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Antimicrobial Resistance Properties And Enterotoxigenic Gene Profile Of Methicillin-Resistant And Methicillin-Susceptible Staphylococcus Aureus Isolates From Raw Milk

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Abstract
Both methicillin-resistant and methicillin-susceptible Staphylococcus aureus bacteria are emerging causes of food-borne diseases. Raw milk of animal species may consider a reservoir of MRSA and MSSA strains. The present survey was done to assess the prevalence and antibiotic resistance properties of MRSA and MSSA strains isolated from raw milk samples of animal species. Three hundred and eighty raw milk samples were collected from cow, sheep, and goat species. S. aureus was identified using culture and biochemical tests. MRSA and MSS identification were done according to cefoxitin and oxacillin antibiotic resistance and mecA gene presence. The pattern of antibiotic resistance was determined by disk diffusion. The distribution of antibiotic resistance genes was determined using PCR. Forty-two out of 380 (11.05%) raw milk samples were contaminated with S. aureus. MRSA and MSSA strains were identified in 64.28% and 35.72% of S. aureus isolates. MRSA isolates harbored the uppermost resistance rate toward tetracycline (100%), penicillin (100%), trimethoprim-sulfamethoxazole (66.66%), erythromycin (66.66%), and ciprofloxacin (66.66%). MSSA isolates harbored the uppermost resistance rate toward tetracycline (46.66%), erythromycin (46.66%), penicillin (40%), azithromycin (40%), and gentamicin (40%). BlaZ (100%), aacA-D (62.96%), tetK (51.85%). cat1 (48.14%), and dfrA1 (44.44%) were the most commonly detected antibiotic resistance genes amongst the MRSA, while aacA-D (26.66%), ermA (26.66%), msrA (26.66%), tetK (26.66%), and gyrA (26.66%) were the most commonly detected amongst the MSSA strains. Raw milk samples may be sources of resistant MRSA and MSSA, which pose a hygienic threat in their consumption. MRSA harbored a higher prevalence of resistance and also the distribution of antibiotic resistance genes.

Keywords: Methicillin-resistant Staphylococcus aureus, Methicillin-susceptible Staphylococcus aureus, Prevalence, Antibiotic resistance, Antibiotic resistance genes.

Introduction

Milk is an integral part of nutrition for all sections of human societies. Milk has many fans due to its variety of vitamins and minerals, such as vitamins B, D, and E and calcium, iron, phosphorus, magnesium, zinc, as well as useful and nutritious fatty acids with high digestibility and absorption (1). However, milk has a risk of contamination in the production and supply stages which may cause dangerous foodborne diseases (2). In this regard, milk contamination with foodborne bacterial pathogens and then its consumption without significant boiling and also in traditional form may cause severe foodborne diseases (3).
Staphylococcus aureus (S. aureus) is a bacterium belonging to the Firmicutes family that originated from the human skin and nose. S. aureus is considered a cause of community-acquired and nosocomial infections, including skin, wound, and burn infections, meningitis, endocarditis, pneumonia and toxic shock syndrome (4). Milk contamination with this bacterium may cause after hand manipulation or through the respiratory droplets after cough and sneezing (5). S. aureus-induced foodborne disease is mostly known for vomiting, weakness, nausea, abdominal cramps and toxic shock syndrome (5). Death may occur in severe cases (6).
S. aureus has an emergence of resistance against several types of antibiotics. S. aureus can be susceptible or resistant to the common antibiotics used to treat it (cefoxitin and oxacillin), namely, methicillin-susceptible S. aureus (MRSA) and methicillin-resistant S. aureus (MSSA), respectively (7). MRSA strains caused more severe diseases and mainly resist several types of antibiotics with significant morbidity, mortality,

length of hospital stay, and economic burden (8). MRSA strains are responsible for around 100,000 morbidities with near to 20% mortality per year in the United States (9). Both MSSA and particularly MRSA strains isolated from environmental and human-based samples harbored a high resistance rate toward several types of antibiotic agents, particularly penicillins, cephalosporins, macrolides, aminoglycosides, tetracyclines, and quinolones (10). The occurrence of antibiotic resistance in such bacteria is mainly associated with the presence of certain specific antibiotic resistance genes, including tetK and tetM (tetracyclines resistance encoding genes), aacA-D (aminoglycosides resistance encoding gene), gyrA and grlA (quinolones resistance encoding genes), cat1 (chloramphenicol resistance encoding gene), ermA and msarA (macrolides resistance encoding genes), rpoB (ansamycins resistance encoding genes), blaZ (penicillin resistance encoding genes), and dfrA1 (folate inhibitors resistance encoding gene) (11).

