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ORIGINAL RESEARCH

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

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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* (SCC*mec*) 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 SCC*mec* 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%), *ermA* (50%) and *gyrA* (42.85%). SCC*mec* 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, SCC*mec* types

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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 fluoroquinolones[17–21] and their foodborne aspects[17–20] 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*), lincosamides (*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* (SCC*mec*)[22] characterized by *mec* and the *ccr* genetic markers. SCC*mec* elements are characteristically divided into 11 different types based on to the positioning *ccr* and *mec* genes.[22] SCC*mec* IV is additionally divided to IVa, IVb, IVc and IVd alleles.[22] A mobile genetic element, SCC*mec*, 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 SCC*mec* 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 *mecA* gene.[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.[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.[23,25]

PCR-Based Amplification of Antibiotic Resistance Genes and SCC*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*mec* types.[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 d test and Fisher's exact two-tailed tests. P value <0.05 was determined as arithmetical significant level.

Results

Incidence of *S. aureus* and MRSA Bacteria

Table 2 signifies the distribution of *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 *S. aureus*. Raw buffalo (9.09%) and bovine (8.46%) milk samples had the maximum incidence of *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 (P <0.05) and bovine and camel (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 *blaZ* (100%), *tetK* (85.71%), *dfrA1* (71.42%) and *aacA-D* (67.85%). Incidence of *ermA* and *gyrA* antibiotic resistance genes were 50% and 42.85%, respectively. Incidence of *msrB* (10.71%), *rpoB* (10.71%), *ermB* (25%), *cfr* (25%), *grlA* (28.57%) and *linA* (28.57%) were lower than other identified resistance genes. Arithmetical important difference was seen between the incidence of *ermA* and *ermB* (P <0.05), *msrA* and *msrB* (P <0.05), *tetK* and *tetM* (P <0.05), and *gyrA* and *grlA* (P <0.05) antibiotic resistance genes.

Table 1 Target Genes, Oligonucleotide Primers and PCR Conditions Used for Detection of Antibiotic Resistance Genes and SCC*mec* 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: TAATCCAAGAGCAATAAGGGC R: GCCACACTATCATAACCACTA	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 $MgCl_2$ 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: AAGCGGTAAACCCCTCTGA R: TTCGCAAATCCCTTCTCAAC	190		
tetK	F: GTAGCGACAATAGGTAATAGT R: GTAGTGACAATAAACCTCCTA	360		
ermB	F: CCGTTTACGAAATTGGAACAGGTAAAGGGC R: GAATCGAGACTTGAGTGTGC	359		
mefA	F: ACTATCATTAATCACTAGTGC R: TTCTTCTGGTACTAAAAGTGG	346		
grlA	F: ACTTGAAGATGTTTTAGGTGAT R: TTAGGAAATCTTGATGGCAA	618		
tetM	F: AGTGGAGCGATTACAGAA R: CATATGTCCTGGCGTGTCTA	158	1 cycle: 94°C ——— 6 min. 34 cycles: 95°C ——— 50 s 55°C ——— 70 s 72°C ——— 60 s 1 cycle: 72°C ——— 8 min	5 µL PCR buffer 10X 2 mM $MgCl_2$ 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: AGTACATCGTCGTATACTATATGG R: ATCACGTAACAGTTCAAGTGTG	280		
msrA	F: GGCACAATAAGAGTGTTTAAAGG R: AAGTTATATCATGAATAGATTGTCCTGTT	940	1 cycle: 94°C ——— 6 min. 34 cycles: 95°C ——— 60 s 50°C ——— 70 s 72°C ——— 70 s 1 cycle: 72°C ——— 8 min	5 µL PCR buffer 10X 2 mM $MgCl_2$ 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: TATGATATCCATAATAATTATCCAATC R: AAGTTATATCATGAATAGATTGTCCTGTT	595		
dfrA1	F: CTCACGATAAACAAAGAGTCA R: CAATCATTGCTTCGTATAACG	201		
linA	F: GGTGGCTGGGGGGTAGATGTATTAACTGG R: GCTTCTTTTGAAATACATGGTATTTTTCGA	323	1 cycle: 94°C ——— 6 min. 30 cycles: 95°C ——— 60 s 57°C ——— 60 s 72°C ——— 60 s 1 cycle: 72°C ——— 10 min	5 µL PCR buffer 10X 2 mM $MgCl_2$ 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: TGAACCGTATGTTAGTGC R: GTCGTGTTAGCGTTGATA	681	1 cycle: 94°C ——— 6 min 30 cycles: 95°C ——— 60 s 59°C ——— 60 s 72°C ——— 60 s 1 cycle: 72°C ——— 10 min	5 µL PCR buffer 10X 2 mM $MgCl_2$ 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 1 (Continued).

