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Research Article

Antimicrobial Susceptibility, Virulence Characteristics, and Incidence of Class 1 and 2 Integrons in *Salmonella* Infantis Isolated from Clinical Cases in Broilers

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Abstract

Salmonella Infantis is a poultry-adapted *Salmonella* enterica serovar that is increasingly reported in broilers and is also regularly identified among human salmonellosis cases. This study aimed to investigate the presence and distribution of virulence determinants and their antimicrobial susceptibilities of *S.* Infantis isolates obtained from clinical cases of broilers. In addition, selected 6 *S.* Infantis isolates were further characterized using whole-genome sequencing (WGS). The majority of the isolates was resistant to at least two or more antimicrobials. Only two isolates were susceptible to all antimicrobials tested. Higher rates of resistance were observed against ciprofloxacin (96.4%), tetracycline (96.4%), sulfamethoxazole-trimethoprim (76.8%), but low resistance rates to chloramphenicol (8.9%), and ampicillin (8.9%) were detected. WGS analysis revealed the presence of different resistome, but *aac(6')-Iaa* and *tetA* genes in all isolates, and mutations in *gyrA* and *parC* genes playing a role in quinolone resistance. WGS also revealed that all isolates were of sequence type 32 (ST32). Based on the presence of virulence genes, the isolates were characterized into five virulence profile. Among the examined virulence genes, *invA*, *sopB*, *pipD*, *sifA*, *stn*, *spaN*, *slyA*, and *hilA* were present in all isolates. Only one isolate had all virulence genes examined. The findings of this study provide valuable information on *S.* Infantis strains isolated from clinical cases of broilers and current antimicrobial resistance levels and virulence determinants. High resistance rates and the widespread occurrence of many virulence genes reveals that the isolates have significant pathogenic potential and pose a threat to public health.

Keywords: Antimicrobial susceptibility, Integron, *Salmonella* Infantis, Virulence, Whole genome sequencing

Introduction

Non-Typhoidal *Salmonella enterica* (NTS) is a foodborne pathogen that causes human gastroenteritis [1], with 93 million cases of gastroenteritis and 155.000 deaths; among them, approximately 85% of the cases are associated with the consumption of contaminated food [2]. In 2022, 65.208 human salmonellosis cases were reported by 27 EU countries, corresponding to an EU notification rate of 15.3 cases per 100.000 population. The three most commonly reported *Salmonella* serovars in 2022 were *S.* Enteritidis (54.6%), *S.* Typhimurium (12.1%) and monophasic *S.* Typhimurium (1,4,[5],12:i:-) (10.4%), representing 77.1% of the 47.122 confirmed human cases. The fourth and fifth serovar were *S.* Infantis (2.3%) and *S.* Newport (1.1%) [3]. NTS infections are frequently characterized with acute onset of diarrhea, abdominal cramps, and fever, which is usually self-limiting, resolving between 1 and 7 days without treatment, depending on the host status. However, NTS infections could cause severe clinical manifestations in individuals, including immune-compromised patients, infants, and older adults, may develop bacteremia or invasive infections such as meningitis, osteomyelitis, endovascular infections, and septic arthritis. In this case, antimicrobial therapy might be needed [1].

Approximately more than 2600 *Salmonella* serovars were identified according to the White–Kauffmann-Le Minor [4], of which *S.* Infantis has emerged as the fourth most common serotype causing human salmonellosis in Europe [3], with 1868 *S.* Infantis related human infection cases [5]. Based on the data of the Ministry of Health in

Türkiye between 2012 and 2016, it was reported *S.* Infantis was among the top three serotypes and the frequency of three serotypes isolated from human clinical samples were reported to be 57.3-74.1% for *S.* Enteritidis, 3.0-8.5% for *S.* Typhimurium and 4.0-6.7% for *S.* Infantis [6].

