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Emergence of *Salmonella* Infantis carrying the pESI megaplasmid in commercial farms of five major integrated broiler operations in Korea

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ABSTRACT Considering *Salmonella* transmission occurs through several routes in integrated broiler operations, control of nontyphoidal *Salmonella* in commercial farms is essential. This study aimed to compare the distribution of persistent *Salmonella* serovars in environments and dead chickens between 5 major integrated broiler operations in Korea. The prevalence of *Salmonella*-positive farms in dust prior to placement by operations was 0 to 25%, but the prevalence in dust and feces at the time of depletion was increased to 16.7 to 41.7% and 16.7 to 66.7%, respectively. Moreover, the prevalence of farms with *Salmonella* in chickens that died within 1 week old and at 4 to 5 weeks old ranged from 8.3 to 58.3% and 16.7 to 41.7%, respectively. The prevalence of *Salmonella enterica* serovar Infantis-positive farms in dust prior to placement and in chickens that died within 1 week old was 5.2 and 3.4%, respectively, but the prevalence in dust and feces at the time of depletion and in chickens that died at 4 to 5 weeks old was significantly increased to 27.6, 41.4, and 20.7%, respectively ($P < 0.05$). Interestingly, the plasmid of emerging *S.* Infantis (**pESI**) was only identified in *S.* Infantis, and the prevalence of multidrug-resistance was significantly higher in pESI-positive *S.* Infantis (99.2%) than in pESI-negative *S.* Infantis (6.7%) ($P < 0.05$). The distribution of pulsotypes between pESI-positive and pESI-negative *S.* Infantis were varied, but a majority of *S.* Infantis were clustered only 2 pulsotypes. Moreover, pESI-positive *S.* Infantis harbored more virulence factors than pESI-negative *S.* Infantis. This study is the first report on characteristics of *S.* Infantis carrying the pESI plasmid in commercial broiler farms in Korea.

Key words: *Salmonella* Infantis, pESI plasmid, broiler, emerging pathogen

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INTRODUCTION

Nontyphoidal *Salmonella* are widely dispersed in broad spectrum of environments, including gastrointestinal tracts of domestic and wild mammals and birds, and *Salmonella* infection in humans may be occur by contact with animals or the environments (Eng et al., 2015). Annually, *Salmonella* causes approximately 200 million to over 1 billion infections worldwide (Chlebicz and Śliżewska, 2018; Castro-Vargas et al., 2020), and in Korea, 136 cases of human salmonellosis were also reported from 2018 to 2022, affecting 7,400 patients (MFDS, 2023).

In particular, many researchers reported that poultry products, including eggs and poultry meat, are the primary sources of *Salmonella* for human (Hoelzer et al., 2011; Antunes et al., 2016). The widespread distribution of *Salmonella* in broiler production stage has been continuously reported in Korea (Choi et al., 2014; Ha et al., 2018; Wei et al., 2021), and the European Union (**EU**) also reported that 70% of all *Salmonella* isolated from foods and animals was from broiler sector (EFSA, 2022). The introduction and dissemination of *Salmonella* in broiler flocks can occur both vertically by infected breeder chickens and horizontally by infected flocks, contaminated feed and water, wild birds, or other biosecurity violations (Davies and Wales, 2010; Cargnel et al., 2023). In particular, *Salmonella*-positive commercial broilers are a major role in the dissemination of *Salmonella* to the slaughterhouse, which directly causes *Salmonella* food poisoning in human, so reducing *Salmonella* in broiler farms is essential for food safety (Van Immerseel et al., 2009; Rivera-Pérez et al., 2014).

Although more than 2,600 *Salmonella enterica* serovars have been identified (Eng et al., 2015), the United States (**US**) and EU reported that the most important serovars associated with poultry and human infections are *S.* Enteritidis, *S.* Typhimurium, *S.* Typhimurium monophasic variant, *S.* Infantis, *S.* Newport, and *S.*

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Derby (CDC; 2018; Collins et al., 2022; EFSA, 2022). In particular, *S.* Infantis is a poultry-adapted *Salmonella* enterica serovar that is increasingly being reported in broilers and frequently identified in human salmonellosis cases worldwide (Vlaanderen et al., 2019; Newton et al., 2020; EFSA, 2022; Kahn et al., 2022; Alvarez et al., 2023; Montone et al., 2023). Moreover, multidrug-resistant (**MDR**) *S.* Infantis carrying the plasmid of emerging *S.* Infantis (**pESI**) or pESI-like plasmid associated with antimicrobial resistance, biofilm formation, and resistance to disinfectant has been continuously reported in Europe, US, Russia, and Japan (Yokoyama et al., 2015; Bogomazova et al., 2020; Mughini-Gras et al., 2021; Srednik et al., 2023). Many researchers reported that the pESI plasmid and pESI-like plasmid could confer an advantage of the rapid spread of *S.* Infantis in a poultry (Aviv et al., 2014, 2016; Franco et al., 2015; Alba et al., 2020; McMillan et al., 2022).

The broiler supply chain is operated as vertically integrated system in many countries (Cesari et al., 2017; Dotas et al., 2021; Solano−Blanco et al., 2023), and in Korea, integrated broiler operations account for approximately 96.5% of broiler meat production (KAPE, 2022). Considering *Salmonella* transmission through a variety of routes, including infected breeder chickens and poor biosecurity practices on commercial farms, there may be difference in the prevalence of *Salmonella* between integrated broiler operations. Therefore, the objective of this study was to compare the distribution of persistent *Salmonella* serovars in environments and dead chickens between 5 major integrated broiler operations in Korea and provide the genetic and phenotypic characterization of the most common serovar, *S.* Infantis.

