Investigation of Integron, Antimicrobial Resistance and Virulence Gene Profiles of *Salmonella enterica* Subspecies *enterica* Serovar Infantis Isolates Obtained from Broiler Chickens

Bekoz Yarar, M.,[1,a] Turkyilmaz, M.K.[2,b] and Turkyilmaz, S.[3,c,*]

[1] Health Sciences Institute, Aydin Adnan Menderes University, Aydin, Türkiye.
[2] Departmant of Animal Science, Faculty of Veterinary Medicine, Aydın Adnan Menderes University, Aydin, Türkiye.
[3] Department of Microbiology, Faculty of Veterinary Medicine, Aydin Adnan Menderes University, Aydin, Türkiye.
[a]: 0000-0002-4431-9553, [b]: 0000-0002-7600-2390, [c]: 0000-0002-1363-4534

* **Corresponding author:** Prof. Dr. S. Turkyilmaz Department of Microbiology, Faculty of Veterinary Medicine, Aydin Adnan Menderes University, Isikli, Aydin-Türkiye. Email: sturkyilmaz@adu.edu.tr

ABSTRACT

Salmonella enterica subsp. *enterica* serovar Infantis (*S*. Infantis) is a bacterium that possesses numerous virulence factors contributing to bacterial invasion and toxicity, leading to challenging situations for treating infections, in view of the continuously rising antibiotic resistance rates. The aim of the study was to investigate integron, antimicrobial resistance and virulence gene profiles in *S*. Infantis isolates obtained from broiler chickens. Two hundred fifty five salmonella isolates obtained from broiler chickens were used in the study. *Salmonella* isolation was carried out according to the ISO 6579-1:2017 standard. Polymerase chain reaction (PCR) was used for the confirmation of the isolates at the genus level (*inv*A) and species level (*sef*A for serovar Enteritidis, *spy* for serovar Typhimurium, *flj*B for serovar Infantis), determination of integron (*int*1, *int*2), and virulence gene (*sop*B, *pip*D, *sop*E, *sif*A, *stn*, *spa*N, *sly*A, *hil*A, *spv*B, *spv*C, *spv*R) profiles. The isolates' resistance profiles against eight antibiotics belonging to eight antimicrobial families were examined using the disk diffusion method. The relationship between multiple antibiotic resistance (MDR) status and the presence of integron genes and virulence genes; in addition, the relationship between the presence of integron genes and the presence of virulence genes and antimicrobial resistance was examined using the Pearson Chi-Square ($\chi 2$) test. By species specific PCR, 159 (62.4%) isolates were identified as *S*. Infantis, 36 (14.1%) isolates as *S*. Enteritidis and 31 (12.1%) isolates as *S*. Typhimurium. Among *S*. Infantis isolates, the highest resistance was observed against trimethoprim sulfamethoxazole, tetracycline and amoxicillin-clavulanic acid; while, the most effective antibiotics were ampicillin and gentamicin.While 61.0% of *S*. Infantis isolates were MDR; 96.8% carried the integron gene. The isolates carried some virulence genes (*sop*B, *pip*D, *sif*A, *stn*, *spa*N, *sly*A, *hil*A) at high rates (94.0%-97.0%) and some virulence genes (*sop*E, *spv*C, *spv*R) at lower rates (12.0%-3.2%). After statistical analysis, a significant relationship was not observed between MDR and the presence of integrons and virulence genes. However, a significant relationship was found between the presence of integron genes and resistance to certain antibiotics (gentamicin and ampicillin) and the presence of certain virulence genes (*sop*B, *pip*D, *spa*N, *spv*C, *sly*A, *hil*A, *spv*R). This study provides up-to-date information on the distribution of antibiotic resistance, virulence, and integron genes in *S*. Infantis strains, as well as their relationships. Investigating and preventing mechanisms that facilitate the transfer of these genes could be crucial for the control and treatment of MDR *S*. Infantis strains.

Keywords: Antimicrobial Resistance; Integron gene; *Salmonella* Infantis; Virulence gen.

INTRODUCTION

Salmonella enterica subsp. *enterica* serovar Infantis is a zoonotic pathogen commonly found worldwide, causing productivity losses, growth retardation and mortality, particularly in poultry animals. It can also be transmitted to humans, posing a significant public health concern (1). The consumption of poultry products is considered to be the most important route of transmission to humans, and *S.* Infantis has been detected at elevated rates (93.1%) specifically in broiler sources (broiler meat and flocks) (2). According to the data from the Ministry of Health in Türkiye, *S.* Infantis was reported as the third most frequently isolated serotype from human clinical samples during 2012-2016, following *S.* Enteritidis and *S.* Typhimurium (3).

Genes responsible for multidrug resistance (MDR) can be transferred among bacteria through transposons, plasmids or integrons (4). These genetic structures enable bacteria to acquire resistance to antibiotics, reducing the effectiveness of antibiotic treatment and leading to the spread of drug resistance (4,5). Integrons are genetic structures that facilitate the transfer of genetic materials horizontally into a bacterial cell (5). Integrons, particularly contribute to the rapid dissemination of beneficial genes such as antibiotic resistance among bacterial populations, making them a significant topic in medical and environmental research (5). So far, more than nine integron classes have been identified based on a conserved 16 amino acid sequence in Gram-negative bacteria (6). Among these, class 1 and class 2 integrons are the most commonly found classes in clinical isolates (7). Studies conducted both globally and in our country have reported that *S.* Infantis isolates commonly exhibit resistance to sulfonamides, tetracyclines, streptomycin, nalidixic acid, and broad-spectrum beta lactams (8,9).

Various virulence factors play different roles in the pathogenesis of salmonella infections. Among these factors are flagella, capsule, plasmids, adhesion systems and type 3 secretion systems (T3SS) (10). Virulence-associated genes found in *S.* Infantis are generally categorized into three groups. In the first group, invasion-related genes (*hil*A, *sop*B, *sop*E, *spa*N, *sif*A, *pip*D) enable *S.* Infantis to adhere to cells and be taken up into the host cell. The second group includes salmonella virulence plasmid genes (*spv*RABCD). In the third group, there are genes (*stn*, *sly*A) responsible for producing toxins that can damage host cells and lead to cell death (1). The most important factor conferring pathogenicity to salmonella species is the T3SS. This system acts like an injector, delivering bacterial virulence proteins into the host cells' cytoplasm (1). Salmonella virulence plasmids encode genes (*spv*A, *spv*B, *spv*C, *spv*D, *spv*R) essential for various functions such as host cell adaptation, antibiotic resistance, and virulence factors. It has been reported that salmonella strains carrying virulence plasmids are necessary for initiating systemic infections in humans and animals (1, 12). The *spv*B proteins are translocated into the host cell via T3SS-1 and also participate in actin polymerization. On the other hand, the *spv*C proteins are translocated into the host cell through both T3SS-1 and T3SS-2, and they exert an anti-inflammatory effect (1,12). *Spv*R is a regulatory gene responsible for the expression of other genes associated with the plasmid (1,12). According to research, two types of exotoxins, salmonella enterotoxin and salmolysin, have been reported, and it has been determined that these toxins are encoded by the *stn* and *sly*A genes, respectively. *Sly*A is known to regulate the virulence genes necessary for extraintestinal systemic infections and has been found to be involved in survival within macrophages and adaptation to oxidative stress (15). On the other hand, the *stn* gene is associated with acute gastroenteritis and is known to cause diarrhea (16).

