

## Molecular Typing and Drug Resistance Analysis of *Salmonella* spp. Isolated from Pig Slaughterhouse in Shandong Province, China <sup>[1]</sup>

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### Abstract

An epidemiological investigation of *Salmonella* enteritidis in pig and pork samples from eight slaughterhouses in Shandong Province, China, was conducted from December 2014 to October 2015. A total of 22.2% (142/640), of the slaughterhouse samples were recovered positive for *Salmonella* spp.. All *Salmonella*-positive were characterized using serotyping, antimicrobial resistance testing, pulsed-field gel electrophoresis (PFGE) and multi-locus sequence typing (MLST). Ten serotypes were shared by all isolates, with the most common serotypes being *Salmonella* Derby, *Salmonella* Typhimurium, and *Salmonella* Thompson. Antimicrobial sensitivity testing revealed that the highest antimicrobial resistance rate was against sulfisoxazole (91.55%) with many multidrug-resistant (MDR) isolates. MLST analysis showed that nine sequence type (ST) patterns were shared, ST40 was the most common (79 isolates) followed by ST19 (26 isolates) and ST26 (24 isolates). PFGE permitted the resolution of *Xba*I macrorestriction fragments of all the isolates, displaying the high similarity. Three clusters and 31 PFGE patterns were generated by PFGE analysis. Our results indicated that *Salmonella* spp. isolates from eight slaughterhouses were phenotypically and genetically homologous. These data could be used for further evolutionary analyses.

**Keywords:** *Salmonella* spp., antimicrobial resistance, pulsed-field gel electrophoresis, multi-locus sequence analysis

## Çin'in Shandong Eyaletinde Domuz Mezbatasından İzole Edilen *Salmonella* spp.'nin Moleküler Tiplendirilmesi ve İlaç Dirençliliği Analizi

### Özet

Çin'in Shandong Eyaletindeki sekiz kesimhaneden Aralık 2014 ile Ekim 2015 tarihleri arasında alınan domuz örneklerinde *Salmonella* enteritisi için epidemiyolojik bir çalışma yürütüldü. Kesimhane örneklerinin %22.2'sinden (142/640) *Salmonella* spp. tespit edildi. Tüm *Salmonella* pozitif örnekler serotiplendirme, antimikrobiyal dirençlilik testi, değişken alanlı jel elektroforez (PFGE) ve multilokus sekans tiplendirmesi (MLST) ile karakterize edildi. En yaygını *Salmonella* Derby, *Salmonella* Typhimurium ve *Salmonella* Thompson olmak üzere toplam on serotip tüm izolatlarda gözlemlendi. Antimikrobiyal hassaslık testi birçok çoklu ilaç direnciyle (MDR) birlikte en yüksek antimikrobiyal direncin sulfisoxazole (%91.55) karşı olduğunu gösterdi. MLST analizi dokuz sekans tipi (ST) şeklinin olduğunu gösterdi. Bu sekans tiplerinden en yaygını ST40 (79 izolat) daha az olarak ise ST19 (26 izolat) ve ST26 (24 izolat) olarak belirlendi. PFGE yüksek benzerlik göstererek tüm izolatların *Xba*I makrorestriksiyon parçalarının rezolüsyonunu sağladı. Üç küme ve 31 PFGE şekli PFGE analizi ile üretildi. Elde edilen sonuçlar, sekiz kesimhaneden izole edilen *Salmonella* spp.'nin fenotipik ve genotipik olarak homolog olduğunu gösterdi. Bu bulgular ileriki analizler için kullanılabilir.

**Anahtar sözcükler:** *Salmonella* spp., Antimikrobiyal direnç, Değişken alanlı jel elektroforezi, Multilokus sekans analizi



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## INTRODUCTION

*Salmonella enteritidis* is an important group of bacterial pathogens which can cause severe foodborne disease in humans and animals, impacting health and productivity in worldwide [1]. In China, an estimated 70% to 80% of foodborne bacterial outbreaks were caused by *Salmonella spp.*[2]. Pigs have been recognized as one important reservoir for *Salmonella spp.*[3]. It also can be transferred to humans via pork along the food chain [4]. As one of the largest pork producers and consumption countries in the world, much more attention to the prevalence of *Salmonella spp.* in pork should be paid present in China.

