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ORIGINAL ARTICLE

Prevalence, antimicrobial resistance, virulence gene distribution and SCCmec typing of methicillin-resistant *Staphylococcus aureus* isolated from raw milk and dairy products

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ABSTRACT

Background and Objectives: Researchers have focused on *Staphylococcus aureus* because it is transmitted through food, such as milk and dairy products, and causes human diseases. Prevalence, antimicrobial resistance, presence, and distribution of methicillin-resistant *S. aureus* (MRSA) virulence genes isolated from raw milk and dairy products were evaluated.
Materials and Methods: 300 samples of dairy products were collected from Shahrekord, Iran. *S. aureus* was identified using biochemical tests and screened for sensitivity to 13 antibiotics to identify resistance genes. In addition, SCCmec typing was performed.
Results: Out of 300, *S. aureus* was found in 82 samples. Raw milk had the highest contamination with *S. aureus* (60 of 82), followed by cheese (15 of 82), and butter (7 of 82). At least one resistance gene was present in every isolate of *S. aureus.* Virulence factors and enterotoxin-coding genes, such as *sea, seb, sec,* and *sed* were highly distributed.
Conclusion: The results of this study revealed the presence of toxin-producing MRSA strains in raw milk and dairy products. MRSA in dairy farms is an important risk factor for the spread of staphylococcal infections; therefore, further studies are needed to find strategies for controlling the presence of *S. aureus*, especially MRSA, in dairy products.

Keywords: Methicillin-resistant *Staphylococcus aureus*; Drug resistance; Pathogenicity; Dairy products; Milk

INTRODUCTION

Staphylococcus aureus has the potential to become an opportunistic pathogen in different host species, including humans and animals. Foods derived from animals are significantly more vulnerable to contamination by *S. aureus* (1). This pathogen is an important factor in the spread of diseases related to food consumption, including milk (2). *S. aureus* is one of the most common foodborne pathogens and is among the leading causes of foodborne outbreaks worldwide. Staphylococcal foodborne poisoning is caused by the ingestion of food containing SEs. Symptoms include nausea, vomiting, abdominal cramps, and diarrhea. The classical antigenic-based classification of SEs includes five serological types: SEA, SEB, SEC,

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SED, and SEE. In recent years, new types of SE and SE-like toxins (SEG, SEH, SEI, SElJ, SElK, SElL, SElM, SElN, SElO, SElP, SElQ, SElR, and SElU) have been reported. These new toxins have been identified based on their sequence similarity with classical SEs, but their emetic properties are still unknown (3). Assessing the potential consequences of this microorganism's presence on food safety and public health requires distinguishing between virulent and nonvirulent strains (4). SEs are highly thermostable and resistant to many proteolytic enzymes and different environmental conditions. The most common SE associated with foodborne illness is SEA. Although pasteurization eliminates staphylococci, it has no effect on SEs. Raw milk, subjected only to filtration, and other unpasteurized dairy products may contain enterotoxigenic *S. aureus* strains (5). The consumption of *S. aureus*-infected milk and dairy products is the leading cause of many gastrointestinal poisonings in humans (6). In general, *S. aureus* can enter milk either by direct excretion from the udder of a cow with clinical and subclinical staphylococcal mastitis or by contamination from the environment during handling and processing of raw milk. Raw milk is a vehicle for the transmission of numerous bacteria of human and animal origin and represents a public health hazard (7). The different groups differ in antibiotic resistance, SCCmec's location and size, and the PVL gene presence. Antimicrobials being misused in dairy farms, whether for therapeutic or preventive purposes, can encourage resistance mechanisms in S. aureus, speeding up the emergence of multidrug-resistant strains such as MRSA (8). Multidrug-resistant (MDR) strains have emerged due to the extensive use of antibiotics in veterinary medicine. One of the concerns of the World Health Organization is the spread of methicillin-resistant *S. aureus* (MRSA). Methicillin-resistant staphylococci (MRS) have a penicillin-binding protein (PBP2a) encoded by *mecA* or *mecC*. This protein makes *S. aureus* resistant to beta-lactam antibiotics (9). Staphylococcal chromosome cassette mec (SCCmec) is a mobile genetic element that contains the *mecA* gene (10). Consuming contaminated dairy products can colonize antibiotic-resistant bacteria in the digestive tract without symptoms. The administration of antimicrobial therapy during the colonization phase can cause severe clinical disease. Antibiotic-resistant foodborne pathogens may overwhelm the beneficial gut microbiota (11). There are two ways to transfer pathogenic microorganisms from farms to raw milk: 1) Milk-producing animals and 2) Environment. The cow's teat skin, nasal cavity, and rectum are contaminated by *S. aureus*. The important sources of transmission of infection in the lactating herd are contaminated udders and teat skin. During milking, infected animals can transmit the bacteria through their milk. The microorganism will then be transferred from one udder to another through contact with contaminated milking machines, farmer's hands, or contaminated litter (12). Therefore, surveillance of MRSA from farm to fork and comparing strains present in livestock with strains sourced from human food is highly recommended globally (13). The aim of this study was to assess the presence of *S. aureus* strains in raw milk and dairy products, to characterize the isolates by testing their antimicrobial susceptibility, presence of SCC*mec* gene in MRSA isolates, and assess the enterotoxigenic activity of raw milk and dairy products isolates from Shahrekord, Iran.