In Türkiye, National *Salmonella* Control Program revealed that 24.5% of broiler flocks were being colonized with *Salmonellae*, among which *S.* Infantis (17.7%) was the most common serotype [6]. In this report, *Salmonella* contamination in broiler carcass samples was determined as 47%, and 72.6% of *Salmonella* isolates were serotyped as *S.* Infantis, followed by *S.* Kentucky (8.6%) and *S.* Enteritidis (7.7%) [6].

The emergence of *Salmonella* antimicrobial resistance (AMR) is a growing public health concern, particularly resistance against the critically important antimicrobials such as fluoroquinolones and third-generation cephalosporins [7]. During the last decade, *S.* Infantis isolated from various sources including animals and humans has been frequently identified as having multidrug resistance (MDR). In 2014, conjugative megaplasmids pESI (also termed "pESI-like", 280 to 300 kb) that harbour virulence, fitness, and MDR genes were first described in Israel [8], and subsequently in Italy, Switzerland, Hungary, Russia, the United States, Latin America, Japan, and in Türkiye [9].

Additionally, some *S.* Infantis strains with pESI-like plasmid have been reported to carry a colistin-resistance gene *(mcr-1),* since colistin is considered as a last resort antibiotic for the treatment of infections caused MDR Gram negative bacteria [10].

Integrons, as one of the mobile genetic elements (MGEs), play a signicant role in the spread of antimicrobial resistance genes (ARGs) among bacteria. Integrons can capture one or more cassette genes and disseminate ARGs via transmissible plasmids and insertion sequences (ISs). Integrons are categorized into three classes according to the sequences of the *intI* gene, and the most prevalent class of integrons is class 1, frequently reported in Gram-negative bacteria [11].

The pathogenicity of *Salmonella* strains has been related to numerous virulence genes, located at different sites of bacteriel genome, plasmids, and prophages. Clusters of chromosomal virulence genes, termed *Salmonella* pathogenicity islands (SPIs), play important roles in adhesion, invasion, intracellular survival, systemic infection, fimbrial expression, antibiotic resistance, toxin production, and Mg^{2+} and iron uptake [12]. For example, genes in SPI-1 (such as *invA*, *orgA*, *prgH*, *sipB*, and *spaN)* encode a type 3 secretion system 1 (T3SS-1) that allows *Salmonella* to invade phagocytic and non-phagocytic cells. Genes such as *spiA* in SPI-2 encode a type 3 secretion system 2 (T3SS-2) that enables *Salmonella* to survive and multiply in host cells [13]. Plasmids also carry virulence genes that contribute to *Salmonella* pathogenicity. Of these, *spvB* is responsible for colonization of deeper tissues [14]. Therefore, virulence genes profile analysis is beneficial for estimating bacterial potential pathogenicity.

This study was designed to determine the antimicrobial resistance phenotypes, virulence gene profile, prevalence of integron 1 and 2 of *S.* Infantis isolated from internal organs of diseased broilers. In addition, selected six strains were further characterized using whole genome sequencing (WGS).

Materials and Methods

S. Infantis Isolates

In this study, 56 *S.* Infantis isolated from the internal organs (liver, heart, spleen) and joint fluid samples of diseased broilers brought to the Microbiology Laboratory of the Department of Microbiology, Faculty of Veterinary Medicine, Aydın Adnan Menderes University between 2021-2023 for diagnosis purposes were used as the material of the study. The isolation method and serotyping was performed according to the ISO 6579-1:2017 and Kauffmann-White scheme, respectively.

DNA Extraction

Genomic DNA for PCR analyses was extracted using boiling method, and the isolated DNA was stored at -20°C until use.

Molecular Confirmation

The isolates phenotypically identified as *Salmonellae*, were molecularly confirmed by amplifying the *invA* gene, which is genus specific [15]. The *fljB* gene was used as a marker for molecular identification of *S.* Infantis, using previously reported species spesific primers [16].

