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Research Article

Characterization and Distribution of *Salmonella* spp. Isolated from Poultry Slaughterhouse-processingin Shandong Province [1]

Wenyan GAI [1] Junwei WANG [1] Juan WANG [1] Zhigang CUI [2]
Zhina QU [1] Jun HONG [1] Jinghua CUI [2] Xiaoli DU [2]
Xiumei HUANG [1] Xumin CAO [1] Jianmei ZHAO 1

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[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 Centerfor Disease Control and Prevention, PulseNetChina, 102206 Beijing - CHINA

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Abstract

Salmonella spp.is the most important food-borne pathogens of public health interest incriminated in poultry meat worldwide. The purpose of this study to estimate the prevalence *Salmonella* spp. contamination in poultry products from 12 different located geographical areas from among the 12 poultry slaughterhouses authorized in China and to characterize all the isolates by serotypes, PFGE patterns, MLST patterns and antimicrobial susceptibility. The prevalence of *Salmonella* spp. in the poultry slaughterhouse was 10.4%. All 100 strains of *Salmonella* spp. comprising 13 majority serotypes were identified. *S. Enteritidis* was the most frequently isolated from samples. The isolates displayed resistance to sulfisoxazole (SF) (79.0%), doxycycline (DOX) (68.0%), tetracycline (TE) (65.0%), florfenicol (FFC) (64.0%), ampicillin (AM) (50.0%), gentamicin (GM) (48.0%), trimethoprim-sulfamethoxazole (SXT) (30.0%), spectinomycin (SPT) (30.0%), enrofloxacin (ENR) (10.0%), ofloxacin (NOR) (10.0%), amoxicillin potassium clavulanat (AC) (1.0%) and polymyxin (PME) (4.0%). The *Salmonella* spp. isolates were not resistant to cefotaxime (EFT). Each isolates were multi-drug resistant as they were resistant to at least 2 groups of antimicrobials. Four clusters and 38 fingerprint-patterns generated by PFGE were identified among strains recovered from various locations, providing information on associations among the strains as well as evidence of the existence of persistent strains in some areas. MLST analysis of isolates identified the 10 STs, the 7 housekeeping genes had the different variation.

Keywords: *Salmonella spp., Poultry slaughterhouse-processing, Serotyping, PFGE, MLST, Antimicrobial susceptibility*

Shandong Eyaletindeki Kanatlı Hayvan Kesimhanelerinden İzole Edilen *Salmonella* spp.'nin Karakterizasyonu ve Yaygınlığı

Özet

Salmonella spp. dünya çapında kanatlı hayvan etlerinden köken alan halk sağlığı bakımından en önemli gıda kaynaklı patojendir. Bu çalışmanın amacı Çin'de 12 değişik bölgede yer alan kesimhanelerde kesilen kanatlı hayvanlara ait ürünlerde *Salmonella* spp. prevalansını tespit etmek ve tüm izolatları serotipleri, PFGE ve MLST görüntüleri ile antimikrobiyal duyarlılıklarına göre karakterize etmektir. Çalışmada *Salmonella* spp. prevalansı kanatlı kesimhanelerinde %10.4 olarak belirlendi. 13 serotipi içeren 100 *Salmonella* suşu tespit edildi. Örneklerden en çok izole edilen etken *S. Enteritidis* olarak kaydedildi. İzolatlar belirtilen antibiyotiklere direnç gösterdi; sulfisozaksol (SF) (%79.0), doksisilin (DOX) (%68.0), tetrasiklin (TE) (%65.0), florfenikol (FFC) (%64.0), ampisillin (AM) (%50.0), gentamisin (GM) (%48.0), trimethoprim-sulfamethoksazol (SXT) (%30.0), spektinomisin (SPT) (%30.0), enrofloksasin (ENR) (%10.0), ofloksasin (NOR) (%10.0), amoksisilin potasyum klavulanat (AC) (%1.0) ve polimiksin (PME) (%4.0). *Salmonella* spp. izolatları cefotaxime (EFT) karşı dirençli değildi. Her bir izolat en az 3 grup antimikrobiyal madde olmak üzere çoklu ilaç direnci gösterdi. PFGE ile elde edilen dört küme ve 38 parmakizi-görüntüsü değişik bölgelerden toplanan suçların arasından belirlendi. Suşlar arası asosiasyon ve bazı bölgelerde persiste suşların varlığı tespit edildi. İzolatların MLST analizi 10 ST belirledi. Yedi housekeeping gen farklı varyasyonlara sahipti.

