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Phenotypic characteristics and genotypic correlation between *Salmonella* isolates from a slaughterhouse and retail markets in Yangzhou, China

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ABSTRACT

An epidemiological investigation of *Salmonella* spp. in pig and pork samples from one slaughterhouse and its downstream retail markets in Yangzhou, Jiangsu Province, China, was conducted from October 2013 to March 2014. A total of 71.8% (155/216) and 70.9% (78/110), respectively, of the slaughterhouse and retail market samples were recovered positive for *Salmonella*. All *Salmonella* isolates were characterized using serotyping, antimicrobial resistance detection, multilocus sequence typing (MLST), and pulsed-field gel electrophoresis (PFGE). Seven serotypes were shared by isolates from the two sources, with the most common serotypes being *Salmonella* Derby, Typhimurium, and Uganda. Antimicrobial sensitivity testing revealed that the highest antimicrobial resistance rate was against tetracycline (49.7% and 37.2% in isolates from the slaughterhouse and retail market, respectively) with many multidrug-resistant (MDR) isolates in both sources. MLST analysis showed that eight sequence type (ST) patterns were shared, and ST40 occupied an absolute superiority among isolates from both sources. PFGE permitted the resolution of *Xba*I macrorestriction fragments of the selected 31 *Salmonella* Derby and 19 *Salmonella* Typhimurium into 30 and 10 distinct pulsotypes, displaying the high similarity between the isolates from the two sources. Our findings indicated that *Salmonella* isolates from a slaughterhouse and its downstream retail markets were phenotypically and genetically homologous. Additionally, *Salmonella* may propagate along the slaughter line and pork production chain from the slaughterhouse to retail markets.

1. Introduction

Salmonella, one of the most important pathogens studied every year in the world, can cause severe foodborne disease in humans and animals, impacting health and productivity (Majowicz et al., 2010). In China, approximately 70 to 80% of foodborne pathogenic outbreaks are caused by *Salmonella* (Wang et al., 2007). The majority of human *Salmonella* infections are associated with the ingestion of contaminated foods, such as pork, poultry, beef, egg, milk, cheese, and vegetables (Zhao et al., 2008).

Pigs have been recognized as one important reservoir for *Salmonella* (Li et al., 2013). *Salmonella* can be transferred to humans via pork along the food chain (Hauser et al., 2011). Therefore, as one of the countries with the largest pork production and consumption around the world, great attention should be paid to the prevalence and control of *Salmonella* with respect to pig slaughtering, pork manufacturing, and retail in China.

In recent years, many studies reported the prevalence and characterization of *Salmonella* along the pork production chain; the contamination of *Salmonella* was 10 to 40% in slaughterhouses, and the major serotypes were *Salmonella* Typhimurium and Derby (Algino et al., 2009; Arguello et al., 2012; Bonardi et al., 2013; Duggan et al., 2010). In retail markets, the prevalence of *Salmonella* isolates from pork samples was 1 to 40% (Li et al., 2014; Mihaiu et al., 2014; Miranda et al., 2009; Thai et al., 2012), and the serovars were diverse.

Though much attention has been focused on *Salmonella* in pig slaughterhouses and retail markets, intensive and simultaneous research regarding the prevalence, serotypes, antimicrobial resistance, multilocus sequence types (STs), and pulsed-field gel electrophoresis (PFGE) profiles of *Salmonella* in both sources is limited, especially in China. Therefore, the objective of this study was to analyze the distribution, antimicrobial susceptibility profiles, and molecular characteristics of *Salmonella* spp. collected from one pig slaughterhouse and its downstream pork retail markets in Yangzhou, Jiangsu Province, China, to determine the clonal relationships between isolates and the possible route of exposure.

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2. Material and methods

2.1. Sample collection

From October 2013 to March 2014, 326 samples were collected from one slaughterhouse, which processed about 600 pigs per workday, and its downstream retail markets in Yangzhou, Jiangsu Province, China. The sampling was separated into two parts.

i) From October 2013 to March 2014, 216 samples were collected at ten different stages including lairage, submitting, scalding water, cooling water, evisceration, visceral processing countertop, waste water, carcass dressing, mesenteric lymph nodes (MLNs), and floor along the slaughter line. Samples were randomly taken during three visits to the slaughterhouse every 2 months. All samples were collected using sterile sponges that were pre-moistened with buffered peptone water (BPW; Difco, BD, Sparks, MD, USA) as described previously (Bonardi et al., 2013). To prevent cross-contamination, gloves were worn during sampling and changed after each sample. Holding pens were sampled using the overshoe method. Carcasses were swabbed in each high-risk contamination area of 100 cm^2 (heel, belly, hip, notum, and jowl). Waste water, scalding and cooling water were sampled using sterile tagged collection tubes once per hour, and the temperature was measured. Ground specimens (visceral processing countertop and floor of each stage) were sampled by swabbing an area of 50 cm × 50 cm within each location. MLNs were collected and processed as previously reported (Anonymous, 2006). Samples were immediately stored in a cooled container after collection and sent to the laboratory for analysis.

ii) During the days after the slaughterhouse sampling, the retail samples were obtained. One hundred ten pork samples were collected from two downstream retail markets. Sampling was performed as described previously (Li et al., 2014). However, it was not possible to obtain both pork types (chop and piece) because of their availability.

2.2. Isolation and identification of Salmonella

For swab samples from the slaughterhouse, the pre-enrichment step was performed by suspending each sample in 50 mL BPW, and incubating samples at 37 °C for 16 to 18 h. Then, 0.1 mL of the BPW suspensions was subcultured in 10 mL subpackaged Rappaport–Vassiliadis (RV) enrichment broth (Difco, BD, Sparks, MD, USA) at 42 °C for 24 h. One loopful of each RV broth culture was then streaked onto xylose lysine tergitol 4 (Difco, BD, Sparks, MD, USA) agar plates, which were incubated at 37 °C for 24 to 48 h. One presumptive *Salmonella* colony per plate was picked and biochemically confirmed using an API-20E test kit (bioMérieux, Marcy l'Etoile, France). All strains were serotyped according to the Kauffmann–White scheme by slide agglutination with O and H antigen-specific sera (Tianrun Bio-Pharmaceutical, Ningbo, China). For pork samples from retail markets, each sample (25 ± 0.5 g) was aseptically weighed and transferred into 225 mL BPW and incubated at 37 °C for 18 h. The pre-enrichment and following isolation and identification were performed as described above.