Antimicrobial Susceptibility Testing

Antimicrobial susceptibilities of the isolates were performed and evaluated according to Clinical Labrotory Standards Institue (CLSI, 2022) criteria using the disc diffusion method. Following discs were used: gentamicin (CN, 10 µg), imipenem (IPM, 10 µg), ceftazidime (CAZ, 30 µg), cefepime (FEB, 30 µg), aztreonam (ATM, 30 µg), ampicillin (AM, 10 µg), amoxycillin-clavulanic acid (AMC, 10/20 µg), sulfamethoxazole-trimethoprim (SXT, 1.25/23.75 µg), chloramphenicol (C, 30 µg), ciprofloxacin (CIP, 5 µg) and tetracycline (TE, 30 µg). *E. coli* ATCC 25922 was used as a control strain for antimicrobial susceptibility testing. The isolates that were resistant to at least one antimicrobial in three or more antimicrobial categories were defined as multi-drug resistant (MDR) [17].

Detection of Virulence Genes by PCR

The eleven virulence genes *(invA*, *sopB*, *sopE*, *sifA*, *spvC*, *pipD*, *spaN*, *stn*, *slyA*, *hilA,* and *spvR)* were examined as previously reported [18].

Detection of Integrons

Class I and II integrons were investigated as previously reported by Bass et al.[19] and Goldstein et al.[20].

A dendrogram was also constructed based on antimicrobial susceptibility, virulence and integron profiles of the isolates. The bands for each isolate were counted using the zero-one manual method, the data was then entered into the following site: *http://insilico.ehu.es/dice_upgma/*, dendrograms were plotted.

Whole-Genome Sequencing

For whole-genome sequencing, 6 isolates were selected based on antimicrobial resistance phenotype, virulence and integron profile. The genomic DNA of selected *S.* Infantis strains was extracted using the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. The quantity and quality of the extracted DNA were measured using a Qubit 3.0 (Thermo Fisher Scientific, Waltham, MA, USA). The sequencing libraries of genomic DNA were prepared with the Illumina Nextera XT DNA Library Preparation Kit (Illumina Inc, San Diego, CA, USA) and the paired-end (2x150 bp) sequencing was run on the NovaSeq 6000 platform (Illumina, San Diego, CA, USA).

Quality Control, Trimming, Assembling and Annotation

After trimming low-quality reads and removing adapter sequences using Trimmomatic v 0.36 [21], the quality of both raw reads and trimmed reads was assessed using FastQC v 0.11.9 *(http://www.bioinformatics.babraham.ac.uk/projects/fastqc/;* accessed on 19 August 2024). The de novo genome assembly was conducted using the SPAdes algorithm (v 3.14.1) by applying the default parameters [22]. The quality of assembly was evaluated using QUAST v.5.0.0 [23], and contigs longer than >200 bp were included in further analysis. Gene predictions and annotations were performed using the National Center for Biotechnology Information's (NCBI) Prokaryotic Genome Automatic Annotation Pipeline (PGAAP) [24].

WGS-Based Characterization of *S.* Infantis Strains

The MLST type and the presence of acquired antimicrobial resistance genes of the strains were searched using the bioinformatic tools available at the Center for Genomic Epidemiology (CGE) platform *(http://www.genomicepidemiology.org/).* Mutations in the topoisomerase II (*gyr*A) and topoisomerase IV *(parC)* genes that mediate fluoroquinolone resistance in the strains were also analyzed using CGE platform.

Phylogenetic Analysis

For phylogenetic analysis, whole genome sequences of 33 *S.* Infantis isolates of chicken and human origin from different countries were retrieved from the PATRIC *S.* Infantis database. The tree was generated with maximum likelihood estimation methods. The evolutionary relationship between the *S.* Infantis strains was inferred by the aligned core-genomes using the program RAxML in PATRIC.

Results

Antimicrobial Resistance, Virulence and Integron Profiles

The isolates revealed various rate of resistance to cipro-floxacin (n=54, 96.4%), tetracycline (n=54, 96.4%), sulfamethoxazole-trimethoprim (n=43, 76.8%), chloramphenicol (n=5, 8.9%), and ampicillin (n=5, 8.9%), but, all isolates were susceptible to amoxycillin-clavulanic acid, imipenem, ceftazidime, cefepime, and aztreonam. Additionally, two isolates were susceptible to all antimicrobials tested.

