Original Article

The examination of some virulence factors in *S. aureus* isolates obtained from the healthy human population, sheep mastitis, and cheese

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Abstract

Background: *Staphylococcus aureus* is responsible for many infections in humans and animals from skin and soft tissue infections to life-threatening diseases. In this study to explore the origin of *S. aureus* infections in humans, the antibiotic resistance profile and the variety of virulence factors in *S. aureus* isolates were examined in three groups: a healthy human population, cheese, and the milk of sheep with mastitis. **Aims:** The examination of some virulence factors in *S. aureus* isolates obtained from the healthy human population, sheep mastitis, and cheese. **Methods:** A total of 400 nasal swab samples from healthy students, 30 cheese samples, and 122 sheep milk samples were collected for the detection of *S. aureus* isolates from January 1, 2018, to March 1, 2018. The frequency of *hla*, *hlb*, *Acme/arcA*, *pvl*, and *tsst-1* virulence genes and *mecA* gene was determined in each group by PCR assay. **Results:** There was a direct relationship between the antibiotic susceptibility profile of the isolates from a healthy population and those from mastitis milk samples. Of 400 nasal samples, 15% (60/400) were positive for *S. aureus*, of which 60% (36/60) were positive for *mecA*. While 50% (15/30) of cheese samples were positive for *S. aureus*. of which 7 cases (46.66%, 7/15) were positive for *mecA*. The prevalence of *S. aureus* among students was dependent on gender (P=0.025). Also, 47.5% (58/122) of milk samples from sheep mastitis were positive for *S. aureus*, and 41.37% (24/58) were positive for the *mecA* gene. Based on PCR results, the highest rate of *hla* (68.33%, 41/60), *hlb* (53.33%, 32/60), and *Acme/arcA* (46.66%, 28/60) genes were related to a healthy population, and the highest frequency of *pvl* (41.38%, 24/58), and *tsst-1* (27.59%, 16/58) was related to milk samples (P<0.05). A significant correlation was observed between the presence of the arginine catabolic mobile element (*ACME)-arcA* gene and resistance to methicillin (P<0.05). **Conclusion:** The high rate of virulence factors in the *S. aureus* isolates obtained from mastitis and dairy products is an alert point, because they could be source of the spreading of *S. aureus* to humans. There is an essential need for continuous monitoring to control staphylococcal food poisoning.

Key words: Dairy products, Healthy population, Mastitis, *S. aureus*, Virulence factors

Introduction

Staphylococcus aureus is a leading cause of different infections from the skin and soft tissue infections to life-threatening diseases (Lindsay and Holden, 2004). The escalating prevalence of multi-drug resistant (MDR) strains is now serious challenges in clinical settings.

In animals, *S. aureus* is a predominant cause of intramammary infections (IMIs), or mastitis (Kwiatkowski *et al*., 2022). Mastitis is responsible for notable economic losses on dairy farms due to decreased milk yield, the production of unsuitable milk for consumption, and treatment costs (Peton and Le Loir, 2014; Aghamohammadi *et al*., 2018). Particularly, milk and milk products such as cheese, are considered as a reservoir of *S. aureus* strains (Szczuka *et al*., 2022). On the other hand, *S. aureus* colonization in healthy individuals is a risk factor for further infections. For this reason, we evaluated and characterized *S. aureus* isolates in milk, cheese, and healthy individuals.

S. aureus can be directly transferred to milk through an animal infected with mastitis and indirectly by an unhygienic environment. *S. aureus* colonization may occur in milk consumers often through the consumption of infected milk and dairy products (Anderson *et al*., 2011). Based on reports, *S. aureus* colonization occurs in 30 to 50% of the healthy carriers (Zeinalpour Ahrabi *et al*., 2019). The ability of contagiousness of the mastitis infection might be associated with the presence and the variation of *S. aureus* virulence factors (Magro *et al*.,

2017). Based on reports, some of the virulence factors produced by *S. aureus* play a key role in the escape of infection from the host immune system (Magro *et al*., 2017; Monistero *et al*., 2018). Alpha-hemolysin (*hla*) gene is usually the cause of clinical symptoms such as brain abscess, pneumonia, and sepsis (Xiao *et al*., 2016). Haemolysin beta (*hlb*) rises cytotoxicity and the adherence of *S. aureus* to bovine mammary epithelial cells (Ito *et al*., 2003), it is mostly the main cause of eye and lung infections (Dehnad *et al*., 2020). Exotoxins (such as leukocidins, enterotoxins, and toxic shock syndrome toxin-1) can support the bacteria versus host immune response. The combination of these factors seems to be essential to the incidence of infection in humans and animals (Fluit, 2012; Koymans *et al*., 2015).