MATERIALS AND METHODS

Sampling. In this cross-sectional descriptive study, 300 dairy product samples were collected from Shahrekord City, Iran. The samples included milk (n=150), traditional cheese (n=90), and butter (n=60).

Isolation of *S. aureus* and investigation of phenotypic characteristics. With culture *S. aureus*, 500 µL of each raw milk sample was combined with 5 ml of Brain Heart Infusion (BHI) (Merck, Germany). For 24 hours, the mixture was kept at 37°C to encourage bacterial growth. To isolate *S. aureus*, the process involved different methods for cheese and butter samples. For cheese, 25 g of the sample was placed in a sterile mortar with sterile white sand. It was crushed and diluted with 225 ml of 2% sterile sodium citrate solution. Each sample of butter was allowed to melt at room temperature or in a thermostatically controlled water bath at 44ºC for under 15 minutes. 5 ml of the resulting sample was inoculated into a 50 ml Trypticase Soy Broth (TSB, Merck, Germany) medium with 10% NaCl and 1% sodium pyruvate for 18 hours. The incubation temperature for these cultures was 35°C. Following this, a loopful of the cultured material was inoculated onto Baird-Parker agar (BPA, Merck, Germany) containing egg yolk

tellurite emulsion. At 37°C, the media were incubated for 24 hours. The identification of *S. aureus* colonies was based on their black and shiny appearance, with clear marginal zones measuring 2-5 mm. Standard identification tests were then performed, which included Gram staining, catalase, oxidase, mannitol fermentation, DNase activity, sugar fermentation tests, and growth on MRSA chromagar. Furthermore, MRSA-positive isolates on MRSA Chromagar were confirmed using PCR amplification of the *mecA* gene (14).

Antimicrobial resistance determination of MRSA strains. The Clinical Laboratory Standard Institute (CLSI, 2021) guidelines were utilized to evaluate the antimicrobial resistance pattern of the isolated MRSA bacteria. Muller Hinton Agar media (MHA, Merck, Germany) were used to culture these bacteria. Antimicrobial disks, including penicillin (10 µg), tetracycline (10 µg), enrofloxacin (5 µg), ciprofloxacin (5 µg), trimethoprim (5 µg), cotrimoxazole (25 µg), cephalexin (30 µg), chloramphenicol (30 µg), nitrofurantoin (300 µg), gentamicin (10 µg), oxacillin (5 µg), cephalothin (30 µg), and erythromycin (15 µg) (HiMedia, India), were placed on the agar plates. The discs were placed on plates containing bacteria with a concentration of 0.5 McFarland. For 24 hours, they were kept at 37°C for incubation. After incubation, the diameter of growth inhibitory zones around the bacteria was measured and compared with the CLSI standard scale. As a control, *S. aureus* ATCC 29213 was used (15).

