Antibiotic Resistance Gene Profiles of *Staphylococcus aureus* Isolated From Foods of Animal Origin

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How to Cite This Article

Hizlisoy H, Ertas Onmaz N, Karadal F, Al S, Yildirim Y, Gonulalan Z, Kilic H: Antibiotic resistance gene profiles of *Staphylococcus aureus* isolated from foods of animal origin. *Kafkas Univ Vet Fak Derg*. 24 (2): 243-249, 2018. DOI: 10.9775/kvfd.2017.18772

Abstract

In this study, the investigation of the antibiotic resistance gene profiles of *Staphylococcus aureus* isolates from foods of animal origin was aimed. Totally, 95 *S. aureus* strains, obtained during a period between 2009 and 2012, from culture collection of the Food Hygiene and Technology Laboratory, were examined. The isolates were confirmed by phenotypic tests and PCR. The antibiotic susceptibilities of the isolates were analyzed by disc diffusion method and the minimal inhibition concentrations of the antibiotics were determined by E test. PCR were also utilized for determining the presence of resistance genes including *blaZ*, *ermA*, *ermC*, *tetK*, *tetM*, mecA, VanA, VanB, VatA, VatB and aacA-aphD genes. Resistance to penicillin, tetracycline, vancomycin, erythromycin, cefoxitin, gentamycin and quinupristin-dalfopristin were evident as 81.1%, 28.4%, 18.9%, 17.9%, 9.4%, 9.4% and 3.2% respectively. E test results were compatible with the disc diffusion method. Multidrug resistance was observed from 29.5% of *S. aureus* isolates. Positive compatibility was observed between conventional methods and PCR for the resistance of the isolates, except for vancomycin. In addition, all of the tested isolates found to include a resistance gene for at least one antibiotic. In conclusion, more efficient interventions must be followed to control the redundant use of antibiotics in veterinary practice. Furthermore, appropriate control measures are needed to be implemented to reduce contamination and the spread of multiresistant *S. aureus* strains.

Keywords: Antibiotic resistance, Animal origin foods, Multidrug resistance, Resistance genes, Staphylococcus aureus

Hayvansal Gidaldardan İzole Edilen *Staphylococcus aureus*’ların Antibiyotik Dirençlilik Gen Profilleri

Öz

Bu çalışmada hayvansal gidaldardan izole edilen *Staphylococcus aureus* izolatlarının antibiotik dirençlilik ve ilgili gen profilinin araştırılması amaçlanmıştır. Besin Hijyen ve Teknoloji laboratuvarımızda yer alan kültür koleksiyonumuzda, 2009 ve 2012 yılları arasında toplanan 95 *S. aureus* izolatları, fenotipik testler ve PCR ile değerlendirilmiştir. Izolatların antibiotiklere duyarlılıkları disk difüzyon testi ile, minimal inhibitör konsantrasyonları ise E test ile incelenmiştir. Ayrıca çalışmada PCR, *blaZ*, *ermA*, *ermC*, *tetK*, *tetM*, mecA, VanA, VanB, VatA, VatB ve aacA-aphD genlerinin varlığını tespit etmek amacıyla kullanılmıştır. Testler sonunda penisilin, tetratsiklin, vankomisin, eritromisin, sefoksitin, gentaminsin ve quinupristin-dalfopristin antibiyotiklerine karşı direnç oranları sırasıyla %81.1, %28.4, %18.9, %17.9, %9.4, %9.4 ve %3.2 olarak bulunmuştur. E test sonuçları ile disk difüzyon bulguları birbiri ile uyumlu bulunmaktadır. *S. aureus* izolatlarının çoğunluğa dirençli polimorfnl %29.5 bulunmuştur. Vankomisin dışında izolatların antibiotiklere dirençlilikleri hussusunda PCR ile konvansiyonel metotlar arasında uyum tespit edilmiştir. Ayrıca test edilen tüm izolatların en az bir antibiotik için gen bulunuridad göstermişdir. Sonuç olarak, veteriner pratikte gerekhus antibiyotik kullanımın kontrol etmek için daha etkin uygulamalar izlenmelidir ve çoğunluğa dirençli sahip *S. aureus* izolatlarının yayılmasını ve buluşlarını azaltmak için uygun kontrol önlemlerinin uygulanması gerekmektedir.

Anahtar sözcükler: Antibiyotik direnci, Çoğunluğa dirençli, Direnç genleri, Hayvansal gıdalar, Staphylococcus aureus
INTRODUCTION

*Staphylococcus aureus* is a pathogen which is usually incriminated for a various kind of diseases ranging from skin infections to serious diseases, such as cellulitis, endocarditis and bacteremia [1-3]. Being commonly found on the skin and mucosae of food producing animal reservoirs, *S. aureus* is one of the most important worldwide food poisoning agents [4].

