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ORIGINAL ARTICLE

Nontyphoid *Salmonella* carriage, serovar profile and antimicrobial resistance phenotypes in slaughter cattle

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Abstract

Current nontyphoid *Salmonella* (NTS) carriage in 200 apparently healthy slaughter cattle by ISO 6579 standard bacteriology (ISO) was 1% (2/200) in carcass and fecal content, and 2% (4/200) in mesenterial lymph nodes. There was no isolation from liver, kidney, spleen, and gallbladder, with an overall prevalence of 4% (8/200). Real-time PCR was in substantial agreement to ISO in confirming *Salmonella*-suspect isolates (Relative Trueness: 93.33%). Predominant serovar was *S.* Enteritidis (50%) followed by *S.* Typhimurium (37.5%), and *S.* Albany (12.5%). Five and three of eight NTS isolates were susceptible (62.5%) and resistant (37.5%) to 18 antimicrobials, respectively. Only three *S.* Enteritidis isolates (37.5%) showed multidrug resistance to 2–3 of 7 antimicrobials (amikacin, cefotaxime, cefoxitin, gentamicin, norfloxacin, pefloxacin, and tobramycin). *S.* Enteritidis predominance over *S.* Typhimurium, first detection of *S.* Albany in cattle in Turkey, and sole resistance in mesenterial lymph node *S.* Enteritidis isolates highlights study findings.

Practical applications

Contaminated carcass and related material, for example, fecal content and mesenterial lymph nodes of apparently healthy slaughter cattle carrying nontyphoid *Salmonella* serovars still pose significant health risk to public in Turkey, where bovine meat covers the highest annual red meat consumption quota with high demand to edible offal. In this study, current predominance of *S.* Enteritidis, particularly in mesenterial lymph nodes, and the MDR pattern identified; the presence of *S.* Typhimurium as the second dominant and pansusceptible serovar; detection of *S.* Albany for the first time in cattle fecal content are new epidemiological findings. This data could be used in revising both bovine meat and offal's actual NTS status, and the control and prevention programs in our country and in the neighboring countries of interest.

1 | INTRODUCTION

Salmonella is the second most commonly reported bacterial pathogen in both gastrointestinal infections and in foodborne outbreaks in the European Union (EU). In 2015, there were 94.625 confirmed salmonellosis cases with a notification rate of 21.2 cases per 100.000 population and confirmed "nontyphoidal" salmonellosis (NTS) cases' most frequent notification rate range of 13.90–38.90 cases per 100,000 population, respectively (EFSA & ECDC, 2016). According to the World Health Organization, foodborne illnesses from NTS results in the largest disease burden, with 59,000 mortalities in 230,000 foodborne deaths from diarrheal disease agents worldwide (WHO, 2015).

Bovine meat is indicated as an important vehicle within animal-derived sources in strong-evidence outbreaks caused by *Salmonella* in the EU (EFSA & ECDC, 2016; Rizzi, 2017). This clearly implies the possible contamination of carcass by fecal content, internal organs and lymph nodes of subclinically infected or intermittent/persistent carriers of *Salmonella* in slaughter cattle, and introduction of this pathogen to the bovine meat-production chain, posing risk for *Salmonella* in related food and products. Accordingly, in our country, since bovine meat has the highest quota within red meat both in the annual production (870,000 tons/1,310,000; 66.4%), and consumption (11.64 kg/17.26 kg *per capita*; 67.4%; FAO, 2013), the absence of NTS in slaughter cattle is of utmost importance for the safety of bovine meat and meat products.

Determination of serovar and antimicrobial resistance profile of NTS in bovine carcass is required for biosecurity and epidemiological purposes. Also, Turkish Food Codex (TFC) Regulation on Microbiological Criteria indicates zero tolerance for *Salmonella* on bovine carcasses tested with the reference method EN/ISO 6579 (ISO, 2002). *Salmonella* serovar data in international platforms not only provide basis in constructing reliable and sustained epidemiological links between host and carrier but also aids in developing efficient control strategies to prevent/reduce infections for healthy global food and animal trade. As in previous years, the two most commonly reported *Salmonella* serovars in EU/ European Economic Area (EEA) in 2015 were *Salmonella* serovar Enteritidis (*S.* Enteritidis) and *Salmonella* serovar Typhimurium (*S.* Typhimurium), representing 45.7 and 15.8%, respectively, of all reported serovars in 69.663 confirmed human cases (EFSA & ECDC, 2016). Similarly, in Turkey, 75.3 and 6.9% of the confirmed salmonellosis cases were linked to *S.* Enteritidis and *S.* Typhimurium, respectively, based on National Enteric Pathogens Network (UEPLA) data (Gulesen, 2016). As source attribution for salmonellosis cases or outbreaks related to food, bovine meat, and products are indicated as important carriers in 2015 in EU, for *S.* Enteritidis and *S.* Typhimurium serovars (Rizzi, 2017).