Anahtar sözcükler: *Salmonella spp., Kanatlı hayvan kesimhane-işleme, Serotiplendirme, PFGE, MLST, Antimikrobiyal duyarlılık*

İletişim (Correspondence)
+86 0532 85633936
yffs2000@sina.com

INTRODUCTION

Salmonella spp. is an important group of bacterial zoonotic pathogens which can cause acute food-borne diseases in humans. Each year, approximately 90 million cases of gastroenteritis due to *Salmonella* spp. occur globally [1]. It multiplies mainly in poutry intestinal tract where it can be detected within two hours after infection [2]. A great increase inhuman food-borne infections caused by *Salmonella* spp. including *Salmonella* Enteritidis and *Salmonella* Typhimurium has been noted in the United States, Europe [3,4] *Salmonella* spp. infections in humans often result from the ingestion of contaminated foods, such as poultry, beef, pork, eggs and produce. Estimates from the Centers for Disease Control and Prevention (CDC) reported that more than a million people have *Salmonella* spp. poisoning every year from a variety of causes. Poultry products have consistently been identified as important sources of *Salmonella* spp. infection in humans, because vertical transfer of infection from breeding hens to progeny is an important aspect of the epidemiology of *Salmonella* spp. infection within the poultry industry [5]. Other study showed that a great number of *Salmonella* spp. contaminate poultry products and have somewhat different genetic types according to the origin of the integrated broiler operation [6]. Nevertheless, there have been no studies following the dissemination of *Salmonella* spp. in the entire poultry industry in China.

The aim of this study was to estimate the prevalence *Salmonella* spp. contamination in poultry products from 12 different located geographical areas from among the 12 poultry slaughterhouses authorized in China and to characterize all the isolates by serotypes, pulse field gel electrophoresis (PFGE) patterns, multi-locus sequence typing (MLST) patterns and antimicrobial susceptibility to investigate possible sources of infection and to provide information which could help strengthen salmonellosis control programs, and minimize the risk of *Salmonella* spp. exposure in the slaughtering line, which can reduce the contamination pressure downstream at retail shops as well as for end consumers.

MATERIAL and METHODS

The Sample Collection and Isolation

The present study was performed in 12 representative slaughterhouses (A ~ L) in 12 different areas in Shandong province from 2014 to 2015. A total of 960 swabs samples were randomly collected from poultry slaughterhouse-processing, every location the sample number is 80. *Salmonella* spp. contamination samples were collected in five critical steps in slaughterhouse-processing.

All samples were shipped in an icebox to the Microbiology Laboratory of Quality and Safety Risk Assessment for Animal Products of Ministry of Agriculture (CAHEC) in Qingdao, China, for *Salmonella* spp. isolation within 24 h. The procedure for the detection and isolation of *Salmonella* spp. was carried out according to the techniques recommended by the International Organization for Standardization [7]. All suspicious *Salmonella* colonies with black center were confirmed biochemically using lysine and triple sugar iron agars and API 20E (bioMérieux, France). The *invA* (F: 5′-GAATCCTCAGTTTTCAGTTTC-3′; R: 5′-TAGCCGTAACAACCAATACAAATG-3′) gene of *Salmonella* was used to further confirm the identity of the presumptive *Salmonella* spp.[8].

Serotyping

Salmonella-positive isolates were randomly serotyped according to the White-Kauffmann-Le Minor scheme by slide agglutination with O and H antigen specific sera (Sifin Diagnostics, Berlin, Germany) [9].

Pulsed-field Gel Electrophoresis

PFGE was performed for *Salmonella enterica* using the Pulse Net protocol procedures described previously [10]. The bacteria genomic DNA was digested with 50 U of *Xba* I (Takara, China) at 37°C for 3 h. The Pulse Net "Universal" standard strain *Salmonella enterica* serovar Braenderup H9812 was used as a reference marker and *Xba* I (Takara, China) was used as the digestion enzyme. PFGE was repeated twice to determine reproducibility. For untypable isolates, 50 μ Mthiourea (Sigma, USA) was added to the 0. 5 XTBE buffer prior to PFGE run as described by Römling and Tümmler [11]. CHEF-Mapper (Bio-Rad, USA) was used for electrophoresis (AutoAlgorithm: 30 kb-low MW, 700 kb-high MW, 19 h). The gel was stained with Gelred (Biofilm) and visualized using a gel imaging system (Bio-Rad, Gel DocXR, USA). PFGE patterns were analyzed using BioNumerics version 5.10; and a dendrogram was constructed using the Dice coefficient and un-weighted pair group methods with the arithmetic mean algorithm (UPGMA). PFGE banding patterns with a similarity index >80% were grouped in the same genotype cluster.