Arginine catabolic mobile element (*ACME*) is known as an enhancing factor of *S. aureus* colonization in the skin and mucous membranes, which act through the neutralization of acidic pH and enhancement of the acid tolerance of pathogen (Thurlow *et al*., 2013). Panton-Valentine leukocidin LukSF-PV (*PVL*), as a pore-forming toxin, has a key role in the incidence of skin and soft tissue infections (Niemann *et al*., 2018). Because of the importance of *S. aureus* infections in humans and animals, there is an essential need for awareness regarding *S. aureus* prevalence and the relationship between the occurrence of virulence factors in healthy populations and dairy products. The present study examined the antibiotic resistance profile, and diversity of virulence factors in *S. aureus* isolates obtained from dairy products, sheep mastitis, and a healthy population of humans.

Materials and Methods

Ethics committee approval

Tabriz University of Clinical Research Ethics Committee (reference No.: IR. TBZMED. REC.1398. 989). The swab samples were obtained after written consent with a brief description of the importance of the study to the participants.

Bacterial identification

A cross-sectional study was conducted from January 1, 2018 to March 1, 2018 in several high schools from Tabriz city. A total of 400 students aged 16 to 17 years were participated in this study. The healthy students without previous antibiotic consumption (during the last three months) were included in this study. The nasal swab samples were collected from 400 students, transferred into tryptic soy broth media, and incubated overnight at 37°C. A total of 122 milk samples related to 53 dairy herds in East Azerbaijan province of Iran were obtained from ewes infected with mastitis. The cheese samples were also obtained from 150 factories producing traditional cheese. The samples were cultured for the identification of *S. aurous* isolates. The *S. aureus* isolates were diagnosed through conventional microbiological and biochemical methods (Ghavghani *et al*., 2019).

Antibiotic susceptibility testing

The antibiotic resistance profile of *S. aureus* isolates was evaluated using the disk diffusion method based on the Clinical Laboratory Standards Institute (CLSI) guidelines (Weinstein and Lewis II, 2020). Antibiotic disks (Biomaxima, Poland) were included cefazolin (30 µg), amoxicillin/clavulanic acid (20/10 µg), chloramphenicol (30 µg), penicillin (6 µg), erythromycin (15 µg), oxacillin (1 µg), clindamycin (2 µg), ciprofloxacin (5 µg), and cefoxitin (30 µg). To perform antibiotic susceptibility tests, the bacterial concentrations of 0.5 McFarland were used to inoculate onto Muller-Hinton agar plates. The inoculated plates containing the antibiotic disks were incubated overnight at 37°C. *S. aureus* ATCC 33591 (oxacillin-resistant) and *S. aureus* ATCC 29213 (oxacillin-susceptible) were utilized as positive and negative controls, respectively.

The detection of virulence factors by PCR reaction

DNA was extracted from *S. aureus* isolates by the boiling method using TE buffer (10 mM Tris, 1 mM EDTA). The quality and quantity of DNA were evaluated by the ratio of absorbance at 260 nm and 280 nm wavelength using Nanodrop (Thermo Scientific NanoDrop™). PCR reaction was performed for detection of *nucA*, *mecA*, *ACME-arc*A, *Tsst-1*, *hla*, *hlb*, and *PVL* genes using designed primers (Table 1) in a 25 µL reaction for 30 cycles (94°C for 1 min, 49°C/53°C for 1 min, 72°C for 1 min) after an initial denaturation at 94°C for 4 min. The final extension was performed at 72°C for 5 min. PCR products were visualized by 1% agarose gel electrophoresis. For further confirmation, PCR products were sequenced.

Statistical analysis

Statistical analysis was performed by SPSS version 16. Demographic and clinical variables were compared by Chi-square test. $P<0.05$ was assumed as the statistical significance.

