

Molecular Characterization and Genotypic and Genotypic Evaluation of Antibiotic Resistance of Methicillin Resistant-staphylococcus Aureus Isolated From Raw Meat

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Data Article

Keywords: Methicillin-resistant Staphylococcus aureus, Raw meat, Panton-Valentine Leukocidin, SCCmec types, Antimicrobial resistance properties.

DOI: <https://doi.org/10.21203/rs.3.rs-82665/v1>

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Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) is considered to be one of the most important causes of food-borne diseases. The present investigation was done to assess the phenotypic and genotypic characterization and distribution of Staphylococcal cassette chromosome *mec* types and Panton–Valentine leukocidin gene in the MRSA strains isolated from raw meat samples. Six-hundred and eighty meat samples were collected and cultured. MRSA strains were subjected to disk diffusion and Polymerase Chain Reaction. One-hundred and thirty-five out of 680 (18.38%) raw meat samples were positive for *S. aureus*. Seventy-nine out of 125 (63.20%) *S. aureus* strains were determined as MRSA. Raw sheep meat samples (75%) had the highest prevalence of MRSA, while raw camel had the lowest (50%). Fifty-eight out of 79 (73.41%) MRSA strains harbored the *PVL* gene. *SCCmec IVa* (39.65%), *V* (22.41%) and *III* (10.34%) were the most commonly detected types in the MRSA strains. MRSA strains harbored the highest prevalence of resistance against penicillin (100%), tetracycline (100%), gentamicin (65.51%) and erythromycin (56.89%). *AadA1* (58.62%), *tetK* (56.89%), *msrA* (41.37%) and *vatA* (36.20%) were the most commonly detected antibiotic resistance genes. Simultaneous presence of *PVL* and antibiotic resistance genes in multi-drug resistant MRSA strains specifies significant public health problem.

Introduction

Staphylococcus aureus (*S. aureus*) is Gram-positive, catalase positive, and cocci-shaped bacterium typically originate from nose and respiratory tract and on the skin. It is mainly associated with nosocomial and community-acquired infections and foodborne diseases (Kadariya et al. 2014). *S. aureus* is a causative agents for about 241,000 food-borne illnesses per year in the United States (Scallan et al. 2011). Meat is one of the most important food stuffs related to the Staphylococcal food-borne diseases all around the world (Beneke et al. 2011; Hanson et al. 2011; Pu et al. 2009; Weese et al. 2010). High prevalence of *S. aureus* strains has also been reported in different types of meat samples (Hasanpour Dehkordi et al. 2017; Madahi et al. 2014; Momtaz et al. 2013; Safarpour Dehkordi et al. 2018; Safarpour Dehkordi et al. 2017a).

Staphylococcal foodborne diseases are primarily related to the emergence of antibiotic resistance (De Boer et al. 2009). It has been documented that about 50% of strains of this bacterium were considered to be methicillin-resistant *S. aureus* (MRSA) (De Boer et al. 2009; Johnson 2011; Morell and Balkin 2010; Shen et al. 2013). A survey which was conducted on the United States revealed an annual estimate of 94,000 MRSA infections with nearly 20% mortality rate (Klevens et al. 2007). Presence of certain antibiotic resistance genes such as *mecA* (methicillin), *linA* (lincosamides), *msrA* and *msrB* (macrolides), *vatA*, *vatB* and *vatC* (acetyl transferase genes and streptogramin A), *ermA*, *ermB* and *ermC* (macrolide–lincosamide–streptogramin B), *tetK* and *tetM* (tetracycline) and *aacA-D* (aminoglycosides) is one of the most important mechanisms for occurrence of antibiotic resistance (Argudin et al. 2010; Momtaz and Hafezi 2014). The gene for methicillin resistance, *mecA*, is carried on a 21- to 67-kb element, the staphylococcal chromosomal cassette *mec* (*SCCmec*), which integrates at a conserved location in the *S. aureus* genome (Shukla et al. 2012). *SCCmec* genetic element characterized by the presence of two essential genetic markers (the *mec* and the *ccr* gene complexes). *SCCmec* elements are classically classified into types I, II, III, IV and V according to the nature of the *mec* and *ccr* genes (Ferreira et al. 2013; Turlej et al. 2011). Type IV of the *SCCmec* genetic element is divided to IVa, IVb, IVc and IVd alleles (Ferreira et al. 2013; Turlej et al. 2011). The frequent recovery of staphylococcal isolates that produce leukocidal toxins from the cases of foodborne diseases and clinical infections, suggests that the Panton-Valentine Leukocidin (PVL) is a virulence marker with significant role in the pathogenicity of staphylococcal diseases (Ogata et al. 2012; Shrestha et al. 2014; Velasco et al. 2014).

