Prevalence and antibiotic resistance profiles of *Salmonella* spp. in chicken meat

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Abstract: This study aimed to determine the *Salmonella* spp. presence in 100 chicken meat samples (25 breasts and 25 drumsticks with skin and 25 breasts and 25 drumsticks without skin) that were collected between February and April, 2021, in Van, Türkiye and investigate the antibiotic resistance profiles of the isolates. The results of the cultivation and PCR analysis revealed that a total of 17 samples comprising four drumstick with skin (16%), five drumstick without skin and breast without skin (20%), and three breast with skin (12%) samples were positive for *Salmonella* spp. The analysis of 17 chicken meat samples yielded a total of 33 *Salmonella* spp. isolates. *S.* Enteritidis and *S.* Typhimurium were not detected in the serotyping of *Salmonella* isolates using PCR. According to the disc diffusion test, the isolates were resistant to amoxicillin/clavulanic acid and streptomycin (33.33%), ampicillin (36.36%), gentamicin and ceftriaxone (24.24%), chloramphenicol (42.42%), cefotaxime (12.12%), tetracycline (30.30%), and trimethoprim-sulfamethoxazole (81.82%). The isolates were intermediately resistant to streptomycin (18.18%) and tetracycline (9.09%). PCR analysis for the detection of resistance genes revealed that 77.78% and 62.96% of the trimethoprim-sulfamethoxazole-resistant isolates harbored *sul*1 and *sul*2 genes, respectively. The *pse*-1 gene was detected in 66.67% of the ampicillin-resistant isolates while the *tet*A and *tet*B genes were detected in 20% and 10% of the tetracycline-resistant isolates, respectively. The *ant*(3")-*la* gene was detected in all (100%) gentamicin-resistant isolates. In conclusion, the absence of *S.* Typhimurium and *S.* Enteritidis in the *Salmonella* spp. isolates from the chicken meats that were obtained from the Van market indicated the compliance of the products with the Turkish Food Codex while the presence of *Salmonella* spp. indicated the poor hygienic quality of the meats. The high antimicrobial resistance of the isolates and the presence of the resistance genes can result in the transmission of resistant species to humans, which may require complicated treatments and cause the emergence of a serious public health issue.

Key words: Antibiotic resistance, chicken meat, foodborne pathogen, *Salmonella* spp.

1. Introduction

Salmonella spp. is an important pathogen of the family *Enterobacteriaceae* and causes foodborne infections. Among different contaminated foods, poultry meat and eggs are important sources of transmission of *Salmonella* to humans [1,2]. *Salmonella* spp. are gram-negative, facultative anaerobic bacteria that are typically 0.7–1.5 × 2.0–5.0 μm in diameter, rod-shaped, catalase-positive, oxidase-negative, generally motile by means of peritrichous flagella, nonspore-forming, and found in the intestines of animals. They grow at temperatures of 5–45 °C with an optimum temperature of 35–37 °C, and at a_w values of 0.95 or greater [3-6].

The resistance to basic antibiotics is increasing in *Salmonella* infections, which has become a major health threat worldwide. This can limit the treatment options for people with severe infections. The appropriate use of antibiotics is among the most important ways of slowing the growing rates of resistance to antibiotics. The appropriate use of antibiotics in humans and animals, especially their timely use and use as specified, can help prevent antibiotic resistance and the spread of resistant bacteria [7,8].

The factors such as the excessive and inappropriate use of antimicrobial agents in the production of food animals, the misuse of antibiotics, irregular antibiotic sales, inappropriate antibiotic prescriptions, the presence of mobile genetic elements (plasmid DNA, transposons, and integrons) lead to the exacerbation of antimicrobial resistance and its spread among *Salmonella* species [9-11].

This study aims to detect the presence of *Salmonella* spp. in chicken meats that are sold in the Van market, identify the isolates, and determine their antimicrobial resistance.

2. Materials and methods

2.1. Sample collection

The material of the research was determined by selecting a total of 100 chicken meat samples, 25 breast (skin) and 25

thigh (skin), 25 breast (skinless), and 25 thigh (skinless). In order to increase the diversity of the samples, taking into account the product information packed using foam board and stretch film were taken at different times (2 for each) from 50 different sales points consisting of markets, butchers, and delicatessens. Samples were collected under aseptic conditions between February and April, 2021, brought to the laboratory in refrigerated thermos boxes set to +4 ℃, and analyzed immediately.

