Research article

Strain typing and antimicrobial susceptibility of *Salmonella enterica* Albany isolates from duck farms in South Korea

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ABSTRACT

Salmonella enterica is distributed worldwide and is a common cause of bacterial food poisoning in humans and a serious public health problem. Although duck meat consumption has recently increased in Korea, studies on the epidemiological relationship between *S. enterica* contamination in duck farms are scarce. *Salmonella enterica* serovar Albany isolates recovered from duck farms were analyzed using two typing methods — IR Biotyper® (IRBT) and multilocus variable-number tandem repeat analysis (MLVA). The clustering results were compared with the epidemiological survey findings and the antimicrobial resistance profiles. From April 2019 to October 2020, 20 individual feces per farm from 5–6-week-old ducks were collected repeatedly from 105 duck farms. *Salmonella* spp. isolated from duck feces were identified using PCR and multilocus sequence typing to investigate the prevalence and distribution of the *Salmonella* serovars. The prevalence of *S. enterica* was 19%, and *S. enterica* Albany was the predominantly recovered isolate. The *S. enterica* Albany isolates underwent antimicrobial susceptibility testing to determine the minimum inhibitory concentration. MLVA and IRBT methods established relatedness and diversity among the *S. enterica* Albany isolates. Multidrug-resistant *S. enterica* Albany was distributed in all the farms. Antimicrobial resistance profiles reflected the duck farm characteristics and isolates recovered from the same farm showed an identical profile. Isolates repeatedly recovered from the same farm also showed identical IRBT clusters and MLVA groups. These findings suggest that the isolates remained on the duck farm and re-infected new duck flocks. Thus, proper cleaning and disinfection is required before the farms are repopulated.

1. Introduction

Salmonella. enterica Albany represents a risk to human and animal health, considering its identification in ducks [1,2]. A previous study reported a high *Salmonella* contamination in duck meat [3]. Another study showed that *S. enterica* Albany contamination of poultry carcasses caused public health problems [1]. However, few studies have investigated the epidemiology of *S. enterica* Albany in ducks.

Strain typing is defined as the epidemiological evaluation of isolates recovered from farms. It can be applied to confirm the characteristics of isolates [4,5]. Strain typing can be performed using multilocus variable number tandem repeat analysis (MLVA) or Fourier transform infrared (FTIR) spectroscopy. Bacterial genomes contain polymorphic tandem repeats at an identical locus (for example, variable-number tandem repeats (VNTRs)), which differ between strains [6]. MLVA takes advantage of these repeats to

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distinguish strains and can be easily performed by conventional PCR with identical primers [7]. VNTR analysis is useful for assessing genetic relatedness between strains and can provide accurate pathogen typing and phylogenetic analysis [8]. The discovery of VNTR loci has enabled genotyping via MLVA of multiple *Salmonella* serovars [6,9] and is considered a highly discriminatory method [6].

FTIR spectroscopy analyzes molecular vibrations caused by infrared light absorption using the principle that different chemical structures vibrate at different frequencies [5]. Microorganisms can be characterized according to their strain-specific absorbance patterns in the infrared spectrum [10]. FTIR spectroscopy has recently become an alternative to bacterial typing due to its ease of use and high discriminatory power in recognizing clonal relationships among bacterial isolates [11]. The IR Biotyper® system (IRBT, Bruker Daltonics GmbH & Co. KG, Bremen, Germany), based on FTIR technology, is a device that enables easy and fast strain typing [12]. For example, following species-level identification using the MALDI Biotyper® (Bruker Daltonics GmbH & Co. Billerica, MA, USA), the strains can be typed and grouped by measuring their specific molecular vibration fingerprints [10]. IRBT technology can be applied to the bacterial surface in a non-destructive manner, facilitating high-resolution analysis to evaluate the similarities between different isolates at the strain level [12].

Antimicrobial susceptibility testing can confirm the current prevalence rate and help identify the characteristics of resistant *S.* Albany strains in duck farms. β-lactams, aminoglycosides, and fluoroquinolones are often used to treat systemic bacterial infections on Korean poultry farms [13,14]. However, continuous antibiotic therapy increases public health problems by fostering antibiotic resistance and increasing the prevalence of multidrug-resistant *Salmonella* [13]. Investigating the prevalence rate and resistance profile of *S. enterica* Albany with antimicrobial resistance is needed to control multidrug-resistant *Salmonella*.

This study was aimed to (1) confirm the distribution of *Salmonella* serovars, the prevalence of *S. enterica* Albany, and the resistance profiles of isolates recovered from ducks; (2) evaluate strain-level typing using IRBT and MLVA for *S. enterica* Albany isolates; (3) compare the discrimination and concordance of IRBT and MLVA; and (4) compare the clustering results of *S. enterica* Albany generated via IRBT and MLVA with the epidemiological relationship and antimicrobial resistance profiles between farms to facilitate the development of prevention and control strategies for *Salmonella* infection in duck farms.

Our study provides a platform for follow-up studies on food safety and public health issues caused by *S. enterica* Albany recovered from duck farms.

2. Materials and methods

2.1. Sample collection

Between April 2019 and October 2020, 20 individual feces from 5–6-week-old ducks per farm were collected from 105 duck farms. The freshly deposited feces were placed in a 50 mL conical tube. A mixed pool of 20 individual feces was applied as one sample, and feces from farms were collected repeatedly each time the ducks were repopulated. Considering the characteristics of duck farming, the

Table 1
Oligonucleotide sequences of primers used for PCR-based serotyping.

Primer	Sequence (5′–3′)		Amplicon size (bp)	Reference
bcfC-F	GGGTGGGCGGAAAACTATTTC	993		[17]
bcfC-R	CGGCACGGCGGAATAGAGCAC			
heli-F	ACAGCCCGCTGTTTAATGGTG	782		
heli-R	CGCGTAATCGAGTAGTTGCC			
steB-F	TGTCGACTGGGACCCGCCCGCCCGC	636		
steB-R	CCATCTTGTAGCGCACCAT			
rhs-F	TCGTTTACGGCATTACACAAGTA	402		
rhs-R	CAAACCCAGAGCCAATCTTATCT			
sdf-F	TGTGTTTTATCTGATGCAAGAG	293		
sdf-R	CGTTCTTCTGGTACTTCAGATGAC			
gly-F	TTCCAATTGAAACGAGTGCGG	170		
gly-R	ACTAACCGCTTGGGTTGTTGCTGT			
fliB-F	GTCTGCTAACAGCACTAACTC	551		
fliB-R	CCTGAGTTTTTGTTACTTCTACC			
aroC-F	CCTGGCACCTCGCGCTATAC	826		[16]
aroC-R	CCACACACGGATCGTGGCG			
dnaN-F	ATGAAATTTACCGTTGAACGTGA	833		
dnaN-R	AATTTCTCATTCGAGAGGATTGC			
hemD-F	ATGAGTATTCTGATCACCCG	666		
hemD-R	ATCAGCGACCTTAATATCTTGCCA			
hisD-F	GAAACGTTCCATTCCGCGCAGAC	894		
hisD-R	CTGAACGGTCATCCGTTTCTG			
thrA-F	GTCACGGTGATCGATCCGGT	852		
thrA-R	CACGATATTGATATTAGCCCG			
sucA-F	AGCACCGAAGAGAAACGCTG	643		
sucA-R	GGTTGTTGATAACGATACGTAC			
purE-F	ATGTCTTCCCGCAATAATCC	510		
purE-R	TCATAGCGTCCCCCGCGGAT			

ducks were first raised communally in one duck house and then moved to other duck houses at an average age of 10.2 days at each farm. Thus, fecal sampling was uniformly based on the first duck house. Feces were collected at each time point and transported directly to the laboratory, where they were processed separately.