Of virulence genes examined, *invA*, *sopB*, *pipD*, *sifA*, *stn*, *spaN*, *slyA*, *hilA* were detected in all *S. Infantis* isolates, but *sopE*, *spvC*, *spvR* were detected in 17.6%, 8.9% and 5.4% of the isolates, respectively. Only one isolate was found to have all virulence genes.

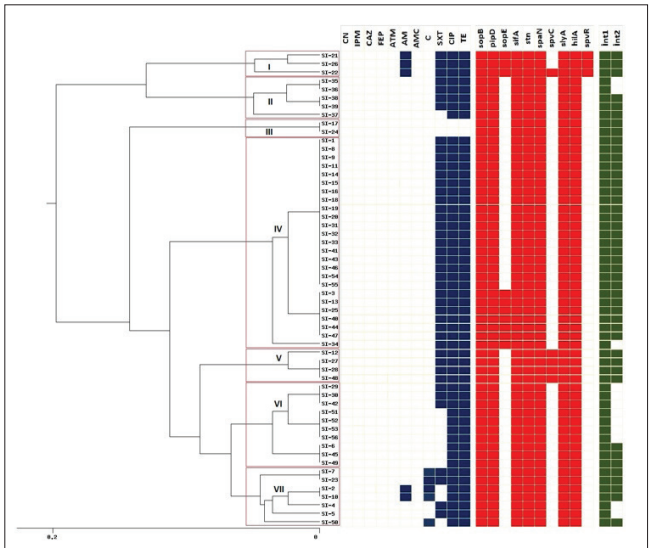

Fig 1. Dendrogram constructed based on antimicrobial susceptibility, virulence and integron profiles. Antimicrobial resistance, virulence and integrons are indicated blue, red and green squares, respectively. CN; gentamicin, IPM; imipenem, CAZ; ceftazidime, FEB; cefepime, ATM; aztreonam; AMP; ampicillin, AMC; amoxycillin-clavulanic acid, C; chloramphenicol, SXT; trimethoprim-sulfamethaxole, CIP; ciprofloxacin, TE; tetracycline

Table 1. *The sequence statistics and genome features of whole-genome sequencing analysis*

Characteristics	Data for the Isolates					
	ADU_VET1	ADU_VET2	ADU_VET3	ADU_VET4	ADU_VET5	ADU_VET6
No of contig	742	92	104	91	93	83
Genome size (bp)	5448326	5104776	4916112	4899282	4910037	4915377
Largest contig	123553	1195269	1195275	1179801	1171637	1195274
N50*	36616	203915	217644	203915	217644	217644
N90**	6623	56592	75578	52688	60144	67113
L50***	47	5	5	6	5	5
L90****	178	24	18	25	20	19
GC percent	51.56	52.11	52.15	52.17	52.16	52
Accession number	JBEOLT010000000	JBEOLU000000000	JBEOLV000000000	JBEOLW000000000	JBEOLX000000000	JBEOLY000000000

** A value that is equal to or greater than 50% of the total length of all the contigs; **A value that is equal to or greater than 90% of the total length of all the contigs; ***Smallest number of contigs whose length sum makes up half of genome size; **** Smallest number of contigs whose length sum makes up 90% of genome size*