2.2. Bacterial strains

Reference *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium) ATCC 14028 and *Salmonella enterica* serovar Enteritidis (*S.* Enteritidis) ATCC 13076 that were obtained from the Food Hygiene and Technology Department of the Veterinary Faculty of Van Yüzüncü Yıl University were used as positive control for cultural cultivation and PCR analyses.

2.3. The isolation and identification of *Salmonella* spp.

The TS EN ISO 6579-1 [12] and EN ISO 6579-1:2017/A1 [13] standards were referred to for the detection of *Salmonella* spp. The purity of the suspicious colonies was examined using Gram staining. Additionally isolates were identified by certain biochemical assays (oxidase, urease, sulfur, indole, glucose, lactose, sucrose fermentation, and lysin decarboxylase) and agglutination test using polyvalent *Salmonella* antiserum (Microgen *Salmonella* Latex M42, England) as *Salmonella* spp. [14]. The agglutination-positive colonies were kept at –20 °C until PCR analysis.

2.4. The confirmation of the presence of *Salmonella* spp. using PCR

A commercial kit (GeneAll, South Korea) was used for the DNA extraction of the *Salmonella* spp. colonies that were isolated from the chicken meats. Following the instructions of the manufacturer, the pure genomic DNA samples were kept at –20 ± 1 °C until analyses. The specific primer pair (5'-AAACGTTGAAAAACTGAGGA-3', 5'-TCGTCATTCCATTACCTACC-3') that was developed by Hoorfar et al. [15] for the Styinva-JHO-2 gene region was used for the PCR confirmation of the *Salmonella* spp. isolates. For the preparation of the PCR mixture, 12.5 μL of mastermix (A.B.T™, Türkiye), 1.5 μL of (10 μM) primer, and 5 μL of genomic DNA were added and the total volume was brought to 25 μL using PCR water. After keeping the mixture at 95 °C for 5 min for predenaturation, a 35-cycle amplification procedure was employed comprising denaturation at 94 °C for 60 s, annealing at 50 °C for 90 s, extension at 72 °C for 60 s, and final extension at 72 °C for 10 min. The gel electrophoresis of the amplicons was carried out using 1.5% agarose gel (Bioshop, Canada)

stained by Biotium brand gel red in horizontal tank at 70-V electric current for 90 min.

2.5. The identification of *S.* Enteritidis and *S.* Typhimurium using PCR

The specific primer pairs for Sdf-ENT (5'-AAATGTGTTTTATCTGATGCAAGAGG-3', 5'-GTTCGTTCTTCTGGTACTTACGATGAC-3', 299 bp) and TYPH (5'-TTGTTCACTTTTTACCCCTGAA-3', 5'-CCCTGACAGCCGTTAGATATT-3', 401 bp) for *S.* Enteritidis [16] and *S.* Typhimurium [17] were used, respectively, for the PCR-confirmation of the isolates. For the preparation of the PCR mixture, 12.5 μL mastermix (A.B.T™, Türkiye), 1.5 μL (10 μM) of each primer, and 5 μL genomic DNA were added and the total volume was brought to 25 μL using PCR water. For *S.* Enteritidis, after predenaturation at 94 °C for 5 min, a 35-cycle amplification procedure comprising denaturation at 94 °C for 60 s, annealing at 57 °C for 90 s, extension at 72 °C for 60 s, and final extension at 72 °C for 5 min was employed while the same procedure except for the temperature and duration of annealing, which was set to 52 °C for 60 s, was employed for *S.* Typhimurium. Next, gel electrophorese of the amplicons was carried out as mentioned above.

2.6. The determination of antibiotic resistance

The antibiotic resistance of the isolates was examined using the disk diffusion method that was proposed by European Committee on Antimicrobial Susceptibility Testing (EUCAST)[1] and Clinical & Laboratory Standards Institute (CLSI) [18]. The amoxicillin/clavulanic acid (AUG), ampicillin (AMP), gentamicin (CN), chloramphenicol (C), cefotaxime (CTX), ceftriaxone (CRO), streptomycin (S), tetracycline (TE), and trimethoprim-sulfamethoxazole (SXT) antibiotic disks were used in the study. As a result of the test, the zone diameters were measured and compared with the values specified in the EUCAST and CLSI to determine the resistance profiles of the isolates (Table 1).