MATERIALS AND METHODS

Sample Collection

According to the standards set by the National Poultry Improvement Plan (**NPIP**) (USDA, 2019), environment dusts were collected from the houses prior to placement and at depletion of birds, and feces were only collected from the houses at depletion of birds from 58 commercial farms of 5 major integrated broiler operations in 2022. Briefly, approximately 10 g of dust samples were obtained by swabbing 15 different spots per house using sterile surgical gauze moistened with buffered peptone water (**BPW**; Difco, Sparks, MD). Additionally, approximately 10 g of feces was sampled from 15 different locations per house using sterile shoe covers. All samples were placed into a sterile bag and transported to the laboratory under 4°C conditions. Moreover, chickens that died within 1 week old and at 4 to 5 weeks old per house of each farm were also transported to the laboratory under 4°C conditions. The liver and spleen samples were collected by necropsy, that is, before sampling, the liver and spleen surfaces were decontaminated using a hot sterile spatula, and incised using scalpel blade, after which sterile cotton-tipped swabs were inserted.

Isolation and Identification of Salmonella spp.

The isolation of *Salmonella* spp. from dust, feces, and organs were performed according to the standards set by the NPIP (USDA, 2019). Briefly, each 10 g of dust and feces samples were inoculated into 100 mL of BPW. After incubation at 37°C ± 2°C for 20 to 24 h**,** 0.1 mL and 1 mL pre-enriched BPW was inoculated into 10 mL Rappaport Vassiliadis broth (**RV** broth; Difco) and 10 mL tetrathionate broth (**TT** broth; Difco), respectively. The RV broth and TT broth were incubated at 42°C for 20 to 24 h and streaked onto Xylose Lysine Tergitol 4 agar (**XLT4** agar; Difco). The liver and spleen swabs were inoculated into 10 mL TT broth, incubated at 37°C ± 2°C for 20 to 24 h and streaked onto XLT4 agar. At least 5 presumptive *Salmonella* colonies were selected from each XLT4 agar and confirmed by identifying the *invA* gene using polymerase chain reaction (**PCR**), as described previously (Rahn et al., 1992). Confirmed colonies were serotyped by agglutination tests using O and H antisera (Difco) according to the Kauffmann and White scheme (Grimont and Weill, 2007). A maximum of 3 houses per farm were sampled; however, if the isolates from the same farm at the same time showed the same serovars and antimicrobial susceptibility patterns, only 1 isolate was randomly selected.

Antimicrobial Susceptibility Testing

According to the guidelines of the Clinical and Laboratory Standards Institute guidelines (CLSI, 2023), all the *S.* Infantis isolates were investigated for antimicrobial resistance by the disk diffusion test using the following antimicrobial disks (BD Biosciences, Sparks, MD): ampicillin (**AM**, 10 μg), amoxicillin−clavulanate (**AMC**, 20/10 μg), cefazoline (**CZ**, 30 μg), cefoxitin (**FOX**, 30 μg), cefotaxime (**CTX**, 30 μg), cefepime (**FEP**, 30 μg), chloramphenicol (**C**, 30 μg), ciprofloxacin (**CIP**, 5 μg), gentamicin (**GM**, 10 μg), imipenem (**IPM**, 10 μg), nalidixic acid (**NA**, 30 μg), tetracycline (**TE**, 30 μg), and trimethoprim−sulfamethoxazole (**SXT**, 1.25/23.75 μg). Multidrug-resistance (**MDR**) was defined as resistance to at least 1 agent of 3 or more antimicrobial classes, as described previously (Magiorakos et al., 2012).

Detection of the pESI Plasmid and Virulence Genes

The presence of 3 target genes (*repA, ipf*, and *K*88-like) for the pESI plasmid and different virulence genes (*cdtB, iron, lpfC, msgA, orgA, pagC, pefA, prgH, sefC, sifA, sipB, sitC, sopB, sopE, spaN, spiA, spvB, stn, tcfA*, and *tolC*) was confirmed by PCR using the primers listed in Table 1. Positive results for all 3 target genes were considered indicative of the pESI plasmid, as described previously (McMillan et al., 2023).

Table 1. Primers used in this study.