*S. aureus* developed resistance shortly after the introduction of penicillin in 1940s, followed by methicillin resistance in 1961. Hitherto, numbers of publication have been reported concerning the resistance of *S. aureus* isolates to diverse spectrum of antibiotics [4,5]. *S. aureus* is causing a concern due to its ability to become resistant to antibiotics via acquired by horizontal transfer of genes and chromosomal mutation [5]. Moreover, enzymatic drug modifications, changes in the target sites and membrane bound efflux pumps are additional mechanisms for the bacteria to combat against antimicrobial agents [6].

Multidrug-resistant (MDR) *S. aureus* isolates are of great public concern as it is possible that these resistant organisms can be transferred to humans via the food chain which in turn leads a limited choice for their control [7]. MDR *S. aureus* strains have frequently been reported from meat, dairy, fishery, poultry, eggs and salads [8-13].

The aim of this study was to characterize the recovered *S. aureus* isolates obtained from various foods of animal origin for their antimicrobial resistance by conventional and molecular methods.

MATERIAL and METHODS

**Bacterial Strains**

A total of 95 *S. aureus* strains, collected during a period between 2009 and 2012, were obtained from culture collection of the Food Hygiene and Technology Laboratory, Faculty of Veterinary Medicine, Erciyes University, Kayseri, Turkey. These isolates were recovered from raw milk (n=12), sheep cheese (n=12), dairy dessert (n=11), chicken meat (n=12), pastrami (n=12), sausage (n=12), salami (n=12) and soudjouk (n=12). All isolates were confirmed as *S. aureus* by Gram staining, catalase activity, tube coagulase test and nuc gene amplification [14,15].

**Antibiotic Susceptibility Test**

In this study, antibiotic susceptibility testing was performed by disc diffusion method. The antibiotics investigated were (Oxoid, UK) gentamicin (CN, 10 μg), erythromycin (E, 15 μg), tetracycline (TE, 30 μg), penicillin G (P, 10 IU), cefoxitin (FOX, 30 μg), vancomycin (VA, 30 μg) and quinupristin-dalfopristin (QD, 15 μg). The minimal inhibition concentrations (MICs) of all above mentioned antibiotics for each isolates were determined by the E test (Oxoid, UK; Biomerieux, France and Liofil chem, Italy). The disc diffusion test results and MICs were interpreted using the criteria published by Clinical and Laboratory Standards Institute [16]. *Escherichia coli* ATCC 25922, *S. aureus* ATCC 29213 (for microdilution method) and *S. aureus* ATCC 25923 (for disc diffusion method) were included as quality control strains in each run. The multidrug resistance was reported whether the single isolate is resistant to three or more unique antimicrobial classes.

**DNA Extraction**

Total genomic DNA was extracted from overnight-grown at 35°C *S. aureus* cultures in Brain Heart Infusion Broth (Acumedia, 7116A, USA) with the Genomic DNA Purification Kit (InstaGene™ Matrix, BIO-RAD, USA) as specified by the manufacturer.

**Amplification of Nuc Gene**

PCR assay conditions were used according to Cremonesi et al. [14] and carried out in a reaction mixture of 50 μL final volume containing, 5 μL of template DNA, 5 μL of 10x PCR buffer (Vivantis, Chino, CA), 2 U Taq polymerase (Vivantis), 2 mM dNTP mix (Vivantis), 1.5 mM MgCl2, (Vivantis) and 30 mM of the primer pairs of each primer (NUC-F166 and NUC-R565). PCR conditions were: 5 min at 94°C for initial denaturation, 30 cycles of 1 min at 94°C, 1 min at 56°C for annealing and 1 min at 68°C for extension. The final extension was achieved 7 min at 72°C (Techne TC-512, Keison Products, Chelmsford, UK).

**Detection of Selected Resistance Genes by PCR**

The *blaZ*, *mecA*, *aacA-aphD*, *ermA*, *ermC*, *vanA*, *vanB*, tetK, tetM, vatA and vatB specific primer pairs were used for the amplifications of antibiotic resistance genes (Table 1). PCR amplifications were performed with 50 μL PCR reaction mixture containing 5 μL of template DNA, 1x PCR Buffer (200 mM TrisHCl (pH 8.4), 500 mM KCl), 10 pmol of each primer, 200 μM dNTPs, 1.5 mM MgCl2, and 2.5 U Taq polymerase. The thermal cycling protocol for PCR was comprised initial denaturation at 94°C for 3 min, followed by 30 cycles of amplification with 94°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 30 s with a final extension of 4 min at 72°C (Techne TC-512, UK) for *aacA-aphD*, *ermA*, *ermC*, tetK, tetM, vatA and vatB genes [17].

