

Prevalence, Characteristics, and Antimicrobial Resistance Patterns of *Salmonella* in Retail Pork in Jiangsu Province, Eastern China

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ABSTRACT

Salmonella is commonly isolated from raw pork and is a leading cause of foodborne illness. Because China has the highest rate of pork consumption and the largest number of pig breeding facilities in the world, an epidemiological analysis of *Salmonella* species from pork in China is warranted. In this study, pork samples ($n = 1,096$) were collected from 20 major free markets in four cities of Jiangsu province from August 2010 to December 2012. A total of 163 *Salmonella* isolates were recovered from 154 *Salmonella*-positive samples. Among 14 *Salmonella* serovars identified, Derby (47.9%) was most prevalent, followed by Typhimurium (10.4%), Meleagridis (9.2%), Anatum (8.6%), and London (6.7%). Antimicrobial sensitivity testing revealed that 134 (82.2%) of the isolates were resistant to at least one antimicrobial agent, and 41 (25.2%) were resistant to more than three antimicrobials. The highest resistance was to tetracycline (66.3% of isolates) followed by ampicillin (39.9%), trimethoprim-sulfamethoxazole (31.3%), and nalidixic acid (30.1%). Multilocus sequence typing analysis revealed 14 sequence type (ST) patterns; ST40 was the most common (77 isolates) followed by ST64 (19 isolates). Our research revealed a high prevalence of *Salmonella* in retail pork. Diversity among the *Salmonella* isolates was high in terms of serovar and genotype, and multidrug resistance was prevalent. Multilocus sequence type was generally associated with serovar and provided a reliable prediction of the most common *Salmonella* serovars.

Salmonella is one of the most important foodborne pathogenic bacteria in the world and can cause a heavy economic burden for health care systems (26). In China, an estimated 70 to 80% of foodborne bacterial outbreaks are caused by *Salmonella* (43). Most human *Salmonella* infection outbreaks are associated with the consumption of contaminated food of animal origin according to the World Health Organization (48). Although attention has been focused on poultry products, pork also remains an important source of human *Salmonella* infections and accounts for 15.0 to 20.0% of all *Salmonella* infection cases in Europe (24, 31). Greater attention on the prevalence of *Salmonella* in pork is needed, especially in China, which is one of the largest pork producers in the world.

Because traditional serotyping methods are time consuming and require a large number of specific antisera (36), various discriminatory methods for subtyping *Salmonella* isolates have been used to characterize its molecular epidemiology, including multilocus enzyme electrophoresis (7), pulsed-field gel electrophoresis (PFGE) (30), and amplified fragment length polymorphism (21). PFGE has

been considered the “gold standard” for subtyping of all major foodborne pathogens because it is highly discriminatory (16). However, this method can be difficult to compare across various laboratories for the same analysis or even among various runs within the same laboratory. To overcome these problems, multilocus sequence typing (MLST) was developed as an alternative method for the analysis of bacterial populations. MLST results are easier to interpret and to compare among laboratories and provide the best inferences of phylogenetic relationships (38). MLST methods have been used extensively to subtype and explore the evolutionary relationships of a variety of bacterial pathogens, including *Campylobacter jejuni*, *Streptococcus agalactiae*, and *Salmonella enterica* (19, 23, 25). Recent research indicates that MLST results generally predict results of serotyping and that genotyping and serotyping can be complementary and provide mutual authentication for *Salmonella* identification (1, 34).

The increase in the number of antimicrobial-resistant *Salmonella* strains, particularly multidrug-resistant (MDR) isolates, has become a global problem. The extensive or inappropriate use of antimicrobial agents is considered the most important factor for the emergence, selection, and dissemination of antimicrobial-resistant bacteria. Recently, an increase in the isolation of MDR *Salmonella* strains has

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been reported in pork from various countries (4, 27, 37). MDR strains of *Salmonella* can be transferred from animals to humans through consumption of contaminated pork, and this transfer has become a significant public health issue (45). However, few reports have been published regarding the prevalence, serovars, multilocus sequence types, and antimicrobial susceptibility of *Salmonella* isolates from pork in China (50).

The aim of this study was to survey the distribution, antimicrobial susceptibility profiles, and population structures of *Salmonella* isolates from pork collected from retail markets in Jiangsu province and provide data that can be used for further evolutionary analyses.

MATERIALS AND METHODS

Sample collection. Jiangsu province in the eastern portion of the People's Republic of China has a population of 70 million (with the highest population density) and is the region where most of the pork is produced. In 2011, 39.9 million hogs were produced in this province, which was 4.0% of the domestic production. In our study, samples were collected from four cities, Huaian, Yangzhou and Taizhou, and Nanjing, located in the north, center, and south areas, respectively, of Jiangsu Province. A total of 1,096 pork samples were collected from August 2010 to December 2012 from four or five major free markets in each city. All pork stands were sampled in turn from each market. Two pork types (chop and piece) were collected at each sampling time. To prevent external contamination of samples, samples were transferred into separate sterile bags immediately after purchase and stored in an icebox at 4°C. Other aseptic measures were taken at all times, e.g., sterile gloves worn by the sample collector were changed between samples. Samples were taken to the laboratory for immediate processing.