Target	Sequence (5′→3′)	Size (bp)	References
Identification			
invA	F: ATGCCCGGTAAACAGATGAG R: CGACAAGACCATCACCAATG	282	Rahn et al., (1992)
repA	F: AAGGCGATGGAGCAACTCAG R: AAGGCGATGGAGCAACTCAG	158	McMillan et al., (2023)
ipf	F: ACTGGTATGCTGTCCCTTGC R: TGCTGCAGTCTTGGCAGTAG	177	McMillan et al., (2023)
*K*88-like	F: TGTATTCCACCCGGATTACTGC R: GGCATTTCTCCCGGAATGAGG	145	McMillan et al., (2023)
Virulence factors			
spvB	F: CTATCAGCCCCGCACGGAGAGCAGTTTTTA R: GGAGGAGGCGGTGGCGGTGGCATCATA	717	Skyberg et al., (2006)
spiA	F: CCAGGGGTCGTTAGTGTATTGCGTGAGATG R: CGCGTAACAAAGAACCCGTAGTGATGGATT	550	Skyberg et al., (2006)
cdtB	F: ACAACTGTCGCATCTCGCCCCGTCATT R: CAATTTGCGTGGGTTCTGTAGGTGCGAGT	268	Skyberg et al., (2006)
msgA	F: GCCAGGCGCACGCGAAATCATCC R: GCGACCAGCCACATATCAGCCTCTTCAAAC	189	Skyberg et al., (2006)
prgH	F: GCCCGAGCAGCCTGAGAAGTTAGAAA R: TGAAATGAGCGCCCCTTGAGCCAGTC	756	Skyberg et al., (2006)
spaN	F: AAAAGCCGTGGAATCCGTTAGTGAAGT R: CAGCGCTGGGGATTACCGTTTTG	504	Skyberg et al., (2006)
orgA	F: TTTTTGGCAATGCATCAGGGAACA R: GGCGAAAGCGGGGACGGTATT	255	Skyberg et al., (2006)
tolC	F: TACCCAGGCGCAAAAAGAGGCTATC R: CCGCGTTATCCAGGTTGTTGC	161	Skyberg et al., (2006)
sitC	F: CAGTATATGCTCAACGCGATGTGGGTCTCC R: CGGGGCGAAAATAAAGGCTGTGATGAAC	768	Skyberg et al., (2006)
lpfC	F: GCCCCGCCTGAAGCCTGTGTTGC R: AGGTCGCCGCTGTTTGAGGTTGGATA	641	Skyberg et al., (2006)
sifA	F: TTTGCCGAACGCGCCCCCACACG R: GTTGCCTTTTCTTGCGCTTTCCACCCATCT	449	Skyberg et al., (2006)
sopB	F: CGGACCGGCCAGCAACAAAACAAGAAGAAG R: TAGTGATGCCCGTTATGCGTGAGTGTATT	220	Skyberg et al., (2006)
iroN	F: ACTGGCACGGCTCGCTGTCGCTCTAT R: CGCTTTACCGCCGTTCTGCCACTGC	1205	Skyberg et al., (2006)
pagC	F: CGCCTTTTCCGTGGGGTATGC R: GAAGCCGTTTATTTTTGTAGAGGAGATGTT	454	Skyberg et al., (2006)
sipB	F: GGACGCCGCCCGGGAAAAACTCTC R: ACACTCCCGTCGCCGCCTTCACAA	454	Skyberg et al., (2006)
stn	F: ATTGAGCGCTTTAATCTCCT R: GCTGTTGAATCTGTACCTGA	543	Choudhury et al. (2016)
sopE	F: GGTAGGGCAGTATTAACCAG R: TTTATCTCCCTAGGTAGCCC	254	Choudhury et al. (2016)
pefA	F: GCCAAAGTACTGGTTGAAAG R: TATTTGTAAGCCACTGCGAA	185	Choudhury et al. (2016)
sefC	F: GGCAGGTCCAAAACTATACA R: GCGATAACGAAACACCATTT	609	Choudhury et al. (2016)
tcfA	F: TCGCTATGTTTGCATGTGGT R: TTCAGGAACAGCCTCGAAGT	335	Suez et al., (2013)

Pulsed-Field Gel Electrophoresis (PFGE)

PFGE was performed by digesting the genomic DNA using the *XbaI* enzyme (Takara Bio Inc., Shiga, Japan) according to a standard protocol of the Centers for Disease Control and Prevention (**CDC**, USA), using a CHEF-MAPPER apparatus (Bio-Rad Laboratories, Hercules, CA), as described previously (Ribot et al., 2006). PFGE profiles were analyzed using the Bionumerics Software (Applied Maths, Sint-Martens-Laem, Belgium). Relatedness was calculated using the unweighted pair-group method with arithmetic averages (**UPGMA**) algorithm based on the Dice similarity index. Isolates that exhibited coefficient of similarity ≥90% were considered genetically closely related (Dionisi et al., 2011).

Statistical Analysis

Statistical analysis was performed using the Pearson's chi-square and Fisher's exact test with Bonferroni correction in IBM SPSS Statistics for Windows, version 25 (IBM Corp., Armonk, NY). Differences were considered significant at $P < 0.05$.

RESULTS

Prevalence and Distribution of Serovars of Salmonella spp.

The distribution of *Salmonella* spp. isolated from 58 commercial farms of 5 integrated broiler operations is shown in Table 2. The prevalence and distribution of

Table 2. Distribution of *Salmonella* spp. isolated from 58 commercial farms of 5 integrated broiler operations.

