Genotypic and Phenotypic-Based Assessment of Antibiotic Resistance and Profile of Staphylococcal Cassette Chromosome mec in the Methicillin-Resistant Staphylococcus aureus Recovered from Raw Milk

Azar Rahi1
Hamidreza Kazemeini2
Sedigheh Jafariaskari3
Ali Seif4
Sahar Hosseini5
Farhad Safarpoor Dehkordi6

1Department of Microbiology, Kazerun Branch, Islamic Azad University, Kazerun, Iran; 2Department of Food Hygiene, Faculty of Veterinary Medicine, Amol University of Special Modern Technologies, Amol, Iran; 3Department of Parasitology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran; 4Doctor Veterinary Medicine, Faculty of Veterinary Medicine, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran; 5Master of Food Science and Technology, Faculty of Agriculture and Food Sciences, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran; 6Halal Research Center of IRI, FDA, Tehran, Iran

Background: Multidrug resistant methicillin-resistant Staphylococcus aureus (MRSA) bacteria are determined to be one of the chief causes of foodborne diseases around the world.

Purpose: This research was done to assess the genotypic and phenotypic profiles of antibiotic resistance and distribution of Staphylococcus cassette chromosome mec (SCCmec) types amongst the MRSA bacteria recovered from raw milk.

Methods: Five-hundred and ninety raw milk samples were collected and examined. MRSA bacteria were recognized using susceptibility evaluation toward oxacillin and cefoxitin disks. Profile of antibiotic resistance genes and SCCmec types were determined using the PCR. Antibiotic resistance pattern of isolates was examined using the disk diffusion.

Results: Thirty-nine out of 590 raw milk samples (6.61%) were positive for S. aureus. Twenty-eight out of 39 (71.79%) bacteria were defined as MRSA bacteria. Raw buffalo (80%) milk samples had the maximum incidence of MRSA, while raw camel (33.33%) had the minimum. MRSA bacteria harbored the maximum incidence of resistance toward penicillin (100%), tetracycline (100%), erythromycin (82.14%), gentamicin (78.57%) and trimethoprim-sulfamethoxazole (78.57%). Incidence of resistance toward more than eight classes of antibiotic agents was 28.57%. The most frequently distinguished antibiotic resistance markers were blaZ (100%), tetK (85.71%), dfrA1 (71.42%), aacA-D (67.85%), ermE (50%) and gyrA (42.85%). SCCmec IVa (29.62%), V (25%), III (14.81%) and IVb (11.11%) were the most frequently distinguished types.

Conclusion: Raw milk of dairy animals maybe sources of multidrug resistant MRSA which pose a hygienic threat concerning the consumption of raw milk in Iran. Nevertheless, further investigations are necessary to understand supplementary epidemiological features of MRSA in raw milk.

Keywords: methicillin-resistant Staphylococcus aureus, raw milk, antibiotic resistance mechanisms, SCCmec types

Introduction

Milk of animal species contains assortment of imperative dietary supplements including proteins, carbohydrate, fats, minerals and vitamins with boost advantageous effects for human life.1 Therefore, their regular daily consumption has been
extensively suggested. However, there is evidence that raw milk of animal species might contain different types of threatening foodborne pathogens.2–5

Most cases of foodborne outbreaks are associated with the consumption of food contaminated with foodborne bacterial pathogens,6–16 especially *Staphylococcus aureus* (*S. aureus*).17–20 *S. aureus* is a bacterium of the Firmicutes family originating from the human nose and skin. *S. aureus* is considered one of the chief causes of hospital and community-acquired infections and foodborne diseases recognized by weakness, vomiting, nausea, abdominal cramps and toxic shock syndrome.17–20