2.2. Isolation and identification of Salmonella spp.

The 20 individual feces collected from each farm at each time point were pooled, and 5 g of the pooled sample was added to 45 mL of sterilized PBS. The suspension was mixed well, and 1 mL of it was added to 9 mL of buffered peptone water (BPW). This mixture was incubated at 37 °C for 24 h [15]. A 0.1 mL aliquot of the cultured BPW was added to 10 mL of Rappaport–Vassiliadis broth, incubated at 42 °C for 24 h, and then inoculated onto xylose–lysine–deoxycholate agar, which was incubated at 37 °C for 24 h. The suspect *Salmonella* colonies (red with a black center) were sub-cultured from a single colony onto 5% sheep blood agar plates. After incubation at 37 °C for 24 h, the cultures were examined in the MALDI Biotyper® (Bruker Daltonics GmbH & Co. Billerica, MA, USA). Isolates identified as *Salmonella* were serotyped using PCR as previously described [16,17] (Table 1). The one-step multiplex PCR used can identify major chicken *S. enterica* subsp. *enterica* serovars – Albany, Enteritidis, Gallinarum, Gallinarum biotype Gallinarum, Gallinarum biotype Pullorum, Heidelberg, Kentucky, and *S. enterica* groups 1 and 2 [17]. Isolates assigned to serovars *S. enterica* groups 1 and 2 via PCR were then examined using a multilocus sequence typing (MLST) method [16] to recognize serovars such as Dublin and Montevideo. The quality control of sequence-based MLST was performed for each run using the pGEM control DNA in the BigDye™ Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). MLST was performed using analysis tools available from the MLST website (https://enterobase.warwick.ac.uk) [18]. Identification of the identical serovars among suspected *Salmonella* colonies was considered as one *Salmonella* spp. Isolates identified as *S. enterica* Albany were sub-cultured on tryptic soy agar for MLVA, IRBT, and antimicrobial susceptibility tests.

2.3. Antimicrobial susceptibility testing

The minimum inhibitory concentration (MIC) was determined with the KRNV5F Sensititre panel (TREK Diagnostic Systems) following the manufacturer's instructions. The antibiotics used in the test 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). The breakpoints for antibiotics used in the MIC test were obtained from the Clinical Laboratory Standards Institute (2019) [19] and the National Antimicrobial Resistance Monitoring System [20] (Table 2). Multiple drug resistance (MDR) was defined as resistance to three or more antimicrobial classes.

2.4. Strain typing via MLVA

Loci for the MLVA were selected by uploading the WGS of ATCC 51960 strain (GenBank accession number CP019177.1) in FASTA format to a tandem repeat finder program (https://tandem.bu.edu/trf/basic_submit). The potential discriminatory ability of the selected loci was evaluated by comparing their sequences with the genomic sequences of *S. enterica* Albany strains deposited in the NCBI database. For the four loci determined as VNTR markers, primer pairs were designed for a product size of 100–600 bp using the

Table 2
Breakpoints of antimicrobials used for MIC testing of *Salmonella* spp.

Antimicrobial agents	Range tested (µg/mL)	Breakpoints[a] (µg/mL)
Amoxicillin/clavulanic acid	2/1–32/16	≥32/16
Ampicillin	2–64	≥32
Cefoxitin	1–32	≥32
Ceftazidime	1–16	≥16
Ceftiofur	0.5–8	≥8
Cefepime	0.25–16	≥16
Meropenem	0.25–4	≥4
Trimethoprim/sulfamethoxazole	0.12/2.38–4/76	≥4/76
Sulfisoxazole	16–256	≥512
Chloramphenicol	2–64	≥32
Ciprofloxacin	0.12–16	≥1
Nalidixic acid	2–128	≥32
Streptomycin	16–128	≥32
Gentamicin	1–16	≥16
Tetracycline	2–128	≥16
Colistin	2–16	≥4

[a] All breakpoints were from Clinical and Laboratory Standards Institute (2019) [19], except for ceftiofur and streptomycin (Centers for Disease Control and Prevention et al., 2014) [20].

Primer Express 3.0 software (Applied Biosystems). The forward primers were labeled with a 5′ fluorescent reporter dye (FAM, NED, VIC, and PET) for analysis in a genetic analysis instrument (Table 3).

PCR was performed using a reaction mixture containing Maxime™ PCR PreMix (iNtRON Biotech), 5 μL of primer mix, and 1–5 ng of DNA template. The final volume (20 μL) was adjusted using RNase-free water. PCR amplification conditions were as follows: 95 °C for 10 min; 40 cycles of 95 °C for 30 s, 56 °C for 30 s, and 72 °C for 30 s; and 72 °C for 10 min. Next, 10 μL of diluted size standard (20 μL size standard mixed in 1 mL HI-DI formamide) was added to the final PCR products (3–5 ng/μL). Following denaturation at 95 °C for 5 min and rapid cooling to 4 °C for 1 min, the samples were injected into a capillary electrophoresis system (Applied Biosystems 3500 XL Genetic Analyzer, Life Technology) to measure the fragment sizes. We included amplified control DNA (CEPH 1347-02 from Applied Biosystems) during every series of capillary runs. The data were analyzed using GeneMapper® (Life Technologies) to determine the size.

2.5. Strain typing via IRBT analysis

The *S. enterica* Albany isolates were incubated on TSA at 37 °C for 24 h. A 1 μL loopful of culture was inoculated into a 1.5 mL microtube containing 50 μL of 70% ethanol and mixed uniformly. After vortexing the mixture for 1 min, 50 μL of sterile water was added, and the resulting 100 μL solution was vortexed for 1 min. After loading three spots of 15 μL each of the well-mixed sample solutions on the target plate, the plate was dried in a 37 °C incubator for 10–30 min. The dried target plate was inserted into the IRBT spectrometer (Bruker Daltonics GmbH & Co. KG, Bremen, Germany), and data were acquired using IRBT software (version 4.0) with the default settings. The quality control was performed for each run using the Infrared Test Standards (IRTS 1 and IRTS 2) from the IR Biotyper. Spectra of the isolates were acquired using OPUS 7.5 software (Bruker Daltonics GmbH & Co. KG, Bremen, Germany). The spectra that met the default quality criteria [0.4 < absorption <2, signal/noise >40, signal/water >20, fringes (× 10^{-6}) < 100] were accepted. All data were analyzed using IRBT software by constructing the dendrograms and 2D scatter plots on average spectra. Dendrograms were constructed using Euclidian distances and the average linkage clustering method. 2D scatter plots for similarity analysis were determined by principal component analysis (PCA). Cutoff values were automatically proposed by the software.