Serotype and bacterial identification are important parts of outbreak investigation and epidemiological surveillance of *Salmonella spp.*[5]. Many DNA genotyping methods can be used to discriminate *Salmonella spp.* isolates beyond subspecies level and species, due to their high discriminative powers [6]. Pulse field-gel electrophoresis (PFGE) has been considered the "gold standard" for subtyping of all major foodborne pathogens because it is highly discriminatory [7]. 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, multi-locus sequence typing (MLST) was developed as an alternative method for the analysis of bacterial populations. MLST results are easier to interpret and compare among laboratories and provide the best inferences of phylogenetic relationships [8]. Recent research indicated that MLST results generally prediction results of serotyping and that genotyping and serotyping can be complementary and also provide mutual authentication for *Salmonella* identification [9].

In recent years, the prevalence and characterization of *Salmonella spp.* along pork production chain were reported in many countries which the contamination rate

of *Salmonella spp.* was 10% to 40% in pig slaughterhouse, the major serotypes were *Salmonella* Typhimurium and *Salmonella* Derby, and the serovars were diverse [10-13].

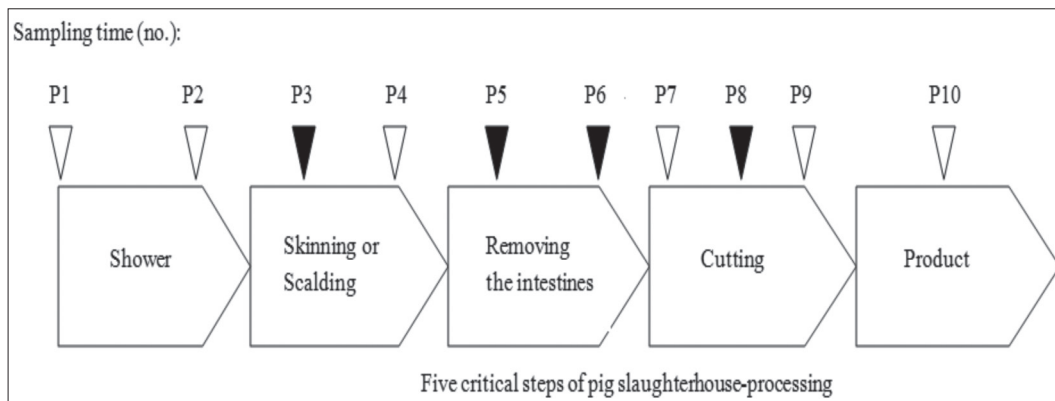
Though much attention has been focused on *Salmonella spp.* in pig slaughterhouse, intensive and simultaneous research regarding the prevalence, serotypes, antimicrobial resistance, pulsed-field gel electrophoresis (PFGE) and multi-locus sequence typing (MLST) profiles of *Salmonella spp.* was limited, 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 eight pig slaughterhouse in Shandong Province, China, to determine the clonal relationships between isolates and provide data that can be used for further evolutionary analyses.

## MATERIALS and METHODS

### *Salmonella* Strains

The 640 fresh samples including five different production stages (n = 640), anal swab (n = 80), shower (n = 80), skinning or scalding (n = 120), removing the intestines (n = 80), cutting (n = 200) and product (n = 80) were collected from eight pig slaughterhouses (A~H) at different areas in Shandong Province, China. Pig slaughterhouse included five critical steps, such as shower, skinning or scalding, removing the intestines, cutting and product. Within a production cycle one compartment on five critical steps was included in the sampling process which was based upon ten time points for collecting *Salmonella spp.* contamination samples as presented (Fig. 1).