2.3. Antimicrobial susceptibility testing

The Kirby–Bauer disk diffusion method was used to determine the isolates' antimicrobial susceptibility (Li et al., 2014). A total of 14 antimicrobial agents were applied: ampicillin (AMP, 10 μg), cefazolin (CFZ, 30 μg), ceftriaxone (CRO, 30 μg), cefotaxime (CTX, 30 μg), gentamicin (GEN, 10 μg), kanamycin (KAN, 20 μg), streptomycin (STR, 10 μg), nalidixic acid (NAL, 30 μg), ciprofloxacin (CIP, 5 μg), ofloxacin (OFX, 5 μg), norfloxacin (NOR, 5 μg), chloramphenicol (CHL, 30 μg), tetracycline (TET, 30 μg), and trimethoprim-sulfamethoxazole (SXT, 1.25–23.75 μg). Results were interpreted according to the established Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2013).

2.4. Multilocus sequence typing (MLST)

Confirmed isolates were grown aerobically in LB broth with shaking overnight at 37 °C. Genomic DNA was extracted with a TIANamp Bacteria DNA Kit (Tiangen, Beijing, China) in strict accordance with the manufacturer's protocol. MLST was carried out as described online (http://mlst.warwick.ac.uk/mlst/dbs/Senterica/documents/primersEnterica_html). All polymerase chain reaction products were purified and sequenced by Nanjing GenScript Biotech Co. (Nanjing, China), and the alleles and STs were assigned according to the MLST scheme at http://mlst.warwick.ac.uk/mlst/dbs/Senterica. A minimum spanning tree was generated using BioNumerics software, version 6.5 (Applied Maths, Kortrijk, Belgium) to analyze the distribution of STs in the slaughterhouse and retail markets.

2.5. PFGE

PFGE was performed according to the protocol by the Centers for Disease Control and Prevention (CDC) (Ribot et al., 2006) with some modifications. In brief, *Salmonella* isolates were streaked onto LB plates and incubated overnight at 37 °C. The pathogen concentration was modulated with bacterial suspensions until a McFarland turbidity of 4.0–4.5 was attained. DNA was digested with 50 U *Xba*I (Takara, Dalian, China) at 37 °C for 3 h. The digested DNA was separated by electrophoresis in 0.5 × TBE buffer at 14 °C for 20 h using a CHEF Mapper electrophoresis system (Bio-Rad, Hercules, CA, USA). The pulse time was ramped from 2.16 to 63.8 s. In addition, a control strain of *Salmonella* Braenderup (H9812), which served as a molecular weight standard, was processed with each batch of isolates. The gels were stained with ethidium bromide, and DNA patterns were visualized on a UV transilluminator (Bio-Rad, Hercules, CA, USA). Dendrograms were created by BioNumerics software version 6.5 (Applied Maths, Kortrijk, Belgium) using the unweighted pair group method with arithmetic means. The band-matching settings with optimization of 0.5% and position tolerance of 1.5% were applied.

2.6. Data analysis

Statistical comparison of prevalence and individual resistance to the 14 antimicrobial agents of *Salmonella* isolates in the slaughterhouse and retail markets were analyzed with the X^2 test, which was performed using the Statistical Package for the Social Sciences (version 15.0, SPSS, Chicago, IL, USA) with $P < 0.05$ considered as statistically significant.

3. Results

3.1. Salmonella prevalence and serotypes

A total of 155 (71.8%) and 78 (70.9%) isolates, respectively, were retrieved and scored as positive for *Salmonella* among the samples from the slaughterhouse and retail markets. Table 1 shows the detailed prevalence of *Salmonella* per visit in the slaughterhouse and retail markets,

Table 1
Prevalence of *Salmonella* isolated from slaughterhouse and retail markets.

Sample source	No. of times	No. of samples	No. of samples (%)positive for *Salmonella*	Total (%)
Slaughterhouse	Visit 1	72	46 (63.9)	155 (71.8)
	Visit 2	72	52 (72.2)	
	Visit 3	72	57 (79.2)	
Retail markets	Visit 1	33	15 (45.5)	78 (70.9)
	Visit 2	35	26 (74.3)	
	Visit 3	42	37 (88.1)	
Total		326	233 (71.5)	233 (71.5)

Table 2
Prevalence of *Salmonella* isolated from different stages of the slaughterhouse.

Source		Visit 1	Visit 2	Visit 3
Environmental samples				
Lairage (5*)		3** (60.0)***	2 (40.0)	3(60.0)
Scalding water (3)		0	0	0
Cooling water (3)		3 (100.0)	3 (100.0)	3(100.0)
Floor (5)		5 (100.0)	4 (80.0)	5 (100.0)
Waste water (1)		0	0	1(100.0)
Visceral processing countertop (1)		1(100.0)	0	1(100.0)
Slaughtering pig				
Lymph node (9)		0	1 (11.1)	0
CCarcass swab	Submitting (15)	5 (66.7)	12 (80.0)	14(93.3)
	Evisceration (15)	15(100.0)	15 (100.0)	15 (100.0)
	carcass dressing (15)	14 (93.3)	15 (100.0)	15 (100.0)

* The number of samples.
** The number of positive.
*** Positive rate.

and no significant difference ($P > 0.05$) was found among the slaughterhouse samples from different visits.

Almost all the three slaughterhouse samplings the prevalence rate of *Salmonella* was higher than 50.0% (Table 2). With regards to environmental samples, *Salmonella* was found most frequently on cooling water (100.0%). And for slaughtered pigs, *Salmonella* was found mostly on carcass swab samples after submitting (100%), which was much higher lymph node samples (11.1%).

