# Species Diversity and Pheno- and Genotypic Antibiotic Resistance Patterns of Staphylococci Isolated from Retail Ground Meats

Husnu Sahan Guran and Serpil Kahya

**Abstract:** The presence and species diversity of staphylococci in 250 ground beef and lamb meat samples obtained from Diyarbakir, Turkey were investigated. The presence of the *16S rRNA* gene, *mecA*, *nuc*, *pvl*, and *femA* was analyzed by multiplex PCR. Pheno- and genotypic antibiotic resistance profiles of 208 staphylococci isolates were established. Of the ground beef and ground lamb samples, 86.4% and 62.4% were positive for staphylococci, respectively. *Staphylococcus aureus*, *S. saprophyticus*, *S. hominis*, *S. lentus*, *S. pasteuri*, *S. warneri*, *S. intermedius*, and *S. vitulinus* made up 40.8%, 28.8%, 11%, 3.8%, 3.8%, 2.4%, 2.4%, and 2.4% of isolates, respectively. Of the 85 *S. aureus* isolates, 40%, 47%, and 5.8% carried *femA*, *mecA*, and *pvl*, respectively, whereas the corresponding rates for the 118 coagulase-negative staphylococci (CoNS) were 0%, 10.1%, and 0%, respectively. We determined from the 208 isolates, the highest antibiotic resistances were to tetracycline and oxytetracycline (85.5%), followed by penicillin (51.4%), novobiocin (45.6%), ampicillin (39.9%), and doxycycline (31.7%), using the Clinical and Laboratory Standards Inst. (CLSI) method. All isolates were sensitive to gentamycin, ofloxacin, and tobramycin, but 2.3% of the *S. aureus* isolates had resistance to vancomycin. The staphylococci isolates carried *tet(K)*, *blaZ*, *tet(L)*, *tet(W)*, *cat*, *tet(S)*, *tet(M)*, *ermB*, *ermA*, and *ermC* antibiotic resistance genes at rates of 59%, 51.7%, 36.9%, 31.8%, 27.2%, 27.2%, 24.4%, 18.1%, 7.9%, and 3.9%, respectively.

**Keywords:** antibiotic resistance, coagulase-negative staphylococci, ground meat, *Staphylococcus aureus*

**Practical Application:** Our study is the 1st to detect *pvl* in MRSA isolates from ground meat samples in Turkey. Moreover, we detected 2 instances of vancomycin resistance in MRSA isolates and also detected a high level of multidrug resistance in staphylococci isolates. The results of this study show that ground meat can be a source of staphylococci of concern to public health because of potential for pathogenicity and as a reservoir for antibiotic resistance genes.



## Introduction

Members of the *Staphylococcus* genus are facultative, Gram-positive cocci and catalase-positive bacteria that are found on the skin and mucosal surfaces of mammals. They are ubiquitous in soil, water, air, dust, and foods such as meat and milk (Marino and others 2011). Frequently, members of the genes are identified genetically based on their *16S* or *23S rRNA* gene regions, and *Staphylococcus aureus* is differentiated from other staphylococci by examining the *nuc* gene that encodes extracellular thermostable nuclease as a marker for *S. aureus* (Stuhlmei and Stuhlmei 2003; Xu and others 2012). Both coagulase-positive and coagulase-negative staphylococci (CoNS) are important public health problems worldwide (Miko and others 2013). Toxins synthesized by staphylococci are involved in food poisoning, postsurgical infections, septicemia, and toxic shock syndrome (Marino and others 2011; Xu and others 2012; Miko and others 2013). One of these toxins, Panton-Valentine Leukocidin (PVL), possesses cytotoxic properties and causes leukocyte fragmentation and tissue necrosis (Argudin and others 2010). Numerous studies have shown *S. aureus* isolates obtained from many different foods to carry the *pvl* gene associated with this toxin. In addition, most community-associated-methicillin-resistant *S. aureus* (CA-MRSA) strains express the PVL toxin (Naimi and others 2003; Rankin and others 2005; Pu and others 2009).

Like other bacteria, antibiotic resistant staphylococci in food, animals, or the environment can be transmitted to humans (Angulo and others 2004; Phillips and others 2004) and in case of pathogenic staphylococci treatment can be complicated when antibiotic resistance is present. Particularly, infections caused by methicillin-resistant *S. aureus* (MRSA), healthcare associated methicillin-resistant coagulase-negative staphylococci (HA-MR-CoNS) (Tiemersma and others 2004), CA-MRSA, and livestock-associated (LA-MRSA) (Golding and others 2010) continue to prevail globally. Methicillin-resistant coagulase negative and coagulase positive staphylococci have been isolated from animals consumed by humans such as cattle, sheep, goats, pigs, and chicken and products associated with these animals (Kawano and others 1996; Zhang and others 2009; Jaglic and others 2010). In addition, some studies have reported that *mecA* and *femA/B/X* (factors essential for methicillin-resistance) also play an important role in methicillin resistance (Hegde and Shrader 2001). In the present study, we aimed (i) to determine the staphylococci species distribution in retail ground beef and lamb, (ii) to detect the staphylococcal *16S rRNA* gene, *mecA*, *nuc*, *PVL-lukS*, and *femA* in a single reaction by multiplex PCR, and (iii) to establish the phenotypic and genotypic antibiotic resistance profiles of the staphylococcal isolates.

*MS 20141967 Submitted 11/28/2014, Accepted 3/27/2015.* *Author Guran is with Dept. of Food Hygiene and Technology, Faculty of Veterinary Medicine, Dicle Univ., 21280 Diyarbakir, Turkey. Author Kahya is with Dept. of Microbiology, Faculty of Veterinary Medicine, Uludag Univ., 16120 Bursa, Turkey. Direct inquiries to author Guran (E-mail: sguran@dicle.edu.tr or sahanguran@yahoo.com).*

© 2015 Institute of Food Technologists®
Further reproduction without permission is prohibited

doi: 10.1111/1750-3841.12893



Journal of Food Science A Publication of the Institute of Food Technologists

## Materials and Methods

### Bacterial strains

In this study, *S. aureus* ATCC 43300, ATCC 33591, ATCC 29213, ATCC 25923, ATCC 6538, ATCC 700699, and ATCC 49775 were used as positive controls. *S. epdermidis* 12228, *Escherichia coli* 0157:H7 ATCC 43895, *Salmonella* Typhimurium ATCC 14028, *Enterococcus feacalis* WHO 3, and *Listeria monocytogenes* ATCC 7644 were used as negative controls.

### Collection of samples

A total of 125 ground beef and 125 ground lamb samples were collected between September 2013 and March 2014 from 25 randomly selected retail stores in Diyarbakir, Turkey. Samples were transported on blue ice packs to the laboratory, and processed within 4 h of purchase.

### Isolation and identification of *Staphylococcus* spp

Twenty-five grams of each ground meat sample were homogenized in 225 mL of sterile buffered peptone water (BPW) (LAB M, Lancashire, U.K.) in a stomacher bag. Homogenized samples ($10^{-1}$) were serially diluted in BPW and the first 2 decimal dilutions were streaked in an amount of 0.1 mL on Baird–Parker agar (BPA) (LAB M) supplemented with egg yolk-tellurite emulsion (LAB M) and incubated at 37 °C for up to 48 h. Up to 5 presumptive staphylococci colonies were transferred to Baird-Parker plates. Following 24 h of incubation at 37 °C, colonies were subcultured on tryptic soy agar (TSA) (LAB M) for further testing. Gram stain, catalase, hemolysis characteristics Voges–Proskauer, anaerobic utilization of mannitol, and coagulase tests were used to identify the isolates as staphylococci. Species-level identification was done with the Vitek 2 Compact identification system (BioMérieux, Durham, N.C., U.S.A.). The isolates were stored at −20 °C in tryptic soy broth (TSB, Merck, Germany) containing 10% glycerol.