Rendering the high importance of MRSA strains as a foodborne pathogen, the current research was done to study the prevalence rate, distribution of *SCCmec* types and *PVL* gene and antimicrobial resistance properties of the MRSA strains isolated from raw ruminant, poultry and fish meat samples in Iran.

Methods

Sampling procedure

From March to September 2017, a total of 680 raw meat samples including beef (n=150), goat (n=80), sheep (n=100), camel (n=60), chicken (n=80), turkey (n=50), quail (n=50), ostrich (n=50) and fish (n=60) were randomly purchased from butchers of several geographic regions of Iran. Samples were immediately transferred to laboratory in cooler with ice-packs.

Isolation and identification of S. aureus and MRSA strains

Each sample was aseptically weighed in an analytical balance and twenty-five grams were transferred into a sterile plastic bag. Then, 225 mL of buffered peptone water (Merck, Germany) was added and homogenized in a Stomacher Bagmixer 400W (Interscience, Saint-Nom, France) for two min. Five milliliter aliquot of the enriched homogenate was transferred into 50 mL Trypticase Soy Broth (TSB, Merck, Germany) supplemented with 10% NaCl and 1% sodium pyruvate. After incubation at 35 °C for 18 h, a loopful of the culture was plated onto Baird-Parker agar supplemented with egg yolk tellurite emulsion (Merck, Germany) and incubated overnight at 37 °C. Black shiny colonies surrounded by 2 to 5-mm clear zones were further identified on the basis of some biochemical tests (Fijałkowski et al. 2016).

Cefoxitin (30 µg) and oxacillin (1 µg) susceptibility tests were used to distinguish the MRSA strains from *S. aureus* isolates of meat samples. All tests were performed using the guidelines of the Clinical and Laboratory Standards Institute (CLSI) (CLSI 2007). MRSA isolates were identified another time using the Polymerase Chain Reaction (PCR)-based amplification of *mecA* gene (Safarpour Dehkordi et al. 2017a). MRSA strains were sub-cultured on TSB (Merck, Germany) and further incubated for 48 h at 37 °C. Genomic Deoxyribonucleic acid (DNA) was extracted from bacterial colonies using the DNA extraction kit (Thermo Fisher Scientific, Germany) according to manufacturer's instruction. After extraction, the DNA samples were quantified (NanoDrop, Thermo Scientific, Waltham, MA, United States (USA)), their purity checked (A260/A280), and their concentrations adjusted to 50 nanogram per microliter (ng/µL).

Antibiotic resistance analysis

Pattern of antimicrobial resistance was studied using the simple disk diffusion technique. The Mueller–Hinton agar (Merck, Germany) medium was used for this purpose. Antibiotic resistance pattern of MRSA strains was studied against 10 commonly used antibiotic agents including penicillin (10 µg/disk), gentamicin (10 µg/disk), azithromycin (15 µg/disk), erythromycin (15 µg/disk), tetracycline (30 µg/disk), doxycycline (30 µg/disk), ciprofloxacin (5 µg/disk), clindamycin (2 µg/disk), trimethoprim-sulfamethoxazole (25 µg/disk), and chloramphenicol (30 µg/disk) (Oxoid, UK). Method was done according to the principles of CLSI guidelines (CLSI 2012). The diameter of the zone of inhibition produced by each antibiotic disc was measured and interpreted using the CLSI zone diameter interpretative standards (CLSI 2012). *S. aureus* American Type Culture Collection (ATCC) 25923 and *Escherichia coli* ATCC 25922 were used as quality control organism in antimicrobial susceptibility determination.

Detection of antibiotic resistance genes and SCCmec types

Table 1 represents the oligonucleotide primers and PCR conditions used for amplification of antibiotic resistance genes and *SCCmec* types amongst MRSA strains isolated from various types of raw meat (Lina et al. 1999; Strommenger et al. 2003; Zhang et al. 2005). Additional PCR method was used to detect the PVL gene amongst the MRSA strains isolated from different types of meat samples (Yamasaki et al. 2005). A programmable DNA thermo-cycler (Eppendorf Mastercycler 5330, Eppendorf-Nethel-Hinz GmbH, Hamburg, Germany) was used in all PCR reactions. All runs included a negative DNA control consisting of sterile PCR grade water (Thermo Fisher Scientific, Germany) and positive DNA control consisting of positive DNA of each target gene. Fifteen microliters of amplified PCR products were subjected to electrophoresis in a 2% agarose gel in 1× Tris-borate-Ethylenediamine tetraacetic acid (EDTA) (TBE) buffer at 90 V for 30-40 min, stained with SYBR Green (Thermo Fisher Scientific, Germany).