Both MSSA and MRSA bacteria have infrequently been examined in raw milk of animal species to assess microbial security, sanitation circumstances through milking, and storage periods. Thus, existing research was done to assess the prevalence rate and antimicrobial resistance properties of the MSSA and MRSA bacteria recovered from raw cow, sheep, and goat milk samples collected from Iran.

Methods

Samples and study procedure

In the present study, the statistical population consisted of raw milk samples of cattle, sheep, and goats that were randomly collected from sales centers located in Alborz province, Iran in the spring and summer of 2021. The sampling method in the present study was simple random sampling. Samples were taken from milk and dairy products sales centers in Alborz province, Iran. Samples included raw milk of cows (120 samples), sheep (130 samples), and goats (130 samples). Totally, 100 ml of each sample was taken separately inside sterile test tubes. Milk samples were physically (color, odor, and consistency) healthy. The samples were transferred to the Microbiological Research Center of the Islamic Azad University of Karaj within 2 hours in a completely sterile condition and on the day of sampling within 2 hours in a refrigerator containing ice at a temperature of about 4 ° C.

Isolation and identification of S. aureus

To isolate S. aureus from milk samples, the samples were first cultured in Tryptic Soy Broth (Merck, Germany) supplemented with 10% NaCl and incubated at 37 °C for 18 h. Colonies grown in Tryptic Soy Broth were then transferred to Baird Parker Agar (Merck, Germany) enriched with tellurite-egg yolk emulsion and incubated at 37 °C for 24 h. Black colonies with a surrounding sedimentary halo were considered typical colonies for S. aureus and were examined by biochemical tests of catalase, oxidase, O/F, urease, phosphatase, coagulase, DNAse, and mannitol fertilization (12).

Identification and MRSA and MSSA strains

MRSA strains were determined using cefoxitin (30 µg) and oxacillin (1 µg) antibiotic susceptibility testing. Isolates that harbored simultaneous resistance against both antibiotics were considered MRSA (13). Confirmation of MRSA isolates was additionally performed using the Polymerase Chain Reaction (PCR)-based detection of mecA gene (14). The S. aureus isolates susceptible to cefoxitin (30 µg) and oxacillin (1 µg) discs and negative for the presence of mecA gene by PCR were considered MSSA strains (14). The experiment was completed under the instructions of the Clinical and Laboratory Standards Institute (CLSI) (12, 15).

Antibiotic resistance examination

MRSA and MSSA pattern of antibiotic resistance was determined using the disk diffusion method after growth on the Mueller–Hinton agar (MHA, Merck, Germany). Principles of CLSI were applied for this purpose (16). Diverse kinds of antibiotic agents including rifampin (5 µg/disk), trimethoprim-sulfamethoxazole (25 µg/disk), chloramphenicol (30 µg/disk), tetracycline (30 µg/disk), penicillin (10 µg/disk), erythromycin (15 µg/disk), clindamycin (2 µg/disk), azithromycin (15 µg/disk), ciprofloxacin (5 µg/disk), levofloxacin (5 µg/disk), gentamicin (10 µg/disk), and amikacin (30 µg/disk) was applied for this goal (Oxoid, UK). At first, a 0.5 McFarland standard concentration of bacteria was prepared and they were cultured on MHA. Antibiotic disks were located at suitable places on the MHGA plates that contained bacteria and plates were incubated at 37 °C for 24 h (17). The diameters of the growth inhibition zone of bacteria were measured and interpreted according to the CLSI (12).

DNA extraction of PCR-based detection of antibiotic resistance genes

MRSA and MSSA isolates were sub-cultured on Trypton Soya broth (TSB, Merck, Germany) media and incubated aerobically for 48 h at 37 ºC. According to the manufacturer's instructions, the genomic DNA was extracted from the isolates using the DNA extraction kit (Thermo Fisher Scientific, St. Leon-Rot, Germany). The purity (A260/A280) and concentration of the extracted DNA were then checked (NanoDrop, Thermo Scientific, Waltham, MA, USA) (12). Furthermore, the DNA's quality was assessed on a 2% agarose gel stained with ethidium bromide (0.5 µg/mL) (Thermo Fisher Scientific, St. Leon-Rot, Germany) (17, 18).

