Isolation and antimicrobial resistance of vancomycin resistant Enterococcus spp. (VRE) and methicillin-resistant S. aureus (MRSA) on beef and chicken meat, and workers hands from slaughterhouses and retail shops in Turkey

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Isolation and antimicrobial resistance of vancomycin resistant *Enterococcus* spp. (VRE) and methicillin-resistant *S. aureus* (MRSA) on beef and chicken meat, and workers hands from slaughterhouses and retail shops in Turkey

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ABSTRACT: The objectives of this study were to determine the presence and antimicrobial resistance of Methicillin Resistant *Staphylococcus aureus* (MRSA) and Vancomycin Resistant Enterococci (VRE) on beef and chicken carcasses and meat, and workers hands' at processing time from a cattle and a poultry slaughterhouse, and beef and chicken meat at retail level. Disk diffusion method was used to determine the antimicrobial resistance profile of the *Enterococcus* spp. and *S. aureus* isolates. Minimum Inhibitory Concentration (MIC) values were determined for vancomycin and oxacillin resistance. Finally, conventional PCR was performed to determine the presence of the *mec*A and *van*A resistance genes in isolates classified resistant to oxacillin and vancomycin according to MIC values. *S. aureus* and *Enterococcus faecium* isolated from 17 (17%) and eight (8%) samples, respectively. *E. faecalis* was not detected in any sample. The highest resistance rates were to ampicillin (3/5, 60 %) and penicillin G (5/5, 100 %) in MRSA and tetracycline (4/5, 80 %) in VRE isolates. While the *mec*A gene was detected in all MRSA isolates, *van*A gene was not detected in any of the phenotypically vancomycin resistant *E. faecium* isolates. The present study provides data for multiple antimicrobial resistance and presence of VRE and MRSA isolated from an ongoing surveillance in humans, livestock and poultry in Turkey.

Keywords: MRSA, VRE, chicken, beef, slaughterhouse, workers

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INTRODUCTION

Staphylococcus aureus is a well known foodborne bacterial pathogen related to foodborne intoxications (Peacock and Paterson, 2015; Haaber et al., 2017). Food handler carriers of enterotoxin-producing *S.aureus* are regarded as the main contamination source of food, via direct manual contact or respiratory secretions. The emergency of *S. aureus* in recent decades, especially the relation between livestock is required to highlight the contamination ways through the food chain starting from slaughtering of animals. Some strains have virulence characteristics which ensure their adaption to different environmental conditions, causing various life-threatening infections and gaining antibiotic resistance (Lowy, 2003). The emergence of methicillin resistance in *S. aureus* strains has become a serious international concern in the treatment and control of Staphylococcal infections. There are several studies reported the presence of MRSA on meat-producing animals including beef and chicken. The scientific report of EFSA (2015), declared that food-producing animals may be contaminated with methicillin-resistant *S. aureus* (MRSA) due to close contact with livestock or by foods of animal origin and lead human illnesses. Unlike penicillinase-related resistance, methicillin resistance affects a broad spectrum of antibiotics, such as the β-lactams, which includes penicillins, cephalosporins, and carbapenems (Chambers and DeLeo, 2009).

Enterococcus species are part of the normal microbiota of humans and warm-blooded animals. Enterococci are found in many foods of animal origin and are able to survive for long time on inanimate surfaces because of their ability to survive in adverse environmental conditions. The most common species identified in food animals are *E. faecium, E. cecorum, E. faecalis* and *E. hirae* (Ahmed and Baptiste, 2018).

Vancomycin-resistant enterococci (VRE) have been an increasing problem worldwide since VRE were first identified in 1980s and *van*A-type VRE was first reported in 1993. The use of avoparcin, a vancomycin analogue as a growth-promoting feed additive, has been linked to an increase in vancomycin-resistant enterococci in food animals (Birkegard et al., 2019). However, 25 years after the ban of avoparcin as a growth promoter in feed, a continuing resistance has been observed to vancomycin in a Danish pig farm (Birkegard et al., 2019). There are different vancomycin resistance mechanisms including acquired resistance (eg. *van*A, *van*B, *van*D, *van*E, *van*G and *van*L) and intrinsic resistance (*van*C in *E. gallinarum* and *E. casseliflavus* / flavescens species) (Çetinkaya et al., 2013). Although *van*A is responsible for the most cases of vancomycin-resistant *Enterococcus* (VRE) in the world, *van*B is emerging in recent years (O'Driscoll and Crank, 2015). Food producing animals carrying VRE have been regarded as a probable source of VRE infections in humans. Besides, there are several studies on the presence of VRE on chicken carcasses and commercial meat products indicates the VRE contamination risk via the food chain.

The aim of this study was to determine the presence and antimicrobial resistance of VRE and MRSA on beef and chicken carcasses, workers' hand surfaces in slaughterhouses and beef and chicken meats at retail.

MATERIAL AND METHODS

Sample collection

Samples (n = 100) were collected between February 2018 and March 2019. The carcass excision (n=10 for each) and the swab samples (n=10 for each) from the brisket of beef cattle and wings of chicken at the pre-chilling stage and workers hands' surfaces (n=10 poultry, n=10 cattle slaughterhouses) were collected at processing time. The samples were obtained from a vertically integrated commercial poultry slaughterhouse where more than 1,000,000 poultry are slaughtered and sold in a year and a large-scale cattle slaughterhouse (with a daily capacity of at least 40 cattle, according to the classification of Turkish slaughterhouses). Retail beef (n=20) and chicken (n=20) meat samples were also purchased from different retailers: 20 samples from 9 modern butcher shops, 12 samples from 8 supermarkets and 8 samples from 6 districts retailers. Swab sampling was performed by modifying the swabbing methods described by Arthur et al. (2004) and Gill et al. (2005) with slight modification. Accordingly, the cotton swabs (Lp Italiana, Italy) moistened with sterile Buffered Peptone Water (BPW) were used to cover an area of 10x10 cm (5 horizontal and 5 vertical passes). Carcass excision samples were taken from brisket of beef cattle and wing of chicken by cutting an area of approximately 5 cm^2 and 2.5 cm^2, respectively (Fromm 1959, Pearce and Bolton 2005). The samples were excised using a sterile scalpel and a sterile forceps and then placed into the sterile stomacher bags. Carcass samples of beef cattle and chicken were taken at the post-intervention stage. The hand surface samples of slaughter-

house workers' were voluntarily taken by swabbing the palm of the right hand as described by Sammarco et al., (1997). The collected samples were immediately transported to the laboratory in a cool box containing ice cubes and analyzed within 2 h.