Results

Frequency of *S. aureus* isolates

In total, 15% (60/400) of nasal samples were positive for *S. aureus*, of which 5.5% (22/400) of the isolates were from female students, and 9.5% (38/400) of them were obtained from male students. Based on the results, the frequency of *S. aureus* was dependent on gender ($P=0.025$). Based on disk diffusion results, 98.33% of the isolates were resistant to a penicillin antibiotic, and 18.33% (11/60) of the isolates were methicillin-resistant *Staphylococcus aureus* (MRSA) based on resistance to cefoxitin (Fig. 1). Among 122 milk samples obtained from mastitis ewes, 58 (47.5%) *S. aureus* isolates were identified, of which 45% (26/58) of them were resistant to cefoxitin and MRSA. Of 15 *S. aurous* in cheese products, 7 cases (46.66%) were MRSA (Fig. 1).

Table 1: The sequence of primers used for the detection of virulence factors by PCR reaction

Primer	Primer sequence (5′→3′)	Annealing Tm (°C)	Size (bp)	Reference
nucA	F: 5′-GCGATTGATGGTGATACGGTT-3′ R: 5′-CAAGCCTTGACGAACTAAAGC-3′	53	276	Dehnad *et al*. (2020)
mecA	F: 5′-AGAAATGACTGAACGTCC-3′ R: 5′-ATTCCACATTGTTTCGGTC-3′	49	305	Dehnad *et al*. (2020)
Tsst-1	F: 5′-ACAAGCGCTATTTTTATTTCAG-3′ R: 5′-CCCATCCCCAACCACTTTT-3′	49	271	Zeinalpour Ahrabi *et al*. (2019)
Hla	F: 5′-GTACAGTTGCAACTACCT-3′ R: 5′-CTTTCCAGCCTACTTTTTTATCAGT-3′	49	253	Dehnad *et al*. (2020)
Hlb	F: 5′-GTGCACTTACTGACAATAGTGC-3′ R: 5′-GTTGATGAGTAGCTACCTTCAGT-3′	49	313	Dehnad *et al*. (2020)
Acme-arcA	F: 5′-CTAGGTGCATAAATGTACGTG-3′ R: 5′-CCAGAAGTACGCGAGAAC-3′	49	577	Sabat *et al*. (2015)
PVL	F: 5′-AGGTAAAATGTCTGGACATG-3′ R: 5′-GCATCAACTGTATTGGATAGC-3′	49	427	Hoppe *et al*. (2018)

