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Analysis of the antibiotic resistance and virulence factors of *Salmonella* strains isolated on a turkey and goose farm

Nurdan Karacan Sever[1*], Neval Berrin Arserim[1] and Nida Özcan[2]

[1] Department of Microbiology, Faculty of Veterinary Medicine, Dicle University, Diyarbakır, Türkiye
[2] Department of Medical Microbiology, Faculty of Medicine, Dicle University, Diyarbakır, Türkiye

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ABSTRACT

The *Salmonella* (*S.*) *enterica* subspecies *enterica* is a major cause of foodborne gastroenteritis associated with the consumption of contaminated animal-derived food products (particularly of poultry origin). It is very important to be aware of the characteristics of *Salmonella* strains of poultry origin for the prevention and treatment of salmonellosis. The present study assesses the antibiotic resistance profiles and virulence factors of 64 *Salmonella* strains isolated from geese and turkeys, in line with the procedure defined in ISO 6579-1:2017. The isolated strains were then serotyped and analyzed phenotypically and genotypically for their resistance to various antibiotics, and their virulence potential was assessed on the basis of an analysis of 11 genes (*sipA, sopD, sopB, sifA, sitC, sipD, sopE, sopE*2, *ssaR, spvC* and *pefA)*. Of the *S*. Senftenberg isolated from the turkey fecal samples, and the *S*. Saintpaul and *S*. Typhimurium from the goose fecal samples, 98.41%, 90.48% and 85.71% were found to be highly resistant to enrofloxacin, gentamicin and amoxicillin, respectively; 85.71% of the *Salmonella* spp. strains were multi-drug resistant and 82.54% carried *int*1. It was further determined that 85.71% of the strains carried bla_{TEM}, 77.78% carried *qnrB* and 74.6% carried *qnrS*. Furthermore, *sopE* (98.41%) and *sipD* (96.82%) were detected in nearly all the *Salmonella* strains, and both of the identified *S*. Typhimurium strains (100%) were found to carry the *sipD, sopE2, sitC, spvC* and *pefA* genes. This analysis of the virulence potential of different *Salmonella* strains and their resistance to various antibiotics can be considered a comprehensive database, with the potential to support future studies addressing the topic. By revealing various characteristics of *Salmonella* strains, such as their virulence and resistance to antibiotics, which can seriously affect human health, the present study provides important data for possible disease or epidemic control.

Key words: antibiotic resistance; turkey; goose; *Salmonella*; virulence

Introduction

Salmonellosis is the second most commonly reported foodborne gastrointestinal infection in humans after campylobacteriosis (EFSA and ECDC, 2022), and the top five *Salmonella* serovars responsible for its development in humans have been reported by the European Food Safety Authority (EFSA) to be: *S*. Enteritidis, *S*. Typhimurium, monophasic *S*. Typhimurium, *S*. Infantis, and *S*. Derby (EFSA and ECDC, 2021; EFSA and ECDC, 2022). *Salmonella* is primarily transmitted to humans through the

* Corresponding author:
Dr. Nurdan KARACAN SEVER, Department of Microbiology, Faculty of Veterinary Medicine, Dicle University, Diyarbakır, Türkiye, e-mail: nurdankaracan@hotmail.com

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consumption of contaminated eggs and poultry products, although it can also be transmitted to poultry meat via fecal contamination, and to eggs through fecal-shell contact (HALD et al., 2016; SEVER and AKAN, 2019). In order to control foodborne salmonellosis, it is, therefore, crucial to monitor for its presence, and characteristics such as virulence and resistance to antibiotics in food-producing animals, especially poultry. The increased antimicrobial resistance to bacterial pathogens, including *Salmonella*, is a global public health concern, and the inappropriate and widespread use of antibiotics in poultry, among other food-producing animals, has been a major factor in the emergence of multi-drug resistant (MDR) *Salmonella* strains (CASTRO-VARGAS et al., 2020; COOPER et al., 2020). EFSA has reported high resistance rates to the antibiotics commonly used to treat salmonellosis in humans, such as ampicillin, chloramphenicol and sulfamethoxazole-trimethoprim, while lower resistance has been observed to fluoroquinolones (e.g., ciprofloxacin) and extended-spectrum cephalosporins (e.g., ceftriaxone) (EFSA and ECDC, 2023). The genes that encode antimicrobial resistance, which poses a significant risk to public health due to reduced treatment effectiveness, can integrate into such genetic elements as plasmids, transposons and integrons, and transpose between strains and different bacterial species, resulting in the emergence of new resistant strains (BENNETT, 1999; MAZEL, 2006; MICHAEL and SCHWARZ, 2016). Antibiotic-resistant strains are transmitted to humans through the consumption of animal-derived food products (FOLEY and LYNNE, 2008; YANG et al., 2020).

Salmonella serovars cause infections in humans and animals through their expression of various virulence factors, and their particular adhesion, invasion, survival and iron acquisition mechanisms (JAJERE, 2019). The genes that encode many of these factors are clustered in specific regions of the *Salmonella* chromosome, known as *Salmonella* Pathogenicity Islands (SPIs). The Type III Secretion System (T3SS)-1 encoded by SPI-1 plays a crucial role in the adhesion and invasion of intestinal epithelial cells, while T3SS-2, encoded by SPI-2, supports the proliferation of *Salmonella* in macrophages and epithelial cells (ZHANG et al., 2018). Effector proteins, such as *sipA, sipD, sopB, sopD, sopE* and *sopE*2 translocated by T3SS-1, are involved in the adhesion and invasion of host cells by *Salmonella*, along with fimbriae, encoded by plasmid-mediated *pefA*, as another important virulence factor at this stage. The *sitC* gene in the *sitABCD* operon within SPI-1 is responsible for the survival and proliferation of *Salmonella* in environments with limited iron (FOLEY et al., 2013; JAJERE, 2019), and the effector proteins *ssaR* and *sifA*, translocated by T3SS-2, are essential in the proliferation and survival phases (MORASI et al., 2022). The *Salmonella* virulence plasmid (*spv*) locus, including *spvC*, is required for *Salmonella* proliferation and survival in the host, and plays a role in the development of systemic infection (JAJERE, 2019; ZHANG et al., 2018).

The transmission and spread of infectious diseases in poultry depends on a combination of many factors, such as the species, the number of breeds present, contact between flocks, and the sanitary measures in place. To minimize the risk of introducing infectious agents into poultry flocks, and to control the spread of existing infections, effective biosecurity measures should be applied (VAN STEENWINKEL et al., 2011). In poultry flocks kept under ineffective biosecurity conditions, poultry of different age groups or different breeds may be raised together, leading to close contact with other poultry, animal keepers and wild birds, while shortfalls in disease control strategies, poor management, inadequate hygiene conditions can lead to the transmission of infectious agents to poultry and the spread of existing diseases (CONAN et al., 2012). For this reason, for the present study we chose to focus on small-scale poultry breeding enterprises with a high circulation of poultry, in which poultry from different sources are raised together, in which effective disinfection processes cannot be applied at the end of each production stage, and where poor biosecurity measures are applied.

The present study assesses *Salmonella* strains isolated from healthy geese and turkeys bred in the Turkish province of Diyarbakır, in terms of their serovar distribution, antibiotic resistance and virulence potential.

Materials and methods

Sample collection. Samples were collected between December 2020 and August 2021 from two small-scale poultry breeding enterprises in the province of Diyarbakır in southeast Türkiye, and both enterprises, engaged in turkey and goose breeding, respectively, kept flocks of 350-450 birds. In December 2020, a total of 100 fecal samples were collected from two flocks of different ages: 6 weeks old (n=45) and 10 weeks old (n=55) from a turkey farm using a sampling method that involved traversing a diagonal path through the flock and collecting the required number of samples in sterile fecal sample cups. For the second sample collection in August 2021, a total of 100 fecal samples were collected from 4-6 week-old geese from a free-range farm close to the Tigris River, using a method as similar as possible to the earlier turkey fecal sampling. All the samples were transferred in their sterile sample cups to the laboratory via a cold chain as soon as possible (approximately 30 min) for analysis.