Isolation and identification of *S.aureus, E. faecium* and *E. faecalis*

Isolation of *S. aureus* was performed in accordance to the procedure for the identification of *S. aureus* in animal feed and food published by the International Organization for Standardization (ISO 6888-3: 2003). Accordingly, the excision samples, and 25 g of retail samples weighed into sterile stomacher bags (VWR, 432-3123) were suspended in 100 ml and 225 ml of BPW, respectively and stirred in a stomacher (Interscience, France) then transferred to sterile glass pyrex bottles. After pre-enrichment at 35 ± 2 º C overnight, 100 µl volume of broth and the swab samples were streaked on to the Baird Parker Agar (Merck 105406) containing 5% Egg Yolk Tellurite (Oxoid, SR0054) and Mannitol Salt Agar (Oxoid, CM 0085) and incubated at 37 ºC for 24-48 hours under aerobic conditions. The suspected colonies were evaluated for gram staining, catalase, oxidase, coagulase and then API Staph (Biomerieux, Ref. 20500) test kit was used for identification of the isolates.

Isolation and identification of *E. faecium* and *E. faecalis* was performed as reported by Klein et al. (1998) with modification. Briefly, the homogenate was prepared as mentioned in *S. aureus* isolation. Then, 100 µl of the homogenate was streaked onto Slanetz Bartley Agar (Oxoid CM 0377) and incubated at 35ºC for 24-48 hours. After the incubation, five suspicious colonies were selected and subcultured onto Bile Esculin (BEA) Agar (Oxoid CM 0888) to discern Enterococci based on it's potential to hydrolyze esculin then incubated at 35 ºC for 24 hours. Colonies with bright black, round, convex shape grown on BEA were considered as suspected *Enterococcus* spp.. Gram staining, catalase, growth in 6.5% NaCl Brain Heart Infusion Broth (BHI, Merck 110493), L-pyrrolidonyl arylamidase activity, motility, hemolysis and API 20 Strep (Biomerieux, Ref. 20600) test were performed to typical colonies.

Antimicrobial susceptibility test

Antimicrobial susceptibility of *E. faecium* isolates to 10 antimicrobials [amikacin (AK-30 µg, Oxoid CT0107B) ampicillin (AMP-10 µg, Oxoid, CT 0003B), erythromycin (E-15 µg, Oxoid, CT 0020B),

gentamicin (CN-120 µg, Oxoid, CT 0794B), chloramphenicol (C-30 µg, Oxoid, CT 0013B), penicillin G (10 units, Oxoid, CT 0043B), ciproflaxocin (CIP-5 µg, Oxoid, CT 0425B), streptomycin (S-300 µg, CT 1897B), teicoplanin (Oxoid-CT 0647B-TEC 30 gg), tetracycline (TE-30 µg, Oxoid, CT 0054B)] and *S. aureus* isolates to 11 antimicrobials [amikacin (AK-30 µg, Oxoid CT0107B), amoxicillin / clavulanic acid (AMC-30 µg, Oxoid, CT 2223B), ampicillin (AMP-10 µg, Oxoid, CT 0003B), erythromycin (E-15 µg, Oxoid, CT 0020B), gentamicin (CN-120 µg, Oxoid, CT 0794B), chloramphenicol (C-30 µg, Oxoid, CT 0013B), penicillin G (10 units, Oxoid, CT 0043B), ciproflaxocin (CIP-5 µg, Oxoid, CT 0425B), clindamycin (DA-10 µg, Oxoid CT0015B), (tetracycline (TE-30 µg, Oxoid, CT 0054B), trimethoprim / sulfamethaxol (SXT-25 µg, Oxoid, CT 0052B)] were determined by disk diffusion method. Accordingly, the isolates were cultured in Mueller Hinton Broth (MHB, Oxoid, CM0405) and the optical density was adjusted to 0.5 Mc Farland (DEN-1B McFarland Densitometer). The broth culture was streaked on to the surface of Mueller Hinton Agar (MHA, Oxoid, CM0337) using a sterile cotton swab. The antibiotic disks were placed on top of the agar surface with sterile forceps. Inhibition zone diameters were measured and evaluated according to the antimicrobial susceptibility testing procedure reported by the Clinical Laboratory Standards Institute (CLSI, 2020) for ampicillin, teicoplanin, tetracyclin, penicillin, erythromycin and chloramphenicol in Enterococcus isolates. The other antibiotics in *Enterococcus* spp. and all the *S. aureus* isolates were evaluated according to European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2020). *E. faecalis* ATCC 29212, *S. aureus* ATCC 25923, *S.aureus* ATCC 29213 were used as the control strains.

Determination of MICs to oxacillin and vancomycin

Minimum Inhibition Concentration (MIC) values to vancomycin (Carbosynth, FV11352) and oxacillin (Carbosynth, AO61591) were determined using microdilution method. Accordingly, antibiotic dilutions were prepared at 10 ml volumes in tubes, diluted at 1/1, 1/2,1/4, 1/8, 1/16, 1/32, 1/64, 1/128, 1/256, 1/512 µg/ml concentrations and then dispensed into the ELISA microplate wells as 100 µl. The bacterial suspensions were adjusted to 0.5 McFarland turbidity standard in MHA tubes (supplemented with 2 % w/v NaCl for *S. aureus* isolates). The suspensions were di-

luted 1:20 and added as 10 µl to each well to yield the concentration approximately 5 x 10^5 CFU / ml. The inoculated microplates were covered and incubated for 24 h at 35 ± 2 ºC under aerobic conditions. The microplates were read on a Spectrophotometric Elisa Reader (MWGt Lambda Scan 200, Bio-Tek Instruments, Winooski, VT, USA) at 600 nm. MIC values were evaluated according to CLSI (2020), and EU-CAST (2020).

Determination of the *mec*A and *van*A genes

DNA extraction

DNA extraction was performed according to the manufacturer's instructions with DNeasy PowerFood Microbial Kit (Qiagen, 21000-100) to the isolates that optical density was adjusted to the Mc Farland 4 in Tryptone Soy Broth.

PCR Mix

A commercially available PCR mix (MyTaq PCR Premix) that consisted of DNA polymerase, dNTP set, reaction liquid, $MgCl_2$, stabilizer and tracking dye was used for the PCR assay. Volumes of 2.5 µl template DNA and 1 µl forward and reverse primers were included to the reaction mix and the total volume was adjusted to 25 µl with nuclease free water (Sigma-Aldrich, LSKNF0500, Germany).

