmonella isolation

We chose two chicken broiler breeding farms, two chicken hatcheries, two chicken abattoirs, three pig farms, and one duck abattoir in eight important farming cities (Linyi, Xintai, Taian, Binzhou, Qingzhou, Feicheng, Zhucheng and Dezhou) in Shandong province in 2009 and 2012 to perform the sample collection. All the investigated farms were the largest in each city. The chicken and duck production line conditions at these facilities were as follows. Poultry breeding stocks were raised in breeding farms, and laid eggs were transferred to hatcheries in the same city to incubate. The 1-day-old chicks or ducklings were sent to a number of farms in various cities, after which the 6- to 7-week-old poultry were transported to several intensive abattoirs for processing. Chicken and duck carcasses were shipped to markets or restaurants all over China.

In this study, a total of 2962 samples were collected. The samples included faeces from 1-day-old chickens (n = 373), caecal contents from 7-week-old broilers (n = 705), 7-week-old broiler carcasses (n = 361), faeces of 1-year-old broiler breeders (n = 386), caecal contents of 6-week-old ducks (n = 385), duck carcasses (n = 60), and rectal swabs of 80- to150-day-old pigs (n = 692) (Table 1). Each sample was collected from a different individual animal. The samples collected at each abattoir represent broilers or ducks from different farms. Fresh faecal samples and caecal contents were transferred into buffered peptone water (BPW; Difco, Cockeysville, MD, USA) at the volume ratio of 1:10, and then incubated for 2 h at 37 °C for pre-enrichment. The carcasses were the 4 °C-chilled final carcasses from abattoirs; samples were collected with large sterile cotton swabs prewetted with 5 ml of BPW, which was used to swab the whole outer skin surface of each carcass. All the carcass swabs and rectal swabs were incubated for 18 h at 37 °C. After pre-enrichment, 300 μl of BPW was added to 3 ml of selenite cystine broth (Oxiod, Basingstoke, UK) and tetrathionate broth (Oxiod), and the cultures were incubated at 37 °C and 42 °C for 20 h, respectively, after which some of the enrichment cultures were simultaneously streaked onto CHROMagar *Salmonella* agar (CHROMagar Company, Paris, France) and bismuth sulphite agar (Oxiod). Colonies were incubated for 24–48 h at 37 °C. One or two colonies (per sample) that were suspected of being infected with *Salmonella* were selected for biochemical analysis using an API 20 E (bioMérieux, Marcy l' Etoile, France) microsubstrate system for genus identification.

2.2. Serotyping

O, H, and Vi antigens from *Salmonella* strains were characterised using the slide agglutination method with *Salmonella* antisera (S & A Reagents Lab Ltd., Bangkok, Thailand) according to the Kauffmann–White scheme (Grimont and Weill, 2007).

2.3. Antibiotic resistance testing

The minimal inhibitory concentration (MIC) of an antibiotic was determined by the manual broth microdilution method according to the criteria recommended by the Clinical and Laboratory Standards Institute (CLSI, 2008, 2013). The ATCC 25922 strain of *Escherichia coli* was used as a control. The following 15 antibiotics were tested (concentration range in mg/l): tetracycline (2–128), ampicillin (2–128), amoxicillin/clavulanic acid (2/1–128/64), cefazolin (4–128), ceftiofur (0.25–128), chloramphenicol (4–128), florfenicol (0.5–128), nalidixic acid (1–128), ciprofloxacin (0.008–128), streptomycin (2–128), gentamicin (2–128), kanamycin (0.5–128), amikacin (0.5–128), sulphafurazole (4–1024), and trimethoprim/sulphamethoxazole (2/38–8/152). No CLSI interpretive criteria were available for streptomycin; hence, for analysis purposes, samples with MIC values ≥ 64 μg/ml were considered to be resistant. All the isolates which were classified as intermediate or resistant were also considered to be resistant (except for ciprofloxacin, MIC values ≥ 1 μg/ml were considered to be resistant).

2.4. Pulsed-field gel electrophoresis (PFGE) analysis

PFGE assays of randomly selected *Salmonella* Enteritidis (30%), *Salmonella* Indiana (60%), *Salmonella* Cremieu (60%), *Salmonella* Typhimurium (100%) and *Salmonella* Derby (100%) isolates were performed. Chromosomal DNA from the *Salmonella* isolates was digested with *Xba*I and subjected to the standardised PulseNet PFGE protocol (http://www.pulsenetinternational.org/protocols/Pages/default.aspx) using the CHEF Mapper XA Pulsed Field Gel Electrophoresis System (Bio-Rad, Hercules, California). Gel electrophoresis was carried out for 18.5 h at 6.0 V/cm at a 120° angle and 14 °C, and an initial switch time of 5 s and a final switch time of 40 s. *Salmonella* serovar Braenderup H9812 was used as a standard size marker. Cluster analysis of pulsotypes was done according to the Dice coefficient method using InfoQuest FP Software/Version 4.5 (Bio-Rad).

2.5. Statistics

Statistical comparisons of the isolation rates and antibiotic resistance rates among the different food-producing animals and serovars were analysed using a Chi-square test and SPSS software (SPSS Inc., Chicago, IL; version 11.5).

3. Results

3.1. Prevalence of Salmonella serotypes in different food-producing animal samples

A total of 465 *Salmonella* strains were identified from 465 out of 2962 (2009 — 319/1425; 2012 — 147/1537) animal samples. The

Table 1
Comparison of the *Salmonella* isolation rates among different animals and different growth stages in chickens.

Year		Chicken				Duck		Pig
		1 day	7 weeks[a]	7 week-C[b]	1 year	6 weeks[a]	6 week-C[b]	80–150 days
2009	No. sample	320	225	–	186	225	–	469
	No. *Salmonella* (%)	206 (64.1)	29 (12.9)	–	9 (4.8)	28 (12.4)	–	47 (10.0)
2012	No. sample	53	480	361	200	160	60	223
	No. *Salmonella* (%)	34 (63)	24 (5.0)	50 (13.9)	11 (5.5)	12 (7.5)	13 (21.7)	3 (1.3)
P value		0.975	0.000	–	0.770	0.117	–	0.000
Total	No. sample	373	705	361	386	385	60	692
	No. *Salmonella* (%)	239 (64.1)	53 (7.5)	50 (13.9)	20 (5.2)	40 (10.4)	13 (21.7)	50 (7.2)

[a] Caecal contents of 7-week-old broiler or a 6-week-old duck.
[b] Carcass of a 7-week-old broiler or a 6-week-old duck.

highest isolation rates among the different animal sources tested for those 2 years were both observed in 1-day-old chickens (64.1% in 2009 and 64.2% in 2012). The lowest isolation rates were in broiler breeders (4.8% in 2009 and 5.5% in 2012) and pigs (10% in 2009 and 1.3% in 2012) (Table 1). No significant difference was found in the *Salmonella* isolation rate in ducks, 1-day-old chickens and chicken breeders between 2009 and 2012 ($P > 0.05$). Analysis of samples from abattoirs showed that 21.7% of the duck carcasses and 13.9% of the chicken carcasses were *Salmonella*-positive. However, only 10.4% of the duck caecal contents samples and 7.5% of those from chickens were *Salmonella*-positive.

