

Research Note

Prevalence and Antimicrobial Resistance of *Salmonella* Isolates Recovered from Retail Pork in Major Village Markets in Tai'an Region, China

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ABSTRACT

The current study was undertaken to evaluate *Salmonella* contamination in retail pork at major village markets of the Tai'an region, China. In total, 200 retail pork samples were collected from four village markets between June 2015 and February 2016, of which 69 samples (34.5%) were determined to be positive for *Salmonella*. Eleven serotypes were identified from the 69 *Salmonella* isolates, and *Salmonella* Derby was the most common (18 of 69, 26.1%), followed by Typhimurium (17 of 69, 24.6%) and Meleagridis (11 of 69, 15.9%). Antimicrobial susceptibility testing showed that antimicrobial resistance against tetracycline was the most prevalent (42 of 69, 60.9%), but antimicrobial resistance against both ceftriaxone and cefotaxime was 1.4% (1 of 69) and 2.9% (2 of 69), respectively. Multilocus sequence typing revealed that the 69 *Salmonella* isolates were divided into 11 sequence types (STs), among which ST40 (18 of 69, 26.1%) was the most common, followed by ST34 (15 of 69, 21.7%) and ST64 (13 of 69, 18.8%). Collectively, retail pork at village markets in the Tai'an region has a high *Salmonella* contamination rate, and these isolates exhibit broad-spectrum antimicrobial resistance. However, the absence of a dominant ST demonstrates that the *Salmonella* isolates from retail pork may be of diverse origins.

Key words: Antimicrobial resistance; Multilocus sequence typing; Retail pork; *Salmonella*; Village markets

Salmonella is one of the most important foodborne pathogens that not only causes huge economic losses to animal husbandry and food processing industries but also threatens public health safety (7, 21, 24). Most of human *Salmonella* infections worldwide are related to the consumption of *Salmonella*-contaminated animal foods, such as chicken, pork, beef, and eggs (24, 25, 33). In the People's Republic of China, 70 to 80% of bacteria-induced food poisoning cases are related to *Salmonella* (28). As one of the top pig-producing and pork-consuming nations (6, 19, 24), the issue of *Salmonella* contamination of pork in China should be examined with greater care because this will be of great significance for the control of foodborne salmonellosis.

Importantly, there has been a gradual increase in antimicrobial resistance in *Salmonella* worldwide as a result of the widespread use of antibiotics in treating animal diseases and promoting animal growth (2, 4, 8, 12, 16, 18). These antimicrobial-resistant *Salmonella* could not only be transmitted to humans via the food chain but also make it increasingly difficult to treat *Salmonella* infections in clinical practice (14, 15, 23, 26). Therefore, understanding the contamination status of *Salmonella* in animal-derived

foods and their antimicrobial resistance characteristics is of utmost importance for effectively controlling the dissemination of *Salmonella*.

Numerous studies have been conducted on *Salmonella* contamination in pork in China (6, 28, 29, 32). For example, the *Salmonella* contamination rate of retail pork in Shaanxi province, China, was 31% in 2007 to 2008 (31). Furthermore, 12.8 to 15.8% of retail pork samples in Jiangsu province, China, were reported to be contaminated with *Salmonella* in 2010 to 2012 (19). However, these studies were mainly carried out in urban areas, and there have been very few reports on the prevalence of *Salmonella* contamination of retail pork in village markets in China. Therefore, in the current study we examined *Salmonella* contamination and molecular epidemiological characteristics in retail pork samples collected from village markets in rural areas of the Tai'an region in China.

MATERIALS AND METHODS

Sample collection. In total, 200 pork samples were collected from four major rural village retail markets in the region surrounding Tai'an city—Daolang, Liangzhuang, Dajinkou, and Fanzhen (50 samples per town)—between June 2015 and February 2016; they are situated 20 to 35 km west, south, north, and east of the Tai'an region, respectively. These village markets are open air, and no refrigerated equipment was in use.

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TABLE 1. Prevalence of *Salmonella* in retail pork samples from four major village markets^a

Market	No. of samples	Prevalence (%)
Liangzhuang	50	19 (38.0)
Fanzhen	50	17 (34.0)
Dajinkou	50	17 (34.0)
Daolang	50	16 (32.0)
Total	200	69 (34.5)

^a One *Salmonella* isolate was collected from each positive sample.

Two main pork types, chop and piece, were randomly collected from various market stalls (two to three samples per stall) and were rapidly transferred into sterile sample collection bags that were subsequently stored in a freezer box. Samples were transported to the laboratory within 6 h after collection for further process.

