

# Clonal dissemination of *Salmonella enterica* serovar *albany* with concurrent resistance to ampicillin, chloramphenicol, streptomycin, sulfisoxazole, tetracycline, and nalidixic acid in broiler chicken in Korea

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**ABSTRACT** The aim of this study was to determine the prevalence, serovar distribution, antimicrobial resistance, and genotypic analyses of the dominating serovars of *Salmonella* in chickens from a national study in Korea. Between 2017 and 2018, a total of 550 chicken samples were collected from the top 12 integrated broiler chicken operations in Korea. *Salmonella* was isolated from 117 (32.5%) chicken feces and 19 (10.0%) retail chicken meat sources. Ten serovars were identified, and the most common *Salmonella* serovar was *Salmonella* ser. Albany (50 isolates, 36.8%), followed by *S. Enteritidis* (38 isolates, 27.9%), and *S. Montevideo* (23 isolates, 16.9%) isolated from 6, 10, and 6 operations, respectively. A total of 35 (25.7%) isolates were with the ACS-SuTN (ampicillin, chloramphenicol, streptomycin, sulfisoxazole, tetracycline, and nalidixic acid) resistance pattern, with high prevalence of this resistance pattern

in *S. Albany* (29 isolates, 58.0%). A total of 35 PFGE types were identified among *Salmonella* isolates of the serovars Albany, Enteritidis, Virchow, Montevideo, and Senftenberg, while 11 distinct types of PFGE patterns were found among *S. Albany* isolates, which showed an overall homology similarity of higher than 85%. Among these 35 PFGE types, 22 PFGE types corresponded to 32 isolates from samples limited to one operation, and the other 13 PFGE types corresponded to 72 isolates from samples widely distributed among different operations. These results highlighted rapid colony dissemination of multidrug-resistant *S. Albany* in chicken all over Korea after it first appeared in 2016; furthermore, the spread of *Salmonella* colonies between various integrated operations was common, and several operations played an important role in *Salmonella* carriage and transmission in Korea.

**Key words:** salmonella, antimicrobial resistance, colony dissemination, *S. Albany*, Integrated chicken operations

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## INTRODUCTION

*Salmonella enterica* is distributed worldwide and is one of the most common pathogens causing bacterial foodborne diseases in human. *Salmonella* infection is a significant public health problem, causing an estimated 93.8 million illnesses and 155,000 deaths each year worldwide (Majowicz et al., 2010). *Salmonella* gastroenteritis is usually a self-limiting disease, and antibiotics like fluoroquinolones and third-generation cephalosporins are reserved for patients with severe disease. As antibiotics have been extensively used, the increasing

prevalence of antibiotic-resistant and multidrug-resistant *Salmonella* adds to the public health burden and is associated with high medical costs, prolonged hospital stays, and increased mortality (Broughton et al., 2010).

*Salmonella* is frequently found in poultry; contaminated broiler chicken and chicken products have been identified as an important source of *Salmonella* infection in humans (EFSA, 2020). Over 2,600 known serovars are found from a variety of hosts. Predominant serovars of *S. Enteritidis*, *S. Typhimurium*, *S. Infantis*, *S. Newport*, and *S. Derby* have repeatedly been recovered from chickens and associated with poultry-related infections or outbreaks in humans in the world (EFSA, 2018; CDC, 2018). On comparing serovar distribution of *Salmonella* between human and chicken sources, the specific serovar distribution in humans could be predicted on the basis of data of chicken sources (Kang et al., 2009). *Salmonella* serovars vary in geographic regions and are always limited to specific geographic areas, except those of *S. Enteritidis* and

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*S. Typhimurium*, which are distributed worldwide (Lu et al., 2017). In addition, shifts in predominant serovars in certain hosts occur over time (Cardinale et al., 2005; Davis et al., 1999). Recently, we noticed that *S. Albany* prevalence dramatically increased in chickens in Korea. Shang et al., (2019) found that *S. Albany* was the most common serovar in one integrated broiler chicken operation in 2016, surpassing *S. Montevideo*, *S. Enteritidis*, and *S. Typhimurium*. Jeon et al., (2019) reported that *S. Albany* was isolated from 4 out of 6 sampled chicken operations, with it being the most predominant type in three operations. Due to the high public health threat, a better understanding the epidemiology of *Salmonella* particularly in chicken-is required. Therefore, the aim of this study was to determine the prevalence, serovar distribution, antimicrobial resistance, and genotypic analyses of the dominating serovars of *Salmonella* in chicken from a national study in Korea.

## MATERIALS AND METHODS

### Statement of Ethics

This study was carried out in accordance with the ethical guidelines of Jeonbuk National University (CBNU). Moreover, before the initiation of this study, a formal approval was obtained by the Ethics Committee for Animal Experiments of the Jeonbuk National University. There were no vulnerable populations involved; sampling was performed according to standard protocols, and prior consent of the farmer/manager of the facilities was taken. Individual written informed consent for the use of data was obtained from the companies and animal owners.

### Sample Collection

Between 2017 and 2018, a total of 550 chicken samples, which included 360 fresh chicken feces and 190 retail chicken meat samples, were collected from the top 12 integrated broiler chicken operations among the 13 integrated broiler chicken operations in Korea. Each operation had separated broiler chicken production chain, including breeder chicken, hatchery, broiler chicken, and slaughterhouse. The sampling locations included all the provinces of Korea, except Jeju Island and Gangwon-do. Pooled feces samples were collected from 72 broiler chicken farms; 5 samples from each farm were collected and tested in this study. In addition, retail meat was collected from 38 farms, and 5 individually packaged chicken meat from each farm were randomly sampled from their final packaging in the slaughterhouse. Collected samples were immediately stored in an ice box after collection and subjected to further processing upon arrival to the laboratory.

### Isolation and Identification of Salmonella

The feces and retail meat samples were examined for the presence of *Salmonella* as recommended by the US Department of Agriculture (USDA-FSIS, 2014). Briefly,

upon arrival to the laboratory, 1 g of each feces sample was separately mixed with 9 mL of buffered peptone water (BPW; BD Difco, Sparks, MD, USA) and incubated at 37°C for 24 h. After incubation, 0.1 mL BPW was transferred to 10 mL of Rappaport–Vassiliadis Broth (RV; Thermo Fisher Scientific, Oxoid Ltd, Basingstoke, UK) and incubated for 24 h at 42°C. A loopful was then plated on xylose–lysine–deoxycholate agar (XLD; BD Difco) and incubated at 37°C for 24 h. Retail meat was aseptically placed into a vacuum bag and rinsed with 400 mL of BPW. After shaking for about 20 times, the suspension of rinse fluid was then cultured at 37°C for 24 h. Then, 0.1 mL of enrichment was further incubated in 10 mL of RV for 24 h at 42°C and subcultured on XLD plate at 37°C for 24 h.