Primers

Forward (5'AAA ATC GAT GGT AAA GGT TGG C 3') and reverse (5'AGT TCT GCA GTA CCG GAT TTG C 3') *mec*A primers were used according to Murakami et al. (1991) to detect the *mec*A gene. Both primers amplify a region of 533 bp length. Primer sequences of *van*A Forward (5'-CAT GAA TAG AAT AAA AGT TGC AAT A 3') and *van*A Reverse (5'-CCCCTTTAACGCTAATACGATCAA-3') were used. The primers amplify a gene region of 1033 bp in length (Kariyama et al., 2000).

Reference strains

S. aureus ATCC 25923, *E. faecium* ATCC 51559 and *E. faecalis* ATCC 29212 were used for quality control strains of antimicrobial susceptibility testing and PCR assays.

RESULTS

In our study, *S. aureus* was isolated from 17 samples (17%). *S. aureus* isolates were isolated from cattle slaughterhouse workers' hands (4), poultry slaughterhouse workers' hands (4), beef cattle carcass swab (1), chicken carcass swab (2), retail beef meat (4) and retail chicken meat (2). *S. aureus* contamination rates of beef and chicken samples were 18% and 16% respectively (Table 1).

Among *S. aureus* isolates the highest antibiotic resistance was found to ampicillin (82.35 %) and penicillin G (94.11%). However, all isolates were susceptible to amoxicillin / clavulanic acid, and chloramphenicol. According to the MIC test to determine oxacillin resistance, five isolates (29.41%) showed resistance at concentrations ranging from 16-128 µg/ml (Table 2).

The oxacillin resistant isolates, according to their MIC value, were found to harbour the *mec*A gene using conventional PCR (Figure 1). These isolates were isolated from beef cattle carcass swab (1), chicken carcass swab (2), retail beef meat (1) and retail chicken meat (1) (Table 1).

E. faecium was isolated from eight (8 %) samples. *E. faecalis* was not detected in any of the samples. *E. faecium* was isolated from cattle slaughterhouse workers' hand (1), poultry slaughterhouse workers' hand (1), chicken carcass swab (2), retail beef meat (1) and retail chicken meat (3), (Table 1).

The highest antibiotic resistance of *E. faecium* isolates was to tetracycline (87.5%) (Table 4). However, most of the isolates were susceptible to ampicillin (62.5 %), penicillin G (75 %) and teicoplanin (75 %). According to the MIC test to determine vancomycin resistance, five isolates (62.5%) displayed resistance at concentrations ranging from 32-64 µg/ml (Table 3 and Table 4). These isolates were obtained from chicken carcass swab (2) and retail chicken meat (3). None of the isolates was determined to harbour the *van*A gene using conventional PCR assay (Figure 2).
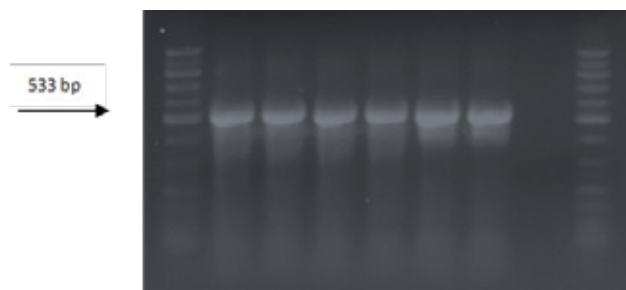

Figure 1. Image of rhe *mec*A gene positive isolates on agarose gel 1:Ladder (100 bp), 2: Positive control (*S. aureus* ATCC 43300), 3-8: Positive isolates, 9:Ladder (100 bp)

Table 1. Distribution of isolates in sample groups (%)

Origin of samples			Number of samples	*S. aureus*	MRSA	*mecA*	*E. faecium*	VRE	*vanA*
Cattle	Slaughter-house	Carcass swab	10	**1** (10 %)	**1** (10 %)	**1**(10 %)	-	-	-
		Carcass excision	10	-	-	-	-	-	-
		Workers	10	**4** (40 %)	-	-	**1** (10 %)	-	-
	Retail		20	**4** (20 %)	**1** (5 %)	**1** (5 %)	**1** (5 %)	-	-
Poultry	Slaughter-house	Carcass swab	10	**2** (20 %)	**2** (20 %)	**2** (20 %)	**2** (20 %)	**2** (20 %)	-
		Carcass excision	10	-	-	-	-	-	-
		Workers	10	**4** (40 %)	-	-	**1** (10 %)	-	-
	Retail		20	**2** (10 %)	**1** (5 %)	**1** (5 %)	**3** (6 %)	**3** (6 %)	-
Total			100	**17**(17 %)	**5** (5 %)	**5** (5 %)	**8** (8 %)	**5** (5 %)	-

Table 2. Antimicrobial resistance and oxacillin MIC values of *S. aureus* isolates

Isolate number	Origin of Sample	OX MIC (μg/ml)	VA MIC (μg/ml)	P	AK	AMC	AMP	CIP	DA	E	CN	C	SXT	TE
1	Bcs 1	16	≤2	R	R	S	R	S	S	S	R	S	S	R
2	Cw 1	≤2	≤2	R	S	S	R	S	R	R	R	S	I	R
3	Cw 2	≤2	≤2	R	S	S	R	S	R	R	S	S	S	R
4	Cw 3	≤2	≤2	R	S	S	R	S	R	R	R	S	S	I
5	Cw 4	≤2	≤2	R	S	S	R	S	S	S	S	S	S	R
6	Br 1	≤2	≤2	S	S	S	S	S	S	S	S	S	S	S
7	Br 2	≤2	≤2	R	S	S	R	S	R	R	R	S	I	R
8	Br 3	≤2	≤2	R	S	S	R	S	R	R	R	S	I	R
9	Br 4	64	≤2	R	S	S	S	R	R	R	S	S	S	S
10	Ccs 1	128	≤2	R	S	S	S	I	R	R	S	S	S	S
11	Ccs 2	32	≤2	R	S	S	R	R	S	S	S	S	S	S
12	Pw 1	≤2	≤2	R	S	S	R	S	S	S	S	S	S	R
13	Pw 2	≤2	≤2	R	S	S	R	S	S	S	S	S	S	S
14	Pw 3	≤2	≤2	R	S	S	R	S	R	R	R	S	I	R
15	Pw 4	≤2	≤2	R	S	S	R	S	R	I	S	S	I	R
16	Cr 1	≤2	≤2	R	R	S	R	S	R	R	R	S	R	R
17	Cr 2	64	≤2	R	R	S	R	R	S	S	R	S	R	R