Isolation and identification of *Salmonella*. Approximately 25 g of each sample was placed into 225 mL of sterile buffered peptone water in an Erlenmeyer flask and grown for 6 h at 37°C with shaking at 100 rpm. After the preliminary enrichment, 10 and 1 mL of the enriched culture was transferred into 100 mL each of the tetrathionate and Rappaport-Vassiliadis broth, respectively, and incubated for 24 h at 42°C with shaking at 100 rpm. The bacterial cultures in tetrathionate and Rappaport-Vassiliadis media were then plated on xylose lysine Tergitol 4 agar and xylose lysine desoxycholate agar, respectively, and incubated at 37°C for 24 to 48 h. Two to three colonies suspected to be *Salmonella* were plated on MacConkey agar plates for purification. Identification of the purified *Salmonella*-like colonies was performed using an API 20E test kit (bioMérieux, Inc., Marcy-l'Étoile, France). Only one *Salmonella* strain from each *Salmonella*-positive sample was randomly selected for serotyping, antimicrobial susceptibility, and multilocus sequence typing (MLST).

Serotyping. O and H antigens of *Salmonella* isolates were characterized using the slide agglutination test with hyperimmune sera (S&A Company, Bangkok, Thailand), and the serotypes were determined based on the manufacturer's instructions.

Antimicrobial susceptibility testing. The Kirby-Bauer disk diffusion method as described by the Clinical and Laboratory Standards Institute was performed (9). A panel of 12 antibiotics were used in this study: ampicillin (10 µg), cefotaxime (30 µg), kanamycin (20 µg), tetracycline (30 µg), gentamicin (10 µg), trimethoprim-sulfamethoxazole (1.25/23.75 µg), ciprofloxacin (5 µg), ofloxacin (5 µg), norfloxacin (5 µg), cefazolin (30 µg), ceftriaxone (30 µg), and streptomycin (10 µg). *Escherichia coli* ATCC 25922 was used as the quality control strain.

DNA extraction. *Salmonella* was grown overnight on Luria-Bertani agar plates, and the colonies were collected using a sterile cotton swab. The swab was washed in sterile normal saline to prepare a bacterial suspension equivalent to a 0.5 McFarland standard. In total, 800 µL of bacterial suspension was then transferred into a 1.5-mL Eppendorf tube, boiled at 100°C for 10 min, and centrifuged at 12,000 × *g* for 5 to 8 min. Then, the supernatant was recovered.

MLST. MLST was performed according to protocols published previously (22). The *Salmonella* isolates were then

TABLE 2. Distribution of *Salmonella* serovars in four major village markets

Serovar	No. of isolates	Village market ^a :			
		LZ	FZ	DJK	DL
Derby	18	6	4	3	5
Typhimurium	17	4	5	4	4
Meleagridis	11	3	3	3	2
Anatum	2	1	1	0	0
Agona	7	0	0	3	4
London	2	0	2	0	0
Rissen	2	0	0	2	0
Goldcoast	3	1	1	1	0
Virchow	2	1	0	1	0
Uganda	3	1	1	0	1
Livingstone	2	2	0	0	0
Total	69	19	17	17	16

^a LZ, Liangzhuang; FZ, Fanzhen; DJK, Dajinkou; DL, Daolang.

grouped by eBURST 3.0 software (Imperial College London, London) based on their allelic properties (11). The isolated strains were considered to belong to the same group when five of the seven alleles were shared between the strains.

Statistical analysis. The isolation rate of *Salmonella* from pork samples collected from different markets was compared by using a chi-square test (SPSS version 15.0, SPSS, Chicago, IL). *P* < 0.05 was considered as statistically significant.

RESULTS

Prevalence of *Salmonella*. In total, 69 *Salmonella* isolates were retrieved from 200 pork samples, with an isolation rate of 34.5% (69 of 200). A slight difference in the isolation rates of *Salmonella* was observed in this study, which may be due to differences in the sample types. However, there was no significant difference in the *Salmonella* isolation rates among the four village markets (*P* > 0.05) (Table 1).

Distribution of *Salmonella* serovars. Eleven serotypes were identified from the 69 *Salmonella* isolates, and *Salmonella* Derby was the most common (18 of 69, 26.1%), followed by Typhimurium (17 of 69, 24.6%) and Meleagridis (11 of 69, 15.9%). Except for these three dominant serotypes that were shared in all four markets, the other eight serotypes were not evenly distributed; for example, *Salmonella* Livingstone was in Liangzhuang only, *Salmonella* London was in Fanzhen only, and *Salmonella* Rissen was in Dajinkou only (Table 2).