A study investigating integron, antimicrobial resistance, and virulence gene profiles in *S.* Infantis isolates could be crucial in understanding the bacterium's antibiotic resistance and infection potential, and in developing effective treatment strategies. Therefore, the aim of this study was to examine integron, antimicrobial resistance, and virulence gene profiles in *S.* Infantis isolates obtained from broiler chickens.

MATERIAL AND METHODS

Ethical Approval

Animal Experiments Local Ethics Committee of Aydın Adnan Menderes University (HADYEK) approved this study (Approval No: 64583101/2020/21).

Bacterial Isolates

In this study, 255 *Salmonella* isolates were used as the material, which were obtained from 59 broiler internal organs, 18 joint fluids, and 178 drag swab samples collected for routine diagnostic purposes and sourced from a single commercial farm of Ross 308 breed chickens aged between 16

Table 1. Antimicrobial resistance rates of isolates.

Antimicrobial Classes (Antibiotic, (Abbreviation))	Disk Content (µg)	≥S	≤R	S (%)	R (%)
Penicillins (Ampicillin (AMP))	10	17	13	155 (97.5)	4 (2.5)
β Lactams (Amoxicillin Clavulanate (AMC))	20/10	18	13	59 (37.0)	100 (63.0)
Aminoglycosides (Gentamicin (CN))	10	18	14	154 (97.0)	5 (3.0)
Tetracyclines (Tetracycline (TE))	30	15	11	17 (11.0)	142 (89.0)
Qinolones (Ciprofloxacin (CIP))	5	26	21	9 (5.6)	20 (12.5)
Folat Pathway (Trimethoprim Sulfamethoxazole (SXT))	1.25/23.75	16	10	14 (9.0)	142 (89.0)
Phenicols (Choramphenicol (C))	30	18	12	153 (96.0)	6 (3.8)
Nitrofurans (Nitrofurantoin (F)	300	17	14	152 (95.6)	7 (4.4)

and 41 days. All isolates were preserved in 20% glycerol-containing Brain Heart Infusion Broth (Oxoid CM 1135, UK) at -20°C.

Sampling of Drag Swabs

The drag swab, were placed in a gauze petri dish, on the poultry house floor, and the entire poultry house was completely traversed by holding the handle of the swab. During this twenty minute process, care was taken to ensure continuous contact of the petri dish with the poultry house floor. The collected samples were transported to the laboratory under cold chain conditions as soon as possible.

Isolation and Identification

The isolation of *S.* Infantis was performed according to ISO 6579-1:2017 (17). In this method, detection of *Salmonella* spp. was carried out through non-selective liquid pre-enrichment, selective enrichment in specific media, identification and confirmation tests using *Salmonella* Omnivalent antiserum (Denka Seiken, Japan).

Antibiotic Susceptibility Test

The resistance profiles of *S.* Infantis isolates against eight antibiotics belonging to eight different antimicrobial families were determined using the Kirby-Bauer disk diffusion method (18). The susceptibility test results were evaluated by measuring zone diameters and categorized as sensitive (S), intermediate (I), and resistant (R) (Table 1). *E. coli* ATCC 25922 strain was used as the quality control strain (18). Bacteria were considered as MDR when resistance to at least three or more antimicrobial families was detected (19). Antimicrobial agents used in the study, disc contents and antibiogram results are presented in Table 1.

Polymerase Chain Reaction (PCR)

DNA Extraction, Purity and Quantity Controls: DNA extraction from *Salmonella* spp. was carried out using the sonication method (20). After the genomic DNAs obtained from the isolates were checked for their integrity by agarose gel electrophoresis, purity controls and quantitation were performed with Nonadrop (Maestro, Taiwan). The OD260/OD280 absorbance ratio of the DNAs was between 1.6 and 2.0, indicating that the DNA was pure.

Primers: Identification of bacteria at the genus and species level was performed using multiplex PCR with specific primers, while the presence of virulence genes and integrons was examined using monoplex PCR. For the genus-level identification of *Salmonella* using PCR, the presence of *inv*A gene was investigated for serovar Enteritidis detection, *sef*A gene for serovar Typhimurium detection, and *flj*B gene for serovar Infantis detection. *S.* Typhimurium ATCC 14028, *S.* Enteritidis ATCC 13076 and a sequenced field isolate of *S.* Infantis were used as positive controls in the PCR, while *Escherichia coli* ATCC 25922 strains were used as negative controls. For *S.* Infantis, the positive control strain was obtained after sequencing the amplicons obtained from PCR using universal 16S rRNA primers (Macrogen, Netherlands). The results were compared with the GenBank (www.ncbi.nlm.nih.gov) using the Nucleotide BLAST program and homologies of each isolate in the GenBank were determined. Primers were utilized in a 25 µl reaction containing 12.5 µl of 5x FIREPol® Master Mix Ready to Load (Tartu, Estonia), 1 µl of each primer of 0.1 mM concentrations, 8.5 µl of water, and 3 µl (20 ηg) of DNA template. The reaction was performed in Boeco (Hamburg, Germany) thermal cycler.

The DNA was amplified using the following protocol: initial denaturation at 95˚C for 5 minutes followed by 30 cycles of denaturation (95˚C for 30 seconds), annealing for 30 seconds [48˚C (*spv*R), 50˚C (*int2*), 54˚C (*inv*A, *sef*A, *spy*, *flj*B, *spv*B, *pip*D,), 55˚C (16SrRNA, *int*1, *stn*), 59˚C (*sly*A), 62˚C (*sop*B, *sop*), 64˚C (*spv*C, *spa*N), 69˚C (*sif*A), 71˚C (*hil*A) 30 sn], and extension (72˚C for 1 minute), with a single final extension for 7 minutes at 72˚C. On electrophoresis, a 2% agarose gel stained with Safe View (100 ml/6 µl) (ABM, Richmond, Canada) was used and the gel was exposed to 100 volts for 45 minutes. After electrophoresis, the gel was placed in the chamber of the transilluminator device, which was connected to the computer and photographed under UV light (Vilbert Lourmat, Collegien, France). When the amplified product formed a band of the expected size (Table 2.), it was assumed to carry the gene examined.

Statistical Analysis

Statistical Package for Social Sciences (SPSS) version 23.0 (SPSS Inc., Chicago, IL, USA) package program was used for the statistical analysis of the data obtained. Pearson Chi-square ($\chi 2$) test was used to compare frequency data. The results were evaluated within a 95% confidence interval. The P value less than 0.05 ($p<0.05$) was considered statistically significant. The $\chi 2$ test was used to investigate in isolates the relationship between [a.]the presence of MDR and the presence of integrons and virulence genes; [b.]the presence of integron genes and the resistance to antibiotics and the presence of virulence genes.

RESULTS

Biochemical Tests and Phenotypic Identification

The 255 isolates, showing Gram-negative rod morphology, were identified as *Salmonella* spp. based on their oxidase-negative, catalase-positive, indole-negative, urease-negative, lactose-negative on MacConkey agar, motile on MSRV agar (Figure 1), H_2S positive on XLD agar, and positive agglutination reaction in serological confirmation tests (17).

