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Prevalence and antimicrobial susceptibility pattern of methicillin-resistant *Staphylococcus aureus* isolates from retail raw meats in Turkey

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Abstract

The transmission of *Staphylococcus aureus* and methicillin-resistant *S. aureus* (MRSA) through food products of animal origin may pose a public health concern. Therefore, the objectives of the present work were to determine the prevalence of *S. aureus* and MRSA in raw meat samples (beef, chicken, turkey, and duck) at retail level, and to determine the antimicrobial resistance profile of the MRSA isolates. Between September 2018 and January 2019, a total of 325 raw meat samples were collected from retail stores, and analysed for the presence of *S. aureus* and MRSA using primary enrichment method as well as using secondary selective enrichment methods for MRSA detection. All the suspected *S. aureus* and MRSA colonies obtained from the samples were confirmed by both phenotypic and genotypic methods. The MRSA isolates were tested against various antimicrobials, and the minimal inhibitory concentration (MIC) of vancomycin was determined. Macrolide-lincosamide-streptogramin B (MLS_B) resistance phenotypes were also screened using the double-disk diffusion test. The overall prevalence of *S. aureus* was 57.2%, whereas the food-specific prevalence in beef, turkey, duck, and chicken samples was 75, 53, 48, and 46%, respectively. The overall prevalence of MRSA was 1.2%, whereas it was 3, 1, 0, and 0% in beef, turkey, chicken, and duck samples, respectively. All MRSA isolates were detected by the secondary selective enrichment method. These MRSA isolates had a variety of MLS_B resistance phenotypes (*i.e.*, $iMLS_B$, $cMLS_B$, and MS-MLS_B) with vancomycin MIC values ranging between 1 - 2 µg/mL. These findings are important for developing interventions to reduce raw meat contamination with *S. aureus* and MRSA, and to improve public health.

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Introduction

Staphylococcus aureus is one of the main causes for both hospital- and community-acquired infections (Klein *et al.*, 2018). This pathogen can cause a wide spectrum of infections such as skin and soft tissue infections, as well as bloodstream infections (Lindsay and Holden, 2004). Furthermore, due to its virulence factors and toxin repertoire, *S. aureus* can cause many toxin-mediated diseases such as staphylococcal food-borne disease, scalded skin syndrome, and toxic shock syndrome (Lindsay and Holden, 2004). Methicillin-resistant *S. aureus* (MRSA) infections can lead to higher mortality rates, longer hospitalisation time, and higher healthcare-associated costs as compared to infections with methicillin-sensitive strains (Klein *et al.*, 2018). Although MRSA was primarily considered a cause for hospital-acquired infections (HA-MRSA), community-acquired MRSA (CA-MRSA) infections were first reported in the 1980s. Nowadays, MRSA is considered a common cause for community-acquired infections (Klein *et al.*, 2018).

According to the European Food Safety Authority (EFSA), foods of animal origin are considered a potential source of MRSA transmission to humans (EFSA, 2009). Several studies have reported *S. aureus* and MRSA prevalence in retail raw pork, beef, and poultry meat in different countries worldwide (Quddoumi *et al.*, 2006; Normanno *et al.*, 2007; De Boer *et al.*, 2009; Agersø *et al.*, 2012; Ge *et al.*, 2017; Tang *et al.*, 2017). In Turkey, while most

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of the studies conducted to detect *S. aureus* and MRSA were mostly in milk and dairy products, few were carried out in retail raw meats (Pamuk *et al.*, 2012; Gocukoglu *et al.*, 2012; Elal Mus *et al.*, 2019; Keyvan *et al.*, 2020). Those studies conducted to detect the pathogen in raw meats reported that its prevalence ranged between 10.4 and 66% (Aydin *et al.*, 2011; Koluman *et al.*, 2011; Ozdemir and Keyvan, 2016), whereas MRSA prevalence ranged between 5 to 28.2% in raw beef, lamb meat, and chicken samples. Nonetheless, MRSA contamination of turkey and duck meat in Turkey was not reported (Gundogan *et al.*, 2005; Aydin *et al.*, 2011; Koluman *et al.*, 2011; Guran and Kahya, 2015; Siriken *et al.*, 2016; Ozdemir and Keyvan, 2016; Can *et al.*, 2017). Additionally, most of these studies focused on isolating *S. aureus* first from the meat samples, and then identifying MRSA, rather than isolating and identifying MRSA directly from the samples without isolating *S. aureus*.

