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 mecA and vanA 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 mecA gene was detected in all MRSA isolates, vanA 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 vanA-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. vanA, vanB, vanD, vanE, vanG and vanL) and intrinsic resistance (vanC in E. gallinarum and E. casseliflavus / flavescens species) (Çetinkaya et al., 2013). Although vanA is responsible for the most cases of vancomycin-resistant Enterococcus (VRE) in the world, vanB 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 for each) 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 cm² and 2.5 cm², 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 (Intercience, France) then transferred to sterile glass pyrex bottles. After pre-enrichment at 35 ± 2 ºC 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 37ºC for 24-48 hours. After the incubation, five suspicious colonies were selected and subcultured onto Bile Esculin (BEA) Agar (Oxoid CM 0888) and Mannitol Salt Agar (Oxoid, CM 0085) and incubated 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 10⁵ 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 EUCAST (2020).

**Determination of the mecA and vanA 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, MgCl₂, 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’) mecA primers were used according to Murakami et al. (1991) to detect the mecA gene. Both primers amplify a region of 533 bp length. Primer sequences of vanA Forward (5’-CAT GAA TAG AAT AAA AGT TGC AAT A 3’) and vanA Reverse (5’-CCCTTTAACGCTAATACGATCAA-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 mecA 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 vanA gene using conventional PCR assay (Figure 2).
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             | Carcass swab     | 20 | 4 (20 %) | 1 (5 %) | 1 (5 %) | 1 (5 %) | - |
| Poultry Slaughter-house | Carcass swab | 10 | 2 (20 %) | 2 (20 %) | 2 (20 %) | 2 (20 %) | - |
|                    | Carcass excision | 10 | - | - | - | - | - |
|                    | Workers          | 10 | 4 (40 %) | - | - | 1 (10 %) | - |
| 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  | R  | S  | S  | R   | 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  | S  | 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  | S  | I   | R  |
| 8              | Br 3            | ≤2             | ≤2             | R  | S  | S   | R   | S   | R  | R  | S  | I   | R  |
| 9              | Br 4            | 64             | ≤2             | R  | S  | S   | S   | R   | R  | S  | S  | S   | R  |
| 10             | Ccs 1           | 128            | ≤2             | R  | S  | S   | S   | I   | R  | R  | S  | S   | S  |
| 11             | Ccs 2           | 32             | ≤2             | R  | S  | S   | R   | R   | S  | S  | S   | S  |
| 12             | Pw 1            | ≤2             | ≤2             | R  | S  | S   | R   | S   | S  | S  | S   | S  |
| 13             | Pw 2            | ≤2             | ≤2             | R  | S  | S   | R   | S   | S  | S  | S   | S  |
| 14             | Pw 3            | ≤2             | ≤2             | R  | S  | S   | R   | S   | 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  | S   | R   | R  |
| 17             | Cr 2            | 64             | ≤2             | R  | R   | R   | R   | S   | S   | 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  | R   | R  | R  |
| 2              | Br 1            | ≤4             | R  | R   | S   | R  | R  | S  | S  | R   | R  | R  |
| 3              | Ccs 1           | 64             | R  | S   | S   | R  | R  | S  | S  | R   | R  | R  |
| 4              | Ccs 2           | 32             | R  | S   | S   | R   | I   | R  | S  | S   | R  | S  |
| 5              | Pw 1            | ≤4             | S  | R   | R   | I   | R   | S  | R  | R   | R  | S  |
| 6              | Cr 1            | 32             | S  | S   | S   | I   | S   | S  | S  | R   | R  | S  |
| 7              | Cr 2            | 64             | S  | S   | I   | S   | R   | S  | S  | R   | S  | S  |
| 8              | Cr 3            | 32             | I   | R   | R   | R   | S   | I   | S   | S   | R  | S  |

*R: Resistance, I: Intermediate, S: Sensitive, OX: 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