Multi-locus Sequence Typing

The multilocus sequence typing method was performed according to the recommendations of the *Salmonella enterica* MLST website *(http://mlst.warwick.ac.uk/mlst/dbs/Senterica)*. Seven housekeeping genes, *aroC*, *dnaN*, *hemD*, *hisD*, *purE*, *sucA* and *thrA*, were amplified using the recommended primers. DNA Taq premix was used with the amplification procedure: 94°C 5 min; followed by 30 cycles: 94°C 1 min, 55°C 30 s, 72°C 1 min; 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 and MEGA4 software. We submitted the sequences to the UCC database for their allele and ST assignments. A minimum

spanning tree based on these STs was generated with BioNumerics version 5.10.

Antimicrobial Susceptibility Tests

Susceptibility of the isolates to antimicrobial agents was evaluated according to Clinical Laboratory Standards Institute (CLSI, 2012) guidelines using the disc diffusion method on Mueller-Hinton agar (Becton Dickinson, USA) [12]. Following 13 antimicrobial agents were tested, including chloramphenicol (florfenicol), penicillins (ampicillin and amoxicillin potassium clavulanat), sulfonamides (sulfisoxazole and trimethoprim-sulfamethoxazole), cephems (cefotaxime), aminoglycosides (gentamicin and spectinomycin), tetracyclines (tetracycline and doxycycline), fluoroquinolones (enrofloxacin and ofloxacin), and other (polymyxin). Results were interpreted using the Clinical and Laboratory Standards Institute (CLSI, 2012) breakpoints when available *E. coli* ATCC 25922 was used as quality control [13].

RESULTS

The prevalence of *Salmonella* spp. from each of the 12 poultry slaughterhouses is presented in *Table 1*. In this study, 100 (10.42%) *Salmonell*a spp. was isolated from poultry slaughterhouses, the 12 (12.0%) *Salmonella* isolated from duck slaughterhouses and the 88 (88.0%) isolated from chicken slaughterhouses. All of the samples from 2 duck slaughterhouses were contaminated with *Salmonella* spp.. The major prevalent serotypes in the duck slaughterhouses were *Salmonell*a Thompson, and 5 different serotypes *(Salmonell*a Agona, *Salmonell*a Montevideo, *S.* Typhimurium, *Salmonell*a Indiana and *Salmonell*a Albany). In the 10 chicken slaughterhouses, the major of *Salmonella* spp. serotypes were *S.* Enteritidis and *S.* Indiana. Other different serotypes,also were found in chicken slaughterhouses *(Fig.1)*. The characteristics of 100 *Salmonella* spp. strains comprising thirteen majority serotypes were identified. *S.* Enteritidis was the most frequently isolated serotype from samples (43.0%, 43 of 100) *(Fig.1)*. *Salmonella* group *S.* Enteritidis (n = 43), *S.* Indinan (n = 14), *Salmonella* Derby (n = 11), *Salmonella* Infantis (n = 8), *S.* Agona (n = 8), *S. Salmonell*a Agona, *Salmonell*a Montevideo, *S.* Typhimurium, *Salmonell*a Indiana and *Salmonell*a Albany (n = 7), *Salmonella* Essen (n = 2), *S.* Typhimurium (n = 2), *S.* Albany (n = 1), *Salmonella* Othmarschen (n = 1), *Salmonella* Schwarzengrund (n = 1), *Salmonella* Abortus equi (n = 1) and *S.* Montevideo (n = 1) were identified during this study *(Fig.1)*.

The results of the antimicrobial susceptibility analysis of the *Salmonella* spp. isolates were summarized in *Fig. 2*. All 100 *Salmonella spp.* isolates were observed to be susceptible to the 13 antimicrobial agents tested in this