Bacterial isolation and identification. All samples were analyzed as follows. A sample of pork (25 ± 0.5 g) was aseptically weighed and transferred into 225 ml of buffered peptone water (BPW; Difco, BD, Detroit, MI) and incubated at 37°C for 18 h. After the preenrichment step, the BPW suspensions were incubated at 37°C for 24 h, and 0.1 ml of this broth culture was subcultured in 10 ml of Rappaport-Vassiliadis enrichment (RV) broth (Difco, BD) at 42°C for 24 h. One loopful of each RV broth culture was then streaked onto xylose lysine Tergitol 4 (Difco, BD) agar plates, which were incubated at 37°C for 24 to 48 h. Two or three presumptive *Salmonella* colonies were picked from each plate, streaked onto biochemical test triple sugar iron slants and lysine iron agar (Hangzhou Microbial Reagent Co., Hangzhou City, China), and incubated for 24 h at 35°C. Isolates with typical color reactions were confirmed as *Salmonella* using an API 20E test kit (bioMérieux, Marcy l'Etoile, France). All strains were serotyped according to the Kauffmann-White scheme (6).

Antimicrobial susceptibility testing. Antimicrobial susceptibility was determined with agar disk diffusion tests on Mueller-Hinton agar plates, according to the guidelines of the Clinical and Laboratory Standards Institute (11). The isolates were tested against 15 antimicrobials: ampicillin (AMP, 10 µg), cefazolin (CFZ, 30 µg), ceftriaxone (CRO, 30 µg), chloramphenicol (CHL, 30 µg), ciprofloxacin (CIP, 5 µg), enrofloxacin (ENR, 5 µg), gentamicin (GEN, 10 µg), kanamycin (KAN, 20 µg), mezlocillin (MEZ, 75 µg), nalidixic acid (NAL, 30 µg), norfloxacin (NOR, 5 µg), ofloxacin (OFX, 5 µg), streptomycin (STR, 10 µg), tetracycline (TET, 30 µg), and trimethoprim-sulfamethoxazole

(SXT, 1.25 and 23.75 µg). Pure cultures of each the *Salmonella* isolate were incubated overnight at 37°C in Luria-Bertani (LB) broth (Merck, Darmstadt, Germany), and the pathogen level was adjusted with sterile saline solution until a McFarland turbidity of 0.5 was attained. One hundred microliters of the culture was then swabbed onto Mueller-Hinton agar with a sterile cotton swab. Antimicrobial disks were placed on the surface of the agar plates far enough apart from each other to avoid overlapping of inhibition zones. The plates were incubated at 37°C for 16 to 18 h, and the results were interpreted as sensitive, intermediate, or resistant according to the Clinical and Laboratory Standards Institute guidelines (12). *Escherichia coli* ATCC 25922 and *Enterococcus faecalis* ATCC 29212 were used as quality control strains. An isolate was defined as resistant after confirmation of resistance to at least one agent tested, and isolates were defined as MDR by resistance to four or more agents.

MLST and sequence data analysis. Strains were grown aerobically in LB broth with shaking at 37°C for 16 to 18 h. Genomic DNA was isolated using the TIANamp Bacteria DNA Kit (Tiangen, Beijing, China) according to the manufacturer's protocol. MLST was carried out based on the published method (28). All PCR products were purified with a High Pure PCR purification kit (Roche, Mannheim, Germany) and sequenced by Nanjing GenScript Biotech Co. (Nanjing, China). To illustrate the evolutionary relatedness of *S. enterica* isolates, a population snapshot of the comparison of the MLST results of our sequence types (STs) with published STs was made using eBURST software, version 3.0 (15). A maximum likelihood tree was constructed with these STs to determine phylogenies. The best-fit substitution model was determined using MEGA 4.0 (35), and the likelihood of each model was measured with the Bayesian information criterion and corrected with the Akaike information criterion. The TrN93 model with a discrete gamma distribution (+G) allowing for invariant sites (+I) was used in the complete genome analysis. A minimum spanning tree was generated using BioNumerics software (Applied-Maths, Kortrijk, Belgium) to study the distribution of STs in the four cities.

Data analysis. Statistical comparison of prevalence, MDR, and individual resistance to the 15 antimicrobials of *Salmonella* isolates in the four cities were analyzed with the χ^2 test, which was performed using the Statistical Package for the Social Sciences (version 15.0, SPSS, Chicago, IL).

RESULTS

Prevalence of *Salmonella*. Of the 1,096 pork samples analyzed, 154 (14.1%) were positive for *Salmonella*. *Salmonella* was slightly more prevalent in pork samples from Huaian (15.8% of samples positive) and Nanjing (15.3%) than in samples from Taizhou (13.2%) and Yangzhou (12.8%), but the differences were not significant ($P > 0.05$). A total of 163 *Salmonella* isolates were recovered from the 154 *Salmonella*-positive samples (1 to 3 isolates per sample): 55 isolates from Yangzhou, 45 from Nanjing, 33 from Huaian, and 30 from Taizhou (Table 1).

Diversity of *Salmonella* serovars. Fourteen serovars were identified among the 163 *Salmonella* isolates (Table 2). The most common *Salmonella* serovar was Derby (78 isolates, 47.9%) followed by Typhimurium (17, 10.4%), Meleagridis (15, 9.2%), Anatum (14, 8.6%), and

TABLE 1. Prevalence of *Salmonella* in pork samples from four cities

Region	No. of samples	No. (%) of samples positive for <i>Salmonella</i>	No. of <i>Salmonella</i> isolates recovered ^a
Nanjing	260	40 (15.3)	45
Yangzhou	400	51 (12.8)	55
Huaian	208	33 (15.8)	33
Taizhou	228	30 (13.2)	30
Total	1,096	154 (14.1)	163

^a More than one *Salmonella* isolate was collected from each positive sample.