For *blaZ* gene, an initial step of 5 min at 94°C was followed by 35 cycles of 1 min at 94°C, 1 min at 54°C and 1 min at 72°C, and a final step at 72°C for 10 min [18]. For the amplification of *mecA* gene, PCR reaction included an initial step at 94°C for 5 min followed by 35 cycles of 92°C for 2 min, 55°C for 2 min, and 72°C for 1 min with a final extension step at 72°C for 7 min [19]. For *vanA* and *vanB* genes an initial denaturation step at 94°C for 2 min; followed by 30 cycles of 94°C for 1, 54°C for 1 min
and 72°C for 1 min and final extension at 72°C for 10 min were done. Amplification products were visualized by electrophoresis in 1.5% agarose gel (100 V for 40 min, EC250-90, Thermo, USA).

**RESULTS**

In the antibiotic susceptibility test, resistance to penicillin G (P), tetracycline (TE), vancomycin (VA) and erythromycin (E) was evident in 81.1%, 28.4%, 18.9% and 17.9% of the isolates used in this study, respectively. A small percentage of isolates demonstrated resistance to gentamycin (CN), cefoxitin (FOX) and quinupristin-dalfopristin (QD) with the rate of 9.4%, 9.4% and 3.2% respectively (Table 2).

The results of E test were compatible with that of disc diffusion method. In the E test, the ranges of MIC values were; gentamycin 0.06-256 µg/mL, erythromycin 0.03-128 µg/mL, cefoxitin 0.5-128 µg/mL, vancomycin 0.03-256 µg/mL, tetracycline 0.03-256 µg/mL, penicillin G 0.015-16 µg/mL and quinupristin-dalfopristin 0.008-32 µg/mL.

**Multidrug Resistance**

In this study, 28 (29.5%) of S. aureus isolates were resistant to three or more antibacterial classes. The majority of MDR S. aureus isolates (22.1%) were found resistant to three antimicrobials whereas resistances to four and six antimicrobials were observed from 5.2% and 2.1% of the isolates respectively (Table 3).

**Relationship Between Antibiotic Susceptibility Testing and PCRs**

In this study, the antibiotic susceptibility test results and the presence of antibiotic resistance genes of 95 S. aureus isolates were compared (Table 4). All nine gentamycin and cefoxitin resistant isolates (100%) were carried aacA-aphD and mecA genes, respectively. Nine (53%) and eight (47%) of 17 erythromycin resistant isolates were shown to have termA and ermC genes, respectively. Seventy of 77 (91%) penicillin G resistant isolates carried blaZ gene. Although none of the isolates were found having vanA gene, two of 18 vancomycin resistant isolates were carried vanB gene. Twelve (44%) of 27 tetracycline resistant isolates had only tetK gene, 10 (37%) had only tetM gene while five (19%) had both tetK and tetM genes. All of three quinupristin-dalfopristin resistant isolates were found to harbour vanB gene (Table 4).

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**Table 1. The oligonucleotide sequence and predicted sizes used in the PCR**

| Primer | Target Gen | Name | Sequence (5’-3’) | Product Size (bp) | Anneling Temperature | Reference |
|--------|------------|------|------------------|-------------------|----------------------|-----------|
| Nuc-F166 | nuc | | AGT TCA GCA AAT GCA TCA CA | 400 | 56°C | [14] |
| Nuc-R565 | aacA-aphD | Gentamycin | TAG CCA AGC CTT GAC GAA CT | | | |
| aacA-aphD-1 | | | TAA TCC AAG AGC AAT AAC GGC | 227 | 55°C | [17] |
| aacA-aphD-2 | | | GCC ACA TCA TCA TAA CCA CTA | | | |
| tetK-1 | tetK | Tetracycline | GTA GCG ACA ATA GGT AAT AGT | 360 | 55°C | [17] |
| tetK-2 | | | GTA GTG ACA ATA AAC CTC CTA | | | |
| tetM-1 | tetM | | AGT GGA GCG ATT ACA GAA | 158 | 55°C | |
| tetM-2 | | | CAT ATG TCC TGG GGT GTG TA | | | |
| vatA-1 | vatA | Quinupristin-Dalfopristin | TGG TCC CGG AAC ACC ATT TAT | 268 | 55°C | [17] |
| vatA-2 | | | TCC ACC GAC AAT AGA ATA GGG | | | |
| vatB-1 | vatB | | GCT GGG AAT TCA GTT GCT ACA | 136 | 55°C | [17] |
| vatB-2 | | | CTG ACC AAT CCC ATT TTA | | | |
| blaZ-1 | blaZ | Penicillin G | TTA AAG TCT TAC CGA AAG CAG | 377 | 54°C | [18] |
| blaZ-2 | | | TAA GAG ATT TGC CTA TGC TT | | | |
| ermA-1 | ermA | Erythromycin | AAG CCG TAA ACC CCT CTG A | 190 | 55°C | |
| ermA-2 | | | TTC GCA AAT CCC TCT TCA AC | | | |
| ermC-1 | ermC | | AAT GGT CAA TCC CTG CAT GT | 299 | | |
| ermC-2 | | | TAA TCG TGG AAT AGG GTT TTG | | | |
| mecA-1 | mecA | Cefoxitin | ACTGCTATCCACCCCCAAA | 163 | 55°C | [19] |
| mecA-2 | | | CTTGGTGAAGTGTAAATCCTGG | | | |
| vanA-1 | vanA | Vancomycin | GTAGGCTGGGATATTCAAGAC | 231 | 54°C | [20] |
| vanA-2 | | | CGATTCAATTGCTGATTTCAA | | | |
| vanB-1 | vanB | | GTAGGCTGGGATATTCAAGC | 330 | 54°C | |
| vanB-2 | | | GCGGACAACTCAATGCTCCTC | | | |
**Antibiotic Resistance Profiles of S. aureus**