3. Results

3.1. Prevalence and distribution of Salmonella serovars on duck farms

A total of 745 samples from 105 duck farms were examined: a mixed pool of 20 individual feces was applied as one sample; the number of sampling events varied in the range of 1–16 because samples were repeatedly collected whenever new duck flocks were placed in the same farm. The positivity rate for *Salmonella* was 19%, and 141 *Salmonella* spp. were isolated from 63 farms. *Salmonella* spp. was not detected in 42 of the 105 duck farms and was detected more than once in 63 farms (Fig. 1A).

The isolates were assigned to 11 serovars (Fig. 1B). *S. enterica* Albany (66.7%; 94 isolates) was the most common serovar, followed by *S.* Enteritidis (12.1%; 17 isolates), *S.* London (4.3%; six isolates), *S.* Hadar (3.5%; five isolates), *S.* Typhimurium (3.5%; five isolates), *S.* Give (2.8%; four isolates), *S.* Indiana (2.8%; four isolates), *S.* Mbandaka (1.4%; two isolates), *S.* Zanzibar (1.4%; two isolates), *S.* Montevideo (0.7%; one isolate), and *S.* Virchow (0.7%; one isolate).

3.2. Antimicrobial susceptibility testing

A total of 94 *S. enterica* Albany isolates were tested for antimicrobial susceptibility. All 94 (100%) were resistant to FIS, 93 (98.9%) were resistant to NAL, 92 (97.9%) were resistant to SXT, 34 (36.2%) were resistant to AMP, 34 (36.2%) were resistant to CIP (36.2%), 30 (32.0%) were resistant to CHL, 28 were resistant to TET (29.8%), and 5 were resistant to STR (5.3%). No isolates were resistant to AUG2, FOX, TAZ, XNL, FEP, MERO, GEN, and COL (Table 4).

Eleven antimicrobial resistance profiles were observed in the isolates (Table 5). All 94 isolates were resistant to two or more

Table 3
Primers for VNTR markers of *Salmonella enterica* Albany.

VNTR markers	Primer sequences (5′–3′)	Repeat sequence	Product size (bp)[b]	Fragment size (bp)[c]	DI[d]	Location[b]
STTR7	**FAM**-CGCGCAGCCGTTCTCACT TGTTCCAGCGCAAAGGTATCTA	39 bp[a]	546	507–585 (4–6)	0.1538	2,979,895–2980,440
SATR1	**NED**-GGATGTTCTGGCGGACATGG CGCCTTCCGGATGTATGTGA	CACGAC	114	114–126 (5–7)	0.5	871,156–871,269
SATR2	**VIC**-AAAATCCCCGTAAATCCCGCT AGGTGCAAAAGTGGCCTCA	TGCCTG	158	158–170 (7–9)	0.2821	1,155,894–1156,051
SATR3	**PET**-CCTCCTGCTGGAAAAATCGC TGGCGATGCAATGCGTCTTA	ATCAATCCGT	129	119–139 (3–5)	0.2821	4,021,834–4021,962

[a] Model of repeats: CAGCAGCCGCAACAGCCGGTAGCGCCGCAACCGCAGTAT (39 bp).
[b] Product size and location in ATCC 51960 (GenBank accession number CP019177.1).
[c] Observed fragment size (repeat copy no.).
[d] Simpson's diversity index, each index was calculated using the results from the 13 unrelated *Salmonella* Albany isolates.
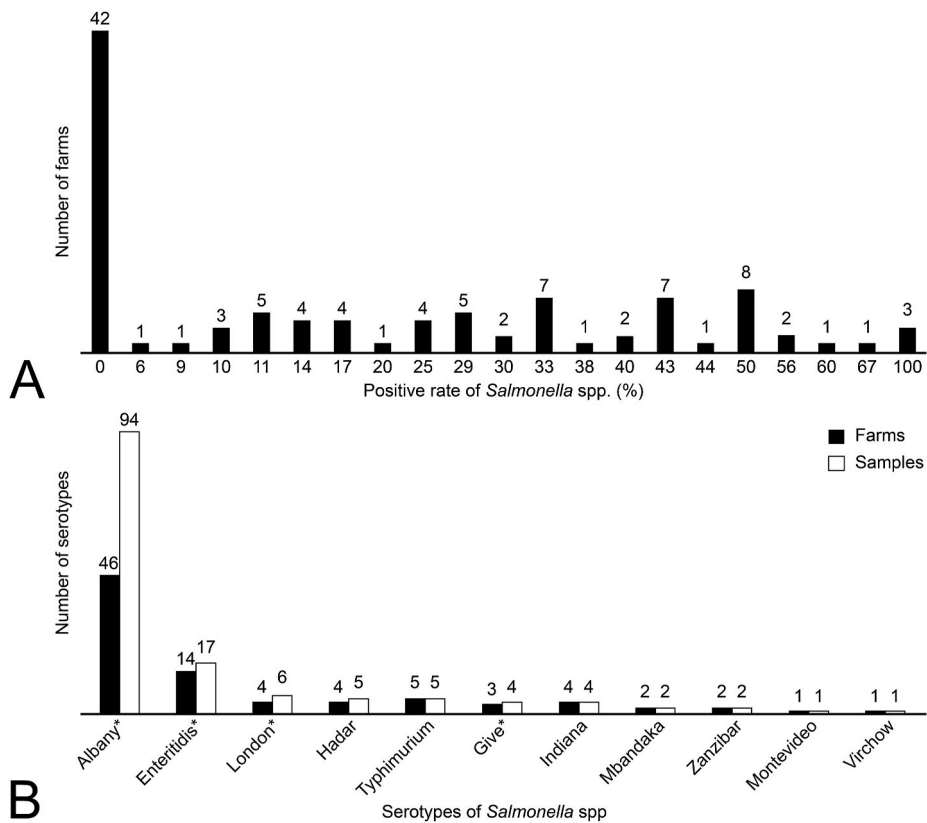
Fig. 1. Prevalence and distribution of *Salmonella* serovars on duck farms. Positive rates of *Salmonella* spp. ranged from 0 to 100% in duck farms (A). A total of 11 serovars were detected, of which *S. enterica* Albany was the most common. The list includes farms where the same serotype was repeatedly recovered from existing and new duck flocks (asterisks) (B).

antimicrobial classes, and 37 were classified as MDR. FIS-SXT-NAL (42/94, 44.7%) was the most prevalent antimicrobial resistance profile, followed by FIS-SXT-NAL-CIP (15/94, 16.0%).

The antimicrobial resistance profiles of *S. enterica* Albany isolates repeatedly recovered from the same farm were similar (Table 6). Twenty-four farms where two or more *S. enterica* Albany isolates were detected had identical antibacterial resistance profiles (Fig. 2). These results confirm that *S. enterica* Albany isolates with identical antimicrobial resistance profiles were repeatedly recovered even when ducks raised on the farm were slaughtered, and a new flock of ducks was introduced to the same farm.