		No. (%) of *Salmonella*-positive farms				
		Environments			Dead chickens	
		Prior to placement	At the time of depletion			
Operation (No. of farms)	Serovar	Dust	Dust	Feces	Within 1 week old	At 4−5 weeks old
A (n = 12)	*S.* Infantis	0 (0.0)	2 (16.7)	2 (16.7)	0 (0.0)	2 (16.7)
	S. Thompson	0 (0.0)	0 (0.0)	0 (0.0)	1 (8.3)	0 (0.0)
	Total	0 (0.0)	2 (16.7)	2 (16.7)	1 (8.3)	2 (16.7)
B (n = 12)	*S.* Agona	0 (0.0)	0 (0.0)	0 (0.0)	1 (8.3)	0 (0.0)
	S. Infantis	1 (8.3)	3 (25.0)	5 (41.7)	1 (8.3)	2 (16.7)
	S. Montevideo	0 (0.0)	0 (0.0)	0 (0.0)	1 (8.3)	0 (0.0)
	S. Senftenberg	0 (0.0)	0 (0.0)	1 (8.3)	0 (0.0)	0 (0.0)
	Total	1 (8.3)	3 (25.0)	6 (50.0)	3 (25.0)	2 (16.7)
C (n = 12)	*S.* Bareilly	1 (8.3)	0 (0.0)	0 (0.0)	1 (8.3)	1 (8.3)
	S. Enteritidis	0 (0.0)	0 (0.0)	2 (16.7)	3 (25.0)	0 (0.0)
	S. Infantis	1 (8.3)	2 (16.7)	6 (50.0)	1 (8.3)	1 (8.3)
	S. Senftenberg	1 (8.3)	2 (16.7)	0 (0.0)	2 (16.7)	0 (0.0)
	S. Thompson	0 (0.0)	0 (0.0)	0 (0.0)	2 (16.7)	1 (8.3)
	Total	3 (25.0)	4 (33.3)	8 (66.7)	7 (58.3)	3 (25.0)
D (n = 10)	*S.* Enteritidis	0 (0.0)	0 (0.0)	1 (10.0)	3 (30.0)	1 (10.0)
	S. Infantis	0 (0.0)	4 (40.0)	4 (40.0)	0 (0.0)	3 (30.0)
	Total	$0\ (0.0)_{B}$	$4\ (40.0)_{A,\ B}$	$5\ (50.0)_{A}$	3 (30.0)	4 (40.0)
E (n = 12)	*S.* Agona	0 (0.0)	0 (0.0)	0 (0.0)	1 (8.3)	0 (0.0)
	S. Enteritidis	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (8.3)
	S. Infantis	$1\ (8.3)_{B}$	$5\ (41.7)_{A,\ B}$	$7\ (58.3)_{A}$	$0\ (0.0)_{D}$	$4\ (33.3)_{C}$
	S. Montevideo	1 (8.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	S. Senftenberg	0 (0.0)	0 (0.0)	0 (0.0)	1 (8.3)	2 (16.7)
	Total	2 (16.7)	5 (41.7)	7 (58.3)	2 (16.7)	5 (41.7)
Total (n = 58)	*S.* Agona	0 (0.0)	0 (0.0)	0 (0.0)	2 (3.4)	0 (0.0)
	S. Bareilly	1 (1.7)	0 (0.0)	0 (0.0)	1 (1.7)	1 (1.7)
	S. Enteritidis	0 (0.0)	0 (0.0)	3 (5.2)	6 (10.3)	2 (3.4)
	S. Infantis	$3\ (5.2)_{B}$	$16\ (27.6)_{A}$	$24\ (41.4)_{A}$	$2\ (3.4)_{D}$	$12\ (20.7)_{C}$
	S. Montevideo	1 (1.7)	0 (0.0)	0 (0.0)	1 (1.7)	0 (0.0)
	S. Senftenberg	1 (1.7)	2 (3.4)	1 (1.7)	3 (5.2)	2 (3.4)
	S. Thompson	0 (0.0)	0 (0.0)	0 (0.0)	3 (5.2)	1 (1.7)
	Total	$6\ (10.3)_{B}$	$18\ (31.0)_{A}$	$28\ (48.3)_{A}$	16 (27.6)	16 (27.6)

Values with different subscript letters ($_{A,\ B}$) represent significant differences between environments by operations, while subscript letters ($_{C,\ D}$) represent significant differences between dead chickens ($P < 0.05$).

serovars in *Salmonella*-positive farms showed the differences between environments prior to placement and environments at the time of depletion, and between chickens that died within 1 week old and chickens that died at 4 to 5 weeks old. In environmental samples, the prevalence in both dust (16.7−41.7%) and feces (16.7 −66.7%) at the time of depletion was higher than in dust prior to placement (0−25.0%) by operations. Although 5 *Salmonella* serovars, *S.* Bareilly, *S.* Enteritidis, *S.* Infantis, *S.* Montevideo, and *S.* Senftenberg were confirmed, the prevalence of *S.* Infantis-positive farms in dust prior to placement was only 5.2%, but the prevalence in dust and feces at the time of depletion was rapidly increased to 27.6 and 41.4%, respectively ($P < 0.05$).

Moreover, the prevalence of farms with *Salmonella* in chickens that died within 1 week old and at 4 to 5 weeks old ranged from 8.3 to 58.3% and 16.7 to 41.7% by operations, respectively. The *Salmonella* serovars isolated from dead chickens were identified as *S.* Agona, and *S.* Thompson including 5 *Salmonella* spp. isolated from environments. Interestingly, *S.* Infantis was only found in each one (8.3%) farm of operations B and C in chickens that died within 1 week old, but it was confirmed in all 5 integrated operations (8.3−33.3%) in chickens that died at 4 to 5 weeks old. Moreover, *S.* Enteritidis at chickens that died within 1 week old was also confirmed in 3 (25.0%) farms and 3 (30.0%) farms by operation C and D, respectively, but at chickens that died at 4 to 5 weeks old, the prevalence of positive farms decreased to 0 and 10.0%, respectively.

Distribution of Salmonella *spp. Carrying the pESI Plasmid*

Distribution of *Salmonella* spp. carrying the pESI plasmid in 58 commercial farms of 5 integrated broiler operations is presented in Table 3. Interestingly, the pESI plasmid was only detected in *S.* Infantis, and was absent in all other *Salmonella* spp., *S.* Agona, *S.* Bareilly, *S.* Enteritidis, *S.* Montevideo, *S.* Senftenberg, and *S.* Thompson. In particular, the prevalence of farms with pESI-positive *S.* Infantis and pESI-negative *S.* Infantis in both dust prior to placement and chickens that died within 1 week old showed no significantly differences; however, in environments at the time of depletion and in chickens that died 4 to 5 weeks old, the prevalence of farms with pESI-positive *S.* Infantis was significantly higher than that of those with pESI-negative *S.* Infantis ($P < 0.05$).

Table 3. Distribution of *Salmonella* spp. carrying the pESI plasmid in 58 commercial broiler farms of 5 integrated broiler operations.