Foodborne *S. aureus* bacteria are typically associated with boost prevalence of antibiotic resistance.17–20 Today, methicillin-resistant *S. aureus* (MRSA) has developed a significant issue in both health care units and the community.17–20 Recognized data described that approximately 70% of *S. aureus* bacteria recovered from the health care units and the community were simultaneously resistant toward penicillins and cephalosporins.17–21 They are responsible for about 100,000 morbidity with near to 20% mortality per year in the United States.21 Higher pathogenicity of MRSA bacteria,17–21 their inclusive levels of resistance toward numerous kinds of antibiotic agents, especially penicillins, aminoglycosides, macrolides, tetracyclines and fluoroquinolones17–21 and their foodborne aspects17–20 have amplified the clinical and microbial importance of MRSA in popularly consumed foodstuffs, particularly milk. Furthermore, foodstuffs containing MRSA bacteria are considered as imperative reservoirs of antibiotic resistance genes.17–21 Boost incidence of the genes encode resistance toward penicillins (*blaZ*), aminoglycosides (*aacA-D*), tetracyclines (*tetK* and *tetM*), macrolides (*ermA*, *ermB*, *msrA*, *msrB* and *mefA*), fluoroquinolones (*gyrA* and *grLA*), lincomamides (*linA*), folate inhibitors (*dfrA1*), phenicols (*cfr*), and ansamycins (*rpoB*) is one of the chief ways for occurrence of severe antibiotic resistance.17–20

The *mecA* gene is another imperative antibiotic resistance marker responsible for resistance toward methicillin. It is associated with a 21- to 67-kb molecular element named staphylococcal chromosomal cassette *mec* (SCCmec)22 characterized by *mec* and the *ccr* genetic markers. SCCmec elements are characteristically divided into 11 different types based on to the positioning *ccr* and *mec* genes.22 SCCmec IV is additionally divided to IVa, IVb, IVc and IVd alleles.22 A mobile genetic element, SCCmec, plays an important role in staphylococci pathogenesis and occurrence of resistance toward penicillins.22 MRSA bacteria have rarely been examined in raw milk to evaluate microbial security, sanitation circumstances through milking, and storage periods. Thus, the existing survey was done to investigate the incidence rate, antimicrobial resistance properties and distribution of SCCmec types of the MRSA bacteria recovered from raw bovine, ovine, caprine, buffalo, and camel milk samples in Iran.

**Materials and Methods**

**Samples**

A total of 590 raw milk samples including bovine (*n*=130), ovine (*n*=120), caprine (*n*=120), camel (*n*=110), and buffalo (*n*=110) were randomly collected during a one-year period (2016 to 2017) from the shopping centers of different parts of Iran. None of the milk samples were not packed. All samples were stored in a refrigerator. Samples of raw milk were distributed by milk carrying specific trucks to shopping centers. A total of 50 mL were collected from each raw milk sample using a sterile laboratory tubes. Samples were proximately transferred to laboratory using cool bags. All milk samples presented usual physical properties such as odor, consolidation and color.

**Isolation and Identification of *S. aureus***

Twenty-five grams of each of the collected samples were blended with 225 mL of buffered peptone water (EMD Millipore, Billerica, MA, USA). At that time, solutions were homogenized using Stomacher (Interscience, Saint-Nom, France). At that point, 5 mL of the achieved solution was transferred into 50 mL trypticase soy broth (TSB; EMD Millipore) supplemented with 10% NaCl and 1% sodium pyruvate and incubated for 18 h at 35°C. At that moment, a loopful of the culture was transferred into Baird-Parker agar supplemented with egg yolk tellurite emulsion (EMD Millipore) and incubated at 37°C for about 24 h. Black shiny colonies enclosed with significant zones were identified using biochemical tests as introduced before.23

**Identification of Methicillin-Resistant *S. aureus* Bacteria**

Antibiotic susceptibility tests were applied for this purpose. Susceptibility of *S. aureus* isolates were tested against cefoxitin (30 µg) and oxacillin (1 µg) antibiotic disks. Experiment was completed by the instructions of the Clinical and Laboratory Standards Institute (CLSI).24
Confirmation of MRSA isolates were additionally performed using the PCR-based detection of \textit{mecA} gene.\textsuperscript{23}