Sixteen different serovars were identified among the 233 positive *Salmonella* isolates (Table 3), which included 14 serovars from the slaughterhouse and nine from retail markets. In total, seven serotypes were shared in the two sources; these serotypes were *Salmonella* Derby, Typhimurium, Meleagridis, Anatum, Uganda, Agona, and London. The most common serotype recovered from both the slaughterhouse and retail market samples was *Salmonella* Derby (27.1% and 38.5%); this was followed by Typhimurium (26.5% and 14.1%). Meanwhile, some serovars were exclusively found in one source; *Salmonella* Goldcoast were found in pork only, and *Salmonella* Kottbus was present in only the slaughterhouse samples.

3.2. Antimicrobial resistance phenotypes

The susceptibility of 233 isolates to 14 antimicrobial agents was assessed (Table 4). Resistance to TET was the most commonly observed resistance in both the slaughterhouse and retail market isolates (49.7% and 37.2%). High rates of resistance were also noted for STR (35.5% and 20.5%), NAL (14.8% and 34.6%) and SXT (26.5% and 11.5%). In contrast, low level of resistance was found for CRO and CTX, which were represented by two and three isolates, respectively.

Table 3
Serovar distribution of 233 *Salmonella* isolates.

Serovar	No. of isolates (%)		Total (%)
	Slaughterhouse	Retail markets	
Derby	42 (27.1)	30 (38.5)	72 (30.9)
Typhimurium	41 (26.5)	11 (14.1)	52 (22.3)
Meleagridis	24 (15.5)	2 (2.6)	26 (11.2)
Anatum	4 (2.6)	14 (17.9)	18 (7.7)
Uganda	13 (8.4)	9 (11.5)	22 (9.4)
Agona	11 (7.1)	2 (2.6)	13 (5.6)
London	4 (2.6)	3 (3.8)	7 (3.0)
4:d,f:-	8 (5.2)	0	8 (3.4)
Senftenberg	2 (1.3)	0	2 (0.9)
Risen	2 (1.3)	0	2 (0.9)
Goldcoast	0	6 (7.7)	6 (2.6)
Worthingten	1 (0.6)	0	1 (0.4)
Kottbus	1 (0.6)	0	1 (0.4)
Infantis	1 (0.6)	0	1 (0.4)
Virchow	0	1 (1.3)	1 (0.4)
Livingstone	1 (0.6)	0	1 (0.4)
Total	155	78	233

Table 4
Antimicrobial resistance phenotypes of 233 *Salmonella* isolates.

Antimicrobial agent	No. of resistant isolates (%)		
	Slaughterhouse (n = 155[a])	Retail markets (n = 78)	Total (n = 233)
β-Lactams			
Ampicillin (AMP)	27 (17.4)	18 (23.1)	45 (19.3)
Cefazolin (CFZ)	5 (3.2)	1 (1.3)	6 (2.6)
Ceftriaxone (CRO)	1 (0.6)	1 (1.3)	2 (0.9)
Cefotaxime (CTX)	2 (1.3)	1 (1.3)	3 (1.3)
Quinolone and fluoroquinolone			
Nalidixic acid (NAL)	23 (14.8)	27 (34.6)	50 (21.5)
Ofloxacin (OFX)	3 (1.9)	1 (1.3)	4 (1.7)
Norfloxacin (NOR)	1 (0.6)	4 (5.1)	5 (2.1)
Ciprofloxacin (CIP)	14 (9.0)	2 (2.6)	16 (6.9)
Tetracycline (TET)	77 (49.7)	29 (37.2)	106 (45.5)
Chloramphenicol (CHL)	18 (11.6)	13 (16.7)	31 (13.3)
Aminoglycosides			
Kanamycin (KAN)	11 (7.1)	3 (3.8)	14 (6.0)
Gentamicin (GEN)	17 (11.0)	5 (6.4)	22 (9.4)
Streptomycin (STR)	55 (35.5)	16 (20.5)	71 (30.5)
Sulfonamides			
Trimethoprim-sulfamethoxazole (SXT)	41 (26.5)	9 (11.5)	50 (21.5)

[a] n, number of *Salmonella*-positive isolates tested.

A significant high level of resistance to penicillins was found in both slaughterhouse and retail market isolates relative to the rate of resistance to cephalosporins ($P = 0.00188$ and 0.00149, respectively). The similar level of resistance was observed for NAL.

A total of 97 (62.6%) and 50 (64.1%) of the *Salmonella* isolates from the slaughterhouse and retail markets, respectively, were identified as resistant to at least one antimicrobial (Table 5), with the most common resistance patterns being TET and STR, and NAL only. The other main resistance patterns were TET only; SXT and TET; and SXT, TET, and STR in slaughterhouse isolates. In isolates from retail markets, the main resistance patterns were TET only; NAL, CHL, and TET; and AMP, STR, and TET. In total, we identified 37 and 23 discrete resistance patterns in isolates from the slaughterhouse and retail markets, respectively.

Of the multidrug-resistant (MDR) strains, 20 and 13 unique MDR patterns were observed in slaughterhouse and retail market isolates (Table 5), respectively, with most of them being *Salmonella* Typhimurium and Derby. Of the slaughterhouse isolates, the most frequent MDR pattern was SXT, NAL, GEN, KAN, AMP, CHL, TET, CIP, and STR, which was represented by eight *Salmonella* Typhimurium isolates, while that of retail market isolates was NAL, CHL, and TET, represented by *Salmonella* Typhimurium as well.

3.3. MLST analysis

An interlinked dataset with partial sequencing of seven housekeeping genes at 399 bp to 501 bp revealed that 18 STs among the 233 isolates were found, which included 15 from the slaughterhouse and 11 from the retail markets. The number of unique alleles for the various housekeeping genes ranged from 10 for *sucA* to 18 for *dnaN* (Table 5). ST40 was the most common ST in this study, both in the slaughterhouse and retail market isolates, and it was represented by 42 and 22 *Salmonella* isolates (Table 5), respectively.

The STs in this study were correlated with specific serovars such as ST40 with *Salmonella* Derby, ST19 with *Salmonella* Typhimurium, and ST463 with *Salmonella* Meleagridis. One ST corresponded to only one serotype. For example, all of the strains that were typed as ST71 were *Salmonella* Derby. In contrast, one serovar could be assigned to multiple STs. For instance, 29 of 41 isolates from the slaughterhouse that were characterized as *Salmonella* Typhimurium belonged to ST19, while the remaining belonged to ST34.