3.3. Strain typing of S. enterica Albany via MLVA

MLVA typing assigned the 94 *S. enterica* Albany isolates to five groups. Most isolates (77 recovered from 39 duck farms) were included in the M1 group, followed by 12 isolates recovered from five duck farms in the M3 group. Three isolates recovered from two duck farms formed the M4 group, while the M2 and M5 groups had one isolate each (Table 7). Of the 26 farms where two or more isolates were recovered, 24 farms yielded one MLVA group in the same farm (Table 6). These results confirmed that isolates of an identical MLVA group were repeatedly recovered from different duck flocks at different times on the same farm.

3.4. Strain typing of S. enterica Albany via IRBT analysis

The 94 *S. enterica* Albany isolates were classified into 16 clusters via IRBT analysis (Fig. 3). Of the 94 isolates, 52 belonged to Cluster 4, forming the largest group; 10 isolates belonged to Cluster 7; nine isolates belonged to Cluster 9; four isolates belonged to Cluster 10; and two isolates belonged to Cluster 3. Clusters 1, 5, and 8 each included three isolates, and eight clusters (Clusters 2, 6, and 11–16) included only one isolate each. *S. enterica* Albany recovered from existing and new flocks of ducks on the same farm exhibited identical IRBT clusters. Of the 26 farms where two or more isolates were recovered, 19 farms yielded only one IRBT cluster, while 5 farms (F1, F3, F4, F10, F45) yielded isolates in more than two IRBT clusters, although some isolates did belong to the same IRBT cluster.

The result of IRBT typing is displayed in 2D scatter plot format. Isolates recovered from the same farm appear as point sets located close together and delimited by ellipses (Fig. 4). These results showed that *S. enterica* Albany isolates included in an identical cluster were repeatedly recovered from a farm even when a new flock was introduced after the existing flock was slaughtered.

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Table 4
Minimum Inhibitory Concentration (MIC) (μg/mL) patterns for *Salmonella enterica* Albany isolated from duck feces.

Antimicrobials	No. of *Salmonella enterica* Albany isolates for each MIC (μg/mL) values												
	≤0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	≥512
Amoxicillin/clavulanic acid	53				9		13		17		2		
Ampicillin	46				10	2	2						34
Cefoxitin		3	12	74	5								
Ceftazidime	94												
Ceftiofur	3		43	47	1								
Cefepime	92	2											
Meropenem	91		2		1								
Trimethoprim/sulfamethoxazole	2										92		
Sulfisoxazole													94
Chloramphenicol				1	15	34	14		30				
Ciprofloxacin	1	9	50	5	12	6	11						
Nalidixic acid					1						93		
Streptomycin	76							13	3	1	1		
Gentamicin	92			1	1								
Tetracycline	58				6	1	1	4	11	2	11		
Colistin	94												

Bold vertical lines indicate breakpoints based on interpretive criteria [19,20], and shaded areas indicate resistance.

3.5. IRBT strain typing compared with MLVA

Table 6 shows a comparison of IRBT spectroscopic typing with MLVA results. There were 16 cluster groups in IRBT and five in MLVA. All isolates in Clusters 1, 3–6, 8, 11–13, 15, and 16 of IRBT corresponded to the M1 group, indicating that IRBT had a higher discriminatory power than MLVA for these isolates. The clustering results of IRBT and MLVA were consistent for Clusters 2 and 10 and M2 and M3. However, IRBT Clusters 7 and 9 corresponded to M1, M3, M4, and M5, indicating that MLVA had a higher discriminatory power than IRBT for these isolates.

Of the 94 isolates from various farms, locations, and companies, 52 (55%) were included in Cluster 4 and M1 group, forming one large group (Fig. 3). Isolates recovered repeatedly from the same farm showed identical MLVA and IRBT typing results; for example, all isolates recovered from farm F2 were included in the M1 group and Cluster 5. Using both typing methods, isolates recovered from the same farm could be distinguished in more detail. Isolates recovered from farm F4 were divided into two isolates included in the M1 group/Cluster 4 and three other isolates included in the M1 group/Cluster 8 (Table 6). A 2D scatter plot of the IRBT typing

Table 5
Antimicrobial resistance profiles of *Salmonella enterica* Albany isolated from duck feces.

Antimicrobial agent (antimicrobial subclass)								No. of resistant CLSI subclasses		No. of resistant isolates
Profiles	FIS (SAs)	SXT (SAs)	NAL (Qs)	CIP (Qs)	AMP (AP)	STR (AMGs)	CHL (PHs)	TET (TETs)		Total 94
A1	R	R	R	S	S	S	S	S	2	42
A2	R	R	R	R	S	S	S	S	2	15
A3	R	R	R	S	R	S	S	S	3	5
A4	R	R	R	S	S	R	S	S	3	2
A5	R	S	S	S	S	R	R	R	4	1
A6	R	R	R	R	R	S	R	S	4	2
A7	R	R	R	S	R	S	R	R	5	9
A8	R	S	R	R	R	S	R	R	5	1
A9	R	R	R	R	R	S	R	R	5	15
A10	R	R	R	S	R	R	R	R	6	1
A11	R	R	R	R	R	R	R	R	6	1

FIS, sulfisoxazole; SAs, sulfonamides; SXT, trimethoprim/sulfamethoxazole; NAL, nalidixic acid; Qs, quinolones; CIP, ciprofloxacin; AMP, ampicillin; AP, aminopenicillin; STR, streptomycin; AMGs, aminoglycosides; CHL, chloramphenicol; PHs, phenicols; TET(s), tetracycline(s); S, susceptible; R, resistance.

demonstrated that three large MLVA groups (M1, M3, and M4) could be defined (Fig. 5).

3.6. Strain typing and antimicrobial susceptibility

IRBT and MLVA typing results of the 94 isolates were statistically correlated with antimicrobial resistance profiles (Fisher's Exact Test, $p < 0.05$) but did not consistently correspond with each other (Fig. 6A and B). However, isolates repeatedly recovered from the same farm showed the same MLVA results, IRBT findings, and antimicrobial resistance profiles (Table 6). Among the 26 farms that yielded multiple *S. enterica* Albany isolates, 20 yielded isolates with identical MLVA results, IRBT findings, and antimicrobial resistance profiles (Fig. 6C).

4. Discussion

In this study, we examined 141 isolates of *Salmonella* spp. obtained from fecal samples of 5–6-week-old ducks from 105 duck farms between April 2019 and October 2020. *S. enterica* Albany isolates, the most recovered serovar, were tested for antimicrobial susceptibility to elucidate drug resistance profiles. The *S. enterica* Albany isolates were typed using IRBT and MLVA.