In the EU summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals, and food in 2016, high proportions of human *Salmonella* isolates were reported as resistant to sulfonamides (34.6%), ampicillin (29.5%), and tetracyclines (29.2%) with and overall MDR of 26.5%. Also, resistance to fluoroquinolones in *Salmonella* from humans was related to consumption of fluoroquinolones in animals (EFSA & ECDC, 2018). Therefore, contamination of these types of resistant *Salmonella* from animal-derived foods and to humans most importantly causes failures in treatment regimes of related infections.

In Turkey, there is a lack of up-to-date prevalence, serogroup/serotype and/or antimicrobial resistance data on *Salmonella* in slaughter cattle (Çalıcıoğlu, Öksüztepe, İlhak, & Dikici, 2005; Küplülü, 1999) and their offal (Genç, 2002), as one of the major sources for this pathogen's introduction site to food chain. Additionally, this limited number of studies, which used varied sampling sites, (dis)similar sampling/isolation–identification methods, may not be reflecting the actual *Salmonella* status in cattle. Determination of current prevalence/serovar profile/antimicrobial resistance of this pathogen in slaughter cattle in Turkey would provide invaluable up-to-date epidemiological data to steer effective control and prevention programs for our country and for the neighboring countries of interest. Thus, this study aimed to determine NTS carriage in slaughter cattle by applying internationally recognized standard methods for sampling, isolation–identification, serotyping and antibiotyping to obtain reliable and actual information with the available optimized conditions.

2 | MATERIALS AND METHODS

2.1 | Sample collection and preparation

In this study, 1,400 samples of seven types comprised of carcass (C), fecal content (FC), mesenterial lymph node (MLN), liver (L), kidney (K), spleen (S), and gallbladder (GB) were collected randomly from 200 cattle (all seven sample types belonged to the same cattle) of various herds and breeding units (representing Marmara, Black sea, Aegean, mid-Anatolia, eastern Anatolia regions) in four slaughterhouses (A: 54; B: 40; C: 46; D: 60 sample/slaughterhouse), with number of samples (*n*) taken in the following months as April (16), May (29), June (15), July (16), August (15), September (15), October (18), November (16), December (16), January (20), February (16), and March (17) between 2013 and 2015. In sampling, the related requirements of TFC were followed as: (1) a non-destructive sponge sampling method was applied to carcasses following the instructions indicated in ISO 17604:2003 (ISO, 2003). For this, the sites with the highest prevalence of contamination/most consistently contaminated by high numbers of microorganisms as indicated in Annex A of the same document as brisket, fore rib, flank, lateral of round, and flank groin were selected. For each site, a sterile square template with hollow internal area of 100 cm^2 (10 cm × 10 cm) was used to enclose the specific location. Then, the whole inner area was wiped for a total of 10 times in vertical and 10 times in horizontal direction with a sufficiently wetted sponge (Whirl Pak, B01351WA) with buffered peptone water (BPW-ISO, Oxoid, CM1049) using sterile sampling techniques. After swabbing, the sponge was placed back and more diluent was added to the sample bag to make a total of 25 mL. (2) Sampling for FC was performed by stripping the whole colonic and rectal content from anus, and placing approximately 25–50 g thoroughly mixed content into a sterile sampling bag. (3) Three to five MLNs were excised by following sterile sampling procedures and were placed into sterile sampling bags as indicated in Alemu and Zewde (2012). (4) L and K samples were taken from the entry site of vena porta, and from the orifice and surrounding of ureter, respectively, by a pre-wetted sterile swab (LP Italiana, L111598) with BPW and placed into tubes containing 10 mL sterile BPW (Genç, 2002). (5) S samples were taken by swabbing an approximately 5 cm^2 area of the arterial and venous orifice in the interior part of S, and the pre-wetted swab was then placed into tubes containing 10 mL sterile BPW. (6) For sampling from the GB, a prewetted sterile swab with BPW was inserted from the neck of the preemptied GB and rubbed against its walls, and then placed into a tube containing 10 mL sterile BPW (Akoachere, Tanih, Ndip, & Ndip, 2009). All the samples were transferred to the laboratory in a +4°C cooler and prepared for analysis within maximum an hour.