PCR

Sequence Analysis

Initially, bacterial presence and DNA extraction were confirmed with amplification of the 16S rRNA gene. Samples with expected size (1371 bp) amplicons were further analyzed by sequencing. Since a positive control strain could not be obtained for *S*. Infantis, three field isolates that tested

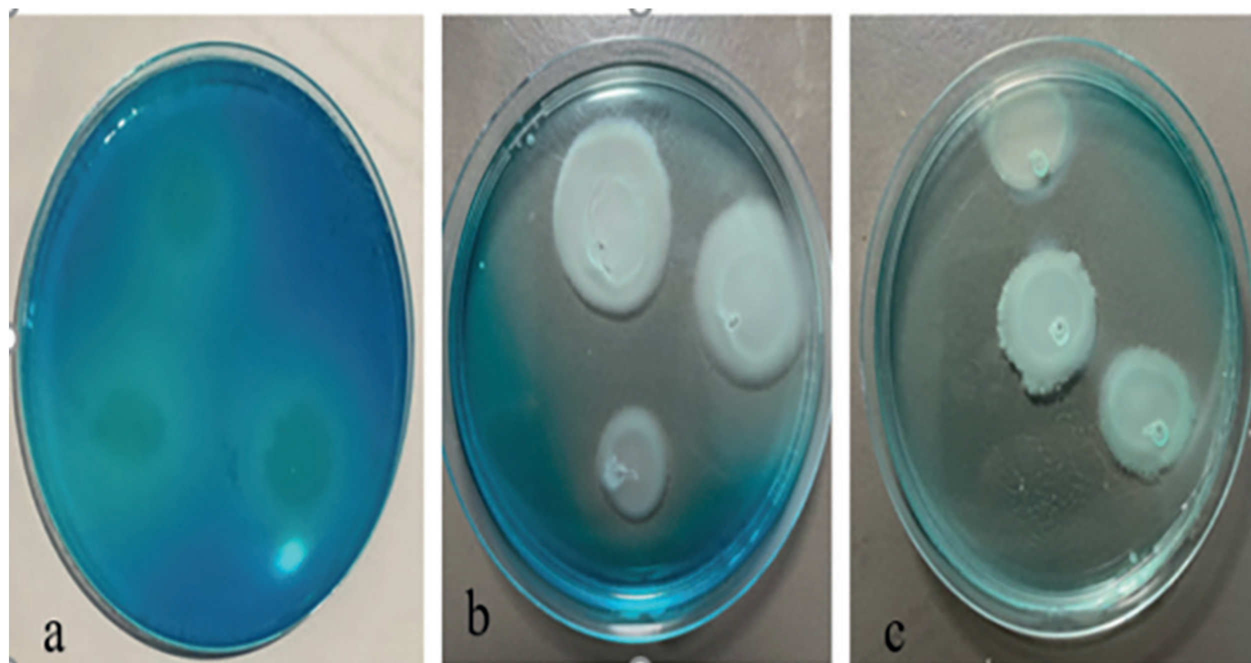

Figure 1. The appearance of negative (a: *E. coli* ATCC 25922) and positive controls (b: *S*. Enteritidis ATCC 1306 (24 hours), c: *S*. Enteritidis ATCC 1306 (48 hours)) after seeding on MSRV medium.

Table 2. Primers used in this study.

Virulenece gene/ Gene Group	Target Gene	Sequence (5'-3')	Tm	Product Length (bp)	Referenece	Result (%)
Salmonella spp.	*inv*A	TTGTTACGGCTATTTTGACCA CTGACTGCTACCTTGCTGATG	55.0 61.0	521	(22)	255 (100.0)
S. Enteritidis	*sef*A	GCAGCGGTTACTATTGCAGC TGTGACAGGGACATTTAGCG	56.0 60.0	330	(22)	36 (14.1)
*S.*Typhimurium	*spy*	TTGTTCACTTTTTACCCCTGAA CCCTGACAGCCGTTAGATATT	56.6 59.4	401	(23)	31 (12.1)
S. Infantis	*flj*B	AACAACGACAGCTTATGCCG CCACCTGCGCCAACGCT	57.3 60.0	727	(24)	159 (62.4)
Universal	16S rRNA	AGAGTTTGATCCTGGCTCAG GACGGGCGGTGTGTACAA	58.0 58.0	1371	(25, 26)	159 (100.0)
Integrase	*int*1	CCTCCCGCACGATGATC TCCACGCATCGTCAGGC	57.2 57.2	280	(27)	150 (94.4)
	int2	TTATTGCTGGGATTAGGC ACGGCTACCCTCTGTTATC	51.6 57.3	233	(28)	126 (79.2)
Salmonella outer membrane protein B	*sop*B	AGCTATAATGCCGAGGCGCTACAT TTTCATGGGCTAACATGGCAAGGC	65.3 65.3	226	(29)	154 (97.0)
T3SS effector gene associated with SPA-1	*pip*D	CGGCGATTCATGACTTTGAT CGTTATCATTCGGATCGTAA	56.4 54.3	350	(30)	152 (96.0)
Salmonella outer membrane protein E	*sop*E	ACACACTTTCACCGAGGAAGCG GGATGCCTTCTGATGTTGACTGG	64.0 64.7	398	(31)	19 (12.0)
Filament A from *Salmonella*	*sif*A	TTTGCCGAACGCGCCCCCACACG GTTGCCTTTTCTTGCGCTTTCCACCCATCT	71.8 72.1	449	(32)	151 (95.0)
Nanphagocytic cellular invasion	*spa*N	AAAAGCCGTGGAATCCGTTAGTGAAGT CAGCGCTGGGGATTACCGTTTTG	66.8 66.4	504	(32)	150 (94.0)
Gene encoding enterotoxin	*stn*	TTGTGTCGCTATCACTGGCAACC ATTCGTAACCCGCTCTCGTCC	57.4 58.4	617	(33)	149 (94.0)
Salmonella virulence plasmid	*spv*C	CGGAAATACCATCTACAAATA CCCAAACCCATACTTACTCTG	65.3 65.8	669	(34)	8 (5.0)
Transcriptional regulator/exotoxin	*sly*A	GCCAAAACTGAAGCTACAGGTG CGGCAGGTCAGCGTGTCGTGC	62.1 69.2	700	(29)	152 (96.0)
Hyperinvasive Locus	*hil*A	CGGAAGCTTATTTGCGCCATGCTGAGGTAG GCATGGATCCCCGCCGGCGAGATTGTG	73.5 75.9	854	(35)	150 (94.0)
Salmonella virulence plasmid	*spv*R	ATGGATTTCATTAATAAAAAATTA TCAGAAGGTGGACTGTTTCAGTTT	49.9 61.8	894	(36)	5 (3.2)
Salmonella virulence plasmid	*spv*B	ATGTTGATACTAAATGGTTTTTCA CTATGAGTTGAGTACCCTCATGTT	55.0 61.0	1776	(37)	0 (0.0)