3.2. Salmonella serovar distribution in different food-producing animal species

Twelve *Salmonella* isolates were untypeable (self-agglutination). Eleven and 15 different serovars were identified in 2009 and 2012, respectively (Table 2). The most common *Salmonella* serotype in 2009 and 2012 was Enteritidis (229/318, 72%, 71/147, 48.3%, respectively). The *Salmonella* serovars showed preference for particular animal reservoirs. The *Salmonella* Enteritidis isolates were more widely disseminated in 1-day-old chickens (193 of 229 total Enteritidis isolates, 84.3%, were from 1-day-old chickens, in 2009, and 34/71, 47.9% in 2012) than other chicken growth stages (30/229, 13.1% in 2009 and 37/71, 52.1% in 2012) and pigs (6/229, 2.6%). Additionally, Pullorum (n = 3) and *Salmonella enterica* subspecies II (n = 2) were identified in 1-day-old chickens. Paratyphi B, Arachavelata, Detmold, Hessarek, Mapo, Schwarzengrund and Waedenswil were identified in 7-week-old chickens. *Salmonella* Indiana were commonly found in 7-week-old chickens (14/12, 100% in 2009 and 31/44, 70.5% in 2012) and 6-week-old ducks (13/44, 29.5% in 2012). The *Salmonella* Cremieu were more commonly recovered from ducks (24/25, 96%) than chickens (1/25, 4%). *Salmonella* Typhimurium were found in both ducks (3/9, 33.3% in 2009 and 6/9, 66.7% in 2012) and pigs (6/9, 66.7% and 3/9, 33.3% in 2012), whilst the Derby serovar was only isolated from pigs (29/29, 100% in 2009).

3.3. Antibiotic resistance in Salmonella

A total of 99.1% of the *Salmonella* isolates in this study were resistant to at least one antibiotic. Resistance was most frequently observed to nalidixic acid (81.4%), sulphafurazole (29.6%) and trimethoprim/sulphamethoxazole (19.8%), whilst ciprofloxacin (4.4%) had the lowest resistance rate in 2009. The overall resistance rates in 2012 were significantly higher than that in 2009 ($P < 0.01$). The highest resistance was to nalidixic acid (95.9%), followed by sulphafurazole (78.2%) and ampicillin (72.3%). The lowest resistance was observed against amikacin (38.1%) (Table 3). When antibiotic resistance was analysed by animal species, a significant increase in the resistance rates for the majority of the agents tested was observed in *Salmonella* of chicken and duck origins in 2012, compared with that in 2009 ($P < 0.01$). *Salmonella* isolates of duck origin only showed high resistance rates to sulphafurazole (92.9%), and showed low resistance rates to trimethoprim/sulphamethoxazole (3.6%), whilst remaining susceptible to the other 13 antibiotics in 2009. However, the overall resistance rate for samples of duck origin increased in 2012 (Table 3). For *Salmonella* of chicken origin, 100% resistance was observed to nalidixic acid, whilst resistance to other agents was relatively low (range: 6.1% to 19.3%) in 2009. Three years later, the resistance rates for all of the other antibiotics tested were significantly higher ($P < 0.01$), with the exception of nalidixic acid, which retained its 100% resistance rate. Among *Salmonella* isolated from pigs in 2009, resistance was commonly observed to tetracycline (78.7%), sulphonamides (76.6%), streptomycin (42.6%) and chloramphenicol (36.2%). Because the number of *Salmonella* isolates from pigs in 2012 was limited (n = 3), a comparison of *Salmonella* of swine origin over the 2-year period was not performed.

As shown in Tables 4 and 5, the *Salmonella* serovars showed strong relationships with antibiotic resistance. The most susceptible serovar was Cremieu, which was susceptible to 14 antibiotics; the MIC values to ciprofloxacin were 0.015 μg/ml. Derby isolates were most likely to be resistant to tetracycline (100%), sulphafurazole (93.1%), trimethoprim/sulphamethoxazole (65.5%) and streptomycin (62.1%), and the MIC values to ciprofloxacin were ≤0.03 μg/ml. All nine *Salmonella* Typhimurium isolates from pigs displayed resistance to tetracycline, ampicillin, amoxicillin/clavulanic acid, florfenicol, kanamycin, sulphafurazole and trimethoprim/sulphamethoxazole; seven of these nine Typhimurium isolates were additionally resistant to chloramphenicol, streptomycin and gentamicin. However, those Typhimurium isolates recovered from ducks were susceptible to over 13 of the antibiotics tested. Multidrug resistance was particularly high in the Indiana isolates. All 58 Indiana isolates were resistant to at least 10 antibiotics (including ciprofloxacin and ceftiofur). The *Salmonella* Enteritidis isolates exhibited high resistance to nalidixic acid (100%), and to a lesser extent, to florfenicol (14.4% in 2009 and 43.7% in 2012), sulphafurazole (8.7% in 2009 and 76.1%) and ampicillin (7.4% in 2009 and 59.2% in 2012). No *Salmonella* Enteritidis was resistant to ciprofloxacin or ceftiofur in 2009. In 2012, 14.1% (10/71) and 16.9% (12/71) Enteritidis isolates were resistant to ceftiofur and ciprofloxacin, respectively. It is noticeable that although only 2.8% (2/71) of *Salmonella* Enteritidis were resistant to both ciprofloxacin and ceftiofur in 2012, 99.3% of (298/300) *Salmonella* Enteritidis in the two isolation years showed MIC vales ≥ 0.125 μg/ml to ciprofloxacin.

Other unusual serovars in 2012; Schwarzengrund, Arachavelata, Westafrica, Fortune, *S. enterica* subspecies II and Treguier, showed resistance to ceftiofur. Furthermore, *S. enterica* subspecies II and *Salmonella* Schwarzengrund were resistant to both ciprofloxacin and ceftiofur. The increased number of multiresistant Indiana, Enteritidis and rare serovars isolated in 2012 accounted for the overall resistance of ciprofloxacin and ceftiofur.

Table 2
Distribution of *Salmonella* serovars in different animal species.

Serovars		2009			2012		
	Total	Chicken	Duck	Pig	Chicken	Duck	Pig
Enteritidis	300	223	0	6	71	0	0
Indiana	58	14	0	0	31	13	0
Derby	29	0	0	29	0	0	0
Cremieu	26	1	25	0	0	0	0
Typhimurium	18	0	3	6	0	6	3
Pullorum	3	3	0	0	0	0	0
Heerlen	2	0	0	2	0	0	0
Schwarzengrund	2	0	0	0	0	2	0
II	2	1	0	0	1	0	0
Arachavelata	1	0	0	0	1	0	0
Detmold	1	0	0	0	1	0	0
Fortune	1	0	0	0	0	1	0
Hessarek	1	0	0	0	1	0	0
Kalamu	1	0	0	0	0	1	0
London	1	0	0	1	0	0	0
Mapo	1	0	0	0	1	0	0
Paratyphi B	1	0	0	0	1	0	0
Treguier	1	0	0	0	0	1	0
Waedenswil	1	0	0	0	1	0	0
Westafrica	1	0	0	0	0	1	0
4, 12: f:—	1	0	0	1	0	0	0
G–S group	1	0	0	1	0	0	0
Rough	12	1	0	1	10	0	0

3.4. Genetic relatedness in Salmonella isolates

As shown in Fig. 1, all of the PFGE patterns of the Enteritidis isolates tested here had similarity scores above 90%. About six PFGE patterns were observed for *Salmonella* Enteritidis. One pattern predominated and comprised at least 64% of the *Salmonella* Enteritidis isolates analysed. The Enteritidis isolates were from different animal origins

Table 3
Antibiotic resistance rates of *Salmonella* isolated from different food-producing animals.