### Phenotypic identification of MRSA

*S. aureus* and CoNS isolates were tested for methicillin resistance using the cefoxitin disk diffusion methods outlined by the Clinical and Laboratory Standards Inst. (CLSI 2011). Cefoxitin disks (30 $\mu$g) were used in this study.

### DNA extraction and Multiplex PCR

DNA was extracted using an InstaGene Matrix (Bio-Rad Laboratories, Hercules, Calif., U.S.A.) following the conditions described by the supplier. Multiplex PCR was carried out with the primers listed in Table 1 and performed in an ABI Veriti Thermal Cycler (Applied Biosystems Asia Pte Ltd., Singapore) in a total reaction volume of 50 $\mu$L containing 6 $\mu$L 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], 8 $\mu$L of 25 mmol/L $MgCl_2$, 8 $\mu$L of 10 mmol/L deoxynucleoside triphosphate mixtures (dNTP, Vivantis Technologies, Selangor, Malaysia), 1 $\mu$L Taq polymerase at 5 U/$\mu$L (MBI Fermentas, Vilnius, Lithuania), 5 $\mu$L of template DNA (5 ng/$\mu$L), and 12 $\mu$L of molecular grade water. The optimized concentration of primer for each gene (1 $\mu$L of 25 pmol each gene) (ELLA Biotech, Germany) was used in the multiplex PCR. Amplification was obtained with one cycle following an initial denaturation step at 94 °C for 5 min, followed by 30 cycles of denaturation at 94 °C for 45 s, annealing at 54 °C for 45 s, 62 °C for 45 s, extension at 72 °C for 45 s and a final extension at 72 °C for 7 min. PCR products were separated in a 1.5% agarose gel containing SafeView (ABM, Canada) and visualized using a gel imaging system (Quantum ST4, Vilber Louma, Germany).

### Antimicrobial susceptibility testing

All isolates were tested for antimicrobial susceptibility by the agar disk diffusion method using disks containing penicillin (10 U), oxacillin (1 $\mu$g), ampicillin (10 $\mu$g), amoxicillin-clavulanic acid (30 $\mu$g), kanamycin (30 $\mu$g,) gentamicin (10 $\mu$g), streptomycin (25 $\mu$g), tobramycin (10 $\mu$g), tetracycline (30 $\mu$g), oxytetracycline (30 $\mu$g), doxycycline (30 $\mu$g), enrofloxacin (5 $\mu$g), ofloxacin (5 $\mu$g), ciprofloxacin (5 $\mu$g), erythromycin (15 $\mu$g), azithromycin (15 $\mu$g), clindamycin (2 $\mu$g), sulfamethoxazole-trimethoprim (25 $\mu$g), chloramphenicol (30 $\mu$g), rifampicin (5 $\mu$g), quinupristin/dalfopristin (15 $\mu$g), mupirocin (200 $\mu$g), novobiocin (5 $\mu$g), or vancomycin (30 $\mu$g). The procedure followed guidelines from the CLSI Inst. (CLSI 2011). *E. coli* (ATCC 25922) and *S. aureus* (ATCC 25923) were used as control strains.

### Detection of antibiotic resistance genes by PCR

The presence of penicillin (*blaZ*), aminoglycosides [*aac(6′) to aph(2″)*], tetracycline [*tet(M)*, *tet(K)*, *tet(L)*, *tet(S)*, *tet(O)*, *tet(W)*], macrolides [*erm(A)*, *erm(B)*, and *erm(C)*], chloramphenicol [*cat(A)*], and vancomycin [(*vanA* and *vanB*)] resistance genes was determined by PCR carried out with DNA isolated from all the staphylococci using the previously reported primers and conditions (Table 1).

## Results and Discussion

### Distribution and diversity of staphylococci in ground meats

More staphylococci species were observed in ground beef (9 species) compared to ground lamb (6 species) (Table 2). While 31% of 250 ground meat samples were contaminated with *S. aureus*, ground lamb had a higher level of contamination with 34.4%. In a study conducted in the United States, of 198 ground beef samples that were collected from 5 different sale locations 28% were contaminated with *S. aureus* (Kelman and others 2011). In Turkey, 66% of 30 lamb samples and 56% of 50 beef samples collected from different supermarkets (Gündoğan and others 2005), and in Jordan, 85.5% of 55 lamb samples and 44% of 50 beef samples collected from slaughterhouses, meat markets, and supermarkets (Al-Tarazi and others 2009) have been reported to be contaminated with *S. aureus*. The prevalence of *S. aureus* reported in the studies above are higher compared to that reported in our study, possibly due to differences in sample size, detection methods, and geographical area. In the present study, the prevalence of *S. aureus* was higher in ground lamb compared to ground beef, which might be associated with higher colonization rates of this bacterium in lamb. Mørk and others (2012) collected swab samples from sheep and cattle body parts and detected *S. aureus* in 394 of 1208 (32.6%) sheep samples and 67 of 420 (16.0%) cattle samples. In the same study, the rate of *S. aureus* positive nasal swabs in cows and in ewes was 56.7% and 13.9%, respectively, while the rate was 58.2% in lamb swab samples.

Although coagulase-positive staphylococci such as *S. aureus*, *S. hyicus*, and *S. intermedius* are considered important pathogens in terms of public health, studies have also shown that CoNS such as *S. epdermidis*, *S. haemolyticus*, *S. hominis*, and *S. saprophyticus* may also cause infections in humans and animals (Zhang and others 2009; Feßler and others 2010). In our study, 41% of the ground meat samples were contaminated with CoNS, and approximately