Antibiotic resistance genes, virulence factors and SCCmec typing. The culture of MRSA strains in TSB was followed by incubation at 37°C for 48 hours. DNA extraction kit (Thermo Fisher Scientific, Massachusetts, USA) was utilized to extract genomic DNA. The extracted DNA was evaluated through nanodrop (Thermo Scientific, USA) and 2% agarose gel electrophoresis. Table 1 details the primer sequences and PCR components used to detect genes related to antibiotic resistance, virulence factors, and SCCmec typing.

SCCmec types. The Zhang et al. (16). method was utilized to detect the SCCmec types. The primer sequences and PCR program are displayed in Table 1.

Statistical analysis. Analysis was done by transferring the data to a Microsoft Excel (version 15). The statistical analysis of the extracted data was done using SPSS (version 16). To achieve this, a chi-square test was used. These tests were utilized to investigate statistical differences in sample types, *S. aureus* prevalence, antibiotic resistance, and virulence genes. The level of $p < 0.05$ was deemed significant.

RESULTS

Isolation and identification of *S. aureus*. Out of the 300 milk and dairy products considered, 82 *S. aureus* isolates were identified (27.33%). Table 2 provides an overview of the incidence of *S. aureus* among the studied samples. *S. aureus* was the most prevalent in milk (60 from 82), followed by cheese (15 from 82) and butter (7 from 82). 57 out of the 82 *S. aureus* isolates from various sample types were confirmed to be MRSA. Among the 57 MRSA isolates, 39 were isolated from milk, 11 from cheese, and 7 from butter.

Antimicrobial resistance determination. Table 3 presents the antibiotic resistance pattern of MRSA strains. The results reveal that MRSA strains exhibited the highest resistance to oxacillin and cefotaxime (100%), followed by tetracycline (94.73%) and penicillin (87.71%). On the other hand, the highest sensitivity among all isolates was observed to nitrofurantoin (89.47%) and chloramphenicol (84.21%).

Antimicrobial resistance and virulence gene determination. Multiple antimicrobial resistance (*aacA-D, tetK, tetM, msrA, vatA, vatB, vatC, ermA, ermC, msrB,* and *linA*) and virulence genes (*sea, seb, sec, sed, Coa, clfA, X-region, IgG* binding region, *tsst-1, etA, etB, agrI, agrII, agrIII,* and *PVL*) were screened among MRSA isolated from milk and dairy products as shown as Fig. 1. Table 4 shows the frequency of antibiotic resistance and virulence genes. Among the findings, the gene *msrA* was identified in a high percentage (100%) of MRSA isolates, with 87.72% of isolates carrying the resistance genes *Coa* and *etA.* Additionally, 85.96% of isolates were found to possess *aacA-D* and *clfA* genes.

SCCmec typing. Table 5 shows the incidence of SCCmec alleles in MRSA strains recovered from different samples. The most prevalent alleles of SCCmec

Table 1. Primers and PCR components to detect antimicrobial resistance, virulence factor genes, and SCCmec typing (15)

