Kafkas Univ Vet Fak Derg
23 (3): 377-384, 2017
DOI: 10.9775/kvfd.2016.16751

Kafkas Universitesi Veteriner Fakultesi Dergisi
Journal Home-Page: http://vetdergi.kafkas.edu.tr
Online Submission: http://vetdergikafkas.org

Research Article

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

Wenyan GAI [1] Junwei WANG [1] Juan WANG [1] Zhigang CUI [2] Zhina QU [1]
Yundong WANG [1] Jun HONG [1] Jinghua CUI [2] Shigan YAN [3]
Xiaoli DU [2] Xiumei HUANG [1] Jianmei ZHAO [1] Liping ZHU [3]

[1] *This work was supported by the project of Ministry of Agriculture Agricultural Product Quality and Safety Supervision of China (GJFP2016007) & the Science and Technology Development Projects of Shandong Province, China (2014GSF120006)*

[1] China Animal Health and Epidemiology Center, Laboratory of Quality & Safety Risk Assessment for Animal Products of Ministry of Agriculture, 266032 Qingdao - CHINA
[2] Chinese Center for Disease Control and Prevention, PulseNetChina, 102206 Beijing - CHINA
[3] School of Food and Bioengineering, Qilu University of Technology, 250353 Jinan - CHINA

Article Code: KVFD-2016-16751 Received: 13.09.2016 Accepted: 01.12.2016 Published Online: 05.12.2016

Citation of This Article

Gai W, Wang J, Wang J, Cui Z, Qu Z, Wang Y, Hong J, Cui J, Yan S, Du X, Huang X, Zhao J, Zhu L: Molecular typing and drug resistance analysis of *Salmonella spp.* isolated from pig slaughterhouse in Shandong province, China. *Kafkas Univ Vet Fak Derg,* 23 (3): 377-384, 2017. DOI: 10.9775/kvfd.2016.16751

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 *XbaI* 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 Mezbahasından 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 XbaI 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*

İletişim (Correspondence)
+86 5328 5623936
yffs2000@sina.com

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 foodbome 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 producersand 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 foodbome 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-

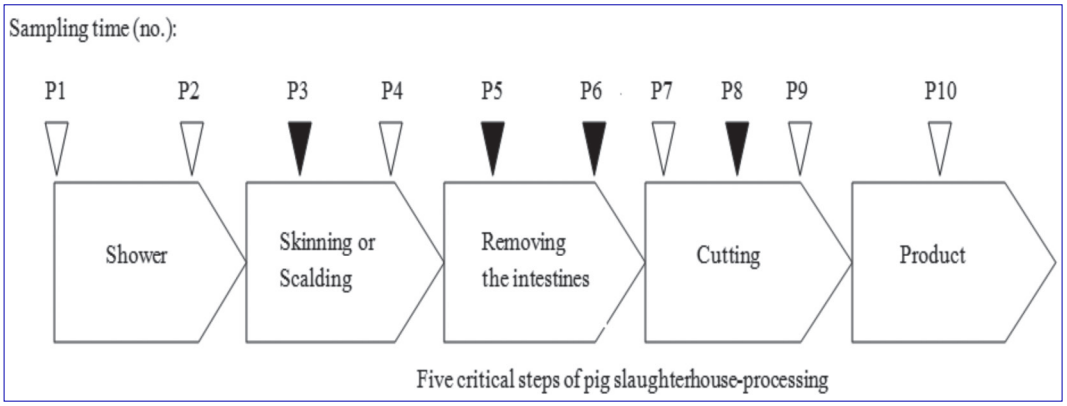


Fig 1. Graphic presentation of the sampling protocol throughout an pig slaughtering production line including one compartment at each production stage of f shower, skinning or scalding, removing the intestines, cutting and product in five China pig slaughtering production line. The numbers (no.) fromP1 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 X 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 carboxylas *(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)