	Environments			Dead chickens	
	Prior to placement	At the time of depletion			
	Dust	Dust	Feces	Within 1 week old	At 4–5 weeks old
No. of *S.* Infantis-positive farms	3	16	24	2	12
No. (%) of pESI- positive	1 (33.3)	14 (87.5)$_{A}$	18 (75.0)$_{A}$	1 (50.0)	12 (100.0)$_{A}$
No. (%) of pESI-negative	2 (66.7)	2 (12.5)$_{B}$	6 (25.0)$_{B}$	1 (50.0)	0 (0.0)$_{B}$
No. of *Salmonella* spp.-positive farms[1]	3	2	4	15	6
No. (%) of pESI-positive	0 (0.0)$_{D}$	0 (0.0)$_{D}$	0 (0.0)$_{D}$	0 (0.0)$_{D}$	0 (0.0)$_{D}$
No. (%) of pESI-negative	3 (100.0)$_{C}$	2 (100.0)$_{C}$	4 (100.0)$_{C}$	15 (100.0)$_{C}$	6 (100.0)$_{C}$

[1]No. of farms with confirmed *Salmonella* spp. (*S.* Agona, *S.* Bareilly, *S.* Enteritidis, *S.* Montevideo, *S.* Senftenberg, and *S.* Thompson) except for *S.* Infantis.Values with different subscript letters ($_{A, B}$ or $_{C, D}$) represent significant differences between pESI-positive and pESI-negative farms, respectively ($P < 0.05$).

Antimicrobial Resistance of S. Infantis

The characteristics of antimicrobial resistance of 136 *S.* Infantis isolated in this study are shown in Table 4. Although total number of isolates was significantly difference between dust prior to placement (n = 3) and dust (n = 40) and feces (n = 61) at the time of depletion, and between chickens that died within 1 week old (n = 2) and at 4 to 5 weeks old (n = 30), resistance against most antimicrobials tested, except for NA, showed higher in isolates from environments at the time of depletion and chickens that died at 4 to 5 weeks old. In particular, the resistance to AM, C, CZ, CTX, SXT, and TE showed significantly higher in isolates from dust (70.0–95.0%) and feces (60.7–85.2%) at the time of depletion and chickens that died at 4 to 5 weeks old (76.7–100%) than dust prior to placement (33.3%) and chickens that died within 1 week old (0–50.0%), respectively ($P < 0.05$). Interestingly, pESI-positive *S.* Infantis showed the significantly higher resistance to AM, C, CZ, CTX, GM, NA, SXT, and TE than pESI-negative *S.* Infantis ($P < 0.05$). Moreover, the distribution of MDR isolates was also significantly higher in pESI-positive *S.* Infantis (99.2%) than in pESI-negative *S.* Infantis (6.7%) ($P < 0.05$).

PFGE Analysis and Characteristics of S. Infantis

The epidemiological genetic relationship and molecular characterization of 136 *S.* Infantis isolates are shown in Figure 1. *S.* Infantis were divided into 20 pulsotypes. Interestingly, 15 pESI-negative *S.* Infantis were identified as PT01 to PT06 and PT14 to PT20. Otherwise, 121 pESI-positive *S.* Infantis were identified as PT07 to PT13. In particular, 59 (43.4%) and 55 (40.4%) among 136 isolates were identified as PT07 and PT08, respectively, which were most prevalent types. Additionally, among the 20 virulence genes tested, 13 genes (*iroN, msgA, orgA, pagC, prgH, sipB, sitC, sopB, sopE, span, spiA, stn*, and *tolC*) were detected in all *S.* Infantis

Table 4. Antimicrobial resistance of 136 *S.* Infantis isolated from 58 commercial farms of 5 integrated broiler operations.

	No. of antimicrobial resistant *S.* Infantis isolates (%)						
	Environments			Dead chickens		Total	
	Prior to placement	At the time of depletion					
Antimicrobial agents	Dust (n = 3)[1]	Dust (n = 40)	Feces (n=61)	Within 1 week old (n = 2)	At 4–5 weeks old (n = 30)	pESI-negative (n = 15)	pESI-positive (n = 121)
Amoxicillin-clavulanate	0 (0.0)	5 (12.5)	1 (1.6)	0 (0.0)	0 (0.0)	0 (0.0)	6 (5.0)
Ampicillin	1 (33.3)^{b}	38 (95.0)^{a}	52 (85.2)^{a,b}	1 (50.0)^{d}	29 (96.7)^{c}	2 (13.3)$_{B}$	119 (98.3)$_{A}$
Cefazolin	1 (33.3)^{b}	38 (95.0)^{a}	50 (82.0)^{a,b}	1 (50.0)^{d}	29 (96.7)^{c}	0 (0.0)$_{B}$	119 (98.3)$_{A}$
Cefepime	0 (0.0)	2 (5.0)	2 (3.3)	0 (0.0)	4 (13.3)	0 (0.0)	8 (6.6)
Cefotaxime	1 (33.3)^{b}	37 (92.5)^{a}	50 (82.0)^{a,b}	1 (50.0)^{d}	29 (96.7)^{c}	0 (0.0)$_{B}$	118 (97.5)$_{A}$
Cefoxitin	0 (0.0)	7 (17.5)	11 (18.0)	0 (0.0)	4 (13.3)	1 (6.7)	21 (17.4)
Chloramphenicol	1 (33.3)^{b}	36 (90.0)^{a}	50 (82.0)^{a,b}	1 (50.0)^{d}	30 (100.0)^{c}	2 (13.3)$_{B}$	116 (95.9)$_{A}$
Ciprofloxacin	0 (0.0)	3 (7.5)	2 (3.3)	0 (0.0)	1 (3.3)	0 (0.0)	6 (5.0)
Gentamicin	1 (33.3)	24 (60.0)	29 (47.5)	1 (50.0)	15 (50.0)	0 (0.0)$_{B}$	70 (57.9)$_{A}$
Imipenem	0 (0.0)	1 (2.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.8)
Nalidixic acid	3 (100.0)	38 (95.0)	56 (91.8)	2 (100.0)	30 (100.0)	9 (60.0)$_{B}$	120 (99.2)$_{A}$
Tetracycline	1 (33.3)^{b}	37 (92.5)^{a}	48 (78.7)^{a,b}	1 (50.0)^{d}	30 (100.0)^{c}	0 (0.0)$_{B}$	117 (96.7)$_{A}$
Trimethoprim/sulfamethoxazole	1 (33.3)	28 (70.0)	37 (60.7)	0 (0.0)^{d}	23 (76.7)^{c}	0 (0.0)$_{B}$	89 (73.6)$_{A}$
MDR	1 (33.3)^{b}	38 (95.0)^{a}	51 (83.6)^{a,b}	1 (50.0)^{d}	30 (100.0)^{c}	1 (6.7)$_{B}$	120 (99.2)$_{A}$