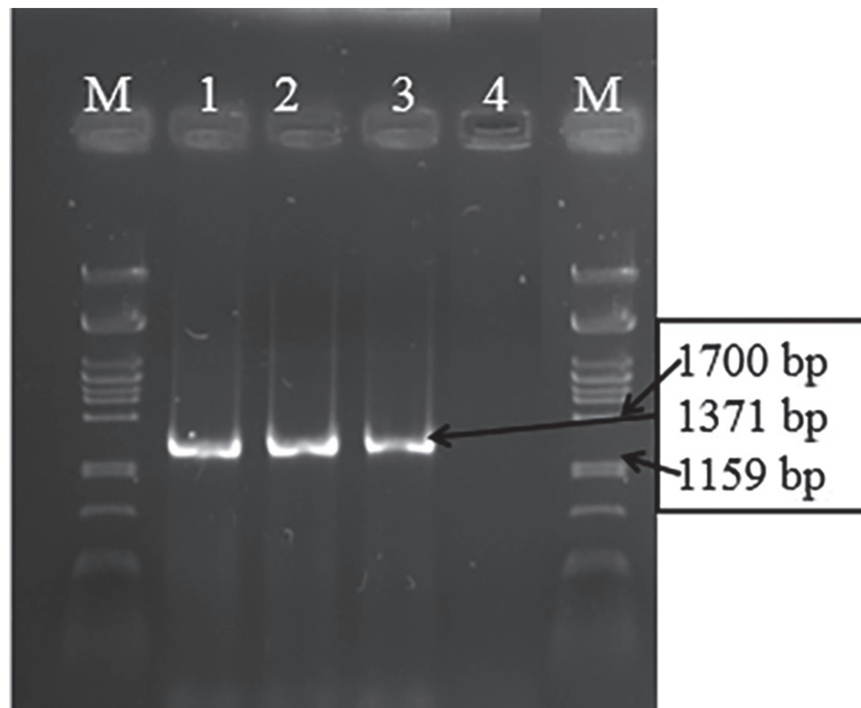

Figure 2. PCR performed by using 16S rRNA universal primers. 1-3: *S.* Infantis field isolates, 4: Negative control (master mix without DNA), M: PCR performed by using 16S rRNA universal primers.

Table 3. Serotypes of *Salmonella* isolates and their sources of isolation.

Serotype	Internal organ	Joint Fluid	Drag swap	Total (%)
S. Enteritidis	11 (4.3)	4 (1.5)	21 (8.2)	36 (14.1)
S. Typhimurium	12 (4.7)	1 (0.4)	18 (7.0)	31 (12.1)
S. Infantis	29 (11.4)	10 (3.9)	120 (47.0)	159 (62.3)
Untypeable	7 (2.7)	3 (1.2)	19 (7.6)	29 (11.5)
TOTAL	59 (23.2)	18 (7.0)	178 (69.8)	255 (100.0)

positive for the target gene, *flj*B, in the PCR, were used to perform PCR with universal 16S rRNA primers. In the PCR with universal 16S primers, a band of 1371 bp in length was observed (Figure 2). The amplicons obtained from the amplification of their DNA were sequenced. According to the sequencing results and BLAST analysis, the DNA with the highest similarity percentage (99.8%) to the amplicon was used as a positive control in subsequent *S.* Infantis PCRs.

Genus and Species Specific PCR

Genotypic identification of salmonella isolates was carried out by multiplex PCR using genus and species specific primers. All 255 isolates phenotypically were identified as *Salmonella* spp. (521 bp) by genus specific PCR. After species specific PCR, 159 (%62.4) isolates were identified as *S.* Infantis (727 bp), 31 (%12.1) isolates as *S.* Typhimurium (401 bp), 36 (%14.1) isolates as *S.* Enteritidis (330 bp) (Figure 3., Table 3.).

The virulence gene, integron and antibiotic resistance profiles of 159 *S.* Infantis isolates identified were analysed.

Virulence Genes

As a result of the detection of 11 virulence genes in a total of 159 *S.* Infantis isolates: The most prevalent virulence genes were *sop*B (96.8%, 154/159=), *pip*D, *sly*A (95.6%, 152/159), *sif*A (95.0%, 151/159), *spa*N, *hil*A (94.3%, 150/159), *stn* (93.7%, 149/159), *sop*E (11.9%, 19/159), *spv*C (5.0%, 8/159), *spv*R (3.1%, 5/159), while there was no detection for *spv*B in any of the isolates with the primers used

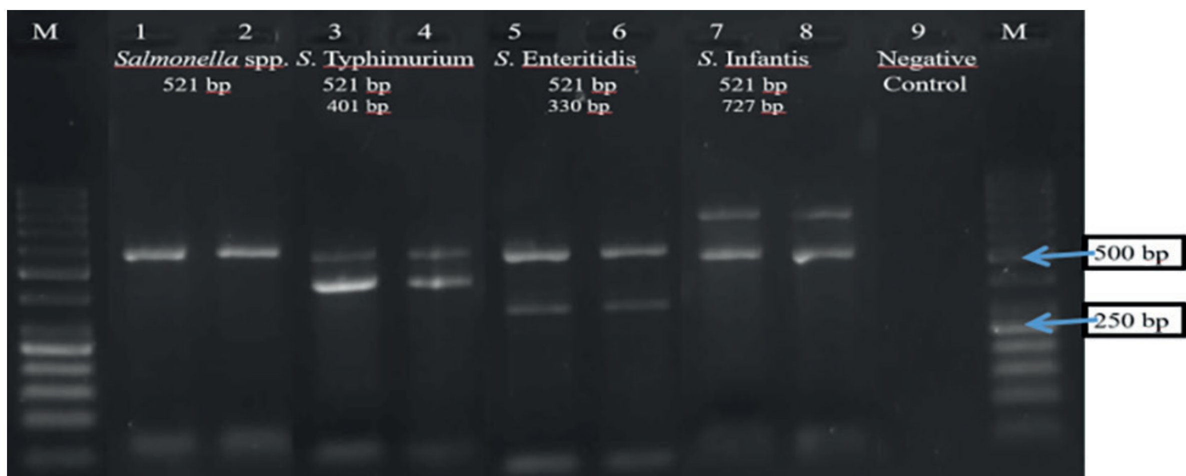

Figure 3. Gel electrophoresis image after PCR of *Salmonella* isolates. 1,2: Unidentified isolates (521 bp), 3: *S.* Typhimurium field isolate (*spy*) (521 bp and 401 bp), 4: Positive Control (*S.* Typhimurium ATCC 14028), 5: *S.* Enteritidis field isolate (*sef*A) (521 bp and 330 bp), 6: Positive Control (*S.* Enteritidis ATCC 1306),7: *S.* Infantis field isolate (*flj*B) (521 bp and 727 bp) 8: Positive Control (sequenced *S.* Infantis field isolate), 9: Negative Control (*E. coli* ATCC 25922), M: Marker (100 bp DNA Ladder).