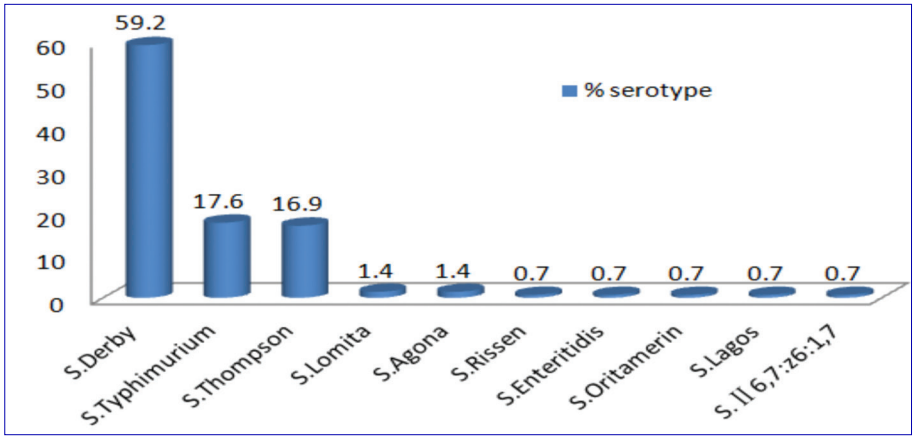
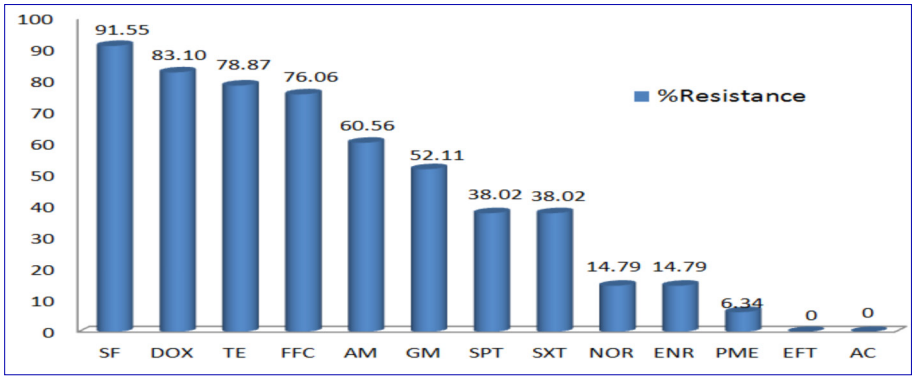
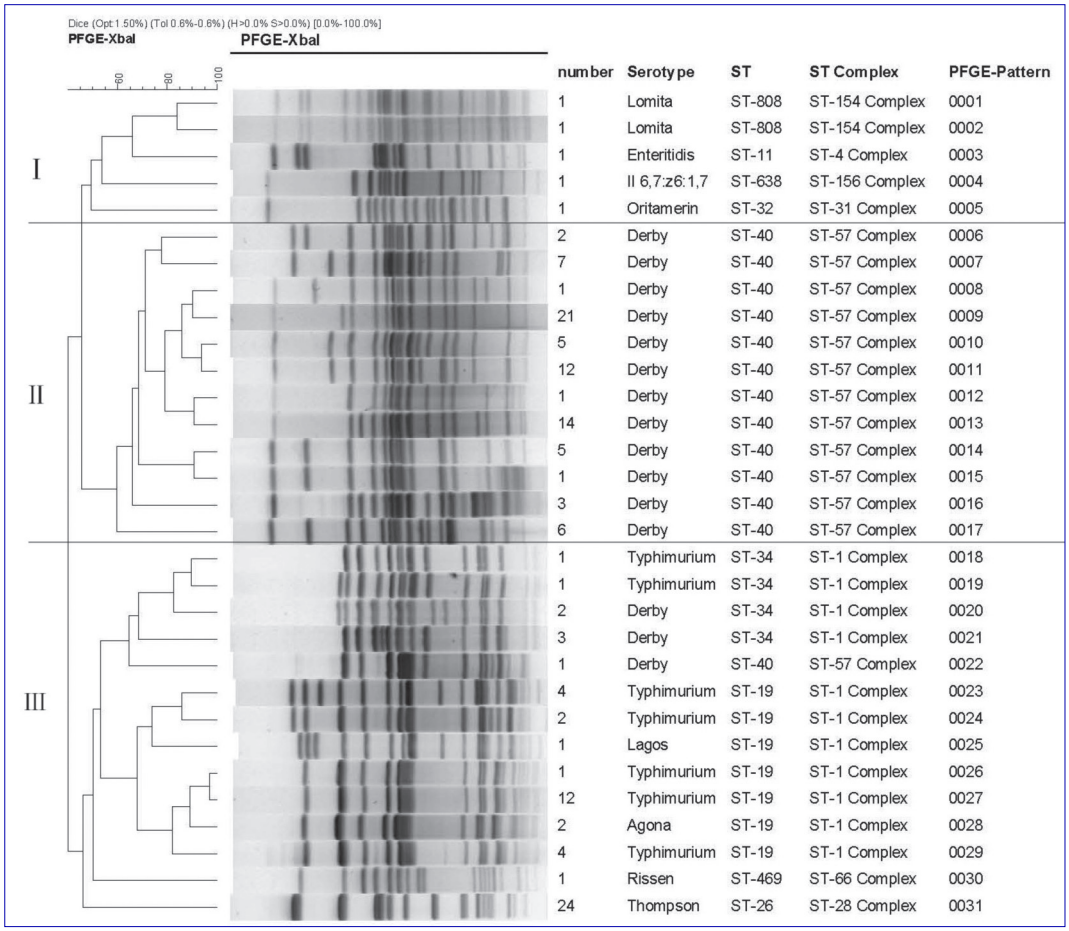