There are several methods which can be used for the detection and characterisation of MRSA directly from meat samples. These include the use of chromogenic agars for isolation (De Boer *et al.*, 2009; Jackson *et al.*, 2013), determination of cefoxitin MICs (via disc diffusion or broth dilution; EUCAST, 2019b), and DNA-based molecular methods for detection of *mecA* gene (Luteijn *et al.*, 2011). Furthermore, some international institutions recommend the use of a method that includes two different consecutive enrichment steps (*i.e.*, 2-S method) to increase the MRSA recovery rate from food animal samples (EFSA, 2012; EURL-AR, 2018). Previous studies from Turkey which investigated MRSA prevalence in retail raw meats have determined the presence of MRSA in *S. aureus* isolates using Baird Parker agar or non-chromogenic agar medium without including a pre-enrichment step (Gundogan *et al.*, 2005; Aydin *et al.*, 2011; Koluman *et al.*, 2011; Guran and Kahya 2015; Ozdemir and Keyvan, 2016; Siriken *et al.*, 2016; Can *et al.*, 2017). This may have underestimated the 'actual' MRSA prevalence in the samples. To the best our knowledge, there is no study that has focused systematically on determining MRSA prevalence in retail raw meats in Turkey by using different enrichment steps. Therefore, the primary objective of the present work was to determine the prevalence of *S. aureus* and MRSA in different types of raw meat sold at the retail level in Turkey. The secondary objectives were to: (1) compare two different enrichment steps for MRSA recovery, and (2) determine both vancomycin minimal inhibition concentration (MIC) values and macrolide-lincosamide-streptogramin B (MLS_B) resistance phenotypes in the MRSA isolates.

Materials and methods

Study design

A total of 325 raw meat samples were collected from supermarkets and butcher shops in Diyarbakir province, Turkey, between September 2018 and January 2019. The required sample size was 325 sample based on the sample size calculation formula for cross-sectional studies, where 'Z' is the standard normal variate (at 5% type I error = 1.96), 'p' is the expected prevalence of *S. aureus* in meats which is 30% based on earlier studies, and 'd' is the absolute error or precision (5% for this study).

The distribution of the 325 meat samples were as follows: chilled chicken parts (n = 100), turkey parts (n = 100), ground beef (n = 100), and frozen duck whole carcasses (n = 25) as shown in Table 1. The supermarkets and meat brands included and selected in the present work were based on the availability of meat type during the store visits. All samples were of Turkish national brands. The sampled meat packs were paired by part type, brand, production date, lot number, and sell-by date. All chilled chicken and turkey part samples were randomly collected from the supermarket stores during five visits. The retail ground beef samples were divided into two types: half of them were vacuumed-sealed samples collected from five supermarkets, and represented two different national beef meat production companies; whereas the other half of samples were chilled non-vacuumed packaged ground beef collected from 15 local butcher shops. The frozen retail whole duck carcasses (represented by one national duck production company) were collected from the five supermarket stores.

All collected samples were raw meats as described in the Legal Food Code Definitions in Turkey (Turkish Food Codex, 2012). The collected samples were stored in an insulated cooler at a temperature below 4°C, and transported within 4 h to the laboratory at the Department of Food Hygiene and Technology of University of Dicle (Diyarbakir province, Turkey) for *S. aureus* and MRSA microbiological analyses.