**DISCUSSION**

*S. aureus* is increasingly developing resistance to formerly effective antimicrobial agents [5,21]. Food is an important vehicle for the transfer of resistant *S. aureus* strains from animals to humans and antimicrobial resistant strains are emerging as a global problem. MDR *S. aureus* isolated from different foods exhibited a various distribution throughout the world [22,23].

In this study, remarkable levels (3.2-81.1%) of resistance to antibiotics which were all broad-spectrum, P, VA, TE, FOX, E, CN and QD (Table 2) were found. These results are not surprising for the antibiotics mentioned above as they are commonly used in both veterinary and human medicine.

In the present study, the resistance of gentamycin was found to be 9.4%. On the contrary, Groves et al. [24] and Gomes et al. [25] found higher results at rates of 83.4% and 26.8% from human isolates than that of ours. All gentamycin-resistant strains had the *aac*\(_A\)−*aph*\(_D\) gene similar to Strommenger et al. [17] (in Germany), Adwan et al. [26] (in Palestine) and Groves et al. [24] (in Australia) from clinical human isolates. In addition, Gomes et al. [25] (in Brazil) and Oksuz et al. [27] (in Turkey) reported that *aac*\(_A\)−*aph*\(_D\) gene was present in 43% and 95% of gentamycin-resistant isolates from clinical samples, respectively.

Erythromycin resistance was determined at rate of 17.9%. Moreover, we found nine (53%) *ermA* and eight (47%) *ermC* positive strains out of 17 erythromycin resistant isolates. In contrast, the frequency of erythromycin resistance (40%) was reported relatively high and related genes (*ermA: 22.8% and *ermC: 17.1%*) was reported relatively low by Zmantar et al. [28] in *S. aureus* strains isolated from auricular infections in Tunisia. Moreover, Gao et al. [29] have also reported that erythromycin-resistant was evaluated as 44% and all erythromycin-resistant isolates to carry *ermA* and *ermC* genes from milk samples. In addition, Adwan et al. [26] stated that the presence of *ermA* and *ermC* genes among MRSA isolates were 30.9% and 74.5% from different clinical samples in Palestine. These disagreements

| Antibiotics       | Target Gen | Resistance (%) | Gene Frequency (%) |
|-------------------|------------|----------------|--------------------|
| Gentamycin        | *aacA*−*aphD* | 9 (9.4) | 9/9 (100) |
| Tetracycline      | *tetK*    | 27 (28.4) | 12/27 (44) |
|                   | *tetM*    | - | 10/27 (37) |
|                   | *tetK + tetM* | 5/27 (19) | |
| Quinupristin-Dalfopristin | *vatA* | 3 (3.2) | - |
| Penicillin G      | *blaZ*    | 77 (81.1) | 70/77 (91) |
| Erythromycin      | *ermA*    | 17 (17.9) | 9/17 (53) |
|                   | *ermC*    | - | 8/17 (47) |
| Cefoxitin         | *meCA*    | 9 (9.4) | 9/9 (100) |
| Vancomycin        | *vanA*    | 18 (18.9) | 2/18 (11) |

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|                   | *tetM*    | - | 10/27 (37) |
|                   | *tetK + tetM* | 5/27 (19) | |
| Quinupristin-Dalfopristin | *vatA* | 3 (3.2) | - |
| Penicillin G      | *blaZ*    | 77 (81.1) | 70/77 (91) |
| Erythromycin      | *ermA*    | 17 (17.9) | 9/17 (53) |
|                   | *ermC*    | - | 8/17 (47) |
| Cefoxitin         | *meCA*    | 9 (9.4) | 9/9 (100) |
| Vancomycin        | *vanA*    | 18 (18.9) | 2/18 (11) |
might be due to the source of isolates and mutation in the genes located in coding or promoter region of the PCR-detected genes or genes in small plasmids, seldomly lost. Moreover, the ermC gene encoding for ERM resistance is located on a small plasmid was reported by Fluit et al.[30]. Some reports indicated ermA to be more dominant factor in *S. aureus* infections [31,32].