* R: Resistance, I: Intermediate, S: Sensitive, OX: oxacillin, AK: amikacin, AMC: amoxicillin / clavulanic Acid, AMP: ampicillin, CIP: ciprofloxacin, DA: clindamycin, E: erythromycin, CN: gentamicin, C: chloramphenicol, P: penicillin G, SXT: sulfamethoxazole / trimethoprim, TE: tetracycline, VA: vancomycin.
**Bcs: Cattle beef carcass swab; Cw: Cattle slaughterhouse workers' hand surface; Br: Retail beef; Ccs: Chicken carcass swab; Pw: Poultry slaughterhouse workers' hand surface; Cr: Retail chicken

Table 3. Antimicrobial resistance and vancomycin MIC values of *E. faecium* isolates

Isolate Number	Origin of Sample	VA MIC (μg/ml)	AK	AMP	CIP	E	CN	C	P	TEC	TE	S
1	Cw 1	≤4	S	S	I	R	R	S	S	S	R	R
2	Br 1	≤4	R	R	S	R	R	S	S	S	R	R
3	Ccs 1	64	R	S	R	R	R	R	S	S	R	R
4	Ccs 2	32	R	S	S	R	I	R	S	S	R	S
5	Pw 1	≤4	S	R	R	I	S	R	R	R	R	S
6	Cr 1	32	S	S	S	I	S	S	R	R	S	S
7	Cr 2	64	S	S	I	I	S	R	S	S	R	S
8	Cr 3	32	I	R	R	R	S	I	S	S	R	S

*R: Resistance, I: Intermediate, S: Sensitive, VA: vancomycin, AK: amikacin, AMP: ampicillin, CIP: ciprofloxacin, E: erythromycin, CN: gentamicin, C: chloramphenicol, P: penicillin G, TEC: teicoplanin, TE: tetracycline, S: streptomycin.
** Cw: Cattle slaughterhouse workers' hand surface; Br: Retail beef; Ccs: Chicken carcass swab; Pw: Poultry slaughterhouse workers' hand surface; Cr: Retail chicken

Table 4. Antimicrobial resistance rates (%) of *S. aureus* and *E. faecium* isolates

	S. aureus							*E. faecium*					
	Cattle			Poultry				Cattle		Poultry			
	Slaughterhouse			Slaughterhouse				Slaughterhouse		Slaughterhouse			
Antibiotic	Carcass swab (n:1)	Workers (n:4)	Retail Beef (n:4)	Carcass swab (n:2)	Workers (n:4)	Retail chicken (n:2)	Total (n:17)	Workers (n:1)	Retail Beef (n:1)	Carcass swab (n:2)	Workers (n:1)	Retail chicken (n:3)	Total (n:8)
VA	0	0	0	0	0	0	0	0	0	1 (50%)	1 (100%)	3 (100%)	5 (62.5%)
P	1 100(%)	3 (75%)	4 (100%)	2 (100%)	4 (100%)	2 (100%)	16 (94.1%)	0	0	1 (50%)	0	1 (33.3%)	2 (25%)
AK	0	0	0	0	2 (50%)	1 (50%)	3 (17.6 %)	0	1 (100%)	1 (50%)	1 (100%)	0	3 (37.5%)
AMP	1 100(%)	3 (75%)	4 (100%)	2 (100%)	2 (50%)	2 (100%)	14 (82.3%)	0	1 (100%)	1 (50%)	0	1 (33.3%)	3 (37.5%)
CIP	0	0	0	0	1 (25%)	2 (100%)	3 (17.6 %)	0	0	2 (100%)	0	1 (33.3%)	3 (37.5%)
CN	1 (100%)	1 (25%)	2 (50%)	1 (50%)	1 (25%)	1 (50%)	7 (41.1%)	1 (100%)	1 (100%)	1 (50%)	0	0	3 (37.5%)
C	0	0	0	0	0	0	0	0	0	2 (100%)	1 (100%)	1 (33.3%)	4 (50%)
SXT	0	0	0	0	1 (25%)	1 (50%)	2 (11.7%)	-	-	-	-	-	-
TE	1 (100%)	2 (50%)	3 (75%)	2 (100%)	2 (50%)	1 (50%)	11 (64.7%)	1 (100%)	1 (100%)	2 (100%)	1 (100%)	2 (66.6%)	7 (87.5%)
OX	0	0	0	0	3 (75%)	2 (100%)	5 (29.4%)	-	-	-	-	-	-
AMC	0	0	0	0	0	0	0	-	-	-	-	-	-
DA	1 (100%)	2 (50%)	2 (50%)	2 (100%)	3 (75%)	0	10 (58.8%)	-	-	-	-	-	-
E	1 (100%)	2 (50%)	2 (50%)	1 (50%)	3 (75%)	0	9 (52.9%)	1 (100%)	1 (100%)	1 (50%)	1 (100%)	1 (33.3%)	5 (62.5%)
TEC	-	-	-	-	-	-	-	0	0	1 (50%)	0	1 (33.3%)	2 (25%)
S	-	-	-	-	-	-	-	1 (100%)	1 (%100)	1 (50%)	0	0	3 (37.5%)

VA: vancomycin, P: penicillin G, AK: amikacin, AMP: ampicillin, CIP: ciprofloxacin, CN: gentamicin, C: chloramphenicol, SXT: sulfamethoxazole / trimethoprim, TE: tetracycline, OX: oxacillin, AMC: amoxicillin / clavulanic Acid, DA: clindamycin, E: erythromycin, TEC: teicoplanin, S: streptomycin

DISCUSSION

Presence of *S. aureus*

In our study, overall *S. aureus* prevalance was 17% (Table 1) and the distribution of the positive samples was 16% in chicken and chicken-related sources, and 18% in beef and beef related samples. Percentage distribution was in line with the study of Hanson et al. (2011) in the United States with the rate of 16.36%, lower of the of Lim et al. (2010) in Korea and Kitai et al. (2005) in Japan, with rates of 43.3% and 65.8%, respectively. These results were far below those observed by Bystron et al. (2005) with no coagulase-positive staphylococci contamination out of 65 samples of chicken parts in Poland. The variability of the contamination rates is thought to be due to factors such as geographical locations, sample size, sampling season, samples analyzed (whole carcasses, parts, different species of animals, etc.) and differences in isolation methods.