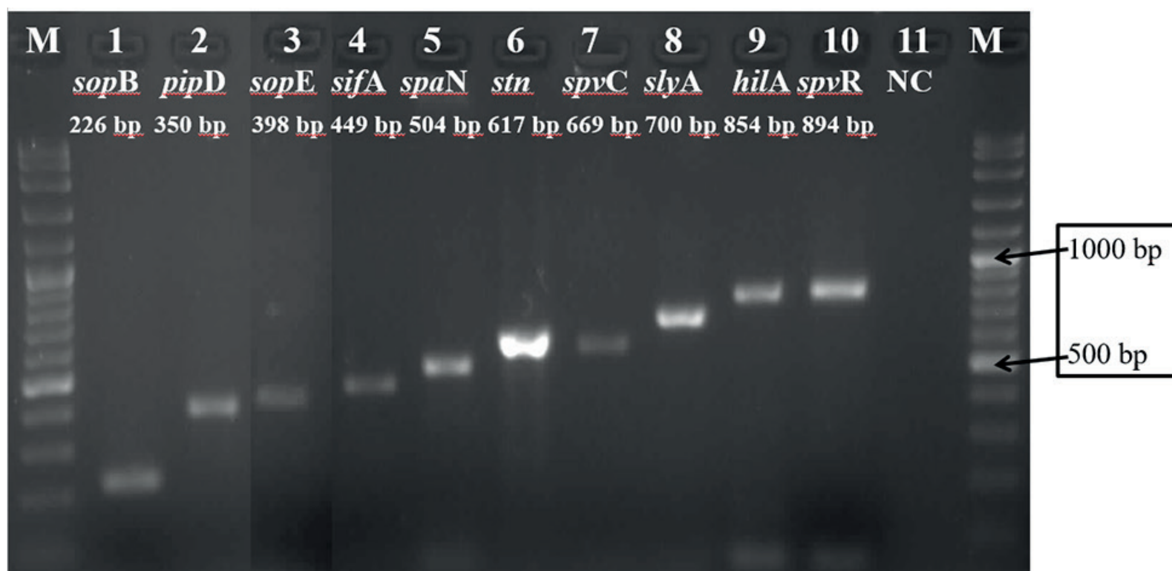

Figure 4. Gel electrophoresis image of *S.* Infantis virulence genes. 1: *sop*B (226 bp) 2: *pip*D (350 bp) 3: *sop*E (398 bp) 4: *sif*A (449 bp) 5: *spa*N (504 bp) 6: *stn* (617 bp) 7: *spv*C (669 bp) 8: *sly*A (700 bp) 9: *hil*A (854 bp) 10: *spv*R: (894 bp) 11: Negative control (master mix without DNA), M: Marker (100 bp DNA Ladder).

(Figure 4). In total, 159 *S.* Infantis isolates had 20 virulence genotypes (Table 4.)

Integron Genes

While 96.8% (154/159) of the isolates carry integrons [17.6% (28/159) of them are only class 1, 2.3% (4/159) is only class 2, 76.7% (122/159) of both int1 and int2]; 3.1% (5/159) did not carry an integron (Figure 5 and Figure 6).

Antimicrobial Resistance

The resistance profiles to eight antibiotics from eight different antimicrobial families were studied. Out of 159 *S.* Infantis isolates, 142 (89.0%) were found as resistant to trimethoprim sulfamethoxazole and tetracycline, 100 (63.0%) to amoxicillin clavulanic acid, 20 (12.5%) to ciprofloxacin, 7 (4.4%) to nitrofurantoine, 6 (3.8%) to chloramphenicol, 5 (3.0%) to gentamicin, 4 (2.5%) to ampicillin (Figure 5). The

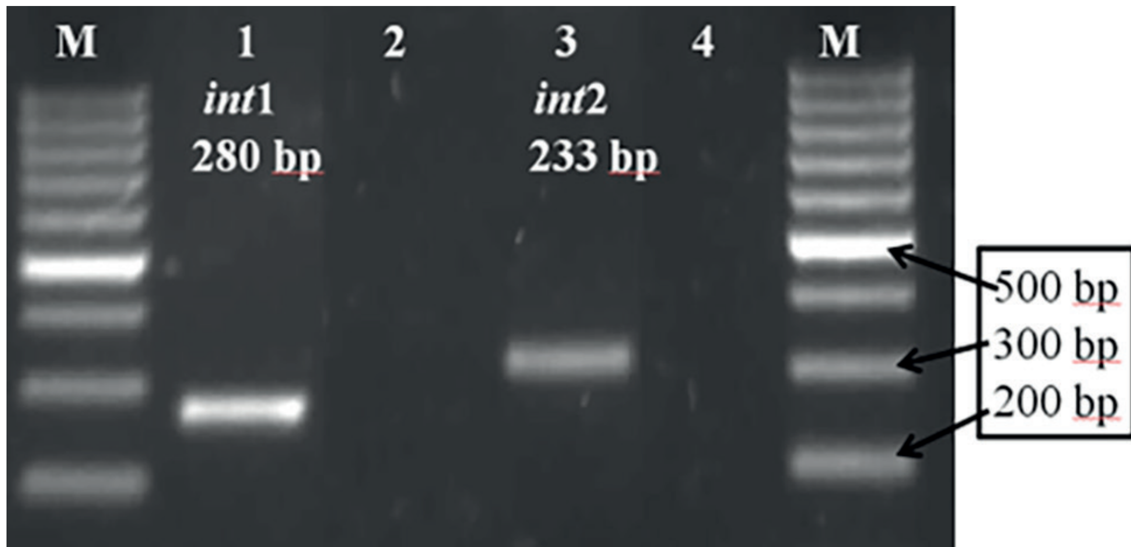

Figure 5. Gel electrophoresis view of *S.* Infantis integron genes. 1: *int*1gene positive *S.* Infantis isolate 2: Negative Control (master mix without DNA) 3: *int*2 gene positive *S.* Infantis isolate 4: Negative Control (master mix without DNA) M: 100 bp Molecular Marker.

Table 4. Virulence gene profiles of *S.* Infantis isolates.

Virulence Genotype	Number of Genes	Number of Isolates	Total (%)
stn, *sly*A, *spv*R	3	1	1 (0.6)
*sif*A, *stn*, *sly*A, *spv*R	4	1	1 (0.6)
*sop*B, *pip*D, *spa*N, *sly*A, *hil*A	5	1	1 (0.6)
*sop*B, *sif*A, *spa*N, *spv*C, *sly*A, *hil*A	6	1	38 (24.0)
*pip*D, *sif*A, *stn*, *spa*N, *sly*A, *hil*A	6	3	
*sop*B, *sif*A, *stn*, *spa*N, *sly*A, *hil*A	6	4	
*sop*B, *pip*D, *stn*, *spa*N, *sly*A, *hil*A	6	5	
*sop*B, *pip*D, *sif*A, *stn*, *spa*N, *hil*A	6	5	
*sop*B, *pip*D, *sif*A, *stn*, *spa*N, *sly*A	6	6	
*sop*B, *pip*D, *sif*A, *stn*, *sly*A, *hil*A	6	7	
*sop*B, *pip*D, *sif*A, *spa*N, *sly*A, *hil*A	6	7	
*sop*B, *pip*D, *sif*A, *stn*, *spa*N, *sly*A, *hil*A	7	94	97 (61.0)
*sop*B, *pip*D, *sop*E, *sif*A, *stn*, *spa*N, *spv*C	7	1	
*sop*B, *pip*D, *sop*E, *sif*A, *spa*N, *spv*C, *hil*A	7	1	
*sop*B, *pip*D, *stn*, *spa*N, *spv*C, *sly*A, *hil*A	7	1	
*sop*B, *pip*D, *sif*A, *stn*, *spa*N, *sly*A, *hil*A, *sop*E	8	14	18 (11.2)
*sop*B, *pip*D, *sif*A, *stn*, *spa*N, *sly*A, *hil*A, *spv*C	8	2	
*sop*B, *pip*D, *sif*A, *stn*, *spa*N, *sly*A, *hil*A, *spv*R	8	2	
*sop*B, *pip*D, *sif*A, *stn*, *spa*N, *sly*A, *hil*A, *sop*E, *spv*C	9	2	3 (2.0)
*sop*B, *pip*D, *sif*A, *stn*, *spa*N, *sly*A, *hil*A, *sop*E, *spv*R	9	1	

most effective antibiotics against isolates are ampicillin and gentamicin (97.5% and 90.7% susceptibility rate) (Figure 7). Ninety-seven (61.0%) of the isolates were found to be resistant to multiple antibiotics and a total of 10 antibiotic resistance phenotypes were present (Table 5).