Statistical analysis

Statistical analysis was done using the Statistical Package for the Social Sciences (SPSS) 25.0 statistical software (SPSS Inc., Chicago, IL, USA). Chi-square test and Fisher's exact two-tailed test were used to assess any significant relationship for

prevalence of MRSA strains and their antibiotic resistance and molecular characters between different types of samples. *P* value <0.05 was considered as statistical significant level.

Results

Incidence of S. aureus, MRSA and PVL gene

Table 2 represents the distribution of *S. aureus*, MRSA strains and *PVL* gene in different types of raw meat samples. One-hundred and thirty-five out of 680 (18.38%) raw meat samples were positive for *S. aureus* strains. Raw sheep samples (28%) had the highest prevalence of *S. aureus* strains, while raw fish samples had the lowest (1.66%). Statistically significant difference was seen between type of samples and prevalence of *S. aureus* strains ($P < 0.05$). *MecA* gene was identified in all MRSA strains. Seventy-nine out of 125 (63.20%) *S. aureus* strains were determined as MRSA strains. There were no MRSA strains in fish samples. Raw sheep samples (75%) had the highest prevalence of MRSA strains, while raw camel meat samples had the lowest (50%). Statistically significant difference was also seen between type of samples and prevalence of MRSA strains ($P < 0.05$). Fifty-eight out of 79 (73.41%) MRSA strains harbored the *PVL* gene. MRSA strains isolated from raw sheep meat samples had the highest prevalence of the *PVL* gene (95.23%), while those of quail had the lowest (33.33%). Statistically significant difference was also seen between type of samples and distribution of the *PVL* gene ($P < 0.05$).

Distribution of SCCmec types

Table 3 represents the distribution of *SCCmec* types in MRSA strains isolated from various types of raw meat samples. *SCCmecIVa* (39.65%), *V* (22.41%) and *III* (10.34%) were the most commonly detected types in the MRSA strains isolated from various types of raw meat samples. Raw sheep meat samples had the highest and most diverse distribution of *SCCmec* types. Statistically significant difference was also seen between type of samples and distribution of the *SCCmec* types ($P < 0.05$).

Antibiotic resistance pattern of MRSA strains

Table 4 represents the antibiotic resistance pattern of MRSA strains isolated from various types of raw meat samples. MRSA strains harbored the highest prevalence of resistance against penicillin (100%), tetracycline (100%), gentamicin (65.51%), erythromycin (56.89%) and trimethoprim-sulfamethoxazole (55.17%). MRSA strains exhibited the lowest prevalence of resistance against chloramphenicol (25.86%) and clindamycin (29.31%). Statistically significant difference was seen in the prevalence of antibiotic resistance between different types of raw meat samples ($P < 0.05$).

Distribution of antibiotic resistance genes

Table 5 represents the distribution of antibiotic resistance genes amongst the MRSA strains isolated from various types of raw meat samples. *AadA1* (58.62%), *tetK* (56.89%), *msrA* (41.37%) and *vatA* (36.20%) were the most commonly detected antibiotic resistance genes amongst the MRSA strains isolated from various types of raw meat samples. Distribution of *vatC* (3.44%), *msrB* (10.34%) and *vatB* (12.06%) were lower than other detected antibiotic resistance genes. Statistically significant difference was seen in the distribution of antibiotic resistance genes between different types of raw meat samples ($P < 0.05$).

Discussion

MRSA was first identified in 1961, immediately after the introduction of methicillin in clinical settings (Barber 1961). MRSA is widely accepted to be the most significant multi-drug resistant human pathogen (Hasanpour Dehkordi et al. 2017; Madahi et al. 2014; Momtaz et al. 2013; Safarpour Dehkordi et al. 2018; Safarpour Dehkordi et al. 2017a).

The present research was done to assess the phenotypic and genotypic characterization of antibiotic resistance and study the distribution of *SCCmec* types and *PVL* gene in the MRSA strains isolated from ruminants, poultries and fish meat samples. Zero prevalence rate of MRSA in fish meat samples is may be due, in part, to the presence of specific primary bacterial flora in fish and low ability of *S. aureus* to compete. Low prevalence of MRSA strains in camel meat samples is may be due the fact that camel's slaughter is done in specific slaughterhouses with high hygienic conditions. Higher pH level of sheep and goat meat