**Table 1–Target gene and expected product length amplified by primers.**

| Gene | Oligonucleotide sequence (5′-3′) | Product size (bp) | Reference |
|---|---|---|---|
| *16S rRNA* | GCAAGCGTTATCCGGATTT<br>CTTAATGATGGCAACTAAGC | 597 | Xu and others (2012) |
| *nuc A* | GCGATTGATGGTGATACGGTT<br>AGCCAAGCCTTGACGAACTAAAGC | 279 | Stuhlmeier and Stuhlmeier (2003) |
| *fem A* | CTTACTTACTGGCTGTACCTG<br>ATGTCGCTTGTTATGTGC | 686 | Vannuffel and others (1995) |
| *pvl* | ATCATTAGGTAAAATGTCTGGACATGATCCA<br>GCATCAAGTGTATTGGATAGCAAAAGC | 433 | Lina and others (1999) |
| *mecA* | TAGAAATGACTGAACGTCCG<br>TTGCGATCAATGTTACCGTAG | 154 | Lima de Castro Nunes and others (1999) |
| *blaZ* | ACTTCAACACCTGCTGCTTTC<br>TGACCACTTTTATCAGCAACC | 173 | Martineau and others (2000) |
| *aac(6′)-aph(2")* | CAGAGCCTTGGGAAGATGAAG<br>CCTCGTGTAATTCATGTTCTGGC | 348 | Vakulenko and others (2003) |
| *tet (K)* | GTAGCGACAATAGGTAATAGT<br>GTAGTGACAATAAACCTCCTA | 360 | Strommenger and others (2003) |
| *tet (M)* | AGTGGAGCGATTACAGAA<br>CATATGTCCTGGCGTGTCTA | 158 | Strommenger and others (2003) |
| *tet (L)* | CATTTGGTCTTATTGGATCG<br>ATTACACTTCCGATTTCGG | 475 | Aarestrup and others (2000) |
| *tet (O)* | GATGGCATACAGGCACAGAC<br>CAATATCACCAGAGCAGGCT | 614 | Aarestrup and others (2000) |
| *tet (S)* | TGGAACGCCAGAGAGGTATT<br>ACATAGACAAGCCGTTGACC | 660 | Aarestrup and others (2000) |
| *tet (W)* | GAGAGCCTGCTATATGCCAGC<br>GGGCGTATCCACAATGTTAAC | 168 | Aminov and others (2001) |
| *erm A* | TCTAAAAAGCATGTAAAAGAA<br>CTTCGATAGTTTATTAATATTAGT | 645 | Sutcliffe and others (1996) |
| *erm B* | GAAAAGGTACTCAACCAAATA<br>AGTAACGGTACTTAAATTGTTTAC | 639 | Sutcliffe and others (1996) |
| *erm C* | TCAAAACATAATATAGATAAA<br>GCTAATATTGTTTAAATCGTCAAT | 642 | Sutcliffe and others (1996) |
| *cat* | GGATATGAAATTTATCCCTC<br>CAATCATCTACCCTATGAAT | 486 | Aarestrup and others (2000) |
| *van A* | CATGAATAGAATAAAAGTTGCAATA<br>CCCCTTTAACGCTAATACGATCAA | 1030 | Ishii and others, (1996) |
| *van B* | GTGACAAACCGGAGGCGAGGA<br>CCGCCATCCTCCTGCAAAAAA | 433 | Radu and others (2001) |

**Table 2–Prevalence of staphylococci isolated from ground meats and distribution of some genes in the isolates.**

| | | No. of isolates obtained from ground meat (*x*/*y*) | | No. of gene positive isolates | | | | |
|---|---|---|---|---|---|---|---|---|
| *Staphylococcus species* | No. of isolates (%) | Beef | Lamb | *16S rRNA* | *nuc* | *femA* | *mecA* | *pvl* |
| *S. aureus* | 85 (40.8) | 35 (125/35) | 50 (125/43) | 85 | 85 | 34 | 40 | 5 |
| *S. saprophyticus* | 60 (28.8) | 40 (125/35) | 20 (125/18) | 60 | – | – | 6 | – |
| *S. hominis* | 23 (11.0) | 18 (125/18) | 5 (125/5) | 23 | – | – | 5 | – |
| *S. lentus* | 8 (3.8) | 8 (125/8) | – | 8 | – | – | – | – |
| *S. pasteuri* | 8 (3.8) | 3 (125/2) | 5 (125/5) | 8 | – | – | – | – |
| *S. warneri* | 5 (2.4) | 5 (125/3) | – | 5 | – | – | – | – |
| *S. intermedius* | 5 (2.4) | – | 5 (125/4) | 5 | – | – | – | – |
| *S. kloosi* | 5 (2.4) | – | 5 (125/3) | 3 | – | – | – | – |
| *S. vitulinus* | 3 (1.4) | 3 (125/2) | – | 5 | – | – | – | – |
| *S. chromogenes* | 3 (1.4) | 3 (125/3) | – | 3 | – | – | 1 | – |
| *S. equorum* | 3 (1.4) | 3 (125/2) | – | 3 | – | – | – | – |
| Total | 208 | 118 (125/108) | 90 (125/78) | 208 | 85 | 34 | 52 | 5 |

–: Not detected.
*x*/*y* : Number of samples analyzed/number of positive samples.

51% of them were contaminated with *S. saprophyticus*, and 14% with *S. hominis* (Table 2). Marino and others (2011) conducted a study with ready-for-consumption animal source foods and isolated *S. saprophyticus* as the dominant species (23.7%), while *S. epdermidis*, *S. pasteuri* as well as other CoNS were isolated less frequently. In another study, Gündoğan and Ataol (2012) reported that 72% of the CoNS isolates obtained from ground meat samples were *S. xylosus*, while 14% were *S. hominis*. The differences between our results and those of others in terms of isolation rates and isolated species are likely due to different sample sizes, sample types, sanitary conditions of work places, personal hygiene of the personnel, and storage times. In addition, in this study we used the Vitek 2 bacterial identification system for differentiation of staphylococci.

**Table 3–Antibiotic resistance pattern of staphylococci isolated from ground meat samples.**

| Antibiotic | Total (n:208) | (%) | *S. aureus* | *S. hominis* | *S. lentus* | *S.saprophyticus* | *S. equorum* | *S. warneri* | *S. chromogenes* | *S. vitulinus* | *S. kloosi* | *S. intermedius* | *S. pasteuri* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Penicillin | 107 | 51.4 | 54 | 8 | 4 | 25 | – | 4 | – | 4 | 3 | – | 5 |
| Oxacillin | 51 | 24.5 | 30 | 3 | – | 15 | 1 | – | – | 1 | 1 | – | – |
| Cefoxitin | 33 | 15.8 | 24 | – | – | 9 | – | – | – | – | – | – | – |
| Ampicillin | 83 | 39.9 | 42 | 4 | 4 | 21 | – | 4 | 1 | – | 1 | – | 4 |
| Amoxicillin–Clavulanic Acid | 8 | 3.8 | 8 | – | – | – | – | – | – | – | – | – | – |
| Kanamycin | 3 | 1.4 | – | – | – | 3 | – | – | – | – | – | – | – |
| Gentamicin | – | – | – | – | – | – | – | – | – | – | – | – | – |
| Streptomycin | 46 | 22.1 | 21 | 8 | – | 12 | – | 4 | – | 1 | – | – | – |
| Tobramycin | – | – | – | – | – | – | – | – | – | – | – | – | – |
| Clindamycin | 20 | 10 | 4 | 6 | – | 5 | – | 5 | – | – | – | – | – |
| Tetracycline | 178 | 85.5 | 75 | 20 | 8 | 52 | 3 | 5 | – | 5 | 3 | – | 7 |
| Oxytetracycline | 178 | 85.5 | 75 | 20 | 8 | 52 | 3 | 5 | – | 5 | 3 | – | 7 |
| Doxycycline | 66 | 31.7 | 43 | 6 | 2 | 12 | – | – | – | 1 | – | – | 2 |
| Enrofloxacin | 9 | 4.3 | 9 | – | – | – | – | – | – | – | – | – | – |
| Ofloxacin | – | – | – | – | – | – | – | – | – | – | – | – | – |
| Ciprofloxacin | 9 | 4.3 | 9 | – | – | – | – | – | – | – | – | – | – |
| Erythromycin | 37 | 17.7 | 12 | 8 | – | 8 | – | 4 | – | 3 | – | 2 | – |
| Azithromycin | 29 | 13.9 | 8 | 8 | – | 8 | – | 4 | – | 1 | – | – | – |
| Sulfamethoxazole–Trimethoprim | 17 | 8.1 | 9 | – | – | 8 | – | – | – | – | – | – | – |
| Chloramphenicol | 4 | 1.9 | – | – | – | – | – | 4 | – | | – | – | – |
| Rifampicin | 19 | 9.1 | 13 | 3 | – | 3 | – | – | – | – | – | – | – |
| Quinupristin/Dalfopristin | 18 | 8.6 | 13 | 3 | – | 2 | – | – | – | – | – | – | – |
| Vancomycin | 2 | 0.9 | 2 | – | – | – | – | – | – | – | – | – | – |
| Novobiocin | 95 | 45.6 | 38 | 9 | 5 | 33 | 1 | 3 | – | 2 | 2 | 2 | – |

