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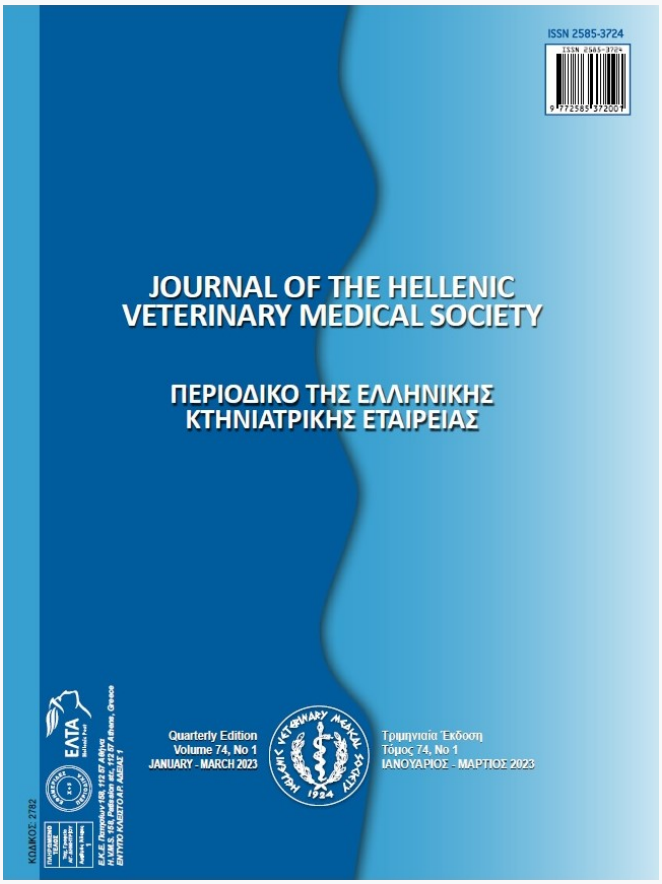

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Research article
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Antibiotic resistance and enterotoxin gene profiles of *Staphylococcus aureus* isolated from raw milk in Iran

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ABSTRACT: *Staphylococcus aureus* is a food-borne pathogen frequently isolated from raw milk all over the world. The emergence of antibiotic resistant strains of *S. aureus* has become an important global public health issue. In spite of this problem, the contamination rate of raw milk with enterotoxigenic and antibiotic resistant *S. aureus* strains has been rarely evaluated in Iran. This study aimed to evaluatethe prevalence and antibiotic resistance of*S. Aureus* strains isolated from bulk tank milk samples in Mashhad, and evaluate the carriage frequency of genes encoding the staphylococcal enterotoxins (SEs). For this, a total of 250 milk samples were tested for *S. aureus* by the conventional plating method and PCR. The disc diffusion method was used to assess the antibiotic susceptibility of the isolated *S. aureus*. Finally, the PCR method was used for the detection of staphylococcal enterotoxin genes.*S. aureus* was detected in 46 (18.4%) of the collected bulk tank milk samples with mean concentrations of 3.5×10^5 CFU/ml**.** Among the tested isolates, 86.96% (40/46) were resistant to at least one antibiotic, and 15% (6/40) were detected as multidrug resistant strains. The highest resistance rate was observed against penicillin (65.22%), ampicillin (41.31%), and methicillin (41.31%). Overall,71.74% of the isolates harbored at least one of the SEs encoding genes.The *sec* and *sea* genes were the prevalent ones.In conclusion, the prevalence of enterotoxigenic and antibiotic-resistant *S. aureus*in the milk samples collected from Mashhad dairy farms shows the need of implementing strict hygiene practices and rational antibiotic usage.

Keywords: Antibiotic resistance; *Staphylococcus aureus*; PCR; enterotoxin;raw milk

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INTRODUCTION

Milk is dietary source of carbohydrates, proteins, fats, vitamins and minerals obtained mainly from cattle dairy farms (Abera et al., 2010).However, this nutrient food can harbor many harmful food-borne pathogens which can lead to food poisonings or illnesses. The source of pathogenic organisms in milk can be livestock, humans or the environment (Hwang et al., 2007).*Staphylococcus aureus* is one of the most prevalent milk-borne pathogens isolated from many bulk tank raw milk of dairy farms in different parts of the world (Khairullah et al., 2020; Mohsenzadeh et al., 2015). This agent is considered as one of the most common pathogens causing food intoxication (Lawrynowicz-Paciorek et al., 2007). Staphylococcal enterotoxins (SEs) are the cause of the *S. aureus*food poisoning (SFP), and include the classical SEA, SEB, SEC, SED and SEE, which are responsible for 95% of SFPs (Rahimi & Safai, 2010). These enterotoxins are highly heat resistant and retain their biological activity even after pasteurization (Khairullah et al., 2020). Consequently,the destruction of bacteria in the pasteurization process can not eliminate the risk of SFP. Moreover, SEs are also resistant to low pH and proteolytic enzymes, allowing them to be active in the gastrointestinal tract (Chen & Xie, 2019).Beside the aforementioned classical SEs, nonclassical SEs, including SEG, SEH, SEI, SER, SES,also act as emetic agents (Hait et al., 2014).