than beef (Lazzaroni et al. 2007) is probably caused higher ability of *S. aureus* strains to growth and survival. Similar prevalence rate of MRSA strains was reported by Pu et al. (2009) (United States) (Pu et al. 2009), Huber et al. (2009) (Switzerland) (Huber et al. 2010), Lin et al. (2010) (Korea) (Lim et al. 2010) and Agwu Ulu et al. (2014) (Nigeria) (Agwu Ulu et al. 2014). Hasanpour Dehkordi et al. (2017) (Hasanpour Dehkordi et al. 2017) reported that the prevalence of MRSA strains in raw beef, sheep, goat and camel meat samples collected from Iran were 16.00%, 24.00%, 20.40% and 10.00%, respectively which was similar to our findings. Raw meat may contain MRSA as a result of carcass contamination during slaughtering. Bhargava et al. (2011) (Bhargava et al. 2011) reported that 65 out of 289 raw meat samples (22.5%) collected from Michigan, USA were positive for *S. aureus*: 32 beef (20.5%), 19 chicken (25.0%), and 14 turkey (24.6%). They showed that 6 samples, consisting of 2 beef (1.30%), 3 chickens (3.90%) and 1 turkey (1.70%), were positive for MRSA which was entirely lower than our results. Febler et al. (2011) (Febler et al. 2011) showed that the prevalence of MRSA strains in turkey, chicken, pork and beef samples were 35.30%, 16%, 15.20% and 10.60%, respectively. Additionally, prevalence of MRSA strains in raw meat samples collected from Turkey (Gundogan et al. 2005), Egypt (Karmi 2013), Germany (Richter et al. 2012) and Denmark (Tang et al. 2017) were 30%, 40.80%, 71.50% and 52.00%, respectively. Role of food handlers in transmission of MRSA strains was reported previously by Agwu Ulu et al. (2014) (Agwu Ulu et al. 2014).

High prevalence of *PVL* gene in the MRSA strains poses an important public health threat. As far as we know, the present study is the first report of detection of the *PVL* gene in the MRSA strains of beef, sheep and chicken meat samples. Previously published data reported that majority of *PVL*-positive *S. aureus* strains were associated with skin and soft tissue infections (Holmes et al. 2005). Thus, *PVL*-positive MRSA strains may originate from infected staffs and meat inspectors in slaughterhouses. Presence of the *PVL* gene in the *S. aureus* strains isolated from food samples has been reported previously (Abdalrahman et al. 2015; Holmes et al. 2005; Lozano et al. 2009).

SCCmec IV and *V* had the highest distribution in the MRSA isolates. MRSA is often sub-categorized as Health-care Associated MRSA (HA-MRSA) and Community Associated MRSA (CA-MRSA). CA-MRSA strains carry *SCCmec* type *IV* or *V*, whereas the majority of HA-MRSA strains carry *SCCmec* type *I*, *II* or *III* (Asghar 2014). Thus, majority of MRSA strains isolated from raw meat samples of the present investigation were CA-MRSA. However, some of them were originate from the hospital environment and are recognized as HA-MRSA. Higher prevalence of *SCCmec* types *IV* in beef and pork samples was also reported by Jackson et al. (2013) (Jackson et al. 2013). In a study which was conducted by Vossenkuhl et al. (2014) (Vossenkuhl et al. 2014) most of the MRSA strains of turkey meat samples carried *SCCmec V* (58.10–71.90%), followed by type *IVa* (19–27%) which was similar to our results. High prevalence of *SCCmec IV* and *V* in foods with animal origin have also been reported previously (Argudín et al. 2010; Bhargava et al. 2011; Kreausukon et al. 2012). Therefore, raw meat samples may be the sources of CA-MRSA with high prevalence of *SCCmec IV* and *V*.

MRSA strains harbored the highest prevalence of resistance against penicillin, tetracycline, gentamicin, erythromycin and trimethoprim-sulfamethoxazole antibiotic agents. Additionally, *aadA1*, *tetK*, *msrA* and *vata* were the most commonly detected antibiotic resistance genes. Thus, phenotypic characters of antibiotic resistance of MRSA strains were approved by the genotypic characterization. Resistance levels found in this study can explain by the indiscriminate use of antimicrobials in humans treating diseases, which is a worrying trend. Hasanpour Dehkordi et al. (2017) (Hasanpour Dehkordi et al. 2017) reported that MRSA strains isolated from raw meat samples had high prevalence of resistance against ampicillin (100%), ceftriaxone (80.00%), amoxicillin-clavulanic acid (50.00%), lincomycin (61.20%), tetracycline (55.00%), gatifloxacin (96.80%), minocycline (51.20%), cotrimoxazole (45.60%), clindamycin (54.30%), azithromycin (48.10%), erythromycin (37.50%), oxacillin (76.20%) and penicillin (100%) antibiotic agents. Momtaz et al. (2013) (Momtaz et al. 2013) reported that the *S. aureus* strains isolated from raw meat samples harbored the highest prevalence of resistance against tetracycline (97.50%), methicillin (75.60%), sulfamethoxazole (31.70%), trimethoprim (31.70%), streptomycin (31.70%), gentamicin (29.20%), enrofloxacin (28.00%), ampicillin (26.8%), chloramphenicol (20.70%), and cephalothin (17.00%) antibiotic agents. They also showed that the prevalence of *mecA*, *msrA*, *msrB*, *aacA-D*, *tetK* and *tetM* antibiotic resistance genes in the *S. aureus* strains were 82.92%, 34.14%, 47.56%, 39.02%, 52.43% and 46.34%, respectively. Shahraz et al. (2012) (Shahraz et al. 2012) revealed that prevalence of antibiotic resistance in the *S. aureus* strains isolated from meat products against methicillin, erythromycin, penicillin G, cefazolin, ciprofloxacin, vancomycin and amoxiclav antibiotic agents were 89.00%, 20.30%, 18.70%, 15.60%, 14.00%, 26.60% and 12.50%, respectively. Another similar study (Udo et al. 2009) revealed that the prevalence of resistance of *S. aureus* strains

