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Determination of antibiotic resistance in *Salmonella* Typhimurium and *Salmonella* Kentucky serotypes of animal origin using conventional and molecular methods

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Abstract: The high incidence of multidrug-resistant *Salmonella* Typhimurium and *Salmonella* Kentucky isolates is a concern for human and animal health. This study aimed to investigate the antibiotic resistance determinants of a total of 150 *S. Typhimurium* and *S. Kentucky* isolates obtained from cows, calves, lambs, and poultry. *Salmonella* isolates were tested against 13 different antimicrobials using the Kirby-Bauer disc diffusion method, and genotypic antimicrobial resistance determinants were investigated by polymerase chain reaction. Significant differences were detected among serovars for gentamicin, streptomycin, ampicillin, sulfonamide, nalidixic acid, ciprofloxacin, and tetracycline resistance, with the rates of resistance to these antibiotics being determined to be 57.8%, 82.2%, 60%, 56.7%, 71.1%, 67.8%, and 50%, respectively for the *S. Kentucky* isolates and 6.7%, 20%, 25%, 33.3%, 8.3%, 0%, and 11.7%, respectively for the *S. Typhimurium* isolates. The rates of multidrug resistance (MDR) of the *S. Typhimurium* and *S. Kentucky* isolates were 16.7% and 62.2%, respectively. MDR for *S. Typhimurium* was detected in lambs, calves, and chickens at the rates of 100%, 40%, and 12.2%, respectively, while it was not detected in geese, turkeys, and gulls. The most prevalent resistance genes were *tetA*, *sulI*, *strA*, and *strB*. Our study has revealed that the strains isolated from livestock have a higher rate of phenotypes and genotypes with multidrug resistance compared to those isolated from poultry. These results emphasize the importance of using antibiotics with greater caution and awareness in food-producing animals.

Key words: Antimicrobial resistance, livestock, poultry, *Salmonella* Kentucky, *Salmonella* Typhimurium

1. Introduction

Salmonella is an important zoonotic microorganism, ranking second only to *Campylobacter* in foodborne infections, and poses a threat to both animal and human health worldwide, including in Türkiye [1,2]. There are more than 2600 serotypes of *Salmonella enterica* that vary in terms of geographic distribution, host diversity, and infectivity in humans [3]. Nontyphoid *Salmonella* (NTS) is a common cause of bacterial gastroenteritis globally, with 153 million cases and 57,000 deaths reported each year [4]. Eighty-five percent of human salmonellosis cases are transmitted through contaminated food, and these foodborne outbreaks are mostly associated with the consumption of contaminated poultry meat, eggs, egg-based food products, red meat and its products, and food made from contaminated milk and dairy products [1,5]. *Salmonella enterica* serovar Typhimurium (*S. Typhimurium*) is the second most common serotype associated with human salmonellosis in the USA, European Union, and Türkiye [2,6,7]. An important characteristic of

S. Typhimurium is the emergence of multidrug-resistant (MDR) phenotypes, which complicate the treatment of NTS infections in humans and animals. In particular, *S. Typhimurium* DT104 [definitive phage type 104], which causes infections in many host species, including humans and food-producing animals, has emerged as a strain resistant to five commonly used antimicrobial drugs (ampicillin, chloramphenicol, streptomycin, sulfonamide, and tetracycline) and has caused significant public health problems worldwide [6,7]. While nalidixic acid (a fluoroquinolone) and ceftriaxone (a third-generation cephalosporin) were considered alternatives to broad-spectrum cephalosporins and fluoroquinolones in the treatment of *Salmonella* infections in the past, resistance to these drugs was reported in the late 1990s following an epidemic in cattle and humans in the USA [8]. In the 2010s, *Salmonella enterica* serovar Kentucky (*S. Kentucky*) ST198-X1 resistant to ciprofloxacin and other antibiotics (ampicillin, streptomycin, gentamicin, sulfonamide, and tetracycline) emerged in Southeast Asian countries

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and spread worldwide [9]. *S. Kentucky*, often exhibiting a multidrug-resistant phenotype, is more commonly associated with poultry farming, although it has also been reported in cattle. Despite being implicated in human clinical cases less frequently compared to other *Salmonella* serovars, *S. Kentucky* has the ability to acquire and spread plasmids that increase virulence and colonization in poultry [10].