London (11, 6.7%). The prevalence of other serovars ranged from 0.6 to 3.7%. Only *Salmonella* Typhimurium and *Salmonella* Derby were present in samples from all cities. *Salmonella* Newport and *Salmonella* Chester were found in pork from only Nanjing, and *Salmonella* Agona and *Salmonella* Limete were isolated from samples collected from only Huaian and Taizhou, respectively. *Salmonella* Schwarzengrund and *Salmonella* Virchow were found in only Yangzhou pork samples.

Antimicrobial resistance phenotypes. The susceptibility of 163 *Salmonella* isolates to 15 antimicrobials was evaluated (Table 3). No isolates were resistant to norfloxacin. Resistance to tetracycline was the most common (66.3% of isolates) followed by resistance to ampicillin (39.9%), trimethoprim-sulfamethoxazole (31.3%), nalidixic acid (30.1%), streptomycin (22.7%), chloramphenicol (17.2%), and mezlocillin (12.3%).

Among the β -lactams studied, a high degree of resistance to ampicillin (39.9% of isolates) was found. Conversely, low levels of resistance to ceftriaxone (1.2%) were observed. Some resistance was found to cefazolin (4.9%).

The aminoglycosides studied had different levels of effectiveness. Resistance to gentamicin (8.6% of isolates) and kanamycin (8.0%) was sporadic. However, higher

resistance (22.7%) was found to spectinomycin ($P < 0.01$). For chloramphenicol, 28 (17.2%) of the isolates were classified as resistant. Representing the sulfonamides, trimethoprim-sulfamethoxazole resistance was found in 31.3% of *Salmonella* isolates.

All isolates were susceptible to norfloxacin. These isolates had a low level of resistance to ciprofloxacin (0.6% of isolates), ofloxacin (1.8%), and enrofloxacin (2.5%), but the resistance to nalidixic acid was higher (30.1%).

A total of 134 (82.6%) of the *Salmonella* isolates were resistant to at least one antimicrobial, and the resistance rate was $>60\%$ in all cities. The rates of resistance to multiple (one to three) antimicrobials were similar (46.7 to 72.7%) among *Salmonella* from the four cities ($P > 0.05$); however, approximately 17.8% of isolates from Nanjing were resistant to seven to nine antimicrobials compared with 10.0 and 3.0% of isolates from Taizhou and Huaian, respectively.

Drug-resistant strains belonged to 14 serovars (Table 4), and for most *Salmonella* serovars resistance was found in $>50.0\%$ of the isolates. Exceptions were *Salmonella* Muenster and *Salmonella* Give. *Salmonella* isolates that were MDR (listed from higher [50.0% of isolates] to lower [6.7%] resistance) were from serovars Typhimurium, Give, Muenster, Derby, Infantis, Anatum, London, and Meleagridis. *Salmonella* Typhimurium also had the highest number of MDR isolates; 25.0% of its isolates were resistant to seven to nine antimicrobials. One *Salmonella* ser. Derby isolate collected from pork in Huaian was resistant to 10 antimicrobials (AMP, CFZ, CHL, CRO, ENR, MEZ, NAL, STR, SXT, and TET).

A total of 134 resistant *Salmonella* isolates with 56 resistance patterns were identified (Table 4); the common pattern was resistance to TET only (24 isolates, 17.3%). For MDR phenotypes, 32 different patterns were found among 42 MDR isolates (Table 4); the most common patterns were AMP, MEZ, SXT, and TET and AMP, GEN, KAN, NAL, STR, SXT, and TET (3 isolates for each pattern, 7.1%).

MLST analysis. We characterized 163 *Salmonella* isolates using MLST analysis. DNA sequence data generated for the seven MLST target genes included 3,336 bp for each isolate. The number of unique alleles for the various housekeeping genes ranged from 26 for *hemD* to 33 for *hisD*, and the sequence variation for each gene ranged from 2.6 to 4.9% (Table 5). Fourteen different ST patterns were identified among the 163 *Salmonella* isolates (Table 4). ST40 was the most common ST in this study, represented by 77 *Salmonella* isolates, followed by ST19, ST64, ST463, ST155, and ST34; ST96 and ST592 were each represented by 1 isolate. Most STs obtained in this study were correlated with certain serovars, such as ST40 with *Salmonella* Derby, ST13 with *Salmonella* Agona, and ST46 with *Salmonella* Newport. However, a few isolates had an ST that did not match well with the serovar. In addition, some serovars could be assigned multiple STs, and some STs were represented by several serovars. For example, 6 of 17 isolates typed as *Salmonella* Typhimurium belonged to ST19, but another isolate

TABLE 2. Serovar distribution of 163 *Salmonella* isolates

<i>Salmonella</i> serovar	No. of isolates				Total no. (%)
	Nanjing	Yangzhou	Huaian	Taizhou	
Derby	20	32	15	11	78 (47.9)
Typhimurium	11	2	2	2	17 (10.4)
Meleagridis	3	4	8		15 (9.2)
Anatum		8		6	14 (8.6)
London	3	1		7	11 (6.7)
Agona			6		6 (3.7)
Infantis		4	1	1	6 (3.7)
Newport	5				5 (3.1)
Chester	3				3 (1.8)
Muenster		2		1	3 (1.8)
Give			1	1	2 (1.2)
Limete				1	1 (0.6)
Virchow		1			1 (0.6)
Schwarzengrund		1			1 (0.6)
Total	45	55	33	30	

