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Characterization of Salmonella species from poultry slaughterhouses in South Korea: carry-over transmission of *Salmonella* Thompson ST292 in slaughtering process

Yewon Cheong [1,†], **Jun Bong Lee** [1,†], **Se Kye Kim** [1], **Jang Won Yoon** [1,2,*]

[1]College of Veterinary Medicine & Institute of Veterinary Science, Kangwon National University, Chuncheon 24341, Korea
[2]The YOON Healthtech Co., Ltd, Chuncheon 24341, Korea

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***Corresponding author:**
Jang Won Yoon
College of Veterinary Medicine & Institute of Veterinary Science, Kangwon National University, 1 Kangwondaehak-gil, Chuncheon 24341, Korea.
Email: jwy706@kangwon.ac.kr
https://orcid.org/0000-0002-6874-5290

[†]Yewon Cheong and Jun Bong Lee equally contributed to this work.

ABSTRACT

Importance: *Salmonella* outbreaks linked to poultry meat have been reported continuously worldwide. Therefore, *Salmonella* contamination of poultry meats in slaughterhouses is one of the critical control points for reducing disease outbreaks in humans.
Objective: This study examined the carry-over contamination of *Salmonella* species through the entire slaughtering process in South Korea.
Methods: From 2018 to 2019, 1,097 samples were collected from the nine slaughterhouses distributed nationwide. One hundred and seventeen isolates of *Salmonella* species were identified using the *invA* gene-specific polymerase chain reaction, as described previously. The serotype, phylogeny, and antimicrobial resistance of isolates were examined.
Results: Among the 117 isolates, 93 were serotyped into *Salmonella* Mbandaka (n = 36 isolates, 30.8%), *Salmonella* Thompson (n = 33, 28.2%), and *Salmonella* Infantis (n = 24, 20.5%). Interestingly, allelic profiling showed that all *S.* Mbandaka isolates belonged to the lineage of the sequence type (ST) 413, whereas all *S.* Thompson isolates were ST292. Moreover, almost all *S.* Thompson isolates (97.0%, 32/33 isolates) belonging to ST292 were multidrug-resistant and possessed the major virulence genes whose products are required for full virulence. Both serotypes were distributed widely throughout the slaughtering process. Pulsed-field gel electrophoretic analysis demonstrated that seven *S.* Infantis showed 100% identities in their phylogenetic relatedness, indicating that they were sequentially transmitted along the slaughtering processes.
Conclusions and Relevance: This study provides more evidence of the carry-over transmission of *Salmonella* species during the slaughtering processes. ST292 *S.* Thompson is a potential pathogenic clone of *Salmonella* species possibly associated with foodborne outbreaks in South Korea.

Keywords: Salmonella; phylogeny; drug resistance, microbial; poultry; slaughterhouse

INTRODUCTION

Salmonellosis is a major foodborne disease in the world. Over 1,500 serotypes of *Salmonella* species have been associated with human infections [1]. The consumption of poultry meat

ORCID iDs
Yewon Cheong
https://orcid.org/0009-0003-4328-7005
Jun Bong Lee
https://orcid.org/0000-0001-9758-9867
Se Kye Kim
https://orcid.org/0000-0002-9161-2533
Jang Won Yoon
https://orcid.org/0000-0002-6874-5290

Author Contributions
Conceptualization: Yoon JW, Cheong Y, Lee JB; Data curation: Cheong Y, Lee JB, Kim SK; Formal analysis: Cheong Y, Lee JB, Kim SK; Funding acquisition: Yoon JW; Investigation: Yoon JW, Cheong Y, Lee JB; Methodology: Cheong Y, Lee JB; Project administration: Yoon JW; Resources: Yoon JW, Cheong Y, Lee JB, Kim SK; Software: Cheong Y, Lee JB; Supervision: Yoon JW; Validation: Cheong Y, Lee JB, Kim SK; Visualization: Cheong Y, Lee JB, Kim SK; Writing - original draft: Cheong Y, Lee JB; Writing - review & editing: Yoon JW.

Conflict of Interest
The authors declare no conflicts of interest.

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contaminated with *Salmonella* spp. is the most common route of *Salmonella* infection [2]. In the United States, 64 *Salmonella* outbreaks in humans were reported to be linked to poultry meats from 2009 to 2015 [3]. In 2022, the multi-country outbreak of *Salmonella* Mbandaka with sequence type (ST) 413 occurred in the European Union, Israel, and the United Kingdom, which was possibly linked to the consumption of chicken meat, resulting in 196 cases of salmonellosis [4].