Table 1 shows the Polymerase Chain Reaction (PCR) conditions used for the detection of toxigenic genes amongst the MRSA and MSSA isolates (19-24). A programmable DNA thermocycler (Eppendorf Mastercycler 5330, Eppendorf-Nethel-Hinz GmbH, Hamburg, Germany) was used in all PCR reactions. In addition, amplified samples were analyzed by electrophoresis (120 V/208 mA) in a 2.5% agarose gel stained with 0.1% ethidium bromide (0.4 µg/ml). Besides, UVI doc gel documentation systems (Grade GB004, Jencons PLC, London, UK) were used to analyze images. MRSA (ATCC 43300) and MSSA (ATCC 25923) were used as positive controls and PCR-grade water (Thermo Fisher Scientific, St. Leon-Rot, Germany) was used as a negative control in all PCR tests (12).

Table 1. PCR conditions (12, 17, 18, 19-24).

Target gene	Primer sequence (5'-3')	PCR product (bp)	PCR programs	PCR volume (50µL)
AacA-D	F: TAATCCAAGAGCAATAAGGGC R: GCCACACTATCATAACCACTA	227	1 cycle: 94 °C ---------- 5 min. 25 cycle: 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 0.5 µM of each primer F & R 1.25 U Taq DNA polymerase 2.5 µL DNA template
ermA	F: AAGCGGTAAACCCCTCTGA R: TTCGCAAATCCCTTCTCAAC	190		
tetK	F: GTAGCGACAATAGGTAATAGT R: GTAGTGACAATAAACCTCCTA	360		
tetM	F: AGTGGAGCGATTACAGAA R: CATATGTCCTGGCGTGTCTA	158	1 cycle: 94 °C ---------- 6 min. 34 cycle: 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 0.5 µM of each primer F & R 1.5 U Taq DNA polymerase 5 µL DNA template
msrA	F: GGCACAATAAGAGTGTTTAAAGG R: AAGTTATATCATGAATAGATTGTCCTGTT	940	1 cycle: 94 °C ---------- 6 min. 34 cycle: 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 0.75 µM of each primer F & R 1.5 U Taq DNA polymerase 3 µL DNA template
blaZ	F: ACTTCAACACCTGCTGCTTTC R: TGACCACTTTTATCA CAACC	490	1 cycle: 94 °C ---------- 5 min. 30 cycle: 94 °C ---------- 20 s 60 °C ---------- 30 s 72 °C ---------- 90 s 1 cycle: 72 °C ---------- 5 min	5 µL PCR buffer 10X 2 mM $MgCl_2$ 150 µM dNTP 0.75 µM of each primer F & R 1.5 U Taq DNA polymerase 3 µL DNA template
cat1	F: AGTTGCTCAATGTACCTATAACC R: TTGTAATTCATTAAGCATTCTGCC	547	1 cycle: 94 °C ---------- 8 min. 32 cycle: 95 °C ---------- 60 s 55 °C ---------- 70 s 72 °C ---------- 2 min 1 cycle: 72 °C ---------- 8 min	5 µL PCR buffer 10X 2 mM $MgCl_2$ 150 µM dNTP 0.75 µM of each primer F & R 1.5 U Taq DNA polymerase 3 µL DNA template
gyrA	F: AATGAACAAGGTATGACACC R: TACGCGCTTCAGTATAACGC	223	1 cycle: 94 °C ---------- 10 min. 25 cycle: 94 °C ---------- 20 s 52 °C ---------- 20 s 72 °C ---------- 50 s 1 cycle: 72 °C ---------- 5 min	5 µL PCR buffer 10X 2 mM $MgCl_2$ 150 µM dNTP 0.75 µM of each primer F & R 1.5 U Taq DNA polymerase 3 µL DNA template
grlA	F: ACTTGAAGATGTTTTAGGTGAT R: TTAGG AAATCTTGATGGCAA	459		

Statistical analysis

Data analysis was performed by SPSS Statistics 21.0 (SPSS Inc., Chicago, IL, USA). Chi-square and Fisher's exact two-tailed tests were performed to assess any significant relationship between the MRSA and MSSA prevalence, enterotoxigenic gene profiles, and antibiotic resistance properties. Besides, P-value < 0.05 was considered statistically significant.