Table 5
Diversity profiles of *Salmonella* isolates based on MLST, serovar and antimicrobialresistance.

ST (n)[a]	Allele profile[b]	Serovar	Source[c]	Antimicrobial resistance[d]	No. (n) ofisolates
ST13 (13)	3, 3, 7, 4, 3, 3, 7	Agona	L3		1
		Agona	S3		1
		Agona	E3		4
		Agona	D3		2
		Agona	F3		2
		Agona	CW3		1
		Agona	R2		1
		Agona	R3		1
ST14 (2)	7, 6, 8, 8, 7, 8, 13	Senftenberg	F1		1
		Senftenberg	D1	CRO STR	1
ST19 (31)	10, 7, 12, 9, 5, 9, 2	Typhimurium	L2		2
		Typhimurium	S2		9
		Typhimurium	E2		6
		Typhimurium	D2		1
		Typhimurium	CW2		2
		Typhimurium	R2		1
		Typhimurium	S2	GEN	1
		Typhimurium	S2	OFX	1
		Typhimurium	D2	NAL	1
		Typhimurium	E2	STR	1
		Typhimurium	E2	AMP	1
		Typhimurium	R3	AMP	1
		Typhimurium	D2	NAL SXT	1
		Typhimurium	E2	AMP CHL SXT TET	1
		Typhimurium	S2	AMP CHL NAL SXT TET	1
		Typhimurium	E2	AMP CFZ CHL STR SXT TET	1
ST34 (21)	10, 19, 12, 9, 5, 9, 2	Typhimurium	S1	AMP STR TET	2
		Typhimurium	R2	AMP STR TET	3
		Typhimurium	R3	CHL NAL TET	4
		Typhimurium	R1	AMP CHL GEN KAN NAL SXT	1
		Typhimurium	S3	AMP CHL CIP GEN KAN NAL STR SXT	1
		Typhimurium	S3	AMP CHL CIP GEN KAN NAL STR SXT TET	8
		Typhimurium	E1	AMP CFZ CHL GEN KAN NAL NOR STR SXT TET	1
		Typhimurium	R3	AMP CFZ CHL CIP CRO CTX KAN NAL SXT TET	1
ST32 (1)	17, 18, 22, 17, 5, 21, 19	Infantis	D3	NAL	1
ST40 (64)	19, 20, 3, 20, 5, 22, 22	Derby	F2		1
		Derby	E3		1
		Derby	R2		1
		Derby	R3		2
		Derby	D2	TET	2
		Derby	E2	TET	1
		Derby	CW2	TET	1
		Derby	S3	TET	2
		Derby	E3	TET	2
		Derby	D3	TET	1
		Derby	F3	TET	1
		Derby	R1	TET	4
		Derby	R2	TET	1
		Derby	R3	TET	1
		Derby	R2	STR	2
		Derby	D2	SXT TET	1
		Derby	F2	SXT TET	1
		Derby	L3	SXT TET	2
		Derby	D2	NAL TET	1
		Derby	E2	NAL TET	1
		Derby	L1	STR TET	1
		Derby	E1	STR TET	2
		Derby	D1	STR TET	3
		Derby	CW1	STR TET	1
		Derby	E2	STR TET	1
		Derby	D2	STR TET	1
		Derby	F2	STR TET	1
		Derby	R1	STR TET	1
		Derby	D2	CFZ TET	1
		Derby	E2	CIP TET	1
		Derby	R2	AMP STR	1
		Derby	R2	CHL TET	1
		Derby	D2	AMP GEN TET	1
		Derby	D2	GEN OFX TET	1
		Derby	LN2	CHL NAL TET	1
		Derby	R1	CHL NAL TET	1
		Derby	E2	STR SXT TET	1
		Derby	D2	STR SXT TET	2
		Derby	F2	STR SXT TET	1

(continued on next page)

Table 5 (*continued*)

ST (n)[a]	Allele profile[b]	Serovar	Source[c]	Antimicrobial resistance[d]	No. (n) ofisolates
		Derby	L1	CTX STR TET	1
		Derby	R1	AMP STR TET	1
		Derby	R2	AMP SXT TET	1
		Derby	R2	NOR STR TET	1
		Derby	E1	AMP STR TET	1
		Derby	CW3	AMP STR TET	1
		Derby	R3	AMP STR TET	1
		Derby	R1	AMP NAL SXT TET	1
		Derby	S1	AMP NAL STR TET	1
		Derby	D3	AMP CIP NAL STR TET	1
		Derby	R1	AMP NAL STR SXT TET	1
		Derby	R2	AMP CHL CIP GEN KAN NAL NOR TET	1
ST71 (8)	39, 35, 8, 36, 29, 9, 36	Derby	R2		1
		Derby	R3		7
ST64 (18)	10, 14, 15, 31, 25, 20, 33	Anatum	D2	NAL	1
		Anatum	E3	NAL	2
		Anatum	R1	NAL	2
		Anatum	R2	NAL	2
		Anatum	R3	NAL	8
		Anatum	E1	NAL STR	1
		Anatum	R1	NAL STR	1
		Anatum	R2	NAL NOR	1
ST155 (7)	10, 60, 58, 66, 6, 65, 16	London	S3	AMP CHL GEN STR SXT TET	1
		London	F3	AMP CHL GEN STR SXT TET	1
		London	D3	AMP CHL GEN KAN STR SXT TET	1
		London	F1	AMP CFZ CHL GEN STR SXT TET	1
		London	R2	AMP CHL GEN NAL STR TET	1
		London	R2	AMP CHL GEN STR SXT TET	1
		London	R2	AMP CHL GEN NAL NOR OFX STR SXT TET	1
ST279 (8)	62, 95, 54, 96, 100, 9, 100	4:d,f:-	S3		1
		4:d,f:-	E3		2
		4:d,f:-	D3		4
		4:d,f:-	D3	STR	1
ST358 (6)	5, 110, 35, 122, 2, 19, 22	Goldcoast	R3		6
ST463 (26)	92, 125, 78, 128, 138, 9, 141	Meleagridis	E1		2
		Meleagridis	D3		2
		Meleagridis	F3		1
		Meleagridis	CW3		1
		Meleagridis	VPC3		1
		Meleagridis	E1	SXT TET	3
		Meleagridis	D1	SXT TET	1
		Meleagridis	F1	SXT TET	1
		Meleagridis	CW1	SXT TET	1
		Meleagridis	R2	SXT TET	1
		Meleagridis	D1	CIP TET	1
		Meleagridis	D1	STR TET	2
		Meleagridis	CW1	STR TET	1
		Meleagridis	L1	STR SXT TET	1
		Meleagridis	E1	STR SXT TET	3
		Meleagridis	F1	STR SXT TET	1
		Meleagridis	E1	OFX STR SXT TET	1
		Meleagridis	D1	CIP STR SXT TET	1
		Meleagridis	R1	AMP CHL STR SXT TET	1
ST469 (2)	92, 107, 79, 156, 64, 151, 87	Rissen	E1	AMP SXT TET	1
		Rissen	VPC1	AMP SXT TET	1
ST592 (1)	189, 70, 68, 132, 175, 9, 172	Worthington	D1	CFZ STR	1
ST638 (1)	47, 98, 36, 152, 174, 7, 171	Livingstone	D3		1
ST684 (22)	147, 13, 15, 123, 15, 19, 17	Uganda	D1		2
		Uganda	E3		4
		Uganda	D3		1
		Uganda	WW3		1
		Uganda	R1		1
		Uganda	R2		2
		Uganda	R3		5
		Uganda	F1	CIP	1
		Uganda	R2	AMP	1
		Uganda	S1	STR	1
		Uganda	D1	STR	2
		Uganda	S1	CTX STR	1
ST808 (1)	10, 71, 43, 12, 190, 20, 18	Kottbus	D2		1
ST1750 (1)	75, 67, 10, 17, 15, 9, 14	Virchow	R2	NAL	1