[1]n = No. of *S.* Infantis isolated from 5 integrated broiler operations.Values with different superscript letters ($^{a, b}$ or $^{c, d}$) represent significant differences between environments or dead chickens, respectively, while subscript letters ($_{A, B}$) represent significant differences between pESI-positive and pESI-negative *S.* Infantis ($P < 0.05$). Multidrug-resistance (MDR) was defined as resistance to 3 or more antimicrobial classes.

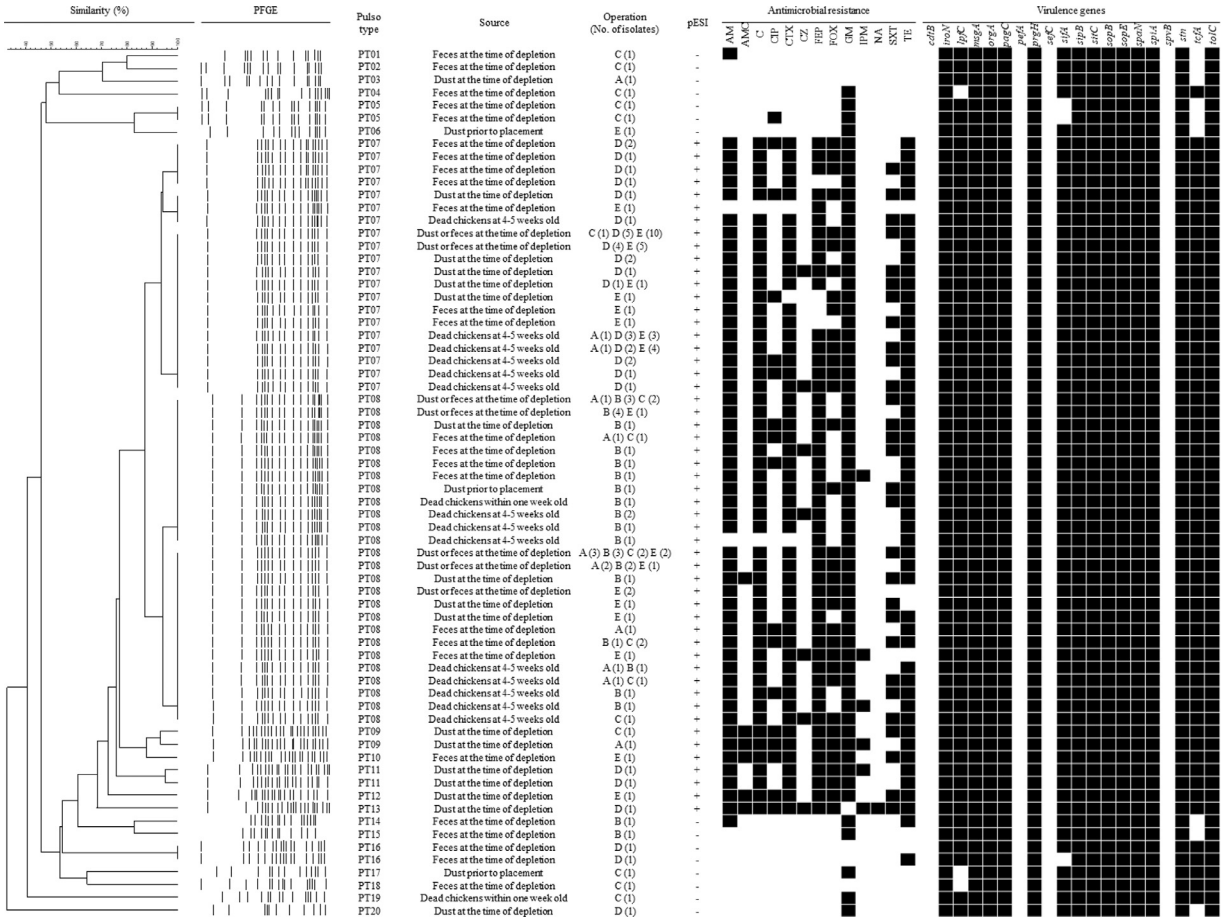

Figure 1. Dendrogram showing genetic relationships among 136 *S.* Infantis isolated from 58 commercial farms of 5 integrated broiler operations characterized by PFGE profiles. *S.* Infantis isolates showing similarities of < 90% in PFGE were considered to be unrelated. Abbreviations: AM, ampicillin; AMC, amoxicillin-clavulanate; C, chloramphenicol; CIP, ciprofloxacin; CTX, cefotaxime; CZ, cefazolin; FEP, cefepime; FOX, cefoxitin; GM, gentamicin; IPM, imipenem; NA, nalidixic acid, SXT, trimethoprim/sulfamethoxazole; TE, tetracycline.

regardless of the presence of the pESI plasmid. Also, 3 genes (*lpfC, sifA*, and *tcfA*) were identified in all 121 pESI-positive *S.* Infantis, but in 12 (80.0%), 12 (80.0%), and 6 (40.0%) among 15 pESI-negative *S.* Infantis, respectively. The *cdtB, pefA, sefC*, and *spvB* genes were not detected in any of the *S.* Infantis isolates.