2.2 | Standard and quality control strains

S. Enteritidis 64K (M.Y. Popoff, Institut Pasteur, Paris Cedex 15, France) and *S.* Typhimurium NCTC 12416 (Refik Saydam National Public Health Agency, Ankara, Turkey) were used as positive controls in *Salmonella* isolation, identification, and serotyping and in *Salmonella* spp.-specific real-time PCR (rPCR) analyses. *Escherichia coli* ATCC 25922 (susceptible, wild-type) was used as the quality control strain following the recommendations of European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2015a) for *Enterobacteriaceae.*

2.3 | Isolation and identification

Samples from C, MLN, L, K, S, and GB are handled as indicated in ISO 6579:2002 (ISO, 2002) as follows: For pre-enrichment (PE); C sponge samples in 25 mL BPW, and approximately 25 g MLN samples, which were diced into smaller pieces, were placed individually into 225 mL BPW in 500 mL stomacher bags (LP Italiana, L177538). Each L, K, S, and GB swab in 10 mL BPW was placed into 90 mL BPW in 100 mL stomacher bags. All samples were homogenized for 2 min at 230 rpm in Stomacher (Seward, 400 C), and incubated at 37°C for 18 hr. Then, for selective enrichment (SE), (1) 1 mL from PE culture was transferred into 10 mL Mueller Kauffmann tetrathionate novobiocin (MKTTn Oxoid, Basingstoke, UK; CM1048) broth with novobiocin supplement (Oxoid, SR0181), and incubated at 37°C for 24 hr. (b) 0.1 mL from PE culture was transferred into 10 mL broth Rappaport Vassiliadis soya peptone (RVS Oxoid, CM0866) broth, and incubated at 41.5°C for 24 hr. Selective plating was performed from each of the SE broths on xylose lysine deoxycholate (XLD, Oxoid, CM0469) agar and brilliance salmonella (BS, Oxoid, CM1092) agar with salmonella selective supplement (Oxoid, SR0194) and incubated at 35°C for 24 hr. Samples of FC were treated as indicated in ISO 6579/A1: 2007 (ISO, 2007) as follows: for PE of the sample, 25 g FC was weighed out from the previously taken 25–50 g sample into a 500 mL stomacher bag, and homogenized in 225 mL of BPW for 2 min at 230 rpm, and incubated at 37°C for 18 hr. After PE, 0.1 mL was transferred onto modified semisolid Rappaport-Vassiliadis (MSRV, Oxoid, CM1112) agar and incubated at 41.5°C between 18 and 42 hr until a defined zone was observed. Selective plating was performed from MSRV agar onto XLD agar, xylose lysine tergitol-4 (XLT-4, Oxoid, CM1061) agar, and BS agar, and incubated at 37°C for 24 hr. After incubation, 1–5 *Salmonella* suspect colonies were selected and streaked onto MacConkey (MC, Oxoid, CM0115) agar for obtaining pure culture to be utilized in rPCR and in biochemical identification. A loopful of pure culture grown on MC agar was homogenized in 500 μL sterile PCR-grade water and stored at −20°C. For initial biochemical identification, pure MC agar culture was initially transferred into brain heart infusion (BHI, Oxoid, CM1135) broth and incubated at 37°C for 18–20 hr. This culture was then used for determining urease activity (Urea Agar Base, Oxoid, CM0053), triple sugar utilization, and H_2S formation (Triple Sugar Iron Agar, Oxoid, CM0277), and lysine decarboxylase activity (Lysine Iron Agar, Oxoid, CM0381). Further identification was performed using API20E (Biomerieux, Marcy-l'Étoile, France, 20,100), and profile results were evaluated accordingly. *Salmonella* spp. positive cultures were stored at −20°C for serotyping.