In our study, the rate of penicillin G resistance was detected at 81.1%, the blaZ gene was present in 70 (91%) of 77 in resistant strains. However, Goa et al.[29] have reported penicillin resistance was 29%, the blaZ gene was present in 81% of them. Moreover, Yang et al.[33] reported that blaZ gene was detected in 94.6% of 37 penicillin resistant *S. aureus* isolates isolated from bovine mastitis.

Regarding the methicillin-resistance and related gene (*mecA*), was detected in 9.4% and 100% of *S. aureus* isolates in this study, respectively. MRSA strains were detected by Pehlivanoglu and Yardimci[34], by Türütoğlu et al.[35] and by Sareyyupoglu et al.[36] in Turkey, by Kumar et al.[37] in India, by Moon et al.[38] in Korea, by Fessler et al.[39] in German. The rates of having *mecA* gene in milk samples have been reported as 77%, 61.9%, 57%, 16.7% and 3.1% by Kumar et al.[37] in India, by Moon et al.[38] in Korea, by Pehlivanoglu and Yardimci[34], by Türütoğlu et al.[35] and by Sareyyupoglu et al.[36] in Turkey. Fessler et al.[39] have also reported that presence of *mecA* gene that has been observed is 37.2% in food and food products of poultry origin in German. The high rate of *mecA* gene obtained in this study, might be due to the horizontal transmission of this gene between the strains found together in food processing environment [40].

In our study, about 18.9% of the isolates were found to be resistant to vancomycin and 11% of the vancomycin resistant isolates harbored vanB gene. Our results demonstrated that the presence of vancomycin resistance gene (*vanB*) in *S. aureus* strains isolated from food in Kayseri is of utmost importance. Therefore, further studies are necessary to keep the emergence and spread of these isolates. Similarly, Abulreesh [41] detected in 14% of 51 *S. aureus* isolates were resistant to vancomycin. Moreover, Pehlivanoglu and Yardimci [34], Turkylmaz et al.[42] and McMillan et al.[43] reported that in milk samples, all strains were susceptible to vancomycin by disk diffusion test. Contrary to our study, Simeoni et al.[44] (from swine meat commodities in Italy), Pehlivanoglu and Yardimci[34] (from milk samples in Turkey), McMillan et al.[43] (from raw milk sources in Australia), and Abulreesh [41] (from potable water samples in Saudi Arabia) reported that none of the *S. aureus* isolates were found to be positive for *vanA/vanB* gene by PCR. The reasons for the different results might be explained with changes in biosynthesis of cell wall of the resistant strains [34,45,46].

The rate of quinupristin-dalfopristin resistant isolates were 3.2% and all resistant isolates had vatB gene in this study. Contrary, Adwan et al.[47] noted the prevalence of vatA 1.8% and vatB gene was not found among MRSA isolates respectively. Fessler et al.[39] reported that from 86 samples originated from food and food products of poultry origin, four MRSA isolates were found to be resistant to quinupristin-dalfopristin, however 11 isolates were detected as intermediate. However, they detected that all isolates tested were negative for vatA or vatB gene.

In our study, no resistance genes were detected in some resistant isolates, which agreed with the findings of Gao et al.[44]. The phenotypic resistance may be caused by other resistance mechanisms, including biofilm formation, rather than gene acquisition [48]. The resistance mechanisms to antibiotics are so complicated that the presence or absence of a particular resistance gene cannot be regarded as a certain evidence for the isolate to be resistant or sensitive to the related antimicrobial agents [49].

In this study, a positive relation was observed between phenotypic and PCR results for the determination of antimicrobial resistance which is in agreement with Gao et al.[29] and Saadat et al.[50]. However, Zmantar et al.[28] and Salih et al.[51] reported no correlation between phenotypic and PCR methods.

According to MDR, 28 (29.5%) of *S. aureus* isolates were found resistant to three or more antimicrobial agents (Table 3). Attention should be given to the fact that all of the isolates demonstrated resistance to more than one antibiotic. MDR has also been reported by Elbargisy et al.[33], Fan et al.[11], and Waters [53], at the rate of 17.1%, 66.3% and 52%, respectively. These findings might be due to the abundant use of antimicrobials for farm animals especially in countries where antibiotic use is not well regulated [11]. Large presence of MDR *S. aureus* in foods causes high risk of infections and a possible transmission of resistances to other pathogens which could lead to failure of antibiotic treatments.