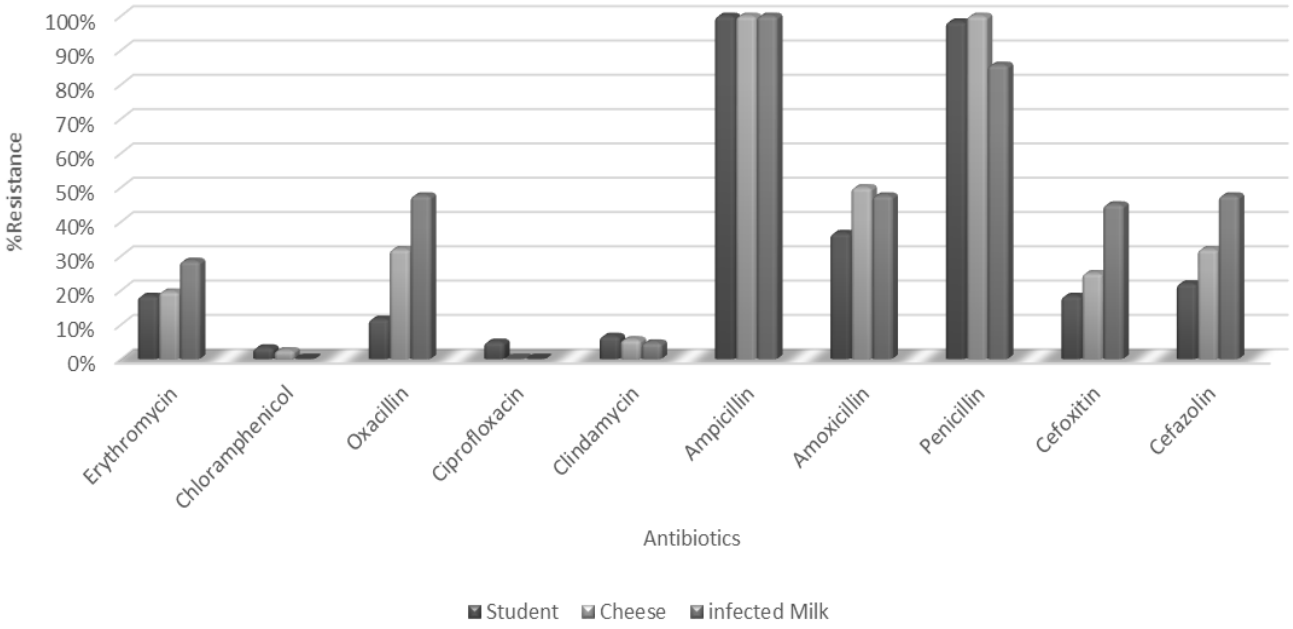

Fig. 1: The comparison of antibiotic susceptibility profile of the *S. aureus* isolates in three groups including health community, ewe's mastitis, and cheese products performed using disc diffusion method

Detection of *mecA* resistance gene and virulence factors by PCR

After the confirmation of *S. aureus* isolates by *nucA* amplification, the presence of *hla*, *hlb*, *ACME/arcA*, *pvl*, and *tsst-1* virulence genes and *mecA* gene was examined by PCR assay (Figs. 2A-F). Based on the results, 54.54% (36/60) of the student isolates were positive for *mecA*, and 46.66% (28/60) were positive for *ACME-arcA* gene. The highest rate of *PVL* was related to the isolates obtained from mastitis samples 41.38% (24/58). Based on the results, 11.66% (7/60) of the student isolates were positive for both *PVL* and *ACME-arcA* genes. A direct relationship was found between resistance to methicillin, and the presence of the *ACME-arcA* gene (P<0.05), whereas 90% (9/10) of the *PVL* positive isolates were sensitive to methicillin (P<0.05). The incidence rate of *PVL* and *ACME-arcA* genes in student isolates was independent of gender (P=0.337, P=0.142, respectively). In mastitis isolates, (27.58%, 16/58) cases were positive for three virulence factors, and (8.62%, 5/58) isolates were positive for both *PVL* and *ACME-arcA* genes. The highest frequency of *PVL* (41.38%, 24/58) and *Tsst-1* (27.59%, 16/58) was related to mastitis samples. Regarding cheese samples, frequency of virulence factors was including *ACME-arcA* (13.33%, 2/15), *Tsst-1* (20%, 3/15), *hla* (26.66%, 4/15), and *hlb* (1/15, 6.66%) (Fig. 3).