We isolated *Salmonella* spp. from 141 (19%) of 745 duck fecal samples, similar to the prevalence reported by a previous study [21]. In addition to the serovars reported in the previous study, we detected *S. enterica* Albany, *S.* Montevideo, *S.* Virchow, and *S.* Zanzibar, with *S. enterica* Albany as the most common serovar. The prevalence of *S. enterica* Albany has recently increased dramatically in poultry farms in Korea [4], but it had not been identified in chickens and ducks in Korea before 2016 [21]. *S. enterica* Albany has been commonly isolated from poultry and other livestock in Southeast Asia and Western countries, and it has recently been reported as an important serovar affecting humans [22,23]. Therefore, we investigated the characteristics of *S. enterica* Albany using phenotypic, genotypic, and spectroscopic techniques to develop prevention and control strategies for *Salmonella* infection.

All *S. enterica* Albany isolates recovered from ducks in this study were resistant to at least three of the antimicrobial agents tested. We detected resistance to FIS, NAL, SXT, AMP, CIP, CHL, TET, and STR in various patterns. In previous studies, *S. enterica* Albany isolated from poultry in Korea [4], Malaysia [24], and Cambodia [25] and from pigs in Taiwan [22] showed antimicrobial resistance, similar to the findings of our study. Our *S. enterica* Albany isolates had high resistance (close to 100%) to FIS, NAL, and SXT antibiotics and a low resistance of 5.3% STR. Approximately 30% of isolates were resistant to AMP, CHL, and TET, which was lower than approximately 90% of the isolates in previous studies [4,22,24,25]. These trends suggest that the amount of antibiotics used in duck production is lower than that used in chicken production in Korea [21]. Furthermore, 36.2% of isolates were resistant to CIP, which is higher than the approximately 6% of isolates found in previous studies in chickens [4,25]. *S. enterica* Albany isolated from ducks showed higher resistance to CIP than those in previous studies, possibly due to the overuse and abuse of fluoroquinolones for disease treatment in ducks [26]. Increased rates of decreased susceptibility to quinolones in *salmonella have been reported with the* prolonged use of excessive doses in poultry [4]. CIP is commonly used to treat non-typhoidal *Salmonella* infections (Bangera et al., 2019; [4,27], and poultry may have played a role in antibiotic-resistant *Salmonella* spp. infections in humans [28].

We found that 39.4% (37/94) of *S. enterica* Albany isolates were MDR strains. In previous studies, a majority of *S. enterica* Albany from both ducks [1] and broiler chickens [4] in Korea were MDR. The indiscriminate use of antibiotics has increased the number of MDR *Salmonella* strains and the antibiotic resistance rate [29]. In addition, the existence of MDR *Salmonella* strains suggests that they may pose a public health problem [30].

ACSuTN (ampicillin, chloramphenicol, sulfisoxazole, tetracycline, and nalicixic acid)-resistant *S. enterica* Albany accounted for 67.6% (25/37) of the MDR isolates. Resistance to these antimicrobials may be due to their long history of use in poultry farms to treat infections [4]. ACSSuT (ampicillin, chloramphenicol, streptomycin, sulfisoxazole, and tetracycline)-resistant *S.* Typhimurium was first

Table 6

Description of *Salmonella enterica* Albany isolates used in the present study.

Farms	Locations	Companies	Isolates	Collection dates	MLVA groups	IRBT cluster groups	Antimicrobial resistance profiles*
F1	Jincheon	C1	CB2	2019.04.18	M1	Cluster 6	A7
			CB39	2019.08.26	M1	Cluster 4	A1
			CB73	2019.11.01	M1	Cluster 4	A1
F2	Eumseong	C2	CB4	2019.04.23	M1	Cluster 5	A9
			CB42	2019.08.26	M1	Cluster 5	A9
			CB70	2019.10.21	M1	Cluster 5	A9
F3	Jincheon	C2	CB6	2019.04.24	M3	Cluster 9	A7
			CB40	2019.08.26	M3	Cluster 9	A11
			CB84	2019.12.20	M3	Cluster 9	A7
			CB92	2020.02.27	M3	Cluster 10	A7
			CB146	2020.08.14	M3	Cluster 10	A7
			CB153	2020.09.08	M3	Cluster 14	A10
F4	Jincheon	C1	CB7	2019.04.29	M1	Cluster 4	A1
			CB41	2019.08.26	M1	Cluster 4	A2
			CB98	2020.03.25	M1	Cluster 8	A2
			CB114	2020.06.08	M1	Cluster 8	A2
			CB141	2020.08.06	M1	Cluster 8	A2
F5	Cheongju	C1	CB8	2019.05.20	M1	Cluster 4	A1
			CB 37	2019.08.19	M1	Cluster 4	A1
			CB48	2019.09.03	M1	Cluster 4	A2
			CB136	2020.07.30	M1	Cluster 4	A1
F6	Jincheon	C1	CB9	2019.05.21	M1	Cluster 3	A2
			CB140	2020.08.03	M1	Cluster 3	A2
F7	Eumseong	C2	CB10	2019.05.22	M1	Cluster 4	A4
			CB76	2019.11.14	M1	Cluster 4	A4
F8	Jincheon	C1	CB21	2019.07.25	M1	Cluster 4	A1
			CB54	2019.09.20	M1	Cluster 4	A1
F9	Jincheon	C1	CB19	2019.07.18	M1	Cluster 4	A1
			CB93	2020.02.27	M1	Cluster 4	A1
F10	Eumseong	C2	CB20	2019.07.24	M1	Cluster 4	A1
			CB52	2019.09.16	M1	Cluster 4	A3
			CB81	2019.12.02	M1	Cluster 4	A3
			CB112	2020.05.18	M1	Cluster 7	A3
			CB132	2020.07.17	M1	Cluster 7	A3
F11	Jincheon	C1	CB7	2019.04.29	M1	Cluster 4	A1
			CB22	2019.07.25	M1	Cluster 4	A1
F12	Eumseong	C1	CB25	2019.07.26	M1	Cluster 4	A1
F13	Jincheon	C1	CB28	2019.08.02	M1	Cluster 4	A1
F14	Eumseong	C2	CB11	2019.05.23	M1	Cluster 4	A8
			CB29	2019.08.02	M1	Cluster 4	A9
			CB127	2020.07.06	M1	Cluster 4	A9
F15	Cheongju	C1	CB30	2019.08.05	M3	Cluster 10	A9
F16	Cheongju	C1	CB31	2019.08.05	M1	Cluster 4	A3
			CB36	2019.09.08	M1	Cluster 4	A1
F17	Cheongju	C1	CB63	2019.09.30.	M1	Cluster 4	A2
			CB69	2019.10.18	M1	Cluster 4	A2
			CB148	2020.08.19	M1	Cluster 4	A1
F18	Eumseong	C2	CB33	2019.08.08	M3	Cluster 9	A2
			CB94	2020.03.12	M3	Cluster 9	A2
F19	Eumseong	C3	CB38	2019.08.20	M1	Cluster 4	A1
F20	Chungju	C1	CB43	2019.08.26	M1	Cluster 4	A1
F21	Jincheon	C4	CB49	2019.09.05	M1	Cluster 7	A9
F22	Jincheon	C1	CB51	2019.09.16	M1	Cluster 4	A1
F23	Cheongju	C1	CB53	2019.09.16	M1	Cluster 4	A1
F24	Eumseong	C1	CB64	2019.10.04	M1	Cluster 1	A1
			CB106	2020.04.16	M1	Cluster 1	A1
			CB149	2020.08.24	M1	Cluster 1	A1
F25	Jincheon	C3	CB65	2019.10.08	M2	Cluster 2	A7
			CB91	2020.02.20	M1	Cluster 16	A1
F26	Jincheon	C1	CB66	2019.10.08	M1	Cluster 4	A1
F27	Eumseong	C6	CB68	2019.10.18	M1	Cluster 7	A1
F28	Cheongju	C1	CB71	2019.10.25	M1	Cluster 4	A1
			CB85	2019.12.27	M1	Cluster 4	A1
F29	Eumseong	C2	CB72	2019.10.31	M5	Cluster 9	A9
F30	Goesan	C1	CB26	2019.07.30	M1	Cluster 4	A1
F31	Goesan	C1	CB79	2019.11.25	M1	Cluster 13	A1
F32	Eumseong	C1	CB82	2019.12.04	M1	Cluster 4	A2
			CB90	2020.01.30	M1	Cluster 4	A2