Salmonella spp. isolation and serotyping. A 25-gram sample was taken from each fecal sample cup and subjected to *Salmonella* isolation according to the procedure defined in ISO 6579-1:2017 (ISO, 2017). Presumptive *Salmonella* isolates were tested serologically by slide agglutination for the presence of Poly (O) antigens using commercially sourced *Salmonella* O Antiserum Poly A-I and Vi (BD Difco, USA) according to the manufacturer's instructions, after which the identified *Salmonella* strains were confirmed by PCR with genus-specific primers targeting the *invA* gene, using the method described by RAHN et al. (1992). The *Salmonella* spp. strains were serotyped according to the Kauffmann-White schemes in the National *Salmonella* Reference Laboratory, Department of Microbiology, Faculty of Veterinary Medicine, Ankara University.

Phenotypic antimicrobial susceptibility testing. The susceptibility of the *Salmonella* strains to 12 different antibiotics from seven antibiotic classes was tested using the Kirby-Bauer disc diffusion method, following the guidelines of the Clinical and Laboratory Standards Institute (CLSI) (CLSI, 2008; CLSI, 2018a; CLSI, 2018b). The antibiotic discs utilized in the present study included: amoxicillin (10 µg), ampicillin (10 µg), ceftriaxone (30 µg), cefoxitin (30 µg), gentamicin (10 µg), ciprofloxacin (5 µg), enrofloxacin (5 µg), nalidixic acid (30 µg), trimethoprim/sulfamethoxazole (25 µg), doxycycline (30 µg), oxytetracycline (30 µg) and florfenicol (30 µg) (Bioanalyse, Ankara, Türkiye). Multidrug resistance (MDR) was defined as resistance to three or more antibiotic classes (TENOVER, 2006; SCHWARZ et al., 2010).

Detection of antibiotic resistance genes and class 1 integrons. The presence of the Integrase gene (*int*1) for detection of class 1 integron, extended-spectrum β-lactamase genes (ESBL), such as bla_{SHV}, bla_{TEM}, bla_{OXA} and bla_{CTX-M}, and plasmid-mediated quinolone resistance determinants (PMQRs), such as *qnrA, qnrB, qnrS* and *qepA* (Sentebiolab, Türkiye), in the *Salmonella* strains was analyzed by conventional PCR (Table 1). The DNA of the *Salmonella* strains extracted by the boiling method was used in all molecular analyses (SAMBROOK and RUSSELL, 2002), and all PCR amplifications were performed under optimized conditions based on the studies presented in Table 1. Amplifications: 2 µL of template DNA, 1µL forward primer (10pmol), 1µL reverse primer (10pmol), 0.5µL dNTPs (10mM dNTP mix), 3µL $MgCl_2$ (25mM), 2.5µL 10x PCR buffer solution, 0.2µL Taq DNA polymerase (ThermoScientific, USA) and a reaction mixture containing nuclease-free water with a final volume of 25 µL. The DNA was amplified in a thermal cycler (T100TM, Bio-Rad, Singapore) for 3 min at 94°C, followed by 30 cycles of 60 s at 94°C, 60 s at 54°C (60°C for bla_{CTX-M}, *qepA* and 50°C for bla_{SHV}) and 60 s at 72°C, and a final extension at 72°C for seven min (SAMBROOK and RUSSELL, 2002).

Detection of virulence genes. The presence of 11 virulence genes, including *sipA, sopD, sopB, sifA, sitC, sipD, sopE, sopE*2, *ssaR, spvC* and *pefA* (Sentebiolab, Türkiye) in all the *Salmonella* strains was analyzed by conventional PCR (Table 1). The reaction mixture for amplification was prepared as mentioned above, and the following amplification conditions were applied: 3 min at 94°C followed by 30 cycles of 1 min at 94°C, 1 min at 54°C (*sipA, sopD, sopB, sifA* and *sitC* at 61°C, *spvC* 51°C and *sopE* at 65°C), 1 min at 72°C and 7 min at 72°C (SEVER and AKAN, 2019).

The amplification conditions for the *pefA* gene were: 5 min at 95°C, 25 cycles of 30 s at 94°C, 30 s at 66.5°C, 2 min at 72°C and 10 min at 72°C (SKYBERG et al., 2006). Positive (*S.* Enteritidis ATCC 13076 and *S.* Typhimurium ATCC 700408) and negative (nuclease-free water) controls were included in each run. All the PCR products were separated through electrophoresis, using a 1.5% agarose gel and SafeView™ Classic (Applied Biological Materials, Canada) at 100 V for 60 min, and were visualized using a gel imaging system (Vilber Lourmat, France).

Table 1. Primer sequences, amplicon sizes and references for antibiotic resistance genes, virulence-associated genes and *int*1

Target genes	(5′-3′) Primer sequences	Amplicon size (bp)	Reference
*int*1	F-GCCTTGCTGTTCTTCTAC	558	GUERRA et al., 2004
	R-GATGCCTGCTTGTTCTAC		
bla_{TEM}	F-TTGGGTGCACGAGTGGGTTA	506	ARLET and PHILIPPON, 1991
	R-TAATTGTTGCCGGGAAGCTA		
bla_{CTX-M}	F-CGATGTGCAGTACCAGTAA	585	BATCHELOR et al., 2005
	R-TTAGTGACCAGAATCAGCGG		
bla_{SHV}	F-TTATCTCCCTGTTAGCCACC	795	ARLET et al., 1997
	R-GATTTGCTGATTTCGCTCGG		
bla_{OXA}	F-ATATCTCTACTGTTGCATCTCC	620	COLOM et al., 2003
	R-AAACCCTTCAAACCATCC		
*qnr*A	F-ATTTCTCACGCCAGGATTTG	516	ROBICSEK et al., 2006
	R-GATCGGCAAAGGTTAGGTCA		
*qnr*B	F-GATCGTGAAAGCCAGAAAGG	469	
	R-ACGATGCCTGGTAGTTGTCC		
*qnr*S	F-ACGACATTCGTCAACTGCAA	417	
	R-TAAATTGGCACCCTGTAGGC		
*qep*A	F-GCAGGTCCAGCAGCGGGTAG	199	YAMANE et al., 2007
	R-CTTCCTGCCCGAGTATCGTG		
invA	F-GTGAAATTATCGCCACGTTCGGGCAA	284	RAHN et al. 1992
	R-TCATCGCACCGTCAAAGGAACC		
sipA	F-ATGGTTACAAGTGTAAGGACTCAG	2055	SHAH et al., 2011
	R-ACGCTGCATGTGCAAGCCATC		
sipD	F-ATGCTTAATATTCAAAATTATTCCG	1029	
	R-TCCTTGCAGGAAGCTTTTG		
sopD	F-GAGCTCACGACCATTTGCGGCG	1291	RAFFATELLU et al., 2005
	R-GAGCTCCGAGACACGCTTCTTCG		
sopB	F-GCTCTAGACCTCAAGACTCAAGATG	1987	
	R-GCGGCCGCTACGCAGGAGTAAATCGGTG		