DISCUSSION

Although *Salmonella* are primary transmitted to commercial chickens by infecting the ovaries, their presence in settled dust in poultry facilities is also a major cause of *Salmonella* colonization of chickens through airborne dust during the rearing period (Chinivasagam et al., 2009; Liu et al., 2022; Pal et al., 2022). Rose et al. (1999) and Volkova et al. (2010) have already reported that *Salmonella* contamination of the previous flock in the grow-out house and the presence of *Salmonella* in house prior to placement of the flock influence *Salmonella* prevalence until the end of next flock rearing. In addition, Janning et al. (1994) also reported that *Salmonella* can survive several weeks even in dry environments, and Wales et al. (2007) reported that the level of environmental contamination of *Salmonella* increases significantly over time. Namely, environmental *Salmonella* can be settled in the cecum via oral route, and finally it is re-transmitted to flocks though feces (Wang et al., 2023). Moreover, continuous fecal shedding from infected broilers can be contributor to increase of the level of environmental contamination (Van Immerseel et al., 2004; Marin and Lainez, 2009). In this study, the prevalence of *Salmonella*-positive farms in dust at the time of depletion was rapidly increased to 16.7 to 41.7% than in dust prior to placement (0−25%) by operations. Therefore, the presence of *Salmonella* in broiler flock was found to be strongly influenced during the rearing period by dust that were already contaminated prior to placement. Moreover, the prevalence of *Salmonella*-positive farms in feces at the time of depletion was also high, ranging from 16.7 to 66.7%.

In this study, the prevalence of farms with *Salmonella* in chickens that died within 1 week old ranged from 0 to 58.3% by operations. Gantois et al. (2009) reported that direct vertical transmission from parent to progeny can occur when breeder chicken with infected reproductive organs lay internally contaminated eggs. In particular, *S.* Enteritidis, which have high potential for vertical transmission, can lead to serious disease and death in broiler chicks (Suzuki, 1994; Liu et al., 2022). Interestingly, in operations C and D, although *S.* Enteritidis was not present in dust prior to placement, the

prevalence of farms with confirmed *S.* Enteritidis in chickens that died within 1 week old was 25 and 30%, respectively, therefore, infection to day old chicks via vertical transmission from breeders is strongly suspected. In Korea, all flock found to be infected with *S.* Gallinarum and *S.* Pullorum, which are host-adapted *Salmonella*, have to be only completely slaughtered according to quarantine management instruction for breeder farm and hatchery by the Ministry of Agriculture, Food and Rural Affairs (MAFRA, 2022). Hence, strategies for *S.* Enteritidis in breeders should also be strengthened by regulatory controls that are available if needed.

But, many *Salmonella* serovars can exhibit pseudovertical transmission, where microbes are initially present on the outside surface of the egg and subsequently penetrate in the shell membranes (Berrang et al., 1999; Cox et al., 2000). Oastler et al. (2022) reported *Salmonella* contamination tends to increase in hatcheries along the workflow from egg areas through to setters. In this study, the presence of *Salmonella* in dead chickens might have been caused by contamination at the hatchery; hence, more intensive and strict management should be implemented in hatcheries to prevent the introduction of infected chicks into broiler farms.

Interestingly, in this study, *S.* Infantis was the most identified of all 5 operations. Namely, *S.* Infantis in dust prior to placement and in chickens that died within 1 week old was only confirmed in 3 and 2 among 5 operations, but *S.* Infantis in dust at the time of depletion and in chickens that died at 4 to 5 weeks old was confirmed in all 5 operations. Moreover, the prevalence of *S.* Infantis-positive farms in dust prior to placement and in chickens that died within 1 week old was only 5.2 and 3.4% among 58 farms, respectively, but the prevalence in dust at the time of depletion and in chickens that died at 4 to 5 weeks old increased rapidly to 27.6 and 20.7%, respectively. In particular, the prevalence of *S.* Infantis-positive farms in feces at the time of depletion was significantly high (41.4%). In Korea, although previous studies revealed that *Salmonella* serovars frequently isolated at broiler farms were *S.* Hadar and *S.* Montevideo (Choi et al., 2014; Ha et al., 2018; Shang et al., 2018), our finding are in accordance with those of other reports which reported that *S.* Infantis is the primary serovar in broilers in Europe, United States, Ecuador and Japan (Vinueza-Burgos et al., 2016; Duc et al., 2019; McMillan et al., 2022; EFSA, 2022). Generally, *S.* Infantis is known to be less invasive than other serovars (Berndt et al., 2007); however, recent studies demonstrated its high ability to colonize the caeca and actively adhere to host cells (Aviv et al., 2019; Drauch et al., 2021). Drauch et al. (2020) also reported that *S.* Infantis is more resistant to disinfectants and was able to persist on farms despite cleaning and disinfection. Therefore, if strongly cleaning and disinfection procedures are not carried out, *S.* Infantis can easily spread between flocks and persist longer in environments including feces.