Three to 5 suspected *Salmonella* colonies from each plate were confirmed by latex polyclonal agglutination test (Thermo Fisher Scientific) and further confirmed through the amplification of the specific *Salmonella* invasive (*invA*) gene by PCR (Cha et al., 2013). After identification, all *Salmonella* isolates were serotyped according to Kauffmann–White scheme by slide agglutination with O and H antigen-specific sera (BD Difco; Denka Seiken Co., Ltd., Japan).

### Antimicrobial Susceptibility Testing

The minimum inhibitory concentrations were determined using the KRVN5F Sensititre panel (TREK Diagnostic Systems, Incheon, Korea). The antimicrobials tested were amoxicillin/clavulanic acid (AUG2, 2/1–32/16 µg/mL), ampicillin (AMP, 2–64 µg/mL), cefoxitin (FOX, 1–32 µg/mL), ceftazidime (TAZ, 1–16 µg/mL), ceftiofur (XNL, 0.5–8 µg/mL), cefepime (FEP, 0.25–16 µg/mL), meropenem (MERO, 0.25–4 µg/mL), trimethoprim/sulfamethoxazole (SXT, 0.12/2.38–4/76 µg/mL), sulfisoxazole (FIS, 16–256 µg/mL), chloramphenicol (CHL, 2–64 µg/mL), ciprofloxacin (CIP, 0.12–16 µg/mL), nalidixic acid (NAL, 2–128 µg/mL), streptomycin (STR, 16–128 µg/mL), gentamicin (GEN, 1–64 µg/mL), tetracycline (TET, 2–128 µg/mL), and colistin (COL, 2–16 µg/mL). *Escherichia coli* ATCC 25922 was used as quality control. The interpretive categories-susceptible, intermediate, or resistant-were used according to the CLSI guidelines, except for colistin, where the MIC value of  $\geq 4$  µg/mL (resistant) was used (CLSI, 2016; Biswas et al., 2012). Multidrug resistance (MDR) was defined as *Salmonella* isolates being resistant to as least 3 antimicrobial categories.

### Pulsed Field Gel Electrophoresis

Pulsed-field gel electrophoresis (PFGE) was used to establish relatedness and diversity among *Salmonella* isolates, and PFGE was conducted according to the Centers for Disease Control and Prevention PulseNet standardized procedure. *Salmonella* genomic DNA was digested with *Xba*I, and PFGE fingerprinting patterns

**Table 1.** Prevalence of *Salmonella* isolates in broiler chicken feces and retail meat from 12 operations in South Korea.

Operation	Total		Feces		Retail meat	
	No. of samples/ farms	Positive No. (%) of samples/farms	No. of samples/ farms	Positive No. (%) of samples/farms	No. of samples/ farms	Positive No. (%) of samples/farms
C1	60/12	15 (25.0)/7 (58.3)	40/8	8 (20.0)/4 (50.0)	20/4	7 (35.0)/3 (75.0)
C2	60/12	11 (18.3)/6 (50.0)	40/8	9 (22.5)/5 (62.5)	20/4	2 (10.0)/1 (25.0)
C3	30/6	2 (6.7)/2 (33.3)	20/4	2 (10.0)/2 (50.0)	10/2	0 (0.0)/0 (0.0)
C4	30/6	14 (46.7)/6 (100.0)	20/4	8 (40.0)/4 (100.0)	10/2	6 (60.0)/2 (100.0)
C5	60/12	15 (25.0)/5 (41.7)	40/8	15 (37.5)/5 (62.5)	20/4	0 (0.0)/0 (0.0)
C6	60/12	29 (48.3)/8 (66.7)	40/8	27 (67.5)/7 (87.5)	20/4	2 (10.0)/1 (25.0)
C7	30/6	10 (33.3)/3 (50.0)	20/4	10 (50.0)/3 (75.0)	10/2	0 (0.0)/0 (0.0)
C8	40/8	8 (20.0)/3 (37.5)	20/4	8 (40.0)/3 (75.0)	20/4	0 (0.0)/0 (0.0)
C9	60/12	15 (25.0)/7 (58.3)	40/8	13 (32.5)/5 (62.5)	20/4	2 (10.0)/2 (50.0)
C10	30/6	6 (20.0)/3 (50.0)	20/4	6 (30.0)/3 (75.0)	10/2	0 (0.0)/0 (0.0)
C11	45/9	10 (22.2)/3 (33.3)	30/6	10 (33.3)/3 (50.0)	15/3	0 (0.0)/0 (0.0)
C12	45/9	1 (2.2)/1 (11.1)	30/6	1 (3.3)/1 (16.7)	15/3	0 (0.0)/0 (0.0)
Total	550/110	136 (24.7)/54 (49.1)	360/72	117 (32.5)/45 (62.5)	190/38	19 (10.0)/9 (23.7)

were analyzed using BioNumerics software (version 5.10 for Windows, Applied Maths, Belgium). The sizes of the fragments were calculated based on the fragments for the *Salmonella* Braenderup H9812 reference standard.

## RESULTS

### Prevalence and Serovar Distribution of *Salmonella*

The prevalence of *Salmonella* in chicken feces and retail chicken meat samples is shown in **Table 1**. Among the 550 samples, 136 (24.7%) samples were found positive for *Salmonella*. Out of 360 feces samples and 190 retail meat samples, 117 (32.5%) feces and 19 (10.0%) retail meat samples were found positive, respectively. The prevalence of *Salmonella* varied from 2.2% to 48.3% among the 12 chicken production operations, and all 12 operations were positive for *Salmonella* at the farm level, and 5 (41.7%) operations were positive at the retail meat level.

Out of 136 *Salmonella* isolates, 131 isolates were assigned to 10 serovars and 5 untyped isolates (**Table 2**). The most common *Salmonella* serovars were *S. Albany* (50 isolates, 36.8%), *S. Enteritidis* (38 isolates, 27.9%),

and *S. Montevideo* (23 isolates, 16.9%) isolated from 6, 10, and 6 operations, respectively. In addition, *S. Virchow*, *S. Senftenberg*, *S. Rissen*, *S. Mbandaka*, *S. Alminko*, *S. Typhimurium*, and *S. Moscow* were also found in this study.

### Antimicrobial Susceptibility in *Salmonella*

The antimicrobial susceptibility test result of *Salmonella* isolates from 12 operations is shown in **Table 3**. Among the 136 isolates, resistance was most frequently observed to nalidixic acid (94.1%), followed by ampicillin (69.9%), sulfisoxazole (67.6%), tetracycline (60.3%), and streptomycin (55.9%); *Salmonella* isolates were less resistant to ciprofloxacin (5.1%), gentamicin (5.1%), and colistin (11.8%). In addition, resistant to cefoxitin (0.7%), ceftazidime (11.8%), ceftiofur (12.5%), and cefepime (11.0%) was also been observed. Meropenem resistance was not found. We also observed that *Salmonella* isolates from operation C4 showed high resistance to third- and fourth-generation cephalosporin and gentamicin.