| Antibiotic | Cattle Slaughterhouse Carcass swab (n:1) | Cattle Workers (n:4) | Poultry Retail Beef (n:4) | Cattle Slaughterhouse Carcass swab (n:2) | Cattle Workers (n:4) | Poultry Retail chicken (n:2) | Total (n:17) | Cattle Slaughterhouse Workers (n:1) | Poultry Retail Beef (n:1) | Cattle Slaughterhouse Carcass swab (n:2) | Cattle Workers (n:1) | Poultry 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%)       | 21 (100%)      | 16 (94.1%)  | 0              | 0              | 1 (50%)        | 0              | 1 (33.3%)       | 2 (25%)     |
| AK         | 0                                      | 0               | 0             | 0              | 0              | 0              | 0           | 0              | 0              | 1 (100%)       | 1 (50%)        | 1 (100%)       | 0            |
| AMP        | 1 (100%)                               | 3 (75%)         | 4 (100%)      | 2 (100%)       | 2 (100%)       | 2 (100%)       | 14 (82.3%)  | 0              | 1 (100%)       | 1 (50%)        | 0              | 1 (33.3%)       | 3 (37.5%)   |
| CIP        | 0                                      | 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              | 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              | 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 prevalence 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 mecA 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 mecA 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 mecA gene and exhibit oxacillin MICs between 4 - 32 µg/ml. A more recent study conducted in Turkey by Sirrken 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 mecA gene. On the contrary the researchers reported that the mecA 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 vancomycin. 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 Montaz 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 amoxicillin / 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 mecA 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; Kasimoglu-Dogru et al., 2010; Kim et al., 2018; Rehman et al., 2018; Stepien-Pyśniak et al., 2016). E. faecalis was not detected in any of the samples. However, there are several con-
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 Kasmoglu-Dogru et al. (2010) in Turkey, Liu et al. (2013) in China, Boulianme 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, Fakhri 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.

Agerse 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, Jarori D, Nou X, Shackelford SD, Wheeler TL, Kent MP, Jarori 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.JFP-67.7.658.

Birkåeg AC, Græsbøll K, Clasen J, Halasa T, Toft, N, Folkesson A (2005) Unraveling antimicrobial resistance genes and antibiotic resistance of vancomycin-resistant enterococci in retail chicken meat. J Food Prot 78: 751-759. https://doi.org/10.4315/0362-028X.JFP-78.4.751.

El-Tawab AA, Mohamed SR, Korb 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/marsnsj.700919.10.

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.

Fellner 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, Bonofoglio L, Gabarro GG, Kaufman S, Mollerach M, Vigliarello L, von Specht M, Toresani I, Lopardo HA (2019) Resistance to ß-lactams in enterococci. Revista Argentina de Microbiologia 51: 179-183. https://doi.org/10.1038/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, Economidou 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.2014.121189.

Guerrero-Ramos E, Molina-Gonzalez D, Blanco-Moran S, Iregjias G, Pota E, 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, 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.2011.11.001.

Hayes JR, English LL, Carter P, Proescholdt T, Lee KY, Wagner DD, White DG (2003) Prevalence and antimicrobial resistance of methicillin-sensitive Staphylococcus aureus in raw poultry meat. Polish Journal of Veterinary Sciences 8:37-40.

International Organization for Standardization (ISO) (2003). Microbiological examination 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:-
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.rvs.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.

Khodabande M, Mohammadi M, Abdolsalehi MR, Hasannejad-Bihan M, Glohani M, Alvandimanesh A, Pourrajab 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: 10.2174/187152651966118113 0095954.

Kim YB, Seo KW, Shin 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, Hori H, Sato E, Nakano C, Uji T, Kitagawa H (2012) 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.

Limi SK, Nam HM, Park HJ, Lee HS, Choi MJ, Jung SC, Lee YJ, 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 JH (2009) Staphylococcus aureus isolated from pork and chicken carcasses in Taiwan: prevalence and antimicrobial susceptibility. J Food Prot 72:608-611. https://doi.org/10.3163/0362-028X.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.

Lowdy FD (2003) Antimicrobial resistance: the example of Staphylococcus aureus. The Journal of Clinical Investigatio, 111:1265-1273. https://doi.org/10.1172/JCI200318355.

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.

MONTAZ H, DEHKORDI FS, RAHIMI E, ASGHARIFAR 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, Minamidate 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-Ormanç F (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/avfd.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, Ebita 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,ucci D, 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.

Rafaaat 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 Enterococcus isolated from broiler chickens. Frontiers in Sustainable Food Systems 2:83. doi: https://doi.org/10.3389/fsufs.2018.00083.

Sammaroo 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/10.3118/jfp.60.4.367.

Siklenka B, Yildirim T, Güney AK, Erol İ, Durmuşan 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.

Stepień-Pysniak D, Marek A, Banach T, Adaszek Ł, Pyzik E, Więczyski 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, Karpsikova R (2011) Virulence factors and antibiotic resistance in Enterococci isolated from food-stuffs. Veterinarni Medicina 56:352-357.

Quddoumi SS, Bsdour SM, Mahasneh AM (2006) Isolation and characterization of methicillin-resistant Staphylococcus aureus from livestock and poultry meat. Annals of Microbiology 56:152-161.

Yılmaz EŞ, Aslantaş Ö, Önen SP, Türkylmaz S, Kürkçü E (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.