Fig 2. Serotype distribution of 142 *Salmonella* spp. isolate

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)





number	Serotype	ST	ST Complex	PFGE-Pattern
1	Lomita	ST-808	ST-154 Complex	0001
1	Lomita	ST-808	ST-154 Complex	0002
1	Enteritidis	ST-11	ST-4 Complex	0003
1	II 6,7:z6:1,7	ST-638	ST-156 Complex	0004
1	Oritamerin	ST-32	ST-31 Complex	0005
2	Derby	ST-40	ST-57 Complex	0006
7	Derby	ST-40	ST-57 Complex	0007
1	Derby	ST-40	ST-57 Complex	0008
21	Derby	ST-40	ST-57 Complex	0009
5	Derby	ST-40	ST-57 Complex	0010
12	Derby	ST-40	ST-57 Complex	0011
1	Derby	ST-40	ST-57 Complex	0012
14	Derby	ST-40	ST-57 Complex	0013
5	Derby	ST-40	ST-57 Complex	0014
1	Derby	ST-40	ST-57 Complex	0015
3	Derby	ST-40	ST-57 Complex	0016
6	Derby	ST-40	ST-57 Complex	0017
1	Typhimurium	ST-34	ST-1 Complex	0018
1	Typhimurium	ST-34	ST-1 Complex	0019
2	Derby	ST-34	ST-1 Complex	0020
3	Derby	ST-34	ST-1 Complex	0021
1	Derby	ST-40	ST-57 Complex	0022
4	Typhimurium	ST-19	ST-1 Complex	0023
2	Typhimurium	ST-19	ST-1 Complex	0024
1	Lagos	ST-19	ST-1 Complex	0025
1	Typhimurium	ST-19	ST-1 Complex	0026
12	Typhimurium	ST-19	ST-1 Complex	0027
2	Agona	ST-19	ST-1 Complex	0028
4	Typhimurium	ST-19	ST-1 Complex	0029
1	Rissen	ST-469	ST-66 Complex	0030
24	Thompson	ST-26	ST-28 Complex	0031

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* II6, 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) Derby (14) Rissen (1)	A A A	ST-40 ST-40 ST-469	0012 0013 0030	20.0
B (19)	II6,7:z6:1,7 (1) Derby (3) Typhimurium (1) Typhimurium (1) Typhimurium (9) Typhimurium (4)	B B B B B B	ST-638 ST-40 ST-34 ST-19 ST-19 ST-19	0004 0017 0019 0026 0027 0029	23.8
C (19)	Lomita (1) Lomita (1) Derby (1) Derby (12) Derby (1) Derby (1) Typhimurium (2)	C C C C C C C	ST-808 ST-808 ST-40 ST-40 ST-40 ST-40 ST-19	0001 0002 0008 0011 0014 0022 0024	23.8
D (14)	Enteritidis (1) Derby (3) Derby (1) Derby (3) Typhimurium (1) Typhimurium (3) Agona (2)	D D D D D D D	ST-11 ST-40 ST-40 ST-40 ST-34 ST-19 ST-19	0003 0014 0015 0017 0018 0027 0028	17.5
E (13)	Oritamerin (1) Derby (2) Derby (2) Derby (3) Typhimurium (4) Lagos (1)	E E E E E E	ST-32 ST-40 ST-34 ST-34 ST-19 ST-19	0005 0006 0020 0021 0023 0025	16.3
F(15)	Derby (7) Derby (5) Derby (3)	F F F	ST-40 ST-40 ST-40	0007 0010 0016	18.6
G(24)	Thompson (24)	G	ST-26	0031	30.0
H(22)	Derby (21) Derby (1)	H H	ST-40 ST-40	0009 0014	27.5