Diversity of antimicrobial resistance in different *Salmonella* serovars was found in this study (**Table 4**). All 50 *S. Albany* isolates were observed to be resistant to

**Table 2.** Distribution of *Salmonella* serovars among 12 operations.

Operation	Serovar (No.)										
	Albany	Enteritidis	Montevideo	Virchow	Senftenberg	Rissen	Mbandaka	Alminko	Typhimurium	Moscow	<i>S. spp</i>
C1 (n = 15)		7	4		1		3				
C2 (n = 11)	6	1	4								
C3 (n = 2)		1									1
C4 (n = 14)		6	2	6							
C5 (n = 15)	9	5	1								
C6 (n = 29)	22	1				3		1			2
C7 (n = 10)	2	7								1	
C8 (n = 8)	8										
C9 (n = 15)		4	8		2						1
C10 (n = 6)	3				1				1		1
C11 (n = 10)		5	4		1						
C12 (n = 1)		1									
Total (n = 136)	50	38	23	6	5	3	3	1	1	1	5

trimethoprim/sulfamethoxazole, sulfisoxazole, and nalidixic acid. Furthermore, they showed high resistance to ampicillin (88.0%), tetracycline (88.0%), chloramphenicol (86.0), and streptomycin (64.0%). All *S. Virchow* isolates were found to be resistant to third- and fourth-generation cephalosporin, and *S. Enteritidis* showed similarly high resistance as well. Gentamicin resistance was found only in *S. Enteritidis* isolates. In addition, *S. Montevideo* isolates showed low resistance to tested antimicrobials, except nalidixic acid; moreover, they showed no resistance to amoxicillin/clavulanic acid, ampicillin, cefoxitin, ceftazidime, cefotiofur, cefepime, meropenem, chloramphenicol, gentamicin, and tetracycline.

A diversity of antimicrobial resistance phenotypes ( $n = 30$ ) was observed among the *Salmonella* isolates from broiler chicken farm and retail meat (Table 5). Except 4 isolates susceptible to all antimicrobials, all *Salmonella* isolates were resistant to at least one antimicrobial. We also found all *S. Albany* and *S. Virchow* isolates were MDR and 35 (92.1%) *S. Enteritidis* isolates were also MDR. While *S. Montevideo* isolates showed less resistance to the tested antimicrobial and 13.0% of *S. Montevideo* isolates were MDR. Meanwhile, a total of 35 (25.7%) isolates were with the ACSSuTN (ampicillin, chloramphenicol, streptomycin, sulfisoxazole, tetracycline and nalidixic acid) resistance pattern. The highest percentage of ACSSuTN resistance was identified in *S. Albany* (29 isolates, 58.0%), followed by *S. Virchow* (3 isolates, 50.0%), *S. Enteritidis* (1 isolate, 2.6%), and other serovars (2 isolates, 14.3%). However, all *S. Montevideo* and *S. Senftenberg* isolates were ACS-SuTN-susceptible.

### Genotypic Determination of Diversity Among *Salmonella* Isolates

The genetic relatedness of the *S. Albany*, *S. Enteritidis*, *S. Virchow*, *S. Montevideo*, and *S. Senftenberg* was evaluated on the basis of the PFGE pattern analysis. Eleven distinct types of PFGE patterns were found among *S. Albany* isolates which showed an overall homology similarity higher than 85% (Figure 1). Six identical PFGE types (A1, A2, A4, A5, A8, and A10) were found with more than one *S. Albany* isolate recovered from 6 operations, whereas 5 types (A3, A6, A7, A9, and A11) were found with only one isolate. We found that identical PFGE type A1 of *S. Albany* isolates was recovered from 3 operations (C6, C7, and C10), type A2 from 4 operations (C2, C6, C8, and C10), type A4 from 2 operations (C6 and C8), type A5 from 3 operations (C6, C7, and C8), and type A8 from 2 operations (C2 and C5). The 12 types of PFGE patterns were found among *S. Enteritidis* isolates (Figure 2), and 2 clusters of *S. Enteritidis* isolates were observed using an 80% cut off value. We found that identical PFGE type E1 of *S. Enteritidis* isolates was recovered from two operations (C4 and C9), type E9 from three operations (C7, C11, and C12), and type E12 from three operations (C3,

C5, and C7). A total of 6 types were found in *S. Montevideo* isolates (Figure 3), except for one isolate of type M6; other *S. Montevideo* isolates showed a homology similarity higher than 85%. Identical PFGE type M2 of *S. Montevideo* isolates recovered from three operations (C1, C4, and C9) and type M5 from 3 operations (C1, C5, and C9) were found.

A total of 35 PFGE types were identified among *Salmonella* isolates of the serovars Albany, Enteritidis, Virchow, Montevideo, and Senftenberg from 12 chicken processing operations. Among these types, 22 PFGE types corresponded to 32 isolates from samples limited to one operation, and the other 13 PFGE types corresponded to 72 isolates from samples widely distributed among different operations. Among these 13 PFGE types, *Salmonella* isolates from one operation were with the identified PFGE types of *Salmonella* isolates collected from at least other two operations (Figure 4). Operation C3 of *Salmonella* isolates with PFGE type E12 was overlapped with the PFGE type of *Salmonella* isolates from other two operations of C5 and C7; in keeping with operation C4 of *Salmonella* isolates with PFGE type M2 overlapped with it of *Salmonella* isolates from operation C1, PFGE types E1 and M2 overlapped with it of *Salmonella* isolates from operation C9; operation C11 of PFGE type E9 with operations C7 and C12, and operation 12 of type E9 with operations C7 and C11. While operations C1 and C9 of *Salmonella* isolates with identified PFGE were overlapped with three operations each of C4, C5, and C9 and C1, C4, and C5, respectively, operations C2, C6, C8, and C10 overlapped with four other operations and operation E overlapped with five operations. It is noteworthy that four PFGEs (A1, A5, E9, and E12) of *Salmonella* isolates from operation C7 were overlapped with seven operations (C3, C5, C6, C8, C10, C11, and C12).

## DISCUSSION

In the present study, a national investigation of the prevalence, serovar distribution, antimicrobial resistance, and genetic characterization of *Salmonella* isolates from 12 integrated broiler chicken operations in South Korea was conducted. The overall prevalence of *Salmonella* was 24.7%, being 32.5% in chicken feces and 10.0% in retail chicken meat. Although *Salmonella* prevalence varied among these operations, all 12 operations are confirmed to be *Salmonella*-positive. This result was in agreement with previous studies stating that *Salmonella* was widely distributed in broiler chicken population (Antunes et al., 2016).