For swab samples from slaughterhouse, the pre-enrichment step was performed by suspending each sample in 50 mL BPW, and incubating samples at 37°C for 16 h to 18 h. Then, 0.1 mL of the BPW suspensions was subcultured in 10 mL subpackaged Rappaport-



**Fig 1.** Graphic presentation of the sampling protocol throughout an pig slaughtering production line including one compartment at each production stage of f shower, skinning or scalding, removing the intestines, cutting and product in five China pig slaughtering production line. The numbers (no.) from P1 to P10 above the white and black arrows are indicating the ten time points for sampling. white arrows: slaughtered each link of the samples (nos.: P1, P2, P4, P7, P9 and P10); black arrows: slaughtering production line used knives (nos.: P3, P5, P6 and P8)

Vassiliadis (RV) enrichment broth (Difco, USA) at 42°C for 24 h. One loopful of each RV broth culture was then streaked onto xylose lysine tergitol 4 (Difco, USA) agar plates, which were incubated at 37°C for 24 h to 48 h. One presumptive *Salmonella* spp. colony per plate was picked and biochemically confirmed using an API-20E test kit (bioMérieux, France). *Salmonella* spp. positive isolates were serotyped according to the White-Kauffmann-Le Minor scheme [14].

### Antimicrobial Resistance

Susceptibility of all *Salmonella* spp. isolates to antimicrobial agents was evaluated according to Clinical Laboratory Standards Institute guidelines using the disc diffusion method on Mueller-Hinton agar [15]. Following 13 antimicrobial agents were tested, including Sulfisoxazole (SF, 30 µg), Doxycycline (DOX, 30 µg), Tetracycline (TE, 30 µg), Florfenicol (FFC, 5 µg), Ampicillin (AM, 10 µg), Gentamicin (GM, 10 µg), Spectinomycin (SPT, 10 µg), Sulfamethoxazole-trimethoprim (SXT, 1.25 and 23.75 µg), Enrofloxacin (ENR, 5 µg), Norfloxacin (NOR, 5 µg), Polymyxin (PME, 30 µg), Cefotaxime (EFT, 30 µg) and Amoxicillinpotassium clavulanate (AC, 30 µg). Results were interpreted using the Clinical and Laboratory Standards Institute (CLSI, 2013) breakpoints when available. *E. coli* ATCC 25922 was used as quality control [16]. Descriptive statistical analysis of the results of these tests was accomplished using Epi Info 7.

### Pulsed field-gel electrophoresis

PFGE was performed for *Salmonella* enterica using the Pulse Net protocol procedures described previously [17]. In brief, the chromosomal DNA of *Salmonella* spp. was digested with 50 U of *Xba* I (Takara, China) in 37°C water bath for 3 h. With a *Salmonella* serotype Braenderup strain (H9812) digested with *Xba* I (Takara, China) as the molecular weight standard, the DNA fragments were separated on a 0.8% agarose gel in 0.5 × TBE using CHEF-Mapper (Bio-Rad, USA). The experimental conditions were set up as follows: the initial and final switch time of 2.16 s and 63.8 s, respectively, an included angle of 120° and a gradient

of 6 V/cm for 19 h at 14°C. The PFGE images were handled using the Gel Doc software (Bio-Rad, USA) according to the operation manual. PFGE was repeated twice to determine reproducibility. For untypable isolates, 50 µM thiourea (Sigma, USA) was added to the 0.5 × TBE buffer prior to PFGE run as described by Römling and Tümmler [18]. The PFGE results of 142 isolates were disposed using the BioNumerics version 5.10 with the uniform marker normalization to record the strip position. A threshold of 85% homology was set to define clonal clustering of PFGE types.

### Multi-locus Sequence Typing

Multi-locus sequence typing (MLST) method was performed according to the recommendations of the *Salmonella* enterica MLST website (<http://mlst.warwick.ac.uk/mlst/dbs/Senterica>). An internal portion of seven housekeeping genes was amplified by PCR, including aspartokinase+homoserine dehydrogenase (*thrA*), phosphoribosylaminoimidazole carboxylase (*purE*), alpha ketoglutarate dehydrogenase (*sucA*), histidinol dehydrogenase (*hisD*), chorismate synthase (*aroC*), uroporphyrinogen III cosynthase (*hemD*), and DNA polymerase III beta subunit (*dnaN*). DNA Taq premix (Promega, USA) was used with the amplification procedure: 94°C 5 min; followed by 30 cycles: 94°C 1 min, 55°C 30 s, 72°C 1min; and a final extension at 72°C 10 min. The amplification products were sent for bidirectional sequencing to Takara, China; and the sequences were analyzed using DNASTar Lasergene Version 7.1. We submitted the sequences to the UCC database for their allele and ST assignments.