The aim of this study was to ascertain the antimicrobial resistance (AMR) profiles and prevalence of the resistance genes of *S. Typhimurium* and *S. Kentucky* serovars isolated from various animal sources. To the best of our knowledge, this is the most comprehensive study to date, encompassing different regions and years and including a large number of samples to investigate differences in the antimicrobial resistance profiles of *S. Typhimurium* and *S. Kentucky* serovars isolated from calves, cattle, lambs, chickens, turkeys, and geese in Türkiye.

2. Materials and methods

2.1. *S. Typhimurium* and *S. Kentucky* strain

Sixty *S. Typhimurium* and 90 *S. Kentucky* strains from the strain collection of the Bacteriological Diagnosis Laboratory of the Veterinary Control Central Research Institute were included in the study. These strains had been isolated from cows, calves, lambs, and poultry houses with different rearing types (95% commercial enterprises and 5% family farms) located in different geographical regions

of Türkiye (Mediterranean, Aegean, Marmara, Central Anatolia, Southeastern Anatolia, Eastern Anatolia, and Western and Central Black Sea) at different times from 2011 through 2020 (Table 1). Therefore, each strain can be considered independent.

All samples were analyzed for *Salmonella* according to the ISO 6579:2002/Amd1:2007 and ISO 6579-1:2017 standards. Serotyping was conducted to confirm *S. Typhimurium* and *S. Kentucky* serotypes and detect surface antigens (LPS, O-antigens) and flagellar antigens (protein, H-antigens). Antigenic combinations were assessed following the Kauffmann–White scheme [11].

2.2. Antimicrobial susceptibility test

The antimicrobial resistance profile of the NTS isolates was determined using a panel of 13 antibiotics, including those listed by the World Health Organization (WHO) as critically important (quinolones, aminoglycosides, carbapenems, penicillins, and cephalosporins) and highly important (tetracyclines, folate pathway inhibitors, and phenicols). Additionally, we prioritized antimicrobials currently approved for use in Türkiye in both veterinary and human medicine.

Antimicrobial susceptibility testing of the 150 *Salmonella* isolates was performed using the Kirby–Bauer disc diffusion method on Mueller–Hinton agar plates following Clinical and Laboratory Standard Institute (CLSI) guidelines [12]. Isolates were tested for sensitivity to ampicillin (AMP: 10 µg), cefotaxime (CTX: 30 µg), ceftazidime (CAZ: 30 µg), chloramphenicol (C: 30 µg),

Table 1. Distribution of *S. Typhimurium* and *S. Kentucky* isolates by origins.

Serotype	Animal	Strain number	Total
<i>S. Kentucky</i>	Chicken	65	90
	Goose	1	
	Calf	23	
	Cow	1	
<i>S. Typhimurium</i>	Chicken	49	60
	Goose	1	
	Turkey	2	
	Gull	1	
	Calf	5	
	Lamb	2	
Total strain number		150	

tetracycline (TE: 30 µg), gentamicin (CN: 10 µg), nalidixic acid (NA: 30 µg), ciprofloxacin (CIP: 5 µg), sulfonamide (S3: 300 µg), trimethoprim/sulfamethoxazole (SXT: 23.75 µg), streptomycin (S: 10 µg), ceftiofur (FOX: 30 µg), and meropenem (MEM: 10 µg). *Escherichia coli* ATCC 25922 served as the reference strain. Multidrug resistance (MDR) was defined based on resistance to three or more different antimicrobial classes.

2.3. Detection of antibiotic resistance genes

DNA extraction was conducted using the boiling method on *Salmonella* strains. A loop and a single colony were taken from strains with pure cultures prepared on nutrient agar and added to Eppendorf tubes containing 200 µL of DNase-free water. Subsequently, the samples were centrifuged at 8000 rpm for 5 min, homogenized, and the upper liquid was discarded. Then, 100 µL of DNase-free water was added. After centrifugation at 8000 rpm for 5 min, the supernatant was discarded, and 50 µL of DNase-free water was added. Finally, the bacterial suspensions were boiled at 100 °C for 10 min using a heating block.