Table 2. *Genetic characteristics of S. Infantis isolates*

Characteritics	ADU_VET1	ADU_VET2	ADU_VET3	ADU_VET4	ADU_VET5	ADU_VET6
Sequence Type (ST)	32	32	32	32	32	32
Incompatibility type	Col, IncFIB	IncFIB	IncFIB	IncFIB	IncFIB	IncFIB
Resistance phenotype	AMP, C, TE, CIP	C, SXT, TE, CIP	SXT, TE, CIP	SXT, TE, CIP	AM, SXT, TE, CIP	AM, SXT, TE, CIP
Antimicrobial resistance genes	*aac(6')-Iaa, blaTEM-1B, floR, qnrS1, tetA*	*aac(6')-Iaa, aph(6)-Id, aph(3")-Ib, aadA1, floR, sul1, sul2, tetA, dfrA14*	*aac(6')-Iaa, aadA1, sul1, tetA, dfrA14*	*aac(6')-Iaa, aadA1, sul1, tetA, dfrA14*	*aac(6')-Iaa, aadA2, aph(3')-Ia, aadA1, bla-TEM-1B, sul1, sul3, tetA, dfrA12*	*aac(6')-Iaa, aadA1, aph(3')-Ia, sul1, tetA, dfrA14*
Amino Acid Substitutions						
QRDR						
gyrA	(S83Y) TCC*TAC	(S83Y) TCC*TAC	(S83Y) TCC*TAC	(S83Y) TCC*TAC	(S83Y) TCC*TAC	(S83Y) TCC*TAC
parC	(T57S) ACC*AGC	(T57S) ACC*AGC	(T57S) ACC*AGC	(T57S) ACC*AGC	(T57S) ACC*AGC	(T57S) ACC*AGC

All isolates harbored integron genes, alone or in combination. In general, both class 1 and 2 integrons were detected in 76.8% (n=43) of the isolates, class 1 integron was only detected in 13 isolates (23.2%) alone. Dendrogram constructed based on antimicrobial susceptibility, virulence and integron profiles of the isolates was given in *Fig. 1*. Accordingly, *S.* Infantis isolates were assigned in seven categories.

Sequencing Statistics

The median length of genome assembly of the isolates was 4.92 Mbp with an average GC% of 52±0.215. The average N_{50} of the assembled contigs was 1.83 Mbp. Detailed sequence statistics and genome features are summarized in *Table 1*.

In Slico Detection of Acquired and Mutation Mediated Resistance

Based on the WGS analyses, 15 different types of acquired resistance genes found against various classes of antimicrobials including beta-lactams *(bla_{TEM-1B})*, aminoglycosides *(aac(6')-Iaa, aph(6)-Id, aph(3")-Ib, aadA1, aadA2,* and *aph(3')-Ia),* amphenicols *(floR),* sulphonamides *(sul1, sul2, sul3),* diaminopyrimidines *(dfr12, dfr14),* quinolones *(qnrS1),* and tetracycline *(tetA).* Amino acid substitutions in quinolone resistance-determining region (QRDR) of *gyr*A and *par*C genes were identified in all representative isolates *(Table 2).*

Sequence Type and Phylogenetic Analysis

WGS data indicated that all *S.* Infantis isolates belonged to ST32. For phylogenetic comparison, genomes of 33 *S.* Infantis strains of human and chicken present in the PATRIC were selected *(Fig. 2).* ADU_VET strains except ADU_VET1 were formed a separate cluster with ERUVET19 (isolated from chicken meat), indicating very high similarity. ADU_VET1 was formed a separate small cluster with Japan human isolates. But, other Türkiye isolate, Chicken/TR01 isolate, was closely related to human clinical isolates from Brazil and Bolivia, and a chicken isolate from Austria.