## RESULTS

The 142 out of 640 *Salmonella* spp. colonies were confirmed as *Salmonella*-positive samples. There into, 17 (21.3%) were recovered from anal swab, 13 (16.3%) from shower, 27 (22.5%) from skinning or scalding, 25 (31.3%) from removing the intestines, 47 (23.5%) from cutting and 13 (16.3%) from product (Table 1). Ten majority serotypes were identified in all *Salmonella* spp. strains. *S. Derby* was

**Table 1.** Prevalence data of *Salmonella*-positive samples by sampling in eight pig slaughterhouses line

Sample from Production Line	No. of Positive Samples (Prevalence in %)								
	Pig Slaughterhouses								
	A n (%)	B n (%)	C n (%)	D n (%)	E n (%)	F n (%)	G n (%)	H n (%)	Weighted Mean n (%)
Anal swab	2 (20.0)	2 (20.0)	3 (30.0)	1 (10.0)	1 (10.0)	2 (20.0)	3 (30.0)	3 (30.0)	17 (21.3)
Shower	1 (10.0)	2 (20.0)	2 (20.0)	1 (10.0)	1 (10.0)	2 (20.0)	2 (20.0)	2 (20.0)	13 (16.3)
Skinning or scalding	4 (26.7)	5 (33.3)	2 (13.3)	3 (20.0)	2 (13.3)	3 (20.0)	4 (26.7)	4 (26.7)	27 (22.5)
Removing the intestines	3 (30.0)	2 (20.0)	4 (40.0)	4 (40.0)	3 (40.0)	2 (20.0)	4 (40.0)	3 (30.0)	25 (31.3)
Cutting	4 (16.0)	6 (24.0)	7 (28.0)	4 (16.0)	4 (16.0)	5 (20.0)	9 (36.0)	8 (32.0)	47 (23.5)
Product	2 (20.0)	2 (20.0)	1 (10.0)	1 (10.0)	2 (20.0)	1 (10.0)	2 (20.0)	2 (20.0)	13 (16.3)
Total	16 (20.0)	19 (23.8)	19 (23.8)	14 (17.5)	13 (16.3)	15 (18.6)	24 (30.0)	22 (27.5)	142 (22.2)

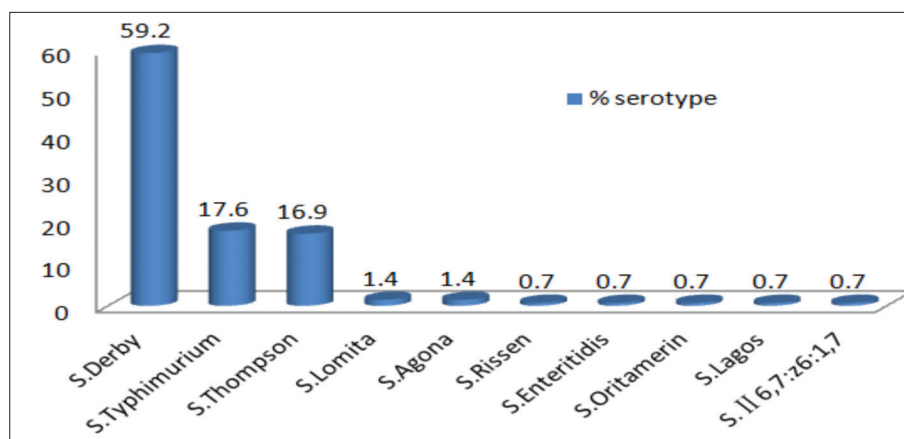


Fig 2. Serotype distribution of 142 Salmonella spp. isolate

**Fig 3.** Overview of antibiotic resistance ability of individual Salmonella strains  
 Antibiotic abbreviation: Sulfisoxazole (SF); Doxycycline (DOX); Tetracycline (TE); Florfenicol (FFC); Ampicillin (AM); Gentamicin (GM); Spectinomycin (SPT); Sulfamethoxazole-trimethoprim (SXT); Enrofloxacin (ENR); Norfloxacin (NOR); Polymyxin (PME); Cefotaxime (EFT); Amoxicillin potassium clavulanate (AC)