In this study, *S. Albany* was the most commonly identified serovar (36.8%), followed by *S. Enteritidis* (27.9%) and *S. Montevideo* (16.9%) in broiler chicken. This is in contrast to previous studies reporting *S. Enteritidis* and *S. Montevideo* as the most common serovars in chicken, perhaps because *S. Albany* was not identified in chicken before 2016 in Korea (Shang et al., 2019; Tamang et al., 2011). From previous reports, *S. Albany*

**Table 3.** Antimicrobial resistance of *Salmonella* isolates among 12 operations (resistance No./%).

Antimicrobial agent	Operation												Total (n = 136)
	C1 (n = 15)	C2(n = 11)	C3(n = 2)	C4(n = 14)	C5(n = 15)	C6(n = 29)	C7(n = 10)	C8(n = 8)	C9(n = 15)	C10(n = 6)	C11(n = 10)	C12(n = 1)	
Amoxicillin/ clavulanic acid	0 (0.0)	2 (18.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (1.5)
Ampicillin	8 (53.3)	10 (90.9)	1 (50.0)	12 (85.7)	14 (93.3)	21 (72.4)	8 (80.0)	8 (100.0)	3 (20.0)	4 (66.7)	5 (50.0)	1 (100.0)	95 (69.9)
Cefoxitin	0 (0.0)	1 (9.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.7)
Ceftazidime	0 (0.0)	1 (9.1)	0 (0.0)	11 (78.6)	0 (0.0)	1 (3.4)	0 (0.0)	0 (0.0)	3 (20.0)	0 (0.0)	0 (0.0)	0 (0.0)	16 (11.8)
Ceftiofur	0 (0.0)	1 (9.1)	0 (0.0)	12 (85.7)	0 (0.0)	1 (3.4)	0 (0.0)	0 (0.0)	3 (20.0)	0 (0.0)	0 (0.0)	0 (0.0)	17 (12.5)
Cefepime	0 (0.0)	0 (0.0)	0 (0.0)	11 (78.6)	0 (0.0)	1 (3.4)	0 (0.0)	0 (0.0)	3 (20.0)	0 (0.0)	0 (0.0)	0 (0.0)	15 (11.0)
Meropenem	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Trimethoprim/ sulfamethoxazole	0 (0.0)	6 (54.5)	0 (0.0)	4 (28.6)	9 (60.0)	26 (89.7)	2 (20.0)	8 (100.0)	0 (0.0)	4 (66.7)	0 (0.0)	0 (0.0)	59 (43.4)
Sulfisoxazole	8 (53.3)	9 (81.8)	1 (50.0)	7 (50.0)	14 (93.3)	26 (89.7)	8 (80.0)	8 (100.0)	1 (6.7)	4 (66.7)	5 (50.0)	1 (100.0)	92 (67.6)
Chloramphenicol	0 (0.0)	5 (45.5)	0 (0.0)	4 (28.6)	9 (60.0)	20 (69.0)	2 (20.0)	8 (100.0)	1 (6.7)	5 (83.3)	0 (0.0)	0 (0.0)	54 (39.7)
Ciprofloxacin	0 (0.0)	1 (9.1)	0 (0.0)	1 (7.1)	1 (6.7)	4 (13.8)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	7 (5.1)
Nalidixic acid	12 (80.0)	11 (100.0)	1 (50.0)	14 (100.0)	15 (100.0)	29 (100.0)	8 (80.0)	8 (100.0)	15 (100.0)	4 (66.7)	10 (100.0)	1 (100.0)	128 (94.1)
Streptomycin	9 (60.0)	7 (63.6)	2 (100.0)	7 (50.0)	10 (66.7)	19 (65.5)	7 (70.0)	5 (62.5)	1 (6.7)	2 (33.3)	6 (60.0)	1 (100.0)	76 (55.9)
Gentamicin	0 (0.0)	0 (0.0)	0 (0.0)	6 (42.9)	0 (0.0)	1 (3.4)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	7 (5.1)
Tetracycline	0 (0.0)	6 (54.5)	1 (50.0)	12 (85.7)	13 (86.7)	22 (75.9)	8 (80.0)	8 (100.0)	3 (20.0)	3 (50.0)	5 (50.0)	1 (100.0)	82 (60.3)
Colistin	2 (13.3)	0 (0.0)	0 (0.0)	1 (7.1)	5 (33.3)	1 (3.4)	4 (40.0)	0 (0.0)	1 (6.7)	0 (0.0)	2 (20.0)	0 (0.0)	16 (11.8)

**Table 4.** Antimicrobial resistance among different *Salmonella* serovar.

Antimicrobial agent	Serovar (No./%)										
	Albany (n = 50)	Enteritidis (n = 38)	Montevideo (n = 23)	Virchow (n = 6)	Senftenberg (n = 5)	Rissen (n = 3)	Mbandaka (n = 3)	Alminko (n = 1)	Typhimurium (n = 1)	Moscow (n = 1)	<i>S. spp</i> (n = 5)
Amoxicillin/ clavulanic acid	2 (4.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Ampicillin	44 (88.0)	34 (89.5)	3 (13.0)	6 (100.0)	1 (20.0)	2 (66.7)	0 (0.0)	1 (100.0)	1 (100.0)	1 (100.0)	2 (50.0)
Cefoxitin	1 (2.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Ceftazidime	1 (2.0)	8 (21.1)	0 (0.0)	6 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (25.0)
Ceftiofur	1 (2.0)	9 (23.7)	0 (0.0)	6 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (25.0)
Cefepime	0 (0.0)	8 (21.1)	0 (0.0)	6 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (25.0)
Meropenem	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Trimethoprim/ sulfamethoxazole	50 (100.0)	1 (2.6)	0 (0.0)	3 (50.0)	0 (0.0)	2 (66.7)	0 (0.0)	1 (100.0)	1 (100.0)	0 (0.0)	1 (25.0)
Sulfisoxazole	50 (100.0)	27 (71.1)	2 (8.7)	6 (100.0)	1 (20.0)	1 (33.3)	0 (0.0)	1 (100.0)	1 (100.0)	1 (100.0)	2 (50.0)
Chloramphenicol	43 (86.0)	2 (5.3)	0 (0.0)	3 (50.0)	0 (0.0)	2 (66.7)	0 (0.0)	1 (100.0)	1 (100.0)	0 (0.0)	2 (50.0)
Ciprofloxacin	3 (6.0)	1 (2.6)	1 (4.3)	0 (0.0)	0 (0.0)	2 (66.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Nalidixic acid	50 (100.0)	36 (94.7)	23 (100.0)	6 (100.0)	5 (100.0)	3 (100.0)	0 (0.0)	1 (100.0)	0 (0.0)	1 (100.0)	3 (75.0)
Streptomycin	32 (64.0)	27 (71.1)	3 (13.0)	6 (100.0)	1 (20.0)	2 (66.7)	1 (33.3)	0 (0.0)	0 (0.0)	1 (100.0)	3 (75.0)
Gentamicin	0 (0.0)	7 (18.4)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Tetracycline	44 (88.0)	25 (65.8)	0 (0.0)	6 (100.0)	0 (0.0)	3 (100.0)	0 (0.0)	1 (100.0)	0 (0.0)	1 (100.0)	2 (50.0)
Colistin	2 (4.0)	11 (28.9)	2 (8.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (100.0)	0 (0.0)