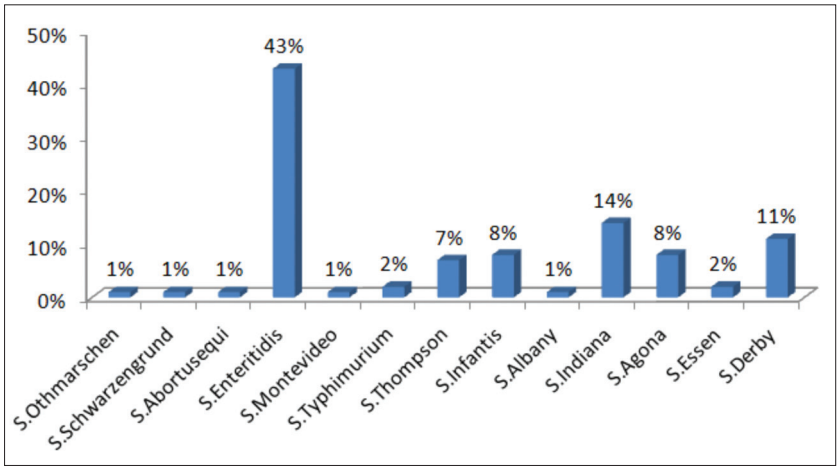


Fig 1. The distinction of serotype of 100 *Salmonella* spp. Isolate

Şekil 1. 100 adet *Salmonella* spp. izolatının serotiplendirmesi

Fig 2. The drug resistance rate of all isolates for 13 kind of antimicrobial agents. Statistical test was only performed in all isolated. Antibiotics abbreviations are: SF: Sulfisoxazole, DOX: Doxycycline, TE: Tetracycline, FFC: Florfenicol, AM: Ampicillin, GM: Gentamicin, SPT; Spectinomycin, SXT: Trimethoprim-sulfamethoxazole, ENR: Enrofloxacin, NOR: Ofloxacin, PME: Polymyxin, EFT: Cefotaxime, AC: Amoxicillin potassium clavulanat

Şekil 2. Tüm izolatların 13 antimikrobiyal madde için ilaç direnci oranları. İstattistiksel test bütün izolatlar için uygulandı. Antibiyotiklerin kısaltmaları; SF: Sulfisozaksol, DOX: Doksisilin, TE: Tetrasiklin, FFC: Florfenikol, AM: Ampisillin, GM: Gentamisin, SPT; Spektinomisin, SXT: Trimethoprim-sulfamethoksazol, ENR: Enrofloksasin, NOR: Ofloksasin, PME: Polymiksin, EFT: Sefotaksim, AC: Amoksisilin potasyum klavulanat

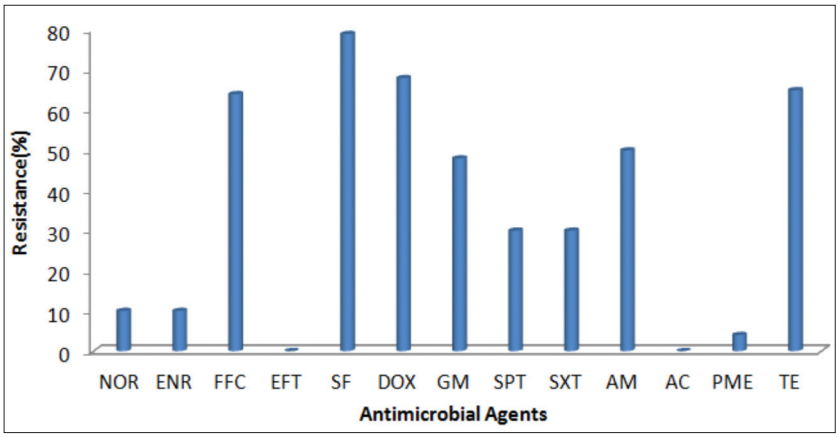


Table 1.*Origin and characterization of Salmonella isolated from twelve poultry slaughterhouse*
Tablo 1. *Oniki kanatlı hayvan kesimhanesinden izole edilen Salmonellaların orjin ve karakterizasyonu*