In conclusion, spreading of MDR *S. aureus* via foodstuffs is a potential hazard for public health and might result in difficulties to treat MDR-related diseases. These results suggest that the incidence of MDR *S. aureus* are steadily increasing and attention needs to be paid to decrease or eliminate the contamination of MDR *S. aureus*. Specified education programs should be supported to define the prudent antibiotic use besides clinical guidelines should be developed and put into practice.

REFERENCES

1. Brook I, Frazier EH: Clinical features and aerobic and anaerobic microbiological characteristics of cellulites. *Arch Surg*, 130, 786-792, 1995. DOI: 10.1001/archsurg.1995.01430070108024
2. Hoen B, Alla F, Selton-Suty C, Bégounet I, Bouvet A, Briançon S, Casalta JP, Danchin N, Delahaye F, Ettiene J, Le Moing V, Leport C, Mainardi JL, Ruymy R, Vandenfesc CJ: Changing profile of infective endocarditis: results of a 1-year survey in France. *JAMA*, 288, 75-81, 2002. DOI: 10.1001/jama.288.1.75
Antibiotic Resistance Profiles of S. aureus

3. Javaloyas M, Garcia-Somoza D, Gudiol F: Epidemiology and prognosis of bacteremia: A 10-years study in a community hospital. Scand J Infect Dis, 34, 436-441, 2002. DOI: 10.1080/03653540110080629

4. Gardete S, Tomasz A: Mechanisms of vancomycin resistance in Staphylococcus aureus. J Clin Invest, 124 (7): 2836-2840, 2014. DOI: 10.1172/JCI68834

5. Kazmierczak Z, Gorski A, Dąbrowska K: Facing antibiotic resistance: Staphylococcus aureus phases as a medical tool. Viruses, 6 (7): 2551-2570, 2014. DOI: 10.3390/v6072551

6. Chambers HF, Deleo FR: Waves of resistance: Staphylococcus aureus in the antibiotic era. Nat Rev Microbiol, 7 (9): 629-641, 2009. DOI: 10.1038/nrmicro2200

7. Pesavento G, Ducci B, Comodo N, Lo Nostro A: Antimicrobial resistance profile of Staphylococcus aureus isolates from raw meat: A research for methicillin resistant Staphylococcus aureus (MRSA). Food Control, 18, 196-200, 2007. DOI: 10.1016/j.foodcont.2005.09.013

8. Belo Sas, Buyukcanaz E, Sherwood JS, Stepan RM, Koslowsky RS: Characterization of Staphylococcus aureus from humans and a comparison with isolates of animal origin, in North Dakota, United States. PLoS One, 10 (10): e0140497, 2015. DOI: 10.1371/journal.pone.0140497

9. Akindilire MA, Babalola OO, Ateba CN: Detection of antibiotic resistant Staphylococcus aureus from milk: A public health implication. Int J Environ Res Public Health, 12, 10254-10275, 2015. DOI: 10.3390/ijerph120910254

10. Visnuvinayagam S, Joseph TC, Murugadas V, Chakrabarti R, Bhattacharya M: Detection of antibiotic resistance in Staphylococcus aureus strains isolated from auricular membranes. J Med Sci, 19 (5): 299-305, 2005. DOI: 10.1016/j.jms.2004.09.002

11. Smith EM, Needs PF, Manley G, Green LE: Global distribution and diversity of ovine-associated Staphylococcus aureus. Infect Genet Evol, 22 (100): 298-213, 2014. DOI: 10.1016/j.meegid.2013.09.008

12. Giffard P, Abraham S, Trott DJ: Molecular epidemiology of methicillin-resistant Staphylococcus aureus isolated from Australian veterinarians. PLoS One, 11 (1): 0146034, 2016. DOI: 10.1371/journal.pone.0146034

13. Gomes MMF, Bomfim MRQ, Trindade MVJ, Farias LM, Auxiliadora M, Carvalho MAR, Serufo JC, Santos SG: Potential spread of methicillin-resistant Staphylococcus aureus recovered from patients with bloodstream infection. Chem. Open Access, 4, 149, 2015. DOI: 10.4172/2167-7700.1000149

14. Adwan GK, Naser J, Alaa A: Molecular detection of nine antibiotic resistance genes in methicillin resistant Staphylococcus aureus isolates. Rom Arch Microbiol Immunol, 73 (1-2): 9-18, 2014.

15. Oktuz L, Dupieux C, Tristan A, Bes M, Etienne J, Gurler N: The high diversity of MRSA clones detected in a University Hospital in Istanbul. Int J Med Sci, 10 (12): 1740-1745, 2013. DOI: 10.7152/ijms.6438

16. Bell JM, Paton JC, Turnidge J: Emergence of vancomycin resistant enterococci in Australia: phenotypic and genotypic characteristics of isolates. J Clin Microbiol, 36, 2187-2190, 1998.