Polymerase chain reaction (PCR) screening for streptomycin resistance genes (*aadA1*, *aadA2*, *strA*, *strB*), sulfonamide resistance genes (*sul1*, *sul2*), tetracycline resistance genes (*tetA*, *tetB*), and chloramphenicol

resistance gene (*flo*) was performed using previously reported primers (Table 2) [13-16].

The PCR reaction mix in a volume of 25 µL for each sample contained 14.8 µL of nuclease-free water, 2.5 µL of 10X PCR buffer solution, 3 µL of MgCl₂ (25 mM), 0.5 µL dNTP (10 Mm) 1 µL of each 10 mM primer, and 0.2 µL of Taq DNA polymerase (Thermo Scientific, USA). Twenty-three microliters of the prepared master mix mixture was distributed into each 0.2 mL tube, to which 2 µL of sample DNA was added. The PCR protocol conditions for amplification were as follows: initial denaturation at 95 °C for 5 min, followed by 35 cycles of 95 °C for 30 s, 51 °C to 60 °C (depending on the primer) for 30 s, and 72 °C for 45 s, and final extension at 72 °C for 5 min. The amplicons were analyzed with 1.5% agarose gel electrophoresis (ThermoScientific, USA). The samples in the gel were visualized using a bioimaging system (Syngene).

2.4. Statistical analysis

The data were analyzed using IBM SPSS Statistics Standard Concurrent User v. 26 (IBM Corp., Armonk, New York, USA). Descriptive statistics were presented as the number of units (n) and percentages (%). Relationships between categorical variables were assessed using the Pearson chi-square and Fisher’s exact tests. A p-value of <0.05 was considered statistically significant.

Table 2. Primary sequences of antimicrobial resistance genes.

Antimicrobial class	Resistance genes	Primer sequence (5'- 3')	Size (bp)	Annealing temperature (°C)	Reference
Aminoglycosides	<i>aadA1</i>	F: TATCAGAGGTAGTTGGCGTCAT R: GTTCCATAGCGTTAAGGTTTCATT	484	56	13
	<i>aadA2</i>	F: TGTTGGTTACTGTGGCCGTA R: GATCTCGCCTTTCACAAAGC	2019	58	14
	<i>strA</i>	F: CTT GGT GAT AAC GGC AAT TC R:CCA ATC GCA GAT AGA AGG C	548	51	15
	<i>strB</i>	F: ATC GTC AAG GGA TTG AAA CC R: GGA TCG TAG AAC ATA TTG GC	509	52	15
Amphenicols	<i>flo</i>	F: CTG AGG GTG TCG TCA TCT AC R: GCT CCG ACA ATG CTG ACT AT	673	56	16
Sulphonamides	<i>sul 1</i>	F: TCA CCG AGG ACT CCT TCT TC R: CAG TCC GCC TCA GCA ATA TC	331	57	16
	<i>sul 2</i>	F: CCT GTT TCG TCC GAC ACA GA R: GAA GCG CAG CCG CAA TTC AT	435	57	16
Tetracyclines	<i>tetA</i>	F: GCG CCT TTC CTT TGG GTT CT R: CCA CCC GTT CCA CGT TGT TA	831	60	16
	<i>tetB</i>	F: CCC AGT GCT GTT GTT GTC AT R: CCA CCA CCA GCC AAT AAA AT	723	59	16

F, forward; R, reverse.

3. Results

3.1. Antimicrobial susceptibility test

The results of the antimicrobial susceptibility testing of *S. Typhimurium* and *S. Kentucky* isolates are presented in Table 3. Statistically significant differences were observed between the *S. Typhimurium* and *S. Kentucky* serovars regarding their resistance to CN, S, AMP, S3, NA, CIP, and TE ($p < 0.05$). The *S. Kentucky* serovars exhibited resistance to CN, S, AMP, S3, NA, CIP, and TE at rates of 57.8%, 82.2%, 60%, 56.7%, 71.1%, 67.8%, and 50%, respectively (Table 3). The resistance rates of the *S. Typhimurium* serovars to CN, S, AMP, S3, NA, and TE were 6.7%, 20%, 25%, 33.3%, 8.3%, and 11.7%, respectively. No CIP resistance was detected among these serovars (Table 3).