Number of Isolates (n)	Serotype	Location	Animal	MLST Pattern	PFGE Pattern	Prevalence (%)
A(6)	Enteritidis(3) Enteritidis(3)	A A	Chicken Chicken	ST-11 ST-11	0031 0032	7.5
B (6)	Agona(3) Indiana(1) Indiana(1) Enteritidis(1)	B B B B	Chicken Chicken Chicken Chicken	ST-13 ST-17 ST-17 ST-11	0003 0005 0017 0031	7.5
C(19)	Abortusequi(1) Schwarzengrund(1) Enteritidis(2) Othmarschen(1) Enteritidis(1) Derby(8) Essen(2) Derby(3)	C C C C C C C C	Chicken Chicken Chicken Chicken Chicken Chicken Chicken Chicken	ND ND ST-11 ND ST-11 ST-40 ND ND	0018 0024 0031 0031 0032 0037 0031 0024	23.8
D(9)	Indiana(2) Indiana(1) Indiana(1) Indiana(2) Enteritidis(1) Indiana(2)	D D D D D D	Chicken Chicken Chicken Chicken Chicken Chicken	ST-17 ST-17 ST-17 ST-17 ST-17 ST-17	0004 0005 0014 0025 0028 0030	11.3
E(13)	Enteritidis(1) Enteritidis(1) Enteritidis(1) Enteritidis(1) Indiana(1) Agona(1) Enteritidis(1) Agona(1) Agona(1) Indiana(1) Enteritidis(3)	E E E E E E E E E E E	Chicken Chicken Chicken Chicken Chicken Chicken Chicken Chicken Chicken Chicken Chicken	ST-11 ST-11 ST-17 ST-11 ST-17 ST-13 ST-11 ST-13 ST-13 ST-17 ST-11	0008 0009 0010 0011 0012 0012 0013 0015 0016 0028 0031	16.3
F(7)	Agona(1) Infantis(2) Indiana(1) Enteritidis(2) Indiana(1)	F F F F F	Chicken Chicken Chicken Chicken Chicken	ST-13 ST-32 ST-17 ST-11 ST-17	0001 0022 0031 0033 0027	8.8
G(9)	Typhimurium(1) Infantis(1) Infantis(1) Enteritidis(3) Enteritidis(2) Enteritidis(1)	G G G G G G	Chicken Chicken Chicken Chicken Chicken Chicken	ST-34 ST-32 ST-32 ST-11 ST-11 ST-11	0006 0021 0023 0032 0034 0036	11.3
H(7)	Infantis(1) Infantis(2) Enteritidis(1) Enteritidis(3)	H H H H	Chicken Chicken Chicken Chicken	ST-32 ST-32 ST-11 ST-11	0021 0022 0029 0032	8.8
I(4)	Agona(1) Montevideo(1) Typhimurium(1) Indiana(1)	I I I I	Duck Duck Duck Duck	ST-13 ST-305 ST-19 ST-17	0003 0007 0019 0020	5.0
J(6)	Enteritidis(2) Enteritidis(3) Enteritidis(1)	J J J	Chicken Chicken Chicken	ST-11 ST-11 ST-11	0026 0031 0032	7.5
K(6)	Enteritidis(5) Enteritidis(1)	K K	Chicken Chicken	ST-11 ND	0033 0035	7.5
L(8)	Thompson(7) Albany(1)	L L	Duck Duck	ST-26 ST-45	0002 0038	10.0

Table 2. *Sequence types (STs)and allele profile of each isolate*
Tablo 2. *Herbir izolatın sekans tipi ve allel profili*

STs	*acroC*	*dnaN*	*hemD*	*hisD*	*purE*	*SucA*	*thrE*	Number of Isolates
11	5	2	3	7	6	6	11	41
13	3	3	7	3	3	3	7	8
17	8	8	11	11	5	11	15	16
19	10	7	12	9	5	9	2	1
26	14	13	18	12	5	18	1	7
32	17	18	22	17	5	21	19	7
34	10	19	12	9	5	9	2	1
40	19	20	3	20	5	22	22	8
45	104	100	54	78	104	1	48	1
305	43	41	16	42	34	13	23	1