2.7. The genotypic characterization of antibiotic resistance

The presence of the ampicillin, gentamicin, tetracycline, and sulfamethoxazole resistance genes in the *Salmonella* spp. isolates, which were determined to be phenotypically resistant using the disk diffusion method, was examined. For this purpose, the specific primer pairs that were developed by Bacci et al. [19] for ampicillin, gentamicin, and tetracycline and by Zishiri et al. [20] for sulfamethoxazole were used (Table 2). For the preparation of the PCR mixture, 10 μL of mastermix (A.B.T™, Türkiye), 1.5 μL (10 μM) of each primer, and 5 μL of genomic DNA were added and the total volume was brought to 25 μL using PCR water. Table 2 shows the primer pairs used in the PCR analyses.

Table 1. Antibiotic discs and evaluation measures used in the study.

Antimicrobial agent	Disk content (µg)	Zone diameter breakpoint (mm)		
		S	I	R
Amoxicillin/Clavulanic acid (AUG)*	20–10	≥19	-***	<19
Ampicillin (AMP) *	10	≥14	-***	<14
Gentamicin (CN)*	10	≥17	-***	<17
Chloramphenicol (C)*	30	≥17	-***	<17
Cefotaxime (CTX)*	5	≥20	-***	<17
Ceftriaxone (CRO)*	30	≥25	-***	<22
Streptomycin (S)**	10	≥15	12–14	≤11
Tetracycline (TE)**	30	≥15	12–14	≤11
Trimethoprim-sulfamethoxazole (SXT)*	1.25–23.75	≥14	-***	<11

* Evaluated according to EUCAST, 2021.
** Evaluated according to CLSI, [18].
*** According to EUCAST, 2021 there is no intermediate level.
R: resistant, I: intermediate, S: susceptible

Table 2. The oligonucleotide sequences used in the identification of antibiotic resistance genes using PCR.

Antibiotics	Gene	Oligonucleotide (5'-3')	bp	Annealing	Reference
Ampicillin	*pse*-1	CGCTTCCCGTTAACAAGTAC CTGGTTCATTTCAGATAGCG	419	51 °C	[20]
Gentamicin	*ant*(3")-la	GTGGATGGCGGCCTGAAGCC ATTGCCCAGTCGGCAGCG	526	58 °C	
Tetracycline	*tet*A	GCTACATCCTGCTTGCCTTC CATAGATCGCCGTGAAGAGG	210	59 °C	
	*tet*B	TTGGTTAGGGGCAAGTTTTG GTAATGGGCCAATAACACCG	659	56 °C	
Sulfamethoxazole	*sul*1	GCGCGG CGTGGGCTACCT GATTTCCGCGACACCGAGACAA	350	64 °C	[21]
	*sul*2	CGGCATCGTCAACATAACC GTGTGCGGATGAAGTCAG	720	57 °C	

The protocol initiated a predenaturation process at 94 °C for 5 min, followed by a 35-cycle amplification procedure comprising denaturation at 94 °C for 60 s, annealing at the appropriate temperatures for each gene (Table 2) for 60 s, extension at 72 °C for 60 s, and final extension at 72 °C for 5 min. The presence of antibiotic resistance genes was determined by the detection of gene-specific bands (Table 2) in the electrophoresis gel.

2.8. Statistical analysis
In the study, SPSS 13.0 package program [21] was used to calculate the analysis results as a percentage.

3. Results

3.1. Results for the isolation and identification of *Salmonella* spp.
Salmonella spp. was isolated from 17 samples (17%) among the 100 samples with or without skin that were procured from the markets, grocery stores, and butchers in Van, Türkiye. *S.* Enteritidis and *S.* Typhimurium were not identified in any sample. Among the samples, four drumstick samples with skin (16%), five drumstick and breast samples without skin (20%), and three breast samples with skin (12%) tested positive for *Salmonella* spp.

Figure 1 shows the PCR results for the positive strains. In the study, a total of 33 *Salmonella* colonies were isolated from 17 chicken meat samples that were detected as *Salmonella* spp.-positive.

3.2. The antibiotic resistance of the *Salmonella* spp. isolates

Table 3 shows the antibiotic resistance and susceptibility of the 33 *Salmonella* spp. isolates that were obtained from 17 chicken meat samples.