Typically, pathogenic *Salmonella* strains invade the gastrointestinal mucosa to cause gastroenteritis [5]. They can disseminate to the bloodstream, leading to systemic disease with severe morbidity and mortality [5]. The five *Salmonella* pathogenicity islands (SPI1 to 5) play an important role in bacterial pathophysiological processes, including invasion, intracellular survival, and inflammation induction [5]. SylA and PhoPQ are associated with *Salmonella* resistance to oxidative stress and innate immunity [6,7]. Other virulence factors, such as *Salmonella* enterotoxin (*stn*), aggregative fimbriae (*agfA*), and *Salmonella* virulence plasmid (*spvC*), are also required for *Salmonella* pathogenesis [8,9].

The prevalence of *Salmonella* in poultry meat has been reported in South Korea since poultry meat was proposed as a primary source of *Salmonella* transmission [10]. The rates of *Salmonella* isolation in poultry carcasses from slaughterhouses during 2014–2016 were 25.8% [11] and 28.0% [12]. The rates of *Salmonella* isolation from retail markets between 2011 and 2018 were 22.4% [13], 3.7% [14], and 17.0% [15]. In particular, the dominant serotypes of *Salmonella* isolates were changed depending on the period. For example, *Salmonella* Enteritidis and *Salmonella* Typhimurium were the dominant serotypes in the early 2010s, whereas *Salmonella* Montevideo and *Salmonella* Virchow appeared to dominate after the mid-2010s [11-15].

Poultry feces is a principal vehicle for *Salmonella* transmission from poultry farms to retail meat [16]. The slaughtering processes under feces-contaminated conditions boost the *Salmonella* contamination of poultry meat [17]. Therefore, many countries use the management system called hazard analysis and critical control points (HACCP) to reduce *Salmonella* contamination in poultry slaughterhouses [18]. This system monitors the contamination status of poultry carcasses at a series of slaughtering processes [19].

Although the HACCP system has been used widely in South Korea, many *Salmonella* outbreaks have been reported during 2015–2019 [20]. Nevertheless, little is known about the carry-over transmission of *Salmonella* species through the entire slaughtering process. Therefore, this study examined whether the carry-over transmission of *Salmonella* species occurs during the slaughtering processes in South Korea.

METHODS

Samples and Salmonella isolation

One thousand and ninety-seven samples were collected from the following six consecutive slaughtering processes: hanging (A), after de-feathering (B), after washing (C), evisceration (D), before chilling (E), and after chilling (F). The nationwide samples were collected from nine poultry slaughterhouses (I to IX) chosen from six geographically different regions in South Korea, including Seoul, Gyeonggi (slaughterhouses I, III, and VI), Jeolla (slaughterhouses II and IV), Gangwon (slaughterhouses V and VII), Gyeongsang (slaughterhouse VIII), and Chungcheong (slaughterhouse IX).

The intestinal contents from the evisceration or swab samples from the other slaughtering processes were subjected to *Salmonella* isolation according to the Ministry of Food and Drug Safety guidelines in South Korea. Briefly, each sample was homogenized with 3.5 mL of buffered peptone water (Becton Dickinson and Company, USA) and incubated at 37°C for 19 h. The enrichment culture (500 µL) was inoculated in Rappaport Vassiliadis broth (Becton Dickinson and Company) and incubated at 42°C for 19 h. One loop of the enrichment culture was then streaked onto xylose lysine deoxycholate agar (Becton Dickinson and Company) and incubated at 37°C for 24 h. The black colonies were transferred to urea agar (Becton Dickinson and Company) and triple sugar iron agar (Becton Dickinson and Company). After incubation at 37°C for 6–8 h, typical *Salmonella* colonies were selected as described elsewhere [21]. All *Salmonella* colonies were identified using the polymerase chain reaction (PCR) with *invA*-specific primers as described elsewhere [22] and kept at −80°C until use.

Serotype determination of *Salmonella* isolates

The serotypes of *Salmonella* isolates were determined using a multiplex PCR method, as described previously [23]. The genomic DNA (100 ng) was amplified with 10 µL 2 × Taq polymerase premix (SolGent Molecular Diagnostics, Korea), 1 pM primer, and distilled water. The primers used in this study are listed in **Supplementary Table 1**. The thermal conditions were 94°C for 5 min, followed by 30 cycles with denaturing at 94°C for 30 sec, annealing at 62°C for 30 sec, and elongation at 72°C for 30 sec. The PCR products were separated on 2% agarose gel by electrophoresis in tris-borate-EDTA buffer (90 mM Tris, 90 mM borate, 20 mM EDTA, pH 8.0). The separated DNA was stained with 1 µg/mL ethidium bromide and visualized using the gel image capture system (Bio-Rad Laboratories, USA). The DNA sizes were determined by comparing them with the molecular size markers (SMOBIO Technology, Taiwan).