Antibiotics are administrated widely to animals to treat bacterial infections and to promote growth (Titouche et al., 2019). However, the emergence of antibiotic resistant bacterial strains is the consequence of excessive usage of these agents.*S. aureus* can acquire antibiotic resistance determinants and hence its isolates often show resistance to many types of antimicrobial agents (Papadopoulos et al., 2018). For instance, methicillin-resistant *S. aureus* (MRSA) is resistant to all common b-lactam antimicrobial agents and represents a grave public health concern due to its capability to infect humans and animals (Papadopoulos et al., 2018). In recent years, the occurrence of antibiotic resistant *S. aureus* like MRSA has increased in bulk tank milk of dairy farms, demonstrating the potential role of milk and dairy products in the spread of those lineages in humans (Giacinti et al., 2017). Despite the importance of the issue, the studies regarding the contamination of raw milk with antibiotic resistant *S. aureus* are rare in Iran.

This study aimed to determine the prevalence and the antibiotic resistance of *S. aureus* in milk samples obtained from bulk tank milkof Mashhad dairy farms and to assess the carriage frequency of genes encodingSEA, SEB, SEC, SED and SEE enterotoxins by polymerase chain reaction (PCR).

MATERIALS AND METHODS

Milk samples

A total of 250 bulk tank milk (BTM) samples were collected from different bovine industrial farms around Mashhad. After homogenization of milk in the tank, the raw bovine milk samples were collected in a sterile 50 ml falcon tube and transferred under refrigeration condition (4 °C) within 1 h of the collection to the food hygiene laboratory of faculty of veterinary medicine, Ferdowsi university of Mashhad for further analysis.

Isolation and enumeration of *S. aureus*

To identify and enumerate *S. Aureus* in the milk samples, the method of Regasa et al. (2019) was used with some modifications (Regasa et al., 2019). In brief, serial dilutions of each samples, were preparedusing0.1% peptone water diluent.Then,0.1 ml of each prepared dilution was spread on Baird-Parker agar (BPA) with 5% egg yolk tellurite emulsion using a bent glass rod and incubated at 35 °C for 30 to 48 h. Presumptive *S. aureus* colonies were circular, convex, smooth, moist, and gray to black appearance surrounded by opaque zone and frequently with an outer thin clear zone on the BPA medium.Plates with 20-200 colonies were chosen for *S. aureus* count and the calculated counts were reported as CFU/mL of BTM samples.

For the identification of *S. aureus*,five colonies of suspected *S. aureus* were selected and transferred on Blood agar (BA) (Cho et al., 2019).The plates were then incubated at 37 °C for 24 hours.The colonies were evaluated based on their morphology and the ability of hemolysis on blood agar,using Gram staining and biochemical tests such ascatalase, coagulase,DNase, VP, and sugar fermentation (sucrose, mannitol and maltose) (Hemamalini et al., 2015). The confirmed *S. aureus* colonies were then transferred to Brain Heart Infusion (BHI) media for further analysis. *S. aureus* ATCC25923 was used as positive control strain. All culture media and diluent were obtained from Merck (Merck, Darmstadt, Germany).

Antibiotic susceptibility

Table 1. Primer sequences, annealing temperatures and predicted sizes of PCR products for detection of staphylococcal enterotoxins.

Target gene	Sequence of primer	Annealing temperature	Amplicon length (bp)	Reference
femA	F: 5-GCAAACTGTTGGCCACTATG -3 R: 5-TCATCACGATCAGCAAAAGT -3	60	594bp	(Riyaz-Ul-Hassan et al., 2008)
sea	F: 5-TTGGAAACGGTTAAAACGAA -3 R: 5-GAACCTTCCCATCAAAAACA -3	52	120bp	(Johnson et al., 1991)
seb	F: 5-TCGCATCAAACTGACAAACG -3 R: 5-GCAGGTACTCTATAAGTCCC -3	50	478bp	(Johnson et al., 1991)
sec	F: 5-GACATAAAAGCTAGGAATTT -3 R: 5-AAATCGGATTAACATTATCC -3	52	257bp	(Johnson et al., 1991)
sed	F: 5-CTAGTTTGGTAATATCTCCT -3 R: 5-TAATGCTATATCTTATAGGG -3	50	317bp	(Johnson et al., 1991)
see	F: 5-AGGTTTTTTCAC AGGTCATCC -3 R: 5-CTTTTTTTTCTTCGG TCAATC -3	54	317bp	(Mehrotra et al., 2000)

The antibiotic susceptibility test of the isolates was assessed by Kirby-Bauerdisc diffusion method on Mueller Hinton agar (Merck) according to the criteria of Clinical and Laboratory Standards Institute (CLSI) (CLSI, 2018).