In the study, a total of 33 *Salmonella* spp. isolates were obtained from 17 chicken meat samples. Of these isolates, 11 were resistant to amoxicillin/clavulanic acid and streptomycin (33.33%), 12 to ampicillin (36.36%), 8 to gentamicin and ceftriaxone (24.24%), 14 to chloramphenicol (42.42%), 4 to cefotaxime (12.12%), 10 to tetracycline (30.30%), and 27 to trimethoprim/sulfamethoxazole (81.82%). Of the isolates, 22 were susceptible to amoxicillin/clavulanic acid (66.67%), 21 to ampicillin (63.64%), 25 to gentamicin (75.76%), 19 to chloramphenicol (57.58%), 29 to cefotaxime (87.88%), 24 to ceftriaxone (72.73%), 11 to streptomycin (33.33%), 14 to tetracycline (42.42%), and 5 to trimethoprim-sulfamethoxazole (15.15%). It was observed that intermediate to streptomycin (18.18%) and tetracycline (9.09%) developed. It was also determined that 16 of the 30 isolates (53.33%) obtained were phenotypically resistant to two or more antibiotics.

## 3.3. The presence of the antibiotic resistance genes in the *Salmonella* spp. isolates

Table 3 shows the resistance genes and their distribution in 33 *Salmonella* spp. isolates with respect to the antibiograms. Table 4 shows the profile of the antibiotic resistance genes in the antibiotic-resistant Salmonella spp. isolates. In addition, Figure 2 shows the agarose gel image of the antibiotic-resistant Salmonella spp. isolates that were identified using PCR.

According to the antibiogram results of 33 *Salmonella* spp. isolates obtained in the study; The *pse*-1 gene was detected in 8 of the 12 isolates (66.67%) resistant to ampicillin, the *ant*(3")-la gene was detected in all of the 8 isolates (100%) resistant to gentamicin, the *tet*A gene was detected in 2 of the 10 isolates (20%) resistant to tetracycline, and the *tet*B gene was detected in one (10%). It was determined that of 27 isolates resistant to trimethoprim-sulfamethoxazole, 21 of them carried the *sul*1 gene (77.78%) and 17 of them *sul*2 (62.96%). It was observed that the isolates containing *tet*A and *tet*B genes were different from each other, and 16 of the 27 trimethoprim-sulfamethoxazole-resistant isolates carried both *sul*1 and *sul*2 genes (59.26%).

4. Discussion and conclusion

The *Salmonella* serotypes are regarded as important causes of foodborne diseases worldwide. The infection is usually transmitted through the consumption of contaminated waters and foods of animal origin. Fruits and vegetables that are contaminated with human and animal feces can also cause *Salmonella* outbreaks [22]. The salmonellosis cases in Europe were reported to be the second most common zoonosis in humans in 2018 [23].

A total of 100 chicken meat samples (breasts and drumsticks with and without skin with 25 samples for each) that were procured from the markets, groceries, and butchers in Van were examined, which revealed that 17 of the samples (17%) contained *Salmonella* spp. This value was lower than those reported by Domínguez et al. [24], Yang et al. [25], Fearnley et al. [26], Süzme [27], Yıldırım et al. [28], Thung et al. [29], Aydın [30], Asal-Ulus [31], and Çadırcı et al. [32] and higher than those reported by Beli et al. [33], Telli [34], and Acaröz et al. [35]. Although it is not in the scope of this study, the differences in the prevalence of *Salmonella* spp. may be attributed to various factors
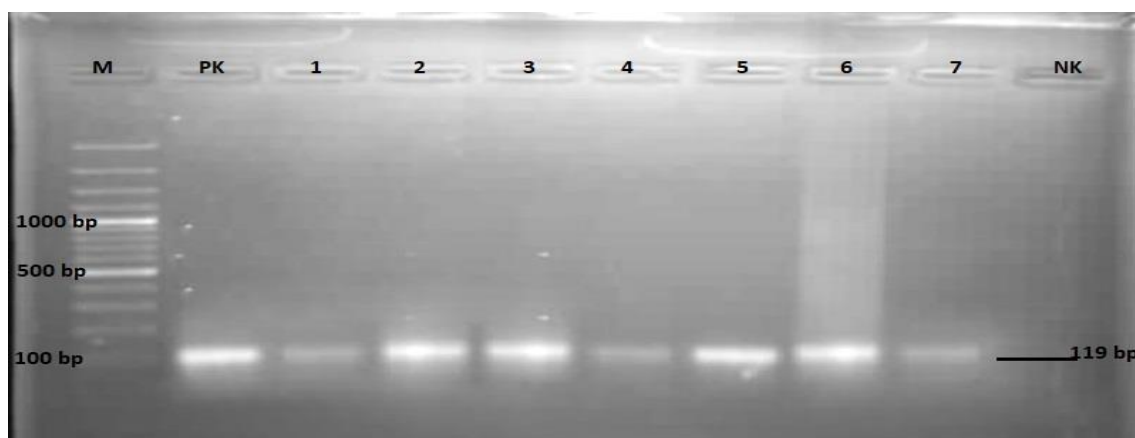
Figure 1. The agarose gel image of the amplicons that were identified in the *Salmonella* spp. isolates using PCR (119bp) (M: 100 bp DNA marker; PK: *Salmonella* Enteritidis ATCC® 13076; 1-7: *Salmonella* spp. isolates; NK: Negative control).