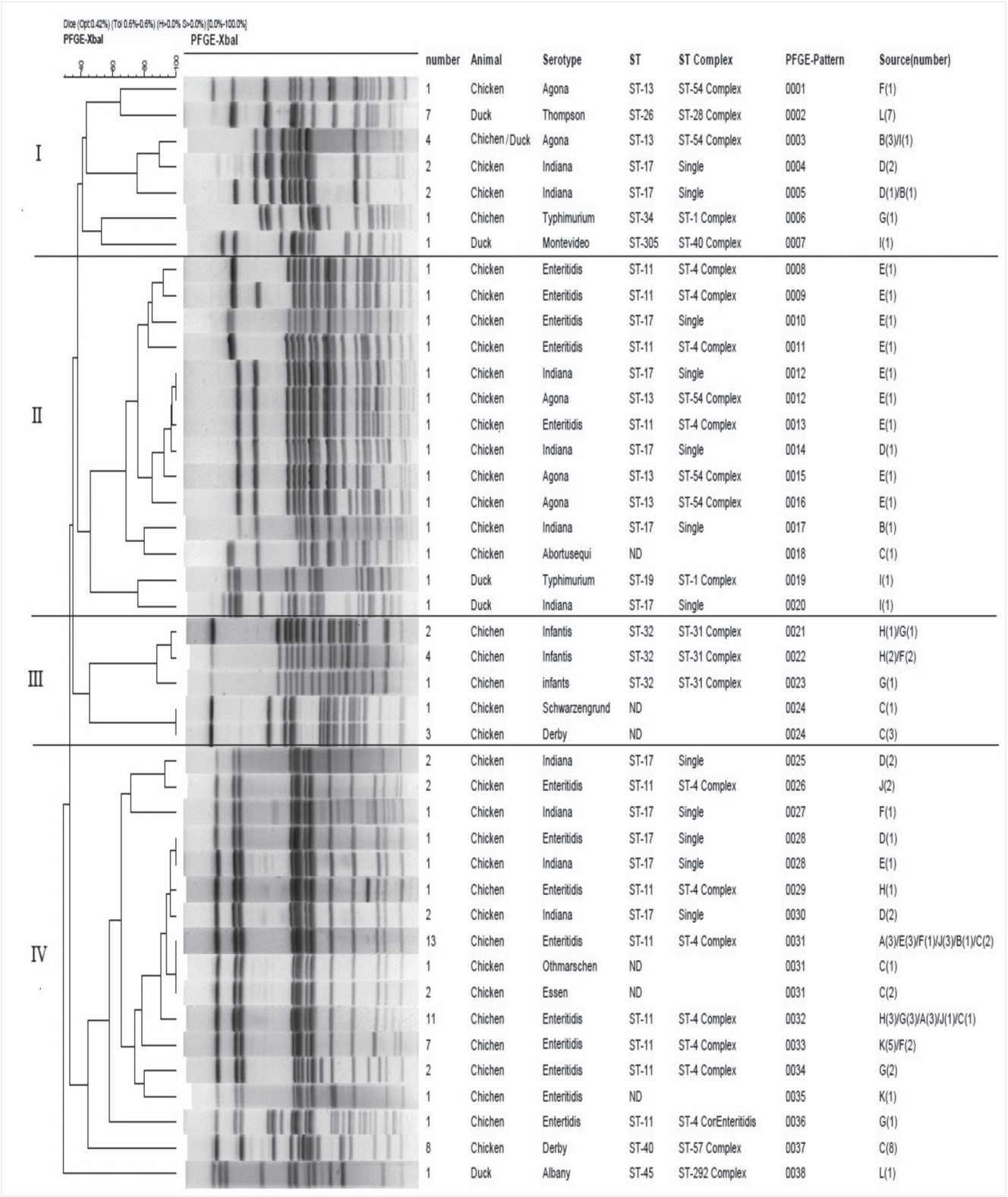
study. The isolates were resistantto sulfisoxazole (SF) (79.0%), doxycycline (DOX) (68.0%), tetracycline (TE) (65.0%), florfenicol (FFC) (64.0%), ampicillin (AM) (50.0%), gentamicin (GM) (48.0%), trimethoprim-sulfamethoxazole (SXT) (30.0%), spectinomycin (SPT) (30.0%), enrofloxacin (ENR) (10.0%), ofloxacin (NOR) (10.0%), amoxicillin potassium clavulanat (AC) (1.0%) and polymyxin (PME) (4.0%). The *Salmonella* spp. isolates were not resistant to cefotaxime (EFT). Each isolates were multi-drug resistant as they were resistant to at least 2 groups of antimicrobials.

The 100 *Salmonella* spp. isolates were analyzed by PFGE using enzymes *Xba*I resulted in 38 distinguishable patterns demonstrating a high level of genetic diversity among the isolates. An UPGMA dendrogram was constructed *(Fig. 3)*, and described in *Table 1*. Ten *Salmonella* spp. isolates were untypeable by PFGE. After the addition of thiourea (Sigma, USA)to the running buffer, all isolates remained typeable. The 100 isolates could be divided into four clusters. All serotypes were divided into groups based on their PFGE patterns. In this study,the most common pattern was 0031, which included 13 strains of *S.* Enteritidis, followed by 0032 which was composed of 11 strains of *S.* Enteritidis, and by 0037 which was composed of 8 strains of *S.* Derby. The origins and characteristics of *Salmonella* spp. Isolates identified in this study are outlined in *(Table 1)*. Looking at the strains in more detail, most pattern were within a single serotype and single source except 0003-pattern, 0005-pattern, 0021-pattern, 0022-pattern, 0031-pattern, 0032-pattern and 0033-pattern. The 0031-pattern was found to be composed of 13 *S.* Enteritidis, all recovered from various processing steps at six slaughterhouse, which were A(3), B(1), C(2), E(3), F(1), and J(3); the 0032-pattern was found to be composed of 11 *S.* Enteritidis, which were from H(3), G(3), A(3), J(1) and C(1) five slaughterhouse. The results showed that different places had the same isolates, these strains maybe come from the same farms, or the slaughterhouse circulation caused by cross contamination.