Table 1. Primer sequences, amplicon sizes and references for antibiotic resistance genes, virulence-associated genes and *int*1 (continued)

sopE	F-ATTGTTGTGGCGTTGGCATCGT R-AATGCGAGTAAAGATCCGGCCT	376	ZOU et al., 2011
*sopE*2	F-TACTACCATCAGGAGG R-GAATGTTTTATGTGACGCAG	995	RAFFATELLU et al., 2005
sitC	F-CAGTATATGCTCAACGCGATGTGGGTCTCC R-CGGGGCGAAAATAAAGGCTGTGATGAAC	768	SKYBERG et al., 2006
ssaR	F-GTTCGGATTCATTGCTTCGG R-TCTCCAGTGACTAACCCTAACCAA	1628	HU et al., 2008
sifA	F-ATGCCGATTACTATAGGCAATGG R-TTATAAAAAACAACATAAACAGCCG	1011	
spvC	F-ACTCCTTGCACAACCAAATGCGGA R-TGTCTCTGCATTTCGCCACCATCA	571	CHIU et al., 2006
pefA	F-GCGCCGCTCAGCCGAACCAG R-GCAGCAGAAGCCCAGGAAACAGTG	157	SKYBERG et al., 2006

Results

Salmonella spp. isolation and serotyping. A total of 94 (94%) *Salmonella* strains were isolated from the 100 fecal samples collected from turkeys, all which were serotyped as *S.* Senftenberg. Since the samples were collected from a single turkey farm and all the strains were *S.* Senftenberg, all further analyses in the study were performed on 54 randomly selected *S.* Senftenberg strains (Table 2). A total of nine (9%) *Salmonella* strains were isolated from the 100 fecal samples collected from the geese, seven (77.78%) of which were serotyped as *S.* Saintpaul, and two (22.22%) as *S.* Typhimurium.

Phenotypic antimicrobial resistance characterization of Salmonella spp. strains. The highest antibiotic resistances were detected against enrofloxacin (98.41%), gentamicin (90.48%), amoxicillin (85.71%), ampicillin (84.13%) and trimethoprim/sulfamethoxazole (79.16%) among the 63 strains, while all *Salmonella* spp. strains were susceptible to ceftriaxone and doxycycline. All *S.* Senftenberg strains were resistant to enrofloxacin, while the resistance rates to amoxicillin, ampicillin, gentamicin and trimethoprim/sulfamethoxazole were in the 88.89-96.3% range, and all were susceptible to ceftriaxone and doxycycline. The highest resistance rates in the *S.* Saintpaul strains were detected against gentamicin (71.43%) and enrofloxacin (85.71%), and all *S.* Saintpaul strains were susceptible to ceftriaxone, ciprofloxacin and doxycycline. Two *S.* Typhimurium strains were susceptible to all antibiotics

Table 2. The number and origin of the samples used for *Salmonella* testing

Origin of the samples		No. of samples	No. of isolates	No. of strains selected
Turkey farm	flock 1	45	41	19
	flock 2	55	53	35
	total	100	94	54
Goose farm	–	100	9	9

Table 3. Phenotypic antibiotic resistance of *Salmonella* strains isolated from geese and turkey fecal samples against seven classes of antibiotics

Antibiotics	*S.* Senftenberg (54)			*S.* Saintpaul (7)			*S.* Typhimurium (2)			Total (63)		
	S	I	R	S	I	R	S	I	R	S	I	R
Penicillins							n (%)					
AX	2 (3.7)	-	52 (96.3)	5 (71.43)	-	2 (28.57)	2 (100)	-	-	9 (14.28)	-	54 (85.71)
AM	3 (5.56)	-	51 (94.4)	5 (71.43)	-	2 (28.57)	2 (100)	-	-	10 (15.87)	-	53 (84.13)
Cephalosporins												
CRO	41 (75.92)	1 (1.85)	12 (22.22)	6 (85.71)	1 (14.29)	-	2 (100)	-	-	49 (77.78)	2 (3.17)	12 (19.05)
FOX	54 (100)	-	-	7 (100)	-	-	2 (100)	-	-	63 (100)	-	-
Aminoglycosides												
CN	2 (3.7)	2 (3.7)	50 (92.59)	-	2 (28.57)	5 (71.43)	-	-	2 (100)	2 (3.17)	4 (6.35)	57 (90.48)
Fluoroquinolones												
CIP	36 (66.67)	16 (29.63)	2 (3.7)	7 (100)	-	-	2 (100)	-	-	45 (71.43)	16 (25.4)	2 (3.17)
ENR	-	-	54 (100)	1 (14.29)	-	6 (85.71)	-	-	2 (100)	1 (1.59)	-	62 (98.41)
NA	1 (1.85)	34 (62.96)	19 (35.18)	5 (71.43)	1 (14.29)	1 (14.29)	2 (100)	-	-	8 (12.7)	35 (55.56)	20 (31.75)
Sulfonamides												
SXT	6 (11.11)	-	48 (88.89)	5 (71.43)	-	2 (28.57)	2 (100)	-	-	13 (20.63)	-	50 (79.36)
Tetracyclines												
DO	54 (100)	-	-	7 (100)	-	-	2 (100)	-	-	63 (100)	-	-
T	47 (87.04)	-	7 (12.96)	6 (85.71)	-	1 (14.29)	2 (100)	-	-	55 (87.3)	-	8 (12.7)
Amphenicols												
FFC	52 (96.3)	-	2 (3.7)	6 (85.71)	-	1 (14.29)	2 (100)	-	-	60 (95.24)	-	3 (4.76)
MDR		52 (96.3)			2 (28.57)			-			54 (85.71)	

R: resistant, I: intermediate, S: susceptible, MDR: multi drug resistance, AX: amoxicillin, AM: ampicillin, CRO: ceftriaxone, FOX: cefoxitin, CN: gentamicin, CIP: ciprofloxacin, ENR: enrofloxacin, NA: nalidixic acid, SXT: trimethoprim/sulfamethoxazole, DO: doxycycline, T: oxytetracycline, FFC: florfenicol

Table 4. Distribution of serovar, virulence and antibiotic resistance characteristics of *Salmonella* strains isolated from geese and turkey fecal samples in this study