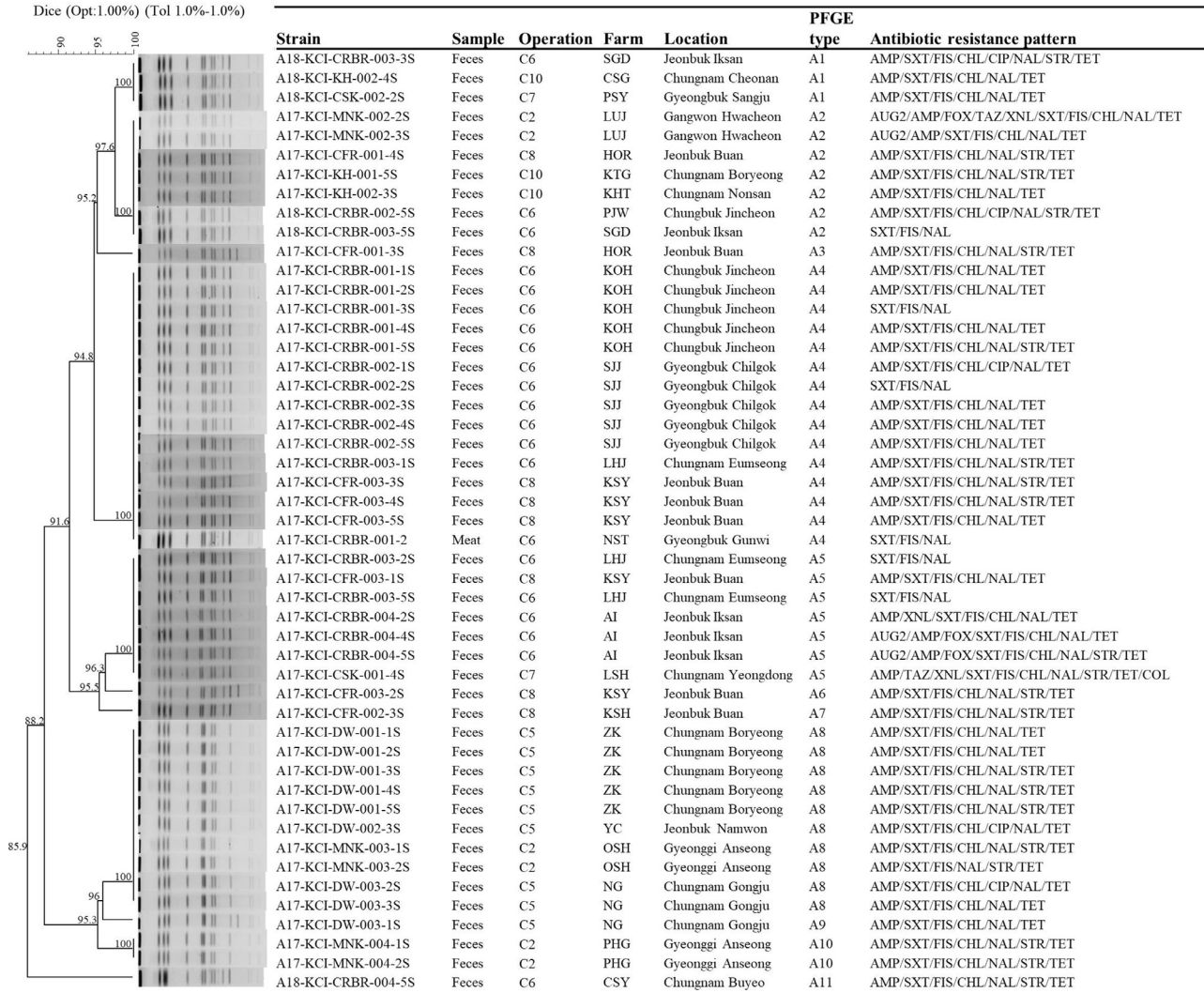
**Table 5.** Resistance pattern of *Salmonella* isolates from chicken.

No.	Antimicrobial resistance pattern	No. of isolates						
		Albany (n = 50)	Enteritidis (n = 38)	Montevideo (n = 23)	Virchow (n = 6)	Senftenberg (n = 5)	Others (n = 14)	Total (n = 136)
0	-		2				2	4
1	AMP			1				1
2	CHL						1	1
3	NAL		1	18		4	1	24
4	STR						2	2
5	NAL/COL			1				1
6	NAL/TET						1	1
7	CHL/NAL/COL		1					1
8	NAL/STR/COL			1				1
9	SXT/FIS/NAL	4						4
10	AMP/FIS/NAL/STR		7	2		1		10
11	AMP/SXT/FIS/CHL						1	1
12	SXT/FIS/NAL/STR	2						2
13	AMP/FIS/NAL/STR/COL		2					2
14	AMP/FIS/NAL/STR/TET		9					9
15	AMP/FIS/NAL/STR/TET/COL		7				1	8
16	AMP/SXT/FIS/CHL/NAL/TET	11					1	12
17	AMP/SXT/FIS/NAL/STR/TET	1						1
18	AMP/TAZ/XNL/FEP/NAL/TET		2					2
19	AMP/SXT/CHL/CIP/NAL/STR/TET						1	1
20	AMP/SXT/FIS/CHL/NAL/STR/TET	25					1	26
21	AMP/SXT/FIS/CHL/NAL/TET/COL	1						1
22	AMP/TAZ/XNL/FEP/NAL/GEN/TET		5					5
23	AUG2/AMP/SXT/FIS/CHL/NAL/TET	1						1
24	AMP/SXT/FIS/CHL/CIP/NAL/STR/TET	3					1	4
25	AMP/SXT/FIS/CHL/NAL/STR/TET/COL	1						1
26	AMP/TAZ/XNL/FEP/FIS/NAL/STR/TET				3		1	4
27	AMP/TAZ/XNL/FEP/FIS/NAL/STR/GEN/ TET		1					1
28	AMP/TAZ/XNL/FEP/SXT/FIS/CHL/NAL/ STR/TET				3			3
29	AUG2/AMP/FOX/TAZ/XNL/SXT/FIS/ CHL/NAL/TET	1						1
30	AMP/XNL/SXT/FIS/CHL/CIP/NAL/STR/ GEN/TET/COL		1					1

was commonly found in poultry and other domestic animals in Southeast Asia and the Western countries (Fuzihara et al., 2000; Ta et al., 2014), and this serovar has gained significant public attention as this serovar has become among the top five serovars affecting humans in recent times (Kuo et al., 2014). Since serovar diversity of *Salmonella* has been recorded from different geographical regions and there have always been certain geography-specific serovars, the emergence of *S. Albany* in Korea suggests that it could have been globally disseminated via international travel or food trade import–export as important vehicles (Huusko et al., 2017; Park et al., 2019). Although imported retail meat has not reported as a possible source for the dissemination of *Salmonella* in Korea, the introduction of foodborne pathogens through imported retail meat has been reported in Korea recently (Kim et al., 2018; Kim et al., 2015). Therefore, as a potential vehicle for *Salmonella* transmission in Korea, extensive monitoring and risk assessment of *Salmonella* in imported chicken meat may be required to estimate the potential public health threat to humans and the environment in the future.