[a] n, number of *Salmonella*-positive isolates tested.
[b] Allele number for *aroC*, *dnaN*, *hemD*, *hisD*, *purE*, *sucA*, and *thrA*, respectively (one for each ST).
[c] L, lairage; S, submitting; E, evisceration; D, carcass dressing; F, floor; CW, cooling water; WW, waste water; VPC, visceral processing countertop; LN, lymph node; R, retail markets. Numbers correspond to different sampling visits.

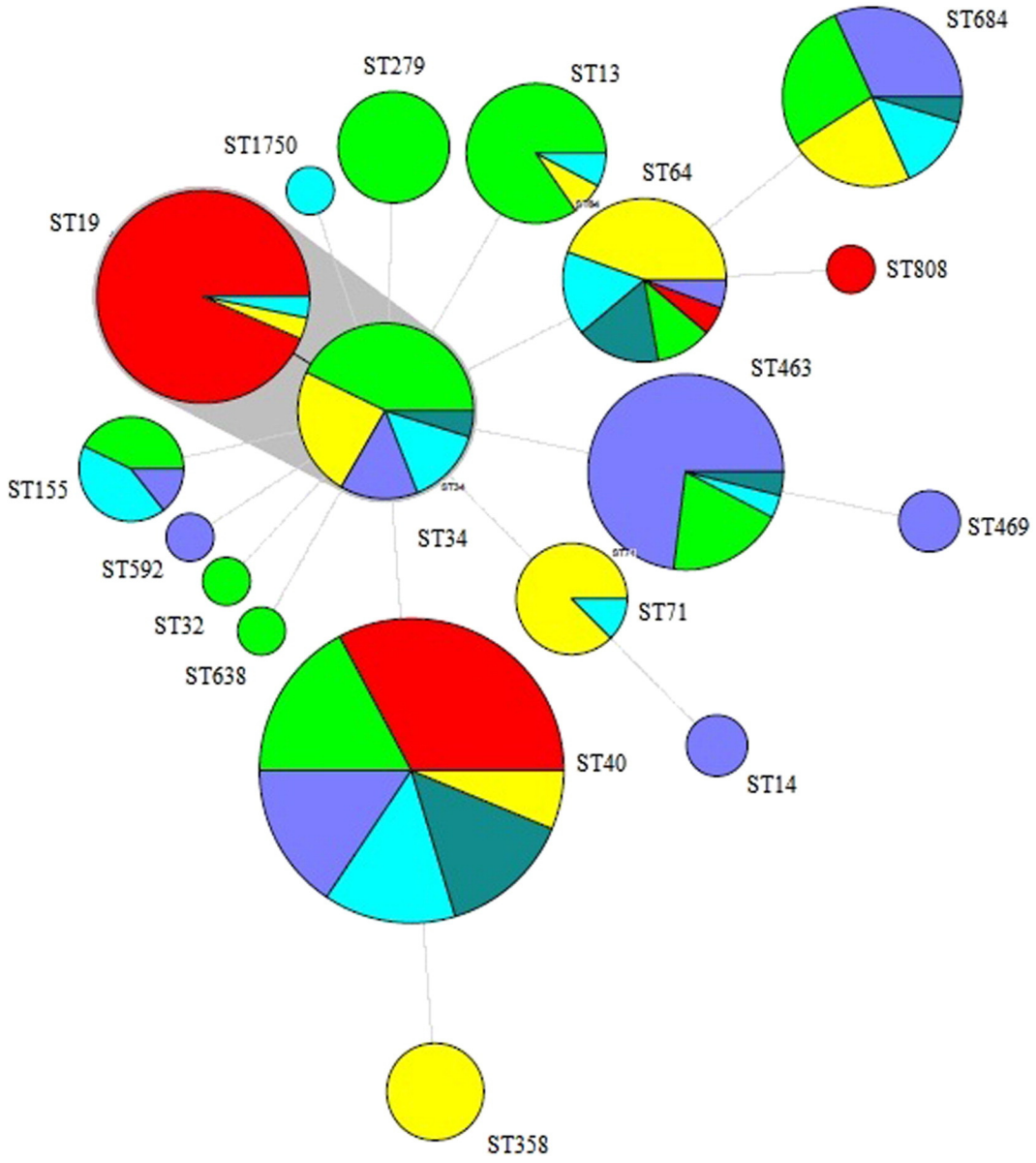

Fig. 1. Minimum spanning tree analysis of *Salmonella* isolated from a slaughterhouse and retail markets. Each circle represents one ST, and the area of the circle corresponds to the number of isolates. STs that were observed in the first, second, and third visit of retail markets are represented by aqua, light blue, and yellow, respectively. STs that were observed in the first, second, and third slaughterhouse visit are represented by purple, red, and green, respectively. The gray region indicates that ST19 and ST34 belong to a clonal complex.