Strain	Serovar	Virulotype	Class 1 Integron, ESBL and PMQR profile	Phenotypic resistance profile	No. of resistant antibiotic classes
1		*sipD, sitC, sopE*	*IntI*1, bla_{TEM}	AX, ENR, NA, T	3
2		*sipD, sopE*	*IntI*1, bla_{TEM}, bla_{CTX-M}, *qnrB, qnrS*	AX, AM, CRO, CN, ENR, SXT	5
3		*sipD, sopD, sitC, sifA, sopE*	*IntI*1, bla_{TEM}, *qnrB, qnrS*	AX, AM, CN, ENR, SXT	4
4		*sipD, sopE*	*IntI*1, bla_{TEM}, *qnrB, qnrS*	AX, AM, CN, ENR, SXT	4
5		*sipD, sopE*	*IntI*1, bla_{TEM}, *qnrB, qnrS*	AX, AM, CN, ENR, SXT	4
6		*sopE*	*IntI*1, bla_{TEM}, bla_{CTX-M}, *qnrB, qnrS*	AX, AM, CRO, CN, ENR, NA, SXT	5
7		*sipD, sopE*	*IntI*1, bla_{TEM}, *qnrB, qnrS*	AX, AM, CN, ENR, SXT	4
8		*sipD, sopD, sitC, sopE, sopB*	*IntI*1, bla_{TEM}, bla_{CTX-M}, *qnrB, qnrS*	AX, AM, CRO, CN, ENR, SXT	5
9		*sipD, sopD, sitC, sopE, sopB*	*IntI*1, bla_{TEM}, *qnrB, qnrS*	AX, AM, CN, ENR, SXT	4
10		*sipD, sopE*	*qnrB*	CN, ENR	2
11		*sipD, sopE*	*IntI*1, bla_{TEM}	AX, AM, CN, ENR, NA, SXT, T	5
12		*sipD, sopE*	*IntI*1, bla_{TEM}, bla_{CTX-M}, *qnrB, qnrS*	AX, AM, CRO, CN, ENR, SXT	5
13		*sipD, sopE*	*IntI*1, bla_{TEM}, *qnrB, qnrS*	AX, AM, CN, ENR, SXT	4
14	*S.* Senftenberg	*sipD, sopE*	*IntI*1, bla_{TEM}, bla_{CTX-M}, *qnrB, qnrS*	AX, AM, CRO, CN, ENR, SXT	5
15		*sipD, sopE*	*IntI*1, bla_{TEM}, bla_{CTX-M}, *qnrB, qnrS*	AX, AM, CRO, CN, ENR, SXT	5
16		*sipD, sopD, sitC, sopE*	*IntI*1, bla_{TEM}, bla_{CTX-M}, *qnrB, qnrS*	AX, AM, CRO, CN, ENR, SXT	5
17		*sipA, sipD, sopD, sitC, sopE, sopB*	*qnr*B	CN, ENR, NA	2
18		*sipD, sitC, ssaR, sopE*	*IntI*1, bla_{TEM}, bla_{CTX-M}, *qnrB, qnrS*	AX, AM, CRO, CN, ENR, SXT	5
19		*sipD, ssaR, sopE*	*IntI*1, bla_{TEM}, *qnrB, qnrS*	AX, AM, CN, ENR, SXT	4
20		*sipD, sopD, sitC, ssaR, sopE, sopB*	*IntI*1, bla_{TEM}	AX, AM, CN, ENR, NA, SXT, T	5
21		*sipD, sitC, sopE, sopB*	*IntI*1, bla_{TEM}, *qnrB*	AX, AM, CN, ENR, SXT	4
22		*sipD, sitC, sopE*	*IntI*1, bla_{TEM}, *qnrS*	AX, AM, CN, ENR, NA, T	4
23		*sipD, sitC, sopE*	*IntI*1, bla_{TEM}, *qnrB, qnrS*	AX, AM, CN, ENR, SXT	4
24		*sipD, sopD, sitC, sopE*	*IntI*1, bla_{TEM}, *qnrB, qnrS*	AX, AM, CN, ENR, SXT	4
25		*sipD, sopE*	*IntI*1, bla_{TEM}, *qnrB, qnrS*	AX, AM, CN, ENR, SXT	4
26		*sipD, sitC, sopE*	*IntI*1, bla_{TEM}, *qnrB, qnrS*	AX, AM, CN, ENR, SXT	4
27		*sipD, sopD, sitC, ssaR, sopE*	*IntI*1, bla_{TEM}, *qnrB, qnrS*	AX, AM, CN, ENR, SXT	4

Table 4. Distribution of serovar, virulence and antibiotic resistance characteristics of *Salmonella* strains isolated from geese and turkey fecal samples in this study (continued)

28		*sipD, sitC, sopE*	*IntI*1, $bla_{TEM,}$ *qnrB, qnrS*	AX, AM, CN, ENR, SXT	4
29		*sipD, sopE*	*IntI*1, $bla_{TEM,}$ *qnrB, qnrS*	AX, AM, CN, ENR, NA, SXT	4
30		*sipD, sopE*	*IntI*1, $bla_{TEM,}$ *qnrB, qnrS*	AX, AM, CN, ENR, NA, SXT	4
31		*sipD, sopD, sitC, ssaR, sopE*	*IntI*1, $bla_{TEM,}$ *qnrB, qnrS*	AX, AM, CN, ENR, NA, SXT	4
32		*sipD, sopD, sitC, ssaR, sifA, sopE*	*IntI*1, $bla_{TEM,}$ *qnrB, qnrS*	AX, AM, CN, ENR, NA, SXT	4
33		*sipD, sitC, sopE*	*IntI*1, $bla_{TEM,}$ *qnrB, qnrS*	AX, AM, CN, ENR, SXT	4
34		*sipD, sitC, sopE*	*IntI*1, $bla_{TEM,}$ *qnrB, qnrS*	AX, AM, CN, ENR, SXT	4
35	*S.* Senftenberg	*sipD, sitC, sopE*	*IntI*1, $bla_{TEM,}$ *qnrB, qnrS*	AX, AM, CN, ENR, NA, SXT, T	5
36		*sipD, sitC, sopE*	*IntI*1, $bla_{TEM,}$ $bla_{CTX-M,}$ *qnrB, qnrS*	AX, AM, CRO, CN, ENR, SXT	5
37		*sipD, sitC, sopE*	*IntI*1, $bla_{TEM,}$ *qnrB, qnrS*	AX, AM, ENR, SXT	3
38		*sipD, sopD, sitC, sopE*	*IntI*1, $bla_{TEM,}$ $bla_{CTX-M,}$ *qnrB, qnrS*	AX, AM, CRO, CN, ENR, SXT	5
39		*sipD, sopE*	*IntI*1, $bla_{TEM,}$ *qnrB, qnrS*	AX, AM, CN, ENR, NA, SXT	4
40		*sipD, sopD, sopE*	*IntI*1, $bla_{TEM,}$ *qnrB, qnrS*	AX, AM, CN, ENR, SXT, FFC	5
41		*sipD, sopE*	*IntI*1, $bla_{TEM,}$ *qnrB, qnrS*	AX, AM, CN, ENR, NA, SXT	4
42		*sipD, sopE*	*IntI*1, $bla_{TEM,}$ *qnrB, qnrS*	AX, AM, CN, CIP, ENR, NA	3
43		*sipD, sitC, sopE*	*IntI*1, $bla_{TEM,}$ *qnrB, qnrS*	AX, AM, CN, ENR, SXT	4
44		*sipD, sitC, sopE*	*IntI*1, bla_{TEM}	AX, AM, ENR, NA, T	3
45		*sipD, sopD, sitC, ssaR, sopE*	*IntI*1, $bla_{TEM,}$ *qnrB, qnrS*	AX, AM, CN, ENR, SXT	4
46		*sipD, sopE*	*IntI*1, $bla_{TEM,}$ $bla_{CTX-M,}$ *qnrB, qnrS*	AX, AM, CRO, CN, ENR, SXT	5
47		*sipD, sopD, sitC, ssaR, sopE*	*IntI*1, $bla_{TEM,}$ *qnrB, qnrS*	AX, AM, CN, ENR, SXT	4
48		*sipD, sopE*	*IntI*1, $bla_{TEM,}$ $bla_{CTX-M,}$ *qnrB, qnrS*	AX, AM, CRO, CN, ENR, SXT	5
49		*sipD, sopE*	*IntI*1, $bla_{TEM,}$ *qnrB, qnrS*	AX, AM, CN, CIP, ENR, NA, SXT, T, FFC	6
50		*sipD, ssaR, sopE*	*IntI*1, $bla_{TEM,}$ *qnrB, qnrS*	AX, AM, ENR, SXT	3
51		*sipD, sopE*	*IntI*1, $bla_{TEM,}$ *qnrB, qnrS*	AX, AM, CN, ENR, NA, SXT	4
52		*sipD, sopE*	*IntI*1, $bla_{TEM,}$ *qnrB, qnrS*	AX, AM, CN, ENR, NA, SXT	4
53		*sipD, sitC, sopE*	*IntI*1, $bla_{TEM,}$ *qnrB, qnrS*	AX, AM, CN, ENR, NA, SXT	4
54		*sipD, sitC, sopE*	*IntI*1, $bla_{TEM,}$ *qnrB, qnrS*	AX, AM, CN, ENR, SXT	4
55	*S.* Saintpaul	*sipD, sopE*	bla_{TEM}	AX, AM, CN, ENR, SXT	4
56		*sipD, sopD, sitC, ssaR, sopE, sopE2*	–	CN, ENR	2
57		*sipD, sitC, ssaR, sopE, sopE2, sopB*	–	–	–

Table 4. Distribution of serovar, virulence and antibiotic resistance characteristics of *Salmonella* strains isolated from geese and turkey fecal samples in this study (continued)