Results

S. aureus, MRSA and MSSA distribution

Table 2 shows the S. aureus, MSSA, and MRSA prevalence amongst the examined raw milk samples. Forty-two out of 380 (11.05%) raw milk samples were contaminated with S. aureus. Raw cow milk samples harbored the highest contamination rate with S. aureus (16.66%), while raw goat milk samples harbored the lowest (8.46%). A significant difference was obtained in the S. aureus prevalence between different raw milk samples (P <0.05). Twenty-seven out of 42 (64.28%) S. aureus isolates were determined as MRSA. However, only 35.71% of S. aureus isolates were determined as MSSA. Raw goat milk samples harbored the highest distribution of MRSA (72.72%). Raw cow milk harbored the highest distribution of MSSA (41.17%). From a statistical view, significant differences were obtained between MRSA and MSSA prevalence and sample type (P <0.05). Additionally, statistically significant differences were obtained between the distribution of MRSA and MSSA isolates (P <0.05).

Table 2. S. aureus, MSSA, and MRSA prevalence amongst the examined raw milk samples.

Raw milk samples	N. collected	N. positive for S. aureus (%)	N. MRSA out of *S. aureus* (%)	N. MRSA out of *S. aureus* (%)
Cow	120	17 (16.66)	10 (71.42)	7 (41.17)
Sheep	130	14 (10.76)	9 (64.28)	5 (35.71)
Goat	130	11 (8.46)	8 (72.72)	3 (27.27)
Total	380	42 (11.05)	27 (64.28)	15 (35.71)

Antibiotic resistance pattern of MSSA and MRSA isolates

Table 3 shows the antibiotic resistance of MRSA and MSSA strains isolated from raw milk samples of animal species. MRSA isolates harbored the uppermost resistance rate toward tetracycline (100%), penicillin (100%), trimethoprim-sulfamethoxazole (66.66%), erythromycin (66.66%), ciprofloxacin (66.66%), clindamycin (62.96%), azithromycin (62.96%), and levofloxacin (59.25%). The lowest resistance rate was found for chloramphenicol (14.81%) and rifampin (29.62%). MSSA isolates harbored the uppermost resistance rate toward tetracycline (46.66%), erythromycin (46.66%), penicillin (40%), azithromycin (40%), and gentamicin (40%). The lowest resistance rate was found toward rifampin (13.33%). From a statistical view, significant differences were obtained between sample type and MSSA and MRSA resistance rate (P <0.05). MRSA isolates harbored a higher prevalence of resistance than MSSA (P <0.05).

Table 3. Antibiotic resistance of MRSA and MSSA strains isolated from raw milk samples of animal species.

Raw milk samples (N. MRSA positive)	N. MRSA isolates harbored resistance against each antibiotic agent (%)											
	Rif*	Tr-Sul	C30	T30	P10	E15	Cln	Az15	Cip5	Lev	G10	Amk
Cow (10)	3 (30)	7 (70)	2 (20)	10 (100)	10 (100)	7 (70)	6 (60)	6 (60)	7 (70)	6 (60)	5 (50)	4 (40)
Sheep (9)	3 (33.33)	6 (66.66)	1 (11.11)	9 (100)	9 (100)	6 (66.66)	6 (66.66)	6 (66.66)	6 (66.66)	5 (55.55)	4 (44.44)	4 (44.44)
Goat (8)	2 (25)	5 (62.50)	1 (12.50)	8 (100)	8 (100)	5 (62.50)	5 (62.50)	4 (50)	5 (62.50)	5 (62.50)	3 (37.50)	3 (37.50)
Total (27)	8 (29.62)	18 (66.66)	4 (14.81)	27 (100)	27 (100)	18 (66.66)	17 (62.96)	17 (62.96)	18 (66.66)	16 (59.25)	12 (44.44)	11 (40.74)
Raw milk samples (N. MSSA positive)	N. MSSA isolates harbored resistance against each antibiotic agent (%)											
	Rif	Tr-Sul	C30	T30	P10	E15	Cln	Az15	Cip5	Lev	G10	Amk
Cow (7)	1 (14.28)	2 (28.57)	-	4 (57.14)	3 (42.85)	4 (57.14)	2 (28.57)	3 (42.85)	2 (28.57)	3 (42.85)	3 (42.85)	2 (28.57)
Sheep (5)	1 (20)	2 (40)	-	2 (40)	2 (40)	2 (40)	2 (40)	2 (40)	2 (40)	2 (40)	2 (40)	2 (40)
Goat (3)	-	1 (33.33)	-	1 (33.33)	1 (33.33)	1 (33.33)	1 (33.33)	1 (33.33)	1 (33.33)	-	1 (33.33)	1 (33.33)
Total (15)	2 (13.33)	5 (33.33)	-	7 (46.66)	6 (40)	7 (46.66)	5 (33.33)	6 (40)	5 (33.33)	5 (33.33)	6 (40)	5 (33.33)