Target Gene	Primer Sequence (5′-3′)	PCR Product (bp)	PCR Programs	PCR Volume (50 µL)
cfr	F: TGAAGTATAAAGCAGGTTGGGAGTCA R: ACCATATAATTGACCACAAGCAGC	746	1 cycle: 94°C ——— 1 min. 34 cycles: 94°C ——— 2 min 48°C ——— 60 s 72°C ——— 3 min 1 cycle: 72°C——— 10 min	5 µL PCR buffer 10X 2 mm $MgCl_2$ 150 µm dNTP (Fermentas) 0.75 µm of each primers F & R 1.5 U Taq DNA polymerase (Fermentas) 3 µL DNA template
rpoB	F: ACCGTCGTTTACGTTCTGTA R: TCAGTGATAGCATGTGTATC	460	1 cycle: 94°C ——— 5 min 40 cycles: 94°C ——— 40 s 45.5°C ——— 40 s 72°C ——— 90 s 1 cycle: 72°C ——— 8 min	5 µL PCR buffer 10X 2 mM $MgCl_2$ 150 µm dNTP (Fermentas) 0.75 µm of each primers F & R 1.5 U Taq DNA polymerase (Fermentas) 3 µL DNA template
SCC*mec*I	F: GCTTTAAAGAGTGTCGTTACAGG R: GTTCTCTCATAGTATGACGTCC	613	1 cycle: 94°C ——— 5 min 35 cycles: 94°C ——— 45 s 65°C ——— 45 s 72°C ——— 90 s 1 cycle: 72°C ——— 10 min	5 µL PCR buffer 10X 2 mM $MgCl_2$ 150 µm dNTP (Fermentas) 0.75 µm of each primers F & R 1.5 U Taq DNA polymerase (Fermentas) 3 µL DNA template
SCC*mec*II	F: CGTTGAAGATGATGAAGCG R: CGAAATCAATGGTTAATGGACC	398		
SCC*mec*III	F: CCATATTGTGTACGATGCG R: CCTTAGTTGTCGTAACAGATCG	280		
SCC*mec*Iva	F: GCCTTATTCGAAGAAACCG R: CTACTCTTCTGAAAAGCGTCG	776		
SCC*mec*IVb	F: TCTGGAATTACTTCAGCTGC R: AAACAATATTGCTCTCCCTC	493		
SCC*mec*IVc	F: ACAATATTTGTATTATCGGAGAGC R: TTGGTATGAGGTATTGCTGG	200		
SCC*mec*IVd	F: CTCAAAATACGGACCCCAATACA R: TGCTCCAGTAATTGCTAAAG	881		
SCC*mec*V	F: GAACATTGTTACTTAAATGAGCG R: TGAAAGTTGTACCCTTGACACC	325		

Note: Fermentas is part of Thermo Fisher Scientific, Waltham, MA, USA.