Among the *S. Kentucky* isolates, statistically significant differences were found in resistance to CN, AMP, S3, CIP, and TE according to animal species ($p < 0.05$). The rates of resistance to CN were 100%, 100%, 91.3%, and 43.3% for dairy cows, geese, calves, and chickens, respectively. AMP resistance was detected at rates of 100%, 100%, and 91.3% among the geese, dairy cows, and calves, respectively. Resistance to S3 was observed in all dairy cows and geese, as well as 95.7% of calves and 40.3% of chickens with *S. Kentucky* strains. The rate of resistance to CIP among the dairy cows, geese, calves, and chickens was found to be 100%, 100%, 100%, and 53.7%, respectively. Lastly, TE resistance was detected at rates of 100%, 100%, 91.3%, and 32.8% among dairy cows, geese, calves, and chickens (Table 3).

There were also statistically significant differences in the resistance to S and TE among the *S. Typhimurium* isolates according to animal species ($p < 0.05$). The S resistance rates were determined to be 40%, 100%, 100%, 100%, and 12.2%, respectively, among the calves, geese, lambs, gulls, and chickens. Additionally, the TE resistance rates were found to be 100%, 100%, 60%, and 6.1% among the geese, lamb, calves, and chickens, respectively (Table 3).

MDR statistically significantly differed between the *S. Typhimurium* and *S. Kentucky* serovars ($p < 0.05$). The rate of MDR was 16.7% for the *S. Typhimurium* strains and 62.2% for the *S. Kentucky* strains (Table 4). The common MDR profiles of the *S. Typhimurium* and *S. Kentucky* serovars were determined to be AMP-C-TE-S-S3 (5%) and CN-S-AMP-S3-NA-CIP-TE (36.7%), respectively.

There were also statistically significant differences between the MDR resistance of the *S. Typhimurium* and *S. Kentucky* serovars according to animal species ($p < 0.05$). In *S. Typhimurium* serovars, MDR was detected at rates of 100%, 40%, and 12.2% among lambs, calves, and chickens, respectively, while it was not present in geese, turkeys, or gulls. When the *S. Kentucky* serovars were

examined, MDR was detected in calves, dairy cows, geese, and chickens at rates of 91.3%, 100%, 100%, and 50.8%, respectively (Table 4).

3.2. Antimicrobial resistance genes results

There were statistically significant differences in the prevalence of the *strA*, *sul1*, and *tetA* genes according to animal species among the *S. Kentucky* isolates ($p < 0.05$). *strA* was found in calves, dairy cows, geese, and chickens at rates of 78.3%, 100%, 100%, and 18.5%, respectively (Table 3). The rates of *sul1* in calves, dairy cows, geese, and chickens were 87%, 100%, 100%, and 18.5%, respectively. *tetA* was found in 87% of calves, all dairy cows and geese, and 29.2% of chickens with *S. Kentucky* isolates.

The *S. Typhimurium* isolates also showed statistically significant differences in the prevalence of *strA*, *strB*, *aadA2*, *flo*, *sul1*, and *tetB* genes according to animal species ($p < 0.05$). The *strA*, *strB*, and *tetB* genes were only detected among the lambs, at rates of 50%, 100%, and 100%, respectively.

Lastly, statistically significant differences were found between the *S. Typhimurium* and *S. Kentucky* isolates in relation to the prevalence of *strA*, *strB*, *aadA2*, *sul1*, and *sul2* genes ($p < 0.05$). Among the *S. Typhimurium* isolates, *strA*, *strB*, *aadA2*, *sul1*, and *sul2* genes were detected at rates of 1.7%, 3.3%, 5%, 5%, and 6.7%, respectively. Among the *S. Kentucky* isolates, the rates of *strA*, *strB*, and *sul1* genes were 34.8%, 25.3%, and 37%, respectively, while *aadA2* or *sul2* were not detected.

4. Discussion

Infections caused by *S. Typhimurium* and *S. Kentucky*, especially ST198, pose a serious threat to human and animal health. It has been reported that food-producing animals are the most common sources of multidrug-resistant *S. Typhimurium* and *S. Kentucky* infections [10].