Statistical Analysis No significant correlation was ob-

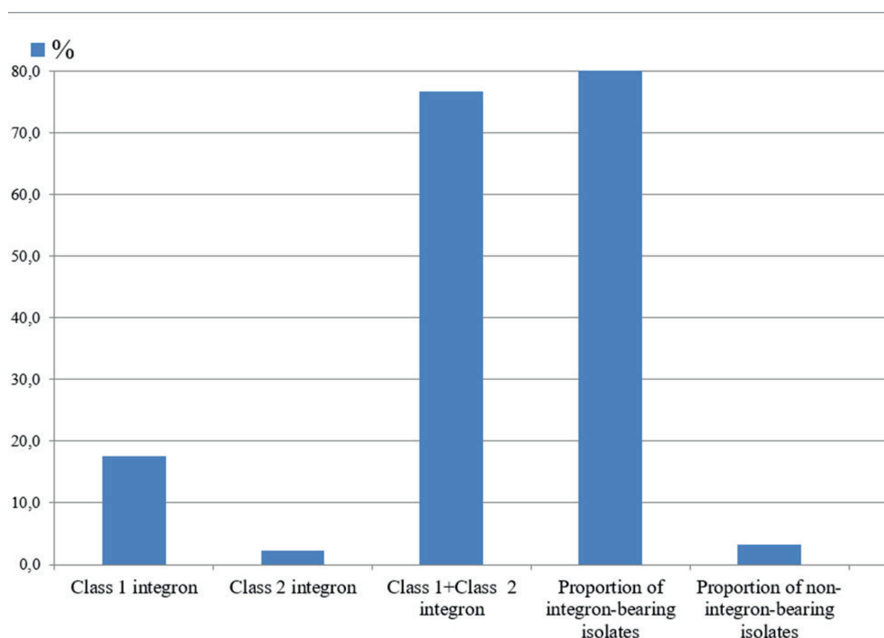

Figure 6. Distribution of integron genes carried by *S.* Infantis isolates.

served between the presence of MDR and integron (Table 6.) and MDR and virulence gene (Table 7.)

While a significant correlation was observed between the presence of integron genes and the presence of *sop*B, *pip*D, *spa*N, *spv*C, *sly*A, *hil*A and *spv*R genes; there was no significant relationship between the presence of the *sop*E, *sif*A and *stn* gene (Table 8.).

While a significant relationship was observed between carrying integron genes and resistance to ampicillin and gentamicin no significant correlation was found between resistance to amoxicillin/clavulanic acid, tetracycline, ciprofloxacin, trimethoprim/sulfamethoxazole, chloramphenicol and nitrofurantoin (Table 9.).

Table 5. Multiple antibiotic resistance phenotypes of *S.* Infantis isolates.

Number of Resistant Antibiotics	Number of Isolates (%)	Total number of isolates (%)
1. AMC, TE, SXT	73 (46.0)	79 (49.8)
2. TE, CIP, SXT	5 (3.2)	
3. AMP, AMC, SXT	1 (0.6)	
4. AMC, TE, SXT, F	1 (0.6)	18 (11.2)
5. AMC, TE, SXT, C	2 (1.2)	
6. AMC, TE, SXT, AMP	1 (0.6)	
7. AMC, TE, SXT, CIP	11 (7.0)	
8. AMC, TE, SXT, CN	1 (0.6)	
9. CN, TE, CIP, SXT	1 (0.6)	
10. TE, CIP, SXT, C	1 (0.6)	
TOTAL	97 (61.0)	97 (61.0)

DISCUSSION

S. Infantis is one of the most common serotypes causing salmonellosis in humans in European Union countries and Türkiye (2,3), posing a risk to human health (1,12). In recent years, the distribution of *Salmonella* serotypes in Türkiye has started to change, with *S.* Infantis becoming the most frequently isolated serotype in poultry (38, 39). This situation

Table 6. Relationship between MDR and integron gene.

Integron Gens	MDR (%) (n=97)	NMDR (%) (n=62)	Total	P	χ2
*Int*1 (+)	93 (58.5)	57 (35.8)	150 (94.4)	0.313	1.093
*Int*1 (-)	4 (2.5)	5 (3.2)	9 (5.6)		
*Int*2 (+)	81 (51.0)	45 (28.3)	126 (79.2)	0.111	2.745
*Int*2 (-)	16 (10.0)	17 (10.7)	33 (20.8)		
Int (+)	94 (59.0)	60 (37.8)	154 (96.8)	1.000	0.002
Int (-)	3 (1.9)	2 (1.3)	5 (3.2)		

NMDR: isolates without multidrug resistance.

Table 7. The relationship between MDR and virulence gene.

Virulence Genes	MDR (%) (n=97)	NMDR (%) (n=62)	Total (%)	P	χ2
*sop*B (+)	95 (59.7)	59 (37.1)	154 (96.8)	0.379	0.952
*sop*B (-)	2 (1,2)	3 (1.9)	5 (3.2)		
*pip*D (+)	93 (58.5)	59 (37.1)	152 (95.6)	1.000	0.046
*pip*D (-)	4 (2.5)	3 (1.9)	7 (4.4)		
*sop*E (+)	11 (7.0)	8 (5.0)	19 (12.0)	0.805	0.088
*sop*E (-)	86 (54.0)	54 (34.0)	140 (88.0)		
*sif*A (+)	92 (57.8)	59 (37.1)	151 (94.9)	1.000	0.008
*sif*A (-)	5 (3.2)	3 (1.9)	8 (5.0)		
stn (+)	92 (57.8)	57 (35.8)	149 (93.7)	0.514	0.540
stn (-)	5 (3.2)	5 (3.2)	10 (6.3)		
*spa*N (+)	92 (57.8)	58 (36.5)	150 (94.4)	0.737	0.118
*spa*N (-)	5 (3.2)	4 (2.5)	9 (5.6)		
*spv*C (+)	6 (3.8)	2 (1.2)	8 (5.0)	0.484	0.689
*spv*C (-)	91 (57.2)	60 (37.7)	151 (94.9)		
*sly*A (+)	93 (58.5)	59 (37.1)	152 (95.6)	1.000	0.046
*sly*A (-)	4 (2.5)	3 (1.9)	7 (4.4)		
*hil*A (+)	92 (57.8)	58 (36.5)	150 (94.4)	0.737	0.118
*hil*A (-)	5 (3.2)	4 (2.5)	9 (5.6)		
*spv*R (+)	2 (1.2)	3 (1.9)	5 (3.2)	0.379	0.952
*spv*R (-)	95 (59.7)	59 (37.1)	154 (96.8)		

is often associated with the antibiotic resistance and presence of virulence genes in this bacterium (1,3,4,5).

S. Infantis has been isolated from broiler chickens in Türkiye at similar rates ranging from 17.7%-95.0% (9, 39, 40, 41, 42), and globally with rates ranging from 13.0%-93.1% (2, 43). In this study, *S*. Infantis (62.3%), *S*. enteritidis (14.1%), and *S*. typhimurium (12.1%) were identified as the most common serotypes, while 11.5% of the isolates could not be typed due to budget limitations. It is recognized that *S*. Infantis can be carried asymptomatically in chickens and is an environmental contaminant (44). In this study, *S*. Infantis was detected in higher proportions (47.0%) in drag swab samples compared to other serotypes (8.2% for *S*. Enteritidis and 7.0% for *S*. Typhimurium). Moreover, it was the most frequently detected serotype in internal organ samples (11.4%) and joint fluid samples (3.9%) compared to other serotypes. This was likely due to the wide distribution of *S*. Infantis in broiler chickens.