TABLE 3. Antimicrobial resistance phenotypes of 163 *Salmonella* isolates

Antimicrobial agent	No. (%) of resistant isolates				
	Nanjing (n = 45) ^a	Yangzhou (n = 55)	Huaian (n = 30)	Taizhou (n = 33)	Total (n = 163)
β-Lactams					
Ampicillin	22 (48.9)	11 (20.0)	23 (76.7)	9 (27.3)	65 (39.9)
Mezlocillin	4 (8.9)	6 (10.9)	9 (30.0)	1 (3.0)	20 (12.3)
Cefazolin	0	5 (9.1)	2 (6.7)	1 (3.0)	8 (4.9)
Ceftriaxone	0	1 (1.8)	1 (3.3)	0	2 (1.2)
Quinolone and fluoroquinolone					
Nalidixic acid	14 (31.1)	9 (16.4)	14 (46.7)	12 (36.3)	49 (30.1)
Oflloxacin	2 (4.4)	0	1 (3.3)	0	3 (1.8)
Enrofloxacin	1 (2.2)	0	2 (6.7)	1 (3.0)	4 (2.5)
Norfloxacin	0	0	0	0	0
Ciprofloxacin	1 (2.2)	0	0	0	1 (0.6)
Tetracycline	33 (73.3)	32 (58.2)	24 (80.0)	19 (57.6)	108 (66.3)
Chloramphenicol	12 (26.7)	6 (10.9)	7 (23.3)	3 (9.1)	28 (17.2)
Aminoglycosides					
Kanamycin	10 (22.2)	0	3 (10.0)	0	13 (8.0)
Gentamicin	9 (20.0)	1 (1.8)	4 (13.3)	0	14 (8.6)
Streptomycin	18 (40.0)	7 (12.7)	12 (40.0)	0	37 (22.7)
Sulfonamides					
Trimethoprim-sulfamethoxazole	18 (40.0)	11 (20.0)	13 (43.3)	9 (27.3)	51 (31.3)

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

characterized as *Salmonella* Typhimurium belonged to ST34. In addition, 9 *Salmonella* Anatum isolates and 10 *Salmonella* Meleagridis isolates were assigned ST14. With the less stringent group definition of five of seven shared alleles, nine clusters of linked ST clonal complexes (CCs) and four unlinked STs were found (Fig. 1). ST13, ST19, ST32, and ST40 were the primary founders of each of their CCs, and ST34 was one of the single-locus variants of ST19. CC-19 was the largest CC, with 22 single-locus variants. Phylogenetic trees revealed that all unique STs were separated into two large clusters and that ST19 was closely related to ST34 in cluster B. ST96 and ST516 were linked by the shortest evolutionary distance of all the STs (Fig. 2), and the other STs were separated by much longer evolutionary distances. With the minimum spanning tree, we found an uneven distribution of STs in the four cities, except for ST40, and every city had its own particular STs, such as ST96 in Yangzhou and ST46 in Nanjing (Fig. 3).

DISCUSSION

In the present study, the overall prevalence of *Salmonella* contamination in pork samples from four cities in Jiangsu province was approximately 14.1%. The result is similar to that of a previous study conducted in the same area of China (44) but lower than those reported in western China (50) and other developing countries, such as Mexico, Vietnam, and Thailand (27, 33, 37). However, the *Salmonella* contamination rate was higher than that in developed European countries and the United States, which range from 2.1 to 4.2% (3, 17, 20). Different sampling seasons, sampling procedures, and isolation methods could

affect the *Salmonella* prevalence results. Better sanitation in slaughterhouses, use of preharvest surveillance and control of *Salmonella*, advanced processing practices, and effective postharvest interventions may account for the lower contamination rates in developed countries (2).

We found 14 *Salmonella* serovars in pork samples, which belonged to four serogroups. The most common *Salmonella* serovar was Derby followed by Typhimurium, Anatum, and Meleagridis, which is consistent with previous reports from other regions and countries (20, 37, 50). *Salmonella* Derby is commonly isolated from pig meat and from breeding and slaughter pigs and is one of the 10 most frequently isolated serovars from human salmonellosis cases in many countries (8, 13). In recent years, the worldwide occurrence of *Salmonella* Derby has become more frequent. For example, *Salmonella* Derby has surpassed *Salmonella* Typhimurium as the leading *Salmonella* serovar in pig holdings in the European Union and was the third most common *Salmonella* serovar causing human infections in China (14, 22, 49). In addition, the high rates of detection of *Salmonella* Typhimurium and *Salmonella* Anatum were consistent with those found in previous studies (37, 40). However, the regional distribution of *Salmonella* Newport, *Salmonella* Chester, *Salmonella* Agona, *Salmonella* Limete, and *Salmonella* Schwarzenbrunn was unbalanced, which may be because of the regional specificity of *Salmonella* and limitations in the sample collection methods. Other *Salmonella* serovars, such as Infantis, Virchow, and Muenster, have been commonly isolated in other countries but are distributed sporadically in China (16, 41). This difference may suggest that the global

TABLE 4. Diversity profiles of *Salmonella* isolates based on MLST, serotyping, and antimicrobial resistance