Table 1. Number of samples collected from retail markets by meat type, brand, market type, and storage temperature in Diyarbakir province, Turkey

Sample	No. of sample	Sample type	Packaging	Brand	Market type	Storage temperature
Chicken	25	Breast	Original packaging	National (three brands)	Two supermarkets	Chilled
Chicken	25	Drumstick	Original packaging	National (three brands)	Two supermarkets	Chilled
Chicken	25	Leg quarter	Original packaging	National (three brands)	Two supermarkets	Chilled
Chicken	25	Wing	Original packaging	National (three brands)	Two supermarkets	Chilled
Beef	50	Ground	Non-vacuumed	Local	Fifteen butcher shops	Chilled
Beef	50	Ground	Original packaging (vacuumed- sealed)	National (two brands)	Five supermarkets	Chilled
Turkey	30	Tenderloin	Original packaging	National (two brands)	Three supermarkets	Chilled
Turkey	30	Diced breast	Original packaging	National (two brands)	Three supermarkets	Chilled
Turkey	40	Thigh	Original packaging	National (two brands)	Four supermarkets	Chilled
Duck	25	Whole carcass	Original packaging	National (one brand)	Five supermarkets	Frozen
Total	**325**					

Detection of S. aureus and MRSA

Staphylococcus aureus

For primary enrichment, 25 g of samples from chicken, turkey, and ground beef were individually added to 225 mL of Mueller Hinton broth (MHB; LABM, UK) containing 6.5% NaCl (w/v), homogenised using a stomacher (Easy Mix-G560E, France), and then incubated at 37°C for 18 - 24 h. Following incubation, a loopful of primary enriched samples were streaked onto Baird Parker Agar (BPA; LABM, UK) that was supplemented with egg yolk emulsion and potassium tellurite, and then incubated at 37°C for 48 h. Thereafter, up to three suspected *S. aureus* colonies were sub-cultured on Tryptone Soy agar (TSA; LABM, UK) plates, and incubated (37°C, 18 - 24 h) for further phenotypic and genotypic analyses. Detection of *S. aureus* in frozen duck was carried out after thawing the whole carcasses at refrigerator temperatures for 24 - 48 h in its original package. Under aseptic conditions, a total of 25 cm^2 breast skin portion was excised using 5 × 5 sterile plates, then the samples were transferred into sterile stomacher bags, and homogenised in 225 mL of MHB containing 6.5% NaCl (w/v). Following incubation (37°C, 18 - 24 h, primary enrichment), the remaining isolation procedure was performed as described earlier.

Methicillin-resistant Staphylococcus aureus (MRSA)

The isolation of MRSA directly from each sample was conducted using two methods (*i.e.*, A and B) as described in the following section. Additionally, MRSA was detected using methicillin-resistance determination as well as via *nuc* and *mecA* genes determination.

MRSA (method A)

Each primary enriched sample was directly streaked onto oxacillin-resistant staphylococci isolation medium agar (ORSIM; LABM, UK) containing 2 µg/mL of oxacillin and 50 units/mL of polymyxin B, and then incubated at 37°C for up to 48 h. Intense blue colonies on the agar plates were considered as presumptive MRSA. Up to three presumptive MRSA colonies were sub-cultured on TSA plates, and incubated at 37°C for 18 - 24 h for further analysis.

MRSA (method B)

One millilitre from each primary enriched sample was transferred into 9 mL of tryptone soy broth (TSB; LABM, UK) tubes supplemented with 3.5 mg/L of cefoxitin (Sigma) and 50 mg/L of aztreonam (Sigma). The solution was then incubated at 37°C for 18 - 24 h as a secondary selective enrichment step. Following the incubation, one loopful of the enriched culture was streaked onto ORSIM agar containing both 2 µg/mL of oxacillin and 50 units/mL of polymyxin B, and then incubated (37°C, up to 48 h). The remaining isolation procedure was carried out as described in method A.

Determination of methicillin-resistance in the isolates

The suspected isolates of *S. aureus* obtained from the BPA medium, as well as from methods A and B, were confirmed and identified as *S. aureus* by Vitek 2 Gram positive cards in Vitek 2 bacterial identification system (bioMérieux, France).

The methicillin-resistance for the confirmed *S. aureus* isolates was determined using the cefoxitin disc diffusion method following the EUCAST guidelines (EUCAST, 2019a) as follows: each *S. aureus* isolate was suspended in TSB, and incubated at 37°C for 24 h. The bacterial suspension was adjusted to 0.5 McFarland turbidity via densitometer (Densichek Plus, bioMérieux), then the adjusted suspension was spread evenly in three directions using a sterile swab on the surface of dried Mueller Hinton agar (MHA; LABM, UK) plates. A cefoxitin disc (30 µg; Oxoid, Basingstoke, UK) was placed on the MHA plate, and incubated at 35 ± 1°C for 18 ± 2 h. According to the EUCAST breakpoints (EUCAST, 2019b), isolates with an inhibition zone diameter < 22 mm were considered as MRSA, and isolates with ≥ 22 mm zones were considered as methicillin-sensitive *S. aureus* (MSSA). *S. aureus* ATCC 43300 served as positive control, and *S. aureus* ATCC 29213 served as negative control.