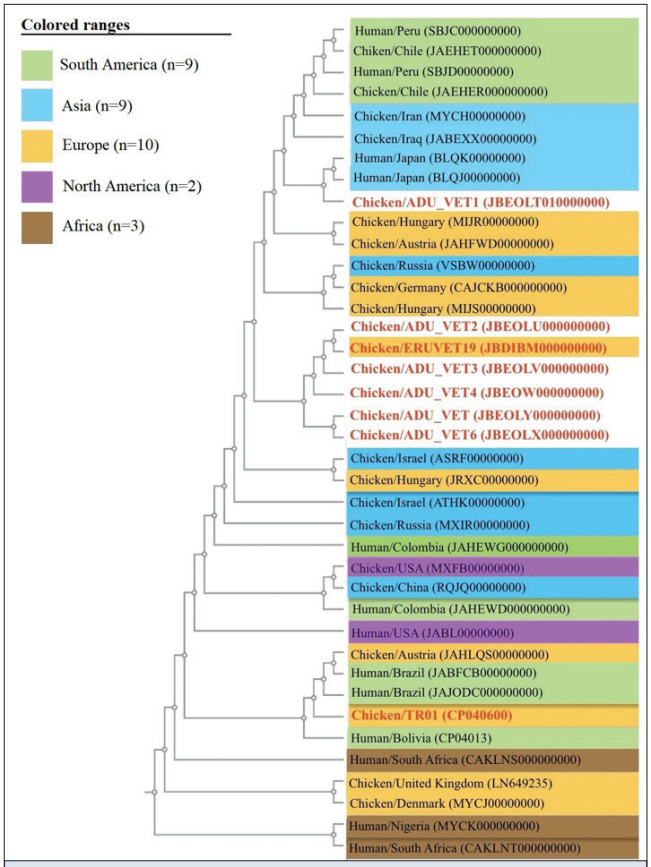

Fig 2. Maximum-likelihood phylogenetic tree based on core genome of 39 *S.* Infantis genomes from different regions of the World. ADU_VET and other Türkiye strains are depicted by bold red font. Geographic source of isolates is highlighted in different colors and GenBank accession numbers of each genome are given in parentheses

Data Availability

The genomic sequences for these isolates are available at NCBI under the BioProject number PRJNA1125001.

Discussion

In recent years, not only *S.* Infantis has become more prevalent serovar among broiler flocks, but also become one of the five serovars most frequently causing human salmonellosis in Europe, Israel, Japan, and United States, are widely related with the consumption of contaminated poultry meat [9]. In addition, among *S.* Infantis isolates from broiler flocks, both increasing rates of resistance and MDR have been reported. In this study, 96.4% (n=54) of *S.* Infantis isolates were found to be resistant to at least two or four antimicrobials, of which 46 (82.1%) were MDR with SXT-CIP-TE phenotype. The highest resistance rates were observed against ciprofloxacin (96.4%), sulfamethoxazole-trimethoprim (76.8%), and tetracycline (96.4%), respectively. In a recent study, Sarıçam İnce and Akan [25] examined a total of 133 *Salmonellae* belonging to four different serovars from chickens for their antimicrobial susceptibilities, and found higher resistance rates against resistance to nalidixic acid, sulphanamid, trimethoprim, sulfamethoxazole-trimethoprim, and tetracycline and high MDR (78.7%, 37/47) rate among *S.* Infantis isolates, compared to other serovars. In a similar study conducted by Kaya et al.[26], higher rate of MDR (89.3%) among 150 *S.* Infantis isolates were also reported, with high resistance rates against to nalidixic acid (94.6%), tetracycline (93.3%), sulphanamide (92.6%), sulphametoxazole-trimethoprim (81.3%), streptomicin (78%), but low resistance rates for chloramphenicol (7.3%), and ampicillin (6.6%). Higher tetracycline, ciprofloxacin and sulphametoxazole-trimethoprim resistance rates detected in this study could be attributed to ongoing the misuse and overuse of these antimicrobials in humans, animals and plants for years.

In abovementioned studies, ciprofloxacin resistance was not reported. However, increased rate of resistance to ciprofloxacin in the current study is important finding because ciproflaxacin is broadspectrum antimicrobial, active in a variety of infectious diseases, considered as one of critically important antimicrobials by WHO [27]. In *Salmonella* isolates, ciprofloxacin resistance is mainly attributed to mutations in quinolone resistance determining regions (QRDR) of *gyrA* and *parC* genes. Indeed, WGS analysis revealed mutation with Ser83Thr in *gyrA* and Thr57Ser in *parC* in ciprofloxaxin-resistant isolates. The co-existence of two single substitutions in these genes (*gyrA*: Ser83Tyr and *parC*: Thr57Ser) have been reported to be primary cause for ciprofloxacin resistance in *Salmonella* isolates by many researchers [28,29].