(continued on next page)

Table 6 (*continued*)

Farms	Locations	Companies	Isolates	Collection dates	MLVA groups	IRBT cluster groups	Antimicrobial resistance profiles*
			CB99	2020.03.27	M1	Cluster 4	A2
			CB137	2020.07.31	M1	Cluster 4	A2
F33	Jincheon	C1	CB101	2020.04.02	M1	Cluster 4	A6
			CB117	2020.06.17	M1	Cluster 4	A6
F34	Eumseong	C1	CB102	2020.04.06	M1	Cluster 4	A1
			CB116	2020.06.16	M1	Cluster 4	A1
F35	Cheongju	C3	CB104	2020.04.13	M1	Cluster 4	A9
F36	Cheongju	C2	CB105	2020.04.13	M1	Cluster 7	A9
			CB122	2020.06.25	M1	Cluster 7	A9
			CB147	2020.08.18	M1	Cluster 7	A9
F37	Jincheon	C1	CB107	2020.04.17	M1	Cluster 4	A1
			CB120	2020.06.25	M1	Cluster 4	A1
F38	Jincheon	C2	CB108	2020.04.20	M1	Cluster 4	A1
F39	Eumseong	C2	CB110	2020.04.23	M1	Cluster 4	A1
			CB124	2020.07.02	M1	Cluster 12	A1
			CB151	2020.08.27	M1	Cluster 11	A4
F40	Eumseong	C2	CB111	2020.04.23	M4	Cluster 9	A7
			CB123	2020.07.02	M4	Cluster 9	A7
F41	Jincheon	C2	CB126	2020.07.06	M3	Cluster 10	A7
F42	Jincheon	C1	CB133	2020.07.20	M1	Cluster 4	A1
F43	Cheongju	C1	CB135	2020.07.30	M1	Cluster 7	A1
F44	Jincheon	C5	CB138	2020.07.31	M4	Cluster 9	A9
F45	Eumseong	C2	CB95	2020.03.17	M1	Cluster 15	A5
			CB139	2020.07.31	M3	Cluster 7	A9
			CB143	2020.08.10	M3	Cluster 7	A9
F46	Eumseong	C2	CB142	2020.08.10	M1	Cluster 4	A1

A1, FIS-SXT-NAL; A2, FIS-SXT-NAL-CIP; A3, FIS-SXT-NAL-AMP; A4, FIS-SXT-NAL-STR; A5, FIS-STR-CHL-TET; A6, FIS-SXT-NAL-CIP-AMP-CHL; A7, FIS-SXT-NAL-AMP-CHL-TET; A8, FIS-NAL-CIP-AMP-CHL-TET; A9, FIS-SXT-NAL-CIP-AMP-CHL-TET; A10, FIS-SXT-NAL-AMP-STR-CHL-TET; A11, FIS-SXT-NAL-CIP-AMP-STR-CHL-TET.
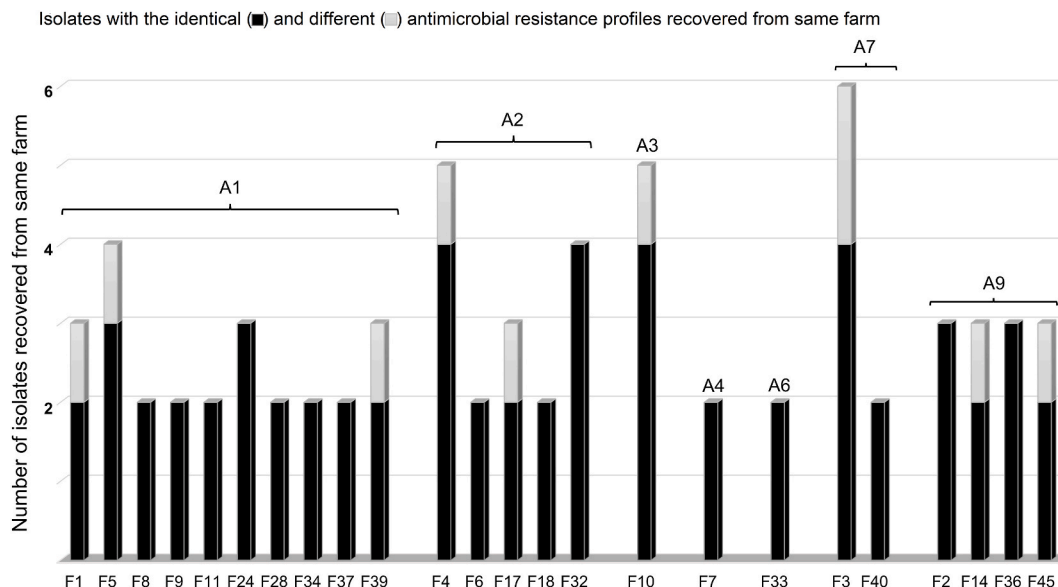

Fig. 2. Isolates of *Salmonella enterica* Albany repeatedly recovered from the same farm indicated identical antimicrobial resistance profiles (A1, FIS-SXT-NAL; A2, FIS-SXT-NAL-CIP; A3, FIS-SXT-NAL-AMP; A4, FIS-SXT-NAL-STR; A6, FIS-SXT-NAL-CIP-AMP-CHL; A7, FIS-SXT-NAL-AMP-CHL-TET; A9, FIS-SXT-NAL-CIP-AMP-CHL-TET). A5, A8, A10, and A11 antimicrobial resistance profiles had one isolate each and were not represented. Two or more isolates from 24 farms had identical antimicrobial resistance profiles.

reported in the United Kingdom in 1984 [31], with many ACSSuT-resistant *Salmonella* isolates reported subsequently [32–34]. ACSSuT *Salmonella* isolates with co-resistance to clinically important antibiotics (fluoroquinolone, cephalosporins, and colistin) complicate treatment and pose a real threat to global public health [4,30,33]. Two of our *S. enterica* Albany isolates showed an ACSSuT resistance profile, with one also showing co-resistance to ciprofloxacin.