## Presence of *mecA*, *nuc*, *pvl*, and *femA* genes in staphylococci

All of the 208 isolates that were identified by the Vitek system as staphylococci were confirmed by PCR and all of the 85 identified *S. aureus* isolates were confirmed as possessing the *nuc* gene (Table 2). The 34 *S. aureus* isolates that were phenotypically identified to be coagulase positive carried the *femA* gene (Table 2) and 3 coagulase-positive *S. intermedius* isolates, but none of the coagulase negative isolates carried the *femA* gene. Similarly, Vannuffel and others (1995) reported a strong relationship between the presence of *femA* in *S. aureus* isolates and coagulase-positivity. Al-Talib and others (2009) detected *femA* in all of their 178 clinical isolates of *S. aureus*, while the *femA* gene was not detected in 52 clinical CoNS isolates. However, Pelisser and others (2009) detected *femA* in 91 of 102 coagulase-positive *Staphylococcus* isolates derived from meat and dairy products, while Kav and others (2011) detected the gene in 40 of 53 coagulase-positive *Staphylococcus* isolates obtained from Urfa cheese. It has been reported that in addition to *S. aureus* and *S. epidermidis*, *femA* positivity was also detected in coagulase-negative *S. saprophyticus* and *S. hominis* and *femA* nucleic acid homology between *S. aureus* and CoNS varied between 75.1% and 78.3% (Vannuffel and others 1999). The relationship between *femA* positivity and CoNS as reported by Veras and others (2008), who determined that 3 CoNS samples that were isolated after a foodborne outbreak of poisoning in Brazil carried *femA*. Based on the results of our study, we also suggest that there is a relationship between coagulase-positive *S. aureus* isolates and *femA* gene positivity. However, one should also remember that *femA* gene positivity can also be detected in CoNS.

MRSA infections may arise due to cross contamination associated with contact with the intestinal contents while cutting raw meat, from the slaughtering environment or through infected handlers. The *mecA* gene is specific for methicillin-resistant staphylococci and the DNA sequences of *mecA* in *S. aureus* and CoNS are identical. Therefore, this gene is used as an important molecular component in PCR-based rapid identification of MRSA and methicillin-resistant CoNS. Particularly, *mecA*-positive CoNS strains are reservoirs of this gene, and can further transfer this gene to *S. aureus* or other staphylococci (Garza-González and others 2010). In the current study, we determined that the correlation between *mecA*-positivity and phenotypic oxacillin resistance is higher in staphylococci (Table 2 and 4). However, many studies reported that the cefoxitin disc diffusion method is more reliable and more sensitive in determining methicillin resistance in staphylococci compared to oxacillin (Boutiba-Ben and others 2004; Cauwelier and others 2004). In our study, based on the suggestion by CLSI, the phenotypic resistance to methicillin was determined by using cefoxitin disc diffusion methods (CLSI 2011). *MecA* was detected in 47% of *S. aureus* and in 10% of CoNS, while phenotypic methicillin resistance was detected in 28.2% of *S. aureus* isolates (MRSA) and 8.3% of CoNS (only *S. saprophyticus*) (MR-CoNS) (Table 2). The direct relationship between the presence of *mecA* and cefoxitin resistance has been previously reported (Swenson and Tenover 2005). However, the proportion obtained in our study was lower than those reported previously. Similar to our study, Pereira and others (2009) reported that 38% of *S. aureus* isolates obtained from different foods were MRSA. However, in a study conducted in Korea with 930 slaughterhouse and retail meat samples only 2 chicken samples were positive for MRSA, while no MRSA was detected in pork or beef samples (Kwon and others 2006). Van Loo and others (2007) detected MRSA strains

**Table 4–Phenotypic multiresistance patterns of staphylococci isolated from ground meat samples.**

| | | No. strains resistant to no. antibiotics | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Species** | **R strains (%)*** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** | **15** | **16** | **17** | **18** | **19** |
| *S. aureus* | 85 (100) | – | 13 | 10 | 18 | 15 | 6 | 6 | – | 4 | – | – | 4 | 4 | – | – | – | 4 | – | 4 |
| *S. saprophyticus* | 52 (86.6) | – | – | 6 | 5 | 18 | 6 | 6 | 2 | 3 | 6 | – | – | – | – | – | – | – | – | – |
| *S. hominis* | 20 (86.9) | – | 2 | – | 3 | 2 | 4 | – | – | 9 | – | – | – | – | – | – | – | – | – | – |
| *S. lentus* | 8 (100) | – | 3 | 1 | – | 2 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – |
| *S. pasteuri* | 8 (100) | – | 2 | 2 | 2 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| *S. warneri* | 5 (100) | – | – | 2 | – | 2 | – | – | 1 | – | – | – | – | – | – | – | – | – | – | – |
| *S. vitulinus* | 5 (100) | – | – | 1 | 3 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| *S. kloosi* | 3 (100) | – | 2 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| *S. equorum* | 3 (100) | – | 1 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| *S. intermedius* | 3 (100) | – | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| *S. chromogenes* | 3 (100) | – | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |

R: Resistance strains
*Percentage of strains.

in 2 of 79 raw beef and pork samples. The studies show that the distribution of MRSA varies based on geographic region, personnel hygiene, and the method used for isolation (Wang and others 2014).

The PVL toxin, a virulence factor produced by some *S. aureus* strains, makes an important contribution to the pathogenicity of *S. aureus*. Rankin and others (2005) reported that 11 MRSA strains isolated from companion animals were positive for the *pvl* gene. In our study, 5 of 40 MRSA isolates carried the *pvl* gene, and all of these isolates were obtained from ground beef samples (Table 2). In a study conducted in the United States, 37 MRSA strains were isolated from 200 pork and beef meat samples and one of them (beef meat) carried the *pvl* gene (Jackson and others 2013). In a study conducted in Turkey with 1.604 sheep and 66 goat half-udder milk samples, 14 methicillin-susceptible *S. aureus* strains of sheep origin were *pvl* positive (Ünal and others 2012). Another study evaluated 81 cow and 40 sheep milk samples in terms of mastitis and CoNS isolates from 3 cow milk samples (3.7%) and 2 sheep milk samples (5.0%) CoNS were reported to carry the *pvl* gene (Ünal and Çinar 2012). Similarly, Sudagidan and Aydin (2010) isolated Staphylococcus strains from 1209 food items and reported that 3 *S. aureus* strains (one from each cake, kashkaval cheese and thin sheet of dough) carried the *pvl* gene. One of the most important features of community-acquired isolates is the presence of the PVL toxin. Recent studies conducted with CA-MRSA isolates reported the PVL toxin occurrence rates varied between 77% and 100% (Naimi and others 2003). In Turkey, CA-MRSA is not common. A multicenter study conducted between 2006 and 2008 investigated 397 MRSA strains isolated from hospitals and reported that *pvl* was present in only 5 (3%) strains (Bozdoğan and others 2013).