Oxacillin resistance and carriage of the *mec*A gene

In our study, MRSA was detected in 5% of the samples. Although the highest *S. aureus* contamination rate in sample groups was noted in the workers' hands both in cattle and poultry slaughterhouses, none of the isolates was MRSA.

The MIC test displayed resistance to oxacillin at different ratios (16-128 µg/ml) among the isolates. There are studies that differ from the present study in terms of sample size and the results of the samples analyzed that MRSA contamination rates were reported lower in Korea, (0.6 %, Lim et al., 2010), Spain (1.6 %, Lozano et al., 2009), Jordan (2.3 %, Quddoumi et al., 2006), and higher in Denmark (16 %, Agersø et al., 2012) in Netherlands (11.9 %, de Boer et al., 2009) and Germany (37.2 %, Feßler et al., 2011).

Determination of the *mec*A gene in all the MRSA isolates was comparable with a study carried out in Germany by Feßler et al. (2011) reported that all MRSA isolates from chicken and turkey products have the *mec*A gene and exhibit oxacillin MICs between 4 - 32 µg/ml. A more recent study conducted in Turkey by Sırıken et al. (2016) reported that 4 of 44 (9.09%) *S. aureus* isolates from beef samples were detected to be MRSA according to their MIC values and all of the isolates confirmed to have the *mec*A gene. On the contrary the researchers reported that the *mec*A gene was not detected in milk isolates which were resistant to oxacillin according to their MIC values.

A high resistance was displayed in *S. aureus* isolates against tetracycline (64.7 %), ampicillin (82.3 %) and penicillin G (94.1 %) antibiotics (Table 4) and a high sensitivity (100 %) against amoxicillin / clavulanic acid, chloramphenicol and vancomycine. Our results were in good agreement with Abdalrahman et al. (2015) in United States of America, which was reported that two of the *S. aureus* (2/114, 1.8%) isolates from retail chicken and turkey meats were determined as MRSA and displayed an antimicrobial resistance against ampicillin (94.6%), tetracycline (72 %) and penicillin (70.8 %). The highest antimicrobial resistance in MRSA isolates against tetracycline with a rate of 100 % was in a similar pattern with Lin et al. (2009) in Taiwan and Momtaz et al. (2013) in Iran stated that *S. aureus* strains from chicken processing plants and raw chicken meats were highly (100% and 97.56%, respectively) resistant to tetracycline. Besides, all the MRSA isolates were sensitive to amoxicillin/clavulanic acid, chloramphenicol and vancomycin. Relatively similar patterns were observed among the methicillin-sensitive *S. aureus* isolates that all of them was sensitive against amoxicillin-clavulanic acid, ciprofloxacin, chloramphenicol and vancomycin. This result was in accordance with a previous study conducted by Osman et al. (2016) in Egypt except the vancomycin resistance (74.1 %) declared to be determined in chicken breast samples.

Resistance rate of MSSA isolates was 91.6 % (11/12) to ampicillin and penicillin but both MSSA and MRSA strains were sensitive against amaoxicillin / clavulanic acid. These results were in accordance with the reports of Peacock and Paterson (2015), suggesting that the most of the MRSA isolates express resistance against β-lactam group heterogeneously. Furthermore, Foster (2017) stated that some isolates would display a high level of resistance could be expressed homogeneously. Conversion of this heterogeneous construct to homogeneously expressed resistance occurs as a result of chromosomal mutations in transcription of the *mec*A gene and PBP2a levels.

Presence of *E. faecalis* and *E. faecium*

Presence of *E. faecium* in eight samples (8 %) seem to be consistent with other research which found that *E. faecium* was detected in varying percentages (Boulianne et al., 2016; Donado-Godoy et al., 2015; Hidano et al., 2015; Kasımoglu-Dogru et al., 2010; Kim et al., 2018; Rehman et al., 2018; Stępień-Pyśniak et al., 2016). *E. faecalis* was not detected in any of the samples. However, there are several con-

trary studies (Donado-Godoy et al., 2015; Hidano et al., 2015; Kasımoglu-Dogru et al., 2010) conducted with poultry and beef samples reported to isolate the microorganism. This difference is thought to be due to the sampling area, sampling method, seasonal variations and individual differences.

Vancomycin Resistance and *van*A Gene

Vancomycin resistance in chicken samples according to MIC values (32-64 µg/ml) in spite of the absence of the *van*A gene was in accordance with the findings of other studies conducted in poultry meats, poultry products, poultry slaughterhouses (Boulianne et al., 2016 in Canada; Donado-Godoy et al., 2015 in Colombia; Hidano et al., 2015 in Japan; Kasımoglu-Dogru et al., 2010 in Turkey; Kim et al., 2018 in Korea; Rehman et al., 2018 in Canada; Stępień-Pyśniak et al., 2016 in Poland) cattle products and cattle slaughterhouses (Çetinkaya et al., 2013 in Turkey; Hayes et al., 2003 in United States of America; Liu et al., 2013 in China; Guerrero-Ramos et al., 2016 in Spain; Yılmaz et al., 2016 in Turkey) report the different resistance profiles and vancomycin MICs of *E. faecium* and *E. faecalis*. The lower values were observed by Hidano et al. (2015) that they stated 1 *E. faecalis* and 1 *E. faecium* isolates were detected as vancomycin resistant but MIC test showed a low resistance level (8µg/ml) in both isolates.

Absence of the *vanA* gene in phenotypically resistant isolates were in aggrement with several studies (Gousia et al., 2015; Raafat et al., 2016; El-Tawab et al., 2019; Onaran et al., 2019). In this context, Gousia et al. (2015) in Greece found that eight out of 30 *E. faecium* and one *E. faecalis* vancomycin-resistant isolates did not harbour the *van*A, Raafat et al. (2016) reported that only one of the 10 vancomycin-resistant isolates was *van*A-positive in foods of animal origin, El-Tawab et al. (2019) reported that *van*A was detected in three out of nine vancomycin-resistant strains isolated from milk and milk products in Egypt and in a recent study by Onaran et al. (2019) with the absence of the *van*A gene in 14 of 20 phenotypically positive VRE strains. Vancomycin resistance is known to be mediated by several van types as *van* A, *van* B, *van* C, *van* D, *van* E etc. although *van*A and *van*B are the most commonly detected clusters. In this context, a major source of limitation in our study is thought to be related to the expression of the resistance by the other van types.