Drugs	2009				2012			
	Total (n = 318)	Chicken (n = 272)	Pig (n = 47)	Duck (n = 28)	Total (n = 147)	Chicken (n = 119)	Pig (n = 3)	Duck (n = 25)
TET	17	6.6	78.7	0	59.2	59.7	100	64
AMP	11.9	12.7	17	0	71.4	72.3	100	76
AMC	5.3	6.1	4.3	0	42.2	37.8	0	72
CFZ	4.7	6.1	0	0	42.2	37.8	0	72
FUR	4.7	6.1	0	0	42.2	37	0	72
CHL	10.1	6.1	36.2	0	55.5	56.3	100	60
FFC	6.9	19.3	14.9	0	66.7	69.7	100	60
NAL	81.4	100	25.5	0	95.9	100	100	72
CIP	4.4	6.1	0	0	41.5	38.7	0	60
STR	11	6.1	42.6	0	44.9	51.3	100	20
GEN	13.2	6.1	10.6	0	50.3	49.6	100	52
KNA	6.3	6.1	14.9	0	40.1	38.7	100	60
AMK	4.7	6.1	0	0	38.1	36.1	0	52
SIZ	29.6	13.9	76.6	92.9	78.2	81.5	100	72
SXT	19.8	13.9	57.4	3.6	61.9	63.9	100	60

Abbreviations: TET, tetracycline; AMP, ampicillin; AMC, amoxicillin/clavulanic acid; CFZ, cefazolin; FUR, ceftiofur; CHL, chloramphenicol; FFC, florfenicol; NAL, nalidixic acid; CIP, ciprofloxacin; STR, streptomycin; GEN, gentamicin; KNA, kanamycin; AMK, amikacin; SIZ, sulphafurazole; SXT, trimethoprim/sulphamethoxazole.

and different cities; some of these isolates exhibited different resistance phenotypes in 2009 and 2012.

Salmonella Indiana strains isolated in the same year and from the same animal species showed the closest genetic relatedness. Using a cutoff value of 91%, three PFGE clusters were found. Two clusters of Indiana isolated from chickens and ducks in 2012 showed 82% similarity. Overall, the similarity of the PFGE patterns of the Indiana strains between 2009 and 2012 was about 78% (Fig. 2). *Salmonella* Typhimurium isolates showed eight patterns and four clusters using a cutoff value of 94% similarity (Fig. 3). Isolates in each cluster showed similar resistance phenotypes. The first cluster contained seven Typhimurium isolates of swine origin from two Chinese cities in 2009 and 2012. Those seven isolates were resistant to10 antibiotics and had the same resistance phenotype (TET/AMP/AMC/STR/KAN/NAD/CHL/FFC/SIZ/SXT). The *Salmonella* Derby isolates could be assigned to six different PFGE patterns (Fig. 4), and 77% of these isolates shared the most common PFGE pattern. For *Salmonella* Derby, using a cutoff value of 96% similarity, three clusters and one individual type were observed. Derby isolates in each PFGE cluster shared similar resistance profiles. In contrast, all of the Cremieu isolates belonged to one PFGE cluster (>95% similarity), consistent with the high similarity of the resistance profiles of Cremieu isolates (Fig. 5). The PFGE profiles of Enteritidis, Indiana and Typhimurium isolated from the carcasses of chicken or duck showed similar molecular "finger prints" with the *Salmonella* isolated from caecal contents from the same abattoir (Figs. 1–3).

4. Discussion

In this study, we collected samples from chickens (at different stages of growth), ducks and pigs in eight cities in the Shandong province of China. The samples were tested for the presence of *Salmonella* and to characterise the *Salmonella* serotype distribution, genetic relatedness and variation in antibiotic resistance over 2 years (2009 and 2012). The average *Salmonella*-positive rate in 7-week-old chickens (7.5%) in Shandong was in accordance with that in Europe (8%) (Jong et al., 2009). Limited epidemiological data are available for *Salmonella* in ducks. In our study, the *Salmonella* isolation rate for caecal samples of duck (12.4% in 2009 and 7.5% in 2012) was similar with that in Mekong Delta, Vietnam (8.7%) (Tran et al., 2004). We found that the *Salmonella* isolation rate for pigs (10.0% and 1.3%) was lower than the isolation rate in Sichuan province of China (16.4%) (Li et al., 2013), whilst consistent with the low isolation rate in Thailand (3%) (Pulsrikarn et al., 2012). The *Salmonella* contamination rate for chicken carcasses (13.9%) is lower than the contamination rates for chicken carcasses in Italy (36.9%) (Bacci et al., 2012), and in Quebec, Canada (21.2%) (Arsenault et al., 2007). The differences in these isolation rates might be caused

Table 4
Antibiotic resistance rates for *Salmonella* serovars.

Drugs	2009						2012			
	Enteritidis	Indiana	Typhimurium	Derby	Cremieu	Others	Enteritidis	Indiana	Typhimurium	Others
	(n = 229)	(n = 14)	(n = 9)	(n = 29)	(n = 26)	(n = 12)	(n = 71)	(n = 44)	(n = 9)	(n = 23)
TET	0	100	66.7	100	0	25.0	46.5	84.1	33.3	69.6
AMP	7.4	100	66.7	3.4	0	16.7	59.2	100	33.3	82.6
AMC	0	100	66.7	0	0	8.3	14.1	100	33.3	50.0
CFZ	0	100	0	0	0	8.3	14.1	100	0	50.0
FUR	0	100	0	0	0	8.3	14.1	100	0	47.8
CHL	0.9	100	44.4	34.5	0	25.0	43.7	100	33.3	30.4
FFC	14.4	100	66.7	0	0	25.0	43.7	100	33.3	30.4
NAL	100	100	66.7	3.4	0	66.7	100	100	33.3	100
CIP	0	100	0	3.4	0	8.3	16.9	100	0	21.7
STR	0	100	55.6	62.1	0	16.7	47.9	54.5	33.3	39.1
GEN	0	100	44.4	0	0	16.7	38.0	97.8	33.3	21.7
KNA	0	100	66.7	0	0	8.3	12.7	100	33.3	21.7
AMK	0	100	0	0	0	8.3	16.9	95.5	0	13.0
SIZ	8.7	100	100	93.1	96.2	50.0	76.1	100	66.7	56.5
SXT	8.7	100	77.8	65.5	0	25.0	47.9	100	33.3	52.2

Abbreviations: TET, tetracycline; AMP, ampicillin; AMC, amoxicillin/clavulanic acid; CFZ, cefazolin; FUR, ceftiofur; CHL, chloramphenicol; FFC, florfenicol; NAL, nalidixic acid; CIP, ciprofloxacin; STR, streptomycin; GEN, gentamicin; KNA, kanamycin; AMK, amikacin; SIZ, sulphafurazole; SXT, trimethoprim/sulphamethoxazole.