Antibiotic resistance profiles. Antimicrobial susceptibility testing showed that resistance against tetracycline was the most prevalent (42 of 69, 60.9%), followed by streptomycin (28 of 69, 40.6%), ampicillin (25 of 69, 36.2%), and trimethoprim-sulfamethoxazole (21 of 69, 30.4%). Resistances against ceftriaxone and cefotaxime were 1.4 and 2.9%, respectively. With regard to most antibiotics tested in this study, similar antimicrobial

TABLE 3. Antimicrobial resistance profiles of 69 *Salmonella* isolates from four major village markets^a

Antimicrobial agent	No. (%) of antimicrobial-resistant <i>Salmonella</i> isolates				Total no. (%) (n = 69)
	LZ (n = 19)	FZ (n = 17)	DJK (n = 17)	DL (n = 16)	
Ampicillin	7 (36.8)	7 (41.2)	5 (29.4)	6 (37.5)	25 (36.2)
Cefazolin	2 (10.5)	1 (5.9)	1 (5.9)	1 (6.3)	5 (7.2)
Ceftriaxone	1 (5.3)	0	0	0	1 (1.4)
Cefotaxime	1 (5.3)	1 (5.9)	0	0	2 (2.9)
Ofloxacin	1 (5.3)	1 (5.9)	1 (5.9)	1 (6.3)	4 (7.2)
Norfloxacin	2 (10.5)	1 (5.9)	1 (5.9)	1 (6.3)	5 (7.2)
Ciprofloxacin	3 (15.8)	3 (17.6)	2 (11.8)	2 (12.5)	10 (14.5)
Tetracycline	12 (63.2)	11 (64.7)	9 (52.9)	10 (62.5)	42 (60.9)
Kanamycin	5 (26.3)	5 (29.4)	2 (11.8)	1 (6.3)	13 (18.8)
Gentamicin	5 (26.3)	3 (17.6)	5 (29.4)	2 (12.5)	15 (21.7)
Streptomycin	9 (47.4)	7 (41.2)	6 (35.3)	6 (37.5)	28 (40.6)
Trimethoprim-sulfamethoxazole	6 (31.6)	5 (29.4)	5 (29.4)	5 (31.3)	21 (30.4)

^a LZ, Liangzhuang; FZ, Fanzhen; DJK, Dajinkou; DL, Daolang.

resistance trends of *Salmonella* isolates from the four village markets were found (Table 3).

In terms of *Salmonella* serovars, isolates most frequently displaying resistance against one to three antibiotics were Rissen (100.0%), Derby (50.0%), Anatum (50.0%), Agona (42.9%), and Typhimurium (41.2%). Isolates exhibiting resistance against four to six antibiotics were London (100.0%), Virchow (100.0%), and Agona (57.1%). Only the Typhimurium (29.4%) isolate showed resistance to more than nine antibiotics. Of note, *Salmonella* Uganda, Livingstone, and Goldcoast isolates displayed susceptibility to all examined antibiotics (Table 4).

MLST. These 69 *Salmonella* isolates were divided into 11 different sequence types (STs), including ST684, ST1750, ST34, ST358, ST638, ST469, ST13, ST64, ST40, ST155, and ST19. The area of black circle corresponds to the number of isolates. ST40 was the most common (18 of 69, 26.1%), followed by ST34 (15 of 69, 21.7%) and ST64 (13 of 69, 18.8%). The snapshot of genetic relatedness was generated using eBURST 3.0. STs grouped based on the definition of five of seven shared alleles are connected by lines. ST19 and ST34 are connected with a line, indicating that they come from the same ancestor bacterium (Fig. 1).

DISCUSSION

In the current study, 34.5% of retail pork was contaminated with *Salmonella*. This value was similar to the contamination rate (31.0%) reported in Shaanxi province, China (31), but higher than the 14.1% reported in Jiangsu province, China (19). Compared with other countries, the *Salmonella* contamination rate in China obtained from this study was higher than the rates reported in Denmark, Ireland, and the United States, which were between 2.1 and 4.2% (3, 13, 17). In this study, the relatively high contamination rate of *Salmonella* may be due to the fact that the retail pork at rural markets is generally from small-scale pig slaughter houses where poor general hygiene and unsuitable storage conditions are common. During the cutting and handling processes of retail pork at rural markets, the absence of cover materials, storage refrigerates, and disinfection treatments in the purchasing areas can increase *Salmonella*-colonizing activity.