ST (<i>n</i>) ^a	Allele profile ^b	Serovar	City	Antimicrobial resistance ^c	No. of isolates
ST13 (6)	3, 3, 7, 4, 3, 3, 7	Agona	Huaian	AMP	6
ST19 (6)	10, 7, 12, 9, 5, 9, 2	Typhimurium	Huaian	AMP, GEN, KAN, NAL, STR, SXT, TET	1
		Typhimurium	Huaian	AMP, CHL, MEZ, SXT, TET	1
		Typhimurium	Taizhou	AMP, GEN, KAN, NAL, STR, SXT, TET	1
		Typhimurium	Taizhou	AMP, CHL, MEZ, SXT, TET	1
		Typhimurium	Yangzhou	CHL, GEN, STR, TET	1
		Typhimurium	Yangzhou	CHL, TET	1
ST32 (7)	17, 18, 22, 17, 5, 21, 19	Infantis	Huaian	AMP, NAL	1
		Infantis	Taizhou	NAL	1
		Infantis	Yangzhou	NAL, SXT, TET	1
		Infantis	Yangzhou	AMP, CHL, MEZ, NAL, SXT, TET	1
		Infantis	Yangzhou	AMP, CHL, MEZ, NAL, SXT	1
		Virchow	Yangzhou	NAL	1
ST34 (12)	10, 19, 12, 9, 5, 9, 2	Derby	Nanjing	STR, TET	1
		Typhimurium	Nanjing	AMP, CHL, KAN, NAL, SXT, TET	1
		Typhimurium	Nanjing	ENR, NAL, TET	1
		Typhimurium	Nanjing	NAL, TET	1
		Typhimurium	Nanjing	SXT, TET	1
		Typhimurium	Nanjing	AMP, CHL, KAN, NAL, SXT, TET	1
		Typhimurium	Nanjing	AMP, CHL, KAN, GEN, STR, SXT, TET	1
		Typhimurium	Nanjing	AMP, CHL, STR, SXT, TET	1
		Typhimurium	Nanjing	AMP, GEN, KAN, NAL, STR, SXT, TET	1
		Typhimurium	Nanjing	AMP, GEN, KAN, SXT, TET	1
		Typhimurium	Nanjing	AMP, CHL, GEN, KAN, NAL, SXT	1
		Derby	Huaian	AMP, CFZ, CHL, CRO, ENR, MEZ, NAL, STR, SXT, TET	1
ST40 (77)	19, 20, 3, 20, 5, 22, 22	Derby	Huaian	ENR, NAL, TET	1
		Derby	Huaian	AMP, NAL, TET	3
		Derby	Huaian	AMP, TET	5
		Derby	Huaian	AMP, CFZ, NAL, TET	1
		Derby	Huaian	AMP, STR, SXT, TET	1
		Derby	Huaian	AMP, MEZ, NAL, OFX	1
		Derby	Huaian	AMP, MEZ, NAL, TET	1
		Derby	Huaian	MEZ, TET	1
		Derby	Taizhou	NAL	1
		Derby	Taizhou		2
		Derby	Taizhou	STR, TET	1
		Derby	Taizhou	AMP, CHL, GEN, STR, SXT, TET	1
		Derby	Taizhou	AMP, CHL, ENR, MEZ, NAL, STR, SXT, TET	1
		Derby	Taizhou	AMP, CHL, GEN, MEZ, NAL, STR, SXT, TET	1
		Derby	Taizhou	CFZ	1
		Derby	Taizhou	NAL, TET	1
		Derby	Taizhou	NAL, TET	1
		Derby	Taizhou	NAL, TET	1
		Derby	Yangzhou	TET	12
		Derby	Yangzhou		7
		Derby	Yangzhou	STR, TET	3
		Derby	Yangzhou	AMP, MEZ, NAL, OFX	1
		Derby	Yangzhou	AMP, CHL, MEZ, NAL, SXT, TET	1
		Derby	Yangzhou	AMP, MEZ, SXT, TET	3
		Derby	Yangzhou	CHL, SXT	1
		Derby	Yangzhou	CHL, TET	1
		Derby	Yangzhou	AMP, CHL, TET, STR, SXT	1
		Derby	Yangzhou	CFZ, TET	1
		Derby	Yangzhou	CRO, NAL	1
		Derby	Nanjing	AMP, CHL, NAL, TET	1
		Derby	Nanjing	STR	1