In this study, *S. Kentucky* isolates exhibited high resistance to S, NA, CIP, AMP, CN, S3, and TE at rates of 82.2%, 71.1%, 67.8%, 60%, 57.8%, 56.7%, and 50%, respectively. These results align with findings reported by other researchers [17-23]. Additionally, we observed that the resistance rates of *S. Kentucky* isolates to CN, AMP, S3, CIP, and TE were higher in cattle than in chickens ($p < 0.05$). It is plausible that the dissemination of *S. Kentucky* strains with MDR resistance among poultry was mitigated by superior hygiene, care, feeding, infrastructure conditions, and control measures in poultry enterprises compared to cattle enterprises.

The rates of S3, AMP, S, TE, NA, CN, and C resistance among the *S. Typhimurium* isolates were found to be 33.3%, 25%, 20%, 11.7%, 8.3%, 6.7%, and 5%, respectively, which is consistent with the findings of previous studies [19,20]. Among these isolates, there were also significant

Table 3. Comparison of the resistance rates of *S. Typhimurium* and *S. Kentucky* serovars according to origin.

Antimicrobials/genes		S. Kentucky (%)					p
		Chicken	Goose	Calf	Cow	Total	
Gentamicin	S	32 (49.2)	0	2 (8.7)	0	4 (4.4)	0.007
	I	4 (6.2)	0	0	0	34 (37.8)	
	R	29 (44.6)	1 (100)	21 (91.3)	1 (100)	52 (57.8)	
Streptomycin	S	12 (18.5)	0	0	0	12 (13.3)	0.385
	I	3 (4.6)	0	1 (4.3)	0	4 (4.5)	
	R	50 (76.9)	1 (100)	22 (95.7)	1 (100)	74 (82.2)	
<i>strA</i>		12 (18.5)	1 (100)	18 (78.3)	1 (100)	32	0.001
<i>strB</i>		15 (23.1)	0	7 (30.4)	1 (100)	23	0.279
Ampicillin	S	36 (55.4)	0	2 (8.7)	0	36 (40)	0.001
	R	29 (44.6)	1	21 (91.3)	1 (100)	54 (60)	
Cefoxitin	S	62 (95.4)	1 (100)	23 (100)	1 (100)	87 (96.7)	0.979
	I	2 (3.1)	0	0	0	2 (2.2)	
	R	1 (1.5)	0	0	0	1 (1.1)	
Ceftazidime	S	64 (98.5)	1 (100)	23 (100)	1 (100)	89 (98.9)	0.945
	I	1 (1.5)	0	0	0	1 (1.1)	
Cefotaxime	S	60 (92.3)	1(100)	20 (87)	1 (100)	82 (91.1)	0.943
	I	4 (6.2)	0	3 (13)	0	7 (7.8)	
	R	1 (1.5)	0	0	0	1 (1.1)	
Meropenem	S	65 (100)	1 (100)	23 (100)	1(100)	90 (100)	-
Chloramphenicol	S	64 (98.5)	1 (100)	22 (95.7)	1 (100)	88 (97.8)	0.873
	R	1 (1.5)	0	1 (4.3)	0	2 (2.2)	
<i>flo</i>		0	0	0	1	1	0.945
Sulfonamide	S	36 (55.4)	0	1 (100)	0	37 (41.1)	0.001
	I	2 (3.1)	0	0	0	2 (2.2)	
	R	27 (41.5)	1 (100)	22 (95.7)	1 (100)	51 (56.7)	
<i>sulI</i>		12 (18.5)	1 (100)	20 (87)	1 (100)	34	0.001
Trimethoprim/ Sulfamethoxazole	S	64 (98.5)	1 (100)	23 (100)	1 (100)	89 (98.9)	0.945
	R	1 (1.5)	0	0	0	1(1.1)	
Nalidixic acid	S	24 (36.9)	0	0	0	24 (26.7)	0.02
	I	2 (3.1)	0	0	0	2 (2.2)	
	R	39 (60)	1 (100)	23 (100)	1 (100)	64 (71.1)	
Ciprofloxacin	S	5 (7.7)	0	0	0	5 (5.5)	0.008
	I	24 (36.9)	0	0	0	24 (26.7)	
	R	36 (55.4)	1 (100)	23 (100)	1 (100)	61 (67.8)	
Tetracycline	S	42 (64.6)	0	1 (4.3)	0	43(47.8)	0.001
	I	1 (1.5)	0	1 (4.3)	0	2 (2.2)	
	R	22 (33.9)	1 (100)	21 (91.3)	1 (100)	45 (50)	
<i>tetA</i>		19 (29.2)	1 (100)	20 (87)	1 (100)	41	0.001

p < 0.05; R, resistant; S, susceptible; I, intermediate.