The *S. aureus* colonies were inoculated in physiological water and incubated at 37°C to get turbidity equal to 0.5 McFarland. A sterile swab was dipped into the bacterial suspension, and the entire surface of Mueller Hinton agar (MHA) plates was swabbed. In this study, the following antimicrobial discs (Padtan-Teb Co., Iran) including streptomycin (10µg), tetracycline (30µg), penicillin (10µg), amoxicillin (25µg), cephalothin (30µg), enrofloxacin (5µg), cloxacillin (1µg), methicillin (1µg), lincomycin (2µg), gentamycin (10µg), ampicillin (10µg), vancomycin (30µg), and trimethoprim/sulfamethoxazole (23.75/1.25µg) were applied on the MHA medium. The plates were incubated at 37°C and examined after 18-24 h.The results were reported as resistant or sensitive by measuring the diameter of the inhibition zone according to CLSI interpretive standards (CLSI, 2018).*S. aureus* ATCC 25923 was used as reference strain.

Extraction of genomic DNA

S. aureus isolates were grown overnight at 37 °C in BHI broth. Chromosome DNA of the isolates was extracted usinga Diatom DNA prep 100 kit following the manufacturer's instructions.

Detection of genes by PCR

The extracted samples were subjected to PCR with six primer pairs as previously described in the literature (Table 1) to detect the *femA* and SEs (*sea*, *seb*, *sec*, *sed*, and *see*) genes.The *femA* was used for the identification of *S. aureus* species.

PCR was performed in a 20µL final volume containing1-unitTaq DNA polymerase, 1.5µL of 10X PCR buffer, 250µM dNTPs, 2µL of primer, 1.5 mM of $MgCl_2$ and 2µL of genomic DNA.

PCR was carried out on a thermal cycler (Bio-Rad, Hercules, CA, USA) using the following steps: initial denaturation at 94 °C for 3 min, then 35amplification cycles with denaturation at 94 °C for 30 s, annealing at the temperature optimum for each primer for 60 s, and a final extension step at 72 °C for 5 min. The PCR products were then detected by electrophoresis in 1.5% non-denatured agarose gel to verify the expected size of the amplicons.

The positive controls (reference strains) were *S. aureus* ATCC 25923 as methicillin-sensitive *S. aureus* (MSSA) strain, *S. aureus* ATCC 33591 as MRSA strain, *S. aureus* ATCC 13565 for gene *sea*, *S. aureus* ATCC 19095 for genes *seb* and *sec*, *S. aureus* ATCC 23235 for gene *sed*, and *S. aureus* ATCC 27664 for gene *see*. Distilled water was used as the negative control.

RESULTS

Out of the 250 BTM samples collected from different dairy farms around Mashhad,92 samples (36.8%) were contaminated with *Staphylococcus* spp., while *S. aureus* was isolated from 46 samples (18.4%).The Staphylococcus counts ranged between6.5×10^4CFU/ml and 2.2×10^6 CFU/ml in BTM samples and mean *S. aureus* count was 3.5×10^5CFU/ml. As a control, the *femA* was detected in all 46 isolates, confirming the presence of *S. aureus* (Fig. 1).

Regarding antibiotic susceptibility results, among the 46 *S.aureus* isolates, 40 (86.95%) isolates were resistant to at least one antibiotic,and multidrug resistance (MDR), defined as resistance to at least one agent in three or more antibiotic classes, was observed in 6 (15%) of the isolates (Table 2).

The highest resistance rate was recorded to penicillin (65.22%), followed by ampicillin (41.31%), methicillin (41.31%), and amoxicillin (26.08%). All isolated *S.aureus* were susceptible to vancomycin and gentamicin (Table 3).

The detection of the genes encoding SEs in *S. aureus* by PCR (Fig. 1) showed that 33 strains (71.74%) of the studied bacteria had at least one of those genes (Table 4). As it can be seen in Table 4, 9.09% had both *sea* and *sec* genes, while *seb* and *see* were not detected in isolates. Among all enterotoxin genes, the *sec* gene was the most prevalent (63.64%).

Table 2. Antibiotic resistance profiles of S. aureus strains (n = 40) isolated from bulk tank milk samples.

Resistance category	Resistance profiles (number)	Number of strains	%
Resistant to one antibiotic	PCN (5), TET (2)	7	17.5
Resistant to two antibiotics	PCN +AMP (2), PCN +AMX (1), PCN +STM (1), PCN+MET (4), TET+LCM (1), CEP+LCM (1)	10	25
Resistant to three antibiotics	PCN +AMP+MET (4), PCN +AMP+TET (1), PCN+CEP+ENF (1), PCN +CEP+LCM (1), AMP+AMX+TET (2), PCN+AMX+MET (2), STM+ENF+CLX (1)	12	30
Resistant to four antibiotics	PCN +AMP+AMX+MET (4), PCN +AMP+CEP+MET (1), AMP+AMX+CLX+MET (1), AMP+CLX+ TMP/SMX +LCM (1), TET+CLX+CEP+ENF (1)	8	20
Resistant to five antibiotics	PCN +AMP+AMX+MET+CEP (1), PCN +AMP+TET+MET+LCM (1)	2	5
Resistant to six antibiotics	PCN +AMP+AMX+CLX+MET+ TMP/SMX (1)	1	2.5

PCN: penicillin, TET: tetracycline, AMP: ampicillin, AMX: amoxicillin, STM: streptomycin, LCM: lincomycin, MET: methicillin, CEP: cephalothin, ENF: enrofloxacin, CLX: cloxacillin, TMP/SMX: trimethoprim /sulfamethoxazole.