Determination of nuc and mecA genes by PCR

Genomic DNA was extracted from all suspected and confirmed *S. aureus* isolates using the boiling method as follows: briefly, a single colony was added into 1.5-mL sterile Eppendorf tubes containing 100 µL of sterile water, mixed well, and then boiled for 15 min. Following centrifugation at 10,000 *g* for 5 min, the supernatant was used as the template for the multiplex PCR assay. The primer pairs *nuc*F 5'-GCGATTGATGGTGATACGGTT-3' and *nuc*R 5'-AGCCAAGCCTTGACGAACTAAAGC-3' were used for the detection of the *nuc* gene (Brakstad *et al.*, 1992); whereas *mec*AF 5'-AAAATCGATGGTAAAGGTTGGC-3' and *mec*AR primer pairs 5'-AGTTCTGCAGTACCGGATTTGC-3' were used for the *mecA* gene detection (Murakami *et al.*, 1991). Multiplex PCR was carried out to detect both genes in a total reaction volume of 50 µL containing 6 µL of 10 × PCR buffer [750 mmol/L Tris-HCl (pH 8.8; 25°C), 200 mmol/L $(NH4)_2SO_4$, and 0.1% Tween 20], 9 µL of $MgCl_2$, 9 µl of 10 mmol/L dNTP mix, 1 µL of 10 pmol of each primer, 1 µL of Taq polymerase at 5 U µ/L, 5 µL of template DNA, and 16 µL of molecular-grade water. For PCR amplification, an initial denaturation step at 94°C for 1 min, followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, 72°C for 1 min, extension at 72°C for 1 min, and a final extension at 72°C for 5 min was carried out using a thermal cycler (ABI Veriti Thermal Cycler, Applied Biosystems Asia Pte Ltd., Singapore). The PCR amplification products were separated in a 1.5% agarose gel containing SafeView (ABM, Canada), and visualised using a UV transilluminator (Spectroline, Model TC-312 E/F). MRSA 27R served as a positive control.

Antimicrobial susceptibility determination in MRSA isolates

The MRSA isolates were tested for antimicrobial susceptibility to multiple antibiotics using BD Phoenix™ 100 Automatic Microbiology Identification System (BD Diagnostic Instrument Systems, Sparks, MD, USA) following the manufacturer's instructions. Phoenix PMIC-87 panel was used for the antimicrobial susceptibility testing. The Phoenix panel was composed of the following 19 antibiotics: ciprofloxacin, clindamycin, daptomycin, erythromycin, fosfomycin/G6P, fusidic acid, gentamicin, levofloxacin, linezolid, oxacillin, penicillin, quinupristin-dalfopristin, rifampicin, teicoplanin, tetracycline, tigecycline, tobramycin, trimethroprim-sulphamethoxazole, and vancomycin. The minimum inhibitory concentrations (MICs) were interpreted according to the Clinical and Laboratory Standards Institute (CLSI, 2017).

The macrolide-lincosamide-streptogramin B (MLS_B) resistance phenotypes of the MRSA isolates

were determined using a double-disk diffusion test (D-test) (EUCAST, 2019a). For this purpose, erythromycin (15 µg; Oxoid, Basingstoke, UK) and clindamycin (2 µg; Oxoid, Basingstoke, UK) disks were placed on MHA plate containing a lawn culture of the MRSA isolates at a 15 mm distance (edge-to-edge), and then incubated at 37°C for 24 h. Following the incubation, if a flattening of the inhibition zone (D shape) around a clindamycin disc that was adjacent to the erythromycin disc was observed, it is considered as inducible clindamycin resistance ($iMLS_B$). Isolates that did not form any inhibition zone around clindamycin and erythromycin discs were considered constitutive resistant ($cMLS_B$), and those that were resistant to erythromycin but sensitive to clindamycin were considered a macrolide and streptogramin B (MS) resistant phenotype.