2.4 | Template preparation for *Salmonella* spp.-specific real-time PCR

Crude template DNA was extracted from each control strain and *Salmonella* isolate culture according to the procedure described by Carli, Unal, Caner, and Eyigor (2001). Briefly, 1 mL of each culture stored at −20°C was thawed to room temperature and centrifuged for 4 min at 4,600 × *g* (Thermo Scientific, Waltham, MA, MicroCL 17). The pellet was suspended in 0.85% saline, was centrifuged, and suspended in 20 μL of deionized water. This bacterial suspension was then boiled at 95°C block heater (Techne, DB-2D-FDB02DD) for 10 min, and centrifuged for 3 min 18,000 × *g*. Two microliters of the supernatant were used as a template in rPCR.

2.5 | Confirmation of *Salmonella*-suspect colonies by rPCR

Sequences of the primers and probe targeting the ttrRSBCA locus located near the *Salmonella* pathogenicity island 2 at centisome 30.5 in rPCR assay, which was developed by Malorny et al. (2004), and a newly designed *Escherichia coli* Lambda phage-specific Internal Amplification Control (IAC), its specific primers (marked in boldface type in IAC sequence), and its probe sequence (underlined in IAC sequence) used in this study are as follows: ttr-6 (forward) 5′-CTCACCAGGAGATTACAACATGG-3′; ttr-4 (reverse) 5′- AGCTCAGACCAAAAGTGACCATC-3′; ttr-5 (target probe) 5′-FAM-CACCGACGGCGAGACCGACTTT-TAMRA-3′; IAC sequence CGTCAGTGTGAAGCGGTTATAAATCTGCTCTTTCGCGG TATCCGTACCGATTTCGGTAAGGTAAACCCCGTTTTTGTTTCGC TTACGTGGCAT; IAC (forward) 5′-CGTCAGTGTGAAGCGGTTA TAA-3′; IAC (reverse) 5′- ATGCCACGTAAGCGAAACA-3′; IAC probe 5′-HEX-TGCTCTTTCGCGGTATCCGTACCGAT-TAMRA-3′ (Way2Gene, BN 15-0001-01, Genmar, Turkey).

Salmonella spp.-specific rPCR was applied based on a protocol defined by Malorny et al. (2007) for capillary-based Light Cycler 2.0 (Roche Diagnostics, Risch-Rotkreuz, Switzerland, 03531414201), with modifications in reaction volume and cycling parameters. The 10 μL rPCR reaction mix (Way2Gene, BN 15-0001-01, Genmar, Turkey) consisted of 2.5 μL *Salmonella* detection mix (0.5 μM each primer and 0.25 μM each probe), 2 μL enzyme mix (0.5 U enzyme; dNTP mix, reaction buffer solution), 1 μL (approximately 100 copies) IAC template DNA, 2.5 μL template DNA (DNA for positive control and samples, PCR-grade water for negative control and 2 μL PCR-grade water. The incubation conditions were: initial denaturation at 95°C for 11 min, followed by 40 cycles of 95°C for 10 s denaturation, 58°C for 30 s annealing and 72°C for 5 s elongation. Fluorescence signals were detected in the 530 nm channel (*Salmonella* target signal) and the 560 nm channel (IAC).

2.6 | Serotyping

Serological identification was performed to the isolates confirmed as *Salmonella* after isolation, identification and rPCR. Serotyping was applied on the basis of reaction with O- and H-group antigen, according to the White–Kauffmann–Le Minor Scheme (Grimont & Weill, 2007) by using commercial antisera (Becton Dickinson, Franklin Lakes, NJ). Slide agglutination and tube agglutination tests were applied for the analyses of somatic and flagellar phase antigens, respectively.