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 was 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%) [12], in Sichuan Province, China (10.7%) [3], and in Thailand (7.22%) [19], but it was lower than that reported in Jiangsu Province, China (71.8%) [13].

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 [18]. Others have previously demonstrated PFGE pattern diversity within a serotype [20-22], 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 [23].

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 [5,24-26], 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 [25]. The limited discriminatory ability of STs may be resulted from the moderate to slow rate of mutation accumulation within seven targeted housekeeping genes [26]. 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 [27]. 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 [12,13,28-31]. 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 [3].

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 [32,33]. Other study also indicated that *Salmonella spp.* had the 'patient-environment-food' transmission circulation [34]. 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.

Acknowledgments

The authors would like to thank Xianxian Liu, Xumin Cao, Xiaojiao Wang, Kun Liu and Lu Sun for their help of samples collection.

REFERENCES

1. Doorduyn Y, Van Den Brandhof WE, Van Duynhoven YT, Wannet WJ, Van Pelt W: Risk factors for *Salmonella* Enteritidis and Typhimurium (DT104 and non-DT104) infections in the Netherlands: Predominant roles for raw eggs in Enteritidis and sandboxes in Typhimurium infections. *Epidemiol Infect*, 134, 617-626, 2006. DOI: 10.1017/S0950268805005406

2. Wang J, Zheng R, Wang J: Risk assessment of Salmonella in animal derived food. *Chin J Anim Q*, 24: 23-25, 2007.

3. Li R, Lai J, Wang Y, Liu S, Li Y, Liu K, Shen J, Wu C: Prevalence and characterization of *Salmonella* species isolated from pigs, ducks and chickens in Sichuan Province, China. *Int J Food Microbiol*, 163, 14-18, 2013. DOI: 10.1016/j.ijfoodmicro.2013.01.020

4. Hauser E, Hebner F, Tietze E, Helmuth R, Junker E, Prager R, Schroeter A, Rabsch W, Fruth A, Malorny B: Diversity of *Salmonella enterica* serovar Derby isolated from pig, pork and humans in Germany. *Int J Food Microbiol*, 151, 141-149, 2011. DOI: 10.1016/j.ijfoodmicro.2011.08.020

5. Noda T, Murakami K, Asai T, Etoh Y, Ishihara T, Kuroki T, Horikawa K, Fujimoto S: Multi-locus sequence typing of *Salmonella enterica* subsp. *enterica* serovar Enteritidis strains in Japan between 1973 and 2004. *Acta Vet Scand*, 53, 38, 2011. DOI: 10.1186/1751-0147-53-38

6. Mürmann L, dos Santos MC, Cardoso M: Prevalence, genetic characterization and antimicrobial resistance of *Salmonella* isolated from fresh pork sausages in Porto Alegre, Brazil. *Food Control,* 20, 191-195, 2009. DOI: 10.1016/j.foodcont.2008.04.007

7. Galanis E, Lo Fo Wong DM, Patrick ME, Binsztein N, Cieslik A, Chalermchikit T, Aidara-Kane A, Ellis A, Angulo FJ, Wegener HC, World Health Organization Global Salm-Surv: Web-based surveillance and global *Salmonella* distribution, 2000-2002. *Emerg Infect Dis*, 12, 381-338, 2006. DOI: 10.3201/eid1205.050854

8. Urwin R, Maiden MC: Multi-locus sequence typing: A tool for global epidemiology. *Trends Microbiol*, 11, 479-487, 2003. DOI: 10.1016/j.tim.2003.08.006