**Antibiotic Susceptibility Test of MRSA Bacteria**

Phenotypic pattern of antibiotic resistance of MRSA bacteria was investigated using the disk diffusion method on the Mueller–Hinton agar (EMD Millipore). Principles of CLSI were applied for this purpose.\textsuperscript{25} Diverse kinds of antibiotic agents including aminoglycosides (amikacin (30 µg/disk) and gentamicin (10 µg/disk)), fluoroquinolones (levofloxacin (5 µg/disk) and ciprofloxacin (5 µg/disk)), lincosamides (clindamycin (2 µg/disk)), macrolides (erythromycin (15 µg/disk) and azithromycin (15 µg/disk)), penicillins (penicillin (10 µg/disk), tetracyclines (doxycycline (30 µg/disk)) and tetracycline (30 µg/disk)), phenicols (chloramphenicol (30 µg/disk)), folate pathway inhibitors (trimethoprim-sulfamethoxazole (25 µg/disk)) and ansamycins (rifampin (5 µg/disk)) were applied for this goal (Oxoid, UK). Method was completed using the protocol labeled beforehand.\textsuperscript{23,25}

**PCR-Based Amplification of Antibiotic Resistance Genes and SCC\textit{mec} Types in MRSA Bacteria**

Table 1 reveals the set of primers and PCR circumstances applied for detection of genotyping pattern of antibiotic resistance and SCC\textit{mec} types.\textsuperscript{26–33} A programmable DNA thermo-cycler (Eppendorf Mastercycler 5330, Eppendorf-Nethel-Hinz GmbH, Hamburg, Germany) was applied for this goal.

**Statistical Analysis**

SPSS 21.0 statistical software (IBM Corporation, Armonk, NY, USA) was applied for arithmetical analysis of data. Significant relations between data achieved from different groups and parameters were analyzed using the chi-square \textit{d} test and Fisher’s exact two-tailed tests. \textit{P} value <0.05 was determined as arithmetical significant level.

**Results**

**Incidence of \textit{S. aureus} and MRSA Bacteria**

Table 2 signifies the distribution of \textit{S. aureus} and MRSA bacteria in diverse kinds of raw milk samples. Thirty-nine out of 590 raw milk samples (6.61%) were positive for \textit{S. aureus}. Raw buffalo (9.09%) and bovine (8.46%) milk samples had the maximum incidence of \textit{S. aureus}, while raw camel (2.72%) milk samples had the minimum. Twenty-eight out of 39 (71.79%) bacteria were defined as MRSA bacteria. Raw buffalo (80%) and ovine (77.77%) milk samples had the maximum prevalence of MRSA bacteria, while raw camel (33.33%) milk samples had the minimum. Arithmetical important difference was seen for the prevalence of MRSA bacteria between buffalo and camel (\textit{P} <0.05) and bovine and camel (\textit{P} <0.05) raw milk samples.

**Antibiotic Resistance Pattern of MRSA Bacteria**

Table 3 signifies the phenotypic pattern of antibiotic resistance of MRSA bacteria recovered from diverse kinds of raw milk samples. MRSA bacteria harbored the maximum incidence of resistance toward penicillin (100%), tetracycline (100%), erythromycin (82.14%), gentamicin (78.57%), trimethoprim-sulfamethoxazole (78.57%), and doxycycline (71.42%) antibiotic agents. MRSA bacteria exhibited lower incidence of resistance toward rifampin (14.28%), amikacin (17.85%), chloramphenicol (28.57%), azithromycin (32.14%), and levofloxacin (32.14%) antibiotic agents.

**Prevalence of Multidrug Resistant MRSA Bacteria**

Figure 1 signifies the incidence of resistance toward multiple groups of antibiotics. We found that all of the MRSA bacteria recovered from diverse kinds of raw milk samples had at least resistance toward four diverse classes of antibiotic agents, though incidence of resistance toward more than eight groups of antibiotics was 28.57%.