Table 3. Continued.

Antimicrobials/genes		S. Typhimurium (%)							p
		Chicken	Goose	Turkey	Gull	Calf	Lamb	Total	
Gentamicin	S	43 (87.8)	1 (100)	2 (100)	1 (100)	5 (100)	2 (100)	54 (90)	0.999
	I	2 (4.1)	0	0	0	0	0	2 (3.3)	
	R	4 (6.7)	0	0	0	0	0	4 (6.7)	
Streptomycin	S	37 (75.5)	0	2 (100)	1 (100)	3 (60)	0	42 (70)	0.026
	I	6 (12.2)	0	0	0	0	0	6 (10)	
	R	6 (12.2)	1 (100)	0	0	2 (40)	2 (100)	12 (20)	
<i>strA</i>		0	0	0	0	0	1 (50)	1 (1.7)	0.001
<i>strB</i>		0	0	0	0	0	2 (100)	2 (3.3)	0.001
<i>aadA2</i>		1 (2)	0	0	0	2 (40)	0	3 (5)	0.015
Ampicillin	S	38 (77.6)	1 (100)	2 (100)	1 (100)	3 (60)	0	45 (75)	0.151
	R	11(22.4)	0	0	0	2(40)	2(100)	15	
Cefoxitin	S	46 (93.9)	1(100)	2(100)	1 (100)	5 (100)	2(100)	57 (95)	0.999
	I	1 (2)	0	0	0	0	0	1 (1.7)	
	R	2 (4,1)	0	0	0	0	0	2 (3.3)	
Ceftazidime	S	48 (98)	1 (100)	2 (100)	1 (100)	5 (100)	2 (100)	59 (98.3)	0.999
	R	1 (2)	0	0	0	0	0	1 (1.7)	
Cefotaxime	S	46 (93.9)	1 (100)	2 (100)	1 (100)	5 (100)	2 (100)	57 (95)	0.999
	I	1 (2)	0	0	0	0	0	1 (1.7)	
	R	2 (4.1)	0	0	0	0	0	2 (3.3)	
Meropenem	S	49 (100)	1(100)	2(100)	1(100)	5(100)	2(100)	60(100)	-
Chloramphenicol	S	47 (95.9)	1 (100)	2 (100)	1 (100)	3 (60)	2 (100)	56 (93.3)	0.16
	I	1 (2)	0	0	0	0	0	1 (1.7)	
	R	1 (2)	0	0	0	2 (40)	0	3 (5)	
<i>flo</i>		1 (2)	0	0	0	2 (40)	0	3 (5)	0.015
Sulfonamide	S	34 (69.4)	1 (100)	1 (50)	1 (100)	3 (60)	0	40 (66.7)	0.356
	R	15 (30.6)	0	1 (50)	0	2 (40)	2 (100)	20 (33.3)	
Trimethoprim/ Sulfamethoxazole	S	47(95.9)	1 (100)	2 (100)	1 (100)	5 (100)	2 (100)	58 (96.7)	0.993
	R	2 (4.1)	0	0	0	0	0	2(3.3)	
<i>sul1</i>		1 (2)	0	0	0	2 (40)	0	3 (5)	0.015
<i>sul2</i>		4 (8.2)	0	0	0	0	0	4 (6.7)	0.966
Nalidixic acid	S	42 (85.7)	1 (100)	2 (100)	1 (100)	5 (100)	2 (100)	53 (88.3)	0.998
	I	2 (4.1)	0	0	0	0	0	2 (3.3)	
	R	5 (10.2)	0	0	0	0	0	5(8.3)	
Ciprofloxacin	S	15 (30.6)	0	2 (100)	0	3 (60)	0	20 (33.3)	0.17
	I	34 (69.4)	1 (100)	0	1 (100)	2 (40)	2 (100)	40 (66.7)	
Tetracycline	S	46 (93.9)	1 (100)	2 (100)	1 (100)	3 (60)	0	53 (88.3)	0.001
	R	3 (6.1)	0	0	0	2 (40)	2 (100)	7 (11.7)	
<i>tetB</i>		0	0	0	0	0	2(100)	2 (3.3)	0.001

p < 0.05; R, resistant; S, susceptible; I, intermediate.