Table 3. The antibiotic resistance, susceptibility of the *Salmonella* spp. isolates that were obtained from the chicken meat samples and the distribution of the antibiotic resistance genes.

Antibiotics	Resistant n (%)	Intermediate n (%)	Susceptible n (%)	Genes	n of genes (%)
Amoxicillin/clavulanic acid	11 (33.33)	-	22 (66.67)	-	-
Ampicillin	12 (36.36)	-	21 (63.64)	*pse*-1	8 (66.67)
Gentamicin	8 (24.24)	-	25 (75.76)	*ant*(3")-la	8 (100)
Chloramphenicol	14 (42.42)	-	19 (57.58)	-	-
Cefotaxime	4 (12.12)	-	29 (87.88)	-	-
Ceftriaxone	8 (24.24)	-	24 (72.73)	-	-
Streptomycin	11 (33.33)	6 (18.18)	11 (33.33)	-	-
Tetracycline	10 (30.30)	3 (9.09)	14 (42.42)	*tet*A	2 (20)
				*tet*B	1 (10)
Trimethoprim-sulfamethoxazole	27 (81.82)	-	5 (15.15)	*sul*1	21 (77.78)
				*sul*2	17 (62.96)

n: number of positive isolates

such as sample size, applications during production, the hygiene of the personnel, tools in the sales outlets, and seasonal differences.

According to the Turkish Food Codex Regulation on Microbiological Criteria[2], 25-g raw chicken meat and chicken meat preparations should not contain *S*. Typhimurium and *S*. Enteritidis. *S*. Enteritidis and *S*. Typhimurium were not identified in any of the 17 *Salmonella* spp. isolates, thus complying with the regulation.

In another study from Türkiye, *S*. Enteritidis and *S*. Typhimurium were not identified on chicken meat [36], chicken meat/internal organs [37], and packaged organic chicken meat [31], which is in agreement with our results. Beli et al. [33] reported *S*. Enteritidis as the most common serotype but they also reported identifying *S*. Senftenberg, *S*. Newport, *S*. Abony, *S*. Agona, *S*. Banana, *S*. Brancaster, *S*. Infantis, and *S*. Oslo. Domínguez et al. [24] found *S*. Enteritidis, *S*. Hadar, and serotype 4,12:b:-(II) to be the most common serotypes in chicken meat samples; they also detected *S*. Mbandaka, *S*. Derby, *S*. Virchow, and *S*. Paratyphi B. Fearnley et al. [26] reported that *S*. Infantis and *S*. Typhimurium phage type 135a were the most common *Salmonella* serotypes in chicken meats. Agbaje et al. [38] found *S*. Haifa to be the most common serotype in chicken meats, followed by *S*. Chomedey, *S*. Saintpaul, *S*. Kainji, *S*. Derby, and *S*. Blockley. The differences

between the present study and others are attributable to the differences in the prevalence of the *S*. Enteritidis and *S*. Typhimurium serotypes, region, the number and type of samples, and methods used in bacterial isolation and identification.

According to the report of the Turkish Ministry of Health for the National *Salmonella* Control Program[3], *S*. Enteritidis was the most isolated *Salmonella* species from human clinical samples between 2012 and 2016, followed by *S*. Typhimurium, *S*. Infantis, *S*. Paratyphi B, and *S*. Kentucky. Moreover, the three most dominant serovars in the poultry slaughterhouse samples (chicken carcasses) were reported to be *S*. Infantis, *S*. Kentucky, and *S*. Enteritidis, respectively. Similarly, *S*. Infantis was reported to be the most common serovar in chicken and chicken meats by the European Food Safety Authority (EFSA) and European Centre for Disease Prevention and Control (ECDC) [23].