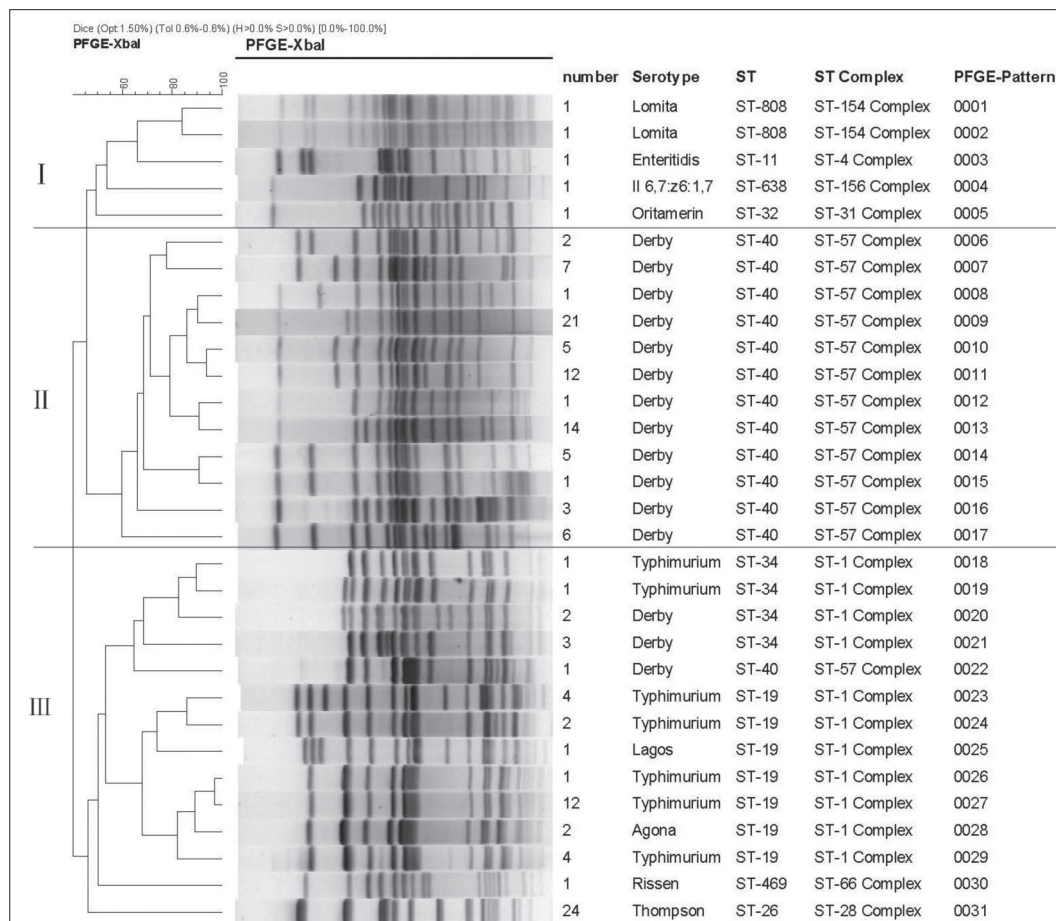
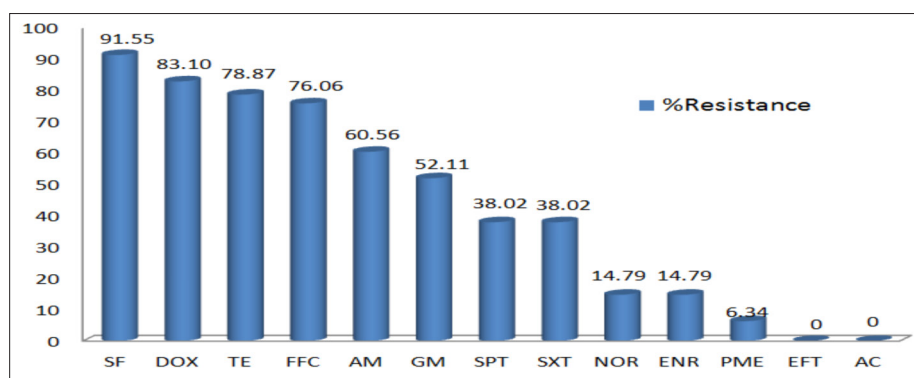