*Rif: rifampin (5 µg/disk), Tr-Sul: trimethoprim-sulfamethoxazole (25 µg/disk), C30: chloramphenicol (30 µg/disk), T30: tetracycline (30 µg/disk), P10: penicillin (10 µg/disk), E15: erythromycin (15 µg/disk), Cln: clindamycin (2 µg/disk), Az15: azithromycin (15 µg/disk), Cip5: ciprofloxacin (5 µg/disk), Lev: levofloxacin (5 µg/disk), G10: gentamicin (10 µg/disk), Amk: amikacin (30 µg/disk).

Distribution of antibiotic resistance genes amongst the MRSA and MSSA isolates

Table 4 shows the distribution of antibiotic resistance genes amongst the MRSA and MSSA isolates. Amongst the MRSA isolates, blaZ (100%), aacA-D (62.96%), tetK (51.85%). cat1 (48.14%), and dfrA1 (44.44%) harbored the highest distribution. Amongst the MSSA isolates, aacA-D (26.66%), ermA (26.66%), msrA (26.66%), tetK (26.66%), and gyrA (26.66%) were the most commonly detected antibiotic resistance genes. From a statistical view, significant differences were obtained between sample type and distribution of antibiotic resistance genes (P <0.05). MRSA isolates harbored a higher distribution of antibiotic resistance genes than MSSA (P <0.05).

Table 4. Distribution of antibiotic resistance genes amongst the MRSA and MSSA isolates.

Raw milk samples (N. MRSA positive)	N. MRSA isolates harbored each antibiotic resistance gene (%)									
	*aacA-D**	*ermA*	*msrA*	*tetK*	*tetM*	*blaZ*	*cat1*	*gyrA*	*grlA*	*dfrA1*	*rpoB*
Cow (10)	7 (70)	3 (30)	3 (30)	6 (60)	4 (40)	10 (100)	1 (10)	3 (30)	2 (20)	5 (50)	4 (40)
Sheep (9)	5 (55.55)	2 (22.22)	2 (22.22)	5 (55.55)	2 (22.22)	9 (100)	1 (11.11)	2 (22.22)	1 (11.11)	4 (44.44)	2 (22.22)
Goat (8)	5 (62.50)	1 (12.50)	3 (37.50)	3 (37.50)	2 (25)	8 (100)	1 (12.50)	2 (25)	1 (12.50)	3 (37.50)	1 (12.50)
Total (27)	17 (62.96)	6 (22.22)	8 (29.62)	14 (51.85)	8 (29.62)	27 (100)	13 (48.14)	7 (25.92)	4 (14.81)	12 (44.44)	7 (25.92)
Raw milk samples (N. MSSA positive)	N. MSSA isolates harbored each antibiotic resistance gene (%)										
	aacA-D	*ermA*	*msrA*	*tetK*	*tetM*	*blaZ*	*cat1*	*gyrA*	*grlA*	*dfrA1*	*rpoB*
Cow (7)	2 (28.57)	2 (28.57)	2 (28.57)	2 (28.57)	1 (14.28)	1 (14.28)	1 (14.28)	2 (28.57)	1 (14.28)	2 (28.57)	2 (28.57)
Sheep (5)	1 (20)	1 (20)	1 (20)	1 (20)	1 (20)	1 (20)	1 (20)	1 (20)	1 (20)	1 (20)	1 (20)
Goat (3)	1 (33.33)	1 (33.33)	1 (33.33)	1 (33.33)	-	1 (33.33)	-	1 (33.33)	-	-	-
Total (15)	4 (26.66)	4 (26.66)	4 (26.66)	4 (26.66)	2 (13.33)	3 (20)	2 (13.33)	4 (26.66)	2 (13.33)	3 (20)	3 (20)

*aacA-D: aminoglycosides, ermA: erythromycin, msrA: macrolides, tetK and tetM: tetracyclines, blaZ: penicillin, cat1: chloramphenicol, gyrA, and grlA: quinolones, dfrA1: trimethoprim, rpoB: rifampin.