Genes	Sequence (5'-3')	Size (bp)	PCR volume (50 µl)	PCR program
mecA	F: AAAATCGATGGTAAAGGTTGGC	533		
	R: AGTTCTGGAGTACCGGATTTGC			
aacA-D	F: TAATCCAAGAGCAATAAGGGC	227		
	R: GCCACACTATCATAACCACTA			
tetK	F: GTAGCGACAATAGGTAATAGT	360		
	R: GTAGTGACAATAAACCTCCTA			
tetM	F: AGTGGAGCGATTACAGAA	158		
	R: CATATGTCCTGGCGTGTCTA			
msrA	F: GGCACAATAAGAGTGTTTAAAGG	156	5 µl PCR buffer 10X, 1.5 mM	(94 °C: 5 min, followed
	R: AAGTTATATCATGAATAGATTGTCCTGTT		MgCl2, 250 µM dNTP, 1 µM of	by 30 cycles of 95°C: 1
ermA	F: AAGCGGTAAACCCCTCTGA	940	each primer, 1.25 U Taq	min, 55°C: 1 min, 72°C:
	R: TTCGCAAATCCCTTCTCAAC		polymerase, 5 µl DNA template	60 s, 72°C: 7 min)
ermC	F: AATCGTCAATTCCTGCATGT	190		
	R: AATCGTCAATTCCTGCATGT			
vatA	F: TGGTCCCGGAACAACATTTAT	299		
	R: TCCACCGACAATAGAATAGGG			
vatB	F: GCTGCGAATTCAGTTGTTACA	268		
	R: CTGACCAATCCCACCATTTTA			
vatC	F: AAGGCCCCAATCCAGAAGAA	136		
	R: TCAACGTTCTTTGTCACAACC			
linA	F: GGTGGCTGGGGGGTAGATGTATTAACTGG	467		
	R: GCTTCTTTTGAAATACATGGTATTTTTCGA			
Sea	F: TTGGAAACGGTTAAAACGAA	323		(94°C: 2 min, followed by
	R: GAACCTTCCCATCAAAAACA			30 cycles of 94°C: 1 min,
Seb	F: TCGCATCAAACTGACAAACG	120		55°C: 2 min, 72°C: 1 min,
	R: GCAGGTACTCTATAAGTGCC			72°C: 8 min)
Sec	F: GACATAAAAGCTAGGAATTT	478		
	R: AAATCGGATTAACATTATCC			
sed	F: CTAGTTTGGTAATATCTCCT	257	2.5 µl PCR buffer 10X, 1 mM	
	R: TAATGCTATATCTTATAGGG		MgCl2, 200 µM dNTP, 1 µM of	
Coa	F: CGAGACCAAGATTCAACAAG	730	each primer, 1 U Taq	
	R: AAAGAAAACCACTCACATCA		polymerase, 3 µl DNA template	
IgG binding region	F: CACCTGCTGCAAATGCTGCG	920		(94°C: 2 min, followed by
	R: GGCTTGTTGTTGTCTTCCTC			30 cycles of 94°C: 1 min,
clfA	F: GGCTTCAGTGCTTGTAGG	980		58°C: 1 min, 72°C: 1 min,
	R: TTTTCAGGGTCAATATAAGC			72°C: 5 min)
X-region	F: CAAGCACCAAAAGAGGAA	320		
	R: CACCAGGTTTAACGACAT			
tsst-1	F: ATGGCAGCATCAGCTTGATA	350		
	R: TTTCCAATAACCACCCGTTT			
etA	F: ACGGCTATATACATTCAATT	119		
	R: TCCATCGATAATATACCTAA			
etB	F: ATGCACATGGTGCACATGC	200	5 µl PCR buffer 10, 2 mM	(94°C: 6 min, followed by
	R: GTCACAAGTACTATAAGCTGCGAT		MgCl2, 200 µM dNTP, 0.4 µM	30 cycles of 94°C: 2 min,
agrI	F: ATGCACATGGTGCACATGC	441	of each primer, 1 U Taq	55 °C: 2 min, 72°C: 1 min,
	R: TATTACTAATTGAAAAGTGGCCATAGC		polymerase, 3 µl DNA template	72°C: 8 Min)

Table 1. Continuing...