9. Sukhnanand S, Alcaine S, Warnick LD, Su WL, Hof J, Craver MP, McDonough P, Boor KJ, Wiedmann M: DNA sequence-based subtyping and evolutionary analysis of selected *Salmonella enterica* serotypes. *J Clin Microbiol*, 43, 3688-3698, 2005. DOI: 10.1128/JCM.43.8.3688-3698.2005

10. Arguello H, Carvajal A, Collazos JA, Garcia-Feliz C, Rubio P: Prevalence and serovars of *Salmonella enterica* on pig carcasses, slaughtered pigs and the environment of four Spanish slaughterhouses. *Food Res Int*, 45, 905-912, 2012. DOI: 10.1016/j.foodres.2011.04.017

11. Bonardi S, Bassi L, Brindani F, D'Incau M, Barco L, Carra E, Pongolini S: Prevalence, characterization and antimicrobial susceptibility of *Salmonella enterica* and Yersinia enterocolitica in pigs at slaughter in Italy. *Int J Food Microbiol*, 163, 248-257, 2013. DOI: 10.1016/j.ijfoodmicro.2013.02.012

12. Li YC, Pan ZM, Kang XL, Geng SZ, Liu ZY, Cai YQ, Jiao XA: Prevalence, characteristics, and antimicrobial resistance patterns of *Salmonella* in retail pork in Jiangsu province, eastern China. *J Food Prot*, 77, 236-45, 2014. DOI: 10.4315/0362-028X.JFP-13-269

13. Cai Y, Tao J, Jiao Y, Fei X, Zhou L, Wang Y, Zheng H, Pan Z, Jiao X: Phenotypic characteristics and genotypic correlation between *Salmonella* isolates from a slaughterhouse and retail markets in Yangzhou, China. *Int J Food Microbiol*, 222, 56-64, 2016. DOI: 10.1016/j.ijfoodmicro.2016.01.020

14. Li W, Raoult D, Fournier PE: Bacterial strain typing in the genomic era. *FEMS Microbiol Rev*, 33, 892-916, 2009. DOI: 10.1111/j.1574-6976.2009.00182.x

15. Clinical and Laboratory Standards Institute: Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard, 8th ed, Document M07-A8. Clinical and Laboratory Standards Institute, Wayne, PA, 2009.

16. Clinical Laboratory Standards Institute (CLSI): Performance standards for antimicrobial susceptibility testing. Twenty-Third Informational Supplement, M100-S23, CLSI, Wayne, PA, 2013.

17. Harbottle H, White DG, McDermott PF, Walker RD, Zhao S: Comparison of multilocus sequence typing, pulsed-field gel electrophoresis, and antimicrobial susceptibility typing for characterization of Salmonella enterica serotype Newport isolates. *J Clin Microbiol*, 44, 2449e57, 2006. DOI: 10.1128/JCM.00019-06

18. Adaska JM, Silva AJ, Berge ACB, Sischo WM: Genetic and phenotypic variability among *Salmonella enterica* serovar Typhimurium isolates from California dairy cattle and humans. *Appl Environ Microbiol, 72*, 6632-6637, 2006. DOI: 10.1128/AEM.01038-06

19. Tadee P, Boonkhot P, Patchanee P: Quantification of contamination levels and particular risk of *Salmonella* spp. in pigs in slaughterhouses in Chiang Mai and Lamphun provinces, Thailand. *Jpn J Vet Res*, 62, 171-199, 2014.

20. Gai W, Wang J, Wang J, Cui Z, Qu Z, Cui J, Du X, Huang X, Zhao J:

Molecular classification and drug resistance analysis of *Escherichia coli* isolated from poultry in China. *Int J Clin Exp Med*, 8, 836-844, 2015.