against penicillin G, tetracycline, erythromycin, clindamycin, trimethoprim, kanamycin, streptomycin and ciprofloxacin were 82%, 19%, 2.50%, 2 %, 7.50%, 2.50%, 1.50% and 1.50%, respectively. High prevalence of resistance of MRSA strains against tetracycline, penicillin, ampicillin, and erythromycin was also reported by Waters et al. 2011 (Waters et al. 2011). Similar antibiotic resistance patterns especially against human-based antibiotic agents were also reported in the *S. aureus* and MRSA strains isolated from foods with animal origins and especially meat samples from Italy (Paludi et al. 2011), Nigeria (Fowoyo and Ogunbanwo 2017), Brazil (Kroning et al. 2016), Iran (Safarpour Dehkordi et al. 2017b), USA (Jackson et al. 2013) and Egypt (Sallam et al. 2015). Chajęcka-Wierzchowska et al. (2017) (Chajęcka-Wierzchowska et al. 2017) reported the high prevalence of tetracycline (*tetK*, *tetM*, *tetL*) and methicillin (*mecA*) antibiotic resistance genes in the *S. aureus* strains isolated from ready to eat food samples. Ma et al. (2018) (Bartter et al. 2018) revealed that *S. aureus* strains isolated from different types of food samples in China harbored the high distribution of β -lactams (*blaTEM*, *blaZ*, *blaOXA* and *mecA*), aminoglycosides (*aac6/aph2*), tetracyclines (*tetA* and *tetM*), macrolides (*ermA*, *ermB*, *ermC* and *msrA*), fluoroquinolones (*griA* and *norA*), glycopeptides (*vanA*) and phenicols (*chlA*) antibiotic resistance genes. Safarpour et al. (2017) (Safarpour Dehkordi et al. 2017a) showed that prevalence of *aacA-D*, *tetK*, *tetM*, *msrA*, *ermA*, *ermC*, *vatA*, *vatB*, *vatC* and *linA* antibiotic resistance genes in the MRSA strains isolated from food samples in Iran were 62.16%, 72.97%, 27.02%, 64.86%, 72.97%, 27.02%, 45.94%, 18.91%, 5.40% and 43.24%, respectively which was similar to our findings. The most imperative mechanism involving resistance against clindamycin is modulated by methylase enzyme which often encoded by *ermA* and *ermC* genes (Zelazny et al. 2005). The literature survey did not indicate any report on the prevalence of *vatA*, *vatB*, *vatC*, *msrA*, *ermA*, *ermC*, *linA*, *aacA-D*, *tetK* and *tetM* genes among the MRSA strains of raw quail, turkey and camel meat samples