Vancomycin resistance of the MRSA isolates was determined using a gradient diffusion E-test method following the manufacturer's instructions (BioMérieux Inc., France). The MIC values were interpreted according to the EUCAST breakpoints (EUCAST, 2019b).

Statistical analysis

Data analyses were conducted using the SPSS statistical software version 24 (IBM SPSS, IBM Corporation, USA). The relationship between the outcomes (presence of *S. aureus* or MRSA per sample) and the sample type (chicken, turkey, duck, and beef) was performed using chi-square (χ^2) test in SPSS. Statistical significance was considered when the *p*-value was < 0.05.

Results

Prevalence of S. aureus and MRSA

Out of the 325 samples analysed, 186 (57.2%) were contaminated with *S. aureus* (Table 2). The prevalence of *S. aureus* in ground beef, turkey, duck, and chicken samples was 75, 53, 48, and 46%, respectively. The *S. aureus* prevalence was significantly ($p < 0.05$) higher in ground beef samples as compared to poultry samples (chicken, turkey, and duck); however, the prevalence between poultry samples (chicken *vs.* turkey *vs.* duck) was not significant different ($p > 0.05$) (Table 2). Since we collected up to three *S. aureus* isolates per sample, a total of 219 isolates out of 186 positive samples were tested. None of these 219 confirmed *S. aureus* isolates were methicillin-resistant; neither phenotypically (based on cefoxitin 30 µg) or genotypically (based on *mecA* gene detection). Hence, these isolates were classified as methicillin-sensitive *S. aureus* (MSSA).

The overall MRSA prevalence was 1.2% (n = 325 samples) based on direct detection of this pathogen from the samples. Additionally, the prevalence in beef, turkey, chicken, and duck samples was 3, 1, 0, and 0%, respectively (Table 2).

The number of suspected MRSA isolates (based on method A) obtained from direct culturing of beef, chicken, turkey, and duck samples were 74, 24, 16, and 4, respectively. None of these suspected MRSA isolates were confirmed as *S. aureus* based on the phenotypic and genotypic analyses. Contrary to method A, fewer colonies were observed on ORSIM agar plates when using method B. Based on method B, four isolates were MRSA out of 73 MRSA suspected isolates originated from three ground beef samples and one turkey sample (Table 2). All four MRSA isolates obtained from method B carried both *nuc* and *mecA* genes, and showed breakpoint of < 22 mm inhibition zone size for cefoxitin (30 µg). The suspected isolates that did not carry the *nuc* gene were identified via the Vitek 2 identification system as *Enterococcus* spp. and coagulase-negative *Staphylococcus* spp.

Antibiotic resistance and MLS_B phenotypes

The results of the antimicrobial susceptibility testing conducted on the four MRSA isolates are shown in Table 3. All the isolates were 100% resistant to oxacillin, penicillin, rifampicin, tetracycline, and tobramycin, but susceptible to daptomycin, vancomycin, linezolid, teicoplanin, levofloxacin, ciprofloxacin**,** trimethoprim-sulfamethoxazole, teicoplanin, and linezolid. All the isolates were multidrug-resistant to at least three different antibiotic classes, and to at least six different antibiotics. One isolate from a turkey sample was resistant to 12 antibiotics, and one isolate from ground beef was resistant to nine antibiotics (Table 3).

One MRSA isolate classified as both erythromycin- and clindamycin-resistant based on the BD Phoenix system was also found $cMLS_B$ by the D-test. Additionally, two MRSA isolates were erythromycin-resistant, but clindamycin-sensitive based on the BD Phoenix system. One isolate was $iMLS_B$, and another was MS-MLS_B via the D-test (Table 3). Out of the four MRSA isolates, two exhibited a vancomycin MIC_{Etest} value of 2.0 µg/mL,

Table 2. Prevalence of *S. aureus* and MRSA in chicken, turkey, duck, and ground beef samples (n = 325) isolated from retail markets in Diyarbakir province, Turkey.