Pulsed-field gel electrophoresis (PFGE)

PFGE was performed using the CHEF MAPPER (Bio-Rad Laboratories) according to the National Institute of Health guidelines. Briefly, bacterial colonies grown in tryptic soy agar (TSA) (Becton Dickinson and Company) were resuspended in Tris-EDTA buffer (100 mM Tris, 100 mM EDTA) at 20% transparency. The resuspended cells were mixed with 1.2% seakem gold agarose (Lonza, Switzerland) and solidified in a plug mold. The agarose plugs were transferred to cell lysis buffer (50 mM Tris, 50 mM EDTA, 1% sodium lauroyl sarcosine) and lysed by 20 mg/mL proteinase K (Invitrogen, USA) at 55°C with shaking at 100 rpm for 1 h. The lysed plugs were transferred to a plug wash buffer (10 mM Tris, 1 mM EDTA) and washed five times at 55°C with shaking at 100 rpm for 20 min. The washed plugs were digested by the 40 unit of *XbaI* (Roche, Switzerland) at 37°C for 4 h. Finally, the plugs were separated on 1% seakem gold agarose gel by PFGE in the chamber containing 0.5 × tris-borate-EDTA buffer plus 1.5 M thiourea (Sigma–Aldrich, USA) at 14°C. The PFGE conditions were 6.0 V for 18 h with initial and final switch times of 2.16 sec and 63.8 sec, respectively. The results were analyzed using the Bionumerics software ver. 5.0 (Applied Maths, Belgium). The dendrogram was generated using the Dice coefficient and UPGMA algorithm (0.5% optimization, 1.0% position tolerance).

Multilocus sequence typing (MLST)

MLST analysis uses the internal fragments of the following seven housekeeping genes: *thrA*, *purE*, *sucA*, *hisD*, *aroC*, *hemD*, and *dnaN*. The PCR products were subjected to Sanger sequencing as described previously [24]. The DNA sequences were submitted to the *Salmonella* MLST website (https://pubmlst.org/organisms/salmonella-spp) to determine the corresponding ST.

Antimicrobial susceptibility test

The minimal inhibitory concentration (MIC) against 16 antibiotics were determined using KRNV5F Sensititre (TREK Diagnostic Systems, USA): ampicillin (AMP, 2–64 μg/mL), amoxicillin/clavulanic acid 2:1 ratio (AUG, 2/1–32/16 μg/mL), ceftazidime (CAZ, 1–16 μg/mL), ceftiofur (XNL, 0.5–8 μg/mL), cefepime (FEP, 0.25–16 μg/mL), cefoxitin (FOX, 1–32 μg/mL), meropenem (MEM, 0.25–4 μg/mL), gentamicin (GEN, 1–64 μg/mL), streptomycin (STR, 16–128 μg/mL), nalidixic acid (NAL, 2–128 μg/mL), ciprofloxacin (CIP, 0.12–16 μg/mL), sulfisoxazole (FIS, 16–256 μg/mL), trimethoprim/sulfamethoxazole (SXT, 0.12/2.38–4/76 μg/mL), colistin (COL, 2–16 μg/mL), chloramphenicol (CHL, 2–64 μg/mL), and tetracycline (TET, 2–128 μg/mL). *Salmonella* colonies grown in TSA agar were suspended in 3 mL of distilled water at 0.5 McFarland standard (~1.5×10^8 CFU/mL). The bacterial suspensions (10 μL) were diluted with 11 mL of cation-adjusted Muller Hinton Broth in a TES tube (TREK Diagnostic Systems). Subsequently, 50 μL of this dilution was added into a KRNV5F panel and incubated at 37°C for 24 h. After incubation, the bacterial susceptibility was determined by interpreting the MIC values according to guidelines of the Clinical and Laboratory Standards Institute and National Antimicrobial Resistance Monitoring System (https://clsi.org/standards/products/microbiology/documents/m100/).