A minimum spanning tree of all STs from both sources was generated using BioNumerics version 6.5 (Fig. 1). ST40 was found in every visit to both the slaughterhouse and retail markets, and it occupied an absolute advantage in number. In total, eight STs were shared among the slaughterhouse and retail market isolates, including ST13, ST19, ST34, ST40, ST64, ST155, ST463, and ST684. ST14, ST32, ST279, ST469, ST592, ST638, and ST808 were exclusive to the slaughterhouse isolates. Among the retail market isolates, ST71, ST358, and ST1750 were unique. In each batch of slaughterhouse and retail market samples, common STs were always discovered in both sources. For instance, during the first visit, ST34, ST40, ST64, and ST684 were shared; in the second visit, ST19, ST40, and ST64 were common.

3.4. PFGE analysis

A total of 31 *Salmonella* Derby and 19 Typhimurium isolates selected randomly from the six visits of both sources were characterized using PFGE. The PFGE patterns of all isolates differed by 1 to 3 bands from 12 to 15 *Xba*I fragments. Fig. 2 shows the PFGE fingerprint profiles of the selected *Salmonella* Derby isolates. For the 31 *Salmonella* Derby isolates, 30 PFGE patterns were identified, which grouped into seven clusters (A–G). Isolates from different sources during the same sampling visit possessed a significantly high similarity (>90%), such as N1-T5 and P1-E8 from the first retail market and slaughterhouse sampling, respectively, in cluster A and N2-J18 and P2-D3 from the second retail market and slaughterhouse sampling in cluster B. Among the chosen *Salmonella* Typhimurium isolates (Fig. 3), 10 PFGE patterns were characterized, and they were grouped into three clusters (A–C). In line with *Salmonella* Derby, *Salmonella* Typhimurium isolates from different sources also had highly similar PFGE patterns, which can be represented by N1-J2 and P1-E15 and by N2-T11 and P2-D8.

4. Discussion

In this study, the prevalence and distribution of *Salmonella* in a slaughterhouse and its downstream retail markets in Yangzhou were

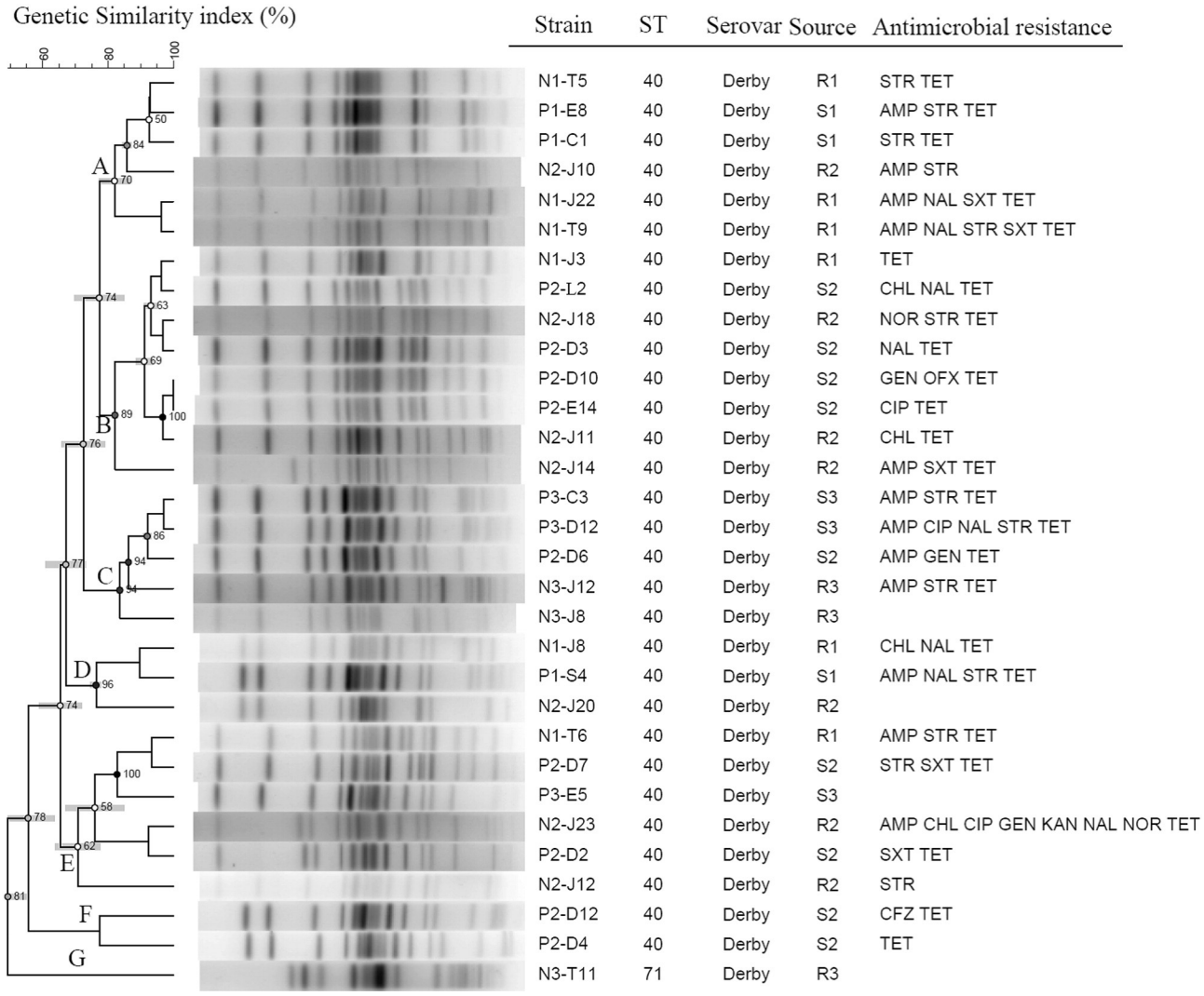

Fig. 2. Dendrogram of PFGE patterns of 31 *Salmonella* Derby isolates recovered from a slaughterhouse and retail markets and their relationship with ST, serotype, source, and antimicrobial resistance. Seven clusters (A–G) were identified. In the strain column, the first letter corresponds to different sources, N for retail market and P for slaughterhouse. The first and second numbers correspond to different sampling times and sample numbers, respectively. For slaughterhouse strains, the second letter represents different stages, E for evisceration, C for cooling water, L for lymph node, D for carcass dressing and S for submitting. While for retail market strains, the second letter represents different markets, T for shitasi and J for sijiyuan.