Table 4. Multidrug resistance models of the *S. Typhimurium* and *S. Kentucky* serovars.

Serotype	Resistance phenotype	Total	Chicken	Goose	Calf	Cow	Lamb	p
Typhimurium	AMP-NA-S3	1	1	-	-	-	-	0.02
	AMP-S3-S	1	1	-	-	-	-	
	AMP-NA-CN	2	2	-	-	-	-	
	AMP-TE-SXT	1	1	-	-	-	-	
	AMP-TE-S-S3	2	-	-	-	-	2	
	AMP-C-TE-S-S3	3	1	-	2	-	-	
	MDR (%)	10 (16.7)	6 (12.2)	-	2 (40)	-	2 (100)	
Kentucky	S-AMP-NA	1	1	-	-	-	-	0.003
	S-S3-NA	1	1	-	-	-	-	
	CN-S-AMP-S3	1	1	-	-	-	-	
	CN-S-S3-TE	1	1	-	-	-	-	
	S-AMP-NA-CIP	1	1	-	-	-	-	
	S-S3-NA-CIP	1	1	-	-	-	-	
	CN-S-AMP-NA-CIP	2	2	-	-	-	-	
	CN-S-S3-NA-CIP	5	5	-	-	-	-	
	S-AMP-NA-CIP-TE	5	5	-	-	-	-	
	CN-S-AMP-S3-NA-CIP	1	1	-	-	-	-	
	CN-S-AMP-FOX-NA-CIP-TE	1	1	-	-	-	-	
	CN-S-AMP-S3-NA-CIP-TE	33	10	1	21	1	-	
	CN-S-AMP-SXT-NA-CIP-TE	1	1	-	-	-	-	
	CN-S-AMP-C-S3-NA-CIP-TE	1	1	-	-	-	-	
	CN-S-AMP-CTX-S3-NA-CIP-TE	1	1	-	-	-	-	
MDR (%)	56 (62.2)	33 (50.8)	1 (100)	21 (91.3)	1 (100)	-		

p < 0.05; MDR, multidrug resistance; AMP, ampicillin; CTX; cefotaxime; CAZ, ceftazidime; C, chloramphenicol; TE, tetracycline; CN, gentamicin; NA, nalidixic acid; CIP, ciprofloxacin; S3, sulfonamide; SXT, trimethoprim/sulfamethoxazole; S, streptomycin; FOX, ceftiofur; MEM, meropenem.

differences in S and TE resistance rates according to animal species (p < 0.05). These findings reveal S and TE as commonly used antibiotics in food-producing animals [7,24].

The resistance rates of the *S. Typhimurium* isolates were notably lower than those of the *S. Kentucky* isolates. Specifically, significant differences were observed in the resistance of the two groups of isolates to CN, S, AMP, S3, NA, CIP, and TE (p < 0.05). The detection of lower resistance rates in *S. Typhimurium* compared to other serovars is consistent with the findings reported by Liljebjelke et al. [20], Mellor et al. [7], and Inbaraj et al. [9].

The reason behind the emergence of this difference may be attributed to control programs and vaccination campaigns implemented for certain serovars (such as *S. Enteritidis* and *S. Typhimurium*) that pose a risk to public health, which may have affected the variation in *S. Typhimurium* and led to a decrease in the dissemination risk of strains with antimicrobial resistance.

Our study revealed that the fluoroquinolone resistance of *Salmonella* isolates was considerably higher than their resistance to cephalosporins and carbapenems. These results are similar to those reported by other researchers [9,23,25]. In particular, *S. Kentucky* isolates were found

to have high resistance rates against NA and CIP. The rates of FOX, CAZ, and CTX resistance were found to be low among both *S. Kentucky* (1.1%, 1.1%, and 1.1%, respectively) and *S. Typhimurium* (3.3%, 1.7%, and 3.3%, respectively) isolates. In the treatment of NTS infections, carbapenems are used as an alternative when resistance to quinolones and cephalosporins is detected. In our study, all *S. Kentucky* and *S. Typhimurium* isolates were found to be susceptible to meropenem.