Discussion

Milk contamination by S. aureus may occur during the milking through the transmission of bacteria from animal skin, wool, and feces and also through milking by the hands of milking room staff. Additionally, it will be contaminated during maintenance and supply through manipulation and bacterial; transmission by airdrops (cough and sneezing). S. aureus can also be originated from sub-clinical mastitis milk which may show normal characteristics of milk. Thus, it is important to assess the main route of S. aureus transmission to raw milk samples.

In the present study, S. aureus was detected in 11.05% of examined raw milk samples. MRSA and MSSA strains were identified in 64.28% and 35.71% of isolates, respectively. Rahi et al. (2020) (25) stated that reported that the S. aureus prevalence amongst the bovine, ovine, caprine, camel, and buffalo milk samples were 8.46%, 7.50%, 5%, 2.27%, and 9.09%, respectively. They showed that the distribution of MRSA strains amongst the S. aureus isolates of raw bovine, ovine, caprine, camel, and buffalo milk samples were 72.72%, 77.77%, 66.66%, 33.33%, and 80%, respectively. Alghizzi and Shami (2021) (26) stated that the distribution of S. aureus amongst the raw cow, horse, camel, and goat milk samples was 80%, 90.90%, 80%, and 100%, respectively. MRSA strains were detected in 75%, 70%, 91.70%, and 100% of S. aureus isolates. MRSA distribution amongst the examined samples of the present survey was 64.28%, which was higher than those reported from Turkey (17%) (27), Italy (20%) (28), United Kingdome (2.30%) (29), and Germany (2.30%) (30), while was lower than that of Turkey (75.40%) (31). A meta-analysis review (32) showed that the pooled S. aureus prevalence rates amongst the raw cow's and caprine milk samples were 33.5% (29.5–37.7%) and 25.8% (17.5–35.0%), respectively. The pooled MRSA prevalence rates were 2.3% (1.3–3.6%), and 1.1% (0.5–1.8%), respectively. According to our findings, MRSA had a higher distribution than MSSA. This finding was not reported in any other survey. Additionally, raw goat milk samples harbored the highest distribution of MRSA strains. The high distribution of MRSA amongst the raw goat milk samples was similarly reported by Tamendjari et al. (2021) (33) and Chai et al. (2020) (34). However, it was not detected amongst the raw goat milk samples collected from Sweden (35). Such a large variation across countries in MRSA prevalence rate from raw milk samples may show real regional differences or may be affected by the use of various detection techniques. Additional developments in the MRSA detection techniques in foods are desirable.