58	*S.* Saintpaul	*sipD, sitC, sopE, sopE2, sopB*	–	CN, ENR	2
59		*sipD, sopD, sitC, ssaR, sifA, sopE, sopE2, sopB*	–	ENR	1
60		*sitC, ssaR, sopE, sopE2, sopB*	*bla*$_{TEM}$	AX, AM, CN, ENR, SXT, T, FFC	6
61		*sipD, sopD, sitC, sopE, sopE2, sopB*	–	CN, ENR, NA	2
62	*S.* Typhimurium	*sipD, sopD, sitC, sopE2, spvC, pefA*	–	CN, ENR	2
63		*sipD, sitC, ssaR, sopE, sopE2, sopB, spvC, pefA*	–	CN, ENR	2

AX: amoxicillin, AM: ampicillin, CRO: ceftriaxone, FOX: cefoxitin, CN: gentamicin, CIP: ciprofloxacin, ENR: enrofloxacin, NA: nalidixic acid, SXT: trimethoprim/sulfamethoxazole, DO: doxycycline, T: oxytetracycline, FFC: florfenicol

Table 5. Antibiotic resistance patterns of *Salmonella* strains isolated from geese and turkey fecal samples

Pattern No	**Pattern**			***S.* Senftenberg (54)**	***S.* Saintpaul (7)**	***S.* Typhimurium (2)**	**Total (63)**
	R	**I**	**S**	**n (%)**			
1	AX, AM, CN, ENR, SXT	NA	CRO, FOX, CIP, DO, OT, FFC	14 (25.92)	1 (14.29)	–	15 (23.81)
2	AX, AM, CRO, CN, ENR, SXT	NA	FOX, CIP, DO, OT, FFC	8 (14.81)	–	–	8 (12.7)
3	AX, AM, CN, ENR, NA, SXT	–	CRO, FOX, CIP, DO, OT, FFC	8 (14.81)	–	–	8 (12.7)
4	AX, AM, CN, ENR, SXT	CIP, NA	CRO, FOX, DO, OT, FFC	5 (9.26)	–	–	5 (7.94)
5	CN, ENR	–	AX, AM, CRO, FOX, CIP, NA, SXT, DO, OT, FFC	–	2 (28.57)	2 (100)	4 (6.35)
6	AX, AM, CRO, CN, ENR,	CIP, NA	FOX, DO, OT, FFC	3 (5.56)	–	–	3 (4.76)
7	CN, ENR, NA	–	AX, AM, CRO, FOX, CIP, SXT, DO, OT, FFC	1 (1.85)	1 (11.11)	–	2 (3.17)
8	AX, AM, CN, ENR, NA,	CIP	CRO, FOX, DO, FFC	2 (3.7)	–	–	2 (3.17)
9	AX, ENR, NA, OT	CN	AM, CRO, FOX, CIP, SXT, DO, FFC	1 (1.85)	–	–	1 (1.59)
10	AX, AM, CN, CIP, ENR,	–	CRO, FOX, DO	1 (1.85)	–	–	1 (1.59)

Table 5. Antibiotic resistance patterns of *Salmonella* strains isolated from geese and turkey fecal samples (continued)

11	AX, AM, ENR, SXT	CIP, NA	CRO, FOX, CN, DO, OT, FFC	1 (1.85)		–	1 (1.59)
12	—	CN	AX, AM, CRO, FOX, CIP, ENR, NA, SXT, DO, OT, FFC	–	1 (11.11)	–	1 (1.59)
13	ENR	CN	AX, AM, CRO, FOX, CIP, NA, DO, OT, FFC	–	1 (11.11)	–	1 (1.59)
14	AX, AM, CN, ENR, SXT, OT, FFC	CRO	FOX, CIP, NA, DO	–	1 (11.11)	–	1 (1.59)
15	AX, AM, CRO, CN, ENR, NA, SXT	CIP	FOX, DO, OT, FFC	1 (1.85)	–	–	1 (1.59)
16	CN, ENR	NA	AX, AM, CRO, FOX, CIP, SXT, DO, OT, FFC	1 (1.85)	–	–	1 (1.59)
17	AX, AM, CN, ENR, SXT	–	CRO, FOX, CIP, NA, DO, OT, FFC	1 (1.85)	–	–	1 (1.59)
18	AX, AM, CN, ENR, NA, OT	–	CRO, FOX, CIP, SXT, DO, FFC	1 (1.85)	–	–	1 (1.59)
19	AX, AM, CN, ENR, NA, SXT	CIP	CRO, FOX, DO, OT, FFC	1 (1.85)	–	–	1 (1.59)
20	AX, AM, CN, ENR, NA, SXT, OT	–	CRO, FOX, CIP, DO, FFC	1 (1.85)	–	–	1 (1.59)
21	AX, AM, ENR, SXT	CRO, CIP, NA	FOX, CN, DO, OT, FFC	1 (1.85)	–	–	1 (1.59)
22	AX, AM, CN, ENR, SXT, FFC	CIP, NA	CRO, FOX, DO, OT	1 (1.85)	–	–	1 (1.59)
23	AX, AM, CN, CIP, ENR, NA	–	CRO, FOX, SXT, DO, OT, FFC	1 (1.85)	–	–	1 (1.59)
24	AX, AM, ENR, NA, OT	CN, CIP	CRO, FOX, SXT, DO, FFC	1 (1.85)	–	–	1 (1.59)

R: resistant, I: intermediate, S: susceptible, AX: amoxicillin, AM: ampicillin, CRO: ceftriaxone, FOX: cefoxitin, CN: gentamicin, CIP: ciprofloxacin, ENR: enrofloxacin, NA: nalidixic acid, SXT: trimethoprim/sulfamethoxazole, DO: doxycycline, T: oxytetracycline, FFC: florfenicol

except gentamicin and enrofloxacin (Table 3). All but two *Salmonella* spp. strains were resistant to at least two antibiotics, and 96.3% of the *S.* Senftenberg strains, 28.57% of the *S.* Saintpaul strains, and 85.71% of all the strains were multi-drug resistant. No multidrug resistance was detected in a total of nine strains, including two *S.* Senftenberg, five *S.* Saintpaul and two *S.* Typhimurium strains (Table 4).

The most common antibiotic resistance phenotype in the *S.* Senftenberg and all *Salmonella* spp. strains was the combination of amoxicillin, ampicillin, gentamicin, enrofloxacin and trimethoprim/sulfamethoxazole, while in the *S.* Saintpaul strains it was the combination of gentamicin and enrofloxacin (Table 5). Only one *S.* Saintpaul strain was susceptible to all the tested antibiotics.

Distribution of antibiotic resistance genes and class-1 integron in Salmonella spp. strains. The *int*1 gene was detected in 82.54% of all the *Salmonella* strains, and almost all (96.3%) of the *S.* Senftenberg strains. None of the *S.* Saintpaul and *S.* Typhimurium strains carried *int*1. The results of the analysis of *int*1 and the antibiotic resistance genes are presented in Table 6. It was determined that 85.71% of the

Salmonella strains carried bla_{TEM}, 19.05% carried bla_{CTX-M}, 77.78% carried *qnrB* and 74.6% carried *qnrS*, while bla_{SHV}, bla_{OXA}, *qnrA* and *qepA* were not detected in any of the *Salmonella* spp. strains, and ESBL and PMQR were not detected in the *S*. Typhimurium strains.

All MDR *S*. Senftenberg strains carried at least one of the ESBL genes, and 48 (92.31%) carried at least one of the PMQR genes, while 12 of the MDR *S*. Senftenberg strains (23.08%) were ESBL and PMQR positive. No ESBL genes were detected in two non-MDR *S*. Senftenberg strains, but the strains were *qnrB* positive, and two of the *S*. Saintpaul strains were ESBL positive but PMQR negative (Table 4).