Table 5
Salmonella serovars that were resistant to the indicated number of antibiotics in 2009 and 2012.

Serovars	2009				Serotypes	2012			
	1–3	4–6	7–9	≥10		1–3	4–6	7–9	≥10
Enteritidis (n = 229)	229	0	0	0	Enteritidis (n = 71)	19	23	11	18
Indiana (n = 14)	0	0	0	14	Indiana (n = 44)	0	0	0	44
Typhimurium (n = 9)	3	1	1	4	Typhimurium (n = 9)	3	0	0	3
Derby (n = 29)	13	15	1	0	Schwarzengrund (n = 2)	0	0	0	2
Cremieu (n = 26)	25	0	0	0	Arachavelata (n = 1)	0	0	0	1
Pullorum (n = 3)	3	0	0	0	Detmold (n = 1)	0	0	1	0
London (n = 1)	1	0	0	0	Fortune (n = 1)	0	1	0	0
4,12: f:− (n = 1)	1	0	0	0	Hessarek (n = 1)	0	1	0	0
Heerlen (n = 1)	1	0	0	0	Kalamu (n = 1)	1	0	0	0
group G–S (n = 1)	1	0	0	0	Mapo (n = 1)	0	1	0	0
II (n = 1)	1	0	0	0	Paratyphi B (n = 1)	0	1	0	0
Rough (n = 2)	2	0	0	0	Treguier (n = 1)	0	1	0	0
					Waedenswil (n = 1)	0	1	0	0
					Westafrica (n = 1)	0	1	0	1
					II (n = 1)	1	0	0	0
					Rough (n = 10)	3	5	1	1

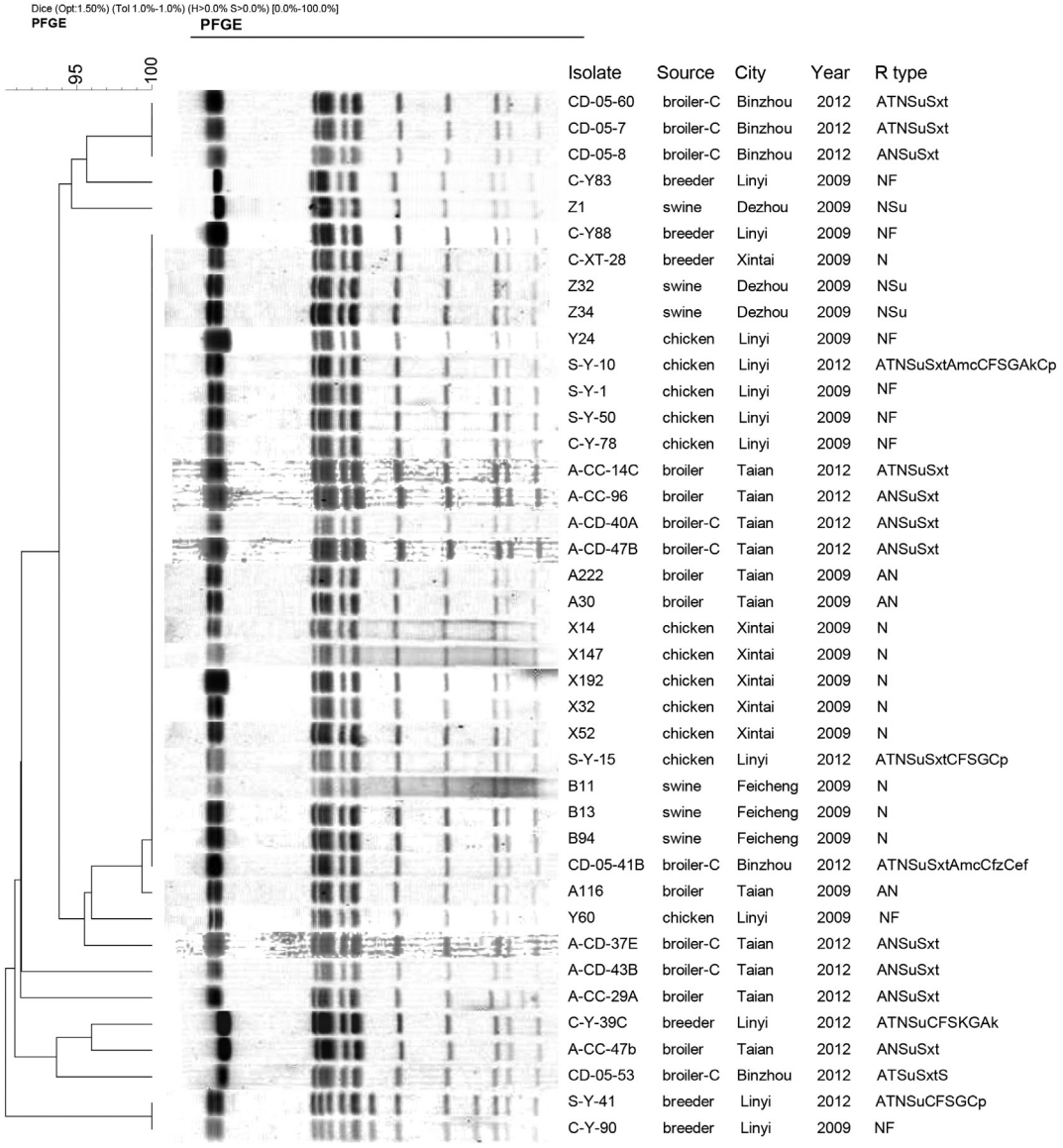

Fig. 1. PFGE profiles of representative Enteritidis isolates from chicken, duck and pigs in Shandong province in 2009 and 2012. Note: "broiler" indicates the caecal contents of a 7-week-old broiler chicken, and "broiler-C" indicates the carcass of a 7-week-old broiler. Abbreviations: A, ampicillin; T, tetracycline; Amc, amoxicillin/clavulanic acid; Cfz, cefazolin; Cef, ceftiofur; C, chloramphenicol; F, florfenicol; N, nalidixic acid; Cp, ciprofloxacin; S, streptomycin; G, gentamicin; K, kanamycin; Ak, amikacin; Su, sulphafurazole; Sxt, trimethoprim/sulphamethoxazole.