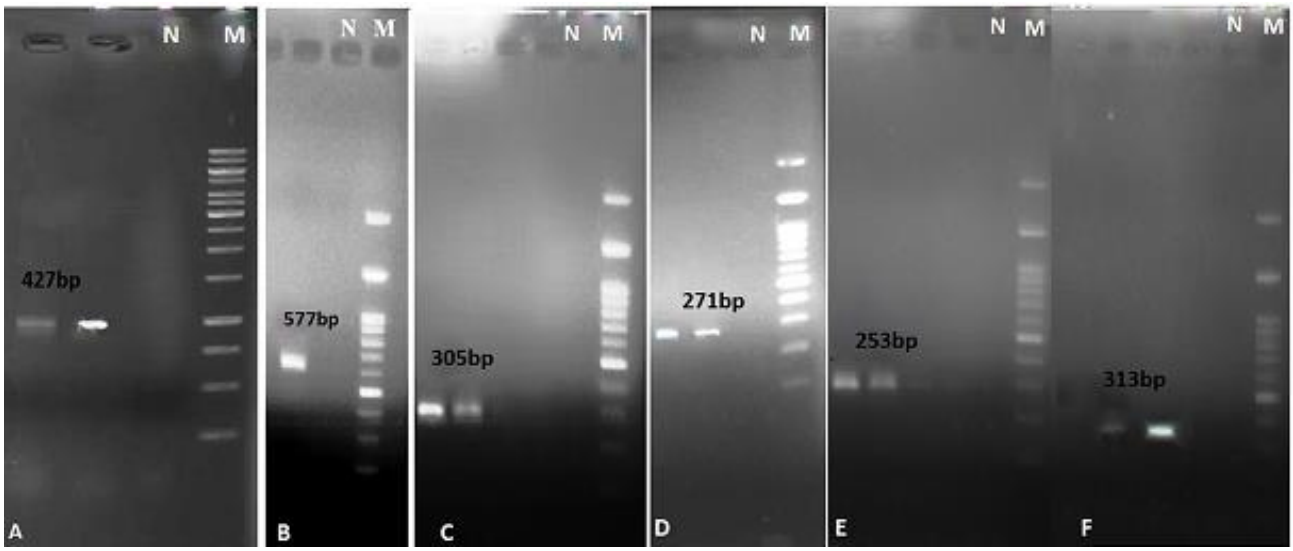

Fig. 2: PCR amplification of *pvl*, *ACME-arcA*, *mecA*, *Tsst-1*, *hla*, and *hlb* gene fragments by specific primers. (**A**) PCR reaction to detect *pvl* gene as a band of 427 bp, (**B**) Amplification of *ACME-arcA* gene fragment as a sharp band of 577 bp, (**C**) A single band of 305 bp related to *mecA* gene fragment, (**D**) A single band of 271 bp related to *Tsst-1* gene, (**E**) A single band of 253 bp related to *hla* gene, and (**F**) A single band of 313 bp related to *hlb* gene displayed in electrophoresis gel. M: Marker 100 bp, P: Positive control, and N: Negative control

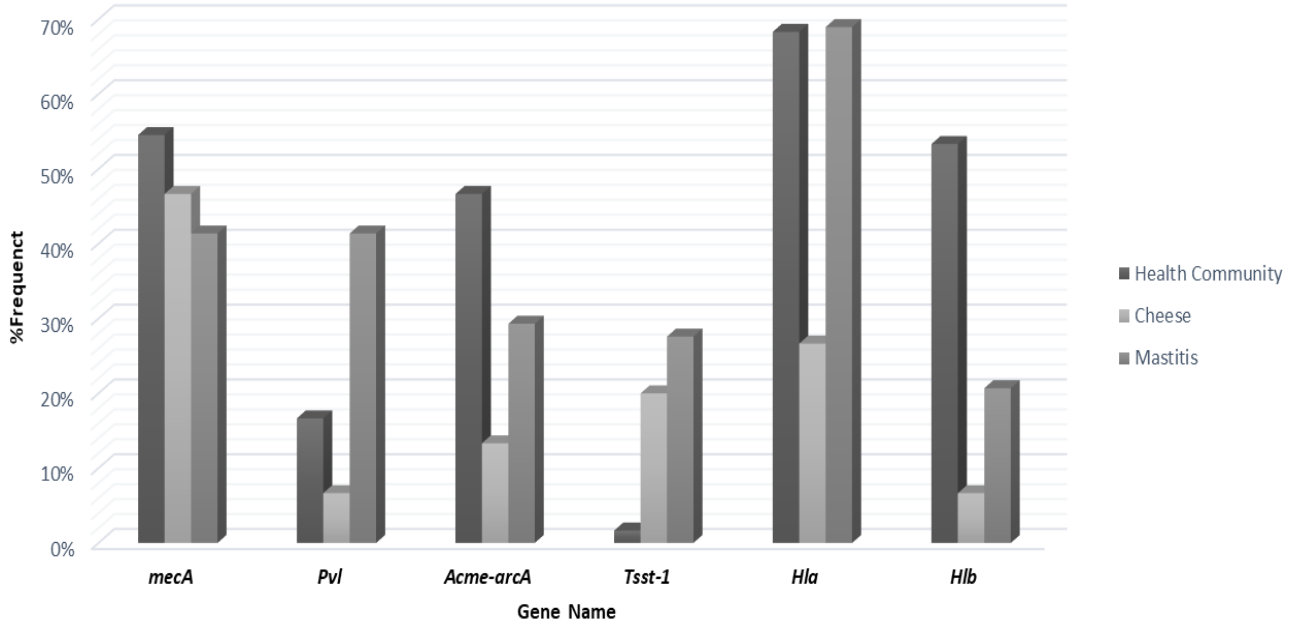

Fig. 3: The comparison of frequency of virulence genes in three groups including health community, ewes mastitis, and cheese products performed by PCR assay