A total of six different ESBL and PMQR resistance patterns were detected in all *Salmonella* strains, with 44.44% of the strains distributed in bla_{TEM}, *qnrB* and *qnrS* combinations, and 28.57% distributed in $bla_{TEM,}$ $bla_{CTX,}$ *qnr*B and *qnr*S combinations. Similarly, the most common patterns in the *S*. Seftenberg strains were bla_{TEM}, *qnrB* and *qnrS* (51.85%) and $bla_{TEM,}$ $bla_{CTX,}$ *qnr*B and *qnr*S (33.33%) combinations (Table 7). It was determined that none of the *S*. Typhimurium strains carried *int1*, ESBL and PMQR, and two of the *S*. Saintpaul strains (28.57%) carried only bla_{TEM}.

Distribution of virulence genes among Salmonella spp. strains. sopE (98.41%) and *sipD* (96.82%) were detected in almost all the *Salmonella* strains, while the presence of other virulence

Table 6. The frequency of the antibiotic resistance genes in *Salmonella* strains isolated from geese and turkeys

Gene	*S.* Senftenberg (54)	*S.* Saintpaul (7)	*S.* Typhimurium (2)	Total (63)
	n (%)			
*int*1	52 (96.3)	–	–	52 (82.54)
bla_{TEM}	52 (96.3)	2 (28.57)	–	54 (85.71)
bla_{CTX-M}	12 (22.22)	–	–	12 (19.05)
bla_{SHV}	–	–	–	–
bla_{OXA}	–	–	–	–
*qnr*A	–	–	–	–
*qnr*B	49 (90.74)	–	–	49 (77.78)
*qnr*S	47 (87.04)	–	–	47 (74.6)
*qep*A	–	–	–	–

Table 7. Antibiotic resistance gene patterns of *Salmonella* strains isolated from geese and turkeys

Pattern	*S.* Senftenberg (54)	*S.* Saintpaul (7)	*S.* Typhimurium (2)	Total (63)
	n (%)			
*int*1, $bla_{TEM,}$ *qnr*B, *qnr*S	28 (51.85)	–	–	28 (44.44)
*int*1, $bla_{TEM,}$ $bla_{CTX,}$ *qnr*B, *qnr*S	18 (33.33)	–	–	18 (28.57)
*int*1, bla_{TEM}	4 (7.41)	–	–	4 (6.35)
bla_{TEM}	–	2 (28.57)	–	2 (3.17)
*qnr*B	2 (3.7)	–	–	2 (3.17)
*int*1, $bla_{TEM,}$ *qnr*B	1 (1.85)	–	–	1 (1.59)
*int*1, $bla_{TEM,}$ *qnr*S	1 (1.85)	–	–	1 (1.59)
–	–	5 (71.43)	2 (100)	7 (11.11)

Table 8. The frequency of the virulence genes in *Salmonella* strains isolated from geese and turkeys

Gene	*S.* Senftenberg (54)	*S.* Saintpaul (7)	*S.* Typhimurium (2)	Total (63)
	n (%)			
sipA	1 (1.85)	–	–	1 (1.59)
sipD	53 (98.15)	6 (85.71)	2 (100)	61 (96.82)
sopD	14 (25.92)	3 (42.86)	1 (50)	18 (28.57)
ssaR	9 (16.67)	4 (57.14)	1 (50)	14 (22.22)
sopB	5 (9.26)	5 (71.43)	1 (50)	11 (17.46)
sopE	54 (100)	7 (100)	1 (50)	62 (98.41)
*sopE*2	–	6 (85.71)	2 (100)	8 (12.7)
sifA	2 (3.7)	1 (14.29)	–	3 (4.76)
sitC	29 (53.7)	6 (85.71)	2 (100)	37 (58.73)
spvC	–	–	2 (100)	2 (3.17)
pefA	–	–	2 (100)	2 (3.17)

genes was in the range of 1.59-58.73% (Table 8). All the *S.* Senftenberg strains were *sopE* positive, with the most frequent genes in these strains being *sipD* (98.15%) and *sitC* (53.7%), while the presence of other virulence genes was in the range of 1.85–25.92%. All the *S.* Senftenberg strains were *sopE*2, *spvC* and *pefA* negative, and all the *S.* Saintpaul strains were *sopE* positive. In the *S.* Saintpaul strains, the *sipD, sitC, sopE*2 (85.71%) and *sopB* (71.43%) genes were detected most frequently, and the presence of other virulence genes was in the range of 14.29-57.14%. All the *S.* Saintpaul strains were *sipA*, *spvC* and *pefA* negative. Both the *S.* Typhimurium strains were positive for *sipD*, *sopE*2, *sitC*, *spvC* and *pefA*, and negative for *sipA* and *sifA*. The virulence gene distribution in the *Salmonella* strains is presented in Table 8.

An analysis of the virulence gene patterns revealed 22 different virulotypes in all *Salmonella* strains, with the most common virulotypes in all *Salmonella* strains and *S.* Senftenberg strains being *sipD, sopE,* and *sipD, sitC and sopE*. The *S.* Saintpaul and *S.* Typhimurium strains each contained different gene combinations (Table 9).

Discussion

Salmonella is one of the leading causes of foodborne outbreaks in humans, and has been responsible for many sporadic cases and epidemics (EFSA and ECDC, 2022). It is very important to be aware of the various genotypic and phenotypic characteristics of *Salmonella* strains of poultry origin, such as their virulence and resistance to antibiotics, for the sake of prevention and treatment of human salmonellosis. In the present study, the *Salmonella* isolation rate from turkey fecal samples was 94%, and all strains were identified as *S.* Senftenberg. PALMEIRA et al. (2016) reported *S.* Senftenberg to be the third most common serovar in turkey carcasses in Brazil, while CAFFREY et al. (2021) and SODAGARI et al. (2023) reported it to be the eighth most common serovar in turkey coops in Canada. *S.* Senftenberg has been isolated from healthy laying hens in Türkiye (DİKER et al., 2020), healthy chickens and ducks in Poland (SKARŻYŃSKA et al., 2017) and pork carcasses in China (LIU et al., 2022). The *Salmonella* isolation rate from geese in the present study was 9%, and the strains were serotyped as *S.* Saintpaul (77.78%) and *S.* Typhimurium (22.22%). In the reports by BINKLEY (2015) and GUVEN et al. (2002), no *Salmonella* was identified in any of the analyzed fecal samples from geese, while studies in China (LIU et al., 2022), Iran (JAMALI et al., 2014) and Canada (JOKNIEN et al., 2011) reported prevalences of *Salmonella* in goose fecal samples of 10%, 22.7% and 10-15%, respectively. SKARŻYŃSKA et al. (2017) reported *S.* Typhimurium to be the most

common serovar in their analyzed goose samples, while WANG et al. (2020) reported it to be the second most common serovar. *S.* Saintpaul is among the four most common serovars in chicken carcasses and strains isolated from turkeys (SKARŻYŃSKA et al., 2017; ZWE et al., 2018), and has also been isolated in duck and goose feces in China (TANG et al., 2023). An analysis of the isolation rates and serovar diversity reported in different studies reveals a number of differences, which may be attributable to such factors as the development level and geographical location of the countries, the temperature-humidity values, the collection season, the origin and size of the analyzed sample, the different production units, and the laboratory conditions and isolation methods (HAN et al., 2020; RETAMAL et al., 2022; SODAGARI et al., 2023).