**Distribution of Antibiotic Resistance Genes**

Table 4 signifies the genotypic pattern of antibiotic resistance amongst the MRSA bacteria recovered from diverse kinds of raw milk samples. The most generally identified antibiotic resistance genes were \textit{blaZ} (100%), \textit{tetK} (85.71%), \textit{dfrA1} (71.42%) and \textit{aacA-D} (67.85%). Incidence of \textit{ermA} and \textit{gyrA} antibiotic resistance genes were 50% and 42.85%, respectively. Incidence of \textit{msrB} (10.71%), \textit{rpoB} (10.71%), \textit{ermB} (25%), \textit{cfr} (25%), \textit{grlA} (28.57%) and \textit{linA} (28.57%) were lower than other identified resistance genes. Arithmetical important difference was seen between the incidence of \textit{ermA} and \textit{ermB} (\textit{P} <0.05), \textit{msrA} and \textit{msrB} (\textit{P} <0.05), \textit{tetK} and \textit{tetM} (\textit{P} <0.05), and \textit{gyrA} and \textit{grlA} (\textit{P} <0.05) antibiotic resistance genes.
### Table 1 Target Genes, Oligonucleotide Primers and PCR Conditions Used for Detection of Antibiotic Resistance Genes and SCCmec Types Amongst MRSA Bacteria Recovered from Raw Milk