Distribution of SCC*mec* Types

Table 5 signifies the incidence of SCC*mec* types amongst the MRSA bacteria recovered from diverse kinds of raw milk samples. SCC*mec* IVa (29.62%), V (25%), III (14.81%) and IVb (11.11%) were the most routinely identified kinds amongst the MRSA bacteria. Incidence of SCC*mec* IVd (3.70%) and II (3.70%) was low. Arithmetical important difference was seen between the incidence of SCC*mec* 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.[34] Furthermore, surveys on

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 (72.723)
	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)

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 al[17] and Safarpoor Dehkordi et al[20] 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] cephems,[19,20,40–42] penicillins,[19,20,40–42] macrolides,[19,20,40–42] tetracyclines,[19,20,40,41] fluoroquinolones,[19,20,40–43] lincosamides,[19,20,40–42] folate inhibitors,[19,20,40–43] phenicols[19,20,40,41] and ansamycins[19,20,40,41] antibiotic agents. Fowoyo and Ogunbanwo[44] 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 al[45] 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 investigation[47] 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 *msrA* antibiotic resistance markers likewise to our survey.

Assess the distribution of SCC*mec* types is a practical method to find presence of HA-MRSA and CA-MRSA bacteria. Findings of epidemiological investigations revealed that presence of SCC*mec* 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 SCC*mec* 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, SCC*mec* 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, SCC*mec* 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 3 Antibiotic Resistance Pattern of the MRSA Bacteria Recovered from Raw Milk Samples

Type of Raw Milk Samples (N of MRSA Bacteria)	Isolates Resistant to Each Antibiotic n (%)													
	Penicillins	Aminoglycosides		Macrolides		Tetracyclines		Fluoroquinolones		Lincosamides	Folate Inhibitors	Phenicols	Ansamycins
	P10	Gen	Amk	Azi	Ert	Tet	Dox	Cip	Lev	Clin	Tr-Sul	C30	Rif
Bovine (8)	8 (100)	7 (87.50)	2 (25)	3 (37.50)	7 (87.50)	8 (100)	6 (75)	3 (37.50)	2 (25)	3 (37.50)	8 (100)	3 (37.50)	1 (12.50)
Ovine (7)	7 (100)	5 (71.42)	1 (14.28)	2 (28.57)	5 (71.42)	7 (100)	4 (57.14)	2 (28.57)	2 (28.57)	2 (28.57)	5 (71.42)	2 (28.57)	–
Caprine (4)	4 (100)	3 (75)	–	1 (25)	3 (75)	4 (100)	3 (75)	1 (25)	1 (25)	1 (25)	3 (75)	1 (25)	1 (25)
Camel (1)	1 (100)	–	–	–	–	1 (100)	–	–	–	–	–	–	–
Buffalo (8)	8 (100)	7 (87.50)	2 (25)	3 (37.50)	8 (100)	8 (100)	7 (87.50)	4 (50)	4 (50)	4 (50)	6 (75)	2 (25)	2 (25)
Total (28)	28 (100)	22 (78.57)	5 (17.85)	9 (32.14)	23 (82.14)	28 (100)	20 (71.42)	10 (35.71)	9 (32.14)	12 (42.85)	22 (78.57)	8 (28.57)	4 (14.28)

Abbreviations: P10, penicillin (10 µg/disk); Gen, gentamicin (10 µg/disk); Amk, amikacin (30 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); Lev, levofloxacin (5 µg/disk); Clin, clindamycin (2 µg/disk); Tr-Sul, trimethoprim-sulfamethoxazole (25 µg/disk); C30, chloramphenicol (30 µg/disk); Rif, rifampin (5 µg/disk).