TABLE 4. *Continued*

ST (<i>n</i>) ^a	Allele profile ^b	Serovar	City	Antimicrobial resistance ^c	No. of isolates
ST46 (6)	10, 7, 21, 12, 35, 12, 12	Derby	Nanjing	AMP, CHL, MEZ, NAL, OFX, STR, SXT, TET	1
		Derby	Nanjing	AMP, CHL, MEZ, NAL, OFX, STR, SXT, TET	1
		Derby	Nanjing	TET	1
		Derby	Nanjing	AMP, MEZ, TET	1
		Derby	Nanjing	AMP, CHL, TET	1
		Derby	Nanjing	AMP, CHL, CIP, GEN, KAN, NAL, SXT, TET	1
		Derby	Nanjing	AMP, NAL	1
		Derby	Nanjing	SXT, TET	1
		Derby	Nanjing	SXT	1
		Derby	Nanjing	SXT	1
		Derby	Nanjing	AMP, STR, TET	2
		Derby	Nanjing	STR, TET	3
		Derby	Nanjing	AMP, STR, TET	2
		Newport	Nanjing	AMP, CHL, GEN, NAL, STR, SXT, TET	1
ST64 (19)	10, 14, 15, 31, 25, 20, 33	Newport	Nanjing	TET	1
		Anatum	Taizhou	AMP, GEN, MEZ, NAL, SXT, TET	1
		Anatum	Taizhou	AMP, CHL, MEZ, NAL, SXT	1
		Anatum	Taizhou	NAL	1
		Anatum	Taizhou	TET	1
		Anatum	Taizhou		2
		Anatum	Yangzhou	NAL	1
		Anatum	Yangzhou	TET	2
		Anatum	Yangzhou	MEZ	1
		Anatum	Yangzhou		4
		Meleagridis	Yangzhou	AMP, STR, TET	1
		Meleagridis	Yangzhou	NAL	1
		Meleagridis	Yangzhou		2
		Meleagridis	Nanjing	AMP, CHL, KAN, NAL, STR, SXT, TET	1
ST96 (1)	43, 47, 49, 49, 41, 15, 3	Schwarzengrund	Yangzhou	TET	1
		London	Taizhou	TET	4
		London	Taizhou		2
		London	Taizhou	CHL, STR, SXT, TET	1
		London	Yangzhou		1
		London	Nanjing	KAN	1
		London	Nanjing		1
		Meleagridis	Huaian	AMP, MEZ, TET	1
		Meleagridis	Huaian	NAL, SXT, TET	5
		Meleagridis	Huaian	SXT, TET	2
ST463 (10)	92, 125, 78, 128, 138, 9, 141	Meleagridis	Huaian	AMP, CFZ, CHL, NAL, SXT, TET	1
		Meleagridis	Nanjing	AMP, CHL, GEN, KAN, NAL, SXT, TET	1
		Meleagridis	Nanjing	STR, TET	1
		Give	Huaian	AMP, KAN, NAL, STR, SXT, TET	1
		Give	Taizhou		1
		Limete	Taizhou	NAL	1
		Muenster	Taizhou	AMP, CHL, KAN, SXT, TET	1
		Muenster	Yangzhou		1
		Muenster	Yangzhou		1
ST516 (2)	84, 11, 16, 42, 40, 71, 4	Chester	Nanjing	STR	1
		Chester	Nanjing	TET	2
ST592 (1)	189, 70, 68, 132, 175, 9, 172				
ST684 (3)	147, 13, 15, 123, 15, 19, 17				
ST1628 (3)	46, 60, 10, 9, 6, 12, 17				

^a *n*, number of *Salmonella*-positive isolates tested.^b Allele numbers for *aroC*, *dnaN*, *hemD*, *hisD*, *purE*, *sucA*, and *thrA*, respectively (one for each ST).^c AMP, ampicillin; GEN, gentamicin; KAN, kanamycin; NAL, nalidixic acid; STR, streptomycin; SXT, trimethoprim-sulfamethoxazole; TET, tetracycline; CHL, chloramphenicol; MEZ, mezlocillin; ENR, enrofloxacin; CFZ, cefazolin; CRO, ceftriaxone; OFX, ofloxacin; CIP, ciprofloxacin.

TABLE 5. Primers used for MLST, number of base pairs analyzed, number of alleles, and number of polymorphic sites per gene

Locus	Portion analyzed (bp)	No. of alleles	No. of polymorphic sites	% polymorphic sites	Primers for amplification (5'-3')
<i>aroC</i>	501	9	13	2.6	F: CCTGGCACCTCGCGCTATAC R: CCACACACGGATCGTGGCG
<i>dnaN</i>	501	12	22	4.4	F: ATGAAATTACCGTTAACGTGA R: AATTCTCATTCGAGAGGATTGC
<i>hemD</i>	432	12	11	2.6	F: ATGAGTATTCTGATCACCG R: ATCAGCAGCTTAATATCTGCCA
<i>hisD</i>	501	12	38	7.58	F: GAAACGTTCCATTCCGCGCAGAC R: CTGAACGGTCATCCGTTCTG
<i>purE</i>	399	10	19	4.8	F: ATGTCTCCCGCAATAATCC R: TCATAGCGTCCCCGCGGATC
<i>sucA</i>	501	10	14	2.8	F: AGCACCGAAGAGAACGCTG R: GGTTGTTGATAACGATACGTAC
<i>thrA</i>	501	12	21	4.2	F: GTACGGTGATCGATCCGGT R: CACGATATTGATATTAGCCCCG

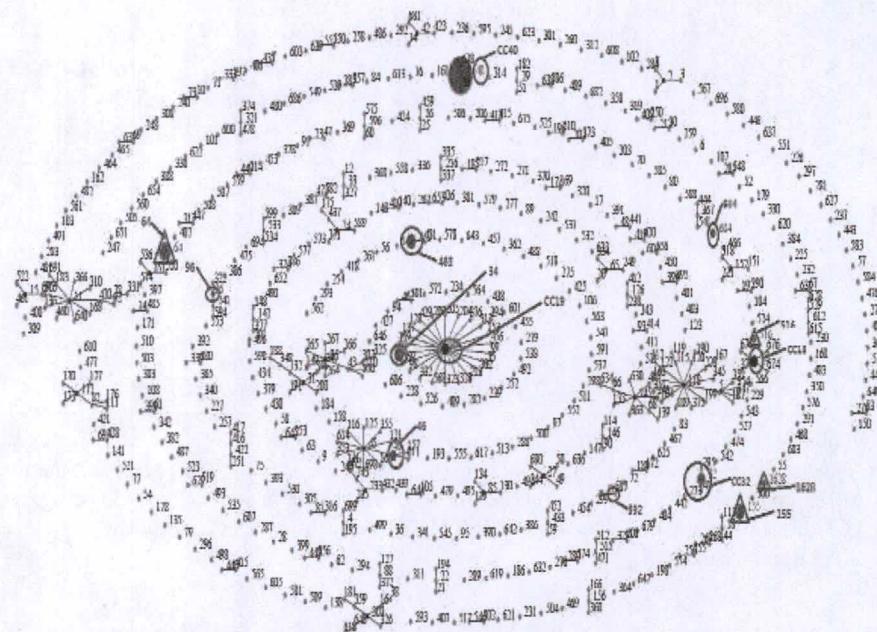
Salmonella serovar distribution is changing because of international travel and the trade of food and animal products worldwide.