In addition, *Salmonella* contamination rate of retail meats can be affected by sampling and isolation methods; therefore, these factors should be taken into account when comparing *Salmonella* contamination rates of meats in different regions. The use of strict quarantine measures, sanitary and cleaning methods, and reasonable slaughter and

TABLE 4. Multidrug resistance observed among *Salmonella* serovars from retail pork

Serovar (no. of isolates)	No. (%) of isolates resistant to indicated no. of antibiotics					Total resistance rate (%)
	0	1–3	4–6	7–9	>9	
Derby (18)	8 (44.4)	9 (50.0)	1 (5.6)	0	0	55.6
Typhimurium (17)	2 (11.8)	7 (41.2)	2 (11.8)	1 (5.9)	5 (29.4)	88.2
Meleagridis (11)	7 (63.6)	3 (27.3)	1 (9.1)	0	0	36.4
Anatum (2)	1 (50.0)	1 (50.0)	0	0	0	50.0
Agona (7)	0	3 (42.9)	4 (57.1)	0	0	100.0
London (2)	0	0	2 (100.0)	0	0	100.0
Rissen (2)	0	2 (100.0)	0	0	0	100.0
Goldcoast (3)	3 (100.0)	0	0	0	0	0
Virchow (2)	0	0	2 (100.0)	0	0	100.0
Uganda (3)	3 (100.0)	0	0	0	0	0
Livingstone (2)	2 (100.0)	0	0	0	0	0