17. Mayrhofer S, Paulsen P, Smulders FJ, Hilbert F: Antimicrobial resistance profile of five major food-borne pathogens isolated from beef, pork and poultry. Int J Food Microbiol, 97, 23-29, 2004. DOI: 10.1016/j.ijfoodmicro.2004.04.006

18. Argudin MA, Mendoza MC, Rodicio MR: Food poisoning and Staphylococcus aureus enterotoxins. Toxins, 2 (7): 1751-1773, 2010. DOI: 10.3390/toxins2071751

19. Pereira V, Lopes C, Castro A, Silva J, Giibs P, Teixeira P: Characterization for enterotoxin production, virulence factors, and antibiotic susceptibility of Staphylococcus aureus isolates from various foods in Portugal. Food Microbiol, 26, 278-282, 2009. DOI: 10.1016/j.fm.2008.12.008

20. Groves MD, Crouch B, Coombs GW, Jordan D, Pang S, Barton MD, Giffard P, Abraham S, Trott DJ: Molecular epidemiology of methicillin-resistant Staphylococcus aureus isolated from Australian veterinarians. PLoS One, 11 (1): 0146034, 2016. DOI: 10.1371/journal.pone.0146034

21. Martin J, Ferrari M, Liu XQ, Chen LB, Su JL, Han B: Development of multiplex polymerase chain reaction assay for rapid detection of Staphylococcus aureus and selected antibiotic resistance genes in bovine mastitic milk samples. J Vet Diagn Invest, 23 (5): 894-901, 2011. DOI: 10.1177/1040638710416964

22. Fluit ADC, Visser MR, Schmitz F: Molecular detection of anti-microbial resistance. Clin Microbiol Rev, 14, 836-871, 2001. DOI: 10.1128/CMR.14.4.836-871.2001

23. Lina G, Quaglia A, Revery ME, Leclercq R, Vandenesc F, Etienne J: Distribution of genes encoding resistance to macrolides, lincosamides, and streptogramins among staphylococci. Antimicrob Agents Chemother, 43 (5): 1062-1066, 1999.

24. Martinez F, Picard FJ, Lansac N, Menard C, Roy PH, Ouellette M, Bergeron MG: Correlation between the resistance genotype determined by multiplex PCR assays and the antibiotic susceptibility patterns of Staphylococcus aureus and Staphylococcus epidermidis. Antimicrob Agents Chemother, 44 (2): 231-238, 2000.

25. Yang F, Wang Q, Wang X, Wang L, Xiao M, Li X, Li H: Prevalence of blaZ gene and other virulence genes in penicillin-resistant Staphylococcus aureus isolated from bovine mastitis cases in Gansu, China. Turk J Vet Anim Sci, 39 (5): 634-636, 2015. DOI: 10.3906/vet-1504-81

26. Pehlivanoglu F, Yardimci H: Molecular detection of antibiotic resistance genes in methicillin resistant Staphylococcus aureus recovered from patients with bloodstream infection. Folia Microbiol, 53 (4): 357-362, 2008. DOI: 10.1007/s12223-008-0055-5

27. Bell JM, Paton JC, Turnidge J: Emergence of vancomycin resistant enterococci in Australia: phenotypic and genotypic characteristics of isolates. J Clin Microbiol, 36, 2187-2190, 1998.

28. Mayrhofer S, Paulsen P, Smulders FJ, Hilbert F: Antimicrobial resistance profile of five major food-borne pathogens isolated from beef, pork and poultry. Int J Food Microbiol, 97, 23-29, 2004. DOI: 10.1016/j.ijfoodmicro.2004.04.006

29. Argudin MA, Mendoza MC, Rodicio MR: Food poisoning and Staphylococcus aureus enterotoxins. Toxins, 2 (7): 1751-1773, 2010. DOI: 10.3390/toxins2071751

30. Pereira V, Lopes C, Castro A, Silva J, Giibs P, Teixeira P: Characterization for enterotoxin production, virulence factors, and antibiotic susceptibility of Staphylococcus aureus isolates from various foods in Portugal. Food Microbiol, 26, 278-282, 2009. DOI: 10.1016/j.fm.2008.12.008
37. Kumar R, Yadav BR, Singh RS: Genetic determinants of antibiotic resistance in Staphylococcus aureus isolates from milk of mastitic crossbred cattle. Curr Microbiol, 60 (5): 379-386, 2010. DOI: 10.1007/s00284-009-9553-1