Antimicrobial resistance in *Salmonella* has become a significant public health concern. The presence of antimicrobial-resistant pathogens in food and food products could enable the bacteria to spread via the food chain to humans, causing infections (46). Our results indicated that 82.2% of the *Salmonella* isolates were resistant to at least one antimicrobial agent, with high levels of drug resistance in all four cities examined. High rates of antimicrobial resistant *Salmonella* in retail meats have also been reported by several other investigators (27, 37, 50). Furthermore, we found that 25.2% of isolates ($n = 41$) were resistant to seven or more antimicrobials. However, these results were slightly lower than those previously reported in China and Vietnam (42, 50). In this study, the serovars *Salmonella* ser. Derby, *Salmonella* ser. Typhimurium, *Salmonella* ser. Meleagridis, and *Salmonella* ser. London showed high rates of antimicrobial resistance and multidrug resistance, similar

to previous observations in retail meats and food animals in China (32, 50). Of particular concern, one *Salmonella* ser. Typhimurium isolate was resistant to 14 antimicrobials. Major health problems may arise if these MDR isolates are transferred to humans.

In the present study, the highest rates of antimicrobial resistance were against tetracycline (66.3%), which is one of the most widely used antimicrobials in feed additives in livestock farming in China and other countries. Thus, this result was somewhat expected and agreed with previous reports from China, Mexico, Vietnam, and the United States (9, 27, 37, 45). However, the high rate of nalidixic acid resistance observed in this study is of concern. Strains of *Salmonella* resistant to nalidixic acid may be associated with clinical failure or delayed response in fluoroquinolone-treated patients, although resistance to fluoroquinolone remains rare in *Salmonella* (11). A large number of *Salmonella* isolates were resistant to trimethoprim-sulfamethoxazole, ampicillin, chloramphenicol, and streptomycin. These findings were consistent with those in other reports from China, Korea, and Vietnam (10, 39, 43, 50).

FIGURE 1. Population snapshot of all STs included in the MLST database for *Salmonella enterica*. The snapshot of clonal complexes (CCs) was produced using eBURST. STs found here belonged to eight CCs (circled). The snapshot displays the evolutionary relationships among STs found in this study and those in the database (ST1 through ST599). The single STs are marked with a solid triangle. The STs grouped based on the less stringent definition of five of seven shared alleles are connected by lines. ST1628, found only in our study, is marked with a shaded triangle.



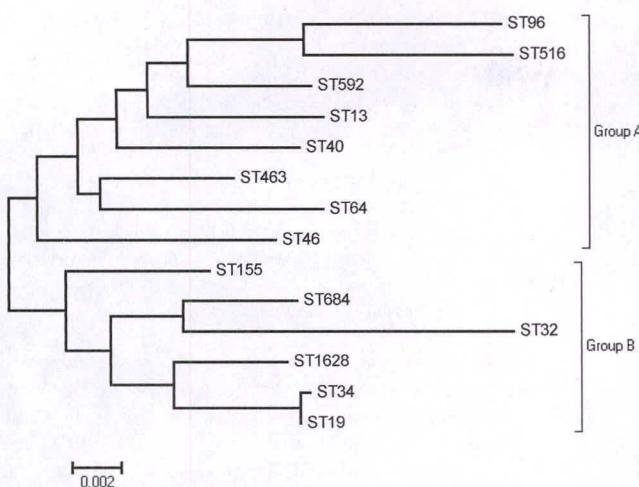


FIGURE 2. Maximum composite likelihood tree showing the genetic relationships among the merged sequences of seven housekeeping gene fragments from 163 *Salmonella* isolates recovered from pork.

These antimicrobial agents are still widely used in human therapy in China because of the low cost and ready availability. Therefore, resistance to these antimicrobials by foodborne pathogens may generate problems for human disease treatment.

The increasing frequency of *Salmonella* resistance to the common antimicrobials has led to the use of third-generation cephalosporins to combat salmonellosis. Ceftriaxone is commonly used to treat children with *Salmonella* infections because of its pharmacokinetic properties and the low prevalence of resistance (45). In the present study, small numbers of *Salmonella* isolates were resistant to ceftriaxone, although a larger number of strains were only slightly susceptible to these drugs. Resistance to four or more of the antimicrobials tested was high in *Salmonella* isolates; 17.2% (28) of the isolates were resistant to four to six

antimicrobials, and 7.4% (20) of the isolates were resistant to seven to nine antimicrobials. This finding was in agreement with those reported previously in Vietnam and China (42, 50). Of the *Salmonella* serovars identified in the present study, Typhimurium, Derby, Meleagridis, and Anatum had the highest rate of antimicrobial resistance and multidrug resistance, which was in agreement with previous findings for *Salmonella* isolates recovered from retail meats and food animals (32, 37, 50). These results highlight the enormous challenges associated with the treatment of *Salmonella* infections in humans and animals, and more legislation about prudent use of antimicrobials should be implemented by the authorities in China.

MLST results revealed that ST40 was the most common genotype in isolates recovered from pork in this study. According to the *Salmonella* MLST database (28), strains belonging to ST40 have appeared in food and swine in Europe and the United States, respectively, and in humans in Asia and Europe. This finding suggests that ST40 isolates may spread from pigs to humans via pork products. ST1628, which has been reported previously only in pigeons in China, was found in pork for the first time in our study. However, ST34 and ST19, which were represented by 18 isolates in our study, have been reported extensively in both human and swine samples from Japan, the United States, and many European countries. According to the *Salmonella* MLST database, ST34 and ST19 have been rarely found in China, but this rarity may be due to the infrequent use of MLST for identification of *Salmonella* sequence types in China.