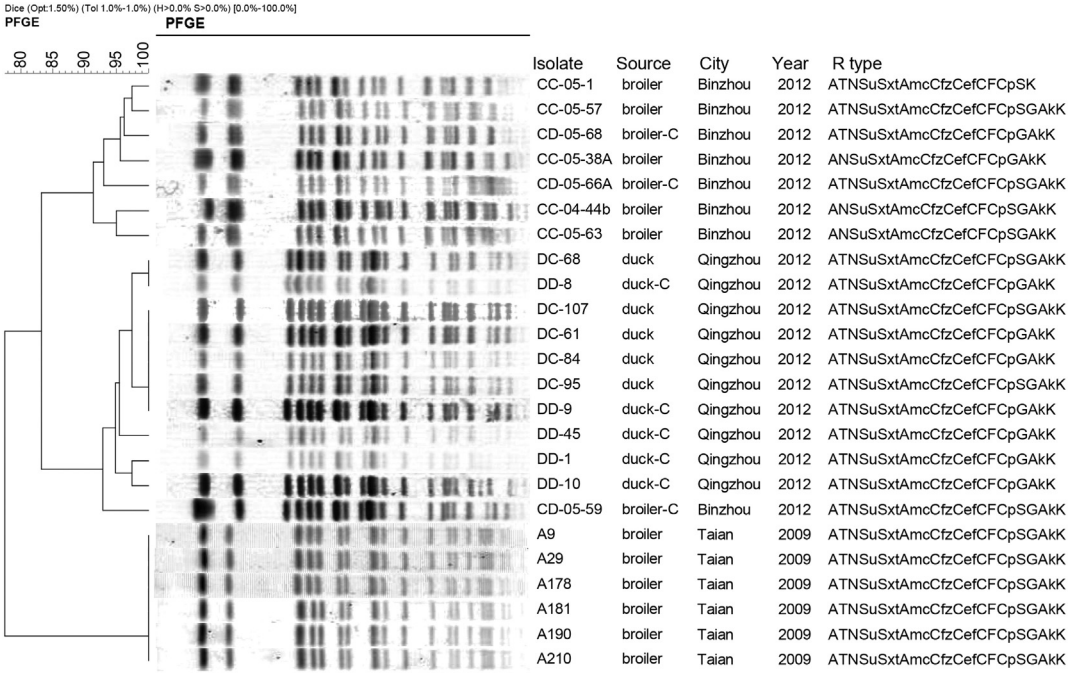

Fig. 2. PFGE profiles of representative Indiana isolates from chickens and ducks in Shandong province in 2009 and 2012. Note: "duck" indicates the caecal contents of a 6-week-old, duck and "duck-C" indicates the carcass of a 6-week-old duck. Abbreviations: A, ampicillin; T, tetracycline; Amc, amoxicillin/clavulanic acid; Cfz, cefazolin; Cef, ceftiofur; C, chloramphenicol; F, florfenicol; N, nalidixic acid; Cp, ciprofloxacin; S, streptomycin; G, gentamicin; K, kanamycin; Ak, amikacin; Su, sulphafurazole; Sx, trimethoprim/sulphamethoxazole.

by differences in the sample types, collection methods, isolation times, and laboratory methodology, as well as the local environmental conditions. The higher *Salmonella*-positive rate in carcass than in caecal contents in this study confirmed other researchers' views that cross-contamination occurred during processing in poultry abattoirs (Henry et al., 2012); and highlights the importance of controlling *Salmonella* infection along the food production chain to prevent the chicken carcass contamination. The highest *Salmonella*-positive rate was observed in 1-day-old chickens, which is in accordance with Lu et al. (2011) who reported that *Salmonella* were frequently isolated from chicken hatcheries in China. As chicken often get *Salmonella* infection through contacting the *Salmonella*-positive eggshells or environments, the low isolation rate in 1-year-old broiler breeders and high *Salmonella* isolation rate in 1-day-old chickens observed in the present study suggest that sanitation in the chicken egg incubators was poor. Hence, in order to reduce *Salmonella* infection in chickens, increased sanitation of eggshells and egg incubators is needed in Shandong, China.

Salmonella Enteritidis was dominant in chicken hatcheries and breeding farms in this study. Fewer samples were collected from chicken hatcheries in 2012 than in 2009, resulting in an overall decrease in the number of *Salmonella* Enteritidis isolated. The finding that Enteritidis was the most common serovar in chickens is in accordance with some reports (Bangtrakulnonth et al., 2004; Van Duijkeren et al., 2002). Serotyping and PFGE analysis revealed a highly similar genetic

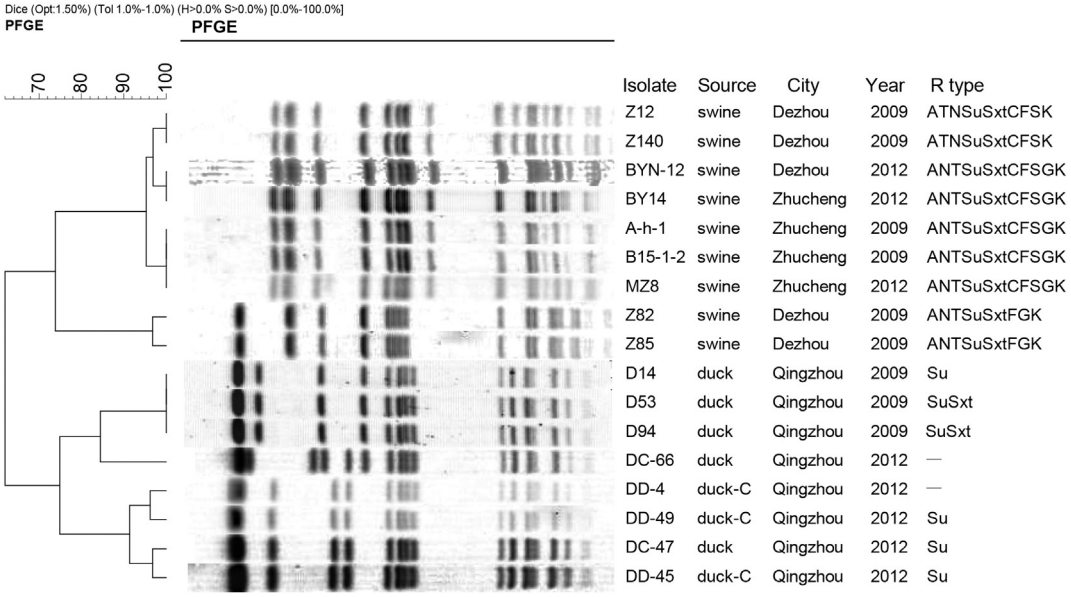

Fig. 3. PFGE profiles of representative Typhimurium isolates from ducks and pigs from Shandong province in 2009 and 2012. Note: "duck" represents the caecal contents of a 6-week-old duck and "duck-C" represents a duck carcass; —, susceptible to all tested antimicrobial agents in this study. Abbreviations: A, ampicillin; T, tetracycline; C, chloramphenicol; F, florfenicol; N, nalidixic acid; Cp, ciprofloxacin; S, streptomycin; G, gentamicin; K, kanamycin; Su, sulphafurazole; Sxt, trimethoprim/sulphamethoxazole.