38. Moon JS, Lee AR, Kang HM, Lee ES, Kim MN, Paik YH, Park YH, Joo YS, Koo HC: Phenotypic and genetic antibiogram of methicillin-resistant staphylococci isolated from bovine mastitis in Korea. J Dairy Sci, 90, 1176-1185, 2007. DOI: 10.3168/jds.S0022-0302(07)71604-1

39. Fessler AT, Kadlec K, Hassel M, Hauschild T, Eidam C, Ehricht R, Schwarz S: Characterization of methicillin-resistant Staphylococcus aureus isolates from food and food products of poultry origin in Germany. Appl Environ Microbiol, 77 (20): 7151-7157, 2011. DOI: 10.1128/AEM.00561-11

40. Chambers HF: Methicillin resistance in staphylococci: Molecular and biochemical basis and clinical implications. Clin Microbiol Rev, 10, 781-791, 1997.

41. Abulreesh HH: Absence of vancomycin-resistance genes in Staphylococcus aureus isolated from potable water. J Public Health Epidemiol, 6 (2): 70-75, 2014. DOI: 10.5897/JPHE2013.0606

42. Turkylmaz S, Tekbiyik S, Oryasin E, Bozdogan B: Molecular epidemiology and antimicrobial resistance mechanisms of methicillin resistant Staphylococcus aureus isolated from bovine milk. Zoonoses Public Health, 57, 197-203, 2010. DOI: 10.1111/j.1863-2378.2009.01257.x

43. McMillan K, Moore SC, McAuley CM, Fegan N, Fox EM: Characterization of Staphylococcus aureus isolates from raw milk sources in Victoria, Australia. BMC Microbiol, 16, 169, 2016. DOI: 10.1186/s12866-016-0789-1

44. Simeoni D, Rizzotti L, Cocconcelli P, Gazzola S, Dellaglio F, Torriani S: Antibiotic resistance genes and identification of staphylococci collected from the production chain of swine meat commodities. Food Microbiol, 25 (1): 196-201, 2008. DOI: 10.1016/j.fm.2007.09.004

45. Geisel R, Schmitza FJ, Thomasa BL, Bernsa G, Zetschec O, Ulrichc B, Flitb AC, Labischinskyd H, Wittee W: Emergence of heterogeneous intermediate vancomycin resistance in Staphylococcus aureus isolates in the Düsseldorf area. J Antimicrob Chemother, 43, 846-848, 1999. DOI: 10.1093/jac/43.6.846

46. Hiramoto K: Vancomycin-resistant S. aureus: A new model of antibiotic resistance. Lancet Infect Dis, 1, 147-155, 2001. DOI: 10.1016/S1473-3099(01)00091-3

47. Gao J, Yu FQ, Luo LP, He JZ, Hou RG, Zhang HQ, Li SM, Su JL, Han B: Antibiotic resistance of Streptococcus agalactiae from cows with mastitis. Vet J, 194, 423-424, 2012. DOI: 10.1016/j.tvjl.2012.04.020

48. Croes S, Deurenb erg RH, Boumans MLL, Beisser PS, Neef C, Stobberingh EE: Staphylococcus aureus biofilm formation at the physiologic glucose concentration depends on the S. aureus lineage. BMC Microbiol, 9, 229, 2009. DOI: 10.1186/1471-2180-9-229

49. Gow SP, Waldner CL, Harel J, Boerlin P: Associations between antimicrobial resistance genes in fecal generic Escherichia coli isolates from cow-calf herds in western Canada. Appl Environ Microbiol, 74 (12): 3658-3666, 2008. DOI: 10.1128/AEM.02505-07

50. Saadat S, Solhjoo K, Norooz-Nejad MJ, Kazemi A: vanA and vanB positive vancomycin-resistant Staphylococcus aureus among clinical isolates in Shiraz, South of Iran. Oman Med J, 29 (5): 335, 2014. DOI: 10.5001/omj.2014.90

51. Salih MA, Abdalla AM, Masri MAR: Detection of erythromycin resistance genes erm (A), erm (B), erm (C) and msr (A) in Staphylococcus Nasal carriers in Khartoum State. Nature Sci, 12 (4): 106-110, 2014.

52. Elbargisy RM, Rizk DE, Abdel-Rahman SH: Toxin gene profile and antibiotic resistance of Staphylococcus aureus isolated from clinical and food samples in Egypt. African J Microbiol Res, 10 (13): 428-437, 2016. DOI: 10.5897/AJMR2015.7927

53. Waters AE, Contente-Cuomo T, Buchhagen T, Liu CM, Watson L, Pearce K, Foster JT, Bowers J, Driebe EM, Engelthaler DM, Keim PS, Price LB: Multidrug-resistant Staphylococcus aureus in US meat and poultry. Clin Infect Dis, 52 (10): 1227-1230, 2011. DOI: 10.1093/cid/cir181