The widespread and inappropriate use of antibiotics in poultry has led to the emergence and spread of drug-resistant *Salmonella* strains, and has become a significant global concern (EFSA and ECDC, 2023). AL et al. (2016), GURAN et al. (2020) and RETAMAL et al. (2022) all reported resistance to enrofloxacin in their analyzed *Salmonella* strains at rates of 9.6-15.3%. In the present study, similar to BABABACAN and KARADENIZ (2019), the resistance

Table 9. Virulence gene patterns of *Salmonella* strains isolated from geese and turkeys

Pattern No	Pattern	*S.* Senftenberg (54)	*S.* Saintpaul (7)	*S.* Typhimurium (2)	Total (63)
		n (%)			
1	sipD, sopE	21 (38.89)	1 (14.29)	–	22 (34.92)
2	sipD, sitC, sopE	14 (25.92)	–	–	14 (22.22)
3	sipD, sopD, sitC, ssaR, sopE	4 (7.41)	–	–	4 (6.35)
4	sipD, sopD, sitC, sopE	3 (5.56)	–	–	3 (4.76)
5	sipD, ssaR, sopE	2 (3.7)	–	–	2 (3.17)
6	sipD, sopD, sitC, sopE, sopB	2 (3.7)	–	–	2 (3.17)
7	sipD, sopD, sitC, sifA, sopE	1 (1.85)	–	–	1 (1.59)
8	sipA, sipD, sopD, sitC, sopE, sopB	1 (1.85)	–	–	1 (1.59)
9	sipD, sitC, ssaR, sopE	1 (1.85)	–	–	1 (1.59)
10	sipD, sopD, sitC, ssaR, sopE, sopB	1 (1.85)	–	–	1 (1.59)
11	sipD, sitC, sopE, sopB	1 (1.85)	–	–	1 (1.59)
12	sipD, sopD, sitC, ssaR, sifA, sopE	1 (1.85)	–	–	1 (1.59)
13	sipD, sopD, sopE	1 (1.85)	–	–	1 (1.59)
14	sopE	1 (1.85)	–	–	1 (1.59)
15	sipD, sitC, ssaR, sopE, sopE2, sopB	–	1 (14.29)	–	1 (1.59)
16	sipD, sitC, sopE, sopE2, sopB	–	1 (14.29)	–	1 (1.59)
17	sipD, sopD, sitC, ssaR, sifA, sopE, sopE2, sopB	–	1 (14.29)	–	1 (1.59)
18	sitC, ssaR, sopE, sopE2, sopB	–	1 (14.29)	–	1 (1.59)
19	sipD, sopD, sitC, sopE, sopE2, sopB	–	1 (14.29)	–	1 (1.59)
20	sipD, sopD, sitC, ssaR, sopE, sopE2	–	1 (14.29)	–	1 (1.59)
21	sipD, sopD, sitC, sopE2, spvC, pefA	–	–	1 (50)	1 (1.59)
22	sipD, sitC, ssaR, sopE, sopE2, sopB, spvC,	–	–	1 (50)	1 (1.59)

to enrofloxacin of the identified *Salmonella* strains was very high (98.41%), while unlike the studies conducted in Brazil (PALMEIRA et al., 2016), Canada (CAFFREY et al., 2021), China (CHEN et al., 2023), Iran (JAMALI et al., 2014) and Nigeria (UGWU et al., 2019; IGBINOSA et al., 2022), the identified resistance to gentamicin (90.48%), amoxi- cillin (85.71%), ampicillin (84.13%) and trimethop- rim/sulfamethoxazole (79.16%) was also very high. This variation in results may be attributable to the origins of the samples (turkey, goose, chicken, duck, etc.), the sample type (feces, meat, dust, etc.), the number and diversity of the samples, and the anal- ysis method (RETAMAL et al., 2022; SODAGARI et al., 2023). The high resistance rate to antibiotics detected in the present study may be due to the in- tensive and inappropriate use of antibiotics in the region, and the collection of samples from a single farm may have further affected the results in terms of strain diversity. bla_{TEM}, one of the ESBL genes, was detected in almost all the *Salmonella* strains (85.71%), coinciding with the high resistance rate to amoxicillin and ampicillin. Similar to the study by CAFFREY et al. (2021), the resistance of *Salmonella* strains to ceftriaxone from third-generation cephalosporins is compatible phenotypically and genotypically (bla_{CTX}:19.05%). A high resistance to tetracycline, cefoxitin and florfenicol has been reported in studies conducted in Türkiye (AL et al., 2016; KAYA et al., 2017), and Tunisia (OUESLATI et al., 2021), although in the present study, the resistance of the strains to oxytetracycline (12.7%) and florfenicol (4.76%) was low, and all strains were found to be susceptible to doxycycline and cefoxitin, from among the second-generation cephalosporins. This may be due to the fact that the antibiotics commonly used in poultry vary from country to country, and from region to region. Among the quinolone group antibiotics, a very high resistance to enrofloxacin was noted, as well as medium-level resistance to nalidixic acid (31.75%) and low-level resistance to ciprofloxacin (3.17%). The resistance to nalidixic acid and ciprofloxacin detected in the present study is lower than that reported in other studies in Türkiye (AL et al., 2016; KAYA et al., 2017; GURAN et al., 2020), China (HAN et al., 2020), Tunisia (OUESLATI et al., 2021) and Nigeria (IGBINOSA et al., 2022). Similar to studies in China (ZHAO et al., 2021), Nigeria (JEMILEHIN et al., 2023) and Korea (KIM et al., 2013), *qnrS* was detected in the majority of the investigated strains (74.6%), while all the strains were *qnrA* and *qepA* negative. The high prevalence of *qnrB* (77.78%) reported in the present study differs from the previously mentioned studies. The low-level reported resistance to ciprofloxacin, which is widely used for the treatment of human infections, and the high resistance to enrofloxacin, which is in common use in poultry, are a matter of concern, along with the high *qnrS* and *qnrB* positivity. There is a concern that these genes, which are associated with antibiotic resistance, may be able to transfer horizontally to antibiotic-susceptible strains or to strains that do not carry these genes, through mobile genetic elements such as integrons in the gastrointestinal tract (BENNETT, 1999; MAZEL, 2006), increasing the potential transmission of resistant strains to poultry products and humans through the food chain.

The present study detected MDR in 85.71% of the *Salmonella* strains, and while this high rate was similar to previous studies in our country (AL et al., 2016; SAHAN et al., 2016; KAYA et al., 2017; GURAN et al., 2020), it is somewhat higher than those reported in studies in China (HAN et al., 2020; ZHAO et al., 2021) and Nigeria (IGBINOSA et al., 2022). The increase in the number and spread of MDR *Salmonella* strains raises serious public health concerns, and poultry products, as well as poultry itself, continue to be a matter of concern in this regard (GURAN et al., 2020). The results of the present study reveal that the majority of *Salmonella* strains (82.54%) carry the *int1* gene, while in previous studies, the reported presence of *int*1 in *Salmonella* strains was 20-100% (KAYA et al., 2017; ELKENANY et al., 2018; ALAM et al., 2020; GURAN et al., 2020; ZHAO et al., 2021; YAPICIER and OZTURK, 2022), while YANG et al. (2019) and OUESLATI et al. (2021) did not detect the *int1* gene in the *Salmonella* strains they analyzed. The Class 1 integron that carries the *int*1 gene contains gene cassettes that encode resistance to many antibiotics in the aminoglycoside, β-lactam, amphenicol and sulfonamide classes, and thus can be considered an important tool for the acquisition of

antibiotic gene resistance in strains or species susceptible to antibiotics (BENNETT, 1999; MAZEL, 2006). In the present study it was determined that all but two MDR *Salmonella* strains carried *int*1, which may point to a significant relationship between MDR and the presence of integron, as reported earlier by YAPICIER and OZTURK (2022).