In this study, significant differences were detected between the MDR rates of the *S. Typhimurium* and *S. Kentucky* serovars ($p < 0.05$). MDR was detected in 62.2% of the *S. Kentucky* isolates. This high rate of MDR in *S. Kentucky* serovars is consistent with the data previously reported from the USA [20], Spain [21], Canada [22], Europe [23], and Türkiye [19]. The dominant antimicrobial resistance profile of the *S. Kentucky* isolates was CN-S-AMP-S3-NA-CIP-TE, at 36.7%. An important finding of our study concerns the resistance profile of the *S. Kentucky* ST198 strain, which was resistant to CIP, AMP, S, S3, CIP, and TE, and showed MDR. In the *S. Kentucky* (ST198) strain, the presence of MDR genes, especially the cephalosporinase and carbapenemase genes on the plasmid, in addition to broad-spectrum beta-lactamase genes, poses a serious threat to public health [23,26]. A multidrug antimicrobial resistance profile was detected in the isolates of *S. Typhimurium* at a rate of 16.7%. The AMP-C-TE-S-S3 resistance profile associated with the *S. Typhimurium* DT104 strain was detected at a rate of 5% in chickens and calves. The resistance profile ASSuT (ampicillin, streptomycin, sulfonamide, and tetracycline) reported for the *S. Typhimurium* DT193 strain associated with human infections in other countries, such as Spain, England, and Wales [27], was only detected in lambs in our study.

The most common antimicrobial resistance patterns of multidrug-resistant *Salmonella* strains related to important therapeutic antimicrobial classes used in human treatment include penicillins, tetracyclines, cephalosporins, and fluoroquinolones. Strategies to prevent human MDR-foodborne *Salmonella* infections have been developed at the primary animal production level. Additionally, national surveillance programs of antimicrobial resistance in the animal food chain have been implemented.

We observed that the MDR resistance of *S. Typhimurium* and *S. Kentucky* serovars statistically significantly differed according to animal species ($p < 0.05$). The MDR phenotype was found to be higher in chickens than in calves and lambs among the *S. Typhimurium* isolates. We were able to reveal the AMR profiles and diversity of *S. Typhimurium* strains in poultry since these isolates were collected with the active surveillance method. However, since the *S. Typhimurium* isolates were collected from calves and lambs using the passive

surveillance method, they may not have fully reflected the AMR profiles and the diversity of the related strains. The *S. Kentucky* isolates were found to have a greater variety of MDR profiles in chickens than in calves and dairy cows. The MDR rates of the *S. Kentucky* isolates in calves, dairy cows, geese, and chickens (91.3%, 100%, 100%, and 50.8%, respectively) showed almost double the rates in the former three groups compared to chickens. The MDR profile of the *S. Kentucky* isolates obtained from calves and dairy cows indicating resistance to antibiotics commonly used to treat cattle suggests that some strains that cause clinical infections in calves may not have been present in chicken enterprises, or even if they were present, they may not have caused persistent infections.

Salmonella contains antibiotic resistance genes found in mobile genetic elements, such as plasmids, transposons, and integrons. These mobile elements can transfer resistance genes among not only *Salmonella* serovars but also different genera. In this study, nine resistance genes in all *Salmonella* isolates were investigated using PCR. The most significant resistance genes detected were *tetA*, *sul1*, *strA*, and *strB* ($p < 0.05$). These results are similar to the findings of other researchers [4,28-30]. In addition, *tetA* and *aadA1* were not detected in *S. Typhimurium* isolates, and *tetB*, *sul2*, *aadA1*, and *aadA2* were not detected in *S. Kentucky*. These results show that there is no horizontal gene transfer between *S. Typhimurium* and *S. Kentucky* serovars.

In conclusion, our study revealed that strains isolated from livestock had a higher rate of phenotypic and genotypic multidrug resistance than those isolated from poultry. Control programs to protect animals from *Salmonella* infections can be summarized into different strategies, including testing, management, sanitation, and gastrointestinal colonization control. The effective methods for controlling *Salmonella* spp. at the farm level will reduce MDR-*Salmonella* infections originating from animal food in humans.

Ethical statement

This study does not raise any ethical concerns.

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Conflict of interest

The authors declare no conflicts of interest.

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