We determined that ST34 may have originated from ST19 and that ST34 and ST19 both belong to CC-19, which included mainly of strains of human clinical origin (Fig. 1). Phylogenetic analyses revealed that the majority of STs in our study represented monophyletic lineages. Although the

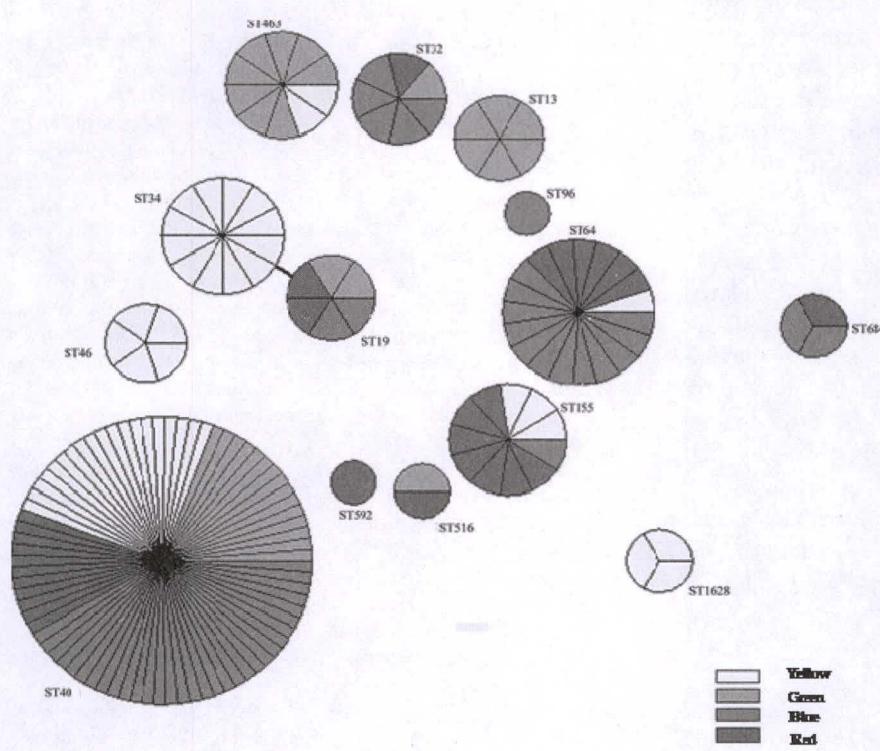


FIGURE 3. Clustering of STs using a minimum spanning tree. Each circle represents an ST, and the area of the circle corresponds to the number of isolates. The four shadings represent the four districts: dark shading, Taizhou; medium shading, Yangzhou; light shading, Huai'an; white, Nanjing.

isolates with these STs were found in various cities, all isolates genotyped as ST19 (allelic profile 10, 7, 12, 9, 5, 9, 2) or ST34 (allelic profile 10, 19, 12, 9, 5, 9, 2) were the same serovar (*Salmonella* Typhimurium), which indicated that these isolates are likely related and that diversity might increase because of recombination, mutational events, or horizontal gene transfer. These findings are consistent with previous research (18).

ST19 and ST34 (a single-locus variant of ST19) have been frequently associated with the ACSSuT type (resistance to AMP, CHL, STR, sulfonamides, and tetracyclines) of *Salmonella* Typhimurium, which is also frequently found in the European Union and China (3, 47). In our study, isolates belonged to ST19 and ST34 that were resistant to TET (16 isolates), SXT (12 isolates), CHL (9 isolates), AMP (7 isolates), and STR (7 isolates) were the most frequently detected. According to previous reports, the ACSSuT type of *Salmonella* Typhimurium was associated with 4.8-fold higher mortality rates than an average *Salmonella* infection (29). Therefore, the increase in the prevalence of the MDR *Salmonella* Typhimurium ST19 clone will pose a considerable threat to control of clinical *Salmonella* infections. Four STs (64, 463, 516, and 684) found in this study were reported for the first time in Asia. For example, ST516 has previously been found only in environmental samples in Africa. Clustering results also revealed that STs in pork were grouped by geographical location and displayed some distribution characteristics. We also found that MLST results were generally associated with serovar, indicating a close relationship between these two characteristics, again in accordance with previous research (25). MLST could be used to overcome the limitations of the traditional serotyping method and provide reliable identification of the most common *Salmonella* serotypes and associated evolutionary information. Therefore, we suggest that *Salmonella* identification by traditional serotyping be replaced by MLST or an optimized method (1, 5).

In summary, our findings revealed a high prevalence of *Salmonella* in retail pork in Jiangsu province, which increases the risk of human infections from eating *Salmonella*-contaminated pork. The *Salmonella* isolates in the four geographical regions examined were both serotypically and genotypically diverse. Many of the *Salmonella* isolates were resistant to multiple antimicrobials, and multidrug resistance was strongly associated with certain serovars. MDR isolates could pose a significant threat to control of clinical *Salmonella* infections in both humans and animals. Further epidemiological surveillance is needed in this region of China, including monitoring the prevalence of foodborne pathogens and their antimicrobial resistance and subtyping profiles. These results will provide useful information for development of science-based public health policies and effective intervention strategies to ensure the safety of our food supplies.

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