Discussion

The variability in virulence factors plays a key role in *S. aureus* pathogenicity and intramammary infections in dairy animals. A 2.75% prevalence of MRSA was found in healthy students in our region (North-West of Iran), which were less than the reported rate from students in the central part of Iran (4.5%) (Japoni-Nejad *et al*., 2013) and the results obtained from farm workers (8.7%) in Turkey (den Heijer *et al*., 2013); (Garipcin and Seker, 2015). Based on the results, the frequency of MRSA colonization in this study was dependent on gender, consistent with similar studies carried out regarding a higher prevalence of MRSA carriage in men (Humphreys *et al*., 2015); (Garoy *et al*., 2019). Based on our results, 16.66% of the student isolates were positive for the *PVL* gene and 46.66% of the cases were positive for the *ACME-arcA* gene. These findings were not consistent with a study completed in the center of Iran, with a prevalence of 17% and 20% for *ACME-arcA* and *PVL* genes, respectively (Fard-Mousavi *et al*., 2015). Also, 11.66% of the *PVL*-positive isolates were positive for the *ACME-arcA* gene. Consistent with previous research (Motamedi *et al*., 2015), in this study, there is a direct

association between the frequency of *mecA* positive strains and the presence of the *ACME-arcA* gene in the student population. In contrast, 85.71% *PVL* positive isolates were MSSA indicating a lack of association between the occurrence of *PVL* and the rate of MRSA.

Regarding the presence of *S. aureus* in dairy products, 50% of cheese samples (15/30) were positive for *S. aureus*, while 46.66% of them were positive for *mecA*. Also, 47.54% of mastitis samples were positive for *S. aureus*, and 41.37% were positive for the *mecA* gene. The prevalence of *S. aureus* in mastitis samples was less than the previous report (60%) from Shahrekord, Iran (Ebrahimi *et al*., 2014) but more than the prevalence of *S. aureus* (13.82%) in the North-West of Iran (Dastmalchi Saei and Panahi, 2020).

The highest rate of *hla* (68.33%, 41/60) and *hlb* (53.33%, 32/60), and *ACME/arcA* genes were related to the students, and the highest frequency of *pvl* (41.38%, 24/58), and *tsst-1* (16/58, 27.59%) was related to the mastitis samples. The prevalence of *ACME*-positive MRSA strains in a healthy population can be one of the leading causes of skin infections. The rate of *tsst-1* in mastitis isolates was less than in a previous study (44.19%) in the North-West of Iran (Dastmalchi *et al*., 2013). Also, the *PVL* rate was more than in the previous studies (Ünal *et al*., 2012; Mistry *et al*., 2016; Wang *et al*., 2018). The frequency of *tsst-1* in cheese samples was almost similar to a report from Brazil (Castro *et al*., 2020). Our findings revealed a high diversity of virulence factors and antibiotic resistance profiles in dairy products and mastitis compared to the healthy population. One of the limitations of this study was related to the small sample sizes.

The presence of *S. aureus* virulence factors in the mastitis and dairy products is an alert point. Because contaminated dairy products are sources of *S. aureus* infection in humans. These results highlight an essential need for continuous monitoring to control staphylococcal food poisoning.

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Conflict of interest

The authors have no conflict of interest.

References

Aghamohammadi, M; Haine, D; Kelton, DF; Barkema, HW; Hogeveen, H; Keefe, GP and Dufour, S (2018). Herd-level mastitis-associated costs on Canadian dairy farms. Front. Vet. Sci., 5: 1-12.

Anderson, H; Hinds, P; Hurditt, S; Miller, P; McGrowder, D and Alexander-Lindo, R (2011). The microbial content of unexpired pasteurized milk from selected supermarkets in a developing country. Pac. J. Trop. Biomed., 1: 205-211.

Castro, R; Pedroso, S; Sandes, S; Silva, G; Luiz, K; Dias, R; Figueiredo, H; Santos, S; Nunes, A and Souza, M (2020). Virulence factors and antimicrobial resistance of *Staphylococcus aureus* isolated from the production process of Minas artisanal cheese from the region of Campo das Vertentes, Brazil. J. Dairy Sci., 103: 2098-2110.

Dastmalchi, SH; Aghdasi, S and Mohammad, ZH (2013). Survey of accessory gene regulator (*agr*) groups and TSST-1 encoding gene (*tst*) in *Staphylococcus aureus* isolated from ewes with mastitis in the northwest of Iran. Iran. J. Vet. Res., 14: 334-340.

Dastmalchi Saei, H and Panahi, M (2020). Genotyping and antimicrobial resistance of *Staphylococcus aureus* isolates from dairy ruminants: differences in the distribution of clonal types between cattle and small ruminants. Arch. Microbiol., 202: 115-125.