Table 3. Antibiotic susceptibility of S. aureus strains (N=46) isolated from bulk tank milk samples.

Antibiotic	Resistant N (%)	IntermediateN (%)	Susceptible N (%)
Penicillin	30 (65.22)	9 (19.56)	7 (15.22)
Ampicillin	19 (41.31)	16 (34.78)	11 (23.91)
Methicillin	19 (41.31)	6 (13.04)	21 (45.65)
Amoxicillin	12 (26.08)	11 (23.91)	23 (50)
Lincomycin	5 (10.87)	14 (30.43)	27 (58.70)
Cephalothin	6 (13.04)	7 (15.22)	33 (71.74)
Enrofloxacin	3 (6.52)	9 (19.57)	34 (73.91)
Tetracycline	8 (17.39)	3 (6.52)	35 (76.09)
Cloxacillin	5 (10.87)	5 (10.87)	36 (78.26)
Streptomycin	2 (4.35)	7 (15.22)	37 (80.43)
Trimethoprim /Sulfamethoxazole	2 (4.35)	0 (0)	44 (95.65)
Vancomycin	0 (0)	0 (0)	46 (100)
Gentamicin	0 (0)	0 (0)	46 (100)

Table 4. Frequency of staphylococcal enterotoxins' encoding genes in *S. aureus* (N=46) isolated from bulk tank milk samples

S. aureus with enterotoxin gene (out of 46 samples)		Enterotoxins' genes					
		sea	*seb*	*sec*	*sed*	*see*	*sea+sec*
Number	33	7	0	21	0	5	3
Percentage	71.74	21.21	0	63.64	0	15.15	9.09