## Pheno- and genotyping antibiotic resistance

Resistant microorganisms or their antibiotic resistance genes are transferred to humans through food, animals, or the environment (Angulo and others 2004; Phillips and others 2004). Antibiotic-resistant strains make it more difficult to treat infections and therefore pose a serious threat to public health. In this study, the staphylococci isolates obtained from ground beef and lamb samples were found to be highly resistant to tetracycline, penicillin, quinolones, macrolides, and aminoglycosides (Table 3). This group of antibiotics is widely used in Turkey mostly to treat infections and as a prophylactic measure in animals; therefore, the fact that we detected a high level of antibiotic resistance to this group of antibiotics in our isolates is not surprising. Previous studies have also reported that staphylococci have high-level resistance to this group of antibiotics (Andre and others 2008; Aydın and others 2011). In the present study, we determined that all of the 24 MRSA strains had multidrug-resistance properties and that these isolates were resistant to between 7 and 19 different antibiotics (Table 4). Similarly, Guven and others (2010) reported that all 52 *S. aureus* strains obtained from beef had multidrug resistance properties. Moreover, multidrug resistance was also reported in all 16 MRSA strains isolated from bovine milk samples (Turkyılmaz and others 2010) and in 10 of 12 MRSA strains isolated form cheese samples (Can and Celik 2012). In previous studies conducted in Turkey, no instances of vancomycin resistance were reported among the MRSA strains isolated from food samples (Citak and Duman 2011; Can and Çelik 2012; Siriken and others 2013); however, in the present study, 2 MRSA strains isolated from ground beef were vancomycin resistant (Table 3). Türkyılmaz and others (2010) have reported that 7 of 16 MRSA strains isolated from milk samples from cows with mastitis had moderate vancomycin resistance. Although vancomycin resistance has not been observed in MRSA isolates obtained from clinical samples in Turkey, studies done in recent years showed diminishing sensitivity to vancomycin (Öngüt and others 2013). Doğan and others (2014) detected 6 instances of vancomycin resistance in their examination of 345 MRSA strains isolated from patient materials between 2011 and 2012. In Turkey and the European Union, the use of avoparcin has been banned in all food production lines and the detection of vancomycin resistance in the ground beef samples suggests either illegal use of avoparcin as anabolic or for prophylactic purposes in cattle or human-associated contamination. In addition, vancomycin-resistant *Enterococcus* strains can be found in humans and animals or animal source foods. These strains are capable of acting as vancomycin resistance determinant reservoirs for other bacteria (Salasia and others 2004). In our study, CoNS isolates were resistant to at least 2 or more antibiotics and multiple drug resistance was widely present in *S. saprophyticus* isolates, an organism that is known as an opportunistic pathogen in humans (Table 4). Multiple drug resistance was detected toward penicillin, tetracycline, novobiocin, and macrolide-group antibiotics. Similar to our results, other studies that examined food, clinical, and environmental samples also determined that multiple antibiotic resistances were common in CoNS (Abulreesh and Organji 2011; Waters and others 2011; Wang and others 2013). Thus, it is tempting to speculate that there is a correlation between the incidence of antibiotic resistances in food-associated CoNS and their connection to (opportunistic) pathogenic staphylococci species. Some staphylococci from this group can

**Table 5–Distribution of antibiotic resistance genes in staphylococci obtained from ground meat samples.**

| Species | No. of isolate | No. of positive isolates[a,b,c] | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *blaZ* | *tet(M)* | *tet(K)* | *tet(L)* | *tet(S)* | *tet(W)* | *Cat* | *ermA* | *ermB* | *ermC* |
| *S. aureus* | 85 | 41 | 30 | 56 | 23 | 19 | 19 | 30 | 4 | 26 | – |
| *S. saprophyticus* | 52 | 24 | 16 | 24 | 18 | 13 | 16 | – | 3 | 8 | – |
| *S. hominis* | 20 | 6 | 2 | 9 | 9 | 4 | 9 | 2 | 4 | 6 | 4 |
| *S. lentus* | 8 | 8 | – | 3 | 3 | 3 | 3 | – | – | – | – |
| *S. pasteuri* | 8 | – | – | – | – | – | – | – | – | – | – |
| *S. warneri* | 5 | 3 | – | 3 | 3 | 3 | 3 | – | 3 | 3 | 3 |
| *S. vitulinus* | 5 | 3 | – | 3 | 3 | – | – | – | – | – | – |
| *S. kloosi* | 3 | 3 | – | 3 | 3 | 3 | 3 | – | – | – | – |
| *S. equorum* | 3 | 3 | – | 3 | 3 | 3 | 3 | – | – | – | – |
| *S. intermedius* | 3 | – | – | – | – | – | – | – | – | – | – |
| *S. chromogenes* | 3 | – | – | – | – | – | – | – | – | – | – |
| *Total* (%) | | 91 (51.7) | 48 (27.2) | 104 (59) | 65 (36.9) | 48 (27.2) | 56 (31.8) | 32(18.1) | 14 (7.9) | 43 (24.4) | 7 (3.9) |

–: Not detected.
[a,b,c] *vanA*, *vanB*, *tet*(*O*), and *aac6* to *aph2* genes were not detected in any of the isolates.

also be isolated from fermented meat products. Therefore, these bacteria can have a potential role as a reservoir for transferring antibiotic resistance genes to commensal or pathogenic bacteria.

Although phenotypic chloramphenicol resistance was detected in 4 *S. warneri* isolates, all 4 of those isolates were negative for the *cat* gene. Meanwhile, 4 *S. hominis* isolates that did not show phenotypical chloramphenicol antibiotic resistance were positive for the *cat* gene (Table 5). In Turkey and in the European Union, the use of chloramphenicol in animals is prohibited, but studies have detected the presence of this antibiotic in urine and to a lesser extent in the muscles of cattle and pigs (Perreten and others 1998). In our study, *S. aureus* isolates contained *tet(K)*, *tet(M)*, *tet(L)*, *tet(S)*, and *tet(W)* tetracycline resistance gene determinants at rates of 65.8%, 35.2%, 27%, 22.3%, and 22.3%, respectively, whereas CoNS contained these genes at 17.1%, 45.7%, 42%, 27.6%, and 35.2%, respectively. None of the isolates had the *tet(O)* gene. Previous studies have shown that staphylococci either did not carry *tet (O)* at all or only infrequently (Schwarz and others 1998; Schmitz and others 2001). In staphylococci, erythromycin resistance is encoded by the *erm* gene. Lina and others (1999) determined that the *ermA* gene was more prevalent than genes, *ermB* and *ermC* in *S. aureus*, while in CoNS the *ermC* gene was more prevalent. Similarly, Martineau and others (2000) reported that *ermC* was more prevalent in CoNS. However, in our study, CoNS carried *ermA*, *ermB*, and *ermC* at rates of 9.3%, 15.8%, and 6.5%, respectively, while *S. aureus* carried *ermA* and *ermB* at 4.75% and 30.7%, respectively, and none of them carried *ermC*. The higher prevalence of *ermB* compared to other *erm* genes might be associated with this gene being frequently carried by conjugative transposons and therefore is increasing its distribution in staphylococci (Khan and others 2002). In the current study, we did not detect vancomycin resistance genes such as *vanA* and *vanB* in any of the isolates (Table 5). There are only few studies that reported the presence of these genes in staphylococci isolated from food samples. Baumgartner and others (2014) reported that only 1% of 267 *S. aureus* isolates obtained from ready-for-consumption food carried the *vanB* gene. Resistance to vancomycin, which is an antibiotic used in humans to treat staphylococcal infections, is more common in clinical isolates than in food isolates and therefore the presence of vancomycin resistance genes could expected to be more prevalent in this type of isolate (Thati and others 2011; Richter and others 2014). In staphylococci, the *blaZ* gene plays an important role in penicillin resistance. Our results showed a wide distribution of the *blaZ* gene in *S. aureus* and CoNS, at 48.4% to 46.2%, respectively. It has been reported that this gene can be transferred between the CoNS and *S. aureus* and that CoNS can serve as reservoirs of *blaZ* genes for *S. aureus* (Al-Masaudi and others 1991). Resistance to aminoglycosides usually develops as a result of enzymatic modification of the antibiotics by aminoglycoside-modifying enzymes. The *blaZ* gene has been reported to be responsible for development of high-level gentamycin resistance as well as resistance to aminoglycosides, except for streptomycin (Vakulenko and others 2003). In this context, none of the isolates obtained in our study had phenotypic gentamycin resistance and in parallel were also negative for the *aac6′-aph2″* gene.