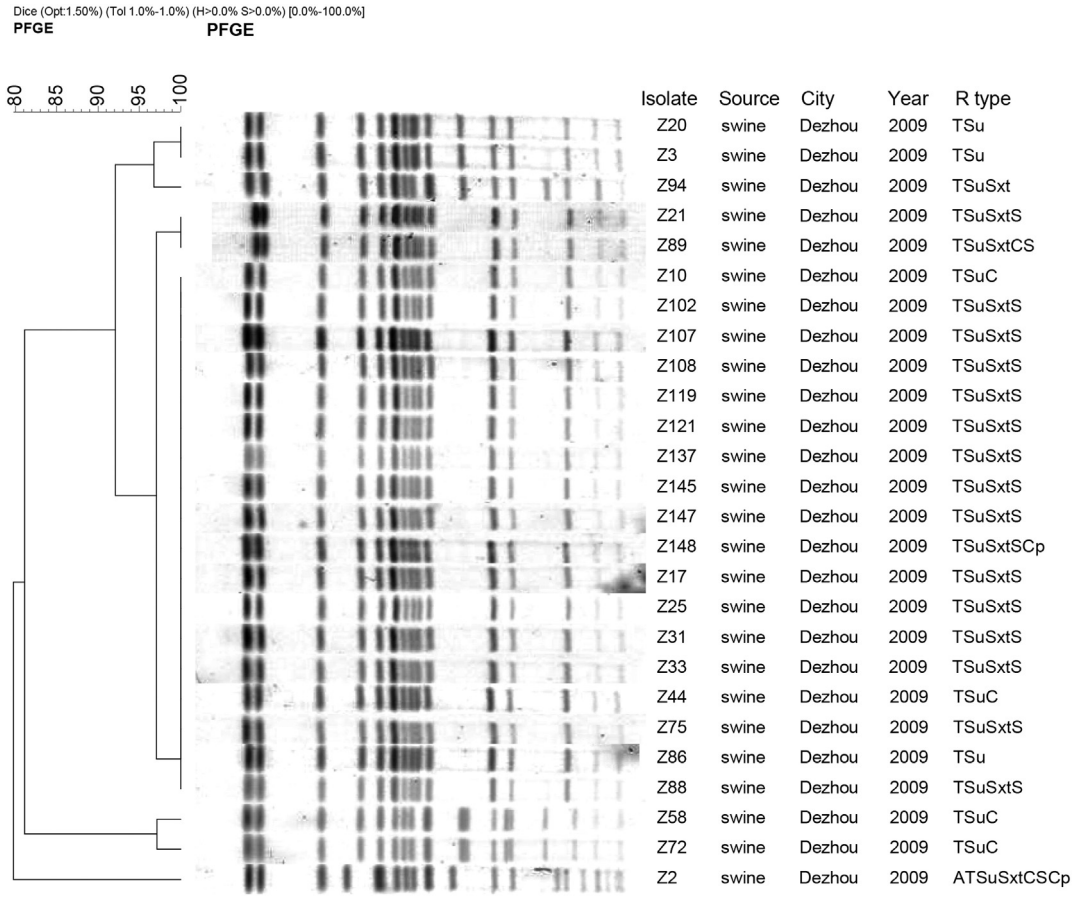

Fig. 4. PFGE profiles of representative Derby isolates from pigs in Shandong province in 2009. Abbreviations: A, ampicillin; T, tetracycline; C, chloramphenicol; N, nalidixic acid; Cp, ciprofloxacin; S, streptomycin; G, gentamicin; Su, sulphafurazole; Sxt, trimethoprim/sulphamethoxazole.

background for Enteritidis isolates from breeding farms, hatcheries and abattoirs in Shandong. The finding that the Enteritidis isolated from pigs was the same clone as that found in chickens suggests that this clone has adaptability to different types of food-producing animals. Enteritidis is a serovar frequently identified in other studies, and is one of the most common serovars that cause human salmonellosis (Thai et al., 2012). For food safety reasons, preventing the dissemination of *Salmonella* Enteritidis in food-producing animals is necessary. In one study, *Salmonella* Indiana was mostly found in poultry and was reported to be the causative agent of a salmonellosis outbreak in humans (Price

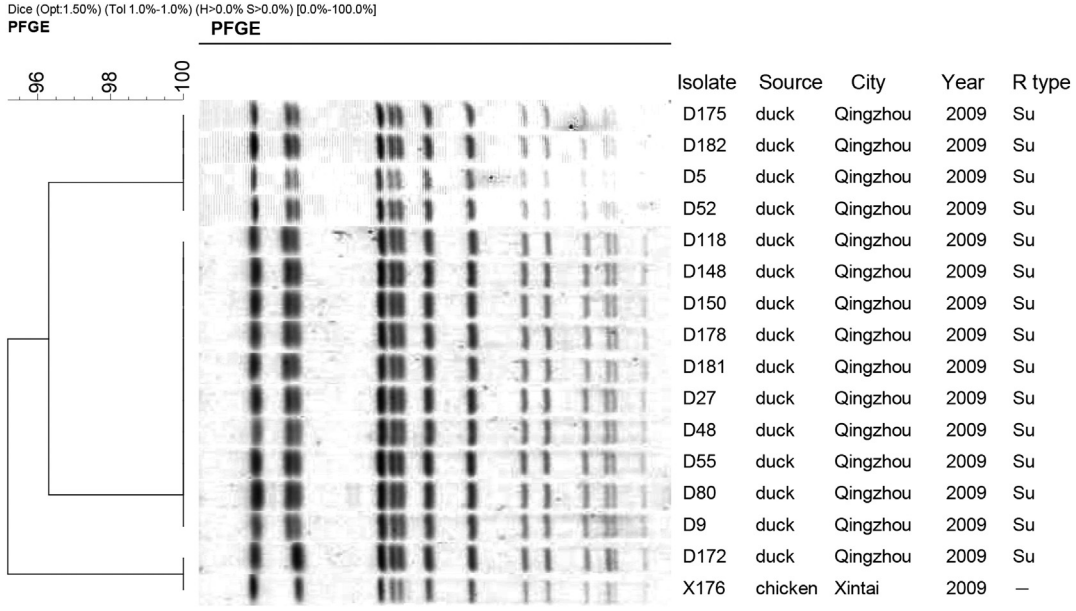

Fig. 5. PFGE profiles of representative Cremieu isolates from chickens and ducks in Shandong province in 2009. Note: —, susceptible to all tested antimicrobial agents in this study. Abbreviations: Su, sulphafurazole.

and Carter, 1967). In the present study, we showed that *Salmonella* Indiana was prevalent in both study years in 7-week-old broiler chickens and 6-week-old ducks. This is the first report of Indiana isolated from ducks used for food in mainland China. Data on the serovar Cremieu is rare; it is not commonly found across the globe. To the best of our knowledge, the 25 Cremieu isolates were the first reported serovar Cremieu isolated from ducks. Typhimurium was isolated from duck and pig farms in both sampling years, and, as found by other researchers, this serovar was readily isolated from pigs and ducks (Adzitey et al., 2012; Jong et al., 2009). However, we did not identify Typhimurium in chickens, a result that differs from studies conducted in many other countries (Lammerding et al., 1988; Van Duijkeren et al., 2002; Yang et al., 2010).