Sample type	No. of sample	No. of sample contaminated with *S. aureus*, and (%)	*S. aureus*†			MRSA						No. of sample contaminated with MRSA, and (%)
						Method A†			Method B			
			No. of isolate‡	*nuc*	*mecA*	No. of isolate	*nuc*	*mecA*	No. of isolate	*nuc*	*mecA*	
Chicken breast	25	17 (68)	18	18	-	8	-	8	4	-	4	-
Chicken drumstick	25	9 (36)	10	10	-	7	-	7	5	-	5	-
Chicken leg quarter	25	12 (48)	13	13	-	6	-	6	4	-	4	-
Chicken wing	25	8 (32)	11	11	-	3	-	3	4	-	4	-
Chicken (total)	100	46 (46)^{A*}	52			24		24	17		17	-
Turkey tenderloin	30	19 (63.3)	22	22	-	2	-	2	6	-	6	-
Turkey diced breast	30	11 (36.7)	14	14	-	6	-	6	4	1	4	1 (3.3)
Turkey thigh	40	23 (57.5)	24	24	-	8	-	8	2	-	2	-
Turkey (total)	100	53 (53)^{A*}	60			16		16	12	1	12	1 (1)
Duck	25	12 (48)^{A*}	13	13	-	4	-	4	2	-	2	-
Ground beef (vacuumed)	50	33 (66)	44	44	-	39	-	39	19	1	19	1 (2)
Ground beef (non-vacuumed)	50	42 (84)	50	51	-	35	-	35	23	2	23	2 (4)
Ground beef (total)	100	75 (75)^{B*}	95			74		74	42	3	42	3 (3)
Total	**325**	**186 (57.2)**	**219**	**219**	**-**	**118**	**-**	**118**	**73**	**4**	**73**	**4 (1.2)**

MRSA: methicillin-resistant *S. aureus*; †None of the samples were contaminated with MRSA; ‡Up to three isolates were obtained from sample; ^{A*,B*}Numbers in the same column with the different superscript are significantly different (p < 0.05).

Table 3. Antimicrobial susceptibility pattern, MLS_B resistance phenotypes, and vancomycin MIC values in MRSA isolates.

Sample type	Isolate	CIP	CLI	DAP	ERY	FOS	FUS	GEN	LVX	LZD	OXA	PEN	QD	RIF	TEC	TET	TIG	TOB	TMP-SUL	VAN	MDR	MLS_B-D test			Vancomycin (mg/mL)
																						ERY	CLI	MLS_B-phenotype	
Beef	MRSA	S	S	S	S	S	R	R	S	S	R	R	S	R	S	R	S	R	S	S	7	S	S	-	1.5
Beef	MRSA	S	S	S	R	R	S	R	S	S	R	R	R	R	S	R	S	R	S	S	9	R	S	$iMLS_B$	1
Turkey	MRSA	S	R	S	R	R	R	R	S	S	R	R	R	R	S	R	R	R	S	S	12	R	R	$cMLS_B$	2
Beef	MRSA	S	S	S	R	S	S	S	S	S	R	R	S	R	S	R	S	R	S	S	6	R	S	MS-MLS_B	2

CIP: ciprofloxacin; CLI: clindamycin; DAP: daptomycin; ERY: erythromycin; FOS: fosfomycin/G6P; FUS: fusidic acid; GEN: gentamycin; LVX: levofloxacin; LZD: linezolid; OXA: oxacillin; PEN: penicillin; QD: quinupristin-dalfopristin; RIF: rifampicin; TEC: teicoplanin; TET: tetracycline; TIG: tigecycline; TOB: tobramycin; TMP-SUL: trimethoprim-sulfamethoxazole; VAN: vancomycin; and MDR: multidrug-resistance.

the third had MIC_{Etest} value of 1.5 µg/mL, and the fourth had MIC_{Etest} value of 1.0 µg/mL (Table 3).