21. Sukhnanand S, Alcaine S, Warnick LD, Su WL, Hof J, Craver MP, McDonough P, Boor KJ, Wiedmann M: DNA sequence based subtyping and evolutionary analysis of selected *Salmonella enterica* serotypes. *J Clin Microbiol*, 43, 3688e98, 2005. DOI: 10.1128/JCM.43.8.3688-3698.2005

22. Liu F, Barrangou R, Gerner-Smidt P, Ribot EM, Knabel SJ, Dudley EG: Novel virulence gene and clustered regularly interspaced short palindromic repeat (CRISPR)multilocus sequence typing scheme for subtyping of the major serovars of *Salmonella enterica* subsp. *enterica*. *Appl Environ Microbiol*, 77, 1946-1956, 2011. DOI: 10.1128/AEM.02625-10

23. Torpdahl M, Skov MN, Sandvang D, Baggesen DL: Genotypic characterization of *Salmonella* by multilocus sequence typing, pulsed-field gel electrophoresis and amplified fragment length polymorphism. *J Microbiol Methods*, 63, 173-184, 2005. DOI: 10.1016/j.mimet.2005.03.006

24. Campioni F, Moratto Bergamini AM, Falcão JP: Genetic diversity, virulence genes and antimicrobial resistance of *Salmonella* Enteritidis isolated from food and humans over a 24-year period in Brazil. *Food Microbiol*, 32, 254-264, 2012. DOI: 10.1016/j.fm.2012.06.008

25. Ben-Darif E, De Pinna E, Threlfall EJ, Bolton FJ, Upton M, Fox AJ: Comparison of a semi-automated rep-PCR system and multilocus sequence typing for differentiation of *Salmonella enterica* isolates. *J Microbiol*, 81, 11e6, 2010. DOI: 10.1016/j.mimet.2010.01.013

26. Van Hoek AHAM, de Jonge R, van Overbeek WM, Bouw E, Pielaat A, Smid JH, Malorny B, Junker E, Löfström C, Pedersen K, Aarts HJ, Heres L: A quantitative approach towards a better understanding of the dynamics of *Salmonella spp.* in a pork slaughter-line. *In J Food Microbiol*, 153, 45-52, 2012. DOI: 10.1016/j.ijfoodmicro.2011.10.013

27. Witte W: Medical consequences of antimicrobial use in agriculture. *Science*, 279, 996-997, 1998. DOI: 10.1126/science.279.5353.996

28. Piras F, Brown DJ, Meloni D, Mureddu A, Mazzette R: Investigation of *Salmonella enterica* in Sardinian slaughter pigs: Prevalence, serotype and genotype characterization. *Int J Food Microbiol*, 151, 201-209, 2011. DOI: 10.1016/j.ijfoodmicro.2011.08.025

29. Miranda JM, Mondragón AC, Martinez B, Guarddon M, Rodriguez JA: Prevalence and antimicrobial resistance patterns of *Salmonella* from different raw foods in Mexico. *J Food Prot*, 72, 966-971, 2009. DOI: 10.4315/0362-028X-72.5.966

30. Thai TH, Hirai T, Lan NT, Yamaguchi R: Antimicrobial resistance profiles of *Salmonella* serovars isolated from retail pork and chicken meat in North Vietnam. *Int J Food Microbiol*, 156, 147-151, 2012. DOI: 10.1016/j.ijfoodmicro.2012.03.016

31. White DG, Zhao S, Sudler R, Ayers S, Friedman S, Chen S, McDermott PF, McDermott S, Wagner DD, Meng J: The isolation of antimicrobial-resistant *Salmonella* from retail ground meats. *N Engl J Med*, 345, 1147-1154, 2001. DOI: 10.1056/NEJMoa010315

32. Tadee P, Boonkhot P, Pornruangwong S, Patchanee P: Comparative phenotypic and genotypic characterization of *Salmonella* spp. in pig farms and slaughterhouses in two provinces in Northern Thailand. *PLo One,* 10, e0116581, 2015. DOI: 10.1371/journal.pone.0116581

33. Zou M, Keelara S, Thakur S: Molecular characterization of *Salmonella enterica* serotype Enteritidis isolates from humans by antimicrobial resistance, virulence genes, and pulsed-field gel electrophoresis. *Foodborne Pathog Dis*, 9, 232-238, 2012. DOI: 10.1089/fpd.2011.1012

34. Römling U, Tümmler B: Achieving 100% typeability of Pseudomonas aeruginosa by pulsed-field gelelectrophoresis. *J Clin Microbiol,* 38, 464-455, 2000.