High resistance of *Salmonella* isolates from chicken to nalidixic acid, ampicillin, sulfonamides, tetracycline, and streptomycin was consistent with the literature from different countries, including Korea (EFSA, 2018; Shang et al., 2019; Tamang et al., 2011). This is not

surprising because these antimicrobials have been widely used for infection treatment in poultry since a long time. We also noticed antimicrobial resistance diversity among different serovars of *Salmonella* isolates. In this study, *S. Albany* isolates showed high resistance to trimethoprim/sulfamethoxazole, sulfisoxazole, nalidixic acid, ampicillin, tetracycline, chloramphenicol, and streptomycin. In agreement with a previous study in Malaysia, Chuah et al., (2018) reported *S. Albany* isolates from wet poultry market that had a high frequency of resistance to these antimicrobials. Similarly, in Taiwan, high resistance to nalidixic acid, ampicillin, tetracycline, chloramphenicol, and trimethoprim/sulfamethoxazole was found in *S. Albany* isolates from humans and pigs (Kuo et al., 2014). Furthermore, it should be noted that all *S. Albany* isolates were multi-drug-resistant, and 29 isolates (58%) showed the ACSSuTN resistance pattern. After the ACSSuT-resistant *S. Typhimurium* was first identified in United Kingdom in 1984, the ACSSuT resistance pattern has attracted significant attention in the world because of the huge public health threat (Threlfall et al., 1996). Along with the prolonged and excessive use of quinolones in food-producing animals in the past, a dramatic increase in resistance to quinolones was reported in *Salmonella* (Antunes et al., 2016). Moreover, of major clinical and public health concerns was the observation that three

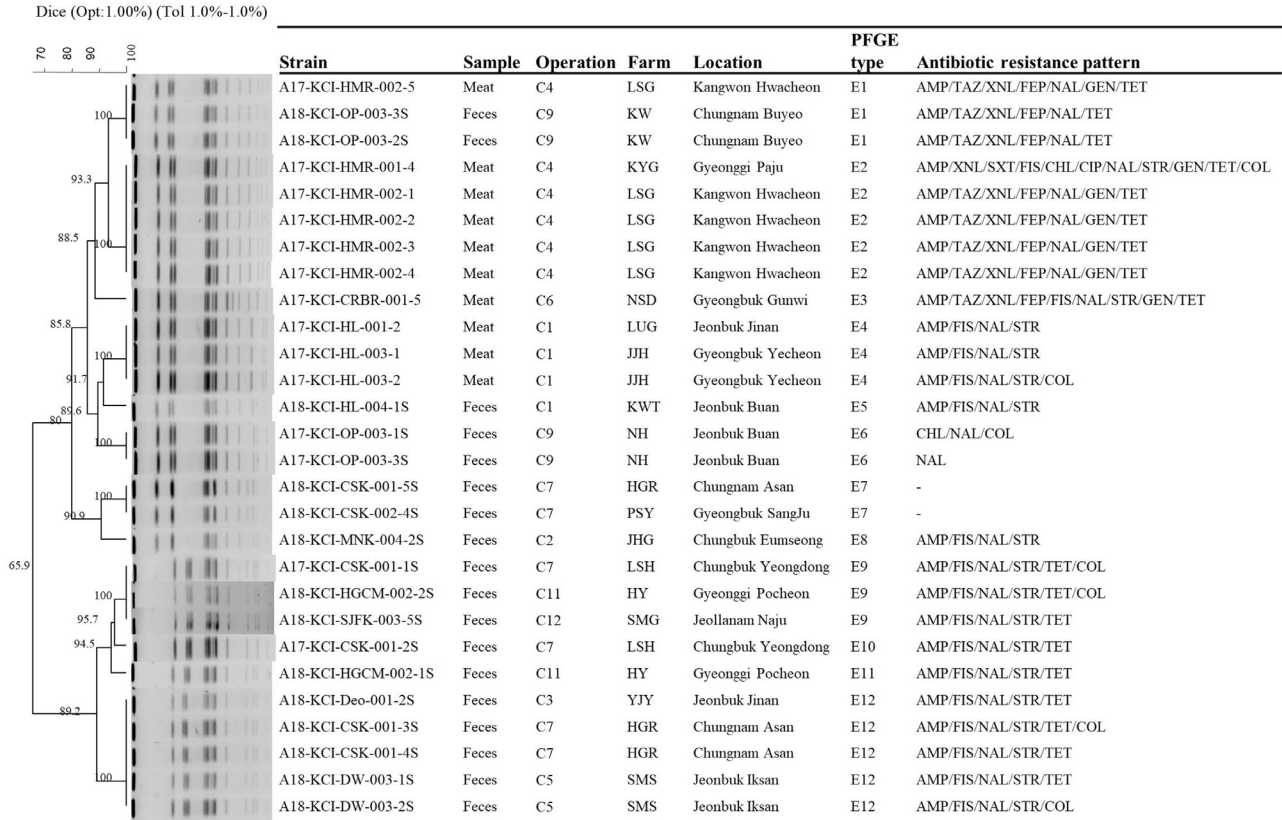


**Figure 1.** Dendrograms based on *Xba* I-pulsed field gel electrophoresis (PFGE) profiles of *Salmonella* ser. Albany isolates from chicken and the corresponding antimicrobial susceptibility patterns to the 16 indicated antimicrobials. The Dice coefficient was used to perform similarity analysis. The antimicrobials shown in the sequence are amoxicillin/clavulanic acid (AUG2), ampicillin (AMP), cefoxitin (FOX), ceftazidime (TAZ), ceftiofur (XNL), cefepime (FEP), meropenem (MERO), trimethoprim/sulfamethoxazole (SXT), sulfisoxazole (FIS), chloramphenicol (CHL), ciprofloxacin (CIP), nalidixic acid (NAL), streptomycin (STR), gentamicin (GEN), tetracycline (TET), and colistin (COL).

ACSSuTN-resistant *S. Albany* isolates were co-resistant to ciprofloxacin and one isolate was co-resistant to colistin, concurrently. Currently, ciprofloxacin is commonly used for the treatment of non-typhoidal *Salmonella* infections, and colistin is considered the last-line of antibiotic defense (Biswas et al., 2012). Co-resistance to these antimicrobials is already a major public health problem because of the possibility of horizontal transmission of the resistant colonies to other humans and horizontal transmission the resistant plasmid between bacterial species. We also identified a multidrug-resistant *S. Albany* isolate that was co-resistant to third-generation cephalosporins, which were considered an alternative drug for treating *Salmonella* infection. Therefore, the spread of multidrug-resistant *S. Albany* isolates co-resistant to these clinically important antibiotics, including fluoroquinolone, third-generation cephalosporins, and colistin, will pose a real threat to global public health resulting in challenges with clinical treatment.