MRSA and MSSA strains isolated from raw milk samples harbored different prevalence of resistance against examined antibiotic agents. MRSA strains harbored the higher resistance rate toward tetracycline, penicillin, trimethoprim-sulfamethoxazole, erythromycin, and ciprofloxacin, while MSSA strains harbored the highest resistance rate against tetracycline, erythromycin, penicillin, azithromycin, and gentamicin. A similar pattern of resistance was reported in Bangladesh (36), Iran (37), and Turkey (38). Improper and unauthorized administration of antibiotics, overuse of antibiotics and disinfectants, and finally self-medication with antibiotics can be possible reasons for the high prevalence of antibiotic resistance. Shrestha et al. (2021) (39) reported that the prevalence of antibiotic resistance of MRSA isolates of raw milk samples against ciprofloxacin, tetracycline, teicoplanin, ampicillin, clindamycin, erythromycin, amikacin, gentamycin, cefotaxime, cefepime, and cefazoline was 69%, 20.60%, 89.70%, 100%, 72.40%, 48.30%, 86.20%, 93%, 44.8%, 69%, and 17.20%, respectively, which was surprising as all of the MRSA strains should resist toward all cephalosporins and penicillins antimicrobials. A Chinese survey (40) reported that the S. aureus strains isolated from raw milk of animal species harbored a high prevalence of resistance against erythromycin (32.30%), clindamycin (30.60%), penicillin (72.60%), trimethoprim-sulfamethoxazole (19.40%), ceftaroline (8.10%), linezolid (25.80%), tetracycline (11.30%), vancomycin (3.20%), gentamicin (30.60%), ciprofloxacin (16.10%), oxacillin (37.10%), and rifampin (11.30%). The higher prevalence of resistance in the present survey compared to that of the Chinese study may be due to the assessment of antibiotic resistance of MRSA strains in the present survey. As some of these antibiotics are only used in the hospital environment, especially for the treatment of human clinical diseases (such as rifampin, trimethoprim-sulfamethoxazole, clindamycin, azithromycin, ciprofloxacin, levofloxacin, and amikacin), resistant S. aureus strains may be originated from cross contamination through hand manipulation and airdrops. The differences reported in the pattern of antibiotic resistance in various studies are probably due to the availability or non-availability of antibiotics, the level of strict rules in prescribing antibiotics, and the opinion of physicians and veterinarians on prescribing antibiotics. The prevalence of resistance to chloramphenicol was lower than that of other antibiotics. Chloramphenicol is an illicit drug with a limited prescription. The use of this antibiotic illegally is done only in poultry farms in Iran. Probably the reason for the low prevalence of antibiotic resistance against chloramphenicol is the keeping of cattle, sheep, and goats traditionally next to poultry and the transfer of chloramphenicol-resistant strains from poultry to farm animals.

MRSA and MSSA strains harbored different distribution of antibiotic resistance genes. blaZ, aacA-D, tetK, cat1, and dfrA1 were the most commonly detected antibiotic resistance genes amongst the MRSA strains, while aacA-D, ermA,

msrA, tetK, and gyrA were the most commonly detected amongst the MSSA isolates. This finding may show that these two phenotypes may have so many additional differences in other microbiological and epidemiological properties, which need further analysis. A similar survey conducted in the United States (41) reported that the prevalence of aadD, blaZ, mecA, ermB, msrA, tetK, and tetM antibiotic resistance genes amongst the S. aureus strains isolated from raw milk samples was 0.8%, 4%, 0.8%, 0.8%, 2.4%, 1.6%, and 0.8%, respectively, which was much lower than our findings. The total distribution of ermA, blaZ, tetK, tetM, aacA, and rpoB antibiotic resistance genes amongst the S. aureus strains isolated from raw milk samples in China was 4.80%, 25.80%, 6.50%, 11.30%, 19.40%, and 61.30%, respectively. Feng et al. (2016) (42) described that the distribution of blaZ, ermA, aacA, tetK, tetM, and rpoB antibiotic resistance genes were 95.45%, 0%, 2.27%, 22.73%, 2.27%, and 100%, respectively. According to our findings, both MRSA and MSSA strains harbored a higher prevalence of resistance in the disk diffusion than those detected by the specific antibiotic resistance genes. this finding may be because the presence of antibiotic resistance genes is one of the ways for the occurrence of antibiotic resistance. Thus, it is not surprising that for example resistance to rifampin was identified in 29.62% of MRSA isolates, while rpoB gene was only detected in 25.92% of MRSA strains.

Food safety and hygiene, especially in foods with animal origins and its relation to the human health have so many importance in the development of the community (44-50).

Conclusion

In conclusion, the considerable prevalence of MRSA strains was accompanied by the high rate of bacterial resistance toward commonly used antibiotic agents, particularly trimethoprim-sulfamethoxazole, erythromycin, and ciprofloxacin. The findings may show the high antibiotic resistance of MRSA and the potential role of raw milk samples in its transmission to the human population. MSSA strains were identified in the lower number of samples. They also harbored the lowest antibiotic resistance. Some MRSA strains harbored different antibiotic resistance genes, particularly blaZ, aacA-D, tetK, cat1, and dfrA1. These findings may show the role of raw milk samples as a source of antibiotic resistance genes. It seems that the consumption of contaminated milk with resistant S. aureus may cause severe food-borne diseases that resist antibiotic therapy. According to the higher distribution of MRSA strains in raw goat milk samples, higher hygienic measurements should perform for their production and supply.

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