Dehnad, A; Agdam, MHG; Rahbarnia, L; Naghili, B and Saffarian, P (2020). Detection of hemolysine genes in methicillin-resistant *S. aureus* isolates obtained from a healthy population in north-west of Iran. Gene Rep., 21: 100874.

den Heijer, CD; van Bijnen, EM; Paget, WJ; Pringle, M; Goossens, H; Bruggeman, CA; Schellevis, FG; Stobberingh, EE and Team, AS (2013). Prevalence and resistance of commensal *Staphylococcus aureus*, including meticillin-resistant *S aureus*, in nine European countries: a cross-sectional study. Lancet Infect. Dis., 13: 409-415.

Ebrahimi, A; Soleimani, F; Motamedi, A; Shams, N and Lotfalian, S (2014). Study on some characteristics of Staphylococci isolated from sheep sub clinical mastitis milk in Shahrekord, Iran. B.J.M., 2: 57-62.

Fard-Mousavi, N; Mosayebi, G; Amouzandeh-Nobaveh, A; Japouni-Nejad, A and Ghaznavi-Rad, E (2015). The dynamic of *Staphylococcus aureus* nasal carriage in Central Iran. Jundishapur J. Microbiol., 8: e20760.

Fluit, A (2012). Livestock-associated *Staphylococcus aureus*. Clin. Microbiol. Infect., 18: 735-744.

Garipcin, M and Seker, E (2015). Nasal carriage of methicillin-resistant *Staphylococcus aureus* in cattle and farm workers in Turkey. Vet. Arh., 85: 117-129.

Garoy, EY; Gebreab, YB; Achila, OO; Tekeste, DG; Kesete, R; Ghirmay, R; Kiflay, R and Tesfu, T (2019). Methicillin-resistant *Staphylococcus aureus* (MRSA): prevalence and antimicrobial sensitivity pattern among patients—a multicenter study in Asmara, Eritrea. Can. J. Infect. Dis. Med. Microbiol., 2019: 1-9.

Ghavghani, FR; Rahbarnia, L; Naghili, B; Dehnad, A; Bazmani, A; Varshochi, M and Agdam, MHG (2019). Nasal and extra nasal MRSA colonization in hemodialysis patients of north-west of Iran. BMC Res. Notes. 12: 1-5.

Hoppe, PA; Hanitsch, LG; Leistner, R; Niebank, M; Bührer, C; von Bernuth, H and Krüger, R (2018). Periorbital infections and conjunctivitis due to Panton-Valentine Leukocidin (PVL) positive *Staphylococcus aureus* in children. BMC Infect. Dis., 18: 371-375.

Humphreys, H; Fitzpatick, F and Harvey, BJ (2015). Gender differences in rates of carriage and bloodstream infection caused by methicillin-resistant *Staphylococcus aureus*: are they real, do they matter and why? Arch. Clin. Infect. Dis., 61: 1708-1714.

Ito, T; Okuma, K; Ma, XX; Yuzawa, H and Hiramatsu, K (2003). Insights on antibiotic resistance of *Staphylococcus aureus* from its whole genome: genomic island SCC. Drug. Resist. Updat., 6: 41-52.

Japoni-Nejad, A; Rezazadeh, M; Kazemian, H;

Fardmousavi, N; van Belkum, A and Ghaznavi-Rad, E (2013). Molecular characterization of the first community-acquired methicillin-resistant *Staphylococcus aureus* strains from Central Iran. Int. J. Infect. Dis., 17: 949-954.

Koymans, KJ; Vrieling, M; Gorham, RD and van Strijp, JA (2015). Staphylococcal immune evasion proteins: structure, function, and host adaptation. Curr. Top. Microbiol. Immunol., 409: 441-489.

Kwiatkowski, P; Masiuk, H; Pruss, A; Łopusiewicz, Ł; Sienkiewicz, M; Wojciechowska-Koszko, I; Roszkowska, P; Bania, J; Guenther, S and Dołęgowska, B (2022). Clonal diversity, antimicrobial susceptibility and presence of genes encoding virulence factors in *Staphylococcus aureus* strains isolated from cut wound infections. Curr. Microbiol., 79: 1-11.

Lindsay, JA and Holden, MT (2004). S*taphylococcus aureus*: superbug, super genome? Trends. Microbiol., 12: 378-385.