In agreement with previous studies in Korea, the top serovars –*S. Enteritidis* and *S. Montevideo*– were commonly found in chickens in this study, whereas the frequencies of *S. Virchow*, *S. Senftenberg*, *S. Rissen*, *S. Mbandaka*, *S. Alminko*, *S. Typhimurium*, and *S. Moscow* were relatively lower (Jeon et al., 2019; Kang et al., 2009; Shang et al., 2019; Tamang et al., 2011). Notably, a relatively high frequency of multidrug resistance was found in *S. Enteritidis* (92.1%) and *S. Virchow* (100.0%) isolates. Of particular interest was high resistance to third-generation cephalosporins in *S. Enteritidis* (23.7%) and *S. Virchow* (100.0%) isolates. After the first report of the isolation of third-generation cephalosporin-resistant *S. Enteritidis* and *S. Essen* strains from chicken, increased resistance in various *Salmonella* has been reported in poultry in Korea (Lee et al., 2016; Park et al., 2017; Tamang et al., 2011). We should particularly focus on all *S. Virchow* isolates with third-generation cephalosporin resistance which were on account of the dramatically increased cefotaxime-resistant *S.*





**Figure 2.** Dendrograms based on *Xba*I-pulsed field gel electrophoresis (PFGE) profiles of *Salmonella* ser. Enteritidis isolates from chicken and the corresponding antimicrobial susceptibility patterns to the 16 indicated antimicrobials.

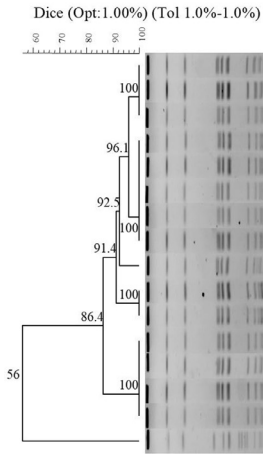
Virchow in human may source from chicken (Kim et al., 2016). In addition, high resistance (28.9%) to colistin in *S. Enteritidis* was found in this study. This result was in agreement with a previous study wherein high colistin resistance was limited to specific *Salmonella* serovars (Chiou et al., 2017). We also noticed that gentamicin resistance was only in *S. Enteritidis* isolates; this result was in contrast to previous studies which reported that mild gentamicin resistance was always present in *Salmonella* isolates (EFSA, 2020). High gentamicin resistance in *S. Enteritidis*; previous studies had showed the persistent distribution of the resistant colonies in Korean poultry industry (Kang et al., 2017). Furthermore, in this study, a multidrug-resistant *S. Enteritidis* strain with the resistance pattern XNL-CIP-GEN-COL was also identified. The findings of this study corroborate the widely held view that poultry is a major source of multidrug-resistant *Salmonella* which is resistant to treatment with several antimicrobials. This study underlines the value of an antibiotic susceptibility survey for selecting appropriate treatment options for salmonellosis caused by strains of poultry origin (Antunes et al., 2016). As the emergence of antimicrobial resistant strains has been linked to the use of antimicrobials in the farm, more prudent and appropriate use of antibiotics in food animals is required.

Based on the PFGE results, all *S. Albany* isolates had a high genetic homology of more than 85% similarity which suggests a colony dissemination of *S. Albany*

in Korea. Furthermore, the high degree of genetic homology may more likely be explained by a common ancestral origin than multiple origins; this is also supported by the fact that most isolates differed by only 1 to 2 bands after *Xba*I digestion (Okoro et al., 2012). We could also assume that the colony does not allow for acquisition of multiple genetic alterations in such a short period after appearance in chicken in Korea. It is possible that same colony was gained different antibiotics treatment pressure in different chicken operations or farms, and resulted in that the *S. Albany* isolates from different farms with high degree of genetic homology and different antibiotics resistance patterns. We also noticed that *S. Albany* was the predominant serovar in 5 among the 6 chicken operations that were positive for *S. Albany* (Table 2). In addition, the rapid dissemination of *S. Albany* in all 6 provinces investigated in this study allowed us to hypothesize that this serovar or certain colonies had some growth advantage over other serovars. In addition, the identification of third-generation cephalosporin-resistant MDR *S. Albany* suggests that MDR *S. Albany* could also acquire extended-spectrum beta-lactamase resistance genes and that these Albany strains may become a great public health concern in Korea. Since studies on the biological and virulence characteristics of *S. Albany* are lacking, further studies that are not limited to advanced surveillance are required to prevent the dissemination of the resistance.

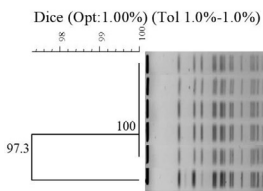
We also noticed that each operation shared the same PFGE types of *Salmonella* isolates with at least 2 other

**A**



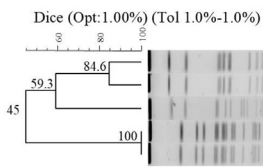
Strain	Sample	Operation	Farm	Location	PFGE	
					type	Antibiotic resistance pattern
A17-KCI-MNK-001-2	Meat	C2	SJS	Chungbuk Cheongju	M1	AMP
A17-KCI-MNK-001-4	Meat	C2	SJS	Chungbuk Cheongju	M1	NAL
A18-KCI-MNK-002-2S	Feces	C2	KKS	Gangwon Cheorwon	M1	AMP/FIS/NAL/STR
A17-KCI-HMR-001-2S	Feces	C4	JY	Gangwon Inje	M2	NAL
A17-KCI-HMR-002-2S	Feces	C4	JCG	Gyeonggi Yangpyeong	M2	NAL
A18-KCI-HL-001-3S	Feces	C1	LKS	Gyeongnam Uiryeong	M2	NAL/COL
A18-KCI-OP-003-5S	Feces	C9	KW	Chungnam Buyeo	M2	NAL
A18-KCI-OP-004-1S	Feces	C9	SJ	Gyeongbuk Sangju	M2	NAL
A17-KCI-HL-002-2	Meat	C1	YUH	Jeonbuk Jeongeup	M3	NAL
A17-KCI-HGCM-002-1S	Feces	C11	KKS	Gyeonggi Pocheon	M4	NAL
A17-KCI-HGCM-002-3S	Feces	C11	KKS	Gyeonggi Pocheon	M4	NAL/STR/COL
A17-KCI-DW-004-1S	Feces	C5	HM	Jeonbuk Gimje	M5	NAL
A17-KCI-OP-004-1	Meat	C9	LMJ	Chungbuk Yeongdong	M5	NAL
A18-KCI-HL-001-5S	Feces	C1	LKS	Gyeongnam Uiryeong	M5	NAL
A18-KCI-OP-002-1S	Feces	C9	HJ	Gyeongbuk Gimcheon	M5	NAL
A17-KCI-OP-002-5	Meat	C9	GYM	Chungnam Buyeo	M6	NAL