Antimicrobials either kill infectious microorganisms or inhibit their growth. Antibiotics such as ciprofloxacin, azithromycin, and ceftriaxone may be needed for the treatment of the severe infections of *Salmonella* spp.[4]

The excessive and inappropriate use of antimicrobials in medicinal and veterinary practices can cause antimicrobial resistance, which is an important public health issue. Furthermore, the resistance mechanisms of bacteria are

[2] Turkish Food Codex Regulation on Microbiological Criteria (2018) ek-1 Gıda Güvenilirliği Kriterleri Ek: RG-9/10/2018-30560 [online]. Website https://www.mevzuat.gov.tr/mevzuat?MevzuatNo=15690&MevzuatTur=7&MevzuatTertip=5e [accessed 26 Agust 2021].

[3] Turkish Ministry of Health for the National *Salmonella* Control Program (2018). National *Salmonella* Control Program [online]. Website https://tuyekad.org.tr/wp-content/uploads/2018/09/ULUSAL_SALMONELLA_KONTROL_PROGRAMI__.pdf [accessed 8 November 2021].

[4] Centers for Disease Control and Prevention (CDC) (2019). Antibiotic Resistance Threats In the United States [online]. Website https://www.cdc.gov/drugresistance/pdf/threats-report/2019-ar-threats-report-508.pdf [accessed 5 August 2021].

Table 4. The profile of the antibiotic resistance genes in the antibiotic-resistant *Salmonella* spp. isolates (AMP/CN/TE/SXT).

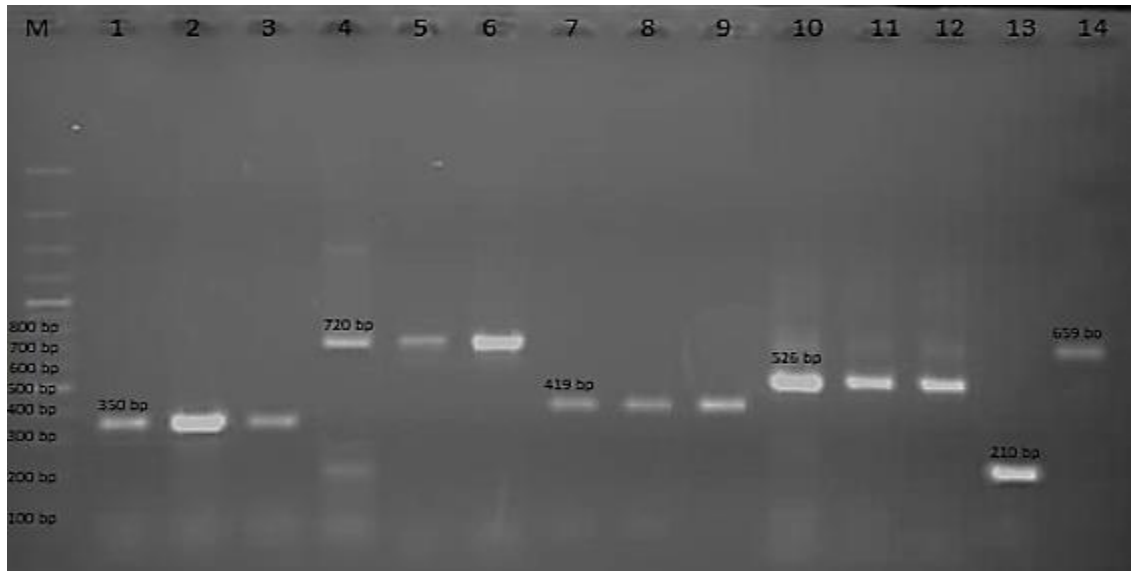| Isolates	Antibiotic resistance	*pse*-1	*ant*(3")-la	*tet*A	*tet*B	*sul*1	*sul*2
Isolate 1	TE/SXT	-	-	-	+	-	-
Isolate 2	SXT	-	-	-	-	+	+
Isolate 3	AMP/CN/TE/SXT	+	+	+	-	+	+
Isolate 4	AMP/CN/TE/SXT	+	+	-	-	+	+
Isolate 5	SXT	-	-	-	-	-	-
Isolate 7	AMP/SXT	+	-	-	-	+	+
Isolate 8	AMP/CN/SXT	-	+	-	-	+	-
Isolate 11	SXT	-	-	-	-	+	-
Isolate 12	CN/SXT	-	+	-	-	+	-
Isolate 13	TE/SXT	-	-	-	-	-	-
Isolate 14	TE	-	-	-	-	-	-
Isolate 15	AMP/TE/SXT	+	-	+	-	+	+
Isolate 16	AMP/TE/SXT	-	-	-	-	-	-
Isolate 17	SXT	-	-	-	-	+	+
Isolate 18	SXT	-	-	-	-	+	+
Isolate 19	AMP/CN/TE	-	+	-	-	-	-
Isolate 20	SXT	-	-	-	-	+	+
Isolate 21	SXT	-	-	-	-	+	+
Isolate 22	SXT	-	-	-	-	+	+
Isolate 23	SXT	-	-	-	-	+	+
Isolate 24	SXT	-	-	-	-	+	+
Isolate 25	AMP/TE	-	-	-	-	-	-
Isolate 26	SXT	-	-	-	-	+	+
Isolate 27	AMP/SXT	+	-	-	-	-	+
Isolate 28	SXT	-	-	-	-	-	-
Isolate 29	SXT	-	-	-	-	+	-
Isolate 30	AMP/SXT	+	-	-	-	+	+
Isolate 31	CN/SXT	-	+	-	-	+	+
Isolate 32	AMP/CN/TE/SXT	+	+	-	-	+	-
Isolate 33	AMP/CN/SXT	+	+	-	-	+	+