Among all tetracycline resistance mechanisms, *tetA* and *tetB* genes encoding efflux pump are recognized as the most common genes associated with resistance in *Salmonella* [29]. The WGS analysis revealed presence of *tetA* gene in tetracycline resistant isolates. Similar observation also reported by Sarıçam İnce and Akan [25], who found *tetA* as the most dominant gene in tetracycline resistant isolates.

The most common mechanism of resistance to sulfonamides is the acquisition of the dihydropteroate synthase enzyme encoded by the *sul1*, *sul2* and *sul3* genes, while main resistance mechanism for trimethoprim is dihydrofolate reductase enzyme encoded by the *dfr* genes [30]. WGS analysis revealed presence of *sul1*, *sul2*, *sul3*, *dfrA12* and *dfrA14* genes among trimethoprim-sulfamethoxazole resistant isolates. Of these genes, *sul1* is associated with class 1 integron, on the contrary, *sul2* genes has been detected on various plasmids but not associated with integrons [31]. Ahmed and Shimamoto [32] also reported *dfrA17*, *dfrA1* and *dfrA12* genes in class 1 integron in *Salmonella* isolated from diseased broilers. Lee et al.[33] reported that *dfr* genes confer to the highest levels of resistance to trimethoprim. The results suggest that presence of *sul* and *dfr* genes in *S.* Infantis isolates could

mainly attributed to the acquisition and dissemination of class 1 integron.

Several factors contribute the emergence and dissemination of antimicrobial resistance among *S.* Infantis strains. The bacteria develop antimicrobial resistance through acquisition of resistance genes via horizontal gene transfer (HGT) or target gene mutation. HGT usually occurs through mobile genetic elements such as plasmids, transposons and integrons, which allow resistance genes to spread rapidly among different species. The misuse and overuse of antibiotics pose selective pressure on bacteria, leading to the selection of resistant strains. This selective pressure also contributes to the maintenance and spread of resistance genes among S. Infantis strains, leading to a serious threat to public health. The combination of these factors increases the likelihood of resistant strains entering the food chain, thereby increasing the potential for transmission and treatment difficulties to humans [7,10].

Integrons are known to disseminate ARGs among bacteria via transmissible plasmids and ISs, posing a threat to public health [11]. In this study, class 1 integron was detected in all isolates, while class 2 integron was detected in 78.6% of the isolates, which is consistent with findings of Kaya et al.[26] who detected class 1 integron in all isolates. This show wide distribution of integrons among *S.* Infantis isolates. Without sequencing PCR products belonged to integrons is not possible to determine gen cassettes. However, when previous studies were evaluated, it has been seen that gene cassettes confer mostly resistance to aminoglycosides *(aadA1*, *aadA2*, *aadA5*, *aadA7*, *aadB*, *aac(3')-IIa*, *aph(4')-Ia*, *aac(4')-IV*, *aph(6')-Id),* beta-lactams *(bla*$_{\text{TEM-1}}$, *bla*$_{\text{CTX-M}}$, *bla*$_{\text{OXA-1}}$, *bla*$_{\text{PSE-1}}$*),* sulfonamides *(sul1, sul2),* amphenicoles *(floR)* and diaminopyrimidines *(dhfr17, dhfrA12, dhfrA1)* [34,35]. It could be speculated that some resistance genes detected in this study by WGS are of integron origin. Further studies are therefore needed to elucidate class 1 and 2 integron-associated gene cassettes from *Salmonella* isolates.