## Conclusion

In this study, we investigated the presence, in ground lamb and beef samples, of staphylococcal pathogens that are important in human and animal health such as MRSA and MR-CoNS. Our study is the 1st to report the *pvl* gene from MRSA isolates obtained from ground meat samples in Turkey. Moreover, we detected 2 instances of vancomycin resistance in MRSA isolates and also detected a high level of multidrug resistance in staphylococci isolates. In particular, we believe that methicillin-resistant staphylococci should be added into surveillance programs, as this step would lead to closer monitoring of these isolates and also make significant contributions to development of preventive-control programs for human and animal health. We also want to emphasize the fact that competent authorities can prevent the development of resistance to MRSA and MR-CoNS by performing strict inspection in order to prevent the uninformed use of antibiotics in humans and animals and use of illegal antibiotics.

## References


Aarestrup FM, Agerso Y, Gerner–Smidt P, Madsen M, Jensen LB. 2000. Comparison of antimicrobial resistance phenotypes and resistance genes in *Enterococcus faecalis* and *Enterococcus faecium* from humans in the community, broilers, and pigs in Denmark. Diagn Microbiol Infect Dis 37:127–37.

Abulreesh HH, Organji SR. 2011. The prevalence of multidrug-resistant staphylococci in food and the environment of Makkah, Saudi Arabia. Res J Microbiol 6:510–23.

Al-Masaudi SB, Day MJ, Russell AD. 1991. Antimicrobial resistance and gene transfer in *Staphylococcus aureus*. J Appl Microbiol 70:279–90.

Al-Talib H, Yean CY, Al-Khateeb A, Hassan H, Singh KKB, Al-Jashamy K, Ravichandran M. 2009. A pentaplex PCR assay for the rapid detection of methicillin-resistant *Staphylococcus aureus* and Panton-Valentine Leukocidin. BMC Microbiol 9:113–20.

Al-Tarazi YH, Albetar MA, Alaboudi AR. 2009. Biotyping and enterotoxigenicity of staphylococci isolated from fresh and frozen meat marketed in Jordan. Food Res Intl 42:374–9.

Aminov RI, Garrigues-Jeanjean N, Mackie RI. 2001. Molecular ecology of tetracycline resistance: development and validation of primers for detection of tetracycline resistance genes encoding ribosomal protection proteins. Appl Environ Microbiol 67:22–32.

Angulo FJ, Nargund VN, Chiller TC. 2004. Evidence of an association between use of antimicrobial agents in food animals and anti-microbial resistance among bacteria isolated from humans and the human health consequences of such resistance. J Vet Med B Infect Dis Vet Public Health 51:374–9.