Conclusion

To put it in a nutshell, we identified high prevalence of antibiotic resistance, antibiotic resistance genes, *SCCmec* types and *PVL* gene in the MRSA strains isolated from raw ruminants, poultrys and fish meat samples. MRSA strains had the higher prevalence in raw sheep and goat meat samples. Additionally, MRSA strains isolated from sheep meat samples harbored higher prevalence of *PVL* gene, antibiotic resistance genes and *SCCmec* types. MRSA strains harbored the highest prevalence of resistance against penicillin, tetracycline, gentamicin, erythromycin and trimethoprim-sulfamethoxazole antibiotic agents. Higher prevalence of *aadA1*, *tetK*, *msrA* and *vatA* antibiotic resistance genes is another important finding. Some MRSA strains exhibited simultaneous resistance against more than one antibiotic agents. Additionally, some of the harbored more than one antibiotic resistance genes. Furthermore, our research demonstrated that most MRSA were found to be resistant to commonly used antimicrobial agents which raised concerns regarding transmission risk following the consumption of raw or undercooked meat. Moreover, raw meat samples were the main sources of CA-MRSA strains with higher prevalence of *SCCmec* IV and V types. MRSA strains isolated from poultry meat samples harbored the highest prevalence of resistance against chloramphenicol antibiotic agents. Prevalence of resistance against human-based antibiotic was also high. Simultaneous presence of *PVL* gene, *SCCmec* types and antibiotic resistance genes in the MRSA strains pose an important public health threat regarding the consumption of raw or undercooked meat samples. As far as we know, the present study is the first report in its field on MRSA strains isolated from camel, turkey and quail meat samples. Our research highlights the importance of monitoring the antimicrobial susceptibility of MRSA in the food chains including raw meat and especially sheep, goat and poultry meat samples and these data could be used proactively to assist government and industries in Iran to develop improved food safety measures, designed to reduce the contamination and transmission of this bacterium. Moreover, a future large-scale and multi-population-based study must be conducted to obtain more comprehensive data on the prevalence and distribution of MRSA strains in different Iranian ethnic populations.

Abbreviations

S. aureus: *Staphylococcus aureus*; MRSA: Methicillin-resistant *Staphylococcus aureus*; PCR: Polymerase Chain Reaction; SPSS: Statistical Package for the Social Sciences

Declarations

Availability of data and materials

All data generated or analyzed throughout this research are included in this published article.

Competing interests

Authors declared that they have no conflict of interest.

Funding

Funding is not applicable for this research.

Authors' contributions

Sample collection, microbial analysis, PCR alignment, writing and drafting of the manuscript were performed by the RC.

Acknowledgements

The authors would like to thank Dr. Manouchehr Momeni Shahraki for his assistance in sample collection and PCR genetic alignments.

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Tables

Table 1. Target enterotoxin genes, oligonucleotide primers, PCR programs and volumes used for detection of antibiotic resistance genes and SCCmec types amongst the MRSA strains isolated from various types of raw meat samples.

Target gene	Primer sequence (5'-3')	PCR product (bp)	PCR programs	PCR volume (50 µL)
<i>AacA-D</i>	F: TAATCCAAGAGCAATAAGGGC R: GCCACACTATCATAACCACTA	227	1 cycle: 94 °C _____ 5 min.	5 µL PCR buffer 10X 2 mM MgCl ₂ 150 µM dNTP
<i>ermA</i>	F: AAGCGGTAAACCCCTCTGA R: TTCGCAAATCCCTTCTCAAC	190	25 cycle: 94 °C _____ 60 s	0.75 µM of each primers F & R 1.5 U Taq DNA polymerase 3 µL DNA template
<i>ermC</i>	F: AATCGTCAATTCCTGCATGT R: TAATCGTGGAATACGGGTTTG	299	55 °C _____ 70 s	
<i>tetK</i>	F: GTAGCGACAATAGGTAATAGT R: GTAGTGACAATAAACCTCCTA	360	72 °C _____ 60 s	
<i>vatC</i>	F: AAAATCGATGGTAAAGGTTGGC R: AGTTCTGCAGTACCGGATTTGC	467	1 cycle: 72 °C _____ 10 min	
<i>tetM</i>	F: AGTGGAGCGATTACAGAA R: CATATGTCCTGGCGTGTCTA	158	1 cycle: 94 °C _____ 6 min. 34 cycle: 95 °C _____ 50 s	5 µL PCR buffer 10X 2 mM MgCl ₂ 150 µM dNTP 0.75 µM of each primers F & R 1.5 U Taq DNA polymerase 3 µL DNA template
<i>vatA</i>	F: TGGTCCCGGAACAACATTTAT R: TCCACCGACAATAGAATAGGG	268	55 °C _____ 70 s 72 °C _____ 60 s 1 cycle: 72 °C _____ 8 min	
<i>msrA</i>	F: GGCACAATAAGAGTGTTTAAAGG R: AAGTTATATCATGAATAGATTGCCTGTT	940	1 cycle: 94 °C _____ 6 min. 34 cycle: 95 °C _____ 60 s	5 µL PCR buffer 10X 2 mM MgCl ₂ 150 µM dNTP 0.75 µM of each primers F & R 1.5 U Taq DNA polymerase 3 µL DNA template
<i>msrB</i>	F: TATGATATCCATAATAATTATCCAATC R: AAGTTATATCATGAATAGATTGCCTGTT	595	50 °C _____ 70 s 72 °C _____ 70 s 1 cycle: 72 °C _____ 8 min	
<i>vatB</i>	F: GCTGCGAATTCAGTTGTTACA	136	1 cycle: 72 °C _____ 8 min	5 µL PCR buffer 10X