In 2012, we found that the overall antibiotic resistance levels were higher than those in 2009. This suggests that the long-term use of antibiotics in animal husbandry exerted a selection pressure on bacteria, thus making it easier for antibiotic-resistant *Salmonella* strains to survive. We found that the *Salmonella* sampled in this study were highly resistant to nalidixic acid and, to a lesser extent, to sulphonamides, ampicillin, tetracycline, florfenicol, gentamicin and streptomycin. The high resistance to sulphonamides, tetracycline, florfenicol and gentamicin observed in the present study was probably due to the use of antibiotic agents, which are incorporated into animal feed and which are present at therapeutic or sub-therapeutic levels to prevent bacteriosis or to promote animal growth. In 2006, the European Union withdrew approval for the use of antibiotics as growth promoters, over concerns about development of antibiotic resistance and transference of antibiotic resistance genes from animals to humans (Castanon, 2007). However, antibiotics are still widely used in food-producing animals in China. Ampicillin and streptomycin are commonly used to treat infectious diseases in veterinary practice. These drugs are inexpensive and easy to obtain; hence, high resistance to these particular antibiotics is not hard to explain. Resistance to ceftiofur was 4.7% in 2009 and 42.2% in 2012; these values are higher than those recorded for ceftiofur resistance in food of animal origins in Vietnam (0.0%) (Thai et al., 2012), and ceftiofur resistance in human clinical *Salmonella* isolates in Henan province of China (2%) (Xia et al., 2008). Ceftiofur is a third-generation cephalosporin approved for use in animal agriculture in China, and has the potential to be selected for resistance to third-generation cephalosporins (Singer et al., 2008). Ceftriaxone is often used to treat children with *Salmonella* infections (Douris et al., 2008). Due to similar or identical resistance mechanisms, cross-resistance between ceftiofur and ceftriaxone is common. Thus, the increased rate of ceftiofur-resistant *Salmonella* of animal origin has important public health implications. Nalidixic acid was one of the first generations of quinolones, and bacteria rapidly developed resistance to it. We found that the resistance rate to nalidixic acid (81.4%, 2009 and 95.9%, 2012; Table 3) was much higher than the resistance rate in Europe (≤10%) (Jong et al., 2009). Ciprofloxacin is a recognised first-line drug for the treatment of invasive salmonellosis in adults (Threlfall et al., 1999), and the reduced susceptibility to it detected in our study might be associated with the overuse of ciprofloxacin or use of enrofloxacin in food-producing animals. The structure of enrofloxacin is similar to ciprofloxacin and has a similar antibiotic spectrum. Because of its excellent bactericidal action on Gram negative and positive bacteria, enrofloxacin has been widely used in intensive farms in China in recent years. Moreover, we found that isolates resistant to ciprofloxacin were multidrug resistant strains (i.e., they were resistant to at least five antibiotics), which was in agreement with other studies (Vo et al., 2006).

Ninety-eight percent of *Salmonella* isolates in the current study were resistant to at least one antibiotic. This rate is much higher than the multidrug resistance reported in Vietnam (78.4%) and the Shaanxi province of China (21%) (Thai et al., 2012; Yang et al., 2010). The surveillance data from 2009 and 2012 showed a dramatic increase in the number of *Salmonella* strains that were resistant to four or more antibiotics (10.4%, 2009; 80.3%, 2012). The increase in antibiotic resistance in Enteritidis from chicken and multidrug-resistant Indiana isolated from ducks contributed to the significant increase in resistance. Unlike other common serovars, Enteritidis is typically susceptible to most antibiotics (Mølbak et al., 2002). In 2009, Enteritidis remained sensitive to most antibiotics (no isolate was resistant to ≥4 antibiotics) in Shandong. However, in 2012, 73.2% of the Enteritidis isolates were resistant to ≥4 antibiotics, whilst 25.3% were resistant to ≥10 antibiotics. Noticeably, most of the Enteritidis strains in 2012 were isolated from the same hatcheries or breeding farms as those in 2009, and all of them shared similar "molecular fingerprints". Multidrug resistance in Indiana was the most serious (100% resistance to ≥10 antibiotics) among the serovars isolated in this study. This multidrug resistance phenotype was similar to the phenotype of clinical Indiana isolates in the Henan province of China (i.e., it was resistant to at least 11 antibiotic agents) (Xia et al., 2008). Seven out of nine Typhimurium isolates of swine origin were resistant to at least 10 antibiotic agents. The common resistance phenotype (ampicillin/chloramphenicol/streptomycin/sulphafurazole/tetracycline) was similar to the phenotype of other Typhimurium isolates in other countries (Cruchaga et al., 2001; Mezali and Hamdi, 2012).

In summary, this study has shown that the *Salmonella* serotype distribution in Shandong is closely related with food-producing animal species, and the isolation rates and serotype distribution differed from other countries. Enteritidis was the dominant serotype in chickens. Derby and Typhimurium were the most common serovars found in pigs. Cremieu, Indiana and Typhimurium were the major serovars in ducks. Each serovar had one dominant PFGE cluster. Our results were consistent with the idea that cross-contamination occurred during poultry slaughter. Antibiotic resistance rates for *Salmonella* in 2012 were much higher than those in 2009, and the resistance phenotype was associated with particular serotypes and animal hosts. As antibiotic resistance in food-producing animals increases, there is a risk that it might be transferred to humans through the food chain and lead to clinical antibiotic chemotherapy failure. Therefore, it is necessary to plan intervention strategies to control the spread of antibiotic-resistant *Salmonella*, and appropriately manage the use of antibiotics in animal husbandry to prevent the further development and spread of antibiotic resistance and protect food safety.

Acknowledgements

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References

Adzitey, F., Rusul, G., Huda, N., 2012. Prevalence and antibiotic resistance of *Salmonella* serovars in ducks, duck rearing and processing environments in Penang, Malaysia. Food Res. Int. 45, 947–952.

Anderson, A.D., Nelson, J.M., Rossiter, S., Angulo, F.J., 2003. Public health consequences of use of antimicrobial agents in food animals in the United States. Microb. Drug Resist. 9, 373–379.

Arsenault, J., Letellier, A., Quessy, S., Boulianne, M., 2007. Prevalence and risk factors for *Salmonella* and *Campylobacter* spp. carcass contamination in broiler chickens slaughtered in Quebec, Canada. J. Food Prot. 70, 1820–1828.

Bacci, C., Boni, E., Alpigiani, I., Lanzoni, E., Bonardi, S., Brindani, F., 2012. Phenotypic and genotypic features of antibiotic resistance in *Salmonella enterica* isolated from chicken meat and chicken and quail carcasses. Int. J. Food Microbiol. 160, 16–23.

Bangtrakulnonth, A., Pornreongwong, S., Pulsrikarn, C., Sawanpanyalert, P., Hendriksen, R. S., Wong, D.M.A.L.F., Aarestrup, F.M., 2004. *Salmonella* serovars from humans and other sources in Thailand, 1993–2002. Emerg. Infect. Dis. 10, 131.

Barza, M., 2002. Potential mechanisms of increased disease in humans from antimicrobial resistance in food animals. Clin. Infect. Dis. 34, S123–S125.

Biedenbach, D.J., Toleman, M., Walsh, T.R., Jones, R.N., 2006. Analysis of *Salmonella* spp. with resistance to extended-spectrum cephalosporins and fluoroquinolones isolated in North America and Latin America: report from the SENTRY Antimicrobial Surveillance Program (1997–2004). Diagn. Microbiol. Infect. Dis. 54, 13.

Castanon, J.I., 2007. History of the use of antibiotic as growth promoters in European poultry feeds. Poult. Sci. 86, 2466–2471.