Regarding the virulence genes that were examined, all isolates carried *sopB*, *pipD*, *sifA*, *stn*, *spaN*, *slyA*, *hilA*, *invA* genes, of which *invA* was used PCR target gene for confirmation of *Salmonellae*, but *sopE* (n=10), *spvC* (n=5) and *spvR* (n=3) were detected in a limited number of the isolates. On the other hand, one isolate had all virulence genes examined. The *sopB* and *sopE* genes promote acute intestinal inflammation and fluid secretion by disrupting tight junctions between epithelial cells and challenging the inositol phosphate signaling pathways that prevent adequate chloride secretion [36]. In a study conducted in Türkiye, prevalence of *sopB* gene in the *S.* Infantis isolates was determined as 92.41% [26]. In other countries, higher prevalence rates of *sopB* above 94.1% have also been reported [37,38]. However lower prevalence of *sopE* (17.9%) was recorded in this study. In conrast, Karacan Sever and Akan [39] reported a higher rate for *sopE* (93.3%) among *S.* Infantis isolates. It was reported that *sopE* gene, which is carried by lysogenic bacteriophage, could contribute to the emergence of new epidemic strains and the epidemic success of strains carrying this gene [40].

The studies investigating the *hilA* gene, which is the transcriptional master regulator of the type III secretion system (T3SS), are very limited in *S.* Infantis is isolates [41]. The frequency of *hilA* gene among *Salmonella* isolates belonging different serovars were reported as 90% [42] and 94.4% [43].

While the *sifA* gene regulates the molecular mechanisms required for *Salmonellae* to enter and replicate in host cells, the *spaN* gene facilitates the entry of bacteria into non-phagocytosing cells and enables intracellular invasion through apoptosis in macrophages. Karacan Sever and Akan [39], in Türkiye, the rate of *sifA* in *S.* Infantis isolates was reported as 90.62%. In Spain, Lamas et al.[44] reported prevalence of *sifA* gene to be 67.16%. The *stn* and *slyA* are the genes responsible for enterotoxin and salmolysin production, respectively. The prevalence of *stn* gene has been reported to be 72.22% in Egypt [43] and 100% in India [45]. However, no study investigating *slyA* gene among *Salmonella* serovars from poultry has been present in Türkiye. However, in studies conducted abroad, the prevalence of this gene for *S.* Infantis *is* was reported as 100% [44,46].

In this study, *spvC* (8.9%) and *spvR* (5.4%) genes were detected in low rates. Chiu et al.[47] reported the *spv* genes are rarely seen in the *Salmonella* genome and are responsible for the systemic infection and multidrug resistance in humans and animals. The *spvC* gene has the ability to inhibit the activation of macrophages and initiate their apoptosis, and giving *Salmonellae* capacity to cause systemic infections [48]. Low prevalence rate of *spvC* gene was also reported by Karacan Sever and Akan [39], who reported a rate of 8.92% in *S.* Infantis isolates. On the other hand, Chaudhary et al.[48] reported the absence of this gene in all isolates. Deguenon et al.[49] therefore claimed that this gene is not systematically present in the *Salmonella* genome but is of paramount importance when present. The *spvR* gene encode a LysR-like transcriptional regulator that positively regulates the independent transcription of its own gene and that of the spvABCD operon [50]. There is no study investigating the *spvC* and *spvR* genes together in *S.* Infantis isolates of poultry origin in Türkiye and abroad. Therefore, it was not possible to compare the results obtained for *spv*R in our study. Lozano-Villegas et al.[38] state that presence of *spv* genes in different strains of *Salmonella* isolated from broilers and humans is associated with increased the possibility of *Salmonella* strains being clinical importance.

To conclude, the present study characterized 56 strains of *Salmonella* belonging to serovar Infantis. The findings of this study indicate higher rate of MDR and virulence genes among *S.* Infantis strains. This imply that these isolates might be able to evolve into a dominant clone with high zoonotic potential. Therefore, regardless of the serovars, continuous monitoring and surveillance of *Salmonella* strains for their antimicrobial resistance and virulence characteristics among the poultry industry is necessary to provides relevant risk assessment data and help to evaluate targeted interventions using advanced molecular technicques.

Declarations

Availability of Data and Materials: The authors declare that data supporting the study findings are also available from the corresponding author on reasonable request.

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Author Contributions: ÖA and ST planned, designed, and supervised the research procedure, ST performed all microbiological and molecular experiments, ÖA performed bioinformatic analyses, and ÖA wrote the manuscript. Both ÖA and ST have read and approved the manuscript.

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