Detection of the virulence genes in *Salmonella* isolates

Multiplex PCR was performed using the primers specific to the genes encoding the virulence factors comprising SPI1-5 (*invE/A*, *orgA*, *ttrC*, *ssaQ*, *mgtC*, *misL*, *spi4R*, *spi4D*, *sopB*, and *pipA*), a transcription factor (*slyA*), a two-component regulatory system (*phoP*), *agfA*, *stn*, and *spvC*. The primers used in this study are listed in **Supplementary Table 2**.

RESULTS

Prevalence and serotypes of Salmonella isolates from slaughterhouses

One hundred and seventeen *Salmonella* strains were isolated from the 1,097 samples collected from the nine slaughterhouses in South Korea (**Table 1**). Serotyping analysis showed that all isolates were classified into nine serotypes: *S.* Mbandaka (n = 36 isolates), *Salmonella* Thompson (n = 33), *Salmonella* Infantis (n = 24), *S.* Virchow (n = 10), *S.* Typhimurium (n = 9), *S.* Enteritidis (n = 2), *Salmonella* Westhampton (n = 1), *S.* Montevideo (n = 1), and untypable (n = 1) (**Table 1**). These results suggest that *S.* Mbandaka, *S.* Thompson, and *S.* Infantis may be the dominant serotypes of *Salmonella* isolates, which are likely associated with slaughterhouses in South Korea.

Table 1. Serotypes of the *Salmonella* isolates in this study

Serotype	No. of isolates	Percentage (%)
Mbandaka	36	30.77
Thompsons	33	28.21
Infantis	24	20.51
Virchow	10	8.55
Typhimurium	9	7.69
Enterititis	2	1.71
Westhampton	1	0.85
Montevideo	1	0.85
Untypable	1	0.85
Total	117	100

Table 2. Serotype distribution of *Salmonella* isolates from six slaughtering processes in the nine slaughterhouses

Slaughterhouse	Serotypes of *Salmonella* isolates from the slaughtering processes (No. of isolates)					
	A (18)	B (22)	C (25)	D (47)	E (2)	F (3)
I	Montevideo (1)		Enteritidis (1)			Westhampton (1)
II	Enteritidis (1) Virchow (5)		Virchow (5)			
III						
IV						
V						
VI				Infantis (2)		Infantis (1)
VII						
VIII				Typhimurium (3)		
IX	Infantis (5) Thompson (2) Mbandaka (4)	Infantis (12) Thompson (4) Mbandaka (4) Typhimurium (2)	Infantis (1) Thompson (5) Mbandaka (11) Typhimurium (2)	Infantis (1) Thompson (22) Mbandaka (16) Typhimurium (2) Untypable (1)	Infantis (1) Mbandaka (1)	Infantis (1)

A, mooring/hanging; B, after defeathering; C, after washing; D, evisceration; E, before chilling; F, after chilling.

Among the 117 *Salmonella* isolates, 97 (82.9%) originated from slaughterhouse IX. The remaining 20 *Salmonella* isolates (17.1%) were found in slaughterhouses I, II, VI, and VIII. In contrast, *Salmonella* was not detected from slaughterhouses III, IV, V, and VII. The isolation rates at each slaughtering process were analyzed to identify the risk factors associated with *Salmonella* contamination. The results revealed the isolation rates in the six slaughtering processes: mooring/hanging (n = 18 isolates, 15.4%), after de-feathering (n = 22, 18.8%), after washing (n = 25, 21.4%), evisceration (n = 47, 46.0%), before chilling (n = 2, 1.7%), and after chilling (n = 3, 2.6%) (**Table 2**). These findings indicated the high prevalence of *Salmonella* from poultry carcasses in the mooring/hanging, de-feathering, washing, and evisceration processes.

In slaughterhouse IX, three dominant serotypes (*S.* Mbandaka, *S.* Thompson, and *S.* Infantis) were distributed widely along the slaughtering processes (**Table 2**). The number of *S.* Mbandaka and *S.* Thompson reached a maximum at evisceration and decreased rapidly before chilling. In contrast, the number of *S.* Infantis reached a maximum after de-feathering and decreased rapidly after washing. These results indicate that evisceration and de-feathering are the main routes of *Salmonella* contamination among the various slaughtering processes. In contrast, *S.* Virchow isolates were detected only in mooring/hanging and after washing in slaughterhouse II (**Table 2**).