| Target Gene | Primer Sequence (5’-3’) | PCR Product (bp) | PCR Programs | PCR Volume (50 µL) |
|-------------|-------------------------|------------------|--------------|--------------------|
| **aacA-D**  | F: TAATCCAAAGCAATAAGGGC  
R: GACCACCTATCATAAACCACAT | 227 | 1 cycle:  
94°C ———— 5 min  
25 cycles:  
94°C ———— 60 s  
55°C ———— 70 s  
72°C ———— 60 s  
1 cycle:  
72°C ———— 10 min | 5 µL PCR buffer 10X  
1.5 mM MgCl<sub>2</sub>  
200 µM dNTP (Fermentas)  
0.5 µM of each primers F & R  
1.25 U Taq DNA polymerase (Fermentas)  
2.5 µL DNA template |
| **ermA**    | F: AAGCGGTGAAACCCCTCTGA  
R: TCCGGCAATCCCTTCTCAAC | 190 | 1 cycle:  
94°C ———— 6 min  
34 cycles:  
95°C ———— 60 s  
55°C ———— 70 s  
72°C ———— 60 s  
1 cycle:  
72°C ———— 8 min | 5 µL PCR buffer 10X  
2 mM MgCl<sub>2</sub>  
200 µM dNTP (Fermentas)  
0.5 µM of each primers F & R  
1.5 U Taq DNA polymerase (Fermentas)  
5 µL DNA template |
| **tetK**    | F: GTAGCGCAATAGGTAAATGT  
R: GTAGTGCAATAAAACCTCCTA | 360 | 1 cycle:  
94°C ———— 6 min  
34 cycles:  
95°C ———— 60 s  
57°C ———— 60 s  
72°C ———— 60 s  
1 cycle:  
72°C ———— 8 min | 5 µL PCR buffer 10X  
2 mM MgCl<sub>2</sub>  
150 µM dNTP (Fermentas)  
0.75 µM of each primers F & R  
1.5 U Taq DNA polymerase (Fermentas)  
3 µL DNA template |
| **ermB**    | F: CCGTTTACGAAAATTGGAACAGTAAAGGCG  
R: GAATCGAGACCTTGAGTGCG | 359 | 1 cycle:  
94°C ———— 6 min  
30 cycles:  
95°C ———— 60 s  
57°C ———— 60 s  
72°C ———— 60 s  
1 cycle:  
72°C ———— 8 min | 5 µL PCR buffer 10X  
2 mM MgCl<sub>2</sub>  
150 µM dNTP (Fermentas)  
0.75 µM of each primers F & R  
1.5 U Taq DNA polymerase (Fermentas)  
3 µL DNA template |
| **mefA**    | F: ACTATCATAATATCACTATGTGC  
R: TTCTTCTGTAACAAAGGTGG | 346 | 1 cycle:  
94°C ———— 6 min  
30 cycles:  
95°C ———— 60 s  
57°C ———— 60 s  
72°C ———— 60 s  
1 cycle:  
72°C ———— 8 min | 5 µL PCR buffer 10X  
2 mM MgCl<sub>2</sub>  
150 µM dNTP (Fermentas)  
0.75 µM of each primers F & R  
1.5 U Taq DNA polymerase (Fermentas)  
3 µL DNA template |
| **grlA**    | F: ACTTGAAGATGTTTTAGGTGAT  
R: TTAGGAAATCTTGATGGCAAA | 618 | 1 cycle:  
94°C ———— 6 min  
30 cycles:  
95°C ———— 60 s  
57°C ———— 60 s  
72°C ———— 60 s  
1 cycle:  
72°C ———— 8 min | 5 µL PCR buffer 10X  
2 mM MgCl<sub>2</sub>  
150 µM dNTP (Fermentas)  
0.75 µM of each primers F & R  
1.5 U Taq DNA polymerase (Fermentas)  
3 µL DNA template |
| **tetM**    | F: AGTGGAGCGATTACAGAA  
R: CATATGCTCCGTGCTCTA | 158 | 5 µL PCR buffer 10X  
1.5 mM MgCl<sub>2</sub>  
200 µM dNTP (Fermentas)  
0.5 µM of each primers F & R  
1.5 U Taq DNA polymerase (Fermentas)  
5 µL DNA template |
| **gyrA**    | F: AGTACATCGTCGTATACTATGTGC  
R: ATACGGTACAGTGGTAGTG | 280 | 5 µL PCR buffer 10X  
1.5 mM MgCl<sub>2</sub>  
200 µM dNTP (Fermentas)  
0.5 µM of each primers F & R  
1.5 U Taq DNA polymerase (Fermentas)  
5 µL DNA template |
| **msrA**    | F: GCCACAATAAGTGTTTAAAGG  
R: AAGTTATATCATAGATGTGTGTCT | 940 | 5 µL PCR buffer 10X  
2 mM MgCl<sub>2</sub>  
150 µM dNTP (Fermentas)  
0.75 µM of each primers F & R  
1.5 U Taq DNA polymerase (Fermentas)  
3 µL DNA template |
| **msrB**    | F: TATGATATCCCTAAAAATATACTCAAC  
R: TTCTATATCATGAAGTGTGTGGTGTCT | 595 | 5 µL PCR buffer 10X  
2 mM MgCl<sub>2</sub>  
150 µM dNTP (Fermentas)  
0.75 µM of each primers F & R  
1.5 U Taq DNA polymerase (Fermentas)  
3 µL DNA template |
| **dfrA1**   | F: CTACAGTAAACAAAGGTGA  
R: CAATCATCCTGTGATAAC | 201 | 5 µL PCR buffer 10X  
2 mM MgCl<sub>2</sub>  
150 µM dNTP (Fermentas)  
0.75 µM of each primers F & R  
1.5 U Taq DNA polymerase (Fermentas)  
3 µL DNA template |
| **linA**    | F: GGTGGCTGGGGGGGTAGATGTATACTGGG  
R: GCTTCTTTTTAAGCATGTTATTTTTCGA | 323 | 5 µL PCR buffer 10X  
2 mM MgCl<sub>2</sub>  
150 µM dNTP (Fermentas)  
0.75 µM of each primers F & R  
1.5 U Taq DNA polymerase (Fermentas)  
3 µL DNA template |
| **blaZ**    | F: TGAACCGTATGTTAGTG | 681 | 5 µL PCR buffer 10X  
2 mM MgCl<sub>2</sub>  
150 µM dNTP (Fermentas)  
0.75 µM of each primers F & R  
1.5 U Taq DNA polymerase (Fermentas)  
3 µL DNA template |

(Continued)
Table 5 signifies the incidence of SCCmec types amongst the MRSA bacteria recovered from diverse kinds of raw milk samples. SCCmec IVa (29.62%), V (25%), III (14.81%) and IVb (11.11%) were the most routinely identified kinds amongst the MRSA bacteria. Incidence of SCCmec IVd (3.70%) and II (3.70%) was low. Arithmetical important difference was seen between the incidence of SCCmec types IVa and II ($P < 0.05$), IVa and IVd ($P < 0.05$), V and II ($P < 0.05$) and V and IVd ($P < 0.05$).