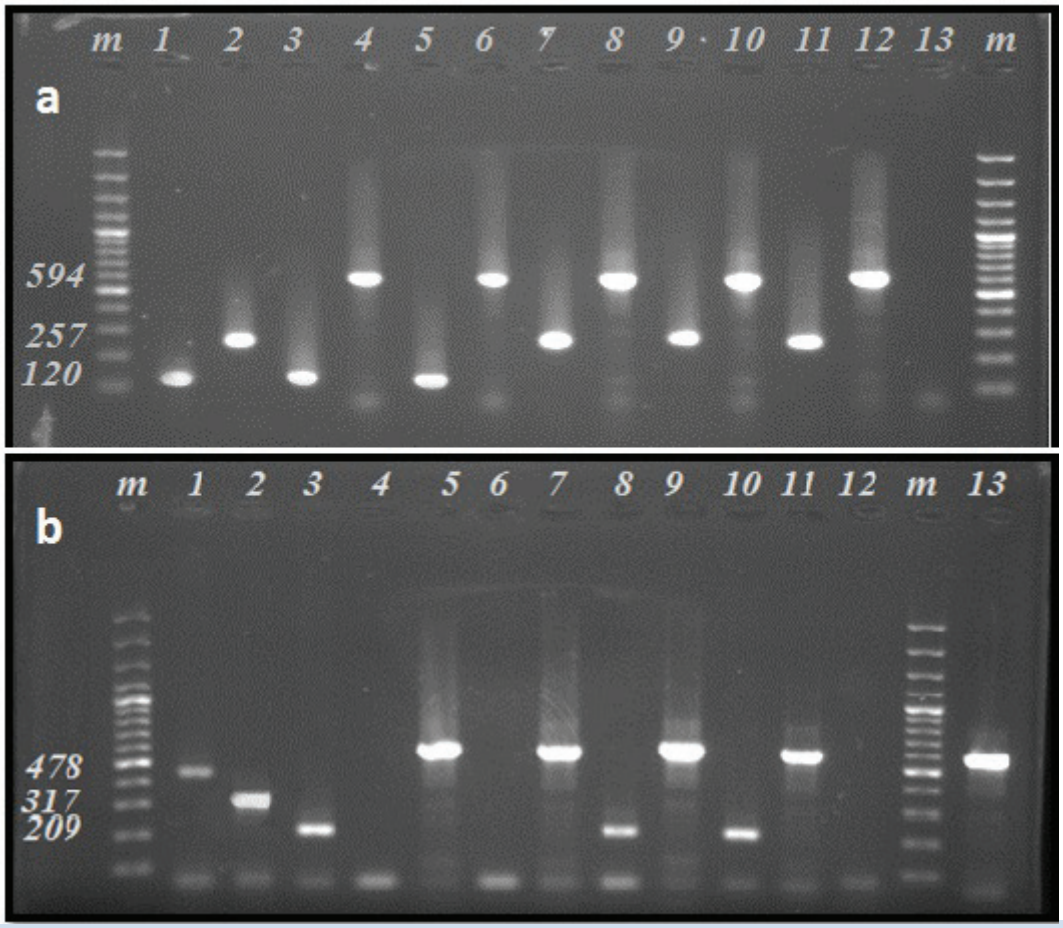

Figure 1. *S. aureus* enterotoxin genes (*sea*,*seb*, *sec*,*sed* and *see*) and *femA*gene detected by gel electrophoresis. a) Lanes: M, Marker, 100 bp ladder; 1, *sea* positive control; 2, *sec* positive control; 3 and 5 *sea* positive isolate; 7, 9 and 11 *sec* positive isolate; 4, 6, 8, 10 and 12 *femA* positive isolate; 13, negative control (Distilled water). b) Lanes: M, Marker, 100 bp ladder; 1, *seb* positive control; 2, *sed* positive control; 3, *see* positive control; 4, *seb* negative isolate; 6, sed negative isolate; 8 and 10, *see* positive isolate; 5, 7, 9 and 11, *femA* positive isolate; 12, negative control (Distilled water) ; 13, *femA*-positive control

DISCUSSION

Food poisoning due to the consumption of raw milk or other milk products, like soft cheese or mashed potato, contaminated with *S. aureus* has been previously reported in several studies (Haghi et al., 2019; Johler et al., 2015; Jørgensen et al., 2005). The contamination of raw milk with *S. aureus* is largely due to the occurrence of clinical and subclinical bovine staphylococcal mastitis, which has been repeatedly reported in many countries including Iran (Monistero et al., 2020; Sahebekhtiari et al., 2011).The risk of the addition of raw milk contaminated with *S. aureus* to a bulk tank is relatively higher when subclinical staphylococcal mastitis occurs. In fact, in this case,the chance of detecting subclinical mastitis is lower compared to clinical mastitis.

In this study, *S.aureus* was isolated in 18.4% of the tested samples. A range of 4-25.6% contamination rates were reported for bovine milk samples collected from different areas including Tabriz, Tehran, Zanjan, São Paulo state, Brazil and Shanghai showing that the level of contamination in present work is comparable to those studies (Fagundes et al., 2010; Fooladi et al., 1970; Haghi et al., 2019; Rahbar Saadat et al., 2014; Song et al., 2015). Different parameters such as season of sampling,post-harvest practices, geographical region, and methods of isolation and identification have been mentioned as probable reasons of the observed variation in the prevalence of *S. aureus* (Haghi et al., 2019).

In the present study, the mean *S. Aureus* count was 3.5×10^5 CFU/ml. This value is higher than the relevant values previously reported for otherdairy products. For instance, in a study by Rahbar et al. (2014), 10^2-10^6 CFU/ml of total *S. aureus* were reported for organic milk collected from bovine milk collection points and farms in Tabriz, Iran (Rahbar Saadat et al., 2014).Similarly, a range of 1×10^2 and 1×10^6 CFU/g *S.*

aureus was reported in different dairy products made from bovine milk like traditional cheese, butter and traditional ice cream (Rahimi, 2013). The higher *S. aureus* contamination in the present study may indicate lower levels of sanitary practices during milking in the studied farms. It should be mentioned that staphylococcal counts should reach ~ 10^6 CFU/g to produce SEs (Pelisser et al., 2009).

The prevalence of the genes encoding SEs in the *S. aureus* isolates was 71.74%which is in good agreement with the data reported in similar studies. In Italy, Morandi et al. (2007) mentioned that 67% of the *S. aureus* detected in ovine, goat, sheep and buffalo's milk and dairy products had SEs encoding genes in their genome (Morandi et al., 2007).In other reports in Japan and Iran, 77.4% and 80.7% of *S. aureus* strains isolated from bovine milk were positive for the presence of toxin genes, respectively (Nazari et al., 2014; Omoe et al., 2002). The prevalence of enterotoxigenic *S. aureus* in bovine raw milk in Iran is important since several Iranian traditional dairy products like cheese, are still produced from unpasteurized milk (Rahimi, 2013).

Concerning the composition of SEs encoding genes in *S. aureus*, different results have been obtained in different studies. In fact, the genetic information of *S. aureus* isolates responsible for food poisoning typically varies by country and even by city within the same country and also by the type of the contaminated food product (Carfora et al., 2015). Despite that variation, SEA is the major *S. aureus* enterotoxin observed in bovine milk samples obtained from different geographical locations including some areas in Iran like Zanjan and Qom (Haghi et al., 2019; Nazari et al., 2014).Moreover, it has been stated that strains isolated from bovine milk products mainly produce SEA and SEE (Nazari et al., 2014). Although the aforementioned reports do not support our findings, there are other studies stating SEC as the most common SE isolated from milk producing animals (Riva et al., 2015).