Discussion

The overall prevalence of *S. aureus* was 57.2% (*n* = 325 meat samples) with the highest percentage in ground beef (75%), followed by turkey (53%), duck (48%), and chicken (46%). All of these *S. aureus* isolates were methicillin-sensitive (MSSA). Similar to our findings, Koluman *et al*. (2011) reported that the prevalence of MSSA was 48, 42, and 58% in chicken, turkey, and minced beef meat samples (*n* = 50 per sample type), respectively. In another study, Tang *et al.* (2017) reported that 66% (total *n* = 145) of chicken, turkey, and pork samples were contaminated with MSSA. Moreover, the authors reported that the prevalence of MSSA was found to be highest in chicken meat (75%), and lowest in turkey meat (35%), somewhat similar to our findings. Contrary to our findings, other studies revealed that the prevalence of *S. aureus* was lower in foodstuffs of animal origin as compared to ours (Ogata *et al.*, 2012; Ozdemir and Keyvan, 2016). This variation might be explained due to the different methods used to detect the presence of *S. aureus* in meat samples. In the present work, three different methods were used to isolate *S. aureus*. In the first method, isolation of *S. aureus* was carried out regardless of the resistance property, while the other two methods specifically targeted the isolation of MRSA directly from the meat samples. Kitai *et al.* (2005) compared the presence of *S. aureus* in 444 retail chicken meat samples using both direct inoculation into solid medium (BPA) with and without enrichment in brain heart infusion broth containing 7% NaCl. The authors reported that *S. aureus* was detected in 40.5% of the samples with the direct method but at a higher percentage (*i.e.*, 65.1%) with the enrichment step.

In the present work, the overall prevalence of MRSA was 1.2% (*n* = 325) with the highest percentage in the ground beef samples (*i.e.*, 3%). Most of the studies conducted to assess MRSA presence in retail meat in Asia, Europe, Canada, USA, and other countries revealed that the prevalence ranged between < 1 and 11.9% with few reported higher percentage (*i.e.*, up to 35%) (Kitai *et al.*, 2005; Normanno *et al.*, 2007; De Boer *et al.*, 2009). Ge *et al.* (2017) found that 1.9% of the beef, chicken, turkey, and pork samples (*n* = 3,520) were contaminated with MRSA. The prevalence of MRSA was highest in turkey meat (3.5%), and lowest in chicken meat (0.3%).

It has been reported that the use of an enrichment step in the broth media (such as MHB or TSB) containing 6.5% NaCl for the detection of MRSA in foods of animal origin enhance the probability of MRSA detection/isolation (Fang and Hedin, 2006; Feßler *et al.*, 2011; Ge *et al.*, 2017). The European Union Reference Laboratory for Antimicrobial Resistance (EURL-AR) and EFSA recommended the use of two different consecutive enrichment steps, known as the 2-S method, for the detection of MRSA from livestock samples (pigs, cattle, and chickens) (EFSA, 2012; EURL-AR, 2018). In the present work, a total of four isolates were determined as MRSA with the use of the second enrichment step (method B) as recommended by EFSA (2012) and EURL-AR (2018) for the detection of MRSA in meat samples. Therefore, we have demonstrated how the use of a second enrichment step increased the selectivity of MRSA detection as compared to method A. Comparable to our study, Fang and Hedin (2006) reported that the use of broth media containing cefoxitin provided a rapid and more sensitive detection method of this pathogen. However, Pauly *et al.* (2019) reported that selective enrichment with broth medium containing 3.5 mg/L of cefoxitin and 50 mg/L of aztreonam may hinder the growth of MRSA and may cause false negative results.

In the present work, all MRSA isolates were found resistant to oxacillin based on Phoenix automated system testing, and resistant to cefoxitin by the disc diffusion method. Quddoumi *et al.* (2006) reported that 19.1% of 157 MRSA isolates were phenotypically resistant to methicillin, and half of those isolates carried the *mec*A gene. In the same study, authors reported that the *mec*A gene was present in the isolates from sheep and poultry meat. Similarly, in the present work, all MRSA isolates from one turkey sample were methicillin-resistant (phenotypically) and carried the *mec*A gene.