quite important in the development of antimicrobial resistance [22, 39]. For example, the genomic element in *S.* Typhimurium that causes resistance to ampicillin, chloramphenicol, streptomycin, sulfonamide, and tetracycline can horizontally spread among other serotypes and gain additional resistance-determining qualities [22].

In the present study, the positive *Salmonella* isolates were resistant to trimethoprim-sulfamethoxazole (81.82%), chloramphenicol (42.42%), ampicillin (36.36%), amoxicillin-clavulanic acid and streptomycin (33.33%), tetracycline (30.30%), gentamicin and ceftriaxone (24.24%), and cefotaxime (12.12%), respectively.

According to the report published by the National *Salmonella* Control Program[5], among the *Salmonella* isolates from broiler houses, 18.5% were resistant to ampicillin, 4.8% were resistant to gentamicin, 7.7% were resistant to chloramphenicol, 0.9% were resistant to cefotaxime, 33.5% were resistant to streptomycin, 29.4% were resistant to tetracycline, 32.2% were resistant to trimethoprim, and 93.1% were resistant to sulfamethoxazole.

[5] Turkish Ministry of Health for the National *Salmonella* Control Program (2018). National *Salmonella* Control Program [online]. Website https://tuyekad.org.tr/wp-content/uploads/2018/09/ULUSAL_SALMONELLA_KONTROL_PROGRAMI__.pdf [accessed 8 November 2021].

Figure 2. The agarose gel image of the antibiotic-resistant *Salmonella* spp. isolates that were identified using PCR M: 100 bp DNA marker; 1-3: *sul*1 gene (350 bp); 4-7: *sul*2 gene (720bp); 7-9: *pse*-1 gene (419 bp); 10-12: the *ant*(3")-la gene (526 bp); 13: the *tet*A gene (210 bp); 14: *tet*B gene (659 bp).

In the present study, 11 *Salmonella* spp. isolates (33.33%) were determined to be resistant to amoxicillin-clavulanic acid. This is higher than the value reported by Yan et al. [40] (10.5%) but lower than that reported by Yang et al. [25] (36%). In their studies, Chaisatit et al. [41] and Acar [36] did not find any amoxicillin-clavulanic acid-resistant isolates. In the present study, 12 *Salmonella* spp. isolates (36.36%) were determined to be resistant to ampicillin (%36.36). This is higher than the values reported in some studies [36, 38, 42, 43] but lower than those reported in others [32, 40, 41, 44]. Among the examined *Salmonella* spp. isolates, eight were resistant to gentamicin (24.24%) in the present study, which differs from the numbers reported in other studies [32, 38, 40-43]. In the present study, 14 *Salmonella* spp. isolates (42.42%) were resistant to chloramphenicol, which is higher than some values reported by other researchers [36, 38, 42, 43, 45], close to those reported by Yan et al. [40] (42.1%) and Çadırcı et al. [32] (40.78%), and lower than the value reported by Miranda et al. [44] (80.5%). In the present study, four isolates (12.12%) were resistant to cefotaxime. Elkenany et al. [46] reported that 13.13% of the isolates from broiler chicken farms and wholesale points were resistant to cefotaxime while Çadırcı et al. [32] reported the value to be 14.47%. Miranda et al. [44] found no isolates that were resistant to cefotaxime. Eight of the *Salmonella* spp. isolates were resistant to ceftriaxone (24.24%) while Yang et al. [25] and Sırıken et al. [43] found this value to be 19% and 1.19%, respectively. Yan et al. [40] and Asal-Ulus