2.7 | Antimicrobial susceptibility test

Taking the recommended antimicrobials in routine analysis for *Enterobacteriaceae* by the EUCAST (2015a) into consideration, antimicrobial susceptibility of the isolates was determined against the following 18 frequently used antimicrobials in veterinary and/or human medicine: amikacin (AK, 30 μg, Oxoid CT0107B), amoxicillin/clavulanic acid

TABLE 1 Prevalence, serovar distribution, and antimicrobial resistance profile of *Salmonella* in slaughter cattle

Sample type (*n*)	Number of positive samples (%)	Sample ID	Serovar	Resistance profile
Carcass (200)	2 (1.0)	C86	Typhimurium	
		C94	Typhimurium	
Fecal content (200)	2 (1.0)	F133	Enteritidis	
		F158	Albany	
Mesenterial lymph node (200)	4 (2.0)	M53	Typhimurium	
		M132	Enteritidis	FOX, NOR, PEF
		M133	Enteritidis	AK, CN, TOB
		M136	Enteritidis	CTX, TOB
Liver (200)	0 (0.0)			
Kidney (200)	0 (0.0)			
Spleen (200)	0 (0.0)			
Gallbladder (200)	0 (0.0)			
Total (1400)	8 (0.6)			

(AMC, 30 μg, Oxoid CT0223B), ampicillin (AMP, 10 μg, Oxoid CT0003B), ampicillin/sulbactam 1:1 (SAM, 20 μg, Oxoid CT0520B), azithromycin (AZM, 15 μg, Oxoid CT0906B), ciprofloxacin (CIP, 1 μg, Oxoid CT0623B), cefepime (FEP, 30 μg, Oxoid CT0771B), cefotaxime (CTX, 30 μg, Oxoid CT0166B), cefoxitin (FOX, 30 μg, Oxoid CT0119B), chloramphenicol (C, 30 μg, Oxoid CT0013B), ertapenem (ETP, 10 μg, Oxoid CT1761B), gentamicin (CN, 120 μg, Oxoid CT0794B), norfloxacin (NOR, 10 μg, Oxoid CT0434B), pefloxacin (PEF, 5 μg, Oxoid CT0661B), piperacillin/tazobactam (TZP, 110 μg, Oxoid CT0725B), sulphamethoxazole/trimethoprim 19:1 (SXT, 25 μg, Oxoid CT0052B), tigecycline (TGC, 15 μg, Oxoid CT1841B), and tobramycin (TOB, 10 μg, Oxoid CT0056B).

For this, a loopful of each *Salmonella* stock culture stored at −20°C was streaked onto nutrient (NA, Oxoid, CM0003) agar and incubated at 37°C for 24 hr. By following the direct colony suspension procedure, 1–1.5 colony was suspended in 5 mL 0.85% NaCl, and was streaked onto Mueller-Hinton (MH, Oxoid, CM0337) agar by 3-way streaking after turbidity adjustment to 0.5 McFarland (Densimat, Biomerieux, 21250) within 15 min. Plates were incubated at 35°C and examined at 16 and 20 hr (EUCAST, 2015b). Isolates were classified as susceptible (S) and resistant (R) based on the measured zone diameters of complete inhibition and interpreted by comparing the zone diameter limit values in EUCAST (2015a).

2.8 | Statistical analysis

Sensitivity for ISO and rPCR, and relative trueness (RT) and false positive ratio (FPR) of rPCR based on sample type were calculated according to the protocol described in ISO 16140 (ISO, 2016). Reliability of the agreement between method results was determined by Cohen's kappa test (Landis & Koch, 1977).

3 | RESULTS

Study results revealed a 4% overall (8/200), 1% (2/200) C and FC (2/200), and 2% (4/200) MLN *Salmonella* prevalence in slaughter cattle examined. There was no *Salmonella* isolation from L, K, S, GB samples. *Salmonella* contamination rate in 1400 samples was calculated as 0.6% (8/1400) (Table 1).

When rPCR and ISO reference method's *Salmonella*-confirmation results from suspect isolates of different sample types were compared, three and two false positives in C and MLN isolates produced a high RT confirmation rate for rPCR as 91.67% and 86.36%, respectively, which lead to a substantial agreement between two methods. Additionally, the absence of any false positivity in rPCR for FC-derived *Salmonella*-suspect isolates indicated that rPCR was in perfect agreement with ISO (RT: 100.00%) in confirming *Salmonella* isolates from this sample type. When all sample types collected from slaughter cattle were considered, the RT of rPCR for detecting *Salmonella*-suspect isolates with respect to ISO was calculated as 93.33%, which resulted a "substantial" overall agreement between two methods (Table 2).