The isolation of highly drug-resistant *S*. Infantis from broilers in Türkiye was first reported in 2011 (45). In some studies conducted in Türkiye, the rates of MDR *S*. Infantis isolates vary between 77.1% and 89.3% (9, 40, 46). In this study, although the rates of MDR isolates were lower compared to other studies, the detection of a considerable proportion (61.0%) of MDR isolates is still of concern. Similar apprehensions have been reported in recent studies conducted in many countries worldwide, such as Slovenia (8), the United Kingdom (47) and Egypt (48). These drug-resistant strains can lead to a reduction in treatment options and treatment failures in humans. Additionally, their contribution to foodborne infections is worrisome for public health and can contribute to the spread of antibiotic resistance. This situation poses a serious threat to the discovery of effective treatments and the preservation of food safety.

In infections caused by *S*. Infantis, the outcomes of antibacterial therapy may be influenced not only by the antibiotic susceptibility of the pathogen but also by the presence of integrons and the ability to produce virulence factors. In this study, virulence genes involved in the pathogenesis of salmonella species, including invasion-related genes (*hil*A, *sop*B,

Table 8. The relationship between integron genes and virulence genes.

Virulence Genes	Integron genes (+) (%) (n=154)	Integron genes (-) (%) (n=5)	Total (%) (n=159)	P	χ2
*sop*B (+)	151 (94.9)	3 (1.9)	154 (96.8)	0.008	22.878*
*sop*B (-)	3 (1.9)	2 (1.2)	5 (3.2)		
*pip*D (+)	149 (93.7)	3 (1.9)	152 (95.6)	0.016	15.445*
*pip*D (-)	5 (3.2)	2 (1.2)	7 (4.4)		
*sop*E (+)	17 (10.7)	2 (1.2)	19 (12.0)	0.109	3.836
*sop*E (-)	137 (86.1)	3 (1.9)	140 (88.0)		
*sif*A (+)	147 (92.4)	4 (2.5)	151 (94.9)	0.230	2.405
*sif*A (-)	7 (4.4)	1 (0.6)	8 (5.0)		
stn (+)	145 (91.2)	4 (2.5)	149 (93.7)	0.280	1.636
stn (-)	9 (5.6)	1 (0.6)	10 (6.3)		
*spa*N (+)	148 (93.1)	2 (1.2)	150 (94.4)	0.001	28.366*
*spa*N (-)	6 (3.8)	3 (1.9)	9 (5.6)		
*spv*C (+)	6 (3.8)	2 (1.2)	8 (5.0)	0.021	13.128*
*spv*C (-)	148 (93.1)	3 (1.9)	151 (94.9)		
*sly*A (+)	149 (93.7)	3 (1.9)	152 (95.6)	0.016	15.445*
*sly*A (-)	5 (3.2)	2 (1.2)	7 (4.4)		
*hil*A (+)	148 (93.1)	2 (1.2)	150 (94.4)	0.001	28.366*
*hil*A (-)	6 (3.8)	3 (1.9)	9 (5.6)		
*spv*R (+)	3 (1.9)	2 (1.2)	5 (3.2)	0.008	22.878*
*spv*R (-)	151 (94.9)	3 (1.9)	154 (96.8)		

*Statistically significant.

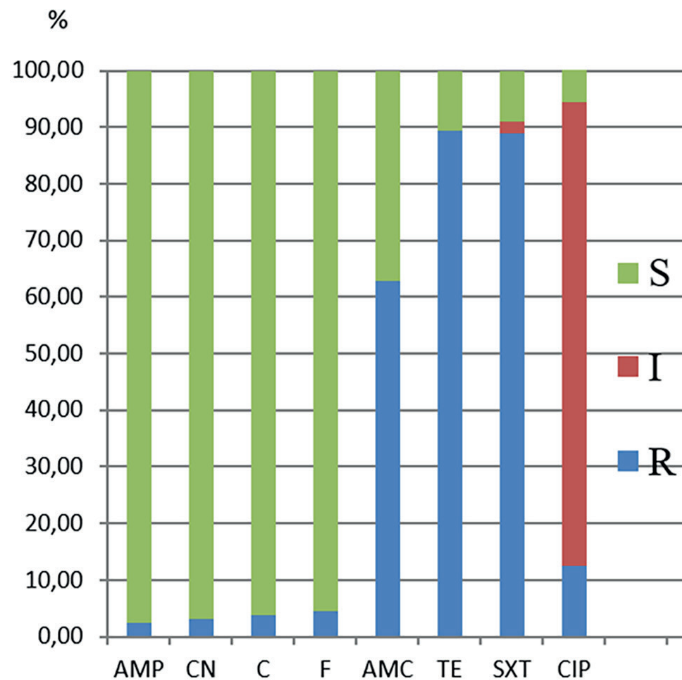

Figure 7. Antimicrobial susceptibility and resistance profiles of *S.* Infantis.

Table 9. The relationship between carrying integron genes and resistance to antibiotics.

Antibiotic Resistance Profile (R/S)	Integron genes (+) (%) (n=154)	Integron genes (-) (%) (n=5)	Total (%) (n=159)	P	χ2
AMP (R)	2 (1.2)	2 (1.2)	4 (2.5)	0.005	29.391*
AMP (S)	152 (95.6)	3 (1.9)	155 (97.5)		
AMC (R)	99 (62.3)	1 (0.6)	100 (62.9)	0.064	4.044
AMC (S)	55 (34.5)	4 (2.5)	59 (37.1)		
CN (R)	3 (1.9)	2 (1.2)	5 (3.2)	0.008	22.878*
CN (S)	151 (94.9)	3 (1.9)	154 (96.8)		
TE (R)	139 (87.4)	3 (1.9)	142 (89.3)	0.089	4.615
TE (S)	15 (9.4)	2 (1.2)	17 (10.7)		
CIP (R)	18 (11.3)	2 (1.2)	20 (12.6)	0.119	3.508
CIP (S)	136 (85.6)	3 (1.9)	139 (87.4)		
SXT (R)	139 (87.4)	3 (1.9)	142 (89.3)	0.089	4.615
SXT (S)	15 (9.45)	2 (1.25)	17 (10.7)		
C (R)	5 (3.2)	1 (0.6)	6 (3.8)	0.177	3.72
C (S)	149 (93.7)	4 (2.5)	153 (96.2)		
F (R)	6 (3.8)	1 (0.6)	7 (4.4)	0.204	2.695
F (S)	148 (93.1)	4 (2.5)	152 (95.6)		

*Statistically significant.