Clinical and Laboratory Standards Institute, 2008. Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals: Approved Standard, 3rd ed. CLSI, Wayne, PA (Informational Supplement. M31–A3).
Clinical and Laboratory Standards Institute, 2013. Performance Standards for Antimicrobial Susceptibility Testing. CLSI, Wayne, PA (Twenty-Third Informational Supplement. M100–S23).
Cruchaga, S., Echeita, A., Aladueña, A., García-Peña, J., Frias, N., Usera, M.A., 2001. Antimicrobial resistance in salmonellae from humans, food and animals in Spain in 1998. J. Antimicrob. Chemother. 47, 315–321.
Douris, A., Fedorka-Cray, P.J., Jackson, C.R., 2008. Characterization of *Salmonella enterica* serovar Agona slaughter isolates from the animal arm of the National Antimicrobial Resistance Monitoring System-Enteric Bacteria (NARMS): 1997 through 2003. Microb. Drug Resist. 14, 55–63.
Grimont, P.A.D., Weill, F.X., 2007. Antigenic Formulae of the *Salmonella* Serovars, World Health Organization Collaborating Centre for Reference and Research on *Salmonella*9th edition. Institut Pasteur.
Gupta, A., Fontana, J., Crowe, C., Bolstorff, B., Stout, A., Van Duyne, S., Hoekstra, M.P., Whichard, J.M., Barrett, T.J., 2003. Emergence of multidrug-resistant *Salmonella enterica* serotype Newport infections resistant to expanded-spectrum cephalosporins in the United States. J. Infect. Dis. 188, 1707–1716.
Henry, I., Granier, S., Courtillon, C., Lalande, F., Chemaly, M., Salvat, G., Cardinale, E., 2012. *Salmonella enterica* subsp. *enterica* isolated from chicken carcasses and environment at slaughter in Reunion Island: prevalence, genetic characterization and antibiotic susceptibility. Trop. Anim. Health Prod. 45, 317–326.
Jong, A., Bywater, R., Butty, P., Deroover, E., Godinho, K., Klein, U., Marion, H., Simjee, S., Smets, K., Thomas, V., Valle, M., Wheadon, A., 2009. A pan-European survey of antimicrobial susceptibility towards human-use antimicrobial drugs among zoonotic and commensal enteric bacteria isolated from healthy food-producing animals. J. Antimicrob. Chemother. 63, 733–744.
Lammerding, A., Garcia, M., Mann, E., Robinson, Y., Dorward, W., Truscott, R., Tittiger, F., 1988. Prevalence of *Salmonella* and thermophilic *Campylobacter* in fresh pork, beef, veal and poultry in Canada. J. Food Prot. 51, 47.
Li, R., Lai, J., Wang, Y., Liu, S., Li, Y., Liu, K., Shen, J., Wu, C., 2013. Prevalence and characterization of *Salmonella* species isolated from pigs, ducks and chickens in Sichuan Province, China. Int. J. Food Microbiol. 163, 14–18.
Lu, Y., Wu, C.M., Wu, G.J., Zhao, H.Y., He, T., Cao, X.Y., Dai, L., Xia, L.N., Qin, S.S., Shen, J.Z., 2011. Prevalence of antimicrobial resistance among *Salmonella* isolates from chicken in China. Foodborne Pathog. Dis. 8, 45–53.
Mezali, L., Hamdi, T.M., 2012. Prevalence and antimicrobial resistance of *Salmonella* isolated from meat and meat products in Algiers (Algeria). Foodborne Pathog. Dis. 9, 522–529.
Mølbak, K., Gerner-Smidt, P., Wegener, H.C., 2002. Increasing quinolone resistance in *Salmonella enterica* serotype Enteritidis. Emerg. Infect. Dis. 8, 514.
Price, J., Carter, H.R., 1967. An outbreak of gastroenteritis caused by *Salmonella* Indiana. Public Health Rep. 82, 551–554.
Pulsrikarn, C., Chaichana, P., Pornruangwong, S., Morita, Y., Yamamoto, S., Boonmar, S., 2012. Serotype, antimicrobial susceptibility, and genotype of *Salmonella* isolates from swine and pork in Sa Kaew Province, Thailand. Thai J. Vet. Med. 42, 21–27.
Ribot, E.M., Wierzba, R.K., Angulo, F.J., Barrett, T.J., 2002. *Salmonella enterica* serotype Typhimurium DT104 isolated from humans, United States, 1985, 1990, and 1996. Emerg. Infect. Dis. 8, 387.
Singer, R.S., Patterson, S.K., Wallace, R.L., 2008. Effects of therapeutic ceftiofur administration to dairy cattle on *Escherichia coli* dynamics in the intestinal tract. Appl. Environ. Microbiol. 74, 6956–6962.
Su, L.H., Chiu, C.H., Chu, C., Ou, J.T., 2004. Antimicrobial resistance in nontyphoid *Salmonella* serotypes: a global challenge. Clin. Infect. Dis. 39, 546–551.
Thai, T.H., Hirai, T., Lan, N.T., Yamaguchi, R., 2012. Antibiotic resistance profiles of *Salmonella* serovars isolated from retail pork and chicken meat in North Vietnam. Int. J. Food Microbiol. 156, 147–151.
Threlfall, E.J., Ward, L.R., Rowe, B., 1999. Resistance to ciprofloxacin in non-typhoidal salmonellas from humans in England and Wales—the current situation. Clin. Microbiol. Infect. 5, 130–134.
Tran, T.P., Ly, T., Nguyen, T.T., Akiba, M., Ogasawara, N., Shinoda, D., Okatani, T., Hayashidani, H., 2004. Prevalence of *Salmonella* spp. in pigs, chickens and ducks in the Mekong Delta, Vietnam. J. Vet. Med. Sci. 66, 1011.
Van Duijkeren, E., Wannet, W.J.B., Houwers, D.J., Van Pelt, W., 2002. Serotype and phage type distribution of *Salmonella* strains isolated from humans, cattle, pigs, and chickens in The Netherlands from 1984 to 2001. J. Clin. Microbiol. 40, 3980–3985.
Vo, A.T.T., Van Duijkeren, E., Fluit, A.C., Heck, M.E.O.C., Verbruggen, A., Maas, H.M.E., Gaastra, W., 2006. Distribution of *Salmonella enterica* serovars from humans, livestock and meat in Vietnam and the dominance of *Salmonella* Typhimurium phage type 90. Vet. Microbiol. 113, 153–158.
White, D.G., Zhao, S., Sudler, R., Ayers, S., Friedman, S., Chen, S., McDermott, P.F., McDermott, S., Wagner, D.D., Meng, J., 2001. The isolation of antibiotic-resistant *Salmonella* from retail ground meats. N. Engl. J. Med. 345, 1147–1154.
Xia, S., Hendriksen, R.S., Xie, Z., Huang, L., Zhang, J., Guo, W., Xu, B., Ran, L., Aarestrup, F.M., 2008. Molecular characterization and antimicrobial susceptibility of *Salmonella* isolates from infections in humans in Henan Province, China. J. Clin. Microbiol. 47, 401–409.
Yang, B., Qu, D., Zhang, X., Shen, J., Cui, S., Shi, Y., Xi, M., Sheng, M., Zhi, S., Meng, J., 2010. Prevalence and characterization of *Salmonella* serovars in retail meats of marketplace in Shaanxi, China. Int. J. Food Microbiol. 141, 63–72.