In this study, *S.* Enteritidis was the most predominant serovar with 50% (4/8 isolates) isolation rate in total from slaughter cattle. One of the FC (50%) and three of the four MLN (75%) isolates were serotyped as *S.* Enteritidis, whereas both C isolates (100%) and one of the four MLN isolates (25%) were identified as *S.* Typhimurium,

TABLE 2 Sensitivity, relative trueness, and false positive ratio of rPCR with respect to ISO in confirmation of *Salmonella*-suspect isolates from different sample types

Source of *Salmonella*-suspect isolate (*n*)	Reference method ISO			Alternative method rPCR					Kappa index values
	Positive (*n*)	Negative (*n*)	SE ISO (%)	False $_{neg}$ (*n*)	False $_{pos}$ (*n*)	SE rPCR (%)	RT (%)	FPR (%)	
Carcass (36)	7	26	70.00	0	3	100.00	91.67[a]	11.54	0.77
Fecal content (23)	2	21	100.00	0	0	100.00	100.00[b]	0.00	1.00
Mesenterial lymph node (22)	4	15	71.43	1	2	85.71	86.36[a]	13.33	0.64
Liver (19)	0	0	ND	0	0	ND	NC	NC	NC
Kidney (8)	0	7	ND	0	1	ND	NC	NC	NC
Spleen (14)	0	13	ND	0	1	ND	NC	NC	NC
Gallbladder (13)	0	12	ND	0	1	ND	NC	NC	NC
Total (135)	13	113	63.64	1	8	95.45	93.33[a]	7.08	0.71

SE, sensitivity; RT, relative trueness; FPR, false positive ratio; ND, not determined; NC, not computed.
[a] Substantial agreement between ISO and rPCR results.
[b] Perfect agreement between ISO and rPCR results.

resulting an overall isolation rate of 37.5% (3/8 isolates) for S. Typhimurium as the second dominant serovar isolated. The third serovar identified was *S.* Albany, as one of the two FC isolates (50%), with an isolation rate of 12.5% in eight *Salmonella* isolates (Table 1).

Antimicrobial resistance findings revealed that only three of eight salmonellae, all of which were *S.* Enteritidis isolates from MLN, were resistant (37.5%) to the 18 antimicrobials tested. In contrast, all *S.* Albany and *S.* Typhimurium were found pansusceptible (62.5%). *S.* Enteritidis isolates showed multiresistance (37.5%) in three different profiles as FOX/NOR/PEF, AK/CN/TOB, and CTX/T OB to seven antimicrobials (Table 1).

4 | DISCUSSION

Salmonella is the number one cause of bacterial foodborne outbreaks in EU with an increasing rate both in overall and bovine meat-related outbreak rates as 21.8% and 5.4% in 2015 compared to 20% and 2.2% in 2014, respectively (EFSA & ECDC, 2016; Rizzi, 2017). Scarcity of recent data on the current *Salmonella* prevalence, serogroup/serotype and/or antimicrobial resistance status in slaughter cattle was the initial driving force of our study.