Strain typing *S. enterica* Albany using MLVA and IRBT classified the isolates into five groups and 16 clusters, respectively. Based on

Table 7
MLVA groups of *Salmonella enterica* Albany isolated from duck feces.

VNTR markers					No. of isolates	No. of farms
Groups	STTR7	SATR1	SATR2	SATR3	Total (94)	Total (48[a])
M1	5	6	7	4	77	39
M2	5	6	8	4	1	1
M3	5	6	6	4	12	5
M4	6	6	6	4	3	2
M5	5	7	6	5	1	1

[a] Isolates of farms F25 and F45 were included in two MLVA groups and counted in duplicate.

the 2D scatter plot assessment of PCA (Fig. 5), IRBT could differentiate MLVA genotypic variation related to strain type due to the three separate data sets (M1, M3, and M4). High discriminating power for the M1, M3, and M4 groups of *S. enterica* Albany isolates was identified, suggesting that IRBT could be used to classify MLVA genotypes. This difference in discrimination power seems to be due to the typing principles – genotyping for MLVA and molecular structure analysis for IRBT. MLVA takes advantage of VNTRs to distinguish strains and can be easily performed at low cost using conventional PCR using identical primers [7]. Therefore, it is a popular method for subtyping for public health surveillance and outbreak investigations of *Salmonella* using DNA-based techniques [35]. On the other hand, IRBT analyzes molecular structures such as carbohydrate composition [36]. IRBT has recently become an alternate method for bacterial typing due to its ease of use and high discriminatory power in recognizing clonal relationships among bacterial isolates [11]. This is an automated system that is simple, quick, and reliable [37]. IRBT was used for transmission route analyses and outbreak investigations of strains [36]. A combination of these two typing methods with different principles could investigate the epidemiological relationship of isolates in more detail.

Strain typing using IRBT and MLVA showed that the *S. enterica* Albany isolates had high genetic and molecular structure homology despite being recovered from various farms with different locations and companies. *S. enterica* Albany isolates had 82% (77/94) similarity, as determined by MLVA, and 55% (52/94) similarity, as determined by IRBT. This suggests clonal dissemination of *S. enterica* Albany across duck farms. These results are consistent with the high genetic homology of *S. enterica* Albany isolated from domestic poultry farms analyzed via PFGE [4].

Moreover, we found that isolates repeatedly recovered from the same farm showed identical IRBT clusters or MLVA groups. Repeated acquisition of an identical strain may represent a risk of persistent infection due to the continued presence of the strain in the farm environment. This suggests that cleaning and disinfection before repopulation were performed improperly or inefficiently, resulting in *Salmonella* remaining in the environment and causing ongoing infection. Duck farms require proper sanitation after all ducks are removed to reduce *Salmonella* contamination of the environment.

Isolates repeatedly recovered from the same farm exhibited identical MLVA and IRBT clusters (Fig. 3). Moreover, isolates from the same farm could be distinguished in more detail using both typing methods (IRBT and MLVA). The two typing methods using different principles showed different results, and selecting one as a universal and ideally applicable typing method was impossible. Isolates could be distinguished more extensively to investigate the epidemiological relationship using both methods.

The typing results of IRBT and MLVA did not consistently correspond with the antimicrobial resistance profiles. However, isolates repeatedly recovered from duck farms did show identical MLVA results, IRBT findings, and antimicrobial resistance profiles (Fig. 6C). We noticed antimicrobial resistance diversity among isolates with identical typing results recovered from different duck farms. Since antibiotic consumption and use and resistance are related, changes in consumption composition may alter resistance patterns. In our study, different duck farms may have used different antibiotic treatment programs, causing different antimicrobial resistance profiles in *S. enterica* Albany isolates with the same strain typing.

The typing results of genotypic (MLVA) and spectroscopic (IRBT) techniques and the phenotypic results of antimicrobial resistance profiles were consistent for the same farm, which suggests that even if the existing duck flock was slaughtered and a new flock was stocked, re-infection with *S. enterica* Albany remaining on the farm could still occur.

5. Conclusions

The prevalence of *S. enterica* was 19%, and *S. enterica* Albany was predominantly distributed in duck farms in Korea. Of these 39.4% of *S. enterica* Albany isolates were classified as MDR. *S. enterica* Albany isolates repeatedly recovered from the same duck farm had identical antimicrobial resistance profiles, IRBT clusters, and MLVA groups. Therefore, re-infection with *S. enterica* Albany remaining in the environment is plausible, even if a new duck flock is introduced after slaughtering all ducks on the same farm. Farms require proper cleaning and disinfection before new duck flocks are repopulated. In this study, the epidemiological investigation of *S. enterica* Albany contamination was performed only in duck farms in Korea. Additional epidemiological analysis of duck carcasses in slaughterhouses is needed to investigate the potential of their transmission to humans via the food chain.