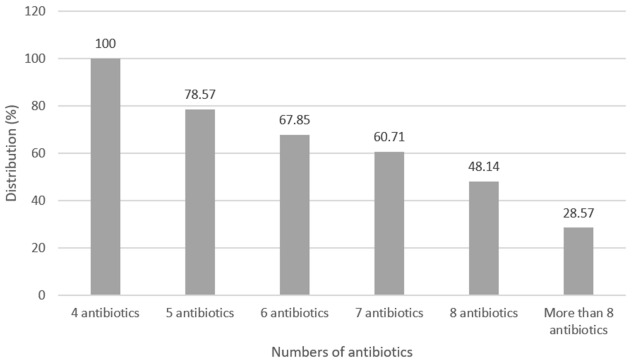

Figure 1 Distribution of multidrug resistant MRSA bacteria recovered from different types of raw milk. Multidrug resistant MRSA bacteria were determined as those who had at least simultaneous resistance toward three or more than three types of antibiotics.

of the milking halls. Johnson[50] reported similar results for the boost incidence of SCC*mec* IV in retail meat samples. In a survey which was carried out by Vossenkuhl et al[51] most of MRSA bacteria recovered from turkey meat samples carried SCC*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[52] reported a the high prevalence of SCC*mec* III in their food samples. Boost incidence of SCC*mec* types IVa and V in food samples with animal origin has also been reported previously.[38,53,54]

Conclusions

By and large, we recognized boost incidence of *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*mec* types. The existing survey is the first report of the genotypic evaluation of antibiotic resistance and SCC*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 *blaZ, tetK, dfrA1, aacA-D ermA* and *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*mec* types IV and V which may have assumed their possible community-acquired origins. However, some of the MRSA bacteria harbored SCC*mec* types I, II, and III which may assume their

Table 4 Distribution of Antibiotic Resistance Genes Amongst the MRSA Bacteria Recovered from Raw Milk

Type of Raw Milk Samples (N of MRSA Bacteria)	Isolates Harbor Each Gene n (%)														
	Penicillins	Aminoglycosides	Macrolides					Tetracyclines		Fluoroquinolones		Lincosamides	Folate Inhibitors	Phenicols	Ansamycins
	blaZ	*aacA-D*	*ermA*	*ermB*	*msrA*	*msrB*	*mefA*	*tetK*	*tetM*	*gyrA*	*grlA*	*linA*	*dfrA1*	*cfr*	*rpoB*
Bovine (8)	8 (100)	6 (75)	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)	2 (25)	7 (87.50)	2 (25)	1 (12.50)
Ovine (7)	7 (100)	5 (71.42)	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)	2 (28.57)	5 (71.42)	2 (28.57)	–
Caprine (4)	4 (100)	2 (50)	2 (50)	1 (25)	1 (25)	–	2 (50)	3 (75)	1 (25)	1 (25)	1 (25)	1 (25)	2 (50)	1 (25)	1 (25)
Camel (1)	1 (100)	–	–	–	–	–		1 (100)	–	–	–	–	–	–	–
Buffalo (8)	8 (100)	6 (75)	5 (62.50)	2 (25)	3 (37.50)	1 (12.50)	4 (50)	7 (87.50)	4 (50)	4 (50)	3 (37.50)	3 (37.50)	6 (75)	2 (25)	1 (12.50)
Total (28)	28 (100)	19 (67.85)	14 (50)	7 (25)	10 (35.71)	3 (10.71)	10 (35.71)	24 (85.71)	10 (35.71)	12 (42.85)	8 (28.57)	8 (28.57)	20 (71.42)	7 (25)	3 (10.71)

Table 5 Distribution of SCC*mec* Types Amongst the MRSA Bacteria Recovered from Raw Milk

Type of Raw Milk Samples (N of MRSA Bacteria)	Isolates Harbor Each SCC*mec* Type n (%)							
	I	II	III	IV				V
				a	b	c	d	
Bovine (8)	1 (12.50)	–	2 (25)	3 (37.50)	1 (12.50)	–	–	1 (12.50)
Ovine (7)	–	–	1 (14.28)	1 (14.28)	1 (14.28)	1 (14.28)	1 (14.28)	2 (28.57)
Caprine (4)	–	–	–	1 (25)	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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