Regarding antibiotic susceptibility testing, 86.96% (40/46) of the *S.aureus* isolates exhibited resistance to the applied antibiotics, while 41.31% (19/46) of them were resistant to methicillin.

MRSA strains are among the most important causes of hospital-acquired infections worldwide, and recently MRSA have been often isolated in farm environment (Carfora et al., 2015). The prevalence rate of MRSA strains in bovine raw milk has been reported in different countries, including Italy (13-20%) and Turkey (17%), and lower percentages of contamination with MRSA were recorded in these studies compared to the present work (Riva et al., 2015). Titouche et al. (2019) and Papadopoulos et al. (2018) also reported very low percentages of MRSA contamination (3-4.1%) in raw milk and dairy product samples collected in Algeria and Greece (Papadopoulos et al., 2018; Titouche et al., 2019).

The high rate of resistance to β-lactam antibiotics like penicillin and ampicillin was in accordance with previous researches on *S. aureus* isolates isolated from food samples in different locations in Iran and other countries (Chen & Xie, 2019; Rahimi, 2013; Sahebekhtiari et al., 2011; Titouche et al., 2019).The high susceptibility to gentamycin, trimethoprim/sulfamethoxazole and vancomycin were similarly observed by Papadopoulos et al. (2018) and Giacinti et al. (2017) in *S. aureus* strains isolated from bovine bulk tank milk and sheep milk respectively (Giacinti et al., 2017; Papadopoulos et al., 2018).

In the present study, multidrug resistance was also found in 15% (6/40) of the resistant *S. aureus* isolates. In recent years, several studies have reported different rates of MDR *S. aureus* isolated from bovine milk samples. For instance, Zhao et al. (2021) reported a high prevalence of MDR *S. aureus* (55.4%) while a lower rate (16%) was observed by Eid et al. (2022) (Eid et al., 2022; Zhao et al., 2021).

The antibiotic resistance profile of the present study corresponds to the antibiotics that are being applied to treat food animals' infections in Iran (Rahimi, 2013). For instance, β-lactam antibiotics, have been widely used for decades to treat staphylococcal infections in Iran and other countries. Therefore, the variations among antimicrobial resistance patterns of *S. aureus* may reflect differences in infection control strategies.

CONCLUSION

In this study,the prevalence, the genes encoding enterotoxins and antibiotic resistance of *S. aureus* strains in bovine milk samples collected from bulk tank of Mashhad farms were evaluated. The prevalence of *S. aureus* showed that the level of contamination in the study area was comparable to other locations in Iran and other countries. The frequency of genes encoding SEs in *S. aureus* (71.74%) was

also in accordance with previous reports in the different areas. However,even though *sea* is mainly the dominant SE encoding gene in enterotoxigenic *S. aureus,sec* was the most prevalent (63.64) in this study.Finally, high rates of resistance to the applied antibiotics (86.96%) were detected in the tested milk samples.In conclusion, the prevalence of enterotoxigenic antibiotic-resistant *S. aureus*in the milk samples collected from Mashhad dairy farms maybe a public health concern,especially because many traditional dairy products are still made by unpasteurized milk in Iran. Therefore, additional efforts must be devoted to implementing hygienic processes in dairy farms and controlling antibiotic usage as well.

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CONFLICT OF INTEREST

None declared.

REFERENCES

Abera, M, Demie, B, Aragaw, K, Regassa, F, & Regassa, A (2010) Isolation and identification of *Staphylococcusaureus* from bovine mastitic milk and their drug resistance patterns in Adama town, Ethiopia. J Vet Med Anim Heal 2 (3) : 29-34.

Carfora, V, Caprioli, A, Marri, N, Sagrafoli, D, Boselli, C, Giacinti, G, Giangolini, G, Sorbara, L, Dottarelli, S, Battisti, A, & Amatiste, S (2015) Enterotoxin genes, enterotoxin production, and methicillin resistance in *Staphylococcusaureus* isolated from milk and dairy products in Central Italy. Int Dairy J 42: 12-15.

Chen, Q, & Xie, S (2019) Genotypes, Enterotoxin Gene Profiles, and Antimicrobial Resistance of *Staphylococcusaureus* Associated with Foodborne Outbreaks in Hangzhou, China. In *Toxins* (Vol. 11, Issue 6).

Cho, YS, Lee, MK, & Hwang, SH (2019) Toxin gene profiles, genetic diversity, antimicrobial resistance, and coagulase type of *Staphylococcusaureus* from cream-filled bakery products. Food Sci Nutr 7 (5) : 1727-1734.

CLSI (2018) M100 Performance Standards for Antimicrobial Susceptibility Testing. In *Clinical and Laboratory Standards Institute*.

Eid, HM, El-Mahallawy, HS, Mohammed, SR, Mohammed, NEY, & Eidaroos, NH (2022) Multidrug-resistant and enterotoxigenic methicillin-resistant *Staphylococcusaureus* isolated from raw milk of cows at small-scale production units. J Adv Vet Anim Res 9 (1) : 113-121.