The current data displayed a high resistance to tetracycline in *E. faecium* and VRE isolates (87,5 %, 80 %, respectively, Table 4), while all isolates were highly susceptible to ampicillin (62.5 %), penicillin G (75 %) and teicoplanin (75 %). A similar pattern of multiple antibiotic resistance was obtained in Kasımoglu-Dogru et al. (2010) in Turkey, Liu et al. (2013) in China, Boulianne et al. (2016) in Canada. In a study conducted by Yılmaz et al. (2016), *Enterococcus* isolates from chicken meat (96 %) and red minced meat (63 %) were resistant to at least one of the 12 tested antibiotics and the highest resistance rate was observed against tetracycline (53%-89.5%). These results were in agreement with the results of the present study as well as Pesavento et al. (2014) in Italy.

For many years β-lactams have been used as one of the first choices in Enterococcal infections, including, ampicillin and penicillin G used in the study showed a relatively lower resistance with rates 37.5% and 25%, respectively. Although ampicillin resistance is generally expressed as rare in *E. faecalis*, it is mostly related to the hospital-associated *E. faecium* isolates which is the result of enhanced production of PBP5 or polymorphisms of this protein (Gagetti et al., 2019).

Another antibiotic class, aminoglycosides are also used generally but resistance against Enterococcal species is alarming over the last few decades (Pesavento et al., 2014). Consistent with the literature, this research found that two of the *E. faecium* (one VSE and one VRE) isolates (25%, 2/8) were resistant to all antibiotics tested of aminoglycoside group (amikacin, gentamicin, streptomycin). These findings were in line with previous findings of Hayes et al. (2003) in USA, Osuka et al. (2016) in Japan and Khodabandeh et al. (2018) in Iran. On the contrary, the relatively lower resistance profiles were determined by Trivedi et al. (2011) in Czechia and Kim et al. (2019) in South Korea.

CONCLUSION

Detection of MRSA and VRE phenotypically and/or genotypically in chicken and beef cattle carcasses and retail products is a noteworthy point for public health surveillance programmes running for antimicrobial resistance. Besides, determination of multiple resistant isolates were also considered to be highly risky in terms of public health. Future research is needed to clarify in monitoring programs whether antibiotic resistant bacterial strains are personnel or animal origin in the slaughtering line and final product.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

Abdalrahman L, Stanley A, Wells H, Fakhr M (2015) Isolation, virulence, and antimicrobial resistance of methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin sensitive *Staphylococcus aureus* (MSSA) strains from Oklahoma retail poultry meats. International Journal of Environmental Research and Public Health 12: 6148-6161. https://doi.org/10.3390/ijerph120606148.

Agersø Y, Hasman H, Cavaco LM, Pedersen K, Aarestrup FM (2012) Study of methicillin resistant *Staphylococcus aureus* (MRSA) in Danish pigs at slaughter and in imported retail meat reveals a novel MRSA type in slaughter pigs. Veterinary Microbiology 157: 246-250. https://doi.org/10.1016/j.vetmic.2011.12.023.

Ahmed MO, Baptiste KE (2018) Vancomycin-resistant Enterococci: a review of antimicrobial resistance mechanisms and perspectives of human and animal health. Microbial Drug Resistance 24:590-606. https://doi.org/10.1089/mdr.2017.0147.

Arias CA, Murray BE (2012) The rise of the *Enterococcus*: beyond vancomycin resistance. Nature Reviews Microbiology 10:266-278. https://doi.org/10.1038/nrmicro2761.

Arthur TM, Bosilevac JM, Kent MP, Jaroni D, Nou X, Shackelford SD, Wheeler TL, Kent MP, Jaroni D, Pauling B, Allen DM, Koohmaraie M (2004) *Escherichia coli* O157 prevalence and enumeration of aerobic bacteria, *Enterobacteriaceae*, and *E.coli* O157 at various steps in commercial beef processing plants. Journal of Food Protection 67:658-665. https://doi.org/10.4315/0362-028X-67.4.658.

Birkegård AC, Græsbøll K, Clasen J, Halasa T, Toft, N, Folkesson A (2019) Continuing occurrence of vancomycin resistance determinants in Danish pig farms 20 years after removing exposure to avoparcin. Veterinary microbiology 232:84-88.

Boulianne M, Arsenault J, Daignault D, Archambault M, Letellier A, Dutil L (2016) Drug use and antimicrobial resistance among *Escherichia coli* and *Enterococcus* spp. isolates from chicken and turkey flocks slaughtered in Quebec, Canada. Canadian Journal of Veterinary Research 80:49-59.

Bystroń J, Molenda J, Bania J, Kosek-Paszkowska K, Czerw M (2005) Occurrence of enterotoxigenic strains of *Staphylococcus aureus* in raw poultry meat. Polish Journal of Veterinary Sciences 8:37-40.

Chambers HF, DeLeo FR (2009) Waves of resistance: *Staphylococcus aureus* in the antibiotic era. Nature Reviews Microbiology 7:629-641. https://doi.org/10.1038/nrmicro2200.

Clinical and Laboratory Standards Institute (CLSI) (2020). Performance standards for antimicrobial susceptibility testing. 30th ed. CLSI supplement M100. Clinical and Laboratory Standards Institute, Wayne, PA.

Çetinkaya F, Muş TE, Soyutemiz GE, Çıbık R (2013) Prevalence and antibiotic resistance of vancomycin-resistant enterococci in animal originated foods. Turkish Journal of Veterinary and Animal Sciences 37:588-593. https://doi.org/10.3906/vet-1211-34.

De Boer E, Zwartkruis-Nahuis JTM, Wit B, Huijsdens XW, De Neeling AJ, Bosch T, van Oosterom RAA, Vila A, Heuvelink AE (2009) Prevalence of methicillin resistant *Staphylococcus aureus* in meat. Int J Food Microbiol 134:52-56. https://doi.org/10.1016/j.ijfoodmicro.2008.12.007.

Donado-Godoy P, Byrne BA, León M, Castellanos R, Vanegas C, Coral A, Arevalo A, Clavijo V, Vargas M, Zuniga JJR, Tafur M, Perez-Gutierrez E, Smith WA (2015) Prevalence, resistance patterns, and risk factors for antimicrobial resistance in bacteria from retail chicken meat in Colombia. J Food Prot 78:751-759. https://doi.org/10.4315/0362-028X.JFP-14-349.