investigated. MLST and PFGE were used to assess the clonal relationship between isolates from the two sources, leading to the conclusion that *Salmonella* in the retail markets originated from the slaughterhouse.

Salmonella spp. were recovered from a slaughterhouse and its downstream retail markets in Yangzhou, Jiangsu Province, with an overall prevalence of 71.8% and 70.9%, respectively. For the slaughterhouse, the prevalence was significantly higher than that reported in Sichuan Province, China (10.7%) (Li et al., 2013), and in Germany (13.2%) (Visscher et al., 2011), but it was lower than that reported in Hong Kong (75%) (Chau et al., 1977). The prevalence in retail markets was higher than that in some districts in China and other countries (Hansen et al., 2010). However, several factors should be considered when comparing the prevalence of *Salmonella* in different areas, including sampling seasons, sample amounts, isolation methods, slaughterhouse management, and slaughtering procedure.

For serotyping, a total of 16 serovars were found among the 233 isolates, including 14 from the slaughterhouse and nine from the retail markets, which incorporated seven that were shared. The most common serotype in both sources was *Salmonella* Derby. To our knowledge, *Salmonella* Derby was commonly isolated from pork and slaughter pigs, and it was one of the most frequent serotypes that cause human salmonellosis in many countries (EFSA, 2008a). In recent years, *Salmonella* Derby has displayed increased prevalence in the world. For instance, *Salmonella* Derby has surpassed Typhimurium as the predominant serovar that is isolated from pig slaughterhouses (Bonardi et al., 2013) and was the most common serotype isolated from infants and toddlers in China (Cui et al., 2009). In addition, high isolation rates of *Salmonella* Typhimurium and Uganda were also noticed from both sources. *Salmonella* Typhimurium remains one of the most important serotypes that can lead to severe human and animal diseases. For example, 4,[5],12:i:-, a variant of Typhimurium, has been an emerging cause of infection around the world in recent years (Madajczak et al., 2014). *Salmonella* Uganda has been continuously isolated in other countries but was found sporadically in China (Kuo et al., 2014).

Though relevant departments have been emphasizing the limited use of antibiotics in animal feeding, the effect was small. Antimicrobial resistance in *Salmonella* is one of threats to human public health. Our results indicated that a similar proportion of slaughterhouse and retail market isolates were resistant to at least one antibiotic (65.2% and 70.5%, respectively). The highest rates of antimicrobial resistance were against TET in both sources (49.7% and 37.2%); this was somewhat expected because of its wide use in animal feed and was consistent with reports from slaughterhouses in Italy (Piras et al., 2011) and retail markets in Mexico (Miranda et al., 2009). Resistance to STR was also

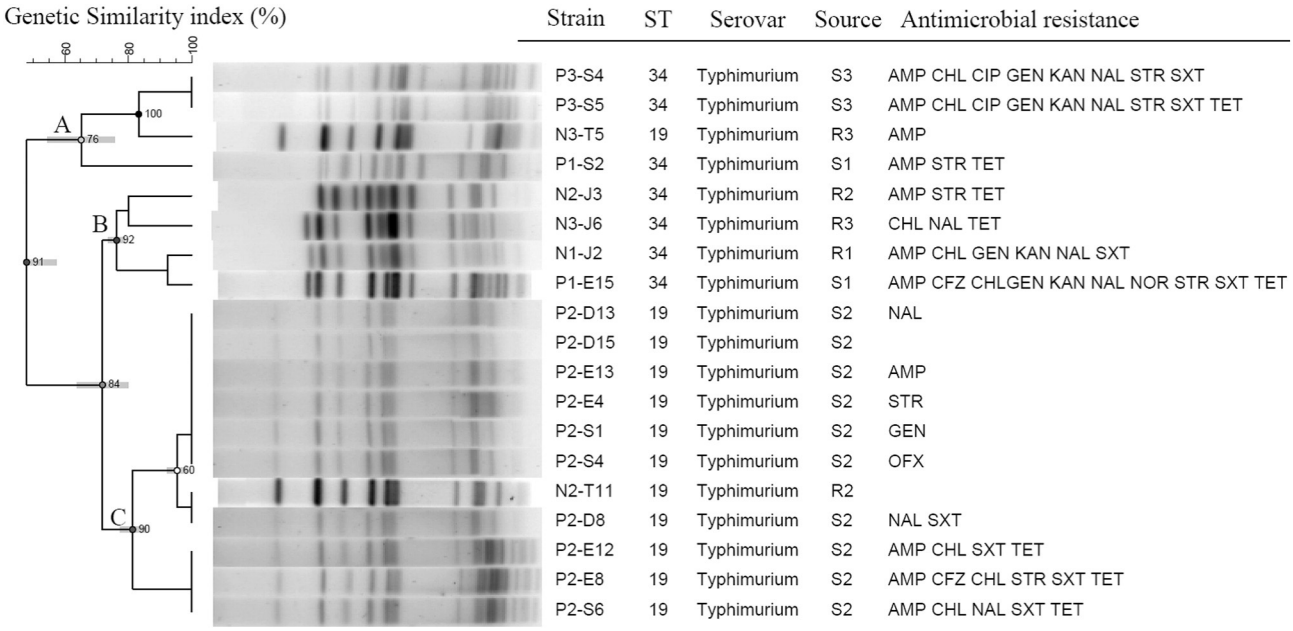

Fig. 3. Dendrogram of PFGE patterns of 19 *Salmonella* Typhimurium isolates recovered from a slaughterhouse and retail markets and their relationship with ST, serotype, source, and antimicrobial resistance. Three clusters (A–C) were identified. In the strain column, the first letter corresponds to different sources, N for retail market and P for slaughterhouse. The first and second numbers correspond to different sampling times and sample numbers, respectively. For slaughterhouse strains, the second letter represents different stages, E for evisceration, D for carcass dressing and S for submitting. While for retail market strains, the second letter represents different markets, T for shitasi and J for sijiyuan.