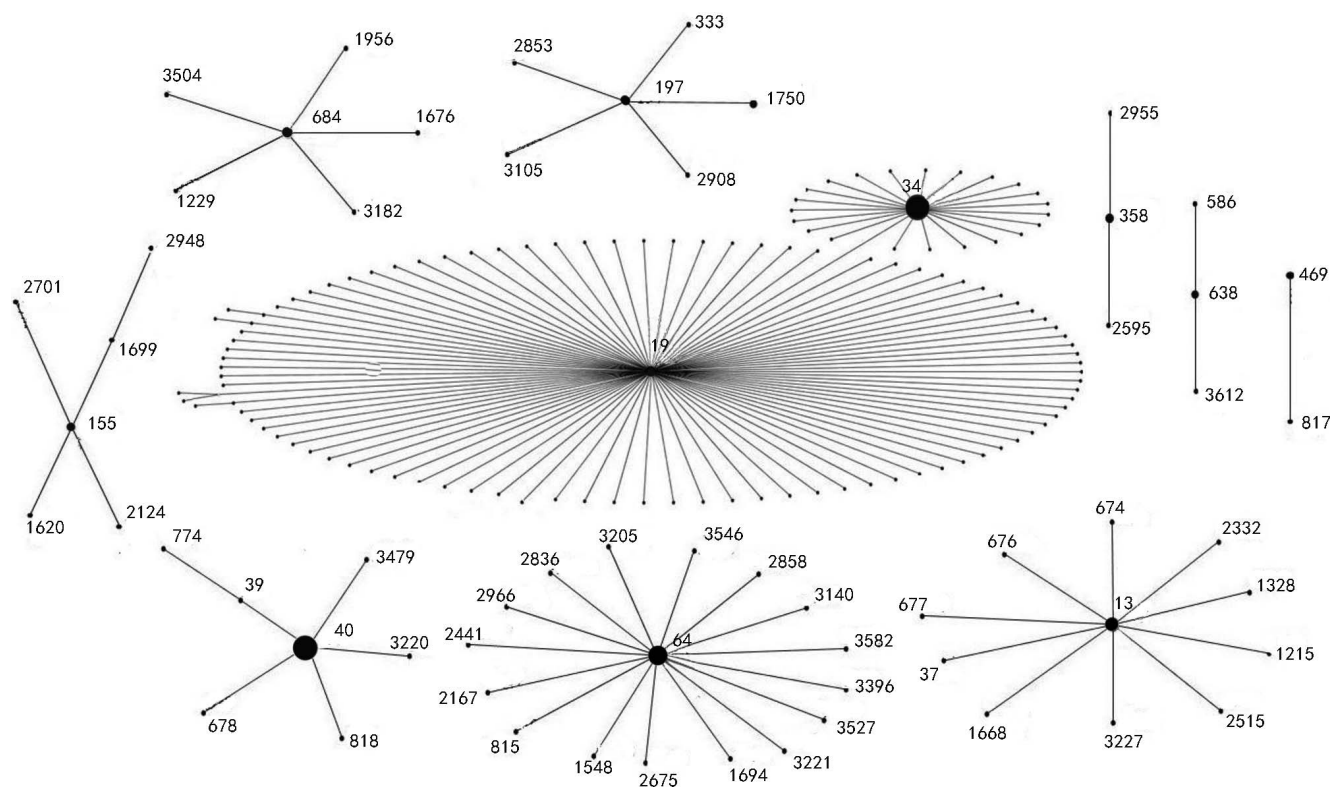


FIGURE 1. Snapshot displaying the evolutionary relationships among STs found in this study and those in the database. The snapshot of genetic relatedness was generated using eBURST 3.0 software. STs grouped based on the definition of five of seven shared alleles are connected by lines. In total, 11 STs (ST684, ST1750, ST34, ST358, ST638, ST469, ST13, ST64, ST40, ST155, and ST19) were detected in this study, and the area of the circle corresponds to the number of isolates. ST19 and ST34 observed in this study are connected with a line, indicating that they come from the same ancestor bacterium.

transport processes have shown to be effective in reduction of *Salmonella* contamination of meats in some countries (1).

In total, 11 *Salmonella* serovars were identified from the 69 *Salmonella* isolates, with *Salmonella* Derby being the most common, followed by Typhimurium. These findings are consistent with the findings in Yangzhou city and Shaanxi province of China as well as those in North Vietnam and the Republic of Ireland (6, 17, 27, 31). The incidence of *Salmonella* Derby contamination has been gradually increasing in recent years. For example, Derby is frequently isolated from retail pork and slaughter pigs and has already surpassed Typhimurium as the most frequently isolated serovar from pigsties in Europe (10). In addition, *Salmonella* Derby is commonly isolated from fecal samples of symptomatic human cases in Europe (5) and has become the third most common *Salmonella* causing human infections in China (20, 30). Other serotypes of *Salmonella* repeatedly identified in this study were *Salmonella* Meleagridis and Agona, which was in agreement with the result described in previous studies conducted in China (6, 19, 24, 31). Other serovars of *Salmonella*, such as Virchow, Rissen, Anatum, and London, were also identified in this study, but the number of isolates was relatively small and may be related to regional differences and limitations in the sampling methods.

Previous studies have demonstrated that antimicrobial resistance genes on conjugative plasmids of *Salmonella* can be readily transferred to other bacteria and therefore pose a

serious threat to public health safety (15, 23, 26). In this study, antimicrobial susceptibility testing showed that 43 *Salmonella* isolates (43 of 69, 62.3%) from retail pork were resistant to at least one antibiotic. The resistance rate against tetracycline was the most prevalent (42 of 69, 60.9%), which may be associated with the fact that tetracycline has been widely used in the pig industry of mainland China. Of note, multidrug-resistant *Salmonella* were observed among the retail pork isolates in this study, particularly *Salmonella* Typhimurium. For example, five *Salmonella* Typhimurium isolates (5 of 17, 29.4%) were resistant to more than nine antibiotics. Fortunately, antimicrobial resistances against both ceftriaxone and cefotaxime were 1.4 and 2.9%, respectively.

To illustrate genetic relatedness of *Salmonella*, a population snapshot of the comparison of the ST results in this study with published ST was made using eBURST 3.0. The results showed that 11 STs were identified and ST40 was the most common *Salmonella* genotype in the current study. ST40 is widely distributed in pigs and pork in Europe and America, and it is often found in Asian and European patients (6, 19). As the second and third most common serotypes, ST34 and ST19 are also widely found in humans and pigs in Japan, the United States, and Europe, indicating that *Salmonella* may be transmitted from pigs to humans via the food chain. In addition, among the 11 STs identified, only ST19 and ST34 belong to one clone complex, and no predominant ST was found in this study, suggesting that

Salmonella in contaminated pork may be derived from diverse sources.

It is an obvious limitation that only one *Salmonella* isolate was obtained to analyze in this study, and this limited the conclusions that can be drawn on distribution over the different markets. But the study to some extent reflected the prevalence and molecular epidemiology of *Salmonella* in retail pork sold in village markets in the Tai'an region of China. Collectively, *Salmonella* isolates in village markets exhibited resistance to multiple antibiotics and antibiotics resistance was strongly related with serotypes. In addition, no predominant ST was found in this study, suggesting that *Salmonella* in contaminated pork originated from diverse sources.

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