## Discussion

Prior to the 1990s, the majority of MRSA bacteria were hospital-associated (HA-MRSA) strains. Then, community-associated MRSA (CA-MRSA) prompted to occur infections outside the health-care and/or hospital environments. Recorded surveys revealed the occurrence of livestock-associated MRSA (LA-MRSA) in animals and/or livestock fields. The extensive developments in LA-MRSA and CA-MRSA have elevated the query as to whether MRSA is certainly a foodborne microbe. Furthermore, surveys on
MRSA are interesting due to their considerable prevalence in diverse kinds of foodstuffs.34

Findings of the existing investigation revealed that the contamination rate of milk samples was 4.74% (28/590). Incidence of the MRSA in raw milk samples in our survey was lower than those of Italy (20%)35 and Turkey (17%),36 while it was higher than those of England (2.30%)37 and Germany (2.30%).38 Investigations conducted in the US along with other countries, including North America, Canada, Africa, Asia and Europe, have recovered MRSA mostly from dissimilar kinds of food and dairy samples.34,39 Some dairy animals are the main sources of MRSA bacteria. The possibility of primary presence of MRSA bacteria in raw milk samples due to the occurrence of sub-clinical mastitis in dairy animals and thus their transmission to raw milk, the opportunity of transmission of multidrug resistant MRSA from the milking halls, and also infected staff into the raw milk are the most important probable reasons for presence of MRSA bacteria.

Irregular and unauthorized prescription of antibiotics are the probable reasons for high prevalence of antibiotic resistance in the current survey. Additionally, boost incidence of antibiotic resistance was attended with boost incidence of specific antibiotic resistance genes. Furthermore, our findings showed that some of the MRSA bacteria exhibited higher incidence of resistance toward antibiotics used for human beings which can indirectly show their anthropogenic source. Conversely, some others exhibited higher incidence of resistance toward antibiotics used for animals which can circuitously demonstrate their animal origins. This conclusion was comparable with those of Hasanpour Dehkordi et al17 and Safarpoor Dehkordi et al20 which were both conducted on Iranian food samples. Comparable resistance of MRSA recovered from dissimilar kinds of foodstuffs and clinical specimens have also been determined toward aminoglycosides,19,20,40–43 cephalosporins,19,20,40–42 penicillins,19,20,40–42 macrolides,19,20,40–42 tetracyclines,19,20,40,41 fluoroquinolones,19,20,40–43 lincomamides,19,20,40–42 folate inhibitors,19,20,40–43 phenicols19,20,40,41 and ansamycins19,20,40,41 antibiotic agents. Fowoyo and Ogunbanwo44 revealed that the S. aureus bacteria recovered from ready-to-eat foodstuffs exhibited the boost incidence of resistance toward trimethoprim–sulfamethoxazole (74.90%), ampicillin (86.70%), cefotaxime (3.50%), amoxicillin–clavulanic acid (52.50%), ciprofloxacin (23.90%), oxacillin (35.70%), gentamicin (11.40%), erythromycin (15.70%), and ofloxacin (7.10%) which was relatively similar to our findings. Boost incidence of resistance toward chloramphenicol (28.57%) maybe due to its unlawful and unselective prescription especially in veterinary medicine. Akanbi et al45 reported that blaZ, mecA, rpoB, ermB and tetM were the most generally identified antibiotic resistance genes amongst the S. aureus bacteria recovered from food samples in South Africa which was relatively similar to our findings. Similar to our findings, high distribution of mecA, gyrA, grlA and cfr was also described in the S. aureus bacteria recovered from chicken meat in Egypt.46 Another Iranian investigation47 showed that oxacillin, gentamicin, penicillin, tetracycline and erythromycin resistant S. aureus bacteria recovered from milk and dairy products carried considerable incidence of blaZ, aacA–aphD, mecA, tetK and tetM, ermB, ermA, ermT, ermC, msrB and mrsA antibiotic resistance markers likewise to our survey.