Ten discrete STs were identified among the 91*Salmonella* spp. isolates, indicating a degree of genotypic diversity, other 9 isolates were not done. Of these 10 STs, 4 were represented by single isolates, 6 were represented by more than one isolate (n = 7 to 41). The predominant STs were ST11, ST17, ST13 and ST26 containing 41 (45.1%), 16 (17.6%), 8 (8.8%) and 8 (8.8%) isolates respectively *(Table 2)*. Isolates characterized as the same STs did not necessarily have the same PFGE pattern. For example, the 41 isolates characterized as ST11 had 11 distinct PFGE patterns *(Fig. 3)*. All of the isolates that shared a PFGE pattern had the same STs. Meanwhile, isolates that shared a PFGE pattern had the same STs *(Fig. 3)*. Showing the detail of the strains in more detail, most STs were within a single serotype.

DISCUSSION

Poultry products have consistently been identified as important sources of *Salmonella* infection in humans, because vertical transfer of infection from breeding hens to progeny is an important aspect of the epidemiology of *Salmonella* spp. infection within the poultry industry. In many countries all over the world including USA, Europe, Korea and China, a wide range of different *Salmonella* serotypes have been found to contaminate the broiler houses, flocks and carcasses of the poultry industry [14]. In this study, 10.4% of the carcasses sampled from 12 poultry slaughterhouses were contaminated with *Salmonella* spp. and showed a lower prevalence than poultry carcasses originating from other countries like Korea (42.1%), Spain (17.9%), Canada (21.2%) and Ireland (26.4%) [15-18]. Although different sampling procedures, sample sizes and bacterial isolation and identification methods could affect the prevalences of *Salmonella* spp., this elevated level of contamination indicates a potential breakdown of hygiene at various stages at poultry farms and processing plants [19]. *S.* Enteritidis, which is responsible for most Salmonella infections in humans, is the major serotypes in this study, and this result supports that contaminated carcasses are the major source of infection in human salmonellosis [20].



number	Animal	Serotype	ST	ST Complex	PFGE-Pattern	Source(number)
1	Chicken	Agona	ST-13	ST-54 Complex	0001	F(1)
7	Duck	Thompson	ST-26	ST-28 Complex	0002	L(7)
4	Chichen / Duck	Agona	ST-13	ST-54 Complex	0003	B(3)/I(1)
2	Chicken	Indiana	ST-17	Single	0004	D(2)
2	Chicken	Indiana	ST-17	Single	0005	D(1)/B(1)
1	Chichen	Typhimurium	ST-34	ST-1 Complex	0006	G(1)
1	Duck	Montevideo	ST-305	ST-40 Complex	0007	I(1)
1	Chicken	Enteritidis	ST-11	ST-4 Complex	0008	E(1)
1	Chicken	Enteritidis	ST-11	ST-4 Complex	0009	E(1)
1	Chicken	Enteritidis	ST-17	Single	0010	E(1)
1	Chicken	Enteritidis	ST-11	ST-4 Complex	0011	E(1)
1	Chicken	Indiana	ST-17	Single	0012	E(1)
1	Chicken	Agona	ST-13	ST-54 Complex	0012	E(1)
1	Chicken	Enteritidis	ST-11	ST-4 Complex	0013	E(1)
1	Chicken	Indiana	ST-17	Single	0014	D(1)
1	Chicken	Agona	ST-13	ST-54 Complex	0015	E(1)
1	Chicken	Agona	ST-13	ST-54 Complex	0016	E(1)
1	Chicken	Indiana	ST-17	Single	0017	B(1)
1	Chicken	Abortusequi	ND		0018	C(1)
1	Duck	Typhimurium	ST-19	ST-1 Complex	0019	I(1)
1	Duck	Indiana	ST-17	Single	0020	I(1)
2	Chichen	Infantis	ST-32	ST-31 Complex	0021	H(1)/G(1)
4	Chichen	Infantis	ST-32	ST-31 Complex	0022	H(2)/F(2)
1	Chichen	infants	ST-32	ST-31 Complex	0023	G(1)
1	Chicken	Schwarzengrund	ND		0024	C(1)
3	Chicken	Derby	ND		0024	C(3)
2	Chicken	Indiana	ST-17	Single	0025	D(2)
2	Chicken	Enteritidis	ST-11	ST-4 Complex	0026	J(2)
1	Chicken	Indiana	ST-17	Single	0027	F(1)
1	Chicken	Enteritidis	ST-17	Single	0028	D(1)
1	Chicken	Indiana	ST-17	Single	0028	E(1)
1	Chichen	Enteritidis	ST-11	ST-4 Complex	0029	H(1)
2	Chicken	Indiana	ST-17	Single	0030	D(2)
13	Chicken	Enteritidis	ST-11	ST-4 Complex	0031	A(3)/E(3)/F(1)/J(3)/B(1)/C(2)
1	Chicken	Othmarschen	ND		0031	C(1)
2	Chicken	Essen	ND		0031	C(2)
11	Chicken	Enteritidis	ST-11	ST-4 Complex	0032	H(3)/G(3)/A(3)/J(1)/C(1)
7	Chicken	Enteritidis	ST-11	ST-4 Complex	0033	K(5)/F(2)
2	Chicken	Enteritidis	ST-11	ST-4 Complex	0034	G(2)
1	Chicken	Enteritidis	ND		0035	K(1)
1	Chichen	Entertidis	ST-11	ST-4 CorEnteritidis	0036	G(1)
8	Chicken	Derby	ST-40	ST-57 Complex	0037	C(8)
1	Duck	Albany	ST-45	ST-292 Complex	0038	L(1)