agrII	F: ATGCACATGGTGCACATGC R: GTAATGTAATAGCTTGTATAATAATACCCAG	575		
agrIII	F: ATGGCAGCATCAGCTTGATA R: TTTCCAATAACCACCCGTTT F:	323		
PVL	TGGTCCCGGAACAACATTTAT R: TCCACCGACAATAGAATAGGG	433		
SCCmec I	F: GCTTTAAAGAGTGTCGTTACAGG R: GTTCTCTCATAGTATGACGTCC	613		
SCCmec II	F- CGTTGAAGATGATGAAGCG R- CGAAATCAATGGTTAATGGACC	398		
SCCmec III	F: CCATATTGTGTACGATGCG R: CCTTAGTTGTCGTAACAGATCG	280		
SCCmec IVa	R: CTACTCTTCTGAAAAGCGTCG F: TCTGGAATTACTTCAGCTGC	776	5 µl PCR buffer 10, 2 mM MgCl2, 200 µM dNTP, 0.4 µM of each primer, 1 U Taq polymerase, 3 µl DNA template	(94°C: 5 min, followed by 30 cycles of 94°C: 45 s, 55°C: 45 s, 72°C: 1 min, 72°C: 4 min)
SCCmec IVb	F: TCTGGAATTACTTCAGCTGC R: AAACAATATTGCTCTCCCTC	493		
SCCmec IVc	F: ACAATATTTGTATTATCGGAGAGC R: TTGGTATGAGGTATTGCTGG	200		
SCCmec IVd	F: CTCAAAATACGGACCCCAATACA R: TGCTCCAGTAATTGCTAAAG	881		
SCCmec V	F: GAACATTGTTACTTAAATGAGCG R: TGAAAGTTGTACCCTTGACACC	325		

Table 2. The prevalence of *S. aureus* and MRSA among the milk and dairy product samples

Samples	*S. aureus* No (%)	MRSA No (%)
Milk	60 (73.17%)	39 (68.42%)
Cheese	15 (18.29%)	11 (19.29%)
Butter	7 (8.53%)	7 (12.28%)
Total	82 (27.33%)	57 (69.51%)

were SCCmec III (50.87%), SCCmec IVa (33%) and SCCmec V (24.56%). Milk samples had the highest prevalence of SCCmec's.

DISCUSSION

In this study, we isolated *S. aureus* strains from 82 (27.33%) of the 300 raw milk and dairy products

Table 3. Distribution of antibiotic resistance pattern in MRSA strains isolates No (%)

Samples	Pen	Tet	Ery	Enr	Cip	Tri	Cot	Cep	Chl	Nit	Gen	Oxa	Cef
Milk (n=39)	32 (82.05%)	36 (92.30%)	21 (53.84%)	22 (56.41%)	20 (51.28%)	23 (58.97%)	20 (51.28%)	18 (46.15%)	7 (17.94%)	3 (7.69%)	18 (46.15%)	39 (100%)	39 (100%)
Cheese (n=11)	11 (100%)	11 (100%)	7 (63.63%)	7 (63.63%)	7 (63.63%)	5 (45.45%)	6 (54.54%)	8 (72.72%)	1 (9.09%)	2 (18.18%)	7 (63.63%)	11 (100%)	11 (100%)
Butter (n=7)	7 (100%)	7 (100%)	3 (42.85%)	4 (57.14%)	4 (57.14%)	4 (57.14%)	3 (42.85%)	4 (57.14%)	1 (14.28%)	1 (14.28%)	3 (42.85%)	7 (100%)	7 (100%)

Pen: penicillin, Tet: tetracycline, Ery: erythromycin, Enr: enrofloxacin, Cip: ciprofloxacin, Tri: trimethoprim, Cot: cotrimoxazole, Cep: cephalexin, Chl: chloramphenicol, Nit: nitrofurantoin, Gen: gentamicin, Oxa: oxacillin, Cef: cephalothin.

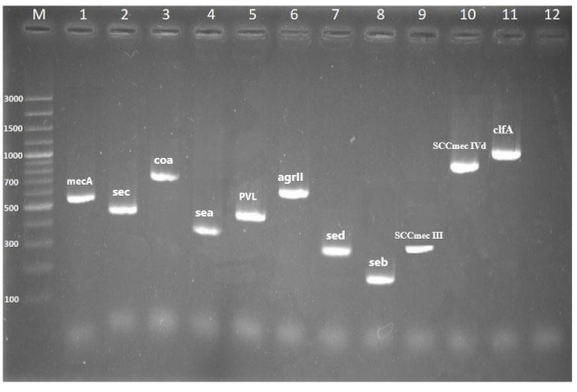

Fig. 1. Polymerase Chain Reaction. M: Marker 100bp, Lane 1: *mecA* (533 bp), Lane 2: *sec* (478 bp), lane 3: *coa* (730 bp), lane 4: *sea* (323 bp), Lane 5: *PVL* (433 bp), Lane 6: *agrII* (57 5bp), *Lane* 7: *sed* (257 bp), Lane 8: *seb* (120 bp), Lane 9: *SCCmecIII* (280 bp), Lane 10: *SCCmecIVd* (881 bp), Lane 11: *clfA* (980 bp), Lane 12: Negative control.