Fagundes, H, Barchesi, L, Filho, A, Menezes, F, & Oliveira, C (2010) Occurrence of *Staphylococcusaureus* in raw milk produced in dairy farms in São Paulo state, Brazil. Brazilian J Microbiol 41:.

Fooladi, AAI, Tavakoli, HR, & Naderi, A (1970) Detection of enterotoxigenic *Staphylococcus aureus* isolates in domestic dairy products. Iran J Microbiol 2 (3 SE-Articles) :

Giacinti, G, Carfora, V, Caprioli, A, Sagrafoli, D, Marri, N, Giangolini, G, Amoruso, R, Iurescia, M, Stravino, F, Dottarelli, S, Feltrin, F, Franco, A, Amatiste, S, & Battisti, A (2017) Prevalence and characterization of methicillin-resistant *Staphylococcus aureus*carrying *mecA*or *mecC* and methicillin-susceptible *Staphylococcus aureus* in dairy sheep farms in central Italy. J Dairy Sci 100 (10) : 7857-7863.

Haghi, F, Daneshamooz, S, Parsadanians, A, & Zeighami, H (2019) The Frequency of *Staphylococcusaureus* Classical Enterotoxin Genes in Raw Milk Samples in Zanjan, Iran TT -. 5 (1) : 32-35.

Hait, J, Tallent, S, Melka, D, Keys, C, & Bennett, R (2014) Prevalence of enterotoxins and toxin gene profiles of *Staphylococcus aureus* isolates recovered from a bakery involved in a second staphylococcal food poisoning occurrence. J Appl Microbiol 117 (3) : 866-875.

Hemamalini, V, Kavitha, V, & Ramachandran, S (2015) In vitro antibiogram pattern of *Staphylococcusaureus* isolated from wound infection and molecular analysis of *mecA* gene and restriction sites in methicillin resistant *Staphylococcus aureus*. J Adv Pharm Technol Res 6 (4) : 170-175.

Hwang, SY, Kim, SH, Jang, EJ, Kwon, NH, Park, YK, Koo, HC, Jung, WK, Kim, JM, & Park, YH (2007) Novel multiplex PCR for the detection of the *Staphylococcus aureus* superantigen and its application to raw meat isolates in Korea. Int J Food Microbiol 117 (1) : 99-105.

Johler, S, Weder, D, Bridy, C, Huguenin, M-C, Robert, L, Hummerjohann, J, & Stephan, R (2015) Outbreak of staphylococcal food poisoning among children and staff at a Swiss boarding school due to soft cheese made from raw milk. J Dairy Sci 98 (5) : 2944-2948.

Johnson, WM, Tyler, SD, Ewan, EP, Ashton, FE, Pollard, DR, & Rozee, KR (1991) Detection of genes for enterotoxins, exfoliative toxins, and toxic shock syndrome toxin 1 in *Staphylococcusaureus* by the polymerase chain reaction. J Clin Microbiol 29 (3) : 426-430.

Jørgensen, HJ, Mathisen, T, Løvseth, A, Omoe, K, Qvale, KS, & Loncarevic, S (2005) An outbreak of staphylococcal food poisoning caused by enterotoxin H in mashed potato made with raw milk. FEMS Microbiol Lett 252 (2) : 267-272.

Khairullah, AR, Sudjarwo, SA, Effendi, MH, Harijani, N, Tyasningsih, W, Rahmahani, J, Permatasari, DA, Ramandinianto, SC, Widodo, A, & Riwu, KHP (2020) A review of methicillin-resistant *Staphylococcusaureus* (MRSA) on milk and milk products: Public health importance. Syst Rev Pharm 11 (8) : 59-69.

Lawrynowicz-Paciorek, M, Kochman, M, Piekarska, K, Grochowska, A, & Windyga, B (2007) The distribution of enterotoxin and enterotoxin-like genes in *Staphylococcusaureus* strains isolated from nasal carriers and food samples. Int J Food Microbiol 117 (3) : 319-323.

Mehrotra, M, Wang, G, & Johnson, WM (2000) Multiplex PCR for detection of genes for *Staphylococcusaureus* enterotoxins, exfoliative toxins, toxic shock syndrome toxin 1, and methicillin resistance. J Clin Microbiol 38 (3) : 1032-1035.

Mohsenzadeh, M, Ghazvini, K, & Azimian, A (2015) Frequency of specific agr groups and antibiotic resistance in *Staphylococcusaureus* isolated from bovine mastitis in the northeast of Iran. Vet Res Forum an Int Q J 6 (4) : 295-299.