El-Tawab AAA, Mohamed SR, Kotb MA (2019) Molecular detection of virulence and resistance genes of Enterococci spp isolated from milk

and milk products in Egypt. Nature and Science 17:77-83. https://doi.org/10.7537/marsnsj170919.10.

EUCAST (2020). https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_10.0_Breakpoint_Tables.pdf. Accessed 20 May 2020.

European Food Safety Authority (2015). Manual for reporting on antimicrobial resistance in the framework of Directive 2003/99/EC and Decision 2013/652/EU for information derived from the year 2014. EFSA publication 2015: EN-771. European Food Safety Authority, Parma, Italy.

Feßler AT, Kadlec K, Hassel M, Hauschild T, Eidam C, Ehricht R, Monecke S, Schwarz S (2011) Characterisation of methicillin-resistant *Staphylococcus aureus* isolates from food and food products of poultry origin in Germany. Applied and Environmental Microbiology 77:7151-7157. https://doi.org/10.1128/AEM.00561-11.

Foster TJ (2017). Antibiotic resistance in *Staphylococcus aureus*. Current status and future prospects. FEMS Microbiology Reviews 41:430-449. https://doi.org/10.1093/femsre/fux007.

Fromm D. (1959) An evaluation of techniques commonly used to quantitatively determine the bacterial population on chicken carcasses. Poultry Science, 38(4):887-893. https://doi.org/10.3382/ps.0380887

Gagetti P, Bonofiglio L, Gabarrot GG, Kaufman S, Mollerach M, Vigliarolo L, von Specht M, Toresani I, Lopardo HA (2019) Resistance to β-lactams in enterococci. Revista Argentina de Microbiologia 51:179-183. https://doi.org/10.1016/j.ram.2018.01.007.

Gill CO, Badoni M (2005) Recovery of bacteria from poultry carcasses by rinsing, swabbing or excision of skin. Food Microbiology, 22:101-107. https://doi.org/10.1016/j.fm.2004.04.005.

Gousia P, Economou V, Bozidis P, Papadopoulou C (2015) Vancomycin-resistance phenotypes, vancomycin-resistance genes, and resistance to antibiotics of enterococci isolated from food of animal origin. Foodborne Pathogens and Disease 12:214-220. doi: https://doi.org/10.1089/fpd.2014.1832.

Guerrero-Ramos E, Molina-Gonzalez D, Blanco-Moran S, Igrejas G, Poeta P, Alonso-Calleja C, Capita R (2016) Prevalence, antimicrobial resistance, and genotypic characterization of vancomycin-resistant enterococci in meat preparations. J Food Prot 79:748-756. https://doi.org/10.4315/0362-028X.JFP-15-390.

Haaber J, Penadés JR, Ingmer H (2017) Transfer of antibiotic resistance in *Staphylococcus aureus*. Trends in Microbiology 25:893-905. https://doi.org/10.1016/j.tim.2017.05.011.

Hanson BM, Dressler AE, Harpe AL, Scheibel RP, Wardyn SE, Roberts LK, Kroeger JS, Smith TC (2011) Prevalence of *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* (MRSA) on retail meat in Iowa. Journal of Infection and Public Health 4:169-174. https://doi.org/10.1016/j.jiph.2011.06.001.

Hayes JR, English LL, Carter P, Proescholdt T, Lee KY, Wagner DD, White DG (2003) Prevalence and antimicrobial resistance of *Enterococcus* species isolated from retail meats. Applied and Environmental Microbiology 69:7153-7160. https://doi.org/10.1128/AEM.69.12.7153-7160.2003.

Hidano A, Yamamoto T, Hayama Y, Muroga N, Kobayashi S, Nishida T, Tsutsui T (2015) Unraveling antimicrobial resistance genes and phenotype patterns among *Enterococcus faecalis* isolated from retail chicken products in Japan. *PLoS One* 10:1-15. https://doi.org/10.1371/journal.pone.0121189.

International Organization for Standardization (ISO) (2003). Microbiology of food and animal feeding stuffs-Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species). https://www.iso.org/obp/ui/#iso:std:iso:6888:-

3:ed-1:v2:en. Accessed at 1 February 2018.

Kariyama R, Mitsuhata R, Chow JW, Clewell DB, Kumon H (2000) Simple and reliable multiplex PCR assay for surveillance isolates of vancomycin-resistant enterococci. Journal of Clinical Microbiology 38:3092-3095.

Kasimoglu-Dogru A, Gencay YE, Ayaz ND (2010) Prevalence and antibiotic resistance profiles of *Enterococcus* species in chicken at slaughter level; absence of *vanA* and *vanB* genes in *E. faecalis* and *E. faecium*. Research in Veterinary Science 89:53-158. https://doi.org/10.1016/j.rvsc.2010.02.005.

Klein G, Pack A, Reuter G (1998) Antibiotic resistance patterns of Enterococci and occurrence of vancomycin-resistant Enterococci in raw minced beef and pork in Germany. Applied and Environmental Microbiology 64:1825-1830.

Khodabandeh M, Mohammadi M, Abdolsalehi MR, Hasannejad-Bibalan M, Gholami M, Alvandimanesh A, Pournajah A, Rajabnia R (2018) High-level aminoglycoside resistance in *Enterococcus faecalis* and *Enterococcus faecium*; as a serious threat in hospitals. Infectious Disorders Drug Targets 20:223-228 doi: 10.2174/1871526519666618113 0095954.

Kim YB, Seo KW, Shim JB, Son SH, Noh EB, & Lee YJ (2019) Molecular characterization of antimicrobial-resistant *Enterococcus faecalis* and *Enterococcus faecium* isolated from layer parent stock. Poultry Science 98:5892-5899. doi: 10.3382/ps/pez288.

Kim YB, Seo HJ, Seo KW, Jeon HY, Kim DK, Kim SW, Lim SK, Lee YJ (2018) Characteristics of high-level ciprofloxacin-resistant *Enterococcus faecalis* and *Enterococcus faecium* from retail chicken meat in Korea. J Food Prot 81:1357-1363. https://doi.org/10.4315/0362-028X.JFP-18-046.

Kitai S, Shimizu A, Kawano J, Sato E, Nakano C, Uji T, Kitagawa H (2005) Characterization of methicillin-resistant *Staphylococcus aureus* isolated from retail raw chicken meat in Japan. Journal of Veterinary Medical Science, 67:107-110. https://doi.org/10.1292/jvms.67.107.