Andre MCD, Campos MRH, Borges LJ, Kipnis A, Pimenta FC, Serafini AB. 2008. Comparison of *Staphylococcus aureus* isolates from food handlers, raw bovine milk and Minas Frescal cheese by antibiogram and pulsed-field gel electrophoresis following SmaI digestion. Food Control 19:200–7.
Argudin MÁ, Mendoza MC, Rodicio MR. 2010. Food poisoning and *Staphylococcus aureus* enterotoxins. Toxins 2:1751–73.
Aydin A, Muratoglu K, Sudagidan M, Bostan K, Okuklu B, Harsa S. 2011. Prevalence and antibiotic resistance of foodborne *Staphylococcus aureus* isolates in Turkey. Foodborne Pathog Dis 8:63–9.
Baumgartner A, Niederhauser I, Johler S. 2014. Virulence and resistance gene profiles of staphylococcus aureus strains isolated from ready-to-eat foods. J Food Prot 77:1232–6.
Boutiba-Ben Boubaker I, Ben Abbes R, Ben Abdallah H, Mamlouk K, Mahjoubi F, Kammoun A, Hammami A, Ben Redjeb S. 2004. Evaluation of a cefoxitin disk diffusion test for the routine detection of methicillin-resistant *Staphylococcus aureus*. Clin Microbiol Infect 10:762–5.
Bozdoğan B, Yıldız Ö, Oryaşin E, Kırdar S, Gülcü B, Aktepe O, Arslan U, Bayramoğlu G, Çoban AY, Coşkuner SA, Güdücüoğlu H, Karabiber N, Öncü S, Otkun MT, Özkütük N, Özyurt M, Şener AG. 2013. t030 is the most common *spa* type among methicillin-resistant *Staphylococcus aureus* strains isolated from Turkish hospitals. Mikrobiyol Bul 47:571–81.
Can HY, Çelik TH. 2012. Detection of enterotoxigenic and antimicrobial resistant S. aureus in Turkish cheeses. Food Control 24:100–3.
Cauwelier B, Gordts B, Descheemaecker P, VanLanduyt H. 2004. Evaluation of a disk diffusion method with cefoxitin (30 $\mu$g) for detection of methicillin-resistant *Staphylococcus aureus*. Clin Microbiol Infect 23:389–92.
Citak S, Duman T. 2011. *Staphylococcus aureus* and coagulase-negative *Staphylococcus* from raw chicken samples in Turkey: Prevalence and antimicrobial resistance. J Food Agric Environ 9:156–8.
CLSI. 2011. Performance standards for antimicrobial susceptibility testing. CLSI approved standard twenty-first informational supplement M100-S21. Wayne, Pa.: Clinical and Laboratory Standards Inst.
Doğan M, Feyzioğlu B, Baykan M. 2014. On yıllık dönemde *S. aureus* suşlarının antibiyotik direnç durumundaki değişim. Abant Med J 3:237–41.
Feßler AT, Billerbeck C, Kadlec K, Schwarz S. 2010. Identification and characterization of methicillin-resistant coagulase-negative staphylococci from bovine mastitis. J Antimicrob Chemother 65:1576–82.
Garza-González E, Morfin-Otero R, Llaca-Diaz JM, Rodriguez-Noriega E. 2010. Staphylococcal cassette chromosome *mec* (SCC*mec*) in methicillin-resistant coagulase-negative staphylococci. A review and the experience in a tertiary-care setting. Epidemiol Infect 138:645–54.
Golding GR, Bryden L, Levett PN, McDonald RR, Wong A, Wylie J, Graham MR, Tyler S, VanDomselaar G, Simor AE, Gravel D, Mulvey MR. 2010. Livestock-associated methicillin-resistant *Staphylococcus aureus* sequence type 398 in humans, Canada. Emerg Infect Dis 16:587–94.
Guven K, Mutlu MB, Gulbandilar A, Cakir P. 2010. Occurrence and characterization of *Staphylococcus aureus* isolated from meat and dairy products consumed in Turkey. J Food Saf 30:196–212.
Gündoğan N, Ataol Ö. 2012. Et örneklerinden izole edilen *Staphylococcus aureus* ve koagülaz negatif stafilokok'ların biyofilm üretimi ve DNaz aktivitelerinin belirlenmesi. Turk Hij Deney Biyol Derg 69:135–42.
Gündoğan N, Citak S, Yucel N, Devren A. 2005. A note on the incidence and antibiotic resistance of *Staphylococcus aureus* isolated from meat and chicken samples. Meat Sci 69:807–10.
Hegde SS, Shrader TE. 2001. FemABX family members are novel nonribosomal peptidyltransferases and important pathogen-specific drug targets. J Biol Chem 276:6998–7003.
Ishii Y, Ohno A, Kashitani S, Iwata M, Yamaguchi K. 1996. Identification of VanB-type vancomycin resistance in *Enterococcus gallinarum* from Japan. J Infect Chemother 2:102–5.
Jackson CR, Davis JA, Barrett JB. 2013. Prevalence and characterization of methicillin-resistant *Staphylococcus aureus* isolates from retail meat and humans in Georgia. J Clin Microbiol 51:1199–1207.
Jaglic Z, Michu E, Holasova M, Vlkova H, Babak V, Kolar M, Bardon J, Schlegelova J. 2010. Epidemiology and characterization of *Staphylococcus epidermidis* isolates from humans, raw bovine milk and a dairy plant. Epidemiol Infect 138:772–82.
Jones TF, Kellum ME, Porter SS, Bell M, Schaffner W. 2002. An outbreak of community-acquired foodborne illness caused by methicillin-resistant. Emerg Infect Dis 8:82–84.
Kav K, Col R, Ardic M. 2011. Characterization of *Staphylococcus aureus* isolates from white-brined Urfa cheese. J Food Prot 74:1788–96.
Kawano J, Shimizu A, Saitoh Y, Yagi M, Saito T, Okamoto R. 1996. Isolation of methicillin-resistant coagulase-negative staphylococci from chickens. J Clin Microbiol 34:2072–7.
Kelman A, Soong YA, Dupuy N, Shafer D, Richbourg W, Johnson K, Brown T, Kestler E, Li Y, Zheng J, McDermott P, Meng J. 2011. Antimicrobial susceptibility of *Staphylococcus aureus* from retail ground meats. J Food Prot 74:1625–9.
Khan AA, Nawaz M, Khan SA, Steele R. 2002. Detection and characterization of erythromycin-resistant methylase genes in Gram-positive bacteria isolated from poultry litter. Appl Microbiol Biotechnol 59:377–81.
Kwon NH, Park KT, Jung WK, Youn HY, Lee Y, Kim SH, Bae W, Lim JY, Kim JY, Kim JM, Hong SK, Park YH. 2006. Characteristics of methicillin- resistant *Staphylococcus aureus* isolated from chicken meat and hospitalized dogs in Korea and their epidemiological relatedness. Vet Microbiol 117:304–12.
Lima de Castro Nunes E, dos Santos KRN, Mondino PJJ, deFreire Bastos MDC, Giambiagi-deMarval M. 1999. Detection of *ileS-2* gene encoding mupirocin resistance in methicillin-resistant *Staphylococcus aureus* by multiplex PCR. Diagn Microbiol Infect Dis 34:77–81.
Lina G, Piémont Y, Godail-Gamot F, Bes M, Peter MO, Gauduchon V, Vandenesch F, Etienne J. 1999. Involvement of Panton-Valentine leukocidin-producing *Staphylococcus aureus* in primary skin infections and pneumonia. Clin Infect Dis 29:1128–32.
Marino M, Frigo F, Bartolomeoli I, Maifreni M. 2011. Safety-related properties of staphylococci isolated from food and food environments. J Appl Microbiol 110:550–61.
Martineau F, Picard FJ, Grenier L, Roy PH, Ouellette M, Bergeron MG. 2000. Multiplex PCR assays for the detection of clinically relevant antibiotic resistance genes in staphylococci isolated from patients infected after cardiac surgery. J Antimicrob Chemother 46:527–34.
Miko BA, Hafer CA, Lee CJ, Sullivan SB, Hackel MAM, Johnson BM, Whittier S, Della-Latta P, Uhlemann AC, Lowy FD. 2013. Molecular characterization of methicillin-susceptible *Staphylococcus aureus* clinical isolates in the United States, 2004 to 2010. J Clin Microbiol 51:874–9.
Mørk T, Kvitle B, Jørgensen HJ. 2012. Reservoirs of *Staphylococcus aureus* in meat sheep and dairy cattle. Vet Microbiol 155:81–7.
Naimi TS, LeDell KH, Como-Sabetti K, Borchardt SM, Boxrud DJ, Etienne J, Johnson SK, Vandenesch F, Fridkin S, O'Boyle C, Danila RN, Lynfield R. 2003. Comparison of community-and health care-associated methicillin-resistant *Staphylococcus aureus* infection. JAMA 290:2976–4.
Öngüt G, Öğünç D, Özhak Baysan B, Ertekin D, Dağlar D, Dönmez L, Turhan Ö, Çolak D. 2013. Kan kültürlerinden izole edilen MRSA suşlarının vankomisin mik değerlerinin beş yıllık bir dönemde değerlendirilmesi. Turkiye Klinikleri J Med Sci 3:1017–21.
Pelisser MR, Klein CS, Ascoli KR, Zotti TR, Arisi ACM. 2009. Ocurrence of *Staphylococcus aureus* and multiplex PCR detection of classic enterotoxin genes in cheese and meat products. Braz J Microbiol 40:145–8.
Perreten V, Giampà N, Schuler-Schmid U, Teuber M. 1998. Antibiotic resistance genes in coagulase-negative staphylococci isolated from food. Syst Appl Microbiol 21:113–20.
Pereira V, Lopes C, Castro A, Silva J, Gibbs P, Teixeira P. 2009. Characterization for enterotoxin production, virulence factors, and antibiotic susceptibility of *Staphylococcus aureus* isolates from various foods in Portugal. Food Microbiol 26:278–82.
Phillips I, Casewell M, Cox T, DeGroot B, Friis C, Jones R, Nightingale C, Preston R, Waddell J. 2004. Does the use of antibiotics in food animals pose a risk to human health? A critical review of published data. J Antimicrob Chemother 53:28–52.
Pu S, Han F, Ge B. 2009. Isolation and characterization of methicillin-resistant *Staphylococcus aureus* strains from Louisiana retail meats. Appl Environ Microbiol 75:265–7.
Radu S, Toosa H, Rahim RA, Reezal A, Ahmad M, Hamid AN, Rusul G, Nishibuchi M. 2001. Occurrence of the *vanA* and *vanC2/C3* genes in *Enterococcus* species isolated from poultry sources in Malaysia. Diagn Microbiol Infect Dis 39:145–53.
Rankin S, Roberts S, O'Shea K, Maloney D, Lorenzo M, Benson CE. 2005. Panton valentine leukocidin (PVL) toxin positive MRSA strains isolated from companion animals. Vet Microbiol 108:145–8.
Richter SS, Diekema DJ, Heilmann KP, Dohrn CL, Crispell EK, Riahi F, McDanel JS, Satola SW, Doern GV. 2014. Activities of vancomycin, ceftaroline, and mupirocin against *Staphylococcus aureus* isolates collected in a 2011 National Surveillance Study in the United States. Antimicrob Agents Chemother 58:740–5.
Salasia SIO, Khusnan Z, Lämmler C, Zschöck M. 2004. Comparative studies on pheno-and genotypic properties of *Staphylococcus aureus* isolated from bovine subclinical mastitis in central Java in Indonesia and Hesse in Germany. J Vet Sci 5:103–9.
Schmitz FJ, Krey A, Sadurski R, Verhoef J, Milatovic D, Fluit AC. 2001. Resistance to tetracycline and distribution of tetracycline resistance genes in European *Staphylococcus aureus* isolates. J Antimicrob Chemother 47:239–40.
Schwarz S, Roberts MC, Werckenthin C, Pang Y, Lange C. 1998. Tetracycline resistance in *Staphylococcus* spp. from domestic animals. Vet Microbiol 63:217–27.
Siriken B, Yildirim T, Erol I, Durupinar B, Ciftci A, Onuk EE. 2013. Prevalence and characterization of coagulase positive staphylococci isolated from salted anchovy. J Aquat Food Prod Technol 22:339–52.
Strommenger B, Kettlitz C, Werner G, Witte W. 2003. Multiplex PCR assay for simultaneous detection of nine clinically relevant antibiotic resistance genes in Staphylococcus aureus. J Clin Microbiol 41:4089–94.
Stuhlmeier R, Stuhlmeier KM. 2003. Fast, simultaneous, and sensitive detection of staphylococci. Am J Clin Pathol 56:782–5.
Sudagidan M, Aydin A. 2010. Virulence properties of methicillin-susceptible *Staphylococcus aureus* food isolates encoding Panton–Valentine Leukocidin gene. Intl J Food Microbiol 138: 287–91.
Sutcliffe J, Grebe T, Tait-Kamradt A, Wondrack L. 1996. Detection of erythromycin-resistant determinants by PCR. Antimicrob Agents Chemother 40:2562–6.
Swenson JM, Tenover FC. 2005. Results of disk diffusion testing with cefoxitin correlate with presence of *mecA* in *Staphylococcus* spp. J Clin Microbiol 43:3818–23.
Thati V, Shivannavar CT, Gaddad SM. 2011. Vancomycin resistance among methicillin resistant *Staphylococcus aureus* isolates from intensive care units of tertiary care hospitals in Hyderabad. Indian J Med Res 134:704–8.
Tiemersma EW, Bronzwaer SL, Lyytikäinen O, Degener JE, Schrijnemakers P, Bruinsma N, Monen J, Witte W, Grundman H. 2004. Methicillin-resistant *Staphylococcus aureus* in Europe, 1999–2002. Emerg Infect Dis 10:1627–34.
Türkyılmaz S, Tekbıyık S, Oryasin E, Bozdogan B. 2010. Molecular epidemiology and antimicrobial resistance mechanisms of methicillin-resistant *Staphylococcus aureus* isolated from bovine milk. Zoonoses Public Health 57:197–203.
Ünal N, Çinar OD. 2012. Detection of stapylococcal enterotoxin, methicillin-resistant and Panton-Valentine leukocidin genes in coagulase-negative staphylococci isolated from cows and ewes with subclinical mastitis. Trop Anim Health Prod 44:369–75.
Ünal N, Askar Ş, Macun HC, Sakarya F, Altun B, Yıldırım M. 2012. Panton-Valentine leukocidin and some exotoxins of *Staphylococcus aureus* and antimicrobial susceptibility profiles of staphylococci isolated from milks of small ruminants. Trop Anim Health Prod 44:573–9.
Vakulenko SB, Donabedian SM, Voskresenskiy AM, Zervos MJ, Lerner SA, Chow JW. 2003. Multiplex PCR for detection of aminoglycoside resistance genes in enterococci. Antimicrob Agents Chemother 47:1423–6.
VanLoo IHM, Diederen BMW, Savelkoul PHM, Woudenberg JHC, Roosendaal R, VanBelkum A, Lemmens-den Toom N, Verhulst C, VanKeulen PHJ, W Kluytmans JAJ. 2007. Methicillin-resistant *Staphylococcus aureus* in meat products, the Netherlands. Emerg Infect Dis 13: 1753–5.
Vannuffel P, Gigi J, Ezzedine H, Vandercam B, Delmee M, Wauters G, Gala JL. 1995. Specific detection of methicillin-resistant *Staphylococcus* species by multiplex PCR. J Clin Microbiol 33:2864–7.
Vannuffel P, Heusterspreute M, Bouyer M, Vandercam B, Philippe M, Gala JL. 1999. Molecular characterization of *femA* from *Staphylococcus hominis* and *Staphylococcus saprophyticus*, and *femA*-based discrimination of staphylococcal species. Res Microbiol 150:129–41.
Veras JF, do Carmo LS, Tong LC, Shupp JW, Cummings C, dos Santos DA, Cerqueira MMOP, Cantini A, Nicoli JC, Jett M. 2008. A study of the enterotoxigenicity of coagulase-negative and coagulase-positive staphylococcal isolates from food poisoning outbreaks in Minas Gerais, Brazil. Intl J Infect Dis 12:410–5.