samples collected in Shahrekord, Iran. The detection of antimicrobial-resistant microorganisms in raw milk and dairy products is a highly important issue for public health because of possible spread of these microorganisms through the dairy food chain. Raw milk is a significant source of oxacillin-resistant, *mecA*-positive strains of *S. aureus* (17). Although the role of food as a vehicle of human infection by MRSA is currently regarded as secondary, MRSA strains are able to evolve very quickly, and characteristics such as virulence and transmissibility can change. Therefore, transmission of MRSA strains from animals to humans and vice versa has the potential to introduce new pathogenic strains into these populations (18). The patterns of multidrug resistance observed in the MRSA strains are an additional pathogenic factor and a risk for the spread of isolates able to cause infections that are difficult to treat. Although MRSA is comparatively rare in food, raw milk and dairy products might be one of the possible sources of MRSA because of wide use of antimicrobials for the treatment of mammary infections in cattle (19). The presence of MRSA strains in raw milk and dairy products has been reported in various countries, including Italy, Germany, England, and Turkey, and these data are consistent with in the results of the present study (20-23). Among the *S. aureus* isolates we obtained from raw milk and dairy products, 69.51% were MRSA, which is more than results reported by Benedetti et al. (20) who found MRSA among 13% of 413 *S. aureus* isolates in the province of Lodi, Italy.

Table 4. Distribution of antibiotic resistance and virulence factor genes in MRSA strains isolates No (%)

Sample	Antibiotic Resistance Genes																							
	mecA	*aacA-D*	*tetK*	*tetM*	*msrA*	*ermA*	*ermC*	*vatA*	*vatB*	*vatC*	*linA*	*sea*	*seb*	*sec*	*sed*	*coa*	*IgG binding region*	*clfA*	*X-region*	*tsst-1*	*etA*	*etB*	*agrI*	*agrII*	*agrIII*	*PVL*
Milk	39	36	21	20	39	30	33	14	13	14	14	21	20	17	19	33	27	33	27	6	35	13	14	13	9	19
(n=39)	(100%)	(92.30%)	(53.84%)	(51.28%)	(100%)	(76.92%)	(84.61%)	(35.89%)	(33.33%)	(35.89%)	(35.89%)	(53.84%)	(51.28%)	(43.58%)	(48.71%)	(84.61%)	(69.23%)	(84.61%)	(69.23%)	(15.38%)	(89.74%)	(33.33%)	(35.89%)	(33.33%)	(23.07%)	(48.71%)
Cheese	11	10	2	4	11	7	9	4	3	5	3	1	1	4	4	11	4	10	4	0	10	3	2	2	3	4
(n=11)	(100%)	(90.90%)	(18.18%)	(36.36%)	(100%)	(63.63%)	(81.81%)	(36.36%)	(27.27%)	(45.45%)	(27.27%)	(9.09%)	(9.09%)	(36.36%)	(36.36%)	(100%)	(36.36%)	(90.90%)	(36.36%)	(0%)	(90.90%)	(27.27%)	(18.18%)	(18.18%)	(27.27%)	(36.36%)
Butter	7	3	2	1	7	3	6	1	2	3	2	1	1	4	1	6	1	6	1	1	5	1	0	1	2	1
(n=7)	(100%)	(42.85%)	(28.57%)	(14.28%)	(100%)	(42.85%)	(85.71%)	(14.28%)	(28.57%)	(42.85%)	(28.57%)	(14.28%)	(14.28%)	(57.14%)	(14.28%)	(85.71%)	(14.28%)	(85.71%)	(14.28%)	(14.28%)	(71.42%)	(14.28%)	(0%)	(14.28%)	(28.57%)	(14.28%)