[31] found no isolates that were resistant to ceftriaxone. In the present study, 11 *Salmonella* spp. isolates (33.33%) were resistant to streptomycin, which is close to the values reported in some studies [38] and different from those in other studies[6] [32, 33, 38, 42, 44]. Ten of the *Salmonella* spp. isolates (30.30%) were resistant to tetracycline, which is higher than those reported by Chaisatit et al. [41] (21.4%) and Çadırcı et al. [32] (18.42%) and lower than those reported by Van et al. [42] (38.9%), Miranda et al. [44] (78%), Yan et al. [40], Sırıken et al. [43] (91.66%), Babacan and Karadeniz [45] (82.85%), and Agbaje et al. [38] (89.3%). In the present study, 27 *Salmonella* spp. isolates (81.82%) were determined to be resistant to trimethoprim-sulfamethoxazole, which is higher than those reported in some studies [32, 36, 40, 41, 43-45].

The analyses revealed that 8 of the 12 ampicillin-resistant isolates (66.67%) harbored the *pse*-1 gen, all 8 gentamicin-resistant isolates (100%) harbored the *ant*(3"%)-la gen, 2 of the 10 tetracycline-resistant isolates (20.00%) harbored the *tet*A gene while 1 tetracycline-resistant isolate (10.00%) harbored the *tet*B gen, and 21 of the 27 trimethoprim-sulfamethoxazole-resistant isolates (77.78%) harbored the *sul*1 gene while 17 (62.96%) harbored the *sul*2 gene. In other studies, Bacci et al. [19] reported the presence of the *pse*-1, *ant*(3")-la, *tet*A, *tet*B, and *sul*1 genes in 2%, 12%, 34%, and 16% of the isolates. Agbaje et al. [38] reported the presence of the *tet*A gene in all isolates (100%), the presence of the *sul*1 gene in 78.9% of the isolates, and the presence of the *sul*2 gene in 10.5%

[6] World Health Organization (WHO) (2020). Food Safety [online]. Website https://www.who.int/news-room/fact-sheets/detail/food-safety [accessed 24 January 2021].

of the isolates. Acar [36] found that 9% of the isolates harbored the *tet*A gene while 81% harbored the *sul*1 gene. Cortes-Vélez et al. [47] reported that 42.5% of the isolates harbored the *tet*B gene while 57.4% harbored the *sul*2 gene. However, Acar [36] did not identify the *tet*B and *sul*2 genes in the isolates.

The examination of antibiotic resistance revealed that 30 of the *Salmonella* spp. isolates (90.90%) were resistant to two or more antibiotics and 20 isolates (66.66%) carried two or more antibiotic resistance genes. Van et al. [42] found that 88.9% of the chicken meat isolates were resistant to at least one antibiotic; Yan et al. [40] reported that 89.47% of the chicken meat isolates were resistant to multiple antibiotics; and Sırıken et al. [43] determined that 92.85% of the isolates were resistant to at least 4 different antibiotics. Bacci et al. [19] found that 52.0% of the 50 *Salmonella* spp. isolates were resistant to multiple antibiotics and 22% of the isolates had multiple resistance genes. In their study in which 166 *Salmonella* spp. isolates were obtained from chicken meats and offal, Abd-Elghany et al. [48] found that 95.18% of the isolates were resistant to multiple antibiotics. Elkenany et al. [46] reported that all 120 isolates (100%) that were obtained from broiler chicken farms and wholesale points were resistant to multiple antibiotics.

The differences in the values reported in the present study and in other studies are attributable to serotype differences, regional and environmental differences, and the use of uncontrolled and inappropriate doses of antibiotics. Moreover, the lack of antibiotic resistance genes in bacteria that are phenotypically resistant to the same antibiotic indicated possibly different mechanisms of resistance to ampicillin, tetracycline, and sulfamethoxazole (such as integron, plasmid, and transposon).

In conclusion, although the analyses revealed that the *Salmonella* spp. isolates from the chicken meats in the market did not contain *S.* Typhimurium or *S.* Enteritidis, thus conforming to the Turkish Food Codex, the presence of *Salmonella* spp. indicated poor hygienic quality. Furthermore, the high antimicrobial resistance of the isolates and the presence of resistance genes in some isolates can cause the transmission of resistant species to humans and complicate treatment, which can lead to a great public health issue.

Conflict of interest
The authors declare that they have no conflicts of interest.

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