In this study, overall *Salmonella* prevalence was 4% (8/200) in slaughter cattle examined. *Salmonella* contamination rate in all samples was calculated as 0.6% (8/1400) (Table 1). Our carcass contamination rate as 1% was lower than the 5% prevalence in 180 carcass swab samples reported by Küplülü (1999) and higher than Çalıcıoğlu et al. (2005), who found no *Salmonella* in their 44 carcass excision samples. In the EU summary report, *Salmonella* prevalence in slaughterhouse bovine carcass swabs from 12 EU countries was 0%, while 3 countries as Denmark, Czech Republic, and Spain indicated the presence of this pathogen as 0.08%, 0.6%, and 2.13%, respectively (EFSA & ECDC, 2016). Worldwide, there are studies from U.S. (Schmidt et al., 2015) with lower prevalence rate as 0% in 74 carcass samples collected from three different plants; a similar rate as 0.93% in one out of 108 carcasses by Loiko et al. (2016) from Brazil; and a higher rate as 44% in 11 out of 25 carcasses by Fauzi, Arshad, Ruhil, and Al-Sultan (2016) from Malaysia. As an important sample type in determining NTS carriage in slaughter cattle, 1% of the FC samples in this study were determined to harbor the pathogen (Table 1). In our country, Genç (2002), Hadimli et al. (2017), and Küplülü (1999) found 1.2% (3/250), 7.5% (33/437), and 14.4% (26/180) of FCs of slaughtered cattle contaminated with *Salmonella*, respectively. Our results are higher than 0% prevalence in 125 fecal samples reported by Bonardi, Bruini, Magnani, Cannistrà, and Brindani (2017) from Italy; and the 0.39% prevalence of Finland in EFSA and ECDC (2016), but lower than the studies by Rene et al. (2017), Ruiz et al. (2017), and Schmidt et al. (2015) with notification rates of 5–66.4% range. There was no *Salmonella* isolation from L, S, K (as edible offal in our country) and GB (possible responsible organ for chronic NTS carriage) samples from slaughtered cattle (Table 1). Within a limited number of studies investigating *Salmonella* prevalence in slaughter cattle including internal organs, Im, Seo, Bae, and Lee (2016), and Lee and Lee (2016) could not detect *Salmonella* in 6 and 8 livers in Korea, respectively, while Moawad et al. (2017) found 10% contamination rate in both of their 10 liver and 10 spleen samples in Egypt. Also, Alemu and Zewde (2012) from Ethiopia and Genç (2002) from Turkey found 1.1% (2/181 samples) and 0.4% (1/250 samples) *Salmonella* in their liver and spleen samples, respectively. We could not come across any study specifically looking for the prevalence of this pathogen in slaughter cattle kidneys. Additionally, a single study by Akoachere et al. (2009), where gallbladders were found to be 32.6% (14/50) contaminated with *Salmonella*, was in contrast to our findings (0%). *Salmonella* prevalence in our MLN samples (2%), with a critical role in determining the possible presence of the bacterium in cattle, was higher than other sample types (Table 1). Also our MLN prevalence rate was higher than indicated in EFSA & ECDC report (2016) for Finland (0.09%), Sweden (0.08%), and Norway (0%), and lower than the 49.48% rate indicated by Ruiz et al. (2017) from Mexico.

Serovar distribution revealed *S.* Enteritidis (50%) as the most predominant serovar, followed by *S.* Typhimurium (37.5%) and *S.* Albany (12.5%), regardless of the sample type (Table 1). Based on *Salmonella* monitoring data and foodborne outbreaks for 2015 in EU, bovine meat and products were responsible for 6.9% of the 116 *S.* Enteritidis-related foodborne outbreaks, while this meat type was found less responsible (3.33%) in a total of 30 *S.* Typhimurium-related outbreaks (Rizzi, 2017). Intriguingly, *S.* Typhimurium was observed as the predominant serovar in samples examined in the studies focused on the determination of up-to-date *Salmonella* serovar profile in cattle. Accordingly, all C (2/2) and one out of four MLN isolates were serotyped as *S.* Typhimurium in our study (Table 1). While our findings are in accordance with Alemu and Zewde (2012), reporting *S.* Typhimurium as the most prevalent serovar in cattle carcasses, are against the studies indicating S. Typhimurium as the predominant serovar in cattle MLNs (Alemu & Zewde, 2012; Küplülü, 1999). One of the two FC and three out of four MLN isolates were serotyped as *S.* Enteritidis (Table 1). While there are similar findings by Genç (2002), Hadimli et al. (2017), Rene et al. (2017), Rizzi (2017) reporting the presence of this serovar within their FC isolates, to the best of our knowledge, this is the first study indicating the presence of S. Enteritidis in slaughter cattle MLNs. Unusually, the other serovar isolated from FC samples was *S.* Albany, and our detailed literature search about this serovar revealed its rare reporting in cattle-related studies as: in bovine meat in Malaysia by Fauzi et al. (2016) and, as a jejunum isolate in USA by Goodman et al. (2017). There is no evidence until this time linking the presence of Albany in cattle to a human salmonellosis case, but our detection of *S.* Albany as one of the serovars in FC, thereof its occurrence in cattle is worth mentioning as an epidemiological contribution for further studies. Additionally, although the contamination source for *S.* Albany to cattle was not investigated in this study, when this serovar's isolated steer's information was retrospectively examined, the farm the steer belonged to was identified as in close proximity to a broiler production unit, which may indicate a possible link between these two farms. Parallel to our idea, Huang et al. (2016) also suspected the contamination source for their *S.* Albany isolate as poultry, poultry meat, or egg-related commercial feed.