Assess the distribution of SCCmec types is a practical method to find presence of HA-MRSA and CA-MRSA bacteria. Findings of epidemiological investigations revealed that presence of SCCmec types I, II and III indirectly showed occurrence of HA-MRSA bacteria, while presence of IV and V types represented the occurrence of CA-MRSA bacteria.48,49 Our findings showed that all of the SCCmec types had diverse distribution in the MRSA bacteria recovered from raw milk samples which may have assumed the presence of both HA and CA-MRSA bacteria. Moreover, SCCmec types IVa (29.62%) and V (25%) had the highest distribution amongst all studied elements. This finding may assume that most of the MRSA bacteria were probably originated from milk of infected animals. In keeping with this, SCCmec type III had also considerable prevalence (14.81%) which may assume that some of the MRSA bacteria had hospital or health-care origin and were probably transmitted from the contaminated workers.

### Table 2 Total Prevalence of S. aureus and MRSA Bacteria in Different Types of Raw Milk

| Types of Samples | Samples Collected n | S. aureus Positive Samples n (%) | MRSA Positive Samples n (%) |
|------------------|---------------------|---------------------------------|-----------------------------|
| Raw milk         |                     |                                 |                             |
| Bovine           | 130                 | 11 (8.46)                       | 8 (72.73)                   |
| Ovine            | 120                 | 9 (7.50)                        | 7 (77.77)                   |
| Caprine          | 120                 | 6 (5)                           | 4 (66.66)                   |
| Camel            | 110                 | 3 (2.72)                        | 1 (33.33)                   |
| Buffalo          | 110                 | 10 (9.09)                       | 8 (80)                      |
| Total            | 590                 | 39 (6.61)                       | 28 (71.79)                  |
of the milking halls. Johnson\textsuperscript{50} reported similar results for the boost incidence of SCC\textit{mec} IV in retail meat samples. In a survey which was carried out by Vossenkuhl et al\textsuperscript{51} most of MRSA bacteria recovered from turkey meat samples carried SCC\textit{mec} V (58.10–71.90%) and IVa (19–27.0%). Type III (0–1.2%) was detected periodically which was comparable to our findings. Zhang et al\textsuperscript{52} reported a the high prevalence of SCC\textit{mec} III in their food samples. Boost incidence of SCC\textit{mec} types IVa and V in food samples with animal origin has also been reported previously.\textsuperscript{38,53,54}

**Conclusions**

By and large, we recognized boost incidence of \textit{S. aureus} and MRSA bacteria in bovine, camel, caprine, ovine, and buffalo milk samples on top of boost incidence of genotypic and phenotypic profiles of antibiotic resistance and SCC\textit{mec} types. The existing survey is the first report of the genotypic evaluation of antibiotic resistance and SCC\textit{mec} typing of the MRSA bacteria in raw buffalo and camel milk samples. High prevalence of MRSA bacteria and substantial incidence of resistance toward erythromycin, penicillin, gentamicin, tetracycline, trimethoprim-sulfamethoxazole and doxycycline antibiotic agents and \textit{blaZ}, \textit{tetK}, \textit{dfrA1}, \textit{aacA-D ermA} and \textit{gyrA} antibiotic resistant genes may pose a possible menace regarding the consumption of raw milk samples in Iran. Presence of multidrug resistant MRSA bacteria may show indiscriminate and unauthorized prescription of antibiotic agents in Iranian dairy animal farms. Most of MRSA bacteria harbored SCC\textit{mec} types IV and V which may have assumed their possible community-acquired origins. However, some of the MRSA bacteria harbored SCC\textit{mec} types I, II, and III which may assume their