Phylogenetic characteristics of *Salmonella* isolates

PFGE analysis was performed to investigate the phylogenetic relatedness between 35 *Salmonella* isolates from the different slaughtering processes because diverse serotypes were identified throughout the slaughtering processes. These 35 isolates included *S.* Virchow (n = 10 isolates), *S.* Infantis (n = 7), *S.* Mbandaka (n = 5), *S.* Thompson (n = 5), *S.* Typhimurium (n = 3), *S.* Enteritidis (n = 2), *S.* Montevideo (n = 1), *S.* Westhampton (n = 1), and untypable (n = 1). PFGE analysis showed that all *S.* Mbandaka isolates shared identical or highly similar patterns (similarity > 85%) regardless of their slaughtering processes (**Fig. 1**), suggesting possible carry-over transmission. These results were observed in *S.* Thompson, *S.* Infantis, and *S.* Virchow isolates, suggesting that sequential transmission of *Salmonella* serotypes occurs during slaughter. The PFGE results also showed that seven *S.* Infantis isolates from two slaughterhouses (VI and IX) shared an identical PFGE pattern. In contrast, three *S.* Typhimurium isolates from two slaughterhouses (VIII and IX) exhibited distinct PFGE patterns (similarity < 75%) (**Fig. 1**).

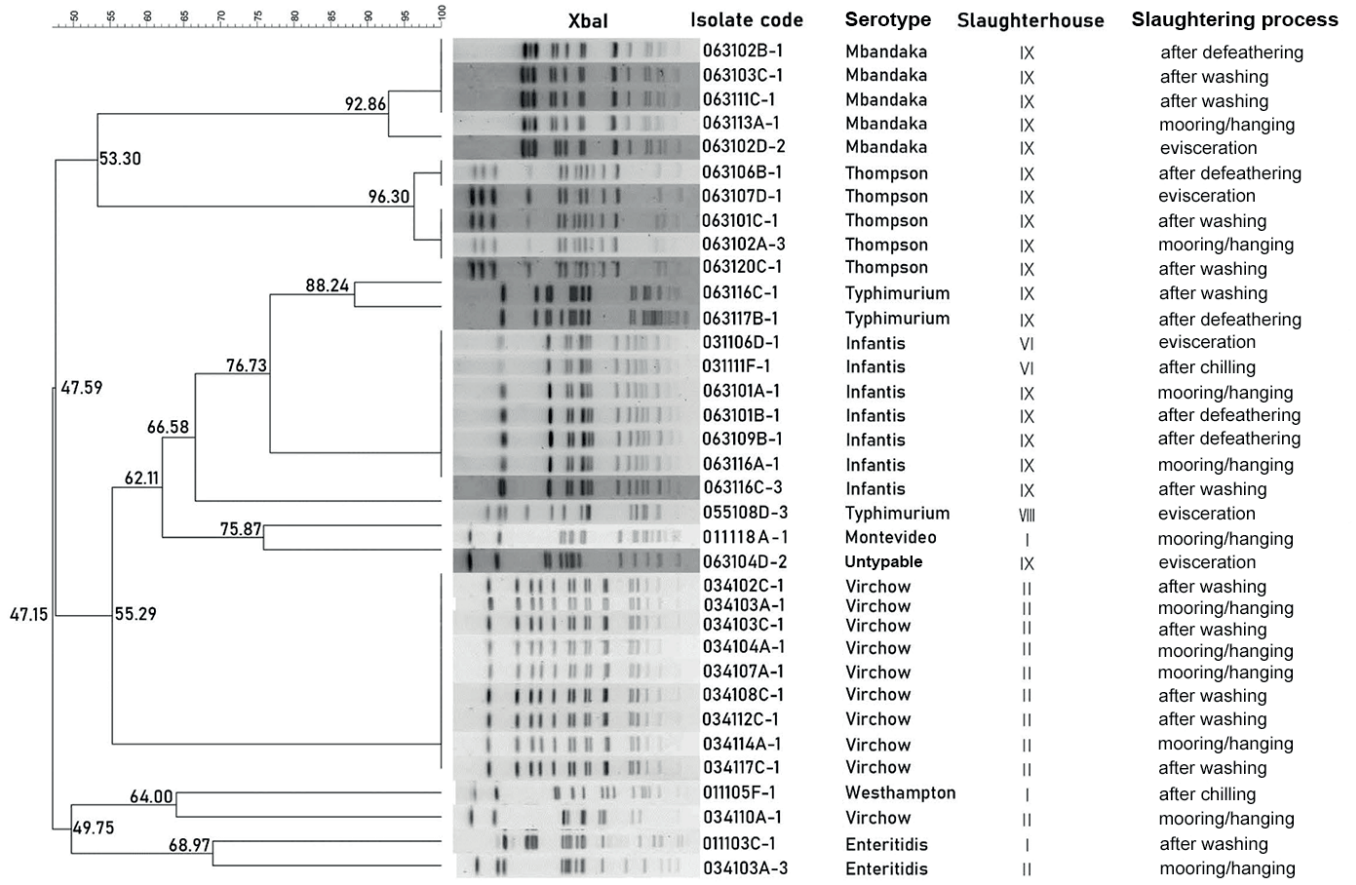

Fig. 1. Phylogenetic relatedness of 35 *Salmonella* isolates from slaughterhouses. Pulsed-field gel electrophoresis represents the genetic relatedness of 35 *Salmonella* isolates belonging to nine serotypes from different slaughtering processes. The percent similarities between *Salmonella* isolates were calculated using the Dice coefficient and UPGMA algorithm (0.5% optimization, 1.0% position tolerance).

The molecular epidemiological characteristics of 69 *Salmonella* isolates belonging to two major serotypes, *S.* Mbandaka and *S.* Thompson, were further investigated using the MLST analysis. All 36 *S.* Mbandaka isolates belonged to ST413, whereas all 33 *S.* Thompson isolates belonged to ST292 (**Supplementary Table 3**).

Antimicrobial resistance profiles of *Salmonella* isolates

Antibiotic-resistant *Salmonella* has become a serious problem in the poultry industry. Therefore, this study investigated the antimicrobial resistance profiles of the 117 *Salmonella* isolates. MIC analysis showed that 63 isolates were resistant to one or more antibiotics, including NAL (n = 54 isolates, 46.2%), FIS (n = 44, 37.6%), TET (n = 40, 34.2%), AMP (n = 39, 33.3%), CHL (n = 39, 33.3%), and SXT (n = 38, 32.5%) (**Fig. 2A**). Low resistance profiles were observed with CIP (n = 2, 1.7%), four cephem antibiotics (CAZ, XNL, FEP, and FOX), two aminoglycosides (STR and GEN), and COL (n = 1, 0.9%). None of the isolates were resistant to AMC and MEM. Forty *Salmonella* isolates (34.2%) were multidrug-resistant (MDR), as shown in **Fig. 2B**.

Virulence potential of the MDR *Salmonella* isolates

With the 40 MDR *Salmonella* isolates, multiplex PCR analysis was performed to detect the virulence genes previously known in *Salmonella* species (**Table 3**). All 40 MDR isolates possess the virulence genes encoding SPI1 (*invE/A*, *orgA*), SPI2 (*ttrC*, *ssaQ*), SPI3 (*mgtC*, *misL*), SPI4

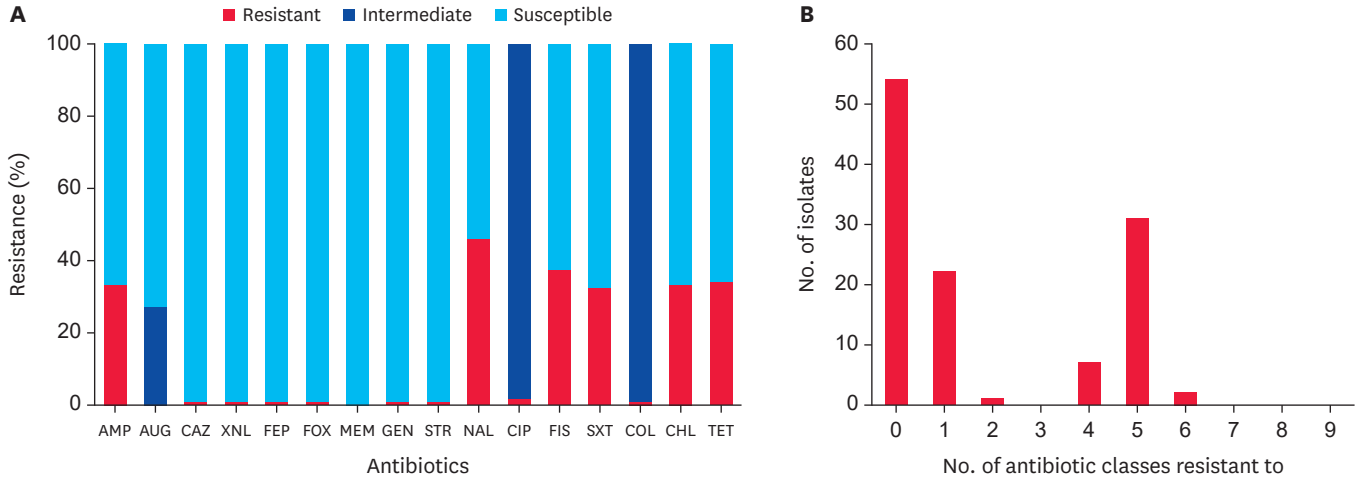

Fig. 2. Antimicrobial resistance profile of the *Salmonella* isolates. (A) Antimicrobial susceptibility of 117 *Salmonella* isolates. The bacterial susceptibility was determined by interpreting the minimal inhibitory concentration values according to the standards of the Clinical and Laboratory Standards Institute and the National Antimicrobial Resistance Monitoring System.(B) Frequency of MDR *Salmonella* isolates. Bacteria resistant to three or more antibiotic classes were referred to as MDR.
AMP, ampicillin; AUG, amoxicillin/clavulanic acid 2:1 ratio; CAZ, ceftazidime; XNL, ceftiofur; FEP, cefepime; FOX, cefoxitin; MEM, meropenem; GEN, gentamicin; STR, streptomycin; NAL, nalidixic acid; CIP, ciprofloxacin, FIS, sulfisoxazole; SXT, trimethoprim/sulfamethoxazole; COL, colistin; CHL, chloramphenicol; TET, tetracycline; MDR, multidrug-resistant.

(*spi4R*, *spi4D*), SPI5 (*sopB*, *pipA*), the virulence regulatory proteins (*slyA*, *phoP*), *agfA*, and *stn*, which are required for the full virulence of *Salmonella* (**Table 3**). Consistent with a previous study [25], the virulence plasmid-encoded *spvC* gene was detected only with two *S.* Enteritidis isolates (**Table 3**).

DISCUSSION

Salmonella was the third most common foodborne pathogen in South Korea during 2002–2017 [26]. *Salmonella* contamination of poultry meat should be tightly controlled during slaughter because poultry meat is often associated with salmonellosis [10,13]. This paper reported the prevalence and serotype distribution of *Salmonella* isolates from the slaughterhouses during 2018-2019. *S.* Mbandaka, *S.* Thompson, and *S.* Infantis were predominant among poultry carcasses. These serotypes might be sequentially transmitted by two main routes: intestinal content leakage and feather contamination. Specifically, *S.* Thompson ST292 isolates displayed both MDR phenotypes and hyper-virulent potentials. Therefore, these results suggest that *S.* Thompson ST292 is likely a potential epidemiological clone in South Korea, which may be associated with foodborne outbreaks.

Table 3. Antimicrobial resistance profiles and virulence genes of the 40 multidrug-resistant *Salmonella* isolates

Antimicrobial resistance profile	Serotype (No. of isolates)	Virulence genes									
		invE/A orgA	*ttrC ssaQ*	*mgtC misL*	*spi4R spi4D*	*spoB pipA*	*slyA*	*phoP*	*agfA*	*stn*	*spvC*
AMP-CAZ-XNL-FEP-GEN-NAL-TET	*S.* Enteritidis (1)	■	■	■	■	■	■	■	■	■	■
AMP-FOX-NAL-FIS-SXT-CHL-TET	*S.* Thompson (1)	■	■	■	■	■	■	■	■	■	
AMP-STR-NAL-FIS-COL-TET	*S.* Enteritidis (1)	■	■	■	■	■	■	■	■	■	■
AMP-NAL-FIS-SXT-CHL-TET	*S.* Thompson (31)	■	■	■	■	■	■	■	■	■	
AMP-FIS-SXT-CHL-TET	*S.* Typhimurium (5)	■	■	■	■	■	■	■	■	■	
NAL-CIP-FIS-CHL-TET	Untypable (1)	■	■	■	■	■	■	■	■	■	

AMP, ampicillin; CAZ, ceftazidime; XNL, ceftiofur; FEP, cefepime; GEN, gentamicin; NAL, nalidixic acid; TET, tetracycline; FOX, cefoxitin; FIS, sulfisoxazole; SXT, trimethoprim/sulfamethoxazole; CHL, chloramphenicol; STR, streptomycin; COL, colistin; CIP, ciprofloxacin.
■, Virulence genes detected.

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In this study, the overall prevalence of *Salmonella* from poultry slaughterhouses was 8.93% (98/1097), which is equivalent to or lower than those of the previous studies. For example, the prevalence of *Salmonella* from poultry slaughterhouses has been reported to be 9.2% [27], 63.89% [28], 13.3% [29], and 28.0% [12] in South Korea. In other countries, it was reported to be 3.6% in Brazil [30], 62.86% in China [31], and 18.05% in Thailand [32]. Therefore, *Salmonella* contamination in poultry slaughterhouses appears to be relatively controlled in South Korea but still needs to be reduced for food safety.

Interestingly, *S.* Mbandaka, *S.* Thompson, and *S.* Infantis are widely distributed along the slaughtering processes in the slaughterhouse IX. These serotypes are transmitted sequentially during slaughter because they share identical PFGE patterns, which concurs with a previous study [12]. These results suggest that intestinal content leakage and feather contamination are important steps for the carry-over transmission of *Salmonella* during the entire slaughtering process. A previous study reported that the leakage of intestinal contents by poultry gut damage during the slaughtering process led to *S.* Thompson and *S.* Mbandaka contamination [33]. Carry-over transmission of *S.* Infantis has also been reported through mooring instruments contaminated with feathers [33].

Previously, diverse serotypes of *Salmonella*, including *S.* Enteritidis, *S.* Typhimurium, *S.* Infantis, *S.* Montevideo, and *S.* Virchow, were prevalent among poultry carcasses in South Korea [11-15,34]. On the other hand, *S.* Mbandaka and *S.* Thompson dominated among the *Salmonella* isolates from poultry carcasses during 2018–2019. The observed changes in the serotype distribution should be noted to prevent foodborne outbreaks. Foodborne outbreaks by *S.* Mbandaka have been reported in other countries. For example, *S.* Mbandaka is one of the 10 major foodborne pathogens in European countries and has been associated with numerous large outbreaks linked with poultry meat [35,36]. In contrast, the epidemiological data of *S.* Thompson are relatively limited. A few studies reported the prevalence of *S.* Thompson from poultry farms in China [37,38]. Despite this, a large foodborne outbreak by *S.* Thompson occurred in South Korea in September 2018 [39]. Two thousand two hundred and seven people were infected with *S.* Thompson by consuming chocolate cakes made from *Salmonella*-contaminated egg whites. Thus, the epidemiological relationship between *S.* Thompson isolates in this study and the recent foodborne outbreaks in South Korea must be analyzed.

All 40 MDR isolates appeared to be highly pathogenic. They all carried the virulence genes encoding SPI1 (*invE/A*, *orgA*), SPI2 (*ttrC*, *ssaQ*), SPI3 (*mgtC*, *misL*), SPI4 (*spi4R*, *spi4D*), SPI5 (*sopB*, *pipA*), the virulence regulatory proteins (*slyA*, *phoP*), *agfA*, and *stn*, which are required for full virulence of *Salmonella*, suggesting their zoonotic potential. One health approach may be needed to reduce salmonellosis in South Korea.

The antimicrobial resistance profiles of *Salmonella* isolates from slaughterhouses exhibited multi-drug resistance to the six antibiotics: NAL, FIS, TET, AMP, CHL, and SXT. The previous studies reported similar resistance profiles among the *Salmonella* isolates from poultry farms and retail poultry meat in South Korea during 2015–2018 [15,40]. These results suggest that the improper use of antibiotics is still problematic in poultry farms in South Korea. Therefore, their use should be restricted to reduce the spread of antimicrobial resistance of *Salmonella* isolates.

In conclusion, *S.* Mbandaka ST413 and *S.* Thompson ST292 are the two dominant clonal lineages of *Salmonella* isolates from slaughterhouses in South Korea. Among the slaughter

process, the de-feathering and evisceration processes should be managed carefully to reduce the carry-over transmission of *Salmonella* in slaughterhouses. Moreover, improper use of the six antibiotics in poultry farms (NAL, FIS, TET, AMP, CHL, and SXT) should be limited. Further studies will be needed to determine if *S.* Thompson ST292 is associated with human infections in South Korea and the genotype-specific *in vivo* host adaptability.

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SUPPLEMENTARY MATERIALS

Supplementary Table 1

Oligonucleotide sequences of primers used for *Salmonella* serotyping

Supplementary Table 2

Oligonucleotide sequences of primers used for *Salmonella* virulence gene detection

Supplementary Table 3

ST and antimicrobial resistance profile of *Salmonella* isolates belonging to *S.* Mbandaka and *S.* Thompson

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