Fig 3. Dendrogram of PFGE profiles

PFGE patterns and the corresponding dendrogram for 100 isolates obtained in the present study are depicted. The 4PFGE clusters were marked on the node as I to IV. The different clusters observed are designated on the left side of the figure. Displayed on the right hand side are number, serotype, PFGE-Pattern, sequence types (STs), ST complex and source (number)

Şekil 3. PFGR profillerinin dendogramı

Çalışmadaki PFGE görüntüleri ve ilgili 100 izolatın dendogramı. 4PFGE kümeleri I'den IV'e kadar işaretlenmiştir. Elde edilen farklı kümeler şeklin sol tarafında belirtilmiştir. Sağ tarafta numara, serotip, PFGR-görüntüsü, sekans tipleri (STs), ST kompleks ve kaynakları (numara)

Each isolates showed antimicrobial resistance to at least one antimicrobial agent. The rate of resistance of *Salmonella* spp. to antimicrobial agents was higher than that previously observed [21,22], but sulfisoxazole, doxycycline and tetracycline were showed the higher resistance in 13 antimicrobial agents. The most of the isolates displayed a high level of susceptibility to most of the antimicrobial agents tested in this study, the antimicrobial susceptibility must will present a challenge for veterinary medicine and farm animal husbandry, and could also pose a threat to public health. *Fig. 2* shows *Salmonella* spp. resistance pattern of ofloxacin (NOR) at 10% is a reflection that it is least resistant to the organism, whereas the fluoroquinolones generally have been previously known to have a good sensitivity to *Salmonella* spp.. 100% resistance of *Salmonella* spp. from poultry sources to amoxicillin potassium clavulanat is in line with previous works [23], even though amoxicillin potassium clavulanat has been known to be of variable resistance. Tetracycline have repeatedly shown high levels of resistance of 60%-65% to which the work was lowed [24].

Some previous studies showed that the PFGE patterns of *S.* Enteritidis in China are similar even when the isolates have different phage types. The present investigation also confirmed that 18 *S. Enteritidis* isolates with different MLST pattern displayed different PFGE patterns in agreement with previous reports [25,26]. The PFGE results showed that different places had the same isolates, these strains maybe come from the same farms, or the slaughterhouse circulation caused by cross contamination.

This study shows that the domestic serotypes are *S. Enteritidis* and *S. Indian* in chicken and duck slaughterhouses in Shandong province, and provides detailed information about *Salmonella* spp. isolates from poultry. In addition, the appearance of resistant *Salmonella* spp. isolates from poultry suggests the need for a more prudent use of antibiotics and the importance of controlling this pathogen in poultry products. As human salmonellosis has been repeatedly related to the consumption of products worldwide, continuous research must be conducted to minimize the *Salmonella* spp. contamination in poultry slaughterhouse.

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