![Figure 1](image-url) Distribution of multidrug resistant MRSA bacteria recovered from different types of raw milk. Multidrug resistant MRSA bacteria were determined as those who had at least simultaneous resistance toward three or more than three types of antibiotics.
Table 4 Distribution of Antibiotic Resistance Genes Amongst the MRSA Bacteria Recovered from Raw Milk

| Type of Raw Milk Samples | Isolates Harbor Each Gene n (%) | Penicillins | Aminoglycosides | Macrolides | Tetracyclines | Fluoroquinolones | Lincosamides | Folate Inhibitors | Phenics | Ansamycins |
|-------------------------|---------------------------------|-------------|-----------------|------------|--------------|-----------------|--------------|------------------|----------|------------|
|                         |                                | blaZ       | oocA-D          | ermA       | msrA         | msrB            | mefA         | tetK             | tetM     | gyrA       |
| Bovine (8)              | 8 (100)                        | 4 (50)     | 2 (25)          | 3 (37.50)  | 1 (12.50)    | 3 (37.50)       | 7 (87.50)    | 3 (37.50)        | 5 (62.50) | 3 (37.50)  |
| Ovine (7)               | 7 (100)                        | 3 (42.85)  | 2 (28.57)       | 3 (42.85)  | 1 (14.28)    | 1 (14.28)       | 6 (85.71)    | 2 (28.57)        | 2 (28.57) | 1 (14.28)  |
| Caprine (4)             | 4 (100)                        | 2 (50)     | 1 (25)          | 1 (25)     | 2 (50)       | 3 (75)          | 1 (25)       | 1 (25)           | 1 (25)   | 1 (25)     |
| Camel (1)               | 1 (100)                        | –          | –               | –          | –            | 1 (100)         | –            | –                | –        | –          |
| Buffalo (8)             | 8 (100)                        | 5 (62.50)  | 2 (25)          | 3 (37.50)  | 1 (12.50)    | 4 (50)          | 7 (87.50)    | 4 (50)           | 3 (37.50) | 3 (37.50)  |
| Total (28)              | 28 (100)                       | 19 (67.85) | 9 (32.15)       | 4 (14.28)  | 8 (28.57)    | 12 (42.85)      | 8 (28.57)    | 20 (71.42)       | 7 (25)   | 3 (10.71)  |

Table 5 Distribution of SCCmec Types Amongst the MRSA Bacteria Recovered from Raw Milk

| Type of Raw Milk Samples (N of MRSA Bacteria) | Isolates Harbor Each SCCmec Type n (%) | I       | II      | III     | IV     | V     |
|---------------------------------------------|----------------------------------------|---------|---------|---------|--------|-------|
|                                            |                                        | a       | b       | c       | d      |       |
| Bovine (8)                                 | 1 (12.50)                              | –       | 2 (25)  | 3 (37.50)| 1 (12.50)| –     |
| Ovine (7)                                  | –                                      | –       | 1 (14.28)| 1 (14.28)| 1 (14.28)| 1 (14.28)| 2 (28.57)|
| Caprine (4)                                | –                                      | –       | –       | 1 (25)  | 1 (25)  | 1 (25)  | –     |
| Camel (1)                                  | –                                      | 1 (100) | –       | –       | –       | –       | –     |
| Buffalo (8)                                | 1 (12.50)                              | –       | 1 (12.50)| 3 (37.50)| –       | –       | 3 (37.50)|
| Total (28)                                 | 2 (7.40)                               | 1 (3.70)| 4 (14.81)| 8 (29.62)| 3 (11.11)| 2 (7.40)| 1 (3.70)| 7 (25) |
possible health care or hospital origins. Incidence of resistance toward human-based and also animal-based antibiotics can indirectly show the origin of MRSA bacteria. Ample boiling of raw milk beforehand consumption and prevention from cross-contamination can diminish the risk of virulent and resistant MRSA bacteria. However, supplementary surveys are necessary to comprehend more advanced epidemiological features of the MRSA bacteria in raw milk of dairy animal species.

**Ethical Criteria**

The contemporary survey was accepted by the ethical research committee of the Department of Food Hygiene, Faculty of Veterinary Medicine, Amol University of Special Modern Technologies, Amol, Iran.

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**Disclosure**

The authors report no conflicts of interest in this work.

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