	R: CTGACCAATCCCACCATTTTA		94 ^{0C} _____ 6 min.	2 mM MgCl ₂ 150 μM dNTP
			35 cycle:	0.75 μM of each primers F & R
			95 ^{0C} _____ 50 s	1.5 U Taq DNA polymerase
			55 ^{0C} _____ 70 s	3 μL DNA template
			72 ^{0C} _____ 80 s	
			1 cycle:	
			72 ^{0C} _____ 10 min	
<i>linA</i>	F: GGTGGCTGGGGGTAGATGTATTAAGTGG R: GCTTCTTTTCAAATACATGGTATTTTTCGA	323	1 cycle: 94 ^{0C} _____ 6 min.	5 μL PCR buffer 10X 2 mM MgCl ₂ 150 μM dNTP
			30 cycle:	0.75 μM of each primers F & R
			95 ^{0C} _____ 60 s	1.5 U Taq DNA polymerase
			57 ^{0C} _____ 60 s	3 μL DNA template
			72 ^{0C} _____ 60 s	
			1 cycle:	
			72 ^{0C} _____ 10 min	
<i>SCCmec I</i>	F: GCTTTAAAGAGTGTCGTTACAGG R: GTTCTCTCATAGTATGACGTCC	613	1 cycle: 94 ^{0C} _____ 5 min.	5 μL PCR buffer 10X 2 mM MgCl ₂ 150 μM dNTP
<i>SCCmec II</i>	F: CGTTGAAGATGATGAAGCG R: CGAAATCAATGGTTAATGGACC	398	10 cycle:	0.75 μM of each primers F & R
<i>SCCmec III</i>	F: CCATATTGTGTACGATGCG R: CCTTAGTTGTCGTAACAGATCG	280	94 ^{0C} _____ 45 s	1.5 U Taq DNA polymerase
<i>SCCmec IVa</i>	F: GCCTTATTCGAAGAAACCG R: CTAATCTTCTGAAAAGCGTCC	776	65 ^{0C} _____ 45 s	3 μL DNA template
<i>SCCmec IVb</i>	F: TCTGGAATTACTTCAGCTGC R: AAACAATATTGCTCTCCCTC	493	70 ^{0C} _____ 90 s	
<i>SCCmec IVc</i>	F: ACAATATTTGTATTATCGGAGAGC R: TTGGTATGAGGTATTGCTGG	200	1 cycle: 72 ^{0C} _____ 10 min	
<i>SCCmec IVd</i>	F: CTCAAATACGGACCCCAATACA R: TGCTCCAGTAATTGCTAAAG	881		
<i>SCCmec V</i>	F: GAACATTGTTACTTAAATGAGCG	325		

Table 2. Total distribution of MRSA and *PVL* gene in various types of raw meat samples.

Types of samples	No. samples collected	No. samples positive for <i>S. aureus</i> (%)	No. samples positive for MRSA (%)	<i>PVL</i> positive (%)
Beef	150	30 (20)	17 (56.66)	12 (70.58)
Goat	80	15 (18.75)	10 (66.66)	7 (70)
Sheep	100	28 (28)	21 (75)	20 (95.23)
Camel	60	8 (13.33)	4 (50)	2 (50)
Chicken	80	20 (25)	12 (60)	10 (83.33)
Turkey	50	11 (22)	7 (63.63)	4 (57.14)
Quail	50	5 (10)	3 (60)	1 (33.33)
Ostrich	50	7 (14)	5 (71.42)	2 (40)
Fish	60	1 (1.66)	-	-
Total	680	125 (18.38)	79 (63.20)	58 (73.41)

Table 3. Distribution of *SCCmec* types in MRSA strains isolated from various types of raw meat samples.

Type of samples (No. MRSA)	N (%) isolates positive for each type							
	<i>SCCmec I</i>	<i>SCCmec II</i>	<i>SCCmec III</i>	<i>SCCmec IVa</i>	<i>SCCmec IVb</i>	<i>SCCmec IVc</i>	<i>SCCmec IVd</i>	<i>SCCmec V</i>
Beef (12)	1 (8.33)	-	2 (16.66)	4 (33.33)	1 (8.33)	-	2 (16.66)	2 (16.66)
Goat (7)	1 (14.28)	-	1 (14.28)	3 (42.85)	-	-	-	2 (28.57)
Sheep (20)	2 (10)	1 (5)	2 (10)	8 (40)	1 (5)	1 (5)	1 (5)	4 (20)
Camel (2)	-	-	-	1 (50)	-	-	-	1 (50)
Chicken (10)	1 (10)	-	1 (10)	3 (30)	1 (10)	1 (10)	1 (10)	2 (20)
Turkey (4)	-	-	-	2 (50)	1 (25)	-	-	1 (25)
Quail (1)	-	-	-	1 (100)	-	-	-	-
Ostrich (2)	-	-	-	1 (50)	-	-	-	1 (50)
Total (58)	5 (8.62)	1 (1.72)	6 (10.34)	23 (39.65)	4 (6.89)	2 (3.44)	4 (6.89)	13 (22.41)

Table 4. Antibiotic resistance pattern of the MRSA isolated from various types of raw meat samples.