An additional output in our study is rPCR's performance in reliable utilization with ISO in the rapid and reliable confirmation of *Salmonella*-suspect isolates from cattle, and particularly from FC samples,

demonstrated by an overall substantial, and a perfect agreement between two methods, respectively (Table 2).

Antimicrobial susceptibility phenotyping indicated majority of salmonellae isolated (62.5%) from cattle as susceptible to all antimicrobials tested, except MDR isolates (37.5%), which were all *S.* Enteritidis (Table 1). Within this most prevalent serovar of Enteritidis isolates, all but one (75%) were particularly resistant to the aminoglycosides (amikacin, gentamicin, and tobramycin), cephalosporins (cefotaxime and cefoxitin) and fluoroquinolones (norfloxacin and pefloxacin) (Table 1). Similarly, without taking sample type into consideration, Hadimli et al. (2017) reported a resistant *S.* Enteritidis in slaughter cattle. In evaluating by sample type, we could not find a study indicating presence/isolation of *S.* Enteritidis from bovine MLNs, and therefore, we were not able to compare our MLN S. Enteritidis isolates' resistance patters to any other previous finding. Apart from this, the profile of our pansusceptible *S.* Enteritidis isolated from FC was in accordance with (susceptible to at least one: chloramphenicol, amoxicillin/clavulanic acid, ampicillin, ciprofloxacin, cefepime, and sulphamethoxazole/trimethoprim) previous works (Rene et al., 2017; Souto et al., 2017). There are also studies indicating resistance of *S.* Enteritidis to tobramycin (Rene et al., 2017), cefoxitin (Souto et al., 2017), and ampicillin and chloramphenicol (Hadimli et al., 2017).

Resistance and MDR to the frequently used fluoroquinolones and aminoglycosides in veterinary medicine detected in our cattle *S.* Enteritidis isolates point out that this animal species is also worth investigating for its role in the introduction of *S.* Enteritidis-related antimicrobial-resistant infections in humans, and the treatment problems thereof, and to be considered for inclusion to control programs, if necessary. Our *S.* Typhimurium serovars isolated from C and MLNs showed no resistance to the antimicrobials tested. Similarly, Alemu and Zewde (2012) reported sensitivity in their carcass *S.* Typhimurium isolates. However, their *S.* Typhimurium isolates from MLN were MDR to three antimicrobials (Alemu & Zewde, 2012). Our FC *S.* Albany isolate was pansusceptible to the antimicrobials tested, and this finding is in contrast to Huang et al. (2016).

As an overall assessment of our findings and related previous data, the divergence in *Salmonella* prevalence rates in different samples could mainly be related to (1) representativeness of the sample (the number of collected samples, their effectiveness in representing the presence of the pathogen, variability in sampling, isolation, and identification methods—variations within traditional culture methods, and inclusion of rapid detection systems); (2) environmental factors (efficiency of the hygiene practices applied in the slaughterhouses, sampling location, time and season, husbandry and management); (3) animal (age, gender, health condition, and breed), while the isolated serovar rates/types/patterns and their antimicrobial resistance profiles could be affected from the above mentioned, and also from the detection method and interpretation criteria used, and may be due to the coexistence of more than one genotype, the origin of the isolate, the intrinsic and extrinsic microenvironmental factors (tissue tropism/affinity of the isolate to one specific site; variation in duration of infection between various sites). Detection of *Salmonella* in carcasses, MLNs, and FCs in similar rates indicated that these cattle, although seemed apparently healthy, were carriers for this pathogen, which is a threat to public health.

5 | CONCLUSION

Slaughter cattle are important carriers for NTS serovars, which pose risk for public health. Predominance of *S.* Enteritidis in cattle in our country, particularly in mesenterial lymph nodes, and the MDR pattern identified; the presence of *S.* Typhimurium as the second dominant and pansusceptible serovar; detection of *S.* Albany for the first time in cattle fecal content, are new epidemiological findings in this study. In view of this information, extension of the national poultry *Salmonella* control program to cattle, with strict and regular monitoring of antimicrobial use in treatment is advised. We believe that this up-to-date epidemiological information regarding the current status of *Salmonella* in slaughter cattle in Turkey would be of use in the revision of control and prevention programs in our country and in the neighboring countries of interest.

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