*sop*E, *spa*N, *sif*A, *pip*D), virulence plasmid genes (*spv*B, *spv*C, *spv*R), and toxin-coding genes (*stn*, *sly*A), were investigated. The transcriptional master regulator of the Type III secretion system, hilA gene, was found to be specific to salmonella species and was detected at high proportions (90.0% to 100.0%) in our study, consistent with previous reports (11,49,50,51). Genes involved in invasion, such as *sop*B, *sop*E, *sif*A, *pip*D, and *spa*N, play a crucial role in the virulence of salmonella species. The *sop*B gene has been reported to be highly prevalent in salmonella isolates, both in Türkiye (52, 53) (92.4%-93.3%) and in Australia, Poland, and Senegal (54, 55, 56) (94.1%-100.0%), similar to our findings (97.0%). The *sop*B gene is responsible for bacterial entry into host cells and fluid secretion, contributing to diarrhea-associated infections. The *sop*E gene has been suggested to play a role in the systemic phase of the infection and may have zoonotic potential (13). The isolation rates of the *sop*E gene in our *S*. Infantis isolates were lower (12%) compared to different studies conducted in Türkiye (37.7% to 93.3%) (52,53) and Senegal and Brazil (33.0% to 98.7%) (56,57). Genetic variations among different *S*. Infantis isolates may lead to differences in the presence or absence of virulence genes. The *sif*A gene regulates molecular mechanisms required for salmonella species to enter and replicate within host cells. In both Türkiye (31.1%-90.62%) (52,53) and Australia, Poland and Spain (67.2%-100.0%) (54, 55, 58), the *sif*A gene detection rate was high, similar to our study (95.0%). The *spa*N gene facilitates the entry of the bacterium into non-phagocytic cells and enables intracellular invasion via apoptosis in macrophages. In our study, a higher detection rate of the *spa*N gene (94.0%) was observed compared to a previous study (53.7%) on broiler litter. The high presence of the *spa*N gene suggests that these isolates may be potentially more infectious and virulent, increasing the risk of causing more severe and widespread infections in humans. The *pip*D gene's function is to enhance the bacterium's effect on causing enteritis and facilitate its survival and spread within host cells.

In our study, the *pip*D gene was highly prevalent (96.0%), consistent with previous studies in Türkiye (95.5%) and globally (92.4%) (56). The *stn* gene in salmonella isolates encodes a toxin called salmonella enterotoxin, contributing to acute gastroenteritis. Many salmonella serotypes from various sources have shown high *stn* gene prevalence (58.8%-100.0%), and strains carrying the *stn* gene have been associated with causing serious infections in humans. In our study, a high prevalence of the *stn* gene (94.0%) was also ob-

served, raising concerns about the potential of these isolates to cause severe gastrointestinal infections and severe disease symptoms. The *sly*A gene has been reported to play a role in producing exotoxins responsible for extraintestinal systemic infections. It was found in all *S*. Infantis isolates from broiler litter. Our study also revealed a high presence of the *sly*A gene (96.0%) in our isolates. The high prevalence of the *sly*A gene in *S*. Infantis isolates suggests the potential for these isolates to be more aggressive and virulent, potentially causing more severe infections in humans. Virulence plasmids carried by salmonella species serve as carriers for important virulence genes (1,12). One of these genes, *spv*C, has been found to be positive at low rates in studies conducted in Türkiye (8.9%), Spain, and Iran, similar to our study (5%) (52, 58, 61).

In our study, when looking at the overall results, the virulence plasmid genes *spv*C, *spv*R, and *spv*B were detected at significantly low rates (5%, 3.2%, and 0%, respectively) in our *S*. Infantis isolates. Therefore, these findings suggest that our isolates may have a lower potential for gene expression related to the plasmid, which could be associated with causing infections with less severe clinical outcomes. Thus, the low expression of plasmid-carried genes in these isolates may lead to reduced virulence and the potential to cause milder infections. However, further studies are required to confirm these results and establish a definitive relationship between gene expression and clinical outcomes.

Integrons have become a focal point in antimicrobial resistance studies due to their ability to carry multiple antibiotic resistance gene cassettes (7). In both Türkiye (42) and worldwide studies (62, 63), similar to the findings in this study, integrons have been reported to be prevalent in *S*. Infantis isolates with multidrug resistance. Integrons are genetic structures that allow the transfer of genetic material and the carriage of antibiotic resistance genes, thus the high presence of integron genes may lead to the further dissemination of antibiotic resistance in bacteria, making the treatment of such infections more challenging. This situation can diminish the effectiveness of antibiotic therapy, complicating the control and treatment of infections, and therefore, posing a serious public health concern.

In this study, it was found that 96.9% (94/97) of MDR isolates and 96.8% (60/62) of NMDR isolates carried integrons. However, no statistically significant relationship was observed between the presence of integrons and MDR. Similar results have been obtained in previous studies (64, 65). Integrons are known to spread among bacteria through gene transfer. Therefore, it is conceivable that initially NMDR *S*. Infantis isolates may acquire resistance genes from surrounding resistant bacteria through contact, leading to the subsequent development of multidrug resistance. The presence of integrons can result in the advantageous dissemination of bacteria carrying genetic material with resistance genes within the population due to natural selection and environmental pressures. Further studies are required to complete detailed genetic analyses and gain a better understanding of the population structures of MDR and non-MDR *S.* Infantis isolates with similar integron carrying potential.

In this study, no statistically significant relationship was observed between the presence of virulence genes and MDR. The high prevalence of virulence genes in isolates without multidrug resistance suggests that these isolates may have high pathogenicity and pose a serious threat to both the poultry industry and public health.

In this study, a statistically significant relationship was observed between the presence of integron genes and the potential transfer of certain virulence genes (*sop*B, *pip*D, *spa*N, *spv*C, *sly*A, *hil*A, *spv*R) in *S*. Infantis isolates, similar to previous studies (66). This suggests that the presence of integron genes may have contributed to the dissemination of virulence factors by enhancing the potential transfer of these genes. Alternatively, the presence of integrons could have increased the transfer potential of these virulence genes by carrying different genes through the same transfer mechanisms. As a result, *S*. Infantis isolates carrying these virulence genes might gain a competitive advantage and become more widespread. Integrons' presence can enhance this advantage by facilitating the transfer of virulence genes. Integrons can be found in genetic contexts containing virulence genes. Therefore, specific virulence genes may be more frequently associated with integrons, and this association may demonstrate a relationship between the presence of integrons and the potential transfer of these virulence genes. Understanding the relationship between integron mobility and virulence genes could be a crucial step in the control of antibiotic resistance and infections.

In this study, there was a significant association between the presence of integron genes and resistance to gentamicin and ampicillin. Similar to previous studies, it has been reported that gene cassettes conferring resistance to amino-

glycosides and beta-lactam antibiotics in salmonella species are commonly found on *int*1 and *int*2 (67). The observed significant relationship between the presence of integron genes and resistance to antibiotics such as gentamicin and ampicillin in *S*. Infantis isolates is important in terms of the impact on the mobility and dissemination of resistance genes. To prevent the spread of antibiotic-resistant bacteria, the role and impact of integrons should be further investigated, and control measures should be considered.

As a result, this study revealed a high prevalence of antimicrobial resistance and virulence gene presence in *S*. Infantis isolates obtained from broiler chickens. Furthermore, the presence of integron genes was significantly associated with resistance to certain antibiotics and the transfer of specific virulence genes. These findings underscore the potential for the dissemination of *S*. Infantis and its resistant virulent features, highlighting the importance of strict hygiene measures and controlled antibiotic usage in poultry farms.

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CONFLICT OF INTERESTS STATEMENT

The authors have no conflicts of interest to declare. All co-authors have seen and agree with the contents of the manuscript. This original work is not under review by any other publication.

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