Magro, G; Biffani, S; Minozzi, G; Ehricht, R; Monecke, S; Luini, M and Piccinini, R (2017). Virulence genes of *S. aureus* from dairy cow mastitis and contagiousness risk. Toxins. 9: 195.

Mistry, H; Sharma, P; Mahato, S; Saravanan, R; Kumar, PA and Bhandari, V (2016). Prevalence and characterization of oxacillin susceptible mecA-positive clinical isolates of *Staphylococcus aureus* causing bovine mastitis in India. PLoS One. 11: e0162256.

Monistero, V; Graber, HU; Pollera, C; Cremonesi, P; Castiglioni, B; Bottini, E; Ceballos-Marquez, A; Lasso-Rojas, L; Kroemker, V and Wente, N (2018). *Staphylococcus aureus* isolates from bovine mastitis in eight countries: genotypes, detection of genes encoding different toxins and other virulence genes. Toxins. 10: 247.

Motamedi, H; Abadi, SSR; Moosavian, SM and Torabi, M (2015). The association of Panton-valentine leukocidin and *mecA* genes in methicillin-resistant *staphylococcus aureus* isolates from patients referred to educational hospitals in Ahvaz, Iran. Jundishapur J. Microbiol., 8: e22021.

Niemann, S; Bertling, A; Brodde, MF; Fender, AC; Van de Vyver, H; Hussain, M; Holzinger, D; Reinhardt, D; Peters, G and Heilmann, C (2018). Panton-Valentine Leukocidin associated with *S. aureus* osteomyelitis activates platelets via neutrophil secretion products. Sci. Rep., 8: 1-15.

Peton, V and Le Loir, Y (2014). *Staphylococcus aureus* in veterinary medicine. Infect. Genet. Evol., 21: 602-615.

Sabat, AJ; Ilczyszyn, WM; van Rijen, M; Akkerboom, V; Sinha, B; Kluytmans, J; Miedzobrodzki, J; Grundmann, H and Friedrich, AW (2015). Genome-wide analysis reveals two novel mosaic regions containing an ACME with an identical DNA sequence in the MRSA ST398-t011 and MSSA ST8-t008 isolates. J. Antimicrob. Chemother., 70: 1298-1302.

Szczuka, E; Porada, K; Wesołowska, M and Łęska, B (2022). Occurrence and characteristics of *Staphylococcus aureus* isolated from dairy products. Molecules. 27: 4649.

Thurlow, LR; Joshi, GS; Clark, JR; Spontak, JS; Neely, CJ; Maile, R and Richardson, AR (2013). Functional modularity of the arginine catabolic mobile element contributes to the success of USA300 methicillin-resistant *Staphylococcus aureus*. Cell Host Microbe. 13: 100-107.

Ünal, N; Askar, Ş; Macun, HC; Sakarya, F; Altun, B and Yıldırım, M (2012). Panton-Valentine leukocidin and some exotoxins of *Staphylococcus aureus* and antimicrobial susceptibility profiles of staphylococci isolated from milks of small ruminants. Trop. Anim. Health Prod., 44: 573-579.

Wang, W; Lin, X; Jiang, T; Peng, Z; Xu, J; Yi, L; Li, F; Fanning, S and Baloch, Z (2018). Prevalence and characterization of *Staphylococcus aureus* cultured from raw milk taken from dairy cows with mastitis in Beijing, China. Front. Microbiol., 9: 1123.

Weinstein, MP and Lewis II, JS (2020). The clinical and laboratory standards institute subcommittee on anti-microbial susceptibility testing: background, organization, functions, and processes. J. Clin. Microbiol., 58: e01864-19.

Xiao, M; Zhao, R; Zhang, Q; Fan, X; O'Sullivan, MV; Li, DF; Wang, XY; Wu, HL; Kong, F and Xu, YC (2016). Genotypic diversity of *Staphylococcus aureus* α-hemolysin gene (*hla*) and its association with clonal background: implications for vaccine development. PLoS One. 11: e0149112.

Zeinalpour Ahrabi, S; Rahbarnia, L; Dehnad, A; Naghili, B; Ghaffari Agdam, MH and Nazari, A (2019). Incidence of oxacillin-susceptible mecA-positive *Staphylococcus aureus* (OS-MRSA) isolates and TSST-1 virulence factor among high school students in Tabriz, Northwest of Iran. Arch. Clin. Infect. Dis., 14: e85341.