Discrepancies between the phenotypic and genotypic detection of MRSA were not seen in the present work. The MRSA-resistant gene, *mec*A, was detected in 100% (4/4) of the phenotypically-confirmed MRSA isolates based on the cefoxitin disc diffusion method as recommended by international authorities such as CLSI and EUCAST (CLSI, 2017; EUCAST, 2017). Contrary to our results,

discrepancies between phenotypic and *mec*A detection of MRSA isolates have been reported in other studies (Aklilu *et al.*, 2013; 2016; Angelidis *et al.*, 2020). These discrepancies were explained due to presence of some other mechanism such as non-*mec*A dependent methicillin-resistance and heterogeneous expression of methicillin-resistance in *S. aureus* rather than the absence of the *mec*A gene (Abdalla *et al.*, 2014).

In the present work, all (100%) of the MRSA isolates were resistant to rifampicin, tetracycline, and aminoglycosides. Kraushaar *et al.* (2017) reported that MRSA isolates from chicken and turkey samples were resistant to tetracycline (96.6%), gentamicin (26.1%), and rifampicin (2.3%). Similarly, Feßler *et al.* (2011) revealed that 96.9% of MRSA isolates from poultry meat samples were resistant to tetracycline. Moreover, Jayaweera and Kumbukgolla (2017) reported that > 60% of MRSA isolates from farm animals and farmers were resistant to doxycycline; whereas, 73% of MRSA isolates from the animal and 100% of those from the farmers were resistant to gentamicin. In contrast to these data, Normanno *et al.* (2007) did not detect tetracycline or aminoglycoside resistance in any of the six MRSA strains isolated from foods of animal origin. Screening MRSA isolates for their resistance profiles is a recommended action for the medical treatment protocols as well as for public health surveillance to control the spread of antimicrobial resistance.

Vancomycin is considered the drug of choice for the treatment of MRSA infections. Therefore, it is important to identify vancomycin-susceptibility in MRSA isolates. In the present work, the vancomycin MIC values determined by the E-test were found to be 1 - 2 µg/mL. Although the reference method for vancomycin MIC determination is broth micro-dilution, it was not used in this study. However, the detected MIC values based on the E-test showed remarkable increased vancomycin resistance in our MRSA isolates.

Macrolide-lincosamide-streptogramin B (MLS_B) antibiotic group is used as one of the treatment options for staphylococcal infections. Although macrolide, lincosamide, and streptogramin belong to different antimicrobial classes, their action mechanism is the same (*i.e.*, through the inhibition of protein synthesis in bacteria). Interestingly, the resistance to one of these antibiotics may also lead to cross resistance to other antibiotics in this group. In the present work, MLS-resistance phenotypes were investigated by both an automated system and disk diffusion methods. One of the four MRSA isolates in the present work was sensitive to the MLS antibiotics, while the other three isolates exhibited various resistance phenotypes (*i.e.*, $iMLS_B$, $cMLS_B$, and MS). One of the interesting findings was that the isolate with $iMLS_B$ phenotype based on the disc diffusion method was sensitive to erythromycin when tested via the Phenoix automated system. Schlegelova *et al.* (2008) found that 3.2% of 1,235 *Staphylococcus* spp. isolates from various foodstuffs were MLS_B-resistant. Moreover, Feßler *et al.* (2011) reported MLS_B-resistance in 87.5% of 32 MRSA isolates from chicken and turkey products. Furthermore, Kraushaar *et al.* (2017) reported that the resistant percentage to MLS group antibiotics in MRSA isolates from chicken and turkey was over 50%; whereas Sudagidan and Aydin (2013) found that 3.2 and 0.64% of 154 *S. aureus* isolates from food had $iMLS_B$- and $cMLS_B$-resistant phenotypes, respectively.

Conclusion

The present work revealed that *S. aureus* was commonly found in raw meats (both beef and poultry) sold at retail in the study region in Turkey. Furthermore, although the prevalence of MRSA was low, the risk of transmission through the food chain cannot be disregarded, especially in foods of animal origin. While the risk to human infection by MRSA via contaminated foods is generally considered low (EFSA, 2009), foodborne outbreaks linked to MRSA-contaminated food have been reported (Kluytmans *et al.*, 1995; Jones *et al.*, 2002). Moreover, the detection of multidrug-resistant MRSA in raw meats and live animals signifies a potential threat for MRSA transmission to consumers via food or close contact with animals. Monitoring the occurrence and distribution of *S. aureus* and MRSA in food products of animal origin is important to reduce the incidence of foodborne disease, and improve public health.

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