Fig 4. Dendrogram of the 31 patterns PFGE-XbaI identified with the frequency of each pattern from ten Salmonella serotypes isolated

**Table 2.** STs and allele profile of isolates

STs	acroC	dnaN	hemD	hisD	purE	SucA	thrE	No. of Isolates
11	5	2	3	7	6	6	11	1
19	10	7	12	9	5	9	2	26
26	14	13	18	12	5	18	1	24
32	17	18	22	17	5	21	19	1
34	10	19	12	9	5	9	2	7
40	19	20	3	20	5	22	22	79
469	92	107	79	156	64	51	87	1
638	47	98	36	152	174	7	171	1
808	10	71	43	12	190	20	18	2

the most serotype (59.2%, 84 of 142) strains. *Salmonella* group *Salmonella* Derby (n = 84), *Salmonella* Typhimurium (n = 25), *Salmonella* Thompson (n = 24), *Salmonella* Lomita (n = 2), *Salmonella* Agona (n = 2), *Salmonella* Rissen (n = 1), *Salmonella* Enteritidis (n = 1), *Salmonella* Oritamerin (n = 1), *Salmonella* Lagos (n = 1) and *Salmonella* Il6, 7:z6:1,7 (n = 1), were identified in this study (Fig. 2).

The individual antibiotic resistance profile of each of the 142 *Salmonella* spp. isolates was measured. Resistance to sulfisoxazole was the most prevalent among the *Salmonella* strains (130 strains, 91.55%) followed by doxycycline (118 strains, 83.10%), tetracycline (112 strains, 78.87%), florfenicol (108 strains, 76.06%), ampicillin (86 strains, 60.56%) and gentamicin (74 strains, 52.11%) (Fig. 3). The isolates were not resistant to cefotaxime and amoxicillinpotassium clavulanate, respectively.

Pulse field-gel electrophoresis (PFGE) generated profiles of three major genotypic clusters(I- III) and 31 fingerprint-patterns with an 80% dice coefficient index cut-off of 12~20 DNA fragment bands (Fig. 4). In this study, the 0031 PFGE pattern was the most common pattern, which included 24 strains of *Salmonella* Thompson, followed was 0009 PFGE pattern, which was composed of twenty-one *Salmonella* Derby isolates, and by 0013 PFGE patterns which was composed of fourteen *Salmonella* Derby isolates. In this study, the prevalence and characteristics of *Salmonella* spp. isolates are outlined in Table 3. Most patterns were within a single serotype and a single source, except 0014, 0017 and 0027 PFGE pattern. The 0014 PFGE pattern was