Table 5. Distribution of SCCmec types in MRSA strains isolates No (%)

Types of Samples	SCCmec types							
	I	II	III	IVa	IVb	IVc	IVd	V
Milk (n=39)	-	-	20 (51.28%)	14 (35.89%)	5 (12.82%)	-	-	8 (20.51%)
Cheese (n=11)	-	-	5 (45.45%)	4 (36.36%)	2 (18.18%)	-	-	5 (45.45%)
Butter (n=7)	-	-	4 (57.14%)	1 (14.28%)	3 (42.85%)	-	-	1 (14.28%)
Total (n= 57)	-	-	29 (50.87%)	19 (33.33%)	10 (17.54%)	-	-	14 (24.56%)

Similar results were reported in Turkey (23): 17% of 93 *S. aureus* isolates were MRSA. The 69.51% prevalence of MRSA in our samples (57 of 82 samples) is more than 2.3% prevalence reported in England (22) and Germany (21). The molecular characteristics of eight MRSA strains were investigated in terms of SCCmec types I, II, III, Iva, IVb, IVc, IVd, and V. 43 (75.43%) of strains had SCC*mec* types Iva, b, and V, typical of CA-MRSA. 29 (50.87%) of strains had SCCmec type III, characteristic of HA-MRSA. Gene cassettes typically found in the health care environment have also been found in other studies. In Japan, Hata et al. (24) detected SCC*mec* types II and III, and in Turkey Türkyılmaz et al. (23). found SCC*mec* III in 87.5% of MRSA strains isolated from bovine milk. The detection of SCC*mec* types IV and V in this study suggests the emergence of CA-MRSA strains in this geographical area, and the presence of SCC*mec* type III might indicate a possible transmission from humans to animals (23). A high percentage of *S. aureus* strains found in our study harbored the genes that encode for enterotoxins involved in staphylococcal food poisoning. We found the *sea*, *seb*, *sec*, and *sed* gene in 40.35%, 38.59%, 43,85%, and 42.10% of the toxigenic isolates. This finding corroborates the data obtained by others who reported that *sec* was the most common SE produced by *S. aureus* isolates from milk-producing animals. The prevalence of toxigenic *S. aureus* in raw milk in Italy is also important because many typical Italian cheeses, both fresh and seasoned, are produced from unpasteurized milk (25-27). Differences in the prevalence of toxigenic *S. aureus* strains have been observed among geographical areas, Jorgensen et al. (28). found a 22.1% prevalence of SE-producing strains in bovine milk in Norway; Zouharova and Rysanek (28) found a 12.9% prevalence of enterotoxigenic *S. aureus* strains in bulk tank milk in the Czech Republic, and Neder et al. (29). detected 11.7% prevalence of enterotoxigenic *S. aureus* strains in bulk tank milk in Argentina. These differences in the prevalence of enterotoxin production might be explained by the techniques used in these studies, the origin of the isolates, and the geographical location. According to the results of the antibiotic resistance pattern, all isolates can resist oxacillin and cefotaxime (100%) and most of them can resist tetracycline (92.3%). The recent findings prove that the rate of antibiotic resistance is alarming. This study's resistance to penicillin was 82.05%, which is very similar to the Liu et al. study (30). This design of multidrug resistance, especially methicillin resistance (MR), is becoming more prevalent worldwide. The use of antibiotics in farming has been associated with the emergence of resistance. Veterinary prescriptions are not required to purchase most antibiotics in developing countries like Iran, which are cheap and easy to find (31).

CONCLUSION

In conclusion, our data suggest that surveillance and monitoring of *S. aureus* in food of animal origin is needed. MRSA strains in livestock and the correct use of antibiotics should be evaluated and monitored to prevent and limit evolutionary pressure that could favor the spread of antimicrobial-resistant microorganisms.

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