Wang X, Li G, Xia X, Yang B, Xi M, Meng J. 2014. Antimicrobial Susceptibility and Molecular Typing of Methicillin-Resistant Staphylococcus aureus in Retail Foods in Shaanxi, China. Foodborne Pathog Dis 11:281–6.
Wang Y, He T, Schwarz S, Zhao Q, Shen Z, Wu C, Shen J. 2013. Multidrug resistance gene *cfr* in methicillin-resistant coagulase-negative staphylococci from chickens, ducks, and pigs in China. Intl J Med Microbiol 303:84–7.
Waters AE, Contente-Cuomo T, Buchhagen J, Liu CM, Watson L, Pearce K, Foster JT, Bowers J, Driebe EM, Engelthaler DM, Keim PS, Price LB. 2011. Multidrug-resistant *Staphylococcus aureus* in US meat and poultry. Clin Infect Dis 52:1227–30.
Xu B, Liu L, Liu L, Li X, Li X, Wang X. 2012. A multiplex PCR assay for the rapid and sensitive detection of methicillin-resistant *Staphylococcus aureus* and simultaneous discrimination of *Staphylococcus aureus* from coagulase-negative staphylococci. J Food Sci 77: M638–42.
Zhang Y, Agidi S, LeJeune JT. 2009. Diversity of staphylococcal cassette chromosome in coagulase-negative staphylococci from animal sources. J Appl Microbiol 107:1375–83.