**Table 3.** Origin and characterization of *Salmonella* spp. isolated from pig slaughter process

No. of Isolates (n)	Serotype	Location	MLST Pattern	PFGE Pattern	Prevalence in %
A (16)	Derby (1)	A	ST-40	0012	20.0
	Derby (14)	A	ST-40	0013	
	Rissen (1)	A	ST-469	0030	
B (19)	Il6,7:z6:1,7 (1)	B	ST-638	0004	23.8
	Derby (3)	B	ST-40	0017	
	Typhimurium (1)	B	ST-34	0019	
	Typhimurium (1)	B	ST-19	0026	
	Typhimurium (9)	B	ST-19	0027	
	Typhimurium (4)	B	ST-19	0029	
C (19)	Lomita (1)	C	ST-808	0001	23.8
	Lomita (1)	C	ST-808	0002	
	Derby (1)	C	ST-40	0008	
	Derby (12)	C	ST-40	0011	
	Derby (1)	C	ST-40	0014	
	Derby (1)	C	ST-40	0022	
	Typhimurium (2)	C	ST-19	0024	
D (14)	Enteritidis (1)	D	ST-11	0003	17.5
	Derby (3)	D	ST-40	0014	
	Derby (1)	D	ST-40	0015	
	Derby (3)	D	ST-40	0017	
	Typhimurium (1)	D	ST-34	0018	
	Typhimurium (3)	D	ST-19	0027	
	Agona (2)	D	ST-19	0028	
	Oritamerin (1)	D	ST-19	0028	
E (13)	Derby (2)	E	ST-40	0006	16.3
	Derby (2)	E	ST-34	0020	
	Derby (3)	E	ST-34	0021	
	Typhimurium (4)	E	ST-19	0023	
	Lagos (1)	E	ST-19	0025	
	Oritamerin (1)	E	ST-19	0025	
F(15)	Derby (7)	F	ST-40	0007	18.6
	Derby (5)	F	ST-40	0010	
	Derby (3)	F	ST-40	0016	
G(24)	Thompson (24)	G	ST-26	0031	30.0
H(22)	Derby (21)	H	ST-40	0009	27.5
	Derby (1)	H	ST-40	0014	

composed of five *Salmonella* Derby isolates. All recovered from various processing steps at three slaughterhouses, which were C (1), D (3) and H (1); The 0017 PFGE pattern was found to be composed of six *Salmonella* Derby, which were from B (3) and D (3) slaughterhouse. Similarly the 0027 PFGE pattern was composed of twelve *Salmonella* Derby, which were from two slaughterhouses of B (9) and D (3).

An interlinked dataset with partial sequencing of seven housekeeping genes at 399 bp to 501 bp revealed that 9 STs among the 142 isolates were found. 4 STs were represented a single isolates, and the others were represented more than one isolates ( $n=2$  to 79). The predominant STs were ST40, ST19 and ST26, which contained 79 strains (55.6%), 26 strains (18.3%) and 24 (16.9%) strains isolates (Table 3). Most of *Salmonella*-positive isolates were assigned to ST40 in the dedicated database (<http://mlst.ucc.ie/mlst/dbs/Senterica>). The isolates possessed identical alleles at all seven loci; *aroC* allele type19, *dnaN* allele type20, *hemD* allele type 3, *hisD* allele type 20, *purE* allele type 5, *sucA* allele type 22, and *thrE* allele type 22 (Table 3). The reproducibility of PFGE and MLST showed that all isolates were consistent with before testing.

*Salmonella* spp. strains characterized as the same STs did not necessarily have the same PFGE pattern. 79 strains characterized as ST40 had 13 different PFGE patterns, the 26 strains characterized as ST19 had 7 different PFGE patterns (Fig. 4). All strains which shared a PFGE pattern had the same ST. *Salmonella* spp. strains which shared a PFGE fingerprint-pattern had the same ST (Fig. 4). Within clonal lineage 79 all *Salmonella* Derby isolates were of an identical MLST type (ST40) and revealed closely related PFGE patterns. The results showed that the character of *Salmonella* spp. strains in more detail, most STs were within a single serotype (Table 2). ST40 distributed in seven slaughterhouses, except G slaughterhouse.

## DISCUSSION

In the study, the overall prevalence of *Salmonella* spp. in pig slaughterhouse in Shandong Province was approximately 22.2%. For the slaughterhouse, the prevalence was significantly higher than that reported in Jiangsu Province, China (14.1%)<sup>[12]</sup>, in Sichuan Province, China (10.7%)<sup>[3]</sup>, and in Thailand (7.22%)<sup>[19]</sup>, but it was lower than that reported in Jiangsu Province, China (71.8%)<sup>[13]</sup>.

Although different methods of sampling, isolation, and identification could affect the overall results, the prevalence of *Salmonella* spp. observed in this study suggested that pig slaughterhouse examined exercised poor hygiene management. Meanwhile, the critical five steps of pig slaughterhouse, cutting is of the highest infection rates. We can focus on purification the step of cutting in pig slaughterhouse, to prevent the spread of salmonella food chain downstream.