Lim SK, Nam HM, Park HJ, Lee HS, Choi MJ, Jung SC, Lee JY, Kim YC, Song SW, Wee SH (2010) Prevalence and characterization of methicillin-resistant *Staphylococcus aureus* in raw meat in Korea. Journal of Microbiology and Biotechnology, 20:775-778. https://doi.org/10.1093/jac/dkq021.

Lin J, Yeh KS, Liu HT, Lin J H (2009) *Staphylococcus aureus* isolated from pork and chicken carcasses in Taiwan: prevalence and antimicrobial susceptibility. J Food Prot 72:608-611. https://doi.org/10.4315/0362-028X-72.3.608.

Liu Y, Liu K, Lai J, Wu C, Shen J, Wang Y (2013) Prevalence and antimicrobial resistance of Enterococcus species of food animal origin from Beijing and Shandong Province, China. Journal of Applied Microbiology 114:555-563. https://doi.org/10.1111/jam.12054.

Lowy FD (2003) Antimicrobial resistance: the example of *Staphylococcus aureus*. The Journal of Clinical Investigatio, 111:1265-1273. https://doi.org/10.1172/JCI200318535.

Lozano C, López M, Gómez-Sanz E, Ruiz-Larrea F, Torres C, Zarazaga M (2009) Detection of methicillin-resistant *Staphylococcus aureus* ST398 in food samples of animal origin in Spain. Journal of Antimicrobial Chemotherapy 64:1325-1326. https://doi.org/10.1093/jac/dkp378.

Momtaz H, Dehkordi FS, Rahimi E, Asgarifar A Momeni M (2013) Virulence genes and antimicrobial resistance profiles of *Staphylococcus aureus* isolated from chicken meat in Isfahan province, Iran. Journal of Applied Poultry Research 22:913-921. https://doi.org/10.3382/japr.2012-00673.

Murakami K, Minamide W, Wada K, Nakamura E, Teraoka H, Watanabe S (1991) Identification of methicillin-resistant strains of *staphylococci* by polymerase chain reaction. Journal of Clinical Microbiology 29:2240-2244.

O'Driscoll T, Crank CW (2015) Vancomycin-resistant enterococcal infections: epidemiology, clinical manifestations, and optimal management. Infection and Drug Resistance 8:217-230. https://doi.org/10.2147/IDR.S54125.

Onaran B, Göncüoğlu M, Bilir-Ormancı FS (2019) Antibiotic resistance profiles of vancomycin resistant *Enterococci* in chicken meat samples. Ankara Univ Vet Fak Derg 66:331-336. https://doi.org/10.33988/auvfd.451328.

Osman KM, Amer AM, Badr JM, Helmy NM, Elhelw RA, Orabi A, Bakry M, Saad AS (2016) Antimicrobial resistance, biofilm formation and mecA characterization of methicillin-susceptible *S. aureus* and non-*S. aureus* of beef meat origin in Egypt. Frontiers in Microbiology 7:222. https://doi.org/10.3389/fmicb.2016.00222.

Osuka H, Nakajima J, Oishi T, Funayama Y, Ebihara T, Ishikawa H, Saito K, Koganemaru H, Hitomi S (2016) High-level aminoglycoside resistance in *Enterococcus faecalis* and *Enterococcus faecium* causing invasive infection: Twelve-year surveillance in the Minami Ibaraki Area. Journal of Infection and Chemotherapy 22:61-63. https://doi.org/10.1016/j.jiac.2015.09.003.

Peacock SJ, Paterson GK (2015) Mechanisms of methicillin resistance in *Staphylococcus aureus*. Annual Review of Biochemistry 84:577-601. doi: 10.1146/annurev-biochem-060614-034516.

Pearce RA, Bolton DJ (2005) Excision vs sponge swabbing-a comparison of methods for the microbiological sampling of beef, pork and lamb carcasses. Journal of Applied Microbiology, 98(4): 896-900. https://doi.org/10.1111/j.1365-2672.2004.02525.x.

Pesavento G, Calonico C, Ducci B, Magnanini A, Nostro AL (2014) Prevalence and antibiotic resistance of *Enterococcus* spp. isolated from retail cheese, ready-to-eat salads, ham, and raw meat. Food Microbiology 41:1-7. https://doi.org/10.1016/j.fm.2014.01.008.

Raafat SA, Abo-Elmagd EK, Awad RA, Hassana EM, Alrasheedy ZE (2016) Prevalence of vancomycin resistant *Enterococci* in different food samples. The Egyptian Journal of Medical Microbiology 38:1-9. doi: https://doi.org/10.12816/0037021.

Rehman M, Yin X, Zaheer R, Goji N, McAllister T, Pritchard J (2018) Genotypes and phenotypes of *Enterococci* isolated from broiler chickens. Frontiers in Sustainable Food Systems 2:83. doi: https://doi.org/10.3389/fsufs.2018.00083.

Sammarco ML, Ripabelli G, Ruberto A, Iannitto G, Grasso GM (1997) Prevalence of Salmonellae, Listeriae, and Yersiniae in the slaughterhouse environment and on work surfaces, equipment, and workers. Journal of Food Protection, 60(4):367-371. https://doi.org/10.4315/0362-028X-60.4.367

Sırıken B, Yıldırım T, Güney AK, Erol İ, Durupınar B (2016) Prevalence and molecular characterization of methicillin-resistant *Staphylococcus aureus* in foods of animal origin, Turkey. J Food Prot 79:1990-1994. https://doi.org/10.4315/0362-028X.JFP-16-161.

Stępień-Pyśniak D, Marek A, Banach T, Adaszek Ł, Pyzik E, Wilczyński J, Stanisław W (2016) Prevalence and antibiotic resistance of Enterococcus strains isolated from poultry. Acta Veterinaria Hungarica 64:148-163. https://doi.org/10.1556/004.2016.016.

Trivedi K, Cupakova S, Karpiskova R (2011) Virulence factors and antibiotic resistance in *Enterococci* isolated from food-stuffs. Veterinarni Medicina 56:352-357.

Quddoumi SS, Bdour SM, Mahasneh AM (2006) Isolation and characterization of methicillin-resistant *Staphylococcus aureus* from livestock and poultry meat. Annals of Microbiology 56:155-161.

Yılmaz EŞ, Aslantaş Ö, Önen SP, Türkyılmaz S, Kürekci C (2016) Prevalence, antimicrobial resistance and virulence traits in enterococci from food of animal origin in Turkey. LWT Food Science and Technology 66:20-26. https://doi.org/10.1016/j.lwt.2015.10.009.