Monistero, V, Barberio, A, Biscarini, F, Cremonesi, P, Castiglioni, B, Graber, HU, Bottini, E, Ceballos-Marquez, A, Kroemker, V, Petzer, IM, Pollera, C, Santisteban, C, Veiga Dos Santos, M, Bronzo, V, Piccinini, R, Re, G, Cocchi, M, & Moroni, P (2020) Different distribution of antimicrobial resistance genes and virulence profiles of *Staphylococcusaureus* strains isolated from clinical mastitis in six countries. J Dairy Sci 103 (4) : 3431-3446.

Morandi, S, Brasca, M, Lodi, R, Cremonesi, P, & Castiglioni, B (2007) Detection of classical enterotoxins and identification of enterotoxin genes in *Staphylococcusaureus* from milk and dairy products. Vet Microbiol 124 (1) : 66-72.

Nazari, R, Godarzi, H, Rahimi Baghi, F, & Moeinrad, M (2014) Enterotoxin gene profiles among *Staphylococcusaureus* isolated from raw milk. Iran J Vet Res 15 (4) : 409-412.

Omoe, K, Ishikawa, M, Shimoda, Y, Hu, D-L, Ueda, S, & Shinagawa, K (2002) Detection of *seg*, *seh*, and *sei* genes in *Staphylococcusaureus* isolates and determination of the enterotoxin productivities of S. aureus isolates Harboring *seg*, *seh*, or *sei* genes. J Clin Microbiol 40 (3)

: 857-862.

Papadopoulos, P, Papadopoulos, T, Angelidis, AS, Boukouvala, E, Zdragas, A, Papa, A, Hadjichristodoulou, C, & Sergelidis, D (2018) Prevalence of *Staphylococcusaureus* and of methicillin-resistant *S. aureus* (MRSA) along the production chain of dairy products in north-western Greece. Food Microbiol 69: 43-50.

Pelisser, MR, Klein, CS, Ascoli, KR, Zotti, TR, & Arisi, ACM (2009) Ocurrence of *Staphylococcusaureus* and multiplex pcr detection of classic enterotoxin genes in cheese and meat products. Braz J Microbiol 40 (1) : 145-148.

Rahbar Saadat, Y, Imani Fooladi, AA, Shapouri, R, Hosseini, MM, & Deilami Khiabani, Z (2014) Prevalence of enterotoxigenic *Staphylococcusaureus* in organic milk and cheese in Tabriz, Iran. Iran J Microbiol 6 (5) : 345-349.

Rahimi, E (2013) Enterotoxigenicity of *Staphylococcusaureus* isolated from traditional and commercial dairy products marketed in Iran. Braz J Microbiol 44 (2) : 393-399.

Rahimi, E, & Safai, HG (2010) Detection of classical enterotoxins of *Staphylococcusaureus* strains isolated from bovine subclinical mastitis in Isfahan, Iran. Vet Microbiol 141 (3-4) : 393-394.

Regasa, S, Mengistu, S, & Abraha, A (2019) Milk Safety Assessment, Isolation, and Antimicrobial Susceptibility Profile of *Staphylococcus aureus* in Selected Dairy Farms of Mukaturi and Sululta Town, Oromia Region, Ethiopia. Vet Med Int 2019: 3063185.

Riva, A, Borghi, E, Cirasola, D, Colmegna, S, Borgo, F, Amato, E, Pontello, MM, & Morace, G (2015) Methicillin-resistant *Staphylococcusaureus* in raw milk: Prevalence, SCCmec typing, enterotoxin characterization, and antimicrobial resistance patterns. J Food Prot 78 (6) : 1142-1146.

Riyaz-Ul-Hassan, S, Verma, V, & Qazi, GN (2008) Evaluation of three different molecular markers for the detection of *Staphylococcusaureus* by polymerase chain reaction. Food Microbiol 25 (3) : 452-459.

Sahebekhtiari, N, Nochi, Z, Eslampour, M, Dabiri, H, Bolfion, M, Khoramian, B, Zali, M, & Emaneini, M (2011) Characterization of *Staphylococcusaureus* strains isolated from raw milk of bovine subclinical mastitis in Tehran and Mashhad. Acta Microbiol Immunol Hung 58: 113-121.

Song, M, Bai, Y, Xu, J, Carter, MQ, Shi, C, & Shi, X (2015) Genetic diversity and virulence potential of *Staphylococcusaureus* isolates from raw and processed food commodities in Shanghai. Int J Food Microbiol 195: 1-8.

Titouche, Y, Hakem, A, Houali, K, Meheut, T, Vingadassalon, N, Ruiz-Ripa, L, Salmi, D, Chergui, A, Chenouf, N, Hennekinne, JA, Torres, C, & Auvray, F (2019) Emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) ST8 in raw milk and traditional dairy products in the Tizi Ouzou area of Algeria. J Dairy Sci 102 (8) : 6876-6884.

Zhao, X, Yuan, X, Hu, M, Zhang, Y, Li, L, Zhang, Q, Yuan, X, Wang, W, & Liu, Y (2021) Prevalence and characterization of *Staphylococcusaureus* and methicillin-resistant *Staphylococcusaureus* isolated from bulk tank milk in Shandong dairy farms. Food Control 125: 107836.