We analyzed 142 *Salmonella* spp. samples to determine diversity within a strain (Fig. 4). Two samples contained isolates with identical properties, suggesting they were the same strain, while the majority of the samples contained isolates belonging to the same sequence type but differing by one or more of the phenotypic or genetic properties tested, indicating that they were variants of the same clone. Most common variations were non-expression of the H antigen, variation in PFGE patterns. Thirty-one unique PFGE patterns were generated. All of the patterns were correlated with one serotype. The reason could also explain the arrangement position of PFGE and the genetically similar profiles<sup>[18]</sup>. Others have previously demonstrated PFGE pattern diversity within a serotype<sup>[20-22]</sup>, but such diversity is a critical observation that is often overlooked. The diversity of PFGE pattern may explain why morbidity and mortality vary within a serotype and could be useful in assessing the effectiveness of control measures.

78 *Salmonella* spp. strains from seven slaughterhouses (A, B, C, D, E, F and H) in Shandong Province and two strains from Shanghai in group III displayed the same PFGE patterns and the same STs, which suggested they have a close genetic relationship. In view of the law, we should be paid to avoid further dissemination of *Salmonella* Derby, which has appeared in some areas<sup>[23]</sup>.

In this study, the most tested isolates of *Salmonella* spp. isolates were assigned to MLST 40, according to the MLST database analysis. In previous studies using the same database<sup>[5,24-26]</sup>, MLST was highly correlated to *Salmonella* serotype. The study targeting seven different housekeeping and virulence genes found that MLST was not able to discriminate clinically relevant serotypes of *Salmonella*<sup>[25]</sup>. The limited discriminatory ability of STs may be resulted from the moderate to slow rate of mutation accumulation within seven targeted housekeeping genes<sup>[26]</sup>. Therefore, the discriminatory performance of MLST needs to be increased if more variable gene targets are examined. Meanwhile, the reproducibility of the PFGE and MLST showed that all isolates were consistent with before testing. This showed that PFGE and MLST were the best method of DNA genotyping methods.

Antimicrobial resistance in *Salmonella* spp. 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<sup>[27]</sup>. Our results indicated that all the *Salmonella* spp. isolates were resistant to at least one antimicrobial agent, with high levels of drug resistance in all eight pig slaughter process examined. This was somewhat expected because of its wide use in animal feed and was consistent with reports from slaughterhouses in China, Italy, Mexico, Vietnam, and the United States<sup>[12,13,28-31]</sup>. In the study, the highest rates of antimicrobial resistance were against sulfisoxazole (91.55%), 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. MDR *Salmonella* isolates were frequently observed among the slaughterhouse and retail market isolates in this study. This was posing great risk to public health if these MDR strains were transferred to humans via pork or pork-derived products<sup>[3]</sup>.

In addition, *Salmonella* Derby was the primary serotype in H and F slaughterhouse, and in G slaughterhouse the main serotype is *Salmonella* Thompson. The strains from the slaughterhouse and tools had the different PFGE patterns as product isolates, except G slaughterhouse (Table 2). Meanwhile, other slaughterhouse isolates have several serotype and PFGE pattern. The diversity of these strains showed that cross contamination among the pig slaughterhouse. This indicated a 'pig-slaughtered each link-product' transmission circulation for *Salmonella* spp. in local area. Previous research has shown that Salmonellosis outbreak is one of multiple *Salmonella* serotypes outbreaks caused by contaminated food<sup>[32,33]</sup>. Other study also indicated that *Salmonella* spp. had the 'patient-environment-food' transmission circulation<sup>[34]</sup>. So we should purify salmonella contamination in pork products, from the source control the spread of *Salmonella*, put an end to the spread of salmonella in the crowd. Study results suggested that *Salmonella* control programs should reduce *Salmonella* spp. loading on carcasses included good practices.

In conclusion, the study attempted to analyze the various molecular classification of *Salmonella* contamination, such as the serotype, PFGE pattern, ST pattern and drug resistance analysis, to minimize the *Salmonella* spp. contamination in pig slaughterhouse. The results of this study provide PFGE fingerprints of *Salmonella* spp. strains in Shandong Province, China and establish a good foundation for the realization of data sharing, which will help to realize active surveillance of Salmonellosis and tracing the source of infection in China.

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