Type of samples (No. MRSA)	N (%) isolates resistant to each antibiotic agent									
	P10*	Gen	Azi	Ert	Tet	Dox	Cip	Clin	Tr-Sul	C30
Beef (12)	12 (100)	9 (75)	7 (58.33)	8 (66.66)	12 (100)	5 (41.66)	6 (50)	5 (41.66)	8 (66.66)	2 (16.66)
Goat (7)	7 (100)	4 (57.14)	3 (42.85)	3 (42.85)	7 (100)	3 (42.85)	3 (42.85)	2 (28.57)	4 (57.14)	1 (14.28)
Sheep (20)	20 (100)	15 (75)	12 (60)	14 (70)	20 (100)	10 (50)	8 (40)	7 (35)	11 (55)	1 (5)
Camel (2)	2 (100)	1 (50)	-	1 (50)	2 (100)	-	-	-	1 (50)	-
Chicken (10)	10 (100)	6 (60)	4 (40)	4 (40)	10 (100)	4 (40)	3 (30)	3 (30)	5 (50)	6 (30)
Turkey (4)	4 (100)	2 (50)	1 (25)	2 (50)	4 (100)	1 (25)	1 (25)	-	2 (50)	3 (75)
Quail (1)	1 (100)	-	-	-	1 (100)	-	-	-	-	1 (100)
Ostrich (2)	2 (100)	1 (50)	1 (50)	1 (50)	2 (100)	-	1 (50)	-	1 (50)	1 (50)
Total (58)	58 (100)	38 (65.51)	28 (48.27)	33 (56.89)	58 (100)	23 (39.65)	22 (37.93)	17 (29.31)	32 (55.17)	15 (25.86)

*P10: penicillin (10 µg/disk), Gen: gentamicin (10 µg/disk), Azi: azithromycin (15 µg/disk), Ert: erythromycin (15 µg/disk), Tet: tetracycline (30 µg/disk), Do: doxycycline (30 µg/disk), Cip: ciprofloxacin (5 µg/disk), Clin: clindamycin (2 µg/disk), Tr-Sul: trimethoprim-sulfamethoxazole (25 µg/disk) and C30: chloramphenicol (30 µg/disk).

Table 5. Distribution of antibiotic resistance genes amongst the MRSA strains isolated from various types of raw meat samples.

Type of samples (No. MRSA)	N (%) isolates positive for each gene										
	<i>aadA1</i>	<i>tetK</i>	<i>tetM</i>	<i>ermA</i>	<i>ermC</i>	<i>vatA</i>	<i>vatB</i>	<i>vatC</i>	<i>msrA</i>	<i>msrB</i>	<i>linA</i>
Beef (12)	8 (66.66)	8 (66.66)	2 (16.66)	5 (41.66)	2 (16.66)	5 (41.66)	2 (16.66)	1 (8.33)	5 (41.66)	2 (16.66)	5 (41.66)
Goat (7)	3 (42.85)	3 (42.85)	1 (14.28)	3 (42.58)	1 (14.28)	3 (42.85)	1 (14.28)	-	3 (42.58)	1 (14.28)	2 (28.57)
Sheep (20)	14 (70)	14 (70)	3 (15)	10 (50)	2 (10)	7 (35)	3 (15)	1 (5)	10 (50)	1 (5)	6 (30)
Camel (2)	1 (50)	1 (50)	-	1 (50)	-	1 (50)	-	-	1 (50)	-	-
Chicken (10)	5 (50)	4 (40)	2 (20)	3 (30)	2 (20)	3 (30)	1 (10)	-	2 (20)	1 (10)	3 (30)
Turkey (4)	2 (50)	1 (25)	-	2 (50)	1 (25)	1 (25)	-	-	2 (50)	1 (25)	1 (25)
Quail (1)	-	1 (100)	-	-	-	-	-	-	-	-	-
Ostrich (2)	1 (50)	1 (50)	-	1 (50)	1 (50)	1 (50)	-	-	1 (50)	-	-
Total (58)	34 (58.62)	33 (56.89)	8 (13.79)	25 (43.10)	9 (15.51)	21 (36.20)	7 (12.06)	2 (3.44)	24 (41.37)	6 (10.34)	17 (29.31)