Funding

Fig. 3. Dendrogram clustered using IRBT for 94 *Salmonella enterica* Albany isolates. The vertical line represents the cutoff value (0.126), and all isolates were classified into 16 clusters via IRBT. For ease of reading, clusters composed of more than two isolates are shaded in orange; two isolates are shaded in yellow; single isolates are shaded in green.
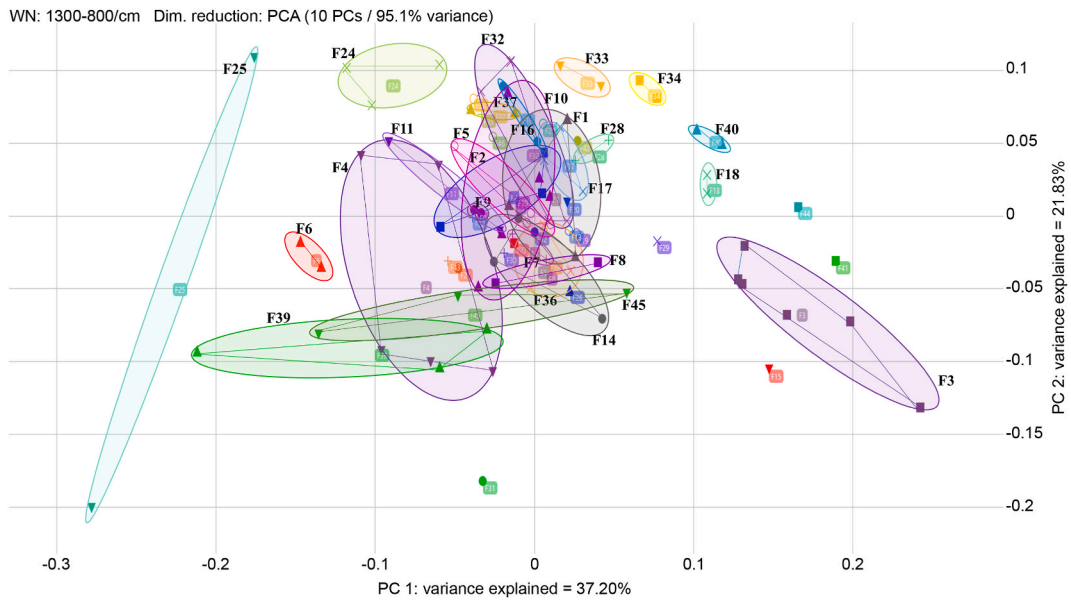
Fig. 4. Assessment of 2D scatter plot for 94 isolates of PCA using IRBT. The target was the farms. Isolates delimited using ellipses show the same farm, and points within the ellipse were located close together. Some farms overlap, but non-overlapping farms were distinguished in the spectra.
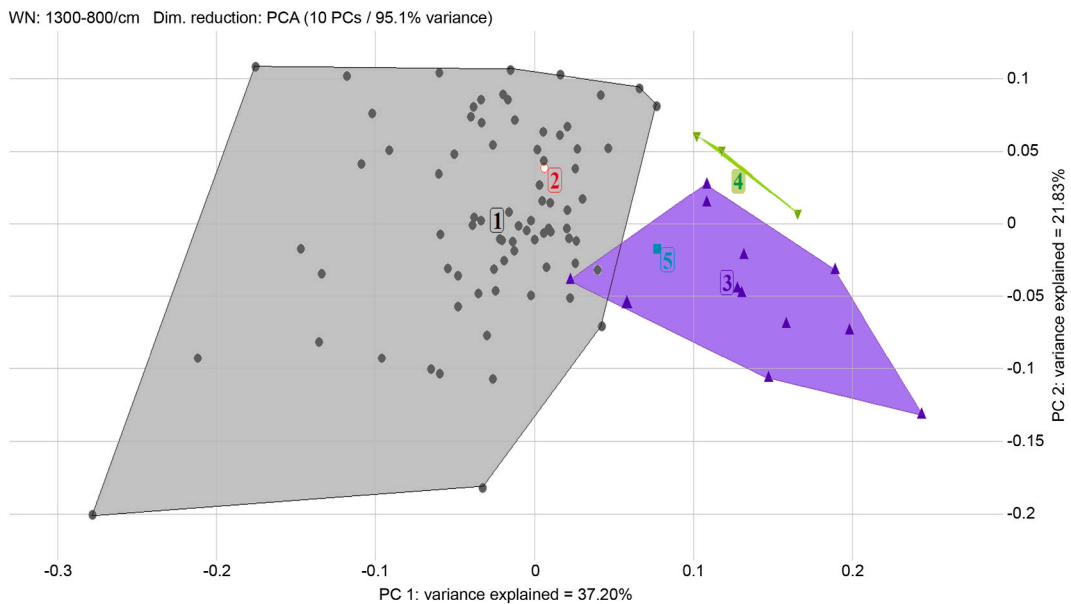
Fig. 5. Assessment of 2D scatter plot for 94 isolates of PCA using IRBT. The target was the MLVA groups. Isolates delimited using lines show the identical MLVA groups (M1, M3, and M4). These groups did not overlap, and a distinction was made in the spectra.

Ethics approval

The present study did not require ethical approval as we collected feces only from normal functioning farms and did not alter their routine.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.
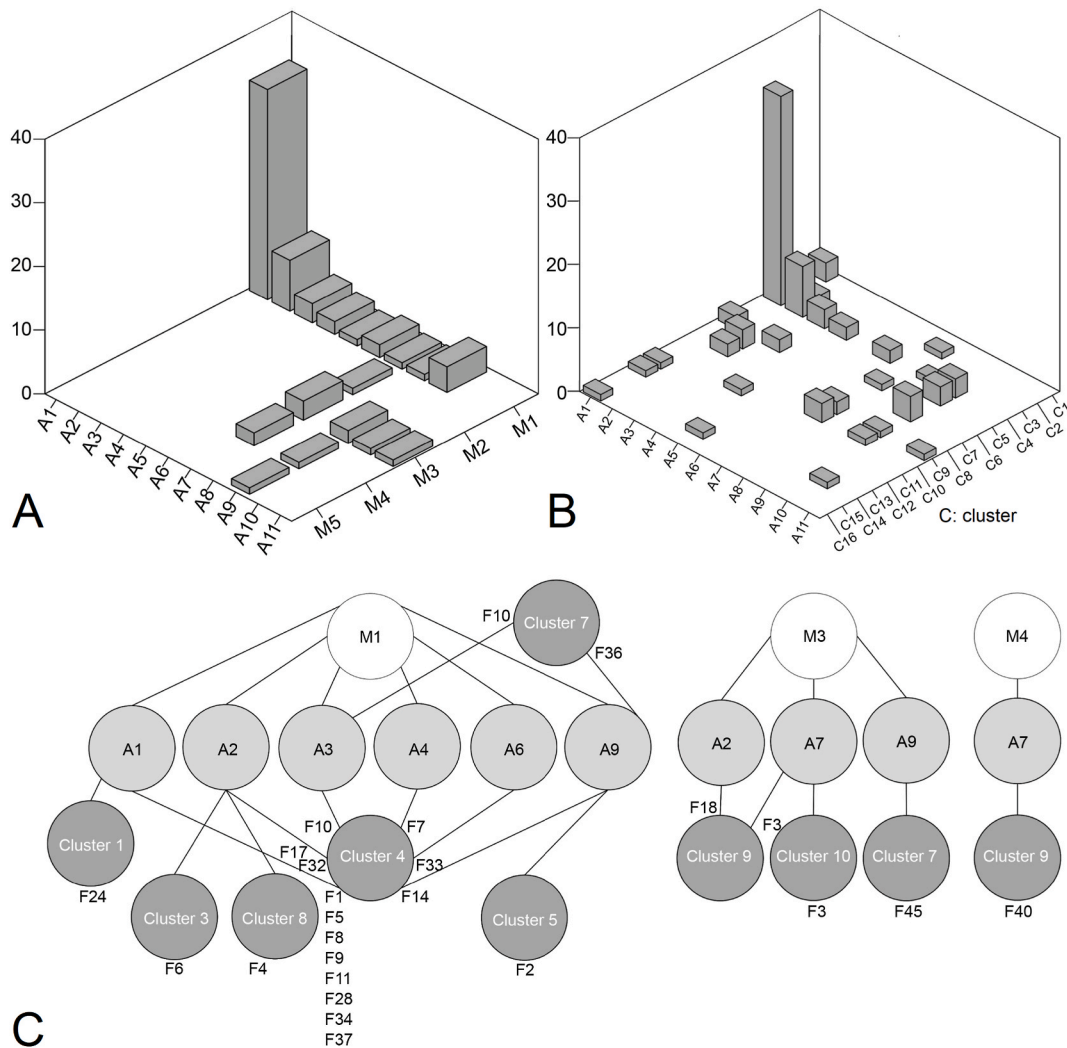
Fig. 6. Comparison of strain typing results and antimicrobial resistance profiles. The typing results of MLVA (A) and IRBT (B) for the 94 isolates did not consistently correspond with the antimicrobial resistance profiles. The isolates recovered from the same duck farms showed identical MLVA results, IRBT findings, and antimicrobial resistance profiles. M2 and M5 groups had one isolate each and were not represented (C).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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