The ability of *Salmonella* serovars to infect animals and humans is a result of the coding and expression of the virulence genes. The effector proteins encoded by virulence genes, which are involved in processes such as adhesion, invasion, proliferation, survival and iron recovery, play an important role in the pathogenesis of salmonellosis (JAJERE, 2019). In the present study, 11 virulence genes were analyzed in the *Salmonella* strains, and similar to previous studies in Türkiye (INCE and AKAN, 2023), Brazil (ALMEIDA et al., 2013; DANTAS et al., 2020), Thailand (UTRARACHKIJ et al., 2016) and Bangladesh (SIDDIKY et al., 2021), almost all carried the *sopE* (98.41%) and *sipD* (96.82%) genes that encode the effector proteins involved in the invasion of the host cell. HOPKINS and THRELFALL (2004) reported that the *sopE* gene, obtained through the lysogeny of the bacteriophage carrying the *sopE* gene, may play a role in the emergence of new epidemic strains, and so the finding that almost all the *Salmonella* strains in the present study carry this gene is somewhat remarkable. Similar to the study by SEVER and AKAN (2019), the *sitC* gene, which encodes the effector proteins required for the survival of *Salmonella* strains in iron-deficient conditions, was detected in more than half of the strains (58.73%) in the present study, while the presence of the *sipA* (1.59%), *sopB* (17.46%), *sopD* (28.57%) and *sopE2* (12.7%) genes that encode the effector proteins that play a role in adhesion and invasion by translocating to the host cell through T3SS-1, was less than reported in earlier studies (ALMEIDA et al., 2013; FARDSANEI et al., 2017; LI et al., 2017; DANTAS et al., 2020; MORASI et al., 2022; INCE and AKAN, 2023). Furthermore, the *ssaR* (22.22%) and *sifA* (4.76%) genes, which encode the proteins that play a role in invasion, replication and survival, by translocating to the host cell via T3SS-2, were also identified more frequently than in previous studies (ALMEIDA et al., 2013; FARDSANEI et al., 2017; GHETAS et al., 2021; INCE and AKAN, 2023). AMAVISIT et al. (2003) reported that the presence of SPIs may vary between different *Salmonella* serovars, and if this is the case, the presence or absence of virulence genes reported in the studies conducted to date may be attributable to the different *Salmonella* serovars analyzed. It is known that *spvC* plays a role in proliferation in the reticuloendothelial system and in the development of systemic infection, while *pefA* plays a role in adhesion, which is the first step of invasion (ZHANG et al., 2018; JAJERE, 2019). These plasmid-mediated genes were determined in only two strains (3.17%) in the present study, both of which were *S.* Typhimurium. In studies investigating the presence of plasmid-mediated virulence genes in different *Salmonella* serovars, *spvC* has been reported in the range of 0-100%, while *pefA* is reported in the range of 0.44–90% (FARDSANEI et al., 2017; LI et al., 2017; QIAO et al., 2017; GHETAS et al., 2021; SIDDIKY et al., 2021; OUESLATI et al., 2021; IGBINOSA et al., 2022; RETAMAL et al., 2022; INCE and AKAN, 2023). The fact that these genes, located on the virulence plasmids in the analyzed *Salmonella* strains, were detected at lower rates than the chromosomal genes may be attributable to the fact that the virulence plasmids are serovar-specific (SEVER and AKAN, 2019), although the chromosomal-plasmid DNA isolation protocol may also be responsible.

S. Typhimurium is one of the main serovars leading to the development of salmonellosis in humans. Both *S.* Typhimurium strains isolated in the present study were susceptible to the antibiotics tested (other than enrofloxacin and gentamicin), and while one of the *S.* Typhimurium strains carried six of the analyzed virulence genes (*sipD, sopD, sitC, sopE*2, *spvC* and *pefA*), the other carried eight (*sipD, sitC, ssaR, sopE, sopE*2, *sopB, spvC* and *pefA*). In the type of small-scale poultry breeding farms from which the samples analyzed in this study were collected, biosecurity cannot be applied effectively, coop hygiene is inadequate, and the uncontrolled entry and exit of people, including children, are known to occur. In this regard, the *S.* Typhimurium strains, which have virulence potential due to

the virulence-associated genes they carry, should raise serious concerns for both poultry and public health.

Conclusions

The present study brings together valuable information about the virulence potential and antibiotic resistance of *Salmonella* spp. strains isolated from healthy geese and turkeys. The *Salmonella* strains were found to have very high resistance to enrofloxacin, gentamicin, amoxicillin, ampicillin and trimethoprim/sulfamethoxazole, and most of the strains carried bla_{TEM} from ESBL and *qnrS* and *qnrB* from PMQR. Furthermore, the majority of *Salmonella* strains analyzed in this study were MDR and had a class-1 integron. The investigated strains were also found to carry many virulence genes, the virulence potential and antibiotic resistance of which pointed to a need for further study into the control of salmonellosis in humans and animals in Türkiye. Furthermore, analyzing *Salmonella* serovars and strains isolated from different regions using various genotypic methods would contribute greatly to the understanding of the pathogenesis and epidemiology of *Salmonella*.

Ethical approval

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. The samples used in the study were collected from small-scale poultry breeding farms in Diyarbakir, and in accordance with the regulation on the working procedures and principles of animal experimentation ethics committees, published in the official gazette dated 15 February 2014 and numbered 289114, 'collecting a fecal or litter sample', are not subject to HADYEK permission.

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Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Declaration of competing interest

The authors have no relevant financial or non-financial interests to disclose.

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KARACAN SEVER, N., N. B. ARSERİM, N. ÖZCAN: Analiza rezistencije na antibiotike i faktora virulencije sojeva bakterije *Salmonella* izoliranih na farmi purana i gusaka. Vet. arhiv 95, 229-250, 2025.

SAŽETAK

Salmonella (*S.*) *enterica* glavni je uzročnik gastroenteritisa povezanog s konzumacijom kontaminiranih prehrambenih proizvoda životinjskog podrijetla (posebno od peradi). Za prevenciju i liječenje salmoneloze iznimno je važno poznavati značajke sojeva navedene bakterije. U istraživanju je procijenjen profil rezistencije na antibiotike i faktori virulencije 64 soja salmonele izoliranih iz gusaka i purana, u skladu s normom ISO 6579-1:2017. Provedena je serotipizacija te fenotipska i genotipska analiza izoliranih sojeva kako bi se ustanovila rezistencija na različite antibiotike, a potencijal virulencije procijenjen je na temelju 11 gena (*sipA, sopD, sopB, sifA, sitC, sipD, sopE, sopE*2, *ssaR, spvC* i *pefA*). Otkriveno je da je 98,41% izolata sojeva *S*. Senftenberg dobivenih iz fecesa purana, zatim 90,48% uzoraka soja *S*. Saintpaul dobivenih iz fecesa gusaka i 85,71% uzoraka soja *S*. Typhimurium dobivenih također iz fecesa gusaka bilo visokorezistentno na enrofloksacin, gentamicin i amoksicilin. Osim toga, 85,71% sojeva *Salmonella* spp. bilo je rezistentno na više antibiotika, a 82,54% uzoraka sadržavalo je gen *int1*. Utvrđeno je zatim da je 85,71% sojeva bilo nositeljem gena bla_{TEM}, 77,78% izolata sadržavalo je gen *qnrB*, a 74,6% izolata bilo je nositeljem gena *qnrS*. Nadalje, geni *sopE* (prisutnost 98,41%) i *sipD* (prisutnost 96,82%) otkriveni su u gotovo svim sojevima bakterije *Salmonella*, a oba identificirana soja *S*. Typhimurium (prisutnost 100%) nositelji gena *sipD, sopE2, sitC, spvC* i *pefA*. Ova analiza potencijala virulencije sojeva *Salmonella* i njihove rezistencije na različite antibiotike može poslužiti kao sveobuhvatna baza podataka za buduća istraživanja. Otkrivanjem različitih značajki sojeva salmonele, kao što su virulencija i rezistencija na antibiotike, mogu se dobiti važni podaci za kontrolu i suzbijanje salmoneloze.

Ključne riječi: rezistencija na antibiotike; puran; guska; *Salmonella*; virulencija