frequently observed in isolates from both sources, which could be explained by the accompany mechanism (Manie et al., 1998). Apart from these, resistance to NAL in 34.6% of retail market isolates and 14.8% of slaughterhouse isolates deserves our attention because resistance to this antimicrobial agent may lead to the delay or failure of fluoroquinolone therapies (Van et al., 2007). MDR *Salmonella* isolates were frequently observed among the slaughterhouse and retail market isolates in this study, especially in *Salmonella* Typhimurium and Derby. Of particular concern, one *Salmonella* Typhimurium slaughterhouse isolate and one *Salmonella* Typhimurium retail market isolate were resistant to 10 antimicrobials, posing great risk to public health if these MDR strains were transferred to humans via pork or pork-derived products (Li et al., 2014).

MLST results revealed that a total of 15 and 11 STs were identified in slaughterhouse and retail market isolates. Among them, ST40 was the most frequent genotype that was recovered in this study, and Fig. 1 shows that ST40 was present in each sampling visit of both sites. Therefore, we speculated that strains of this ST persist in both sites and are continually propagated (Piras et al., 2011). Moreover, ST40 has been widely found in pigs and pork from Europe and the United States and in humans from Asia and Europe (Li et al., 2014). To some extent, this observation indicates that *Salmonella* can spread from swine to humans via pork and pork-derived products (Hauser et al., 2011).

ST19 and ST34 have continually been reported to cause human salmonellosis in recent years (Garvey et al., 2013). And these two STs were also in great diversity in the two sources, which should be treated cautiously. Additionally, isolates that were characterized as ST19 and ST34 belong to the same serotype, *Salmonella* Typhimurium, and the same circumstance was also true for *Salmonella* Derby, which was represented by ST40 and ST71. These findings suggest that serovars and STs were tightly coupled (Achtman et al., 2012).

Another case that should be considered is ST358, an emerging ST in our study and a new ST that appeared in China that corresponds to *Salmonella* Goldcoast, which caused an unusual increase of human cases of *Salmonella* Goldcoast infection in Hungary in 2009 (Horvath et al., 2013). Therefore, it is urgent for healthcare sectors to supervise and prevent these cases.

PFGE, a valid and in-depth tool to investigate bacterial characterizations, has been frequently used to determine the genetic relationships between *Salmonella* isolates (Angelo et al., 2015). PFGE typing allowed more precise tracing of *Salmonella* Typhimurium and Derby isolates, distinguishing 20 *Salmonella* Typhimurium and 16 Derby genotypes from 390 and 285 isolates from two plants (Giovannacci et al., 2001). In our study, the selected 31 *Salmonella* Derby isolates characterized as ST40 or ST71, and 19 *Salmonella* Typhimurium typed as ST19 or ST34 were subdivided into 30 and 10 pulsotypes, respectively, via PFGE, which strengthened the discriminatory power and traceability of *Salmonella* Derby and Typhimurium at the chromosome level.

Salmonella transmission from the slaughterhouse to retail markets was frequently observed in almost all of the sampling visits except visit 3. This transmission was represented by *Salmonella* isolates P1-C1 and N1-T5 in cluster A and P2-L2 and N2-J11 in cluster B during visit 1 and 2, respectively, in the dendrogram of *Salmonella* Derby. Analogous circumstances also appeared in the dendrogram of *Salmonella* Typhimurium, which were represented by N1-J2 and P1-E15 in cluster B and N2-T11 and P2-D8 in cluster C. These isolates, whose PFGE patterns were highly consistent (>90% similarity), demonstrated that *Salmonella* clones spread along the pork production chain (Hauser et al., 2011, 2012; Li et al., 2013). However, no isolates from visit 3 had PFGE fingerprints that were similar to those from visit 1 and 2, which could be explained by insufficient samples and different outlets from the slaughterhouse (Sandt et al., 2013).

Salmonella transmission along the slaughter line has also been widely discovered in slaughterhouses (Keelara et al., 2013). On the three slaughterhouse samplings, prevalence rate of *Salmonella* of all the stages was almost beyond 50%, especially in cooling water and evisceration stages. Cooling water was easily contaminated by dirt, feces and ingesta carried by the slaughtering pigs and it could be contamination sources of subsequent stages, represented by *Salmonella* Derby P1-E8 and P1-C1, P3-C3 and P3-D12. *Salmonella* transmission occurred in other stages along the slaughter line, such as lymph node and carcass dressing, submitting and evisceration, were also obvious from *Salmonella* Derby P2-L2 and P2-D3 and *Salmonella* Typhimurium P2-E8 and P2-S6, respectively. The direct or indirect correlation of these isolates

with final retail pork-derived products deserves special attention. Also, further studies are required to prove the conclusions as the sampling intervals were somewhat too long to provide sufficient data.

5. Conclusion

In summary, we examined the epidemiology of *Salmonella* in one slaughterhouse and its downstream retail markets in Yangzhou, China. Our results indicated a high prevalence of *Salmonella* in both sources. *Salmonella* isolates from the two sources were demonstrated to be genetically similar by MLST and PFGE, and *Salmonella* was transferred along the slaughterhouse line and from the slaughterhouse to retail markets. Furthermore, many serovars that were reported in humans in other countries and MDR *Salmonella* were recovered in this study. Therefore, it is imperative for healthcare departments in China to manage, supervise, and prevent these cases. Also, more legislation about slaughtering procedures and prudent use of antimicrobials in animal feeds should be enacted by the authorities to control the spread of *Salmonella* from pigs to humans.

Acknowledgments

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