**B**



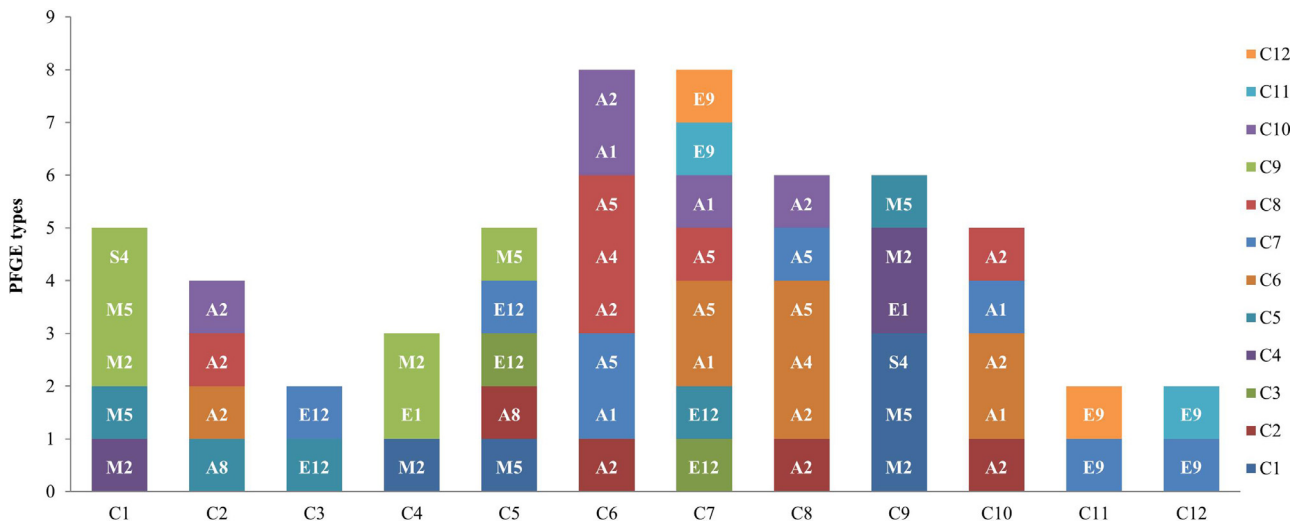
Strain	Sample	Operation	Farm	Location	PFGE	
					type	Antibiotic resistance pattern
A18-KCI-HMR-001-1S	Feces	C4	HJ	Gyeonggi Yeouju	V1	AMP/TAZ/XNL/FEP/FIS/NAL/STR/TET
A18-KCI-HMR-001-3S	Feces	C4	HJ	Gyeonggi Yeouju	V1	AMP/TAZ/XNL/FEP/SXT/FIS/CHL/NAL/STR/TET
A18-KCI-HMR-001-4S	Feces	C4	HJ	Gyeonggi Yeouju	V1	AMP/TAZ/XNL/FEP/FIS/NAL/STR/TET
A18-KCI-HMR-002-2S	Feces	C4	LJ	Gyeonggi Paju	V1	AMP/TAZ/XNL/FEP/FIS/NAL/STR/TET
A18-KCI-HMR-002-3S	Feces	C4	LJ	Gyeonggi Paju	V1	AMP/TAZ/XNL/FEP/SXT/FIS/CHL/NAL/STR/TET
A18-KCI-HMR-002-5S	Feces	C4	LJ	Gyeonggi Paju	V2	AMP/TAZ/XNL/FEP/SXT/FIS/CHL/NAL/STR/TET

**C**



Strain	Sample	Operation	Farm	Location	PFGE	
					type	Antibiotic resistance pattern
A18-KCI-HGCM-003-5S	Feces	C11	JS	Gyeonggi Yangpyeong	S1	NAL
A18-KCI-KH-002-5S	Feces	C10	CSG	Chungnam Cheonan	S2	NAL
A17-KCI-OP-003-5S	Feces	C9	KW	Chungnam Buyeo	S3	NAL
A17-KCI-OP-004-2S	Feces	C9	SJ	Gyeongbuk Sangju	S4	NAL
A18-KCI-HL-004-3S	Feces	C1	KWT	Jeonbuk Buan	S4	AMP/FIS/NAL/STR

**Figure 3.** Dendrograms based on *Xba* I-pulsed field gel electrophoresis (PFGE) profiles of *Salmonella* ser. Montevideo (A), *S. Virchow* (B), and *S. Senftenberg* (C) isolates from chicken and the corresponding antimicrobial susceptibility patterns to the 16 indicated antimicrobials.



**Figure 4.** Overlap each of the PFGE types of *Salmonella* ser. Albany, *S. Enteritidis*, *S. Montevideo*, *S. Virchow*, and *S. Senftenberg* from one operation to other operations.

operations (**Figure 4**). This result suggests that the dissemination of the same *Salmonella* colonies between different chicken operations is common. The rapid and wide spread of these *Salmonella* colonies across different chicken operations may indicate an increasing public health concern with increased chance for these colonies to acquire antibiotic resistance and virulence genes in the presence of different environment stresses in different chicken operations (Andino et al., 2015). Our results were contradictory to those of other studies that had demonstrated phenotypic and genetic diversity of *Salmonella* isolates from different chicken farms and operations (Ha et al., 2018). The dissemination between different operations may indicate a common origin within these broiler chicken operations. This result suggests that external environmental factors play an important role in the dissemination of colonies among these integrated chicken production operations wherein each vertical integrated operation has a separate supply chain that includes broiler breeder, broiler hatchery, broiler, and slaughterhouse, among others. Furthermore, we cannot rule out that the contamination in broiler chicken is vertically infected with *Salmonella* from broiler breeder chickens. This is because vertical transmission of *Salmonella* to broiler chicken could result from infected breeder chicken, and it is common for different broiler chicken production operations to share the same breeder chicken company (Davies et al., 2001; Oh et al., 2010). Among these operations, we should specifically focus on operation C7 which shared four PFGE types with seven operations (E12 with operations C3 and C5, A1, and A5 with operation C6, A5 with operation C8, A1 with operation C10, and E9 with operations C11 and C12). This data suggests operation C7 as the original source of *Salmonella* for these genotypes or the important intermediate route of *Salmonella* transmission, thus emphasizing the importance to control the spread of *Salmonella* in operation C7. In addition, attention needs to be paid to multiple interchange activities among operations C1 and C9 as three genotypes in 2 serovars were identified among these two operations. Therefore, to speed up the development of intervention strategies, further epidemiological studies are needed to identify the sources of *Salmonella* infection for each operation, particularly for the common infection route among these operations.

In conclusion, this nationwide surveillance study presents findings on serovar distribution, antibiotic resistance, and genetic diversity of *Salmonella* source from 12 integrated broiler chicken operations across Korea. The results obtained the current epidemiological state of *Salmonella* isolates present in chicken and revealed that the multidrug-resistant serovar *S. Albany* has distributed all over Korea and suggested that the nationwide occurrence of this serovar during the study period was due to increased circulation of *S. Albany* colonies and establishment of a specific colony that took place after it first appeared in 2016. In addition, we also noted that the spread of *Salmonella* colonies between different integrated operations was common, and several

operations played a part in *Salmonella* carriage and transmission in Korea.

## ACKNOWLEDGMENTS

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## DISCLOSURES

The authors have no conflicts of interest to declare.

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