Moreover, several researchers reported that one of the most significant virulence factors of *S.* Infantis is the acquisition of a conjugative megaplasmid, called pESI or pESI-like plasmid, which provides the bacteria with various genetic properties (Aviv et al., 2014, 2016; Franco et al., 2015; Srednik et al., 2023). In this study, interestingly, the presence of the pESI plasmid was only detected in *S.* Infantis, and was absent in all other *Salmonella* spp.. Moreover, the prevalence of farms with pESI-positive *S.* Infantis showed significantly higher in environments at the time of depletion and chickens that died 4 to 5 weeks old compared to farms with pESI-negative *S.* Infantis. Aviv et al. (2014) reported that acquisition of the pESI plasmid played an important role in efficient dissemination and successful spread of emergent *S.* Infantis, which replaced the local *S.* Infantis population in the short time of only 2-3 yr in Israel. Moreover, Papić et al. (2022) also reported that in Slovenia, pESI-negative *S.* Infantis isolated from broiler farms has been completely replaced by pESI-positive *S.* Infantis since 2010. Therefore, the introduction of *S.* Infantis carrying the pESI plasmid can lead to the rapid spread of this serovar in broiler farms despite the short rearing period of commercial broilers, as demonstrated in this study. Moreover, Cohen et al. (2022) reported that the pESI plasmid could transfer from *S.* Infantis to other *Salmonella* serovars via conjugation. Although, in this study, the presence of the pESI plasmid was only identified in *S.* Infantis, Cohen et al. (2022) and Santos et al. (2022) reported the presence of the pESI and pESI-like plasmid in *S.* Agona, *S.* Muenchen, *S.* Schwarzengrund, and *S.* Senftenberg. Therefore, constant surveillance is necessary to determine if the pESI plasmid is present or emerges in other *Salmonella* serovars in broiler farms.

The pESI plasmid and pESI-like plasmid are also closely associated with the MDR phenotype (Aviv et al., 2014; Franco et al., 2015; Bogomazova et al., 2020; Lee et al., 2021; Papić et al., 2022; Alvarez et al., 2023). In this study, the prevalence of MDR was significantly higher in pESI-positive *S.* Infantis (99.2%) than that of in pESI-negative *S.* Infantis (6.7%). In particular, all pESI-negative *S.* Infantis except for one isolate showed susceptibility to cephalosporin, but the prevalence of pESI-positive *S.* Infantis resistant to CTX representing third-generation cephalosporin was 97.5%. Third-generation cephalosporins are one of the treatment options for severe human salmonellosis cases (WHO, 2023), and several researchers reported that the pESI-like plasmid harboring the extended-spectrum beta-lactamase genes, a major cause of third-generation cephalosporin resistance, were observed in *S.* Infantis isolated from broilers and humans (Franco et al., 2015; Lee et al., 2021; Pietsch et al., 2021). Therefore, these results indicate that MDR *S.* Infantis carrying the pESI plasmid is the most widespread in the country, and further studies regarding the epidemiology and control of this serovar are required to prevent transmission from poultry to humans through the food chain.

In this study, although all *S.* Infantis were divided into 20 pulsotypes, the distribution of pulsotypes between pESI-positive and pESI-negative *S.* Infantis were clearly different. Moreover, the major pulsotypes of

pESI-positive *S.* Infantis were PT07 (43.4%) and PT08 (40.4%). These results are consistent with other studies, which showed high genetic homogeneity among the analyzed *S.* Infantis isolates (Vinueza-Burgos et al., 2016; Pate et al., 2019). Alba et al. (2020) also reported that pESI-positive *S.* Infantis, which is widespread in Europe, were high genetically homogeneous as in this study.

Furthermore, in this study, all pESI-positive and pESI-negative *S.* Infantis harbored 13 virulence genes (*iroN, msgA, orgA, pagC, prgH, sipB, sitC, sopB, sopE, spaN, spiA, stn*, and *tolC*), simultaneously. These virulence genes, which are associated with host recognition and invasion, intracellular survival, filamentous structure formation, iron metabolism, and enterotoxin, are known to be frequently found in *Salmonella* serovars including *S.* Infantis (Choudhury et al., 2016; Kim and Lee, 2017; Amini et al., 2018; Karacan Sever and Akan, 2019). However, 2 genes (*lpfC* and *sifA*), which plays a role in biofilm formation and bacterial replication (Beuzón et al., 2000; Ledeboer et al., 2006), were detected in all pESI-positive *S.* Infantis, but in 12 (80.0%) and 12 (80.0%) of the 15 pESI-negative *S.* Infantis, respectively. Moreover, *tcfA*, which is related with intestinal colonization of *S.* Infantis (Azriel et al., 2017), was also detected in 121 (100.0%) pESI-positive *S.* Infantis, but in 6 (40.0%) pESI-negative *S.* Infantis. These results are consistent with other reports that *S.* Infantis carrying the pESI and pESI-like plasmid have increased virulence factors, which provide significant advantages in host infection and persistence on environments (Aviv et al., 2014; Franco et al., 2015; Srednik et al., 2023). But, in this study, *cdtB,* which is typhoid-associated virulence gene (Haghjoo and Galán, 2004), and *sefA, spvB,* and *pefA*, which are closely associated with specific serovars, such as *S.* Enteritidis and *S.* Typhimurium (Karasova et al., 2009; Amini et al., 2018; Borges et al., 2019), were not detected. To the best of our knowledge, this study is the first report on emergence of *S.* Infantis carrying the pESI plasmid from commercial broiler farms in Korea. Therefore, this study could provide valuable information for strategies to control *Salmonella* in the broiler industry in Korea